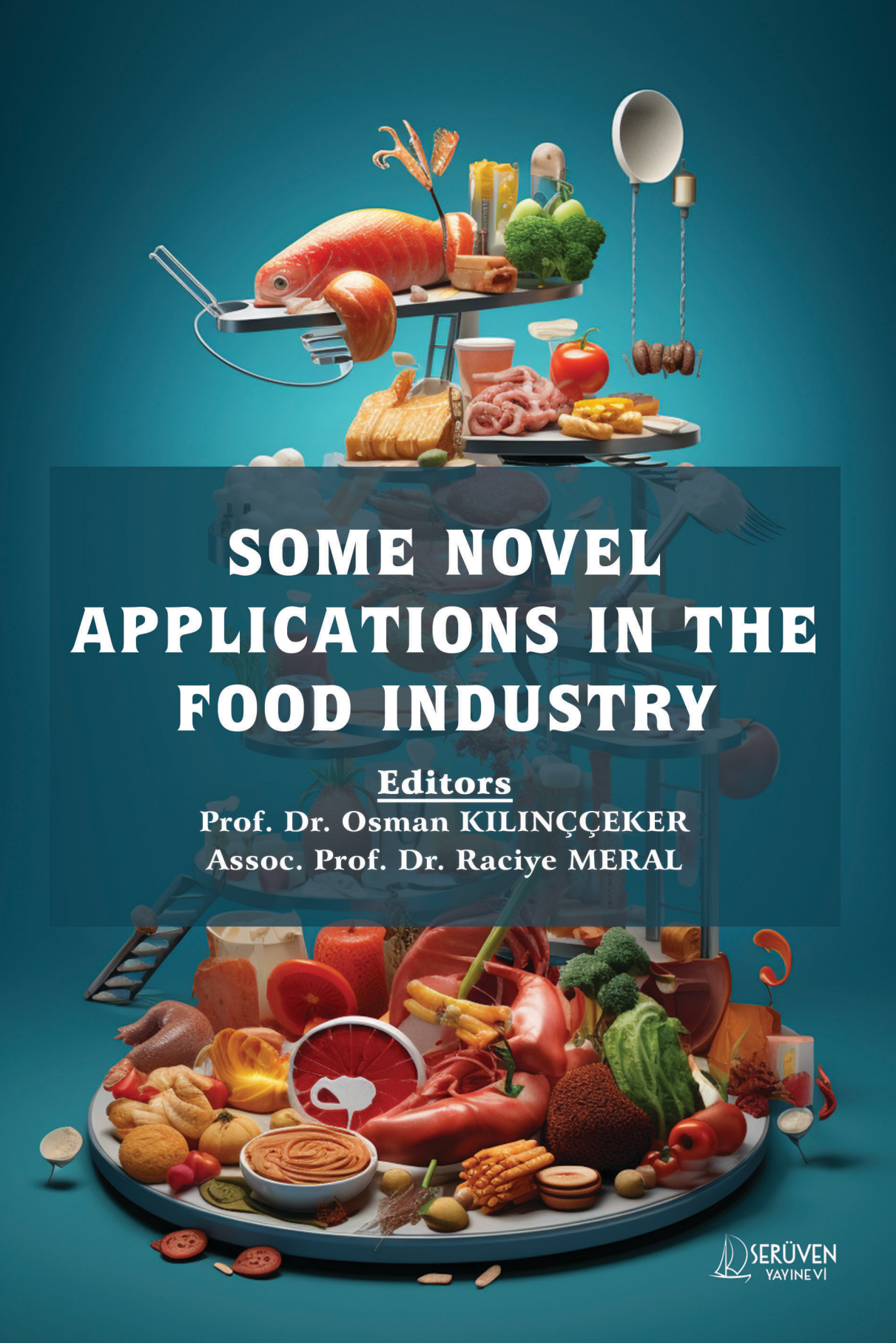

SOME NOVEL APPLICATIONS IN THE FOOD INDUSTRY

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PREFACE

The rapid increase in the world's population and developments in the food sector lead both producers and consumers to seek alternative food. In addition, the development of awareness of healthy nutrition accelerates this search. As a result of the aforementioned developments, products that can meet the demands of consumers in terms of economy and health are being put forward. Especially in food raw materials or production processes, new products and methods are tried to be developed by going beyond the traditional methods. In this way, while protecting the nutrition and health of the consumers, an economic balance is tried to be achieved. It is aimed to develop maximum quality products by using new methods. When we look at the literature in line with the aforementioned, it is understood that while many studies stand out, it is understood that the researches on some subjects are insufficient and these subjects should be examined. In this book, some of these topics are discussed and their applications in the food industry are emphasized. In this way, it is desired to give an idea about the mentioned applications to both producers and consumers. In addition, it is aimed to present an alternative reference book to academics who conduct research on these subjects. In the hope that this book will be useful for the food industry and the academic community, we would like to thank all the authors of the chapters and the staff of the Serüven Publishing House who contributed to the writing of the book

CONTENTS

Chapter 1

POTENTIAL USE OF WILD LACTIC ACID BACTERIA IN CHEESE PRODUCTION

Tuğba KARABEKMEZ ERDEM[1]

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1. INTRODUCTION

Cheese is the general name of a group of fermented milk products with high nutritional value obtained by processing the milk in different ways. It is estimated that there are over 2,000 different types of cheese around the world. The production of all varieties of cheese involves a generally similar protocol, such as coagulating the milk with rennet or harmless organic acids, cutting the curd, draining the whey, shaping the curd, salting the cheese, adding harmless substances to give it taste and smell, and maturing (Yetişemiyen, 1997; Hayaloğlu & Özer, 2011; Tekinşen & Akar, 2017; Tilocca, Costanzo, Morittu, Spina, Soggiu, Britti, Roncada, & Piras, 2020).

As a historical aspect, it is believed to have evolved 8,000 years ago in the half-crescent area (Mesopotamia) between the Euphrates and Tigris rivers, now known as Iraq, when certain plants and animals were domesticated as food sources during the so-called agricultural evolution (Fox & McSweeney, 2017).

When the development process of cheese production is examined, it is seen that the production was generally made from raw milk until the 1940s. However, in these years, many important diseases and many deaths, including tuberculosis and scarlet fever, were seen, and this situation was associated with raw milk consumption. In addition to these, it is also stated that in cheeses produced from raw milk, bacteria-contaminated with milk can remain in the clot and cause defects in the cheese. Therefore, since the beginning of the 20th century, pasteurization of milk has become widespread, primarily in Denmark, France, and the United States (USA), due to the risk of obtaining high-quality and safe products and the risks of raw milk-borne diseases (Fox, 2011a; Hayaloğlu & Özer, 2011; Donelly, 2014; Fox & McSweeney, 2017).

A great deal of today's cheese is made from pasteurized milk, utilizing specialized microorganisms called starter cultures. As a result, characterized, predictable, and controllable cheeses can be produced on an industrial scale (Donelly, 2014).

Lactic acid bacteria (LAB) are important components of starter cultures used in the manufacture of many fermented dairy products (Peng, Koubaa, Bals, & Vorobiev, 2020). *Lactococcus, Lactobacillus*, and *Streptococcus* are the most commonly used LAB starter cultures in cheese production. Their primary role in fermentation is the consistent generation of lactic acid, along with metabolic products that regulate the flavor, texture, and nutritional properties of dairy products (Powell & Broome, Limsowtin, 2011; Li, Huang, Zeng, Ge, Lin, Zhang, Chen, Wang, & Shi, 2020; Arrioja-Bretón, Mani-Lopez, Palou, & Lopez-Malo, 2020; Margalho,

Kamimura, Brexo, Alvarenga, Cebeci, Janssen, Dijkstra, Starrenburg, Sheombarsing, Cruz, Alkema, Bachmann, & Sant'Anaa, 2021). However, these wild LABs play an important role in determining the characteristics of artisanal cheeses. And accordingly, the characterization and selection of these bacteria are in demand for the design of new industrially important cultures as well as for the exploration of natural biodiversity (Margalho & Feliciano, Silva, Abreu, Piran, Sant'Ana, 2020). This article will focus on the characterization of wild LAB from artisanal raw milk cheeses and their applicability for their potential use in cheese production.

2. CHARACTERIZATION AND SELECTION OF WILD LAB

A group of Gram-positive, microaerophilic, acid-tolerant, non-spore-forming cocci or rods is commonly referred to as LAB. They are distinguished by the formation of L (+) and/or D (-) lactic acid as well as the fermentation of carbohydrates, including lactose. The major genera of LAB are *Lactobacillus, Lactococcus, Streptococcus, Leuconostoc, Pediococcus, Bifidobacterium, Carnobacterium, Weissella,* and *Enterococcus* (Fox, 2011b).

LAB used as starter cultures provide a wide range of sensory and textural products with properties such as acidifying, proteolytic, lipolytic, antagonistic, and exopolysaccharide production (Peng et al., 2020) (Figure 1.).

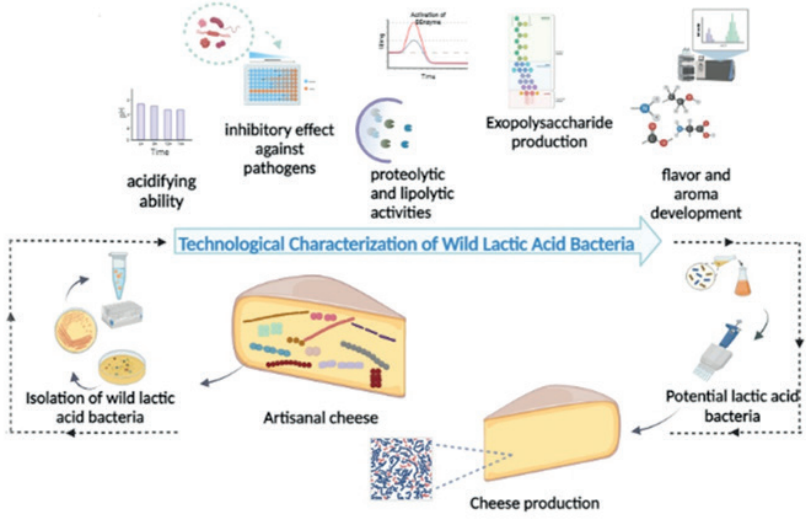

Figure1. Technological characterization of wild lactic acid bacteria for cheese production.

As was previously noted, starter cultures' main function is to convert lactose into lactic acid via lactose metabolism. The presence of lactic acid has effects such as the coagulation of milk caseins to form curd and syneresis (Vázquez-Velázquez, Salvador-Figueroa, Adriano-Anaya, DeGyves–Córdova, & Vázquez-Ovando, 2018). Several isolates belonging to *Lactobacillus, Leuconostoc*, and *Enterococcus* from artisanal Pico cheese demonstrated high acidification capacity by reducing the pH of pasteurized milk below 5.3 after 6 hours of incubation at 30 °C (Câmara, Dapkevicius, Riquelme, Elias, Silva, Malcata, & Dapkevicius, 2019). Similarly, *Enterococcus* species isolated from artisanal Carpathian cheese demonstrated an acceptable ability to produce lactic acid (pH 4.99-5.10) (Slyvka, Tsisaryk, Musii, Kushnir, Koziorowski, & Koziorowska, 2022).

In addition to lactic acid, the metabolism of lactose also results in the production of compounds that give food its flavor and odor, including acetic acid, acetaldehyde, acetoin, diacetyl, and ethanol. Another starter culture metabolism that directly affects the flavor and aroma development of cheese is citrate metabolism. LABs metabolize citrate, which is found in small amounts in milk and cheese, and is responsible for the formation of pyruvate, also called a key component in the formation of many flavor compounds (Broome & Powell, Limsowtin, 2011; Vázquez-Velázquez et al., 2018).

One of the most important biochemical events in cheese is the lipolysis and metabolism of fatty acids, proteolysis, and amino acid catabolism. It is well known that starter cultures utilize a wide range of enzymes through the release of intracellular and extracellular enzymes to carry out their roles (Fox, 2011a; Hill & Kethireddipalli,2013).

In these enzyme systems, which support the formation of aromatic compounds besides the development of texture in cheese, milk lipids are converted to free fatty acids, glycerol, monoacylglycerides, or diacylglycerides, while casein is hydrolyzed to peptides, and peptides are degraded into amino acids (Yilmaz, Ayar, & Akin, 2005; Broome et al., 2011; Johnson, 2017; Hickey, Fallico, Wilkinson, & Sheehan, 2018).

In artisanal Serpa cheese, it is indicated that FFAs (Free fatty acids), peptides, and FAAs (Free amino acids) resulting from both lipolytic and proteolytic activities are related to organoleptic compounds and are required for starter culture design. And strains *Lactiplantibacillus plantarum* PL1 and *Lactiplantibacillus plantarum* PL4 were determined to exhibit both lipolytic and proteolytic properties (Araújo-Rodrigues, dos Santos, Ruiz-Moyano, Tavaria, Martins, Alvarenga, & Pintado, 2021).

LABs, through the production of lactic acid and thus lowering pH, inhibit the growth of many undesirable microorganisms. In addition to lactic acid,

several organic acids (acetic acid, propionic acid, etc.), and antimicrobial substances such as hydrogen peroxide, diacetyl, inhibitory enzymes, and bacteriocins are produced by LABs and exhibit antagonistic activity against pathogenic bacteria (Hernandez & Cardell, Zarate, 2005; Molloy, Hill, Cotter, & Ross Hernandez, 2017; Vázquez-Velázquez et al., 2018).

Diacetyl, a volatile product of citrate metabolism in LABs, is also responsible for the desired butter flavor or butterscotch notes in dairy products, along with its inhibitory effect on foodborne pathogens. The strains isolated from traditional Italian cheeses (Pecorino and Ragusano cheese) and traditional Brazilian cheeses (Marajó and Pará cheese) were *Lactobacillus delbrueckii*, *Lactobacillus rhamnosus*, *L. plantarum*, *Pediococus acidilactici*, and *Lactococus lactis*, which synthesized varying amounts of diacetyl (Rincon-Delgadillo, Lopez-Hernandez, Wijaya, & Rankin, 2012; Nicosia, Pino, Maciel, Sanfilippo, Caggia, de Carvalho, & Randazzo, 2023). The *Wiessella parameseneteroides* isolated from Brazilian artisanal cheese also showed this ability (Teixeira, Fusieger, Martins, de Freitas, Vakarelova, Nero, & de Carvalho, 2021). Also among the LAB isolates, *Enterococus faecium* strains, which are isolated from Artisanal White cheese, were found to be good candidates for utilization in industrial cheese production with diacetyl production ability (Albayrak & Duran, 2021).

Bacteriocins are proteins or peptides ribosomally synthesized by Gram-positive and Gram-negative bacteria. And it is commonly known that artisanal cheeses offer an important source of bacteriocin (Hosken, Melo Pereira, Lima, Ribeiro, Magalhães Júnior, & Martin, 2023). According to Gutiérrez-Cortés, Suarez, Buitrago, Nero, & Todorov (2018), artisanal cheeses offer an important source of bacteriocin, such that P. pentosaceus strains isolated from artisanal Minas cheese can produce bacteriocins that have potential use as preservatives in food. It has been reported that bacteriocin-producing LAB (*L. plantarum* TF711) isolated from raw Tenerife goat cheese may exert inhibitory effects against pathogenic *Bacillus cereus, Clostridium sporogenes*, and *Staphylococcus aureus* when used as a bioprotective (Hernandez et al., 2005).

The *E. faecium* strains from artisanal Carpathian cheese showed moderate antagonistic activity against *Escherichia coli*, *Salmonella enteritidis, Enterococcus aerogenes, Proteus mirabilis,* and *Pseudomonas aeruginosa* (Slyvka et al., 2022). In a previous study, *L. plantarum* strains isolated from an artisanal Brazilian cheese were selected due to their ability to inhibit common cheese spoilage caused by *Penicillium* species (Mareze, Ramos-Pereira, Santos, Beloti, & López-Díaz, 2022). *E.faecium* strains derived from artisanal Tulum cheeses indicated antagonistic activity against both *Listeria monocytogenes* ATCC 7644 and *S. aureus*

ATCC 25923 (Özkan, Demirci & Akın, 2021) while *E. faecium* CRL 1879 isolated from Northwestern Argentina artisanal cheeses demonstrated inhibitory capacity against only *L. monocytogenes* (Suárez, Weckx, Minahk, Hebert, & Saavedra, 2020).

Exopolysaccharides (EPSs) are known as high molecular weight long-chain polymers consisting of repeating sugar units or sugar derivatives in a branched structure (Gezginç, Karabekmez-Erdem, Tatar, Ayman, Ganiyusufoğlu, & Dayısoylu, 2022). These biopolymers, produced mainly by LAB, are stated to protect them from bacteriophages, antibiotics, physical stressors, and toxic compounds. As it contributes to food preservation, flavor, and texture improvement and is characterized by several biological effects, EPS-producing LAB is used as a starter culture in the production of a broad variety of fermented milk products (Malesevic, Stanisavljevic, Miljkovic, Jovcic, Filipic, Studholme, & Kojic, 2021; Moradi, Guimarães, & Sahin, 2021). In cheese production, it has been reported that the use of EPS-producing LAB has a positive effect on the moisture content and texture of the cheese (Mende, Rohm, & Jaros, 2016). A study conducted by Özkan et al. (2021) has indicated that all the studied *E. faecium* strains, derived from artisanal goatskin Tulum cheeses, showed high EPS production. Similarly, according to Margalho et al. (2020), *Lactococcus garvieae* isolated from Brazilian artisanal cheeses exhibited high EPS production from glucose, sucrose, fructose, and lactose.

3. APPLICATION OF SELECTED WILD LAB IN CHEESES

Wild LABs are derived from naturally fermenting artisanal cheeses and contribute a wide array of flavors, aromas, and textures to artisanal cheeses. In parallel, the characterization of wild LAB strains obtained from their natural sources and the investigation of natural biodiversity are considered promising strategies for their use in cheese production and the derivation of new cultures (Albayrak & Duran, 2021; Arrioja-Bretón et al., 2020).

Hadef & Idoui, Sifour, Genay, Dary-Mourot (2023) noticed that among LAB isolated from traditional three types of cheese (dry Klila, french Klila, and Bouhezza), *L. plantarum* B15 and *Lactobacillus casei* BM10 were found to be good candidates with their potential as starter cultures. Among a wide number of strains studied for starter or adjunct cultures, *L. plantarum* and *Lactobacillus paracasei* were characterized for their acidifying capacity, enzymatic activities, and production of flavor compounds. As a result, they promised technological potential in cheese production (Abarquero, Renes, Combarros-Fuertes, Fresno, & Tornadijo, 2022). Further, the isolates *Lactococcus lactis* subsp. *lactis* L1C21M1, *L.*

paracasei L1B1E3, *Leuconostoc pseudomesenteroides* L1C1E6, *L. casei* L1A1E5, and *L. casei* L1C1E8 were dedicated as the most promising strains for the development of starter cultures or adjunct cultures (Câmara et al., 2019). Nicosia et al. (2023), suggested that *L. lactis*, *L. delbrueckii*, and *L. rhamnosus* from Italian and Brazilian cheeses be used as adjunct cultures for their technological characteristics consisting of acidifying ability, lipolytic activities, the ability to produce diacetyl and EPS, as well as aminopeptidase activities.

Artisanal Pico cheese is distinguished from other cheeses by its strong aroma. Adjunct cultures with appropriate qualities are critical in cheese manufacturing for predictable and improved cheese quality. Domingos studied the applicability of LAB strains obtained from Pico cheese in cheese manufacturing in a study. It was determined that the two strains of *Lactobacillus paracasei* subsp. *paracasei* (L3C21M6 and L2B21R3) may be utilized as adjunct cultures to achieve Pico cheese's unique properties (Domingos-Lopes, Stanton, Ross, Dapkevicius, & Silva, 2017).

CONCLUSION

Using selected wild LAB derived from artisanal cheeses as starter cultures or adjunct cultures in cheese production offers numerous benefits and possibilities. These indigenous LAB strains, naturally present in the environment and on raw materials, contribute unique flavors, aromas, and textures to cheeses, resulting in rich diversity.

Additionally, recent studies have demonstrated the potential for the industrial application of wild LAB, such as acidification activity, proteolytic activity, lipolytic activity, antagonistic activity, and ability to produce exopolysaccharides, in cheese production.

Ongoing technological advances in cheese production have focused on discovering natural biodiversity and unique qualities as a promising strategy, uncovering new industrially important cultures, and improving the selection of starter cultures or adjunct cultures. So, the application of wild LAB as adjunct cultures alongside commercial starters presents an exciting opportunity to modulate cheese characteristics.

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Chapter 2

GREEN ANALYTICAL TECHNIQUES FOR FOOD INDUSTRY

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1. INTRODUCTION

In response to the problems of the twenty-first century, green technologies have been offered as a creative approach to protect consumers and the environment as well as to create an industrial perspective that is both ecologically and commercially feasible. The creation, harvesting, storage, and sale of food raw materials are all covered by this environmentally friendly strategy. Green technology approaches can be used to analyze parameters such as food quality control, macro and micronutrients, bioactive components, geographical origin and adulteration detection, microbial contamination, and pathogen presence. Traditional analytical techniques for analyzing food quality and composition take a long time, call for sample preparation, and frequently produce toxic waste that pollutes the environment. To decrease its energy, water, and carbon footprints, the food industry must create innovative technology.

The main advantages of green technology-based methods are;

i) no solvents are used in the preliminary preparation phase,

ii) using a single device to detect all components of the sample to be analyzed,

iii) significantly reduced analysis costs,

iv) no environmental toxic waste,

v) reduced water and energy consumption,

vi) to get results quickly and safely (Kılınç & Bağdatlıoğlu, 2022)

Particularly in green analytical chemistry (GAC), efforts are made to avoid using toxic or dangerous reagents and to replace them with non-toxic ones. Additionally, energy, reagent, and solvent consumption are drastically reduced, and waste is decontaminated, or at the very least, laboratory residues are reduced (Moros, Garrigues, & Guardia, 2010). By using traditional analytical methods, which frequently result in environmental contamination (toxic wastes) and require lengthy, tedious sample preparation, it is possible to determine the quality of food (Pallone, Caramês, & Alamar, 2018). It's crucial to create and execute green viewpoints for these techniques to strike a balance between economic expansion, resource sustainability, and environmental preservation.

2. GREEN CHEMISTRY

The principles of green chemistry are now widely used in a variety of contexts, from governmental policy to industrial management to educational practice and technology development. One could argue that environmentally friendly and sustainable chemistry is a way to

alter perceptions and paradigms in the creation and manufacture of chemicals. Additionally, the overall idea of "green chemistry" permeates all of its subfields, forcing a revaluation of methods and the emergence of fresh paradigms. Green analytical chemistry is aimed to minimize or completely avoid the use of solvents, reagents, and other materials that are detrimental to people or the environment while offering speedy and energy-saving solutions (Armenta, Garrigues, & de la Guardia, 2008).

Anastas & Warner, (1998) discussed the 12 principles of green chemistry in 1998, firstly. Green chemistry is the use of safer, more environmentally friendly chemicals as well as the design of chemical products or methods that employ chemicals in an effort to minimize or completely do away with the usage of potentially harmful compounds. Green chemistry is not an organic or inorganic chemistry subfield. It entails adopting a fresh perspective on conventional chemistry. The greatest approach for us to guarantee a safer, more benign future for us all is to think about how to include sustainability in their experimental design. Galuszka et al.(2013) recently reinterpreted these 12 principles to meet analytical chemistry procedures after realizing that virtually all of them were not suitable for organic chemical processes (Galuszka, Migaszewski, & Namieśnik, 2013). In Table 1, a comparative description of Green Chemistry and Green Analytical Chemistry principles has been given.

The application of green chemistry and the focus on it in the laboratory are two themes that are becoming more prevalent in the chemistry community. Chemists all over the world are working to replace conventional processes and reactions with more eco-friendly, energy-efficient substitutes. Green chemistry can take many different shapes, from actively managing waste to redesigning experiments with more environmentally friendly ingredients. Finding a balance between affordable methods and environmentally friendly materials can be challenging, especially when weighing the pros and cons of several alternative technologies. For instance, swapping out a reagent in a process can result in less waste, but it might also produce more harmful waste. All aspects of a given analytical approach must be grasped in order to comprehend its overall greenness. Many harmful substances used in laboratories have less harmful or harmless substitutes. For instance, a more secure substitute can be used in place of ethidium bromide. Numerous pieces of laboratory equipment frequently contain hazardous substances. Mercury is present in conventional fluorescent lighting sources, for instance. A smart strategy to lower chemical use is to share chemicals or add them to a chemical inventory. Every person who utilizes chemicals should work to minimize their exposure to dangerous substances. Green

chemistry isn't only for chemists. To lessen the production of hazardous trash and your exposure to dangerous chemicals, you must make greener choices. Case studies are conducted to examine the advantages of green transformation. For example, each of the 25 researchers in the group used 1 L of solvent, typically acetone, each week to clean and/or rinse glassware, spatulas, and other tools used in their processes, according to a pollution prevention assessment of one organic chemistry research laboratory at a university. To speed up drying after washing with soap and water or to rinse a spatula after treatment, a researcher can use a solvent. The justifications for utilizing the solvent ranged from not having enough glassware available (thus needing to speed up drying) to not having good brushes for removing residue to simply cutting corners with the cleaning process. More glassware, finer brushes, and an ultrasonicator that utilizes a gentle detergent were all purchases made by the lab. Within three months, the cost of the new purchases was covered by the savings in solvent procurement and disposal. The lab then put in under-the-bench lab dishwashers, which led to even more cuts in cleaning solvent usage.

Table 1. Green chemistry and green analytical chemistry principles

Principle	Green Chemistry (Anastas & Warner, 1998)	Green Analytical Chemistry (Galuszka et al., 2013)
1	Avoid producing waste	Use direct analysis when possible.
2	Create efficient and atom economic reactions	Use a minimal sample size and a minimal number of samples.
3	Modify methods to use less dangerous materials	Make in-situ measurements
4	Produce a safer and more sustainable final product	Reduces the need for reagents and conserves energy.
5	Modify methods to use as few substances as possible	Chose miniaturized and automated procedures
6	Carry out protocols at ambient temperature and pressure	Avoid derivatization.
7	Use sustainable materials	Prevent the production of a lot of analytical waste and ensure that it is managed properly
8	Avoid or decrease derivatization	Prefer multi-analyte methods
9	Use selective catalytic reactions	Minimize the energy usage
10	Design safely degrades the final product	Prefer reagents derived from renewable sources
11	Monitor waste production	replace or remove toxic reagents.
12	Minimize danger and chemical accidents	Improve the operator's safety

Green sample preparation is the easiest way to use green analytical chemistry because it doesn't call for changing existing instrumental techniques while still having a noticeable green impact on laboratory procedures. Every analytical chemist must utilize some kind of sample

preparation, and every day, huge numbers of samples are examined in labs all around the world. Techniques for preparing samples should be improved to the greatest extent possible in order to reduce the amount of energy used, the amount of solvent used, the amount of trash produced, and the amount of operator exposure. Several methods exist for doing this, including limiting the size and amount of samples, using reusable sample extraction equipment, and avoiding harmful organic solvents (Billiard, Dershem, & Gionfriddo, 2020). Solvents play an important role from a green chemistry perspective. Availability, biodegradability, low toxicity, recyclability, and nonflammability are just a few prerequisites that a green solvent must meet (Zheng et al., 2014).

Large amounts of solvents are used in reactions and purification processes. However, neither the product's composition nor the formulation's active ingredients are determined by the solvents. The chemical industry sees the choice of solvent as being essential to a manufacturing process's overall sustainability profile (Bhandari & Raj, 2017). A few pharmaceutical corporations and institutions have created data-rich solvent selection guides with graphic assistance for speedy selection to assist chemists in choosing more environmentally friendly solvents. Every guide uses the "traffic light" approach and takes into account industrial, financial, environmental, occupational health, and safety constraints. The choice of solvents cannot be based on a single strategy. To choose the best solvent for their particular chemistry, chemists should consult solvent guidelines. For a particular chemical process, the "greenest" solvent with the lowest toxicity, fewest safety concerns, and least negative environmental effects can be chosen from these visual aids. These guides differ from one another since they are each customized for the institution or company's culture and policies. For instance, a well-publicized internal solvent selection tool was created by a pharmaceutical producer (Dunn & Perry, 2008). The tool divides solvents into categories based on worker safety (acute and chronic toxicity), process safety (flammability, and reactivity), as well as environmental and regulatory issues. A solvent replacement table is also provided, which can be used to direct "greener" solvent selections. Solvents are classed into "preferred," "usable," and "undesirable" solvents. During the years 2004 to 2006, the manufacturer research department used the solvent selection tool internally and saw a 50% decrease in the use of chlorinated solvents, a 97% decrease in the use of undesired ether, and a shift to the less toxic heptane over hexane and pentane (Beyond Benign, 2020). In Table 2, a solvent replacement table is given as an example.

Table 2. An example solvent replacement table (Dunn & Perry, 2008)

Undesirable Solvent	Alternative
Pentane	Heptane
Hexane	Heptane
Di-isopropyl ether or diethyl ether	2-MeTHF or tert-butyl methyl ether
Dioxane or dimethoxyethane	2-MeTHF or tert-butyl methyl ether
Chloroform, dichloroethane, or carbon tetrachloride	Dichloromethane
Dimethyl formamide, dimethyl acetamide or N-methylpyrrolidinone	Acetonitrile
Pyridine	Et3N (if pyridine is used as a base)
Dichloromethane (extractions)	EtOAc, MTBE, toluene, 2-MeTHF
Dichloromethane (chromatography	EtOAc/heptane
Benzene	Toluene

Another step in making the analytical processes suitable for green transformation is the use of the proper equipment with the proper strategies. The use of energy-efficient equipment is one of these solutions. Appliances including freezers, refrigerators, ice makers, vortexes, drying ovens, and water baths can all be labeled with the energy star, EU Energy, or other appropriate energy efficiency program. Making wise, environmentally responsible purchases can be facilitated by utilizing these energy efficiency programs. Ultra-low temperature freezers can use as much energy as an average household every day. Refrigerators and freezers can have their cooling systems operate more effectively if there is a one-foot clearance all the way around them. Best practices for cold storage management include defrosting freezers, vacuuming freezer and refrigerator coils, and lowering the set point of -80 °C freezers to -70 °C. Two key advantages come from cooling down your ultra-low freezer from -80 °C to -70 °C: first, it can use 30–40% less electricity, and second, it can last longer. Small water baths can use as much energy per hour as a dishwasher, while large water baths can use as much energy per hour as a window air conditioner. Because the ventilation system requires a large volume of air to circulate through the chemical fume hood in order to function, one fume hood can consume as much energy each day as 3.5 homes. By regulating the height of a movable sash that serves as a partition between the fume hood's interior and the rest of the lab, one may control the amount of air flowing through the hood. To protect the safety of lab workers, the sash should be raised when working in the hood, and it should typically be dropped once work in the hood is finished. Lowering the sash in a Variable Air Volume (VAV) fume hood also reduces the amount of air being vented by the VAV ventilation system and the speed of the exhaust fan. In a VAV fume hood, lowering the sash can result in energy savings of up to 40%. All this translates to greater green transformation and less energy use.

3. GREEN ANALYTICAL TECHNIQUES FOR FOOD ANALYSIS

As food samples frequently contain a complex mixture of proteins, fatty acids, minerals, carbs, vitamins, etc., food analysis is a challenging process. The physico-chemical composition of foods, the measurement of macro- and micronutrients (carbohydrates, proteins, lipids, fibers, minerals, and vitamins, among others), the measurement of nutritional and bioactive components, the presence of microbial contamination and pathogens, the identification of the country of origin of food products or the detection of adulteration in food matrices are some parameters that can be used to assess food quality. Food quality can be determined using traditional analytical methods, however, these methods frequently result in environmental pollution (toxic wastes) and require lengthy, laborious sample preparation (Pallone et al., 2018).

Major food components are also present in large amounts, however other analytes, such as pollutants or dietary biomarkers, are typically present at very low concentrations (nanogram per liter or gram level, and much lower). In most analytical instruments, direct food analysis is typically not feasible because of these problems. Thus, it is necessary to use analytical sample preparation techniques, especially when those techniques include features like excellent clean-up capabilities and pre-concentration for the target analytes (Saura-Cayuela et al., 2023). The green perspective in the selection of these methods has been frequently included in the studies conducted in recent years.

3.1. Green Extraction Technique

The two aspects of developing an analytical method that can be most easily modified to adhere to the green analytical chemistry principles are sample preparation and extraction methods. By using green extraction procedures, it is necessary to safeguard not only the extracted chemicals from the negative impacts of extraction but also the environment. Employing innovative green extraction techniques that have been developed as alternatives to traditional techniques aims to reduce solvent use and extraction time. Early attempts towards greener techniques aided the extraction process using various forms of heat, pressure, or radiation. These techniques, which include ultrasound-assisted extraction (UAE) pressurized solvent extraction (PSE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE), successfully enhanced the extraction process's eco-friendliness in comparison to their forebears (Kutlu, Yeşilören, İşçi, & Şakiyan, 2017).

The principle behind UAE methods is that by applying acoustic vibrations to the sample, the liquid it passes through will become cavitated, causing the particles to break free (Cheok, Chin, Yusof, Talib, & Law, 2013). According to studies, UAE is more efficient at extracting phenolics, antioxidants, and anthocyanins than other extraction methods that call for high temperatures and extended processing times (Chen, Zhao, & Yu, 2015). UAE works by subjecting the material to ultrasonic vibrations, which stir the molecules and accelerate the extraction process. Although the efficiency of extraction is improved by this procedure, a lot of solvent is still needed.

Pressurized liquid extraction is also included in the literature as accelerated solvent extraction. The technique in which water is used as a solvent is called pressurized water or sub-critical water extraction. The mechanism of this extraction takes place in four successive sequential steps in an extraction cell filled with a highly inert material (mostly sand) and sample. The first step is the separation of solutes from the various active sites in the sample matrix under pressurized and elevated temperature conditions. The second step involves the diffusion of the extraction solvent in the matrix. In the next step, the dissolved substances depending on the sample matrix pass into the extraction solvent, and finally, the extracted analyte is collected in the collection vessel. The term pressurized hot water is used to denote the condensed phase region of water between 100 °C and 374 °C. In order to keep the water in the condensed phase during the extraction, pressure application at values such as 15 bar at 200 °C and 85 bar at 300 °C is required. The dielectric constant of water, which is about 80 at 25 °C, drops to a value between the dielectric constant of methanol and ethanol at 25 °C, at the pressure required to keep the water in the liquid phase at high temperature (250 °C and 50 bar) (ε= 27). Under these conditions, water acts as an organic solvent that can dissolve analytes with a wide range of polarities (Kutlu et al., 2017; Teo, Tan, Yong, Hew, & Ong, 2010).

Microwaves are used in MAE for rapid heat of the solvent and the sample in a sealed container. This technique offers a more effective extraction and lowers the amount of solvents used. Additionally, water can be utilized as the extracting solvent instead of an organic solvent due to the temperature and pressure requirements for MAE. It is safer for the user and more environmentally friendly to do without the need for an organic solvent (Billiard et al., 2020).

In simple terms, SFE is defined as the dissolution of a substance in a fluid under supercritical conditions and then the separation of the product from the fluid by reducing the pressure (Kutlu et al., 2017). Through the use of supercritical CO_2 as the extraction fluid, SFE facilitates extraction

by directing the supercritical CO_2 flow through the sample. Because it is non-toxic, non-flammable, abundant, renewable, and waste-free, supercritical CO_2 is referred to in this context as a "green solvent"(Billiard et al., 2020).

The use of more environmentally friendly green solvents as an alternative to the solvents used in extraction has also been preferred along with green extraction techniques. These solvents are accessible non-volatile, recyclable, low or no toxicity, and environmentally friendly solvents. In the category of green solvents, water is the most frequently used solvent. Using water as a solvent is crucial for environmentally friendly procedures. Water's non-toxic, non-flammable, dependable, affordable, and able to enable quick extraction at high pressure and temperature (Paes, Dotta, Barbero, & Martínez, 2014). In addition, it has become quite popular in recent years for the extraction of bioactive chemicals from food products using deep eutectic solvents. With the use of these solvents, significant improvements in extraction study effectiveness were found.

Many researchers investigated common extraction solvents for food analyses using microextraction techniques. The chosen liquid solvents include ionic liquids (ILs) (Zhang, Cagliero, Pierson, & Anderson, 2017) and deep eutectic solvents (DESs) (Faraji, Afsharsaveh, & Shirani, 2022). Due to their low flammability and minimal vapor pressure at room temperature, ionic liquids, and their derivatives have historically been regarded as green solvents. Because of this, they are less dangerous than the traditional organic solvents employed in the laboratory for analytical extraction. In addition to this significant benefit, ILs have gained a lot of popularity due to their strong solvation qualities and impressive tunability. When a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD) are combined, DESs are created. When these two species interact, the HBD-HBA interactions lower their respective melting points, which leads to the creation of a liquid under ambient circumstances. (Makoś-Chełstowska, Kaykhaii, Płotka-Wasylka, & de la Guardia, 2022). From a green chemistry perspective, DESs share some of the characteristics of ILs, such as non-flammability and low to negligible vapor pressure at ambient temperature. They are therefore regarded as being safe solvents. When used in analytical microextraction methods, they also have excellent tunability and the capacity to interact with a variety of solutes, which is certainly advantageous. According to their solubility in aqueous media, three categories for DESs have recently been established: There are three types of DESs: (i) hydrophilic DESs, which have both HBD and HBA that are water-soluble (for example, choline chloride and phenol) (Zounr, Tuzen, & Khuhawar, 2018); (ii) quasi-hydrophobic DESs, which have only one component that is water non-soluble (for example, choline

chloride and n-dodecanol) (Triaux, Petitjean, Marchioni, Boltoeva, & Marcic, 2020); and (iii) hydrophobic DESs, in the case that both HBD and HBA are water non-soluble components (e.g., thymol and heptanoic acid (Y. Wang et al., 2022). These techniques have been investigated in the analysis of pesticides, PAHs, phenols, metals, and additives from various foods (juices (Su et al., 2017), honey (Yang, Ran, Xu, Ren, & Yi, 2019), milk (Zhang et al., 2017), beverages (Shishov, Terno, Moskvin, & Bulatov, 2020))

3.2. Green Spectroscopy Technique

Fourier Transform Infrared Spectroscopy (FT-IR), mid-infrared spectroscopy (MIR, 4000-400 cm-1), near-infrared spectroscopy (NIR, 14290-4000 cm-1), and Raman spectroscopy are some of the vibrational spectroscopy-based techniques. These techniques are classified as green analytical chemistry methods because they do not damage the sample, do not create toxic waste, and do not use solvents (Pallone et al., 2018). An additional factor is that vibrational spectroscopy techniques are typically non-destructive and could allow extremely rapid examination without any prior sample preparation, in contrast to standard methods of analysis based on ultraviolet-visible spectroscopy or electrochemistry (Moros et al., 2010).

Infrared radiation was discovered by William Herschel in the 1800s, and identification of functional groups in organic compounds was a common tool that is accomplished using (IR) infrared spectroscopy by the 1940s. In the 1970s, near-infrared spectroscopy (NIR) reflectance tools were developed that allow rapid quantitative determinations of moisture, protein, and fat in cereals and other foods (Rodriguez-Saona, Ayvaz, & Wehling, 2017). Infrared spectroscopy-based techniques are being utilized more frequently in a variety of sectors, including the clarification of organic substance structures, qualitative and quantitative analysis, and the detection of food fraud and adulteration. Using novel methods like genetic modification, NIR spectroscopy has tremendous potential for food monitoring and detection of chemical, microbiological, and physical dangers in many food types. Raman spectroscopy works by measuring the strength of an intense infrared laser beam that is directed at the sample. In physico-chemical research on foods and quantitative analysis, many researchers have combined Raman spectroscopy data with chemometric techniques.

Vibrational spectroscopy in combination with chemometrics (NIR, MIR, Raman) requires no solvent use, has little sample preparation requirements, and is quick, inexpensive, and user-friendly (Pallone et al., 2018). It has been successfully applied to investigate bioactive substances

(Caramês, Alamar, Poppi, & Pallone, 2017; Hu et al., 2016), microbiology (Cho, Bhandari, Patel, & Irudayaraj, 2015; Davis, Irudayaraj, Reuhs, & Mauer, 2010; J. Wang et al., 2015), macro-compounds (Alamar, Caramês, Poppi, & Pallone, 2016; Hell et al., 2016; Silveira Jr et al., 2016), physical properties, and frauds (Black, Haughey, Chevallier, Galvin-King, & Elliott, 2016; Márquez, López, Ruisánchez, & Callao, 2016).

3.3. Green Chromatography Technique

Gas chromatographic or liquid chromatographic techniques are used for the vast majority of organic chemical determinations. Thus, it must be ensured that these techniques have little effect on the environment. In all phases of the analysis, from sample collection and preparation to separation and conclusion, chromatographic procedures have the potential to be more environmentally friendly (Płotka et al., 2013).

Various strategies for greening chromatographic techniques are given in Table 3.

It is important to consider the environmental impact of the solvents used in chromatographic analyses. One liquid chromatograph has the capacity to produce 1-1.5 L of liquid waste every day (Welch et al., 2010). Due to the necessary solvent qualities for the eluting solvent, the choice of chromatography solvent may be limited. Resources abound that can direct scientists toward solvents that are more environmentally friendly. Simple solvent substitutions, particularly reducing the usage of chlorinated solvents, can have a significant impact. The table below lists commonly used solvents in chromatography (Table 4).

Table 3. Various strategies for greening chromatographic techniques (Płotka et al., 2013)

Liquid Chromatography (LC)		**Gas Chromatography**
Green Techniques	**Green Approach in LC**	• If possible, use hydrogen as the carrier gas rather than helium. • Heating of capillary columns by resistance • Application of fast GC • Application of GC for on-site measurements
Supercritical Fluid Chromatography (SFC) Enhanced Fluidity Liquid Chroma. (EFLC)	• Fewer solvents are utilized in the mobile phase • The greener alternative of the mobile phase • Solvent recovery • Use of direct injection techniques	

Table 4. Lists the most frequently used chromatographic solvents and their relative solvent rankings

Chemical Name	Solvent ranking based on solvent selection guides
Hexane	Hazardous
Heptane	Problematic
Dichloromethane	Problematic or hazardous
Ethyl acetate	Recommended

In food analysis, for instance, to identify degradation chemicals, HPLC is the most popular chromatographic technique. In contrast, the chromatographic procedure known as HPLC is inexpensive, popular, straightforward, and simple to use. However, using significant amounts of volatile organic solvents results in waste that are linked to environmental contamination and pose a risk to human health in order to achieve a chromatogram with narrow peaks and decreased tailing. In the majority of HPLC procedures, significant quantities of volatile organic solvents like acetonitrile (ACN) and methanol (MeOH) are utilized to reduce peak tailing and peak broadening. This produces a lot of waste that needs to be disposed of. MeOH and ACN can also be highly volatile, damage water and soils, endanger human health, and raise disposal expenses for laboratory waste.

One option is to switch out dangerous organic solvents with safer ones. With its low vapor pressure, low toxicity, and cheap disposal costs, ethanol (EtOH) is now one of the greenest organic solvents. Additionally, in the chromatographic system, EtOH's polarity is quite close to that of ACN and MeOH, providing satisfactory performance for green liquid chromatography. The greater UV cut-off (210 nm) wavelength and increased viscosity of EtOH make it unsuitable for use in the mobile phase of HPLC. By increasing the mobile phase's constant temperature to 40 C and the UV wavelength to 260 nm, these limitations can be eliminated (Duan et al., 2020). There has been numerous research where greener HPLC alternatives have been investigated and have achieved positive results (Abid et al., 2022; Duan et al., 2020).

CONCLUSION AND FUTURE ASPECTS

Analytical chemistry is one of the chemistry fields that has adopted green chemistry principles as a result of ongoing concern for the ecosystem and awareness of its state. Analytical techniques can have a severe impact on the environment and seriously endanger its users. Determining the

effects and implications of researchers' and users' usage of analytical tools is therefore crucial. It is extremely desirable to create methodologies based on the idea of "green analytical chemistry" (GAC), which promotes environmentally friendly practices by avoiding or minimizing the use and production of hazardous compounds throughout research and development operations. To limit the production of environmental contaminants, it suggests using green organic solvents, small sample sizes, fewer sample preparation procedures, quick analytical times, and less energy (Ballesteros-Vivas, Socas-Rodríguez, Mendiola, Ibáñez, & Cifuentes, 2021). As the interest in green analytical chemistry grows, new perspectives on metrics that allow the evaluation of analytical procedures have been proposed, such as the National Environmental Method Index (NEMI), Modified NEMI, analytical Eco-Scale, HPLC-EAT (Environmental Assessment Tool), Analytical Method Volume Intensity (AMVI), Green Analytical Procedure Index (GAPI), Complementary Green Analytical Procedure Index (Complex GAPI), and Analytical Method Greenness Score (A) (Imam & Abdelrahman, 2023). By using these methods to evaluate the overall protocol's greenness, it is possible to see at a glance where the procedures under consideration differ and which areas need special attention to avoid specific problems (Płotka-Wasylka & Wojnowski, 2021). Additionally, compared to traditional analysis approaches, the strategy of combining green technology-based methods with multivariate statistical analysis methods has important advantages in terms of findings interpretation. Analytical techniques have a promising future for both academia and industry, according to ongoing advancements in green technology and effectively used techniques. Although new methodologies, approaches, and ideas have recently been established for Green Food Analysis, more study is still required to bring about a significant change in our society. There is still a lot more to be done in this era to achieve sustainable development goals by 2030, and as food analytical scientists, we have to think a different way about greener food analysis.

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Chapter 3

MILLET IN TERMS OF USAGE AS FOOD

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1. INTRODUCTION

Cereals play important roles in human nutrition, particularly in developing countries as they are more economically and easily grown than animal foods. They are considered one of the essential food groups for a healthy lifestyle (Köten, 2022).

Cereals and cereal-based products are regarded as primary food sources in many parts of the world. Cereals make significant contributions to the economies of most countries, providing national income, employment, trade, and raw materials for agriculture-based industries. Additionally, they meet people's daily nutritional requirements (Köten, 2023).

Millet is a group of cereals belonging to the Poaceae family, which has been used as a food source for humans for approximately 10000 years. It includes various species of small grains. (Dias-Martins, Pessanha, Pacheco, Rodrigues, & Carvalho, 2018). Millet is cultivated worldwide, mainly for human food consumption and feed purposes. The term "millet" is widely used to refer to species belonging to the five genera "*Panicum, Setaria, Echinochloa, Pennisetum*, and *Paspalum*" of the tribe "Paniceae" and various small-seeded annuals belonging to the genus "*Eleusine*" of the tribe "*Chlorideae*" (Sunil, Rawson, & Anandharamakrishnan 2022). Millets are classified into major and minor groups based on their usage and size. The major millets are Pearl millet (*Pennisetum glaucum*), Proso millet (*Panicum miliaceum*), Finger millet (*Eleusine coracana*) and Foxtail millet (*Seratia italica*). The Minor millets include Barnyard millet (*Echinochloa colane*), Little millet (*Panicum miliare*), kodo millet (*Paspalum scrobiculatum*), Black fonio (*Digitaria iburua*), White fonio (*Digitaria exilis*), and Teff (*Eragrostis tef*) (Mahajan, Bera, Panesar, & Chauhan, 2021). Pearl millet and Fonio are believed to have originated in West Africa, while Finger millet and Teff are thought to have originated in Northeast Africa (Taylor, 2017).

Millets are crops that mainly rely on rain-fed water in drylands, which are generally water deficient. They can be cultivated in less fertile soils and require less moisture (thus they are drought-tolerant). In addition, millets have multiple advantages such as resistance to pests and diseases, shorter growth periods, and high yields even in dry conditions (Devi, Vijayabharathi, Sathyabama, Malleshi, & Priyadarisini, 2014).

Millet is a staple food in developing regions and countries, like Africa and Asia (specifically India and China), and is also used to produce traditional alcoholic and non-alcoholic beverages. In more developed countries, like the USA, Argentina, Brazil, Australia, and South Africa, millet is widely grown for feed production. Millet is produced in large

quantities as bird feed for both domestic and wild birds in both developing and developed countries. Pearl millet production has also increased in the USA and South America, especially for poultry feed (Taylor, 2017). Developing countries have enormous potential to convert millet grains into value-added foods and beverages. Millet is gluten-free, making it suitable for consumption by celiac patients Devi, Vijayabharathi, Sathyabama, Malleshi, & Priyadarisini, 2014). In addition, the United Nations has declared the year 2023 as the International Year of Millet (Kumar & Kotwal, 2023).

This section of the book provides fundamental information on millet, including its status in world production, varieties, grain structure, chemical composition, potential health benefits, and traditional and novel food applications.

2. WORLD PRODUCTION STATUS

Various types of millet are cultivated in different parts of the world. Pearl millet (*Pennisetum glaucum*) is the most produced type, accounting for 40% of global millet production. Following Pearl millet, Foxtail millet (*Setaria italica*), Proso millet (*Panicum miliaceum*), and Finger millet are the next most produced types. (Selladurai, Pulivarthi, Suprabha-Raj, Iftikhar, Vara Prasad, & Siliveru, 2023)

The Food and Agriculture Organization of the United Nations data displays the global millet production metrics, which include the area under cultivation in hectares and production amount in tons. The data is for 2017 to 2021 and is shared in Table 1. Table 2 shows the distribution of millet production across continents. By the data gathered, the year with the highest millet production amount was 2018. Asia and Africa are the primary geographic regions of millet production. By 2021, more than 96.72% of total millet production worldwide occurred in Asia and Africa (FAO, 2023).

Table 1. World Millet Cultivation Area and Production (2017-2021) (FAO, 2023)

Year	Area Harvested (ha)	Production Quantity (tone)
2017	31337031	28913011.89
2018	32458976	31471641.33
2019	30563470	28273641.59
2020	31136763	30825051.54
2021	30934728	30089625.23

Table 2. Millet Production by Continents (tons) (FAO, 2023)

Continent	2017	2018	2019	2020	2021
Africa	12814470.69	15781455.89	13512129.47	13557827.93	12105491.58
America	343008	265445	381076.45	222830.02	358503.81
Asia	15205224.68	15069280.08	13714708.39	16338770.17	16997317.16
Europe	513506.27	318636.56	629300.58	669099.51	591691.55
Oceania	36802.25	36823.8	36426.7	36523.92	36621.13

Table 3. Top 10 millet-producing countries and as a percentage of total millet production (FAO, 2023)

Country	2019		2020		2021	
	Production (tone)	Percentage of world production	Production (tone)	Percentage of world production	Production (tone)	Percentage of world production
India	10235830	36.20	12488470	40.51	13210000	43.90
China	2300500.8	8.14	2807498.77	9.11	2700000	8.97
Niger	3270453	11.57	3508902.54	11.38	2146706	7.13
Nigeria	1925075	6.81	1905000	6.18	1922000	6.39
Sudan	1133000	4.01	422107	1.37	1500000	4.99
Mali	1878527	6.64	1921171	6.23	1487683	4.94
Senegal	807044.07	2.85	1144854.92	3.71	1039859.75	3.46
Ethiopia	1125957.87	3.98	1218581.54	3.95	1000000	3.32
Burkina Faso	970175.88	3.43	957253	3.11	718000	2.39
Chad	717621	2.54	686584.39	2.23	621367.26	2.07

Millet was produced in 75 countries worldwide in 2021. Table 3 displays the top 10 countries that produced millet between 2019 and 2021. India (43.90%) is the largest producer of millet globally. China, Niger, and Nigeria follow India, respectively. The top 10 countries account for 84.24% of the total millet production. The remaining 15.76% of millet was produced by the other 65 countries (FAO, 2023).

3. MILLET TYPES

Millets are typically grown and cultivated in dry regions in temperate, subtropical, and tropical zones. These crops are classified into African types (finger millet, pearl millet, fonio, and teff), and Asian types (foxtail millet, proso millet, little millet, barnyard millet, and Kodo millet) based on their origin (Narciso & Nystrom, 2023). Details about millet species can be found in Table 4.

3.1. Pearl Millet

Pearl millet (*Pennisetum glaucum* L.) originated approximately 4000 years ago in tropical Western Africa. It is a diploid (2n=2x=14), cross-

pollinated plant that is annual in nature. Its chromosomes contain only the A genome (Joshi, Jain, Malhotra, & Kumari, 2021; Andrews & Kumar, 1992; Devos, Hanna, & Ozias-Akins, 2006).

Pearl millet has been widely cultivated in the Indian subcontinent and Africa since ancient times. Drained light sandy soils are most suitable for cultivating Pearl millet. Although it grows better than most cereals under poor fertility and low moisture, pearl millet can easily adapt to more favorable growing conditions (Das & Rakshit, 2016; Devos, Hanna, & Ozias-Akins, 2006).

3.2. Finger Millet

Finger millet, also known as *Eleusine coracana* L., belongs to the Poaceae family. The crop was cultivated approximately 5,000 years ago in the highlands of Western Uganda and Ethiopia. The cultivated E. Coracana is a tetraploid with a chromosome number of 36, represented as 2n=4x=36. It is extensively grown in India and Africa, serving as a staple food for a substantial population. The seeds are drought and insect-resistant, which makes it possible to store them for extended periods (Meena, Buvaneswaran, Byresh, Sunil, Rawson, & Venkatachalapathy, 2023; Ganapathy, 2017; Abioye, Babarinde, Ogunlakin, Adejuyitan, Olatunde, & Abioye, 2022).

Table 4. Place of origin and common names of millets (Aruna Reddy, 2017)

Crop	Scientific name	Common names	Place of origin	Chromosome no.
Pearl millet	*Pennisetum glaucum*	Cumbu, spiked millet, bajra, bulrush millet, candle millet, dark millet	West Africa	2n=2x=14
Finger millet	*Eleusine coracana*	African millet, koracan, ragi, wimbi, bulo, telebun	East Africa, India	2n=4x=36
Foxtail millet	*Setaria italic*a	Italian millet, German millet, Hungarian millet, Siberian millet	Eastern Asia	2n=2x=18
Proso millet	*Panicum miliaceum*	Common millet, hog millet, broomcorn millet, Russian millet, brown corn	Central and Eastern Asia	2n=4x=36
Barnyard millet	*Echinochloa frumentacea* *Echinochloa utilis*	Indian barnyard millet, sawa millet, Japanese barnyard millet	India Japan	2n=6x=54
Kodo millet	*Paspalum scrobiculatu*m	Kodo millet	India	2n=4x=40
Little millet	*Panicum sumatrense*	Little millet	Southeast Asia	2n=4x=36
Teff	*Eragrostis tef*	Teff, lovegrass, annual bunch grass, warm season annual bunch grass	Ethiopia	2n=4x=40
Fonio	*Digitaria exilis*	Fonio, hungry rice, white fonio (En.), fonio blanc, petit mil	West Africa	2n=6x=54

3.3. Foxtail Millet

Foxtail millet (*Seratia italica*) is an annual herb in the Poaceae family and self-pollinates. It contains only the AA genome and has 2n=2x=18 chromosomes. Around 2700 BC, it originated in China and was domesticated in the mountainous regions of Central Asia. It is primarily cultivated in China and other Asian countries. This millet, adapted to hot and dry environments, can also withstand climate change. As a result, it is a crucial grain for food and nutrition security (Karpagapandi, Kalaiselvan, & Baskaran, 2023; Taylor & Emmambux, 2008a; Kumar Singh, Muthamilarasan, & Prasad, 2017).

3.4. Proso Millet

Proso millet (*Panicum miliaceum* L.) belongs to the Poaceae family. Its chromosome number is 2n=4x=36. Despite natural cross-pollination being possible, it is still a widely self-pollinated plant. First cultivated in China 10,000 years ago, Proso millet is currently widely grown in both semi-arid and arid regions across Australia, Asia, and Europe. Proso millet is a salt-tolerant and drought-resistant crop. Because of its ability to reach maturity quickly and tolerate low agricultural input and limited water use, Proso millet is an alternative crop (Kumar, Tangsrianugul, Sriprablom, Wongsagonsup, Wansuksri, & Suphantharika, 2023; Upadhyaya, Vetriventhan, Dwivedi, Pattanashetti, & Singh, 2016; Narciso & Nystrom, 2023; Rose & Santra 2013).

3.5. Barnyard Millet

Barnyard millet comprises two different species: *Echinochloa frumentacea* and *Echinochloa esculent*; they are cultivated in various parts of the world. The crop has been cultivated in China since 4100 BC, according to evidence. *E. frumentacea* is predominantly grown in India and Africa. *E. esculenta* is an annual crop grown in temperate regions of Japan, Korea, China, Russia, and Germany. In areas where paddy cannot be grown, such as in India, Japan, and China, barnyard millet is used as a substitute for rice. It is grown as a fodder crop in the United States and Japan (Joshi, Jain, Malhotra, & Kumari, 2021).

3.6. Kodo Millet

Kodo millet (*Paspalum scrobiculatum*) is a plant with 40 chromosomes arranged in 4 sets, denoted by the formula 2n=4x. This plant is grown in African and Asian regions with poor soil fertility. The origin of this plant is unknown, but it was domesticated in India during the Neolithic and post-Neolithic eras and is now mostly used by local tribes. This plant is

widely cultivated in the highlands of India, Indonesia, the Philippines, Thailand, and Vietnam. This invasive plant is considered a noxious weed in North America (Upadhyaya, Vetriventhan, Dwivedi, Pattanashetti, & Singh, 2016; Williams, Williamson, & Real, 2011; Ravikesavan, Jeeva, Poornima Jency, Muthamilarasan, & Francis, 2020).

3.7. Little Millet

Little millet has a chromosome number of 2n=4x=36. Little millet (*Panicum sumatrense*) can thrive in regions with low to moderate rainfall. In India, it is cultivated mainly for food in arid and semi-arid regions, particularly by low-income populations. Additionally, it is found in Nepal, West Burma, and several African countries (Upadhyaya, Vetriventhan, Dwivedi, Pattanashetti, & Singh, 2016; Guha, Sreerama, & Malleshi, 2015).

3.8. Teff

Teff is a self-pollinated, annual cereal belonging to the Poaceae family and has a chromosome number of 2n = 4x = 40. It is a C4 tetraploid cereal. It is believed that Teff was domesticated in Ethiopia around 4000 BC. Teff is the primary cereal crop grown by more than 5 million small-scale farmers in Ethiopia and is the staple food for more than half of its population. Teff demonstrates high resilience to various soil conditions, including waterlogged soils, insects, and diseases. Teff can yield more than one crop in a season under favorable climatic and environmental conditions. Although Teff grains are cultivated for human consumption, the straw obtained from its harvest is used as livestock feed (Bultosa, 2016; Zhu, 2018; Small, 2015).

3.9. Fonio

Fonio is commonly grouped as a "minor millet" with some small-seeded members of the Poaceae family, such as finger millet (*Eleusine coracana*), foxtail millet (*Setaria italica*), and proso millet (*Panicum miliaceum*). Fonio millet has a basic chromosome number of x=9 and can be diploid (2n=2x=18), tetraploid (2n=4x=36), or hexaploid (2n=6x=54). Two species of fonio exist, white fonio (*Digitaria exilis*) and black fonio (*Digitaria iburua*). Fonio is also commonly referred to as 'Acha'. The black fonio is called "Ibura" in Nigeria. Both fonio species are annual plants with numerous seeds. White fonio (*Digitaria exilis*) is more broadly cultivated from Senegal to Chad in comparison to the other fonio species. Research has revealed that fonio is the oldest grain cultivated in Africa and has been growing for over 7,000 years (Animasaun, Adedibu, Olawepo, & Oyedeji, 2022; Small, 2015).

4. GRAIN STRUCTURE and CHEMICAL COMPOSITION

The kernel structure of millet is like that of sorghum, in general. Specifically, the kernel consists of three parts: pericarp (fruit shell), embryo, and endosperm, as depicted in Figure 1 (Hassan, Sebola, & Mabelebele, 2021).

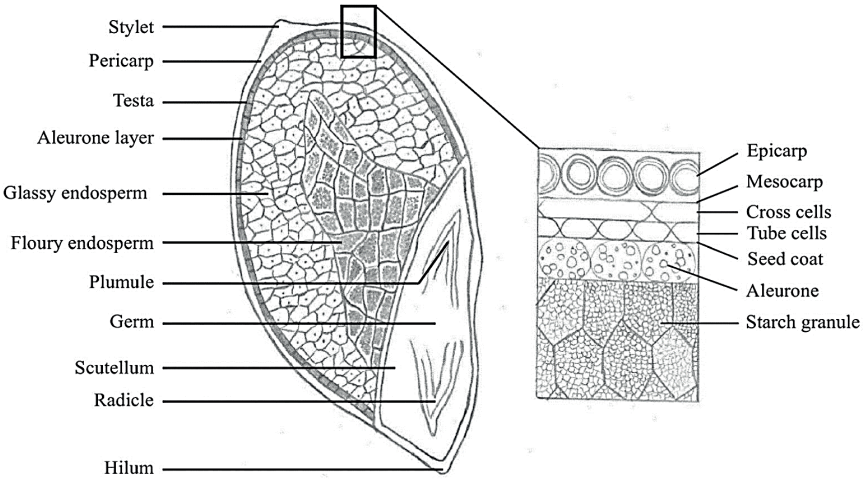

Figure 1. The grain structure of pearl millet

4.1. Properties and Appearance of Millets

4.1.1. Pearl millet

The structure of pearl millet grains is very similar to that of sorghum. Compared to sorghum, pearl millet grains are smaller, and their 1000-grain weight ranges from 3-15 g. Additionally, pearl millet has a proportionally larger embryo and a smaller endosperm. The composition of pearl millet grains is approximately 71.0-76.2% endosperm, 15.5-21.0% embryo, and 7.2-10.6% pericarp (Abdelrahman, Hoseney & Varriano-Marston, 1984).

A thin, waxy cutin layer is present on the surface of the pericarp, which can potentially reduce the effects of weathering. The pericarp is composed of the epicarp, mesocarp, and endocarp, which differ in thickness. All cultivars have a partial or thin seed coat (0.44 mm), which is usually more predominant in gunpowder-colored cultivars (Rooney & McDonough, 1987).

The aleurone layer is a layer containing single cell-thick, blocky cells ranging from 16 to 30 mm in length and 14-33 mm in width. Aleurone cell walls are thick and shiny. They appear dark blue under fluorescence microscopy. This suggests that the aleurone cells contain large amounts

of protein and spherosomes (McDonough & Rooney, 1989; Rooney & McDonough, 1987).

The endosperm can be divided into three regions: the peripheral, the glassy, and the floury areas. The peripheral endosperm contains a dense protein matrix with small starch granules in the first one or two cell layers. The glassy endosperm area contains uniformly sized polygonal starch granules embedded in a protein matrix with minimal protein content (McDonough & Rooney, 1989).

The color of the grain is a result of pigmentation in the pericarp, aleurone layer, and endosperm, as well as pericarp thickness. Pearl millet grains usually display a range of color shades from greenish-yellow to gray (Serna-Saldivar & Espinosa-Ramirez, 2019).

4.1.2. Foxtail millet

Foxtail millet has small grains with an average weight of 2 g per 1000 grains and a length of approximately 2 mm. The grains are covered with thin husks that are removed during threshing. The grain color may be white, red, yellow, brown, or black (Serna-Saldivar & Rooney, 1995). The pericarp of foxtail millet comprises two characteristic epidermal layers tightly bound together, is thin, and contains numerous starch granules that disappear after ripening. Foxtail millet has a single layer of aleurone consisting of cells that contain a dense cytoplasm. The aleurone layer surrounds the endosperm and embryo and consists of 25-50 mm long cells containing specialized protein-packed spherosomes. Like in other millets, the starch granules in this millet variety's glassy and floury endosperm are a mixture of spherical and angular shapes. The dimensions of the starch granules range from 0.8 mm to 11.8 mm. The protein content is more concentrated in the peripheral cells of the endosperm but becomes sparser towards the interior (Serna-Saldivar & Espinosa-Ramirez, 2019). The embryo also contains inactive protein particles. The scutellum consists of cells with irregular shapes. Upon maturation, the chalazal region of the embryo integrates with the scutellum. In contrast to other cereals, the embryo lacks a separate epiblast (Serna-Saldivar & Rooney, 1995).

4.1.3. Proso millet

Proso millet grains vary in color from white to cream, yellow, orange, red, and brown to black. The grains are oval-shaped, about 3 mm long and 2 mm in diameter with a 1000-grain weight of about 5.0-7.1 g (Baltensperger, 2002). Proso millet grains are covered by a smooth, hard, and lustrous husk. Proso millet is classified as an utricle grain, rather than a true caryopsis grain. The utricles' pericarp is loosely attached to the

kernel, which enables its easy removal. The cavities are tightly attached to the utricle and are challenging to remove using conventional milling. The prolamins in the kernel represent 80% of the protein (Kohama, Nagasawa, & Nishizawa, 1999; Kumari & Thayumanavan, 1998).

4.1.4. Finger millet

Finger millet varies greatly in color and has a 1000-grain weight of 2.5 g (Serna-Saldivar, 2010). The grain structure is small, round, or spherical, and 1.2-1.8 mm in diameter. Like sorghum, finger millet has a utricle grain structure, not a true caryopsis. Therefore, the pericarp is easily separated during harvest. Finger millet has a five-layered testa that ranges in color from red to purple (McDonough, Rooney, & Earp, 1986). The aleurone is located under the testa and has a single layer. Grains are small (18x7.6 mm) and rich in aleurone bodies ranging from 0.9 to 2.2 mm in diameter. The endosperm cell walls are highly fluorescent, indicating the presence of phenolic compounds. The protein particles in finger millet are spherical and have a diameter of about 2 mm. Finger millet contains a small embryo (270-980 mm) in a cavity surrounded by a specialized scutellum. The scutellum is separated from the floury endosperm by the scutellar epithelium (Serna-Saldivar & Espinosa-Ramirez, 2019).

4.1.5. Barnyard millet

There is limited knowledge about the microstructure of Indian and Japanese barnyard millet. Veena, Chimmad, Naik, & Shanthakumar (2005) reported that the color of dehulled Indian barnyard millet grains varies from dull cream to brownish cream. The inflorescences of barnyard millets, which belong to the genus Echinochloa, produce one fertile and one sterile flower positioned below an irregular spike. The cavities fully enclose the kernel. The mature pericarp comprises two layers of the epidermis. The cells of the inner epidermis are denser than those of the outer layer. Barnyard millet features an aleurone layer containing cell walls with strong cutinization. While the seed is developing, the testa consists of multiple layers. However, the only permanent component within the mature caryopsis is the inner testa margin, which consists of tangentially compressed elongated cells. The endosperm structure is analogous to that of other members belonging to the Paniceae family. These millets have a 1000-grain weight that varies between 3.3 and 5.0 grams. The husks, or husks and bran, make up approximately 23% of the total weight, with a weight equivalent to 5.0 grams. The small caryopses have a length of 2 mm and a width of 1.8 mm (Malleshi & Hadimani, 1994; Sheahan, 2014; Yabuno, 1987). Most starch granules have a spherical

or polygonal morphology with diameters ranging from 1.2 to 10 mm (Kumari & Thayumanavan, 1998).

4.1.6. Kodo millet

Kodo millet is an annual grass that typically reaches a height of 90 cm. The color of Kodo millet grain varies from light red to dark gray, and it is encased in a hard shell that is challenging to detach. Kodo grains comprise 8.35% protein, 1.45% fat, 65.65% carbohydrates, and 2.95% ash. Each Kodo millet weighs about 6.7 g, and the husk or bran makes up roughly 37% of the total weight. Kodo millet is a monocotyledonous plant with light brown to dark gray seeds covered with a shell that is hard to remove. The seeds are 1.5 mm wide and 2 mm long. Among all small millets, Kodo millet is recognized as the most drought-tolerant and yields favorably in a brief growing season of 80-135 days (Malleshi & Hadimani, 1994; Bunkar & Goyal, Meena, Kamalvanshi, 2021).

4.1.7. Fonio

Fonio plants generate tiny grains, approximately 0.5 g per 1000 grains, that grow in open panicles. The grains are enveloped by a hull that is 1.5 to 1.8 mm long and about 0.9 mm wide. The hull accounts for about 23% of the total grain weight (Hulse & Laing, Pearson, 1980). The naked caryopsis, or grain, comprises three primary anatomical parts: the pericarp, endosperm, and embryo. The outer layer of the caryopsis comprises the pericarp and the aleurone layer which contains lipids and proteins. Beneath the aleurone layer, the starchy endosperm constitutes the primary anatomical structure of the kernel. It is composed of simple, polyhedral-shaped starch granules that range in diameter from 2 to 13 mm and are mixed with proteins and a continuous protein matrix. The embryo is partitioned into the endosperm and the scutellum, which is adjacent to the embryo. These tissues constitute roughly one-third of the caryopsis and are abundant in lipids, proteins, and phytic acid (Ballogou, Soumanou, Toukourou, & Hounhouigan, 2013; Irving & Jideani, 1997).

4.1.8. Teff

Teff is classified by seed color as white, red/brown, and mixed (Tefera, Ayele, & Assefa, 1995). White-seeded varieties are typically preferred for food production, such as the popular dish injera. The teff grain is oval-shaped, measuring 0.9-1.7 mm in length and 0.7-1.0 mm in diameter. The hectoliter and 1000 grain weights for teff fall between 85-87 kg/hL and 0.2-0.4 g, respectively. (Belay, Zemede, Assefa, Metaferia, & Tefera, 2009; Bultosa, 2007; Bultosa, 2016; Hulse, Laing, & Pearson, 1980; McDonough, Rooney, & Serna-Saldivar, 2000; Serna Saldivar, 2010).

Teff has a caryopsis structure without glumes, comprising of pericarp, endosperm, and embryo. The pericarp is separated into cuticle, mesocarp, and endocarp. The endocarp and mesocarp are fused and contain starch granules, similar to certain sorghum species. The seed coat or testa, which consists of thick cells, is located below the epicarp and above the aleurone. Teff has only one layer of aleurone, characterized by thick cell walls and a high protein and oil content. Similar to most cereals, Teff's starchy endosperm is the bulk of the grain and comprises glassy and floury regions. The floury endosperm contains more starch granules and less protein. Teff, like rice, contains compound starch granules that are relatively smaller than those found in other commercially available cereals. The bulk of the grain consists of the embryo, which is rich in lipids and proteins (Bultosa, 2016).

4.2. Chemical Composition of Millets

From a nutritional perspective, millets may have a higher energy value, protein content, and macronutrient composition than traditional cereals. Millets are important contributors to human and animal nutrition because of their high content of energy, calcium, iron, zinc, lipids, and protein of high quality. Additionally, millets are rich sources of dietary fiber and micronutrients (Hassan, Sebola, & Mabelebele, 2021).

Millets significantly contribute to the energy needs of low- and middle-income populations in most parts of Africa, Asia, and Europe. However, in North America, millets are often used as animal feed. Millets' gluten-free nature makes them a viable substitute for individuals with celiac disease and allergies triggered by consuming wheat-based products (Taylor & Emmambux, 2008b). Pearl millet is regarded as nutritionally superior to major cereals such as maize, wheat, and rice. According to reports, pearl millet contains 9.2-13.6% protein, 3.4-7.1% fat, 61.0-70.3% starch, and 1.1-2.4% ash (Hadimani, Ali, & Malleshi, 1995; Malleshi & Klopfenstein, 1998). According to Vasan, Dutta, Mandal, Sharma, & Kadam (2008), pearl millet contains 10.95% protein. The pericarp has the majority of fiber and minerals, while the embryo primarily comprises crude protein and oil. Over 95% of millet's non-starch polysaccharides are fibers. These fibers may help prevent constipation and lower blood cholesterol levels. They also offer hypoglycemic benefits by slowing glucose release during digestion. Consistently consuming millet could help decrease the occurrence of cardiovascular, gastrointestinal, and diabetes diseases. Pearl millet, in particular, is an excellent source of fiber (Rai, Gowda, Reddy, & Sehgal, 2008). Phenolic acids are among the polyphenols present in the pericarp of pearl millet. Pearl millet also contains caffeic, cinnamic, gentisic, p-hydroxybenzoic, protocatechuic, syringic, vanillic, p-coumaric, ferulic,

and sinapic acids, as reported by Dykes & Rooney (2006) and Dykes & Rooney (2007). Unsurprisingly, cereals and millets are low in lysine. In contrast, millets are good sources of other amino acids, particularly those that contain sulfur. The amino acid composition of pearl millet is superior to that of sorghum and maize (Abdalla, El-Tinay, Mohamed, & Abdalla, 1998; Malleshi & Klopfenstein, 1998). Millets have a similar chemical composition to other cereals, as shown in Table 5. Millets are the primary source of carbohydrates (60-73%), protein (6-13%), fat (1-5%), crude fiber (1%-10%), and phytochemicals that possess nutraceutical properties. Pearl millet is a good source of proteins (11-13%) and lipids (4-6%), whereas finger millet contains a lower proportion of protein (6-8%) and fat (1.5-2%) (Dayakar, Karthikeyan, Seetharama, & Hyma Jyothi, 2004). Millets contain crude fiber content that is 10-50 times higher compared to fine cereals, with the highest value found in barnyard millet (Table 5).

Table 5. Chemical composition of millets compared to fine cereals (per 100 g) (Rao, Malleshi, Annor, & Patil, 2016)

Millets/cereals	Carbo-hydrates (g)	Protein (g)	Fat (g)	Energy (kcal)	Crude fiber (g)	Mineral matter (g)	Ca (mg)	P (mg)	Fe (mg)
Pearl millet	67.5	11.6	5	361	1.2	2.3	42	296	8
Finger millet	72	7.3	1.3	328	3.6	2.7	344	283	3.9
Foxtail millet	60.9	12.3	4.3	331	8	3.3	31	290	2.8
Proso millet	70.4	12.5	1.1	341	2.2	1.9	14	206	0.8
Kodo millet	65.9	8.3	1.4	309	9	2.6	27	188	0.5
Little millet	67	7.7	4.7	341	7.6	1.5	17	220	9.3
Barnyard millet	65.5	6.2	2.2	307	9.8	4.4	20	280	5
Rice (raw, milled)	78.2	6.8	0.5	345	0.2	0.6	10	160	0.7
Wheat (whole)	71.2	11.8	1.5	346	1.2	1.5	41	306	5.3

Proteins in millet have high levels of essential amino acids, and the content of these amino acids is 1.2-1.5 times higher than that in rice, especially in finger millet. Pearl millet and gin millet have a histidine content 1.2 times higher than that of rice. The level of phenylalanine is 1.3 times higher in pearl millet, and methionine content is 1.4 times higher in finger millet compared to rice. Cystine levels are comparable in all millets as compared to rice. Proso millet and finger millet have a 1.3 times higher level of isoleucine. The amino acid, lysine, is the most limiting one in both millets and fine grains. The presence of amino acids in all millets is 1.2-1.9 times higher than in wheat (see Table 6).

Millet also contains important vitamins such as riboflavin, thiamine, folic acid, and niacin. Compared to rice, all millets have 2.5-5.2 times higher levels of riboflavin and 1.1-5.6 times higher levels of folic acid. The

highest value is in pearl millet. Compared to rice, the thiamine content is 1.4 times higher in foxtail millet and similar in finger millet. The niacin content in proso millet is like that in rice. All vitamins are similar to the content found in wheat (Table 7). The micronutrient composition of different millets is shown in Table 8. Copper content is 11 times higher in Proso and Kodo millet and 7 times higher in Pearl millet, compared to fine cereals. The magnesium content in millets is 1.4-1.9 times higher, and the manganese content is 9.3 times higher. Finger millet has 2.2 times more zinc. Table 9 shows that millet lipids provide a rich source of unsaturated fatty acids. Table 10 reveals significant variations in the starch content of millet, which is determined based on the proportion of amylose and amylopectin (16-28% and 76-84%, respectively). These differences apply not only to various types of millet but also to individual varieties within a millet type. The amylose content in pearl millet is 1.1 times higher than in fine grains. Other millets have amylose and amylopectin values like fine grains. Pearl millet is more nutritious than fine grains due to these additional nutritional benefits (Rao, Malleshi, Annor, & Patil, 2016).

Table 6. Essential amino acid profile of millets compared to fine cereals (mg/g of N) (Rao et al., 2016)

Millets/ cereals	Arginine	Histidine	Lysine	Tryptophan	Phenylalanine	Tyrosine	Methionine	Cystine	Threonine	Leucine	Isoleucine	Valine
Pearl millet	300	140	190	110	290	200	150	110	140	750	260	330
Finger millet	300	130	220	100	310	220	210	140	240	690	400	480
Foxtail millet	220	130	140	60	420	–	180	100	190	1040	480	430
Proso millet	290	110	190	50	310	–	160	–	150	760	410	410
Little millet	250	120	110	60	330	–	180	90	190	760	370	350
Barnyard millet	270	120	150	50	430	–	180	110	200	650	360	410
Rice	480	130	230	80	280	290	150	90	230	500	300	380
Wheat	290	130	170	70	280	180	90	140	180	410	220	280

Phenolic compounds in plants act as a natural defense against pests and phytopathogens. These compounds have been extensively studied for their antioxidant activity and potential health benefits. Millet has the potential to contain high levels of these compounds. Millet is rich in phenolics and flavonoids, and some species contain significant levels of tannins (Awika & Rooney, Waniska, 2004). Phenolic compounds are concentrated in the outer layers of millet, particularly in the pericarp and testa. Therefore, common practices such as manual pounding or

mechanical decortication significantly reduce the amounts of these antioxidant phytochemicals.

Table 7. Vitamin contents of millets and major cereals (mg/100 g) (Rao et al., 2016)

Millets/ cereals	Thiamin	Niacin	Riboflavin	Vit. A (carotene)	Vit. B6	Folic Acid	Vit. B5	Vit. E
Pearl millet	0.38	2.8	0.21	132	–	45.5	1.09	19
Finger millet	0.42	1.1	0.19	42	–	18.3	–	22
Foxtail millet	0.59	3.2	0.11	32	–	15	0.82	31
Proso millet	0.41	4.5	0.28	0	–	–	1.2	–
Kodo millet	0.15	2	0.09	0	–	23.1	–	–
Little millet	0.3	3.2	0.09	0	–	9	–	–
Barnyard millet	0.33	4.2	0.1	0	–	–	–	–
Rice	0.41	4.3	0.04	0	–	8	–	–
Wheat	0.41	5.1	0.1	64	0.57	36.6	–	–

Table 8. Micronutrient contents of millets compared to fine cereals (mg/100 g) (Rao et al., 2016)

Millets/ cereals	Mg	Na	K	Cu	Mn	Mb	Zn	Cr	Si	Cl
Pearl millet	137	10.9	307	1.06	1.15	0.069	3.1	0.023	147	39
Finger millet	137	11	408	0.47	5.49	0.102	2.3	0.028	160	44
Foxtail millet	81	4.6	250	1.4	0.6	0.07	2.4	0.03	171	37
Proso millet	153	8.2	113	1.6	0.6	–	1.4	0.02	157	19
Kodo millet	147	4.6	144	1.6	1.1	–	0.7	0.02	136	11
Little millet	133	8.1	129	1	0.68	0.016	3.7	0.18	149	13
Barnyard millet	82	–	–	0.6	0.96	–	3	0.09	–	–
Rice	90	–	–	0.14	0.59	0.058	1.4	0.004	–	–
Wheat	138	17.1	284	0.68	2.29	0.051	2.7	0.012	128	47

Phenolic compounds in various millets have also been extensively studied for their antioxidant properties and potential health benefits. Millet has been acknowledged to be a rich source of phenolic acids (benzoic and cinnamic acid derivatives) and flavonoids. Only certain finger millet varieties contain tannins (Hithamani & Srinivasan, 2014). Millet with pigmented testa and pericarp have a higher phenolic content than millet with white or yellow testa and pericarp. Kodo millet has the highest total phenolic content among millets (Chandrasekara & Shahidi, 2011).

Table 9. Fatty acid composition of millet lipids compared to fine cereals (mg/100 g) (Rao et al., 2016)

Millets/ cereals	Palmitic	Palmoleic	Stearic	Oleic	Linoleic	Linolenic
Pearl millet	20.85	–	–	25.4	46	4.1
Finger millet	–	–	–	–	–	–
Foxtail millet	6.4	–	6.3	13	66.5	–
Proso millet	–	10.8	–	53.8	34.9	–
Little millet	–	–	–	–	–	–
Rice	15	–	1.9	42.5	39.1	1.1
Wheat	24.5	0.8	1	11.5	56.3	3.7

Table 10. Amylose and amylopectin content of millet starches (Rao et al., 2016)

Millets/Cereals	Amylose (%)	Amylopectin (%)
Pearl millet	21.1	78.9
Finger millet	16	84
Foxtail millet	17.5	82.5
Proso millet	28.2	71.8
Kodo millet	24	76
Short grain rice	12-19	88-81
Wheat	25	75

Flavonoids are one of the most studied groups of phenolic compounds due to their health benefits, including antioxidant, anticancer, anti-inflammatory, and gastroprotective properties (Chandrasekara & Shahidi, 2010). These important compounds exist in free (soluble) and bound forms. Except for Teff, millet flavonoids are mostly present in free form (Shumoy & Raes, 2016). Total flavonoid content varies according to millet type. Kodo millet is known to have the highest total flavonoid content, followed by finger millet and teff.

Finger millet is considered the only millet that contains a considerable amount of tannins. Its tannin content is reported to range from 5.9 mg catechin equivalent/g or 0.2% to 0.54% of total phenolic compounds (Shashi, Sharan, Shittalamani, Shankar, & Nagarathna, 2007; Dykes & Rooney, 2006; Hithamani & Srinivasan, 2014).

For different millets, Chandrasekara & Shahidi (2011) identified and quantified 59 phenolic compounds classified as hydroxybenzoic acids/derivatives, hydroxycinnamic acids/derivatives, and flavonoids. Hydroxybenzoic acids such as gallic, protocatechuic, p-hydroxybenzoic, gentisic, vanillic, and syringic acids and their derivatives were identified, while hydroxycinnamic acids such as chlorogenic, caffeic, p-coumaric, sinapic, trans-ferulic, cis-ferulic acids, and their derivatives were also

identified. Ferulic, p-coumaric, and caffeic acids were the primary phenolic compounds identified. Typically, these phenolics are found in the grain's outer layers. Therefore, up to 80% of the total phenolics found in whole grains may be lost when hulled (Chandrasekara, Naczk, & Shahidi, 2012).

Carotenoids are a group of lipophilic yellow-orange plant pigments with various health benefits. These compounds are divided into two main classes: carotenoids and xanthophylls. Xanthophylls containing at least one hydroxyl group mainly include lutein, zeaxanthin, and cryptoxanthin (Serna-Saldivar, 2010). The content of carotenoids in millet varies depending on the species and variety. According to Khangura & Gill, Phul, (1980), pearl millet contains 227-229 mg/100 g total carotenoids. Asharani, Jayadeep, & Malleshi (2010) determined 78-316 mg/100 g, 126-191 mg/100 g, and 249-518 mg/100 g of total carotenoids in finger, gin, and proso millet, respectively. Panwar, Dubey, & Verma, (2016) found that total carotenoids ranged from 36.7 to 50.8 mg/g and 20.0 to 26.0 mg/g in different Indian barnyard and finger millet varieties, respectively. Lutein and zeaxanthin are the dominant xanthophylls in millet. Foxtail millet has lutein and zeaxanthin contents of 6.2 and 14.0 mg/kg (dwb) and 0.8-2.0 mg/kg (dwb), respectively (Shen, Yang, Zhao, Shen, & Diao, 2015). Kim, Kim, Uddin, Park, Kim, Chung, Lee, & Park (2014) evaluated the lutein, zeaxanthin, and β-carotene levels in proso millet and found lutein content as 2 mg/g, zeaxanthin content as 0.8 mg/g and β-carotene content as 0.04-0.06 mg/g. Khangura, Gill, & Phul, (1980) reported a β-carotene content of 36-38 mg/100 g in pearl millet.

5. POTENTIAL HEALTH BENEFITS

Millets are a significant source of bioactive phytochemicals, especially phenolics. They are also rich in macro- and micronutrients, similar to the major cereals widely consumed worldwide. Additionally, millets are rich in protein, fiber, calcium, and iron. Millets contain flavonoids, which provide therapeutic properties, like anti-inflammatory, diuretic, anti-cancer, and antihypertensive activities. Consumption of millet can help lower the risk of conditions like type 2 diabetes, breast cancer, and childhood asthma. Due to high dietary fiber content, millets have a low glycemic index value. Additionally, they exhibit beneficial prebiotic activity that supports digestion. Consuming millet is beneficial in preventing cardiovascular diseases. Millets are alkaline and can be easily digested. The magnesium content of millet aids in the prevention of heart attacks and migraines, while niacin (vitamin B3) assists in decreasing cholesterol levels. Millet is a gluten-free cereal, making it a suitable option for individuals who suffer from celiac disease. The presence of phosphorus in millet contributes

to body tissue repair, fat metabolism, and energy formation (Shahidi & Chandrasekara, 2013; Selladurai, Pulivarthi, Suprabha-Raj, Iftikhar, Vara Prasad, & Siliveru, 2023; Das, Pegua, & Arya, 2021; Arya & Shakya, 2021; Ratnavathi, 2017).

Pearl millets are rich in omega-6 fatty acids, which may reduce the risk of cardiovascular disease. They are also a good source of fiber, making them effective in treating obesity, constipation, and type II diabetes. Phytates and polyphenols found in seeds help control aging and metabolic diseases. Phenolics, which are more concentrated in the pericarp and testa layers, have anticarcinogenic properties (Selladurai, Pulivarthi, Suprabha-Raj, Iftikhar, Vara Prasad, & Siliveru, 2023).

The consumption of finger millet, with its phytochemicals, fibers, and phytates, reduces the risk of developing diabetes by delaying the digestion of carbohydrates and regulating postprandial glucose levels. In addition to lowering the glycemic index, consuming finger millet can also increase the level of antioxidants in the blood plasma. Finger millet can reduce blood glucose and cholesterol levels. The phenolic compounds found in finger millet can inhibit the glycation and cross-linking of collagen, as well as intestinal α-glucosidase and aldose reductase enzymes, which are the primary factors of glycation that cause diabetes complications. This inhibition can significantly reduce hyperglycemia and related disruptions. Finger millet, with its high calcium content and better bioavailability, can be used as a therapeutic food to prevent complications arising from calcium deficiency (Devi & Rajendran, 2023; Malleshi, Agarwal, Tiwari, & Sood, 2021).

Foxtail millet is rich in amino acids, resistant starch, dietary fiber, minerals, and antioxidants, making it a highly nutritious food choice. Its consumption shows benefits in the treatment of cardiovascular disease, blood pressure, diabetes, and immune support, as well as hypolipidemic, low glycemic index, and antioxidant properties (Zhang, Shen, Yang, Zhang, Wang, Liu, Zhao, Wang, Diao, & Cheng, 2023; Singh, Khan, Chauhan, Singh, Jaglan, Yadav, Takahashi, & Juneja, 2019).

Proso millet promotes health by preventing the development of cardiovascular diseases, type 2 diabetes, cancer, and obesity because it contains several beneficial antioxidant compounds such as α-tocopherol, carotenoids, polyphenols, and active polysaccharides. These compounds protect human tissues and organs from oxidation. Studies have found that feeding proso millet can increase plasma high-density lipoprotein cholesterol (HDL cholesterol) and adiponectin levels. It also effectively reduces glucose and insulin levels in mice under high-fat diet conditions and may play a role in the prevention of cardiovascular diseases by

reducing plasma triglycerides (Xiang, Yuan, Du, Zhang, Li, & Beta, 2023; Yuan, Xiang, Zheng, Sun, Luo, Li, & Fan, 2022).

Barnyard millet is a nutritious option suitable for patients with celiac disease (Gomashe, 2017). Kodo millet grains have traditionally been used in treating diabetes. It is also useful in treating bleeding, inflammation, and general weakness (Gaurav, Shweta, Ashutosh, Nema, Ankur, & Sonia, 2010).

Little millet is a rich source of nutraceuticals, including phenolics, gamma-aminobutyric acid (GABA), lignans, resistant starch, sterols, and phytates. Due to the additive and synergistic effects of bioactive nutraceuticals in millet-based foods, they can provide health benefits, with hypoglycemic, hypocholesterolemic, hypolipidemic, and anti-glycation properties (Guha, Sreerama, & Malleshi, 2015).

Foods prepared from teff have nutritional and functional properties such as gluten-free, high dietary fiber content, low glycemic index, and very high mineral content, especially calcium and iron (Zhu, 2018; Arendt & Zannini, 2013).

Fonio has positive effects on human health due to its gluten-free, low glycemic index, rich phytochemical content, amino acid profile with a high content of essential amino acids, the majority of the fatty acids it contains are unsaturated fatty acids, high mineral, dietary fiber, resistant starch (Ballogou, Soumanou, Toukourou, & Hounhouigan, 2013; Bassey, Chinma, Ezeocha, Adedeji, Jolayemi, Alozie-Uwa, Adie, Ofem, Adebo, & Adebo, 2023).

6. USAGE IN TRADITIONAL AND NEW FOODS

Millets are classified as 'nutri-cereals' because they contain significant amounts of protein, carbohydrates, fatty acids, fiber, minerals, phytochemicals, and balanced amino acid content. Therefore, millets are a suitable ingredient for various foods, particularly gluten-free functional products for celiac disease, which is widespread and can only be treated with a strict gluten-free diet (Tomar, Bhardwaj, Verma, Singh, Dahuja, Krishnan, Kansal, Yadav, Praveen, & Sachdev 2022; Kumar, Kaur, Rudra, & Arora, 2023).

Flour from finger millet grains is used to make bread, cakes, pastries, and baby foods (Abioye, Babarinde, Ogunlakin, Adejuyitan, Olatunde, & Abioye, 2022).

Arya & Shakya (2021) used foxtail, kodo, and barnyard millet to produce a non-dairy multigrain beverage with high fiber and low glycemic index. Fructooligosaccharides, galactooligosaccharides, and

maltitol were used to sweeten the beverage. At the end of the study, they found that the optimal mixture of barnyard, foxtail, and Kodo millets was 7:10:8, respectively, and the addition of 1.2 g/100 g fructooligosaccharide improved the taste of the beverage, increased the soluble fiber content and thus the prebiotic activity. It was revealed that the produced multigrain beverage would provide at least 10 g/100 g of the soluble fiber recommended for daily intake in a 200 g portion and contained 96.46 mg of phenolic substances. The glycemic index of the beverage was also determined as 45.07. Thus, without adding sugar, a healthy drink suitable especially for diabetic patients was produced.

Kodo millet has organoleptic properties similar to those of rice so the husked millet can be cooked and eaten in the same way as rice. Due to the lower glycemic index value of Kodo millet compared to rice can be used as a replacement for rice in traditional Indian dishes (Annapure, Kalaivendan, Mishra, & Eazhumalai, 2023).

Flours from white teff and proso millet in particular are becoming more widely used in Western countries as a source of gluten-free flour for baked goods and pasta production. Breakfast cereals are also produced from proso millet. It is also malted and used to make gluten-free beer (Taylor, 2017).

Although other cereals can also be used in the production of boza, a pale yellow, thick liquid with a characteristic acid-alcoholic aroma, also called "Busa" or "Bouza", produced in Balkan countries, Egypt and Turkey, proso millet is mostly preferred (Taylor & Emmambux, 2008a).

Teff has enormous potential for use in formulating foods and beverages as part of the diet for people with celiac disease due to the absence of gluten. Teff flour is utilized to add dietary fiber, starch, protein, and mineral content to gluten-free foods while also improving the iron and antioxidant content of bread made with wheat flour. Ethiopia and Eritrea have a long tradition of producing foods and beverages like injera (flatbread), kitta (unleavened bread), and tella (opaque beer) (Bultosa, 2016; Zhu, 2018). Although teff flour is gluten-free, it can produce high-quality leavened flatbread that stays fresh longer than flatbread made from other cereals. (Adebowale, Emmambux, Beukes, & Taylor, 2011).

Fonio makes a good substitute for wheat semolina in making spaghetti and other pasta. This plant can be consumed steamed, in porridge form, and can even be used to make alcoholic beverages. Furthermore, in some West African countries, this plant is also widely consumed as couscous (Small, 2015; Ayo & Okoye, 2020).

CONCLUSION

Millet is the oldest plant that humans have domesticated traditionally. It has been a staple food for people throughout history. During economic progress and modernization, millet production and consumption have decreased significantly due to the increased consumption of refined carbohydrates and processed foods. Other factors contributing to its decline include the taboo of "food for the poor" lack of awareness of its health benefits, and lack of knowledge about different ways to prepare millet. Historical data and scientific evidence suggest that millet has great potential to enhance people's health and socioeconomic status. Regular intake of millet can help stabilize blood sugar and HbA1c levels. Furthermore, its antioxidants can help reduce insulin resistance and lower the risk of atherosclerotic cardiovascular disease (ASCVD), thus providing better glycemic control. It is important to raise awareness about the nutritional and therapeutic benefits of millet and revitalize its usage. It is necessary to encourage people to consume more millet and promote awareness of their health benefits. Researchers in the field should be optimistic about this traditional food revolution and work to build a solid scientific evidence base.

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Chapter 4

SMART PACKAGING APPLICATIONS FOR FOOD

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1. INTRODUCTION

The packaging sector has become one of the most developing commercial industries in the world due to the high demand in other industries besides the food industry. It is stated that the packaging industry grows by 5.6% per year and the global food packaging market, which was approximately 394 billion dollars in 2018, is estimated to reach approximately 606 billion dollars in 2026 (Cheng, Xu, McClements, Chen, Jiao, Tian, Miao, & Jin, 2022).

Packaging ensures that food is protected from external environments such as heat, light, humidity, water, pressure, gases, microorganisms, dust, etc. and that information about the packaged food is transmitted to consumers. As it is known, it is inevitable for packaged foods to deteriorate during storage due to factors such as humidity, heat, light, O_2, $CO_{2,}$ and microorganisms. The relative humidity of foods such as packaged dairy products, meat, and dry foods is particularly important for the stability and preservation of food structure (Yousefi, Su, Imani, Alkhaldi, Filipe, & Didar, 2019). Changes in the a_w (water activity) value of packaged foods as a result of damage to the packaging or extreme temperature fluctuations during storage may cause irreversible degradation of food quality and the proliferation of pathogenic microorganisms. (Bibi, Guillaume, Vena, Gontard, & Sorli, 2016).

Changes in the oxygen level in the packaging cause lipid and pigment oxidation as well as browning reactions, especially in oily and/or pigment-containing foods, resulting in reduced food quality (Lee, Lee, Choi, & Hur, 2015). As a result, microbial infections that occur due to the presence of O_2 in some ready-made fresh products can pose health risks and quality defects such as softening may occur in these products (Lee et al., 2015). Besides, O_2 can cause an increase in ethylene synthesis due to raised respiration in vegetables and fruits (Idumah, Zurina, Ogbu, Ndem, & Igba, 2020).

Changes in pH significantly affect the characteristics and stability of foods. For example, organic acids synthesized throughout the growth and ripening of vegetables and fruits (McAtee, Karim, Schaffer, & David, 2013) can be modified by any microorganism present in foods, thereby reducing the product quality by changing the pH (Nopwinyuwong, Trevanich, & Suppakul, 2010). Similarly, carbon dioxide produced by microorganisms dissolves in food and forms carbonic acid, lowering the pH and causing quality losses in food (Puligundla, Jung, & Ko, 2012).

Preventing microbial contamination is crucial for food safety and maintaining food quality (Aloui & Khwaldia, 2016). Pathogenic microorganisms and their toxins can cause foodborne illness (Nummer,

Shrestha, & Smith, 2012). Apart from these, some substances such as dimethylamine, trimethylamine, cadaverine, and putrescine produced due to microbial contamination during storage and ripening are also chemically toxic (Sharoba, Morsy, Zor, Kostesha, Alstrom, Heiskanen, El-Tanahi, Sharoba, Papkovsky, Larsen, Khalaf, Jakobsen, & Emneus, 2016).

Since many packaging materials, mainly plastic-based, which are widely used in traditional packaging, the primary purpose of which is to protect food, cause environmental pollution (Napper & Thompson, 2019), research is conducted on the use of components isolated from food waste to produce food packaging materials (Cazon, Velazquez, Ramírez, & V´azquez, 2017). The biggest challenges in food packaging, both now and in the future, include healthier and safer food, convenience, longer shelf life, regulations, global markets, authenticity, food waste, and environmental protection (Kerry, 2014; Realini & Marcos, 2014). In addition to the increasing demands of people to consume more natural and fresh foods, changes in industrial, retail, and distribution levels due to globalization create problems in terms of food safety and quality. These conditions present the packaging industry with a unique opportunity to meet the demands of both consumers and the food industry and meet regulatory requirements. The search for innovative solutions to eliminate the above-mentioned problems (Firouz, Mohi-Alden, & Omid, 2021) and to monitor the changes in food in the process from farm to fork has revealed active and smart packaging applications (Muller & Schmid, 2019).

Active packages, which are prepared by adding some auxiliary components to the headspace of the packages or to the packaging materials to increase the effectiveness of the packaging (Robertson, 2006), are widely used to protect foods, especially against moisture and O_2 (Saha, 2015).

Indicators placed in smart packages provide information to consumers about food's properties and storage conditions (Robertson, 2006; Ghoshal, 2018). A smart system with various smart functions (such as tracking, identification, recording, and communication) improves the quality and safety of food, extends the shelf life of food, and alerts consumers to potential problems (Yam, Takhistov, Miltz, 2005; Kerry & O'Grady, M. N., Hogan, 2006).

2. ACTIVE PACKAGING APPLICATIONS

In these applications, which are developed to protect the quality of foods and extend their shelf life, the desired result is achieved by releasing some chemical, physical, or biological substances placed in the headspace

of packages, package or/and the food the desired result is achieved by releasing some chemical, physical, or biological substances placed in the headspace of packages, package or/and the food (Yam et al., 2005). There are two types of systems in active packaging; the first is the pads and bags placed in the packages, and the second is the active ingredients added directly to the packaging materials.

2.1. Sachets and Pads

Very commonly used bags and pads for absorbing gases or releasing them into a package or headspace were manufactured in Japan in the late 1970s.

2.1.1 Oxygen scavengers

O_2 scavenger compounds are mostly substances that reduce the amount of oxygen in the environment by reacting with oxygen (Dobrucka & Cierpiszewski, 2014). O_2 scavenging systems operate on the principle that iron (Fe) compounds rust or oxidize in the presence of water and O_2. Ascorbic acid or powdered Fe is usually used to manufacture O_2 absorbers. Ascorbic acid is more advantageous than Fe as Fe-based scavengers fail the metal detector examination on packaging lines (Saha, 2015).

The market of O_2 scavengers, which is seen as one of the major subcategories of active packaging, has been enlarging continuously in recent years. There are two concepts in oxygen capture systems: first, loose bags, packages containing self-adhesive labels or adhesive devices, and second, active ingredients incorporated into single-layer or multi-layer packaging material or caps of jars and bottles (Rooney, 2005).

Various types of O_2 scavengers used today successfully remove the O_2 in the package, prolonging the shelf life of the products. Although metal-based scavengers pose some disadvantages such as potential health risks to consumers due to accidental ingestion, arcing during microwave heating, and detection by metal detectors, the most common O_2 scavengers are those based on the oxidation of Fe powder. Therefore, organic-type scavengers are being researched as an alternative to metal-based scavengers (Realini & Markos, 2014). These scavengers include organic reducing agents such as catechol, ascorbate, or ascorbate salts, as well as enzymes (ethanol oxidase or glucose oxidase) that can be fixed on packaging film surfaces, attached to adhesive labels, or incorporated into bags (Gün, 2003; Dobrucka & Cierpiszewski, 2014).

In addition, systems using microorganisms as O_2 scavengers, which can provide advantages in terms of sustainability and meet the demands of consumers for natural food preservation, have also been developed

(Anthierens, Ragaert, Verbrugghe, Ouchchen, De Geest, Noseda, Mertens, Beladjal, De Cuyper, Dierickx, Du Prez, & Devlieghere, 2011).

It has been reported that adding unsaturated functional substances to polymer films could enhance the oxygen barrier property (Ferrari, Carranza, S., Bonnecaze, Tung, Freeman, & Paul, 2009). A strong non-metallic O_2 scavenger was manufactured and evaluated by Byun & Whiteside (2012). This complex, consisting of an ascorbyl palmitate-β-cyclodextrin with chemical and thermal stability, can be used in heat treatment applications such as autoclave bags and can also be extruded to produce a film for O_2 capture.

Different O_2 scavenging films are produced by incorporating titanium nanoparticles into various polymers (Xiao, Green, Haque, Mills, & Durtant, 2004; Mills, Doyle, G., Peiro, & Durrant, 2006; De Azeredo, 2009). Nanocrystalline titanium dioxide (TiO_2) on the surface of these films can oxidize organic materials by showing photocatalytic activity, thus consuming O_2 and producing CO_2. Since these films inhibit microorganisms, they can also be used as antimicrobial packaging materials (Lee, 2014; Realini & Marcos, 2014).

Oxygen trap sachets can also contain activated carbon as well as Fe powder to provide odor adsorption (Rooney, 1995). Although a mixture of activated charcoal and CaO is used to remove CO_2 in polyethylene coffee bags, double-acting O_2 and CO_2 scavenging bags used in canned and foiled coffee bags are preferred in the USA and Japan (Day, 1989; Rooney, 1995).

Since the sachet cannot be used in liquid foods, studies have been conducted to include O_2 scavenging systems on the container lid. For this purpose, liquid salts of ascorbic acid or isoascorbic acid prepared alone or in combination with sulfite are applied as residue on the lids or bottle seals. After a container is closed with a lid or gasket on a metal lid, pasteurization or sterilization processes enable these compounds to become active (Zenner & Benedict, 2002; Ekkert, 2008; Pereira de Abreu, Cruz, & Losada, 2012).

2.1. 2. Moisture absorbers

Bacteria and molds that develop in foods with high a_w cause quality losses along with the shortening of the shelf life of the foods. Therefore, excessive moisture in food packaging should be controlled (Realini & Marcos, 2014). For this purpose, moisture absorbers produced in various forms such as pads, bags, sheets, or blankets are used.

While tear-resistant permeable plastic bags containing desiccants such as CaO, silica gel, minerals, and active clays are preferred in dry food packaging, moisture-absorbing pads, blankets, and/or sheets are utilized in foods with high aw value. Moisture absorbers placed between two layers of plastic films consist of a superabsorbent polymer that can absorb approximately 500 times its weight in water. A superabsorbent polymer contains salts such as polyacrylate salts, starch copolymers, and CMC (carboxymethyl cellulose) with extraordinarily strong water-holding properties.

Moisture-absorbing pads are often used to absorb liquid dripping from the tissue of fresh fish, meat, and poultry. Larger blankets and sheets are preferred during shipping to control the sweating of horticultural products or to absorb melted ice in refrigerated seafood.

2.1.3. Carbon dioxide emitters

Carbon dioxide generators, which have an antimicrobial effect, are also used in active packaging applications. Increasing the CO_2 level in the package (10-80%) is used as one of the food preservation methods because it creates an antimicrobial effect (Vermeiren, Devlieghere, F., van Beest, de Kruijf, & Debevere, 1999). This method is generally applied with modified atmosphere packaging (MAP) systems (Coma, 2008). By using CO_2 diffusers, packaging headspace can be reduced in MAP applications. Thus, the efficiency of MAP can be increased, and the quality of the products may be maintained without changing the shelf life during transportation.

Food preservation technology with CO_2 is often used in seafood poultry, and meat packaging (CO_2 Technologies, 2014). The water dripping from the muscle is absorbed by the pads and reacts with the sodium bicarbonate and citric acid in the pad and causing the formation of carbon dioxide (Kerry et al., 2006). Pads with dual antimicrobial effects have been developed by adding an antimicrobial agent to CO_2 generators. These pads are mostly used for poultry, fish, and fresh meat (Paper Pak Industries, 2014). For fillets packed with MAP, the SUPERFRESH system with a carbon dioxide emitter is used. The CO_2 diffuser in this system is activated when it absorbs liquids from the fillets. The advantages of the system are that the product occupies less volume, has a longer shelf life, protects the environment, and has no bloating and vacuum effect.

2.1.4. Ethylene scavengers

Ethylene, one of the phytohormones, which is secreted after harvest in vegetables and fruits, provides the initiation and acceleration of ripening,

also causes the breakdown of chlorophyll, and softens these products. To preserve the post-harvest freshness of respiring fresh products for a longer period, the ethylene they produce during storage should be controlled (Terry, Ilkenhans, Poulston, Rowsell, & Smith, 2007). Ethylene scavengers are often used on ethylene-sensitive products such as apples, bananas, kiwis, mangoes, carrots, onions, tomatoes, and asparagus.

The first of the ethylene cleaning systems works according to the principle of oxidation of ethylene to CO_2 and water by potassium permanganate ($KMnO_4$). Various substances such as clays, alumina, activated carbon, and silica gel can be included as catalysts in this system (Abe & Watada, 1991), which has a permanganate content of 4-6%. Purple-colored potassium permanganate turns brown due to the oxidation of ethylene acetate and ethanol in the environment (Day, 2008). The most important problem in this system is the necessity of using potassium permanganate, which is a toxic substance, in a way that it does not go into direct contact with food.

In other systems, ethylene is absorbed using a mineral such as activated carbon, silicates, and zeolites. Studies have shown that palladium has a higher ethylene-retaining capacity than permanganate-based cleaners (Terry et al., 2007; Smith, Poulston, Rowsell, Terry, & Anderson, 2009). It has been stated that palladium used with charcoal prevents ethylene accumulation, reduces the softening rate of bananas, kiwis, and tomatoes, and reduces chlorophyll loss in spinach leaves (Abe & Watada, 1991; Pereira de Abreu, Cruz, & Losada, 2012; Bailen, Guillen, Castillo, Serrano, Valero, & Martinez-Romero, 2006).

2.1.5. Ethanol emitters

Ethanol, which is used especially to prevent the development of molds, also has an antimicrobial effect on yeast and bacteria. Ethanol spreaders used in active packaging systems are usually in the form of bags. Studies have shown that ethanol sprayed on bakery products is stored without molding throughout the shelf life of the products. Although, it is reported that the use of bags that emit ethanol is a safer and more practical method (Day, 2003; Rooney, 1995). The size of the ethanol-emitting bag to be placed in the package varies according to the shelf life, aw, and amount of the food. Ethanol vapor, which is released when the food in the package absorbs the moisture in the ethanol diffuser bag, spreads to the top space of the package. Ethanol spreaders used in Japan have been reported to delay mold growth in high-humidity cakes and other baked goods, thereby prolonging their shelf life (Rooney, 1995; Day, 2003; Dobrucka & Cierpiszewski, 2014).

2.2. Antimicrobial Packaging Systems

As a result of microbial contamination, color, texture, and aroma changes are accelerated, shelf life is shortened and the risks of foodborne diseases increase in foods, which are an excellent environment for the development of microorganisms. Microbiological contamination from pathogenic or spoilage bacteria can occur as a result of manufacturing defects or disruption of packaging integrity, such as perforation recesses, a torn seal, or incomplete glass finishes (Cutter, 2002). Conventional food preservation methods include heat treatment, cooling, freezing, drying, MAP, irradiation, and the addition of food preservatives (Pereira de Abreu et al., 2012). Developed antimicrobial packaging systems actively control the proliferation of microorganisms, improve food quality, extend the shelf life of foods, and thus ensure food safety (Han, 2000; Kerry et al., 2006; Kerry, 2014).

In these packaging systems, bioactive substances can be dispersed into the package, the surface of the package may be coated with antimicrobial agents, a bag can be added to the package, or edible film-forming antimicrobial macromolecules can be used (Coma, 2008). Although there is a lot of research on the antimicrobial effects of certain substances such as antibiotics, CO_2, ethanol, silver (Ag) ions, ClO_2, essential oils, organic acids, and spices on spoilage microorganisms, commercial use of such systems is extremely limited (Campillo, Sanchez, Garai, &Nerin, 2009; Pereira de Abreu et al., 2012).

The system called "BioSwitch" (De Jong, Boumans, Slaghek, van Veen, Rijk, & van Zandvoort, 2005) works on the principle of releasing an antimicrobial agent with a command given depending on bacterial growth. The basic principle in activating the system is that the antimicrobial reacts to changes in ambient conditions (UV light, temperature, pH, etc.). In this system, the amount of chemicals used in foods can be reduced.

The best-known example of antimicrobial food packaging is antimicrobial compounds encapsulated in polysaccharides. Bacteria that develop due to any contamination in packaged food digest the polysaccharide capsules as they grow, and antimicrobial compounds are released, preventing microbial growth (Saha, 2015).

Commercially available silver-based additives in Japan and the USA are used to slow or inhibit the growth of *Escherichia coli, Campylobacter,* and *Salmonella* in fresh meats (LINPAC, 2012). Some companies (Mitsubishi-Kagaku Foods Corporation, 2002) produce packaging materials such as sheets, labels, and films using allyl isothiocyanate, which has antibacterial and antifungal effects (Realini & Marcos, 2014).

Others have extended the shelf life of vacuum-packed meat products with the antimicrobial film they have developed (Nanopack, 2014).

2.3. Antioxidant Packaging

Oxidation of lipids in foods causes changes in aroma, tissue deterioration in foods containing muscle, and reduced nutritional value, thus shortening shelf life (Pereira de Abreu, Losada, Maroto, & Cruz, 2010b). The antioxidants contained in active packages with antioxidant properties protect food from oxidation better than conventional packages (López-de-Dicastillo, Gómez-Estaca, Catalá, Gavara, & Hernández-Muñoz, 2012). The oxidation of fats in foods can be slowed or prevented by the antioxidants and oxygen scavengers added to the packaging (Pereira de Abreu, Paseiro, Maroto, & Cruz, 2010a; Pereira de Abreu et al., 2010b). As known, oxidation is initiated by radicals formed in the presence of oxygen. Oxidation can be prevented if radicals are removed as soon as they form. This is the working principle of antioxidants, and they stop the progress of oxidation by trapping radicals. In order to prevent oxidation in packages produced with this principle, oxidation of food can be prevented by using radical scavengers instead of high-barrier packaging materials (Nerin, Tovar, & Salafranca, 2008). The scavenger-containing materials used in this application do not need to be activated or protected before use. Antioxidants in the form of labels and sachets can be added to packaging systems by incorporating them into the polymer matrix, coating them on the surface of the packaging material, or placing them between multi-layer films (Realini & Marcos, 2014).

The use of natural extracts with high phenolic content such as rosemary, barley, thyme, cloves, ginger, and cinnamon, and additives such as vitamin E, which is rich in antioxidants, for food preservation is becoming more and more popular day by day (Bentayeb, Rubio, Batlle, & Nerin, 2007; Bentayeb, Vera, P., Rubio, & Nerin, 2009; Nerin et al., 2008; Camo, Beltran, & Roncales, 2008; Pereira de Abreu et al., 2010a, b;). As is known, spices rich in phenolic compounds have antioxidant and antimicrobial properties (Suppakul, Miltz, Sonneveld, & Bigger, 2003; Matan, Rimkeeree, Mawson, Chompreeda, Haruthaithanasan, & Parker, 2006). When rosemary extract is used as an oxygen scavenger, there is no need to add any other antioxidant to food or packaging (Gardes, Nerin, Beltran, & Roncalés, 2004; Nerin et al., 2008). Antioxidant packaging systems can be applied for bakery products, fresh meat and meat derivatives, butter, nuts, vegetables and fruits, oil, and other products containing fat (Pereira de Abreu et al., 2012). Nerín, Tovar, Djeane, Camo, Salafranca, Beltrán, & Roncalés (2006) stated that fresh meat packaged with the antioxidant active film is protected from oxidation, and the

stability of myoglobin in meat increases. It has been reported that the oxidation of whole milk powders (WMP) is delayed due to the migration of α-tocopherol, which is utilized as an antioxidant in the active packaging of WMP (Granda-Restrepo, Soto-Valdez, Peralta, Troncoso-Rojas, Vallejo-Cordoba, Gamez-Meza, & Graciano-Verdugo, 2009). It has been reported that apple slices covered with cellulose films containing cysteine and sulfites are brighter and brown less (De Oliveira, Soares, de Paula, Viana, 2008).

2.4. Other Active Packaging Systems

Apart from the ones described above, aroma-absorbing active packages are also used in products where the aroma is important (Robertson, 2006).

2. 5. Application of Active Packaging Materials

2.5.1 Fresh vegetables and fruits

To extend the shelf life of inhaling fresh products respiration rates of them can be reduced or CO_2 and O_2 concentrations in the atmosphere can be balanced. For this purpose, while ethylene scavengers or diffusers have been used effectively in the distribution of packages, their use in films as primary packaging has been limited. In addition to preserving the product quality in fresh products, it is aimed to prevent mold formation. Bacteria that cause foodborne outbreaks due to post-harvest contamination have also been a cause for concern.

It has been reported that the quality of fresh tomatoes packaged with MAP combined with activated carbon is better than those packaged with MA without activated carbon (Bailen et al., 2006). This treatment improved the quality and organoleptic properties of fresh tomatoes and reduced their spoilage. It has been determined that this situation is related to the fact that the activated carbon used with palladium acts as an ethylene absorber.

Almenar, Valie, Catala, & Gavara (2007) found that fungal growth was inhibited in wild strawberries due to the synergistic effect of antifungal nonanon placed in the lid of a polypropylene container and high CO_2 partial pressures.

Valero, Valverde, J.M., Martynez-Romero, Guillen, Castillo, & Serrano, (2006) determined that table grapes' shelf life packaged with an MA package developed by adding thymol or eugenol is up to 56 days in the refrigerator. While the decay rate of grapes in MA packages without thymol or eugenol was 50%, it was reported that the decay rate of grapes

in MA packages containing 150 µL of eugenol was 12%. It was determined that the texture, color, weight loss, and sensory properties of grapes in MA packages containing essential oils were better.

It was determined that *Botyris cinereal*, which is common in grapes packaged with sulfur dioxide-releasing active packaging, decreased. The principle of this system is that sodium metabisulfite ($Na_2S_2O_5$), which can be added to a sachet, plastic, or paper sheet is activated by reacting with the moisture produced in the packaging (Scully & Horsham, 2007).

It has been stated that the shelf life of table grapes, which are commercially available and stored at 0°C using an SO_2-releasing paper pad called grape preserves, can be up to 8-10 weeks (Mustonen, 1992). However, the sudden release of high SO_2 as a result of changes in humidity and temperature can be a problem, causing the grapes to turn pale. Another disadvantage of this system is that the maximum SO_2 level to be used is limited to 10 ppm by the US Environment 360 Chapter 10 Protection Agency (EPA) as it causes severe asthmatic reactions in susceptible individuals. (Scully & Horsham, 2007).

2.5.2. Dairy products

Active packaging is used to prevent mold and moisture control in dairy products, especially cheese, and to extend their shelf life. Some of the compounds used for this purpose might cause undesirable aromas in dairy products. Research continues on active ingredients that may only extend shelf life without affecting the sensory organoleptic properties of products.

Pantaleao, Pintado, & Pocas (2007) determined that a Portuguese cheese (Soloio) wrapped in a polystyrene (PS) based polyvinyl chloride PVC shell (Humidipaks) can be stored for approximately 84 days at 8˚C changing its sensory and textural properties.

It has been reported that natamycin added to the cellulose-based film used in the active packaging of Gorgonzola cheese reduces the number of *Penicillium roqueforti*. They stated that the level of natamycin released from the package during the ripening period (45 days) was also below the amount allowed by the legislation (Oliveria, Pintado, & Pocas, 2007).

Pires, Soares, Andrade, Silva, Camiloto, & Barnardes (2008) found that the shelf life of Mozzarella cheese packaged with a cellulose-based active film coated with nisin (50%) and natamycin (8%) was 6 days longer than the control sample. Researchers have found that nisin is less effective against mold and yeast when used alone.

2.5.3. Bakery products

Silveira, Soares, Geraldine, Andrade, & Goncalves (2007) compared the microbiological properties of dough packed with the cellulose-based film of different thicknesses (70 μm and 25 μm) containing sorbic acid at different rates (3% and 7%) and stored at 8°C during 40 days with dough packed with low-density polyethylene (control). While there was a decrease in the psychotropic, mesophilic, and *Staphylococcus* spp numbers of the doughs packaged with active films containing sorbic acid, it was determined that there was an increase in the doughs packaged with control films. Researchers reported that the amount of sorbic acid transferred from the packaging to the product was below the legal limits.

Neilson & Rios (2000) used various oleoresins and essential oils from herbs and spices to prevent mold growth in high-barrier MAP hot dog buns. The researchers determined that the hot dogs packaged in allyl isothiocyanate-added packages provided the desired shelf life, but the sensory bad taste and odor were detected in the samples.

Franke, Wijimma, & Bouma (2002) found that the ethanol release sachet added to the packaging of pre-baked buns extended their shelf life. The ethanol sachet increased its shelf life from 2-3 days to 13 days. It has been reported that the bad taste of the products can be reduced as a result of the evaporation of ethanol by heating the buns before serving.

Guynot, Sanchis, & Romas, Marin (2003) investigated mold growth during storage (28 days at 25°C) of sponge cakes with a water activity of 0.8 and 0.9 and packed in MAP with O_2 absorbent bags with 100 and 200 mL absorption capacity. Although the use of MAP alone is beneficial in preventing mold growth, MAP combined with O_2 traps completely inhibited mold growth in packaged cakes even on the 28th day of storage. The treatment was found to be more beneficial for cakes with higher aw (0.9) compared to low aw (0.8).

2.5.4. Fish, meat, and poultry products

MAP is utilized as an active packaging system for taste, color, and spoilage control in meat, fish, and poultry. However, a combination with other active packaging ingredients is required to achieve the desired effect. Various outbreaks due to contamination in ready-to-eat meat products have led to numerous studies on antimicrobial packaging (Çağrı, Usumol, & Ryzer, 2004; Aymerich, Picouet, & Monfort, 2008; Coma, 2008). The use of antimicrobials such as carbon dioxide diffusers, oxygen scavengers, nisin, chlorine dioxide, chitosan, natural plant extracts, and oils, triclosan, and grape seed extract in active packaging of poultry, meat, and fish have been investigated.

Martinez, Djenane, Cilla, Beltran, & Roncales (2006) determined that the fresh pork sausages' shelf life packaged by adding O_2 scavengers combined with 80% nitrogen and 20% carbon dioxide, extended up to 20 days. Besides, the number of psychotropic aerobic bacteria in the sausages decreased and the color and lipid stability of the sausages were better.

Neetoo, Ye, & Chen (2008) determined that sodium diacetate and sodium lactate added as antimicrobial agents to the package of smoked salmon fillets stored in the refrigerator for 4 weeks inhibited the growth of *L. monocytogenes*.

It has been determined that pathogens such as *Clostridium botulinum, C. perfringens*, and *L. monocytogenes* are not affected by 50% or fewer CO_2 levels in fresh meat products packaged with active packaging with carbon dioxide diffusers added (Coma, 2008). The author reported that pathogenic bacteria may develop in these products where bacteria that cause spoilage do not grow, and this may pose a risk in terms of food safety.

Ku, Hong, & Song (2008) used an edible active film produced from a kind of red algae (*Gelidium corneum*) by adding catechin to the wrap of sausages inoculated with *L. monocytogenes* and *E. coli* O157:H7. It was determined that the pathogen population and oxidation decreased in sausages stored for 5 days, and it was reported that this active film could extend the sausage's shelf life.

Calcium alginate film containing two different lysozyme species (oyster and chicken) combined with nisin was utilized as an antimicrobial active package in the packaging of smoked salmon inoculated with *Salmonella anatum* and *L. monocytogenes*. It was determined that the number of *S. anatum* and *L. monocytogenes* decreased by 2.8–2.2 logcfu/g^{-1}, respectively, in salmon covered with antimicrobial film and stored at 4°C for 35 days, and the effect of lysozyme type on the number of bacteria was not significant (Datta & Janes, M.E., Xue, Losso, La Peyer, 2008).

3. INTELLIGENT PACKAGING SYSTEMS

Intelligent packaging systems perform functions such as improving the quality of food by monitoring its characteristics, informing the consumer, providing convenience, and providing resistance against tampering or theft (Robertson, 2006). With intelligent packaging technology, both the quality of the food inside the package and the conditions outside the package can be monitored. To achieve this, the quality mark must be in direct contact with the food or the top of the package.

A smart system not only increases the shelf life, safety, and quality of the product but can also alert the consumer to potential problems. This system is an excellent tool to monitor the problems encountered from production to consumption of food. Recently, research has been conducted to develop smart packaging systems that notify the consumer if a package has been tampered with. In this system, seals or labels that are transparent before the package is opened, undergo permanent color changes after the package is tampered with, and may even write "opened" or "stop". In this way, it is thought that smart packaging may even save a consumer's life because it warns that the package has been tampered with (Cheng et al., 2022).

3.1. Principles of Intelligent Food Packages

Intelligent packages are systems consisting of special materials that be able to inform consumers about the safety and quality of food, as well as help their consumption and purchasing decisions. Inputs from a wide range of disciplines such as materials and food sciences, food microbiology, and sensor technology are needed to develop smart packaging materials that can detect and record changes in the packaging or its external environment and transmit this information to the consumer (Sohail, Sun, & Zhu, 2018). Thanks to smart packaging technology, food waste can be reduced, and food quality can be improved (Poyatos-Racionero, Ros-Lis, & Vivancos, Martínez-Manez, 2018) so that consumers can reach safe food (Sohail et al., 2018). This section discusses packaging materials that contain detection mechanisms that respond to certain changes in order to ensure food safety and/or quality.

3. 1. 1. Time-temperature indicators (TTIs)

Factors such as temperature and time affect the shelf life and quality of foods (Zhang, Sun, Xiao, Liu, & Zheng, 2016). Among these factors, it is particularly important to examine the variation of temperature depending on time. Time-temperature indicators (TTIs), pioneers of intelligent packaging design, have been produced to continuously monitor the temperature and time changes that refrigerated and frozen foods are exposed to throughout the supply chain (Lee & Rahman, 2014; Vanderroost, Ragaert, Devlieghere, & De Meulenaer, 2014). Prepared in the form of a small self-adhesive label, TTIs can be put in individual packages or shipping containers, and when faced with inappropriate conditions, an irreversible change, such as discoloration, will occur (Soltani Firouz, Mohi-Alden, & Omid, 2021). TTIs, which provide continuous monitoring of storage conditions, provide information about whether the cold chain has been broken and can therefore also be utilized as an indicator of

shelf life. Temperature indicators show that the temperature of the food rises or falls below the critical temperature during the freezing and thawing processes, thus alerting consumers to the risk of pathogenic microorganisms and the possibility of protein denaturation (Mehauden, Cox, Bakalis, Simmons, Tucker, & Fryer, 2007; Tucker, Brown, Fryer, Cox, Poole, & Lee, 2007; Tucker, Hanby, & Brown, 2009; Dobrucka & Cierpiszewski, 2014).

TTIs according to different working principles; can be grouped into physical TTIs, chemical TTIs, microbial TTIs, enzymatic reaction TTIs, and other TTIs. The working principle of physical TTI is based on electrons, diffusion, and nanoparticles. In electronic TTIs, the temperature signal, which is converted into an electrical signal via the thermal sensor, is then converted into visual output as an indicator (Wang, Liu, X., Yang, Zhang, Xiang, & Tang, 2015). Diffusion-based physical TTIs operate based on a phase transition or diffusion reaction depending on the temperature change in colored substances (Wang et al., 2015). Physical TTIs based on nanoparticles (NPs), works according to the principle that the wave number moves to the visible region as a result of the change in the surface morphology of the heat-absorbing metal nanoparticles (Ag NPs/Au NPs), and then their color changes.

The indicators used in chemical TTIs are based on redox reaction, photochromism, and polymerization. In TTIs based on a redox reaction, the color change occurs as a result of the redox reaction between the compound in the indicator and the O_2 in the air or the redox reaction caused by the light effect. Since the reaction rate is dependent on temperature and time in these TTIs, the shelf life of foods can also be estimated (Wang et al., 2015). Photoluminescence-based chemical TTIs work on the principle that thermally induced photoluminescent compounds fade as a result of the reverse reaction. Depending on time and temperature, the degree of wilting and reaction rate may directly show the change in the shelf life of the food. In chemical TTIs based on polymerization, monomers excited by high temperatures are irreversibly converted into polymer compounds (Wang et al., 2015), and the absorption spectrum changes from the high waveband to the low waveband, causing the color to change (Gou, Guo, Zhang, Men, Song, Luo, Zhao, Qian, & Wei, 2010).

Microbial TTIs work with the principle that the metabolites produced by microorganisms at a certain temperature and time cause a color change in the pH indicator (Wang et al., 2015).

Enzymatic TTIs work with the principle of changing color in the indicator as a result of the hydrolysis reaction between the substrate and the enzyme (Wang et al., 2015). Since the degree of enzymatic reaction

depends on time and temperature change, different concentrations of enzymes, substrates, activators, or inhibitors can be used to determine quality changes in different foods (Siddiqui, Guo, Zhang, Men, Song, Luo, Zhao, Qian, & Wei, 2021).

3. 1. 2. Gas indicators

The gas composition of packaged foods can easily change as a result of respiration or the interaction of foods with their environment. Gas indicators that change color as a result of an enzymatic or chemical reaction are used to monitor these changes (De Jong et al., 2005). Gas indicators that can be used to verify the gas permeability of the package or the effectiveness of an oxygen scavenger must be in contact with the gas atmosphere in the package to be able to measure. Gas gauges measure the absence or presence of CO_2 and/or O_2 in the package. Various gas indicators are also being developed to measure the presence of ethyl alcohol, water vapor, and hydrogen sulfide in the packaging.

The most common among the various types of gas meters, O_2 indicators can change color in the presence of O_2, alerting the consumer to a leak or tampering with the package, or indicating a package has been incorrectly sealed. (Vu & Won, 2013; Vanderroost et al., 2014). The working principle of O_2 indicators is based on the color change of a strong reducing agent such as glucose by oxidizing a redox dye (e.g. methylene blue) in an alkaline environment (Mills, 2005; Dobrucka & Cierpiszewski, 2014). The disadvantage of this type of oxygen indicator is that the color can be restored to its original state depending on the oxygen concentration. Since the microorganisms in the packaged food can consume oxygen, reversibility is not required for the control of the packaging permeability (Hurme, 2003). To overcome this disadvantage, O_2 indicators are used with oxygen scavenging systems (Kuswandi, Wicaksono, Jayus, Abdullah, Lee, & Ahmed, 2011). O_2 scavengers added to the package will prevent the oxygen indicator from reacting with the residual O_2 contained in the package (Realini & Marcos, 2014).

3.1.3. Freshness indicators and sensors

Freshness and/or maturity indicators indicate staleness or spoilage of products by measuring the number of volatile metabolites such as hydrogen sulfide and ammonia (NH_3) (Smolander, Hurme, Latva-Kala, Louma, Alakomi, & Ahvenainen, 2002), amines, CO_2, and diacetyl (Nopwinyuwong et al., 2010), formed during ripening of products. pH indicators that change color depending on the amount of microbial metabolite and the maturity of the product have great potential for use

(Hong & Park, 2000; Nopwinyuwong et al., 2010; Smolander, 2008). The following ripeness and/or freshness indicators are commercially available:

Eo® (Cryolog, 2010), Timestrip® (Timestrip Plc., 2010), FreshTags® (Betsy, 1999), Sensor QTM (Food Quality Sensor International Inc., 2009) and ripeSense® (Ripesense Limited, 2010).

Biosensors are also used to determine the freshness of the products. Biosensors included in food packages to determine target metabolites are specially designed according to the packaged product and detect spoilage products. Therefore, they are more specific than freshness indicators. Biosensors identify, record and conduct information about biochemical reactions. There are generally two main parts in the device: the bioreceptor and the transducer. The bioreceptor recognizes the target analyte, while the transducer converts them into an electrical response. Biosensors are used in a barcode system (Food Sentinel System) developed to identify food pathogens. If the product contains pathogenic bacteria, a localized dark bar is formed during scanning, making the barcode unreadable (Yam et al., 2005; Realini & Marcos, 2014).

3.1.5. Humidity sensors

Both high aw foods (fish, meat, vegetables, and other fresh produce) and low aw foods (dried or powdered products) are sensitive to moisture. If the amount of moisture in the package is too low, foods with high water activity will dry out because they will lose their moisture, on the contrary, if the humidity inside the package is too high, foods with low water activity will deteriorate because they absorb moisture (Gaikwad, Singh, & Ajji, 2019). For this reason, it is particularly important to monitor the moisture content in food packages in real time in order to prevent food spoilage and ensure the preservation of its quality. The humidity sensors used for this purpose work by measuring the dielectric properties of the crystals, the changes in capacitance, and resonance frequency. Bibi et al. (2016) and Pereia et al. (2020) prepared films with wheat gluten and nanocomposite containing a layer of gelatine-based semiconductor ZnO, respectively, and they followed the relative humidity changes in food packages. In both packaging types, changes in relative humidity due to changes in dielectric properties were measured.

Sensors embedded in packaging materials can also be used to measure changes in food moisture. In this system, the biodegradable materials on which the interdigitated electrode is placed function as an induction layer. These materials, consist of materials such as polypropylene composite, planarized polyethylene terephthalate, polylactic acid, starch, and glossy photo paper or polyvinyl alcohol (Raju & Bridges, 2021; Wawrzynek,

Baumbauer, & Arias, 2021), absorb moisture from the atmosphere as the dielectric constant increases, and the capacitance between the electrode's changes depending on the relative humidity of the environment. In case of increased humidity in the package, the induction layer absorbs water vapor, causing a change in the capacitance of the capacitor and the resonance frequency of the sensor (Wawrzynek et al., 2021).

3.1. 6. Oxygen sensors

In oxygen-sensitive foods (such as oils and pigments), it is necessary to control the amount of oxygen in the package to prevent oxidative deterioration. For this reason, research has focused on the development of smart packaging materials that can detect O_2 levels in foods. Two types of sensors are used to detect changes in the amount of O_2 in food packaging: colorimetric redox-based and phosphorescence-based sensors. Colorimetric redox sensors are manufactured using redox dyes (e.g., methylene blue) that change color according to the presence or absence of O_2 in the environment. The disadvantage of these sensors; they need to be produced under anaerobic conditions and do not work if reducing agents are depleted (Won, Jang, & Jeon, 2016). This problem can be solved by the use of various UV-active colorimetric redox dyes. Redox paints used in this application lose their color when exposed to ultraviolet light and only return to their original colors with the effect of O_2 (Yılmaz & Altan, 2021; Vu & Won, 2013; 2014). Since these dyes can leak from the packaging material and pass into the food when they come into contact with food, they must be kept in a biopolymer gel net. It is possible to prevent this leakage by using glycerol, titanium dioxide, and redox dye-added polyvinyl alcohol (PVOH) nanofibers as an oxygen indicator (Yılmaz & Altan, 2021). On the other hand, it is an important problem that some components such as alkalis and redox dyes used in this system are synthetic chemicals that may pose a food safety hazard. To solve the problem, it is recommended to use natural substances (for example, laccase as a natural biocatalyst and guaiacol as a substrate) instead of synthetic substances (Won et al., 2016).

3.1.7. pH sensors

Changes in the pH values of foods during storage give important clues about their freshness, quality, and/or reliability. From this point of view, smart food packaging systems containing pH-sensitive sensors have been developed (Pourjavaher, Almasi, Meshkini, Pirsa, & Parandi, 2017). pH sensors are generally of two types: First, sensors using substances whose electrochemical potentials vary with pH; secondly, sensors using substances whose color changes with pH. pH sensors, in which

electrochemical potentiometers are used, convert the electrochemical potential produced by the electrodes into the frequency with the change of the ambient pH and then transmit it to a wireless reader (Huang, Deb, Seo, Rao, Chiao, & Chiao, 2012). These sensors have limited usage area due to their high cost and complex equipment.

On the other hand, pH sensors using synthetic or natural dyes whose colors change according to the pH of the environment are more widely used because they are easy and inexpensive. The concern here is that dyes used as pH-sensitive color markers can have toxic effects due to leaching into foods and food safety. (Kobylewski & Jacobson, 2012; El-Wahab & Moram, 2012; Roy & Rhim, 2020; Musso, Salgado, & Mauri, 2016). For this reason, research on the production of intelligent packaging materials in which pH-sensitive natural dyes are used instead of synthetic dyes has gained importance. Natural dyes that might be used in pH sensors include curcumin (Ezati & Rhim, 2020), green tea extracts (Wen, Hsu, Asoh, & Uyama, 2020), betaine (Kanatt, 2020), alizarin (Ezati, Tajik, & Moradi, 2019), shikonin (Dong, Ling, Z., Zhang, Zhang, Ramaswamy, & Xu, 2020) and anthocyanins (Choi, Lee, Lacroix, & Han, 2017).

Among these dyes, anthocyanins, which are the most widely used, are preferred due to their reliability as well as their antioxidant and antimicrobial effects (Roy & Rhim, 2020). Research continues maintaining the stability of the above-mentioned natural dyes in different environments (humidity, temperature, etc.), reducing or preventing their migration from various polymers, and improving their stability with nanotechnology (Balbinot-Alfaro, Craveiro, Lima, Costa, Lopes, & Prentice, 2019).

3.1.8. Microorganism sensors

The number and variety of microorganisms in foods are of great importance in terms of food safety and quality. Microbial contamination can lead to spoilage of food, as well as various diseases, poisoning, and even death. However, microorganisms are used in the production of foods such as yogurt, bread, and wine. For this reason, analytical methods such as colony counting, bacterial culture, ELISA tests, PCR, and isothermal amplification are used to detect microorganisms in food (Kim & Kim, 2020). Because these methods are highly sensitive and provide accurate results, but are laborious, time-consuming, and expensive, researchers are working to develop methods to quickly determine whether food is contaminated with microorganisms.

Smart food packaging materials are produced using two basic technologies for the detection of bacteria: lateral flow test strip (LFTS)

technology and DNAzyme probe technology. In LFTS technology, labeled antibodies (Abs) containing palladium (PdNPs), or gold (AuNPs) nanoparticles are loaded onto a test strip and when food is contaminated with bacteria, bacteria bind to these Abs and form a complex. This complex then moves along the strip and binds to another antibody fixed to the other end of the strip. Microbial contamination is detected with the colored lines and dots produced by this antibody (Tominaga, 2018). It has been reported that no other equipment is required for this test and the test is completed in only 15 minutes, but it is not sensitive when compared to methods such as cultural enumeration or PCR (Tominaga, 2018). However, the flow test strip test is reported to hold promise for application in intelligent packaging materials in detecting microbial contamination (Tominaga, 2017). Xia, Yu, Z. Liu, Xu, & Lai (2016) used this method to identify bacteria in liquid foods and milk.

DNAzyme probe technology uses a DNAzyme probe that covalently binds to a transparent, flexible polymer film containing epoxy groups and reacts specifically with *E. coli*. DNAzyme, located at the cleavage point of a single ribonucleotide and surrounded by a quencher and a fluorophore, has a very low fluorescence signal as it does not react with the substrate in an *E.coli* uncontaminated product. If the product is contaminated with *E. coli*, the substrate is cleaved by the action of DNAzyme, which discriminates between the quencher-labeled sequence and the fluorophore, providing a strong fluorescent signal. It has been reported that DNAzyme technology can detect target bacteria, including *E. coli*, in a variety of foods, such as apple juice, apple slices, and meat, without opening the package. The use of this technology is not widespread due to its high cost, complex setup time, and difficulties such as long identification time, as well as the need for other monitoring equipment (Yousefi, Ali, Su, Filipe, & Didar, 2018).

3.1.9. Specific chemical sensors

There are sensor methods developed to detect substances such as biogenic amines, volatile basic nitrogenous substances, biotoxins, and sulfurous compounds formed during the food spoilage process: (i) semiconductor gas sensors; (ii) metal nanoparticle plasma sensors; and (iii) pH-sensitive colorimetric gas sensors. Semiconductor gas sensors work on the principle of measuring the changing electrical conductivity of the sensor material after the absorption of amines. With this method, the amount of amine in the environment is determined indirectly (Park, Choi, Bae, Yoon, Jang, & Lee, 2013). Its high cost limits its use. For the detection of amines, low-cost metal nanoparticle plasma sensors are used. In this system, the amines formed in the product adhere to the surface of the metal nanoparticles and change their refractive index. Due to this

change, the redshift and increase in the resonance absorption wavelength are detected by an optical system such as a barcode reader. (Tseng, Li, Yi, Sun, Gao, & Wan, 2017). The working principle of sensors using pH-sensitive dyes is based on the fact that the change in pH changes the color of the dye since amines formed in food are alkaline (Zhou, Yang, Wang, & Chen, 2019; Huang, Li, Zou, Shi, Mao, Zhao, Hao, & Holmes, 2016a; Huang, Aguilar, Xu, Lai, & Xiong, 2016b).

Hydrogen sulfide (H_2S), which is formed during the degradation of foods containing sulfurous amino acids such as meat, milk, and eggs, is one of the main volatiles that causes undesirable aromas and reduces food quality and acceptance. There are two main approaches in smart food packaging materials developed for the determination of H_2S levels in foods: (i) metal nanoparticle plasma sensors and (ii) electrochemical sensors. The working principle of metal nanoparticle plasma sensors is based on the reaction of H_2S formed in the product with metal nanoparticles to produce metallic sulfides. Silver nanoparticles are generally used for this purpose. The Ag_2S produced changes in color by creating a change in the local surface plasmon resonance, thus detecting H_2S (Zhai, Li, Shi, Huang, Sun, Zhang, Zou, Sun, Zhang, Holmes, Gong, Povey, & Wang, 2019). The working principle of electrochemical sensors is based on the transmission of the electrical resistance, which occurs as a result of the reaction of H_2S-sensitive substances printed on a substrate, with H_2S in the environment, measured by the sensor and transmitted to a wireless reader (Koskela, Sarfraz, Ihalainen, Maattanen, Pulkkinen, Tenhu, Nieminen, Kilpela, & Peltonen, 2015).

Efforts are also being made to develop smart food packaging that can detect aflatoxins, organophosphate pesticides, and other toxic compounds formed in foods. Huang et al. (2019) developed a simple colorimetric paper sensor that works with a degradable nano enzyme and color indicator and can quickly and sensitively detect the activity of acetylcholinesterase and organophosphorus pesticides. Smartphone-based optical bionic sensors developed for the detection of aflatoxins consist of molecularly imprinted polymer films that can produce a measurable response via a fluorescent sensor that selectively identifies target analytes. In this method, it is possible to use the smartphone camera for recording and image analysis (Sergeyeva, Yarynka, Piletska, Linnik, Zaporozhets, Brovko, Piletsky, & El'skaya, 2019). Optical bionic sensors provide precise and real-time detection of aflatoxin B1.

3. 1.10. Thermochromic inks

Thermochromic inks, which have become a popular technology for beverages, are dynamic ink that can change color when exposed to different temperatures. These inks are printed on the label or package

and convey a message to the consumer about the status of the product according to the color of the ink (Robertson, 2006). There are two types of thermochromic inks: those whose color change is irreversible and those which are reversible. Initially invisible, irreversible thermochromic inks create an intense color that remains constant when exposed to a certain temperature, alerting the consumer that the temperature of the product is changing. The color of reversible thermochromic inks changes when the temperature of the product rises and returns to its original color when the temperature drops (Sarley, 2011).

There are various thermochromic inks with different activation temperatures:

- *Cool activated thermochromic ink* changes color when cooled

- *Touch-activated thermochromic ink* becomes transparent when touched or rubbed, revealing another color or image imprinted underneath

- *Touch-activated liquid crystal ink* changes color inside the visible spectrum when touched or rubbed.

- *High-temperature thermochromic ink* changes color in hot beverages and alerts consumers to a safety hazard.

Because the inks used are affected by very high temperatures (above 121°C) and UV light, consumers should not completely rely on the ink messages that a beverage is "too hot" or a beverage is "totally chilled" (Vanderroost et al., 2014).

3.1.11. Radiofrequency identification tags (RFID)

RFID technology, which works with electronic information, is not included in the indicator or sensor classes of the smart packaging system. (Dobrucka & Cierpiszewski, 2014). RFID tags used in this system make use of RF electromagnetic fields to automatically identify and track products, and transmit and store real-time information (Lee & Rahman, 2014). The chip placed on the tags stores the information and the integrated circuit connected to an antenna transmits this information to a reader. RFIDs are much more advantageous than barcodes due to their features such as being able to be controlled remotely, tracking more than one item at the same time, and storing various information (process parameters, origin, commercial) (Kuswandi et al., 2011).

RFID tags can be divided into two classes: active and passive tags. Battery-powered active tags are active up to a distance of approximately 50 m. Passive tags, which work with the energy provided by the reader, have an unlimited life and are active at a shorter distance (up to about 5

m). RFID frequencies typically range from microwave frequencies (~2.45 GHz), low (~125 kHz), and Ultra High Frequency (UHF) (850–900 MHz). Used mostly for meat products and products with high-water content, low-frequency tags perform better, use less power, are cheaper, and can better penetrate non-metallic objects (Kerry et al., 2006).

To make RFID systems smarter, RFID tags can be connected to sensors that measure relative humidity, temperature, pressure, pH, light exposure, concentrations of gas molecules, and volatile compounds. Thus, it can provide information about both the quality and integrity of the food, and the environmental conditions throughout packaging and storage or transportation (Abad, Zampolli, Marco, Scorzoni, Mazzolai, Juarros, & 2007; Sample, Yeager, Powledge, Mamishev, & Smith, 2008).

3.2. Types of Intelligent Food Packaging

3.2.1. Films

The most common packaging materials developed for smart packaging are blended and coated films because they are inexpensive and simple. In the preparation of blended films, the sensor is mixed with a natural or synthetic film-forming polymer material. The crucial point here is that the polymer and the sensor are compatible.

Three main methods are used to prepare blended films: thermal compression, solution casting, and extrusion. In film production with the thermocompression method, which is more suitable for commercial applications, the sensors are mixed with polymers and additives and then compressed in a hot press for a few minutes (Uranga, Etxabide, Guerrero, &de la Caba, 2018). Anthocyanins in the fish gelatine matrix (Uranga et al., 2018) and blueberry in the tapioca starch matrix were used in pH indicator films produced by this method (Andretta, Lucchese, Tessaro, &Spada, 2019). The solution pouring method, which is a simple, inexpensive, and easily applicable method in the laboratory, is not very economical for large-scale film production and is not very suitable for commercial applications. For example, in the preparation of a smart food packaging material that uses anthocyanins as a pH-sensitive sensor, film-forming gelatine solutions are poured onto a plate and dried (Musso, Salgado, &Mauri, 2019). Similarly, ZnO nanoparticles are used as sensors in the preparation of moisture-sensitive food packaging materials, and glycerine used as a plasticizer is mixed into a gelatine solution and dried (Pereira, Picciani, Calado, & Tonon, 2020). In the production of the film with the extrusion method, all components are mixed in an extruder at the appropriate pressure, screw speed, and temperature, and the produced material is converted into a film through hot presses (Gutierrez

& Alvarez, 2018). An ammonia monitoring packaging material produced by the extrusion method was prepared by adding curcumin (sensor) to the LDPE matrix (Zhai, Wang, Zhang, Yang, Sun, Li, Huang, Holmes, Gng, Povey, Shi, & Zou, 2020). This film has been reported to be stable at various pHs. This method is not suitable for the production of smart food packaging materials prepared with heat-sensitive colorants because high temperatures can cause thermal degradation of these colorants (Roy & Rhim, 2020). Indeed, Gutierrez & Alvarez (2018) reported that the pigments in an extruded cornstarch film prepared with blueberry extract lost their ability to detect pH changes due to chemical degradation at high temperatures.

The indicator used in coating films is placed on the film surface. The sensor or indicator used in the preparation of coating films is first dispersed in a suitable medium and then printed or coated on a polymer film surface. It has been reported that a film containing nanoparticles produced by this method and sprayed onto the PET surface can be used to monitor the freshness of minced meat by measuring the H_2S gas (Sukhavattanakul & Manuspiya, 2021).

3.2.2. Bar codes

Today, the potential use of barcodes in smart food packaging applications is being investigated. For example, photosensitive printing inks have been developed that can be used as pH indicators in barcodes. These inks increase or decrease the density of one or more black lines in the barcode depending on the pH change and can be read with a suitable barcode reader (Zhang et al., 2016).

Chen, Fu, Zilberman, Ruan, Ameri, Zhang, Miller, & Sonkusale (2017) used tetraphenyl porphyrin, nil red and methyl red, and silica beads in a barcode system they developed, and with this barcode, chicken quality was evaluated in real-time using an application on a smartphone.

3.2.3. Labels

On traditional food labels, only the place of origin, content, production date, and expected shelf life of the food are given to the consumers, while the labels of smart food packages also provide information such as the maturity, quality, or safety of the food. For example, labels can be created that change color when food spoils. For this purpose, labels consisting of three layers can be produced. In such labels, the innermost layer is semipermeable and controls the diffusion of metabolites, the middle layer contains an indicator that changes color in the presence of metabolites, and the outermost layer acts as a shield (Lee, Baek, Kim, & Seo, 2019). In

this type of label, the design of the semi-permeable layer is particularly important because this layer should not only allow the passage of metabolites but also prevent the indicator dye from passing into the food (Lee et al., 2019). The most preferred semipermeable polymers are spun PO (polyolefin) (Tyvek®) (Lee et al., 2019), PE (polyethylene) (Chen, Zhang, Bhandari, & Guo, 2018), and PEBA (poly ether-block-amide) films (Baek, Maruthupandy, Lee, Kim, & Seo, 2018). Colorimetric sheets have previously been produced by coating, impregnation, or lamination methods using filter paper (Lee et al., 2019), cellulosic polymers (Nopwinyuwong et al., 2010), or various superabsorbent materials (Kim, Lee, Lee, Baek, & Seo, 2017). Bromothymol blue, bromocresol green, and methyl red were used as colorants (Rukchon, Nopwinyuwong, Trevanich, Jinkarn, & Suppakul, 2014; Chen et al., 2018). Films such as PET (polyethylene terephthalate) and LDPE low-density polyethylene) were used to form the outer layer (Lee et al., 2019; Baek et al., 2018). The advantage of this type of label is that the real-time status of the food can be monitored without the need for any other equipment. However, the complexity and high cost of their production pose a disadvantage (Firouz et al., 2021). In addition, attention should be paid to the identification of target metabolites, the selection of appropriate label material, and safety considerations (Firouz et al., 2021).

RFID tags used in smart packaging systems can store up to 1 MB of data out of sight and contactless (Muller & Schmid, 2019). An improved passive RFID tag (Fiddes & Yan, 2013) was able to identify various vapors such as water, ethanol, toluene, and ammonia due to changes in electrical resistance. RFID tags can also be used to detect biogenic amine production (Fiddes, Chang, & Yan, 2014) and changes in humidity (Feng & Xie, L., Chen, Zheng, 2015).

3.3. Applications of Intelligent Food Packaging

3.3.1. Fruits and vegetables

Kuswandi et al. (2013) developed a colorimetric film using a sensor (bromophenol blue) embedded in a bacterial cellulose matrix to measure the freshness of guava. In this film, volatile compounds such as acetic acid, formed as a result of the over-ripening of the guava, lowered the pH and changed the color from blue to green.

Chen et al. (2018) used a pH-sensitive indicator label to measure the freshness of green pepper. The sensors (bromothymol blue and methyl red) on this label change color as a result of the pH change due to the increased carbon dioxide concentration as a result of the deterioration of the green pepper.

3.3.2. Dairy products

Since dairy products, which are the most consumed and highly nutritious in the world, tend to spoil quickly, their packaging should be done very carefully (Roy & Rhim, 2021; Alizadeh, Masoomian, Shakooie, Khajavi, & Farhoodi, 2020). Thanks to smart packaging technology, the quality of dairy products can be monitored throughout the supply chain. pH indicators are generally used in smart packaging used in dairy products (Cheng et al., 2022). Li, Wu, & Wang, Li (2021) and Moazami Goodarzi, Moradi, Tajik, Forough, Ezati, & Kuswandi (2020) produced various smart packages that change color depending on pH change by adding vegetable pigment and anthocyanins to the polymer matrix. Roy & Rhim (2021), with the packaging they developed by adding shikonin to carrageenan/gelatine-based films, both monitored the freshness of milk and added antioxidant and antimicrobial properties to the packaging. Ziyaina, Rasco, Coffey, Ünlü, & Sablani (2019) also developed a colorimetric sensor to detect the shelf life of pasteurized milk. Similarly, a colorimetric sensor that changes color depending on the acid concentration in milk has been developed in smart milk packaging (Weston, Kuchel, Ciftci, Boyer, & Chandrawati, 2020).

Apart from these, pH labels based on essential oils and anthocyanins have been developed by Bandyopadhyay, Saha, Zandraa, Pummerova, & Saha (2020) to monitor the quality of cheese throughout storage.

3.3.3. Meat

Proteins in meat can be broken down by microorganisms into volatile basic nitrogen compounds that cause pH changes. Therefore, smart packaging materials may be used to determine changes in meat and meat product quality throughout storage. For example, Zhang, Zou, Zhai, Huang, Jiang, & Holmes (2019) measured the deterioration of meat through storage using intelligent packaging materials containing pH-sensitive dyes. Zhai et al. (2019) also used silver nanoparticles to monitor the deterioration of chicken breast meat in real time. A colorimetric hydrogen sulfide (H_2S) sensor is used in this application. Yousefi et al. (2018) used a DNAzyme probe to monitor *Escherichia coli* contamination in raw beef in real time during storage.

3.3.4. Seafood

Seafood with high nutritional value tends to deteriorate chemically and microbiologically (Mohammadalinejhad, Almasi, & Moradi, 2020). Therefore, the production of smart packaging materials developed to monitor the quality and safety of these products throughout their

shelf life has gained popularity. Since methylamine, dimethylamine, trimethylamine ammonia, and similar volatile compounds formed during the deterioration of seafood products cause changes in pH value, pH indicators have been started to be used in developed smart packaging materials (Dudnyk, Janecek, Vaucher-Joset, & Stellacci, 2018). For example, anthocyanin extracts from mulberry have been used as pH sensors in films designed to monitor fish quality (Zeng, Chen, Qin, Zhang, Wang, Wang, Ning, Ruan, & Zhang, 2019). The researchers reported that the film color changed from purple to greyish purple to dark green due to spoilage of the fish throughout storage.

Sulfur nanoparticles and curcumin were used in pH-sensitive films developed to monitor the quality of shrimps. It has been reported that the initially yellow color turns orange during storage (Ezati & Rhim, 2020). The researchers determined that the curcumin and nanoparticles in the film had an antimicrobial effect and inhibited the growth of *E. coli* and *L. monocytogenes*.

Bhadra, Narvaez, Thomson, & Bridges (2015) utilized a wireless-based sensor that measures ammonia concentration to monitor fish spoilage.

3.3. 5. Other foods

Apart from these, there are also smart packages in which moisture indicators in cereals (Tan, Ng, Shao, Pereles, & Ong, 2007) and pH indicators in desserts (Nopwinyuwong et al., 2010) are used.

CONCLUSION and FUTURE PROSPECT

Active and smart packaging, which is increasingly used for foods, has become a very dynamic and popular sector with environmentally friendly packaging solutions. develops in connection with the search. Smart packaging contributes to improving consumers' quality of life. Smart packaging is more focused on food safety, food quality, shelf life, monitoring, and sustainability of foods. Advances in technology allow the sensors used in smart packaging to be read with our portable smart devices such as mobile phones. As a result, all consumers will have easy access to detailed information on food quality and condition, thus protecting themselves from adulteration or food fraud.

Despite all the benefits it provides, there are some problems that need to be overcome before smart food packaging technology may be used commercially. The biggest problem of smart food packaging is production costs. The studies are mostly laboratory-scale, and the cost is expected to be very high in commercial productions. Normally, the smart packaging cost should make up a small fraction of the total packaging

cost, such as 10%. However, the currently developed smart packaging cost is approximately 50% higher than the total cost (Cheng et al., 2022; Ghaani, Cozzolino, Castelli, & Farris, 2016). Another issue is that smart packaging does not harm the environment and is recyclable. This floating work needs to focus on cost reduction and the recyclability of smart packaging. Research on smart food packaging has only just begun, but it is estimated that developments in this field will ensure the production of healthy, reliable, and sustainable foods and reduce costs.

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Chapter 5

FONIO (*DIGITARIA SPP.)* AND ITS POTENTIAL FOR USE IN FOODS

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1. INTRODUCTION

Societies around the world continue to depend on cereal crops and rely on cereal-based foods. Widely grown and consumed globally, cereals such as wheat, maize, and rice are known as the oldest crops cultivated by humankind. In addition to these main cereal crops, some so-called rare cereals also play a significant role in the food supply in low-income communities. Adapted to harsh weather conditions, these crops are drought-resistant and contribute to environmental protection by preserving vegetation in ecologically sensitive areas. One of these crops, Fonio (*Digitaria spp.*), is a small grain cereal that has long been grown as a staple food for many rural communities in West Africa and is being rediscovered by city consumers around the world.

Fonio is one of the most ancient indigenous cereals of West Africa. Ibn Battuta first mentioned fonio in the middle of the 14th century in his book "Journey to Sudan", where he described cooking with fonio, which is similar to a mustard seed and better than rice. The French explorer René Kayle mentioned fonio in the 19th century and called it "Fiogne", meaning a small herbaceous species. McIntosh & McIntosh (1988) reported that in communities living on the west coast of Africa, fonio was a staple food along with rice and could be a solution to food shortages (Zargaran Khouzani, 2023).

Fonio has common names in the different languages of the communities where it is grown, such as acha, afio, fonio, fundi millet, foundé, foni, fundi, findo, findi, founié, fonyo, fundenyo, fini, hungry rice, hungry millet, hungry koos, ipoga, kpendo, petit mil, pende, pounié and pene. This sub-Saharan grass, which has been used as a food source in Africa for 1000s of years, has been little recognized until today. However, among agronomists interested in the future of agriculture, it has become as popular as other cereals and ancient grains in recent years. Fonio has outstanding ecological qualities, not only in food quality but also in its ability to help agricultural products adapt to the world's diverse ecosystems and their wild species (Jideani, 1999).

Fonio (*Digitaria spp.*), is the sixth most important cereal in arid regions of Africa after rice, wheat, maize, sorghum, and pearl millet and one of the four millet species grown in semi-arid tropical savannas. This crop is preferred by farmers in the region due to its resistance to drought (Kanlindogbe Sekloka, & Kwon-Ndung, 2020). It is important among the crops grown in the region and in the diet of the people of the region (Ezekiel, Sulyok, Warth, & Krska, 2012).

The Food and Agriculture Organization (FAO) recognizes fonio as the "grain of life" in many communities in West Africa and its sub-regions

(Figure 1), Nigeria, Guinea, Benin, Togo, Mali, Burkina Faso, Senegal, and many other countries (Adoukonou-Sagbadja, Wagner, Dansi, Ahlemeyer, Daïnou, Akpagana, Ordon, & Friedt, 2007).

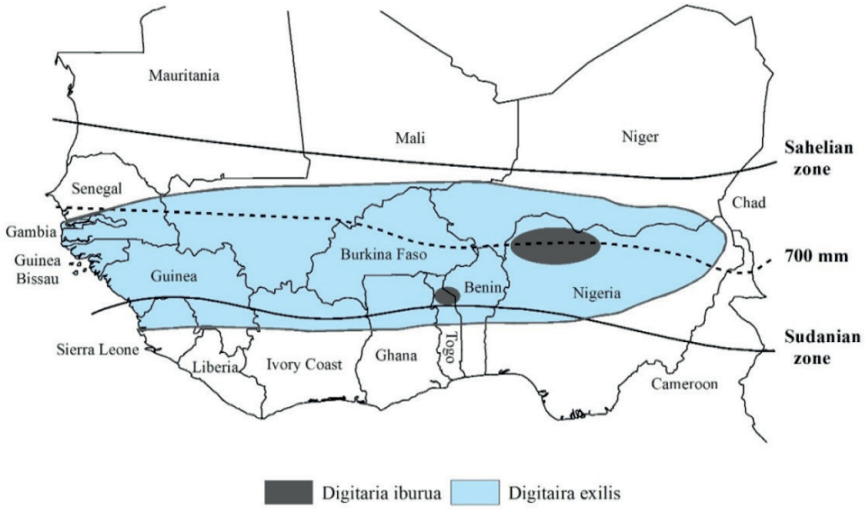

Figure 1. Major fonio plantations in Africa (Kanlindogbe et al., 2020).

Approximately 250,000 tons/year of fonio is produced in West Africa and the world fonio production is estimated at 720,000 tons/year (520,000 ha) (URL-1, 2020). Fonio is an important source of income, especially for low-income farmers, and a kilo of fonio can be sold for 1.5 to 2 times the value of rice (Vodouhe Dako, Dansi, & Adoukonou-Sagbadja, 2007).

Fonio is also of interest in some other countries, for example, in the United States it has been commercially promoted as a "healthy" cereal. Because its cultivation to date has been limited to specific geographical areas and not linked to international agricultural trade, it was included in the US Academy of Sciences priority list of underutilized African plants of economic value in 1974 (Zhu, 2020). The European Commission approved the commercialization of Fonio as a "Novel Food" in Europe through EU Regulation L 323/1 of 19 December 2018, due to its nutritional quality, authenticity, healthier properties, and environmental friendliness, especially for European and American consumers (Cruz & Béavogui, 2016; EFSA, 2018).

2. FONIO IN AFRICAN CULTURE and MYTHOLOGY

Fonio, cultivated in parts of Africa for more than five thousand years, is one of the oldest cereals, full of legends and superstitions and considered sacred by some communities. These legends are part of the

rich oral traditions of various West African cultures. They are evidence of the cultural importance and respect given to fonio grain in the region (National Research Council, 1996; URL-2, 2019).

- In the legends of the Dogon people of Mali, "Amma", the supreme creator of the universe, created the entire universe by exploding a single grain of fonio in a "world egg".

- In Senegal, some people grow fonio around their settlements to ward off evil spirits and curses. Sometimes mothers put raw fonio grains in their children's school bags for protection and good luck.

- In West Africa, fonio can be used as a bride price at weddings.

- Fonio is eaten during the Muslim holy month of Ramadan or at celebrations such as weddings and baptisms.

- Especially in Senegal, Burkina Faso, Mali, and Togo, it is served at times of celebration or to guests returning from a long journey and is also included in rituals and ceremonies related to harvest, weddings, and births, symbolizing good luck and fertility.

- Archaeologists have found fonio buried in the tombs of the Egyptian pyramids, and it is seen as a sign that a person has something of value that who would want with them in the afterlife.

- According to the legend of the Fulani people, a blind old woman found a grain of fonio in the grass and mistook it for a pebble. She put it in her mouth and immediately regained her sight. Since then fonio has been recognized as a miraculous grain.

- In some cultures, fonio is believed to have a spiritual connection to ancestors, and offerings of fonio are made at ceremonies and celebrations to honor them.

3. ORIGIN OF FONIO

Cereals are edible seeds or grains from the *Poaceae* genus. A large number of cereals such as rye, oats, barley, maize, triticale, millet, and sorghum are grown in various regions. Botanically, Fonio (*Digitaria spp.*) is a monocot in the grass family (*Gramineae* or *Poaceae*) and the genus *Digitaria*, which includes hundreds of subspecies, is sometimes cultivated as a forage crop, with three or four species considered cereals. *Digitaria spp.* is generally considered one of the nine species of millet. Today, white fonio (*Digitaria exilis*) and black fonio (*Digitaria iburua*) are economically important in West Africa (Figure 2) (Karahan, Köten, & Akın, 2009).

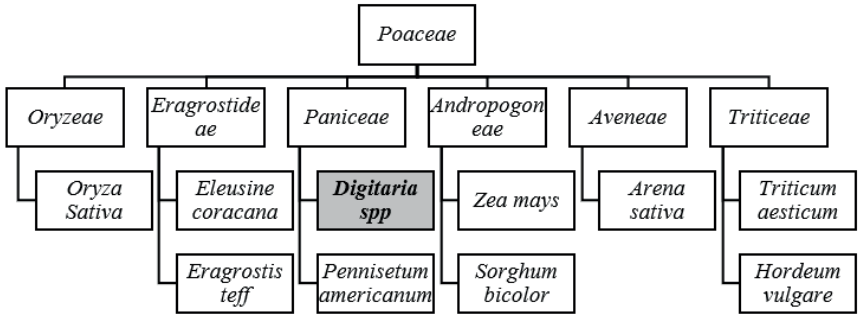

Figure 2. Taxonomy of fonio (Digitaria spp.) (Clayton & Renvoize, 1986).

4. MORPHOLOGY OF FONIO

All cereals have certain structural similarities; the embryo contains the genetic material, the single or multilayered husk, and the starch-filled endosperm. Cereals are widely used and consumed because of their availability, energy, nutrients, flavor, and neutral taste (Elgün & Ertugay, 1997).

Figure 3. Comparison of (a) white (Digitaria exilis) and (b) black fonio (Digitaria iburua) (Small, 2015).

Two different species of fonio are common based on their color variation. *Digitaria exilis* (acha), called white fonio, has a light yellow-brown seed coat, while *Digitaria iburua* (iburu), called black fonio, has a darker seed coat. Despite their morpho-botanical similarities, these species also have some differences. For example, the stems and leaves of black fonio are longer and wider than those of white fonio (Kanlindogbe et al., 2020).

Fonio is a plant that can grow on nutrient-poor and low fertility acidic and sandy soils without the need for fertilization and has the potential to help prevent problems such as erosion and desertification in areas with

insufficient annual rainfall of 200-500 mm. The seeds germinate within a week after sowing. Adult plants grow to about 50 cm in height, while white flowers appear after about 6 to 8 weeks. The grain is harvested after 60 to 120 days. Due to its short growth cycle, it is harvested during the critical famine season, which precedes the main food crops of the region (millet, sorghum and maize, etc.). Harvesting is usually done with a knife or sickle, tied in bundles, dried, and stored under cover. With low cultivation inputs, fonio can be harvested three times a year under favorable climatic conditions, making it an early food source (Echendu, Obizoba, Anyika, & Ojimelukwe, 2009; Koréissi, 2015).

Fonio grain is usually stored as husks and is sometimes called "paddy fonio". Optimal storage requires lower moisture content (11%-13%) in fonio grain than in other cereals. If dried and stored properly, fonio grain can be kept for several years (Koréissi, 2015). Harvested fonio grains are surrounded by a layer of cellulose-rich husk. The shell makes up about 23% of the grain. Fonio grains in hulled or paddy form are 1.5 to 1.8 mm long and about 0.9 mm wide. When the husk is separated, the peeled grain is obtained. The cereal grain is small, oblong to spherical to ellipsoid, about 0.5 mm long, white to pale brown or purplish. Fonio is one of the smallest known cereals, with a 1000-grain weight of about 0.3-0.5 g. These values are significantly lower than other cereals and pseudo cereals (Cruz & Béavogui, 2016).

The husked grain is divided into three main parts: pericarp, endosperm, and germ. The starchy endosperm, located below the aleurone layer, forms the main structure of the kernel and consists of simple, polyhedral, polygonal starch granules ranging from 3 to 13 μm in diameter, merged with a protein matrix. The germ is adjacent to the endosperm and embryo and is rich in protein groups, lipids, and phytic acid (Figure 4) (Cruz, Beavogui, & Dramé, 2011).

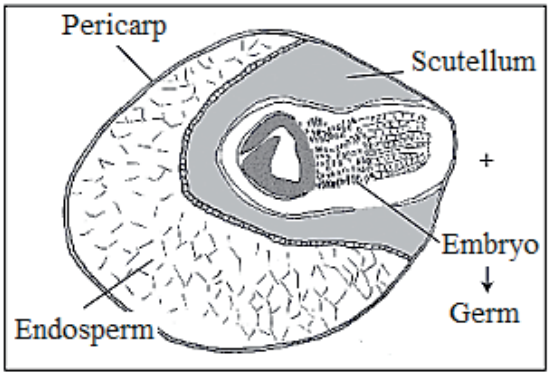

Figure 4. Cross section of fonio grain (Cruz et al., 2011).

After harvest, the grain is still surrounded by the husk. The hulling and blanching of fonio grain are traditionally done by hand, usually with a large pestle. The very small size of the grain makes fonio processing time-consuming (2 kg fonio/1 hour). In addition, the grains need to be washed several times to remove all hulls and husks. Similar to rice, there are two stages of fonio processing. The first stage is the removal of the outer husks from the fonio in paddy (coarse grain) to produce hulled grain, and the second stage is bleaching to remove the bran (pericarp and germ) from the hulled grain (Figure 5) (Vodouhe et al., 2007).

Figure 5. Fonio in the husking process (Cruz & Béavogui, 2016)

5. CHEMICAL AND NUTRITIONAL PROPERTIES OF FONIO

The arrangement and granular structure of the chemical constituents found in fonio grain are similar to other cereals such as millet and wheat. The seeds are rich in methionine and cysteine, which are lacking in some important cereals such as rice, wheat, sorghum, barley, and rye. Fonio is a source of many bioactive compounds and is gluten-free, indicating that it has significant potential for celiac patients and consumers (Adoukonou-Sagbadja et al., 2007; Chukwurah, Uyoh, Usen, Ekerette, & Ogbonna, 2016).

Table 1. The chemical composition of fonio

Nutrient Composition	**Average**
Energy (kcal/100 g fonio)	463.7
Carbohydrates (%)	79.1
Starch (%)	68
Amylose (%)	25.1
Soluble sugars (%)	1
Crude fibre (%)	5.85
Protein (%)	8.05
Albumin	3.5
Globulin	1.8

Prolamin	5.5
Glutelin	14
Lipid (%)	3.25
Ash (%)	3.5
Vitamins (mg/100 g)	
Vitamin A	0.007
Vitamin B1	0.24
Vitamin B2	0.14
Vitamin B3	2.03
Vitamin B5	0.46
Vitamin B6	0.46
Vitamin B9	0.018
Vitamin C	0.036
Vitamin D	0.00001
Vitamin E	0.76
Vitamin K	0.004
Amino acids (%) (g/100 g protein)	
Essential Amino Acids	
Phenylalanine	3.72
Histidine	1.71
Isoleucine	2.68
Leucine	7.10
Lysine	2.30
Methionine	4.30
Threonine	2.90
Tryptophan	0.92
Valin	4.07
Non-essential Amino Acids	
Aspartic Acid	5.00
Glutamic Acid	13.55
Alanin	6.60
Arginine	2.55
Cysteine	2.90
Glycine	2.55
Proline	5.15
Serine	3.60
Tyrosine	2.25
Fatty acids (%)	
Myristic Acid	0.1
Palmitic Acid	16.8
Palmitoleic Acid	0,3
Stearic Acid	4,1
Oleic Acid	30,6
Linoleic Acid	45,7
Linolenic Acid	1.2
Arachidonic Acid	1,1

Behenic Acid	0,4
Macro elements (%)	
Calcium (Ca)	0.018
Phosphorus (P)	0.17
Potassium (K)	0.14
Sodium (Na)	0.017
Magnesium (Mg)	0.46
Sulphur (S)	0.16
Microelements (ppm)	
Iron (Fe)	84,8
Copper (Cu)	8.25
Manganese (Mn)	25.8
Zinc (Zn)	36.15
Phenolics (mg/100 g d.m.)	
Phenolic acids	134.42
Flavonoids	3.79
Antinutrients (mg/100 g d.m.)	
Phytic acid	19.48
Tannin	34.65
Oxalate	80.10

*The data received from the authors are compiled and presented. (Temple & Bassa, (1991); Jideani & Akingbala, (1993); Irving & Jideani, (1997); Serna-Saldivar, (2003); Fliedel & Ouattara, Grabulos, Drame, Cruz, (2004); Chukwu & Abdul-Kadir, (2008); Cruz et al., (2011); Babarinde & Adeyanju, Ogunleye, Adegbola, Ebun, Wadele, (2020); Jocelyne & Béhiblo, Ernest, (2020)).

5.1. Carbohydrates

Carbohydrates are the major components of cereals and are used by living organisms as a primary energy source. Studies have shown that the average carbohydrate content of fonio grain is 79.1% (Table 1). Furthermore, the average starch content was reported to be 68%. The starch percentage found was less than that of sorghum (73.8%) and rice (77.2%) (Serna-Saldivar, 2003). Jideani & Akingbala (1993) found the amylose content of fonio to be 25.1%. This high amylose content value indicates that the fonio endosperm is not waxy. Waxy starch structure affects many properties of flour. Waxy flour structure absorbs more water during dough formation and requires less time and energy for dough development (Yalçin, Masatcioğlu, & Cindik, 2020).

The sugars (sucrose, glucose, and fructose) detected in the fonio grain have an average soluble sugar content of 1% (Cruz et al., 2011). Fonio is regarded as a viable food for people with type 2 diabetes (diabetes mellitus) due to its low glycemic index. In a study by Traore & Ndoye, Hamaker, Stoecker, Betts, & Guiro, (2009) on the effects of different cereals consumed

in Mali on glycemia, fonio was reported to have a lower glycaemic index value with GI=66 than sorghum (GI=72) and rice (GI=95).

5.2. Protein and Amino Acids

The average crude protein content of Fonio grain samples was 8.05% (Table 1). Fonio also has four protein fractions that can be found in most cereals, namely albumin (3.5%), globulin (1.8%), prolamin (5.5%), and glutelin (14%). These results suggest that glutelin is the most significant protein fraction in fonio grain; prolamin and glutelin are storage proteins, while albumin and globulin are metabolic proteins. Fonio does not contain gliadin protein from the prolamins group, which is a suitable feature especially for the use of celiac patients (Adoukonou-Sagbadja et al., 2007; Karahan et al., 2009). As in many cereals, lysine is low in fonio. However, fonio is richer in methionine and cysteine, which are low in major cereals such as sorghum, rice, wheat, or barley (Ballogou, Soumanoud, Toukourou, & Hounhouigan, 2013).

5.3. Lipids

Lipids are the third major macronutrient in human and animal nutrition following protein and carbohydrates. Lipids are relatively small constituents of cereal grains but play an important role in the quality of cereals during their storage. The average lipid content determined for fonio was 3.25% (Table 1). This average value obtained for fonio is higher than sorghum (3.2%) and rice (2.5%) but lower than millet (5.1%) (Serna Saldivar, 2003).

5.4. Vitamins

Vitamins are trace molecular nutrients essential for growth, metabolism, reproduction, and general health. Cereals are a good source of vitamins other than vitamins B12, C and D. Fonio is a rich source of B-group vitamins such as thiamine, riboflavin, and niacin, which are essential for the growth, development, and function of cells as well as for energy production. Serna Saldivar (2003) reported that vitamin B3 (niacin) was found in higher concentrations in fonio than other vitamins (2.03 mg/100 g).

5.5. Minerals

In general, cereals with a similar grain structure are composed of three main parts: the outer shell or bran (10-14%), the endosperm (80-85%), and the germ (2.5-3%). The bran is the part that surrounds the grain and protects it from external factors and has rich fiber content. The

pericarp, the germ, and aleurone are rich in vitamins and minerals, but as these layers are separated from the grain during refining, the grains lose some of their minerals and vitamins. According to the results obtained in the studies on fonio, the average ash content was determined as 3.5% (Table 1). Jocelyne et al., (2020) studied the nutritional values of wheat, maize, sorghum, millet, and fonio. According to their results, fonio was found to be richer in calcium (19.6 mg/100 g km) and zinc (2.27 mg/100 g km) than other samples. Similarly, Temple & Bassa, (1991) reported that compared to most cereals, fonio was higher in calcium, magnesium, iron, and copper, but lower in potassium, sodium, and manganese.

5.6. Fibers

The indigestible portion of food consumed by humans consists largely of dietary fibers, hemicellulose, cellulose, and lignin. The average value of crude fiber content reported for fonio grain is 5.85% (d.m.). According to Cruz et al., (2011), the hemicellulose, cellulose, and lignin contents of fonio are 3.0%, 4.0%, and 0.5%, respectively. Fonio is a highly digestible food that provides short-term satiety in whole grain form. A high-fiber diet may reduce the risk of cardiovascular disease, colon cancer, and diabetes.

5.7. Energy value

According to studies, the average energy value of fonio was reported to be 463.7 (kcal/100 g) (Table 1). These results are higher than other cereals such as wheat (329.6 kcal/100 g), rice (430 kcal/100 g), maize (410 kcal/100 g), and sorghum (399 kcal/100 g) (Serna-Saldivar, 2003).

5.8. Phenolic Compounds

Phenolic compounds include a group of compounds defined by the aromatic ring formed by one or more hydroxyl groups and various groups. The main phenolic compounds are phenolic acids, flavonoids, and lignans. According to Pietta, (2000), polyphenolic compounds such as flavonoids, phenolic acids, and proanthocyanidins are essential for radical scavenging activities and are effective in the prevention of many important diseases. Jocelyne et al., (2020) investigated the phenolic acid content of wheat, corn, sorghum, millet, and fonio samples in their study. As a result of the study, fonio was close to wheat in terms of phenolic compounds, higher than sorghum but lower than maize and millet. In terms of flavonoid content, fonio was higher than millet and sorghum but lower than maize and wheat. Similarly, Bello, Ogbesejana, Balkisu, Osibemhe, Musa, & Oguntoye, (2022) investigated polyphenolic fractions in three millet species (Fonio, finger millet, and pearl millet). The extract obtained from

fonio showed the highest antioxidant activity compared to other samples and at the end of the study, they stated that these polyphenolic extracts obtained from millet species have therapeutic potentials that can play important roles in the prevention and management of type 2 diabetes, and especially fonio has a higher potential for use as a functional food.

5.9. Anti-Nutrient Ingredients

Anti-nutrients are defined as synthetic or natural compounds that inhibit the absorption of nutrients in foods and include saponins, tannins, lectins, protease inhibitors, and amylase inhibitors (Samtiya, Aluko, & Dhewa, 2020). Jocelyne et al., (2020) examined the anti-nutrient component ratios of wheat, maize, sorghum, millet, and fonio samples. According to the results of the study, fonio was reported to have high phytic acid, medium oxalate, and low tannin content.

6. POTENTIAL USES OF FONIO IN FOOD PRODUCTS

Similar in taste to rice, fonio can be cooked into porridge and cream and eaten directly. It can be made into desserts and fermented drinks. Its flour can also be mixed with other flour to make biscuits. It is also prepared as a staple food, eaten with vegetable soup, or made into an oat-like breakfast cereal (Morales-Payán, Ortiz, Cicero, & Taveras, 2002). Fonio has a slightly earthy and nutty flavor and is good at absorbing flavors. It goes well with both savory and sweet recipes and is a gluten-free alternative to couscous. Cooking fonio is quick and simple. Cooked fonio can be eaten with a sauce, such as rice or pasta, or used to make a cereal or salad bowl (Figure 6).

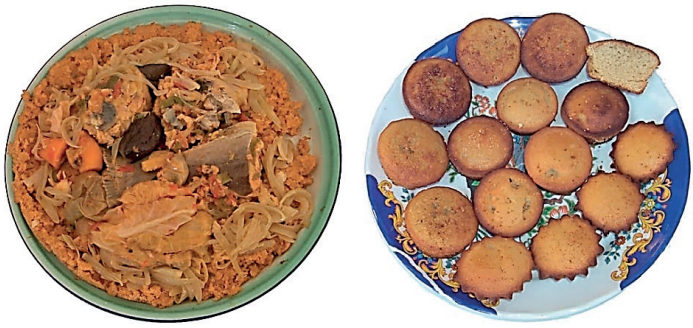

Figure 6. Traditional fonio foods (Cruz & Béavogui, 2016).

Fonio, a cereal that is believed to be a healthy and inexpensive addition to diets with a variety of products available to consumers in Europe, while at the same time generating income for local producers

in Africa, is being introduced to the European market through a project managed by the French Agricultural Research Center for International Development (Figure 7) (Cruz et al., 2011).

Figure 7. Commercially available boiled or pre-cooked fonio products (Cruz & Béavogui, 2016).

Fonio is mostly produced by smallholders and sold in local urban markets but is also sold to Africans migrating to Europe and the United States. Export markets are dominated by high consumer demand, partly due to its nourishing qualities and to help meet the demand for a more varied cereal diet. With the increase in research and development activities, it is expected that exportable value-added acha and iburu products will be available in the European and United States markets in the near future. Fonio is already used in the production of many types of food but is also recommended and attracted in the production of healthy or specialty foods such as alcoholic and non-alcoholic beverages, bakery products, biscuits, breakfast cereals, cookies, flour, porridge, sweets (dambu), granulated dough products, pasta, sourdough, traditional beverages, and wheat-free fonio bread, (Obizoba & Anyika, (1994); Nzelibe, Obaleye, Chidozie, & Onyenekwe, (2000); Ayo & Nkama, (2004); Jideani & Ibrahim, (2005); Jideani et al., (2007); Agu, Jideani, & Yusuf, (2008); Babarinde et al., 2020)).

Švec & Hrušková, (2018) studied the effect of fonio flour in combination with bread wheat flour on bread quality. Commercial fonio flour was mixed with wheat flour in three different proportions (2.5%, 5.0%, and 10.0%). In the Farinograph test, the addition of fonio flour shortened the dough stability (from 12.0 to 3.5 minutes). In the fall number test, the 2.5% wheat flour- fonio flour mixture gave the highest value (425 s). With the same additive rate, bread volume increased from 337 to 402 ml/100 g. At the end of the study, it was reported that 2.5% to 5% fonio flour

additives gave the best values in terms of dough workability and bread quality.

Olapade & Oluwole, (2013) investigated the bread-making potential of cowpea flour (*Vigna unguiculata*) in addition to wheat-fonio blend flour. Wheat and fonio blend flour were prepared at a ratio of 9:1 (w/w) and then mixed with cowpea flour at 95:5, 90:10, and 85:15 (w/w) respectively. As a result of the study, it was revealed that the protein content of bread samples prepared by adding 10% cowpea flour to wheat-fonio blend flour was improved, water retention capacity was increased and breads with acceptable sensory qualities could be produced.

Ayo, (2004) studied the effects of fonio (*Digitaria spp.*) and millet (*Pennisetum typhoideum*) cereals on Kunun-zaki (a cereal-based non-alcoholic fermented drink). Sorted fonio and millet were boiled in water, washed, and ground with spices, dry, granulated sweet potato, and sugar to produce syrup for making kanun-Zaki drink. As a result of the study, the effect of fonio and millet on the physico-chemical and sensory quality of kanun-Zaki did not make much difference. However, in terms of taste and aromatic characteristics, fonio-based kunun-zaki was found to be more acceptable than millet-based kunun-zaki. These results suggest that fonio grain is suitable for use in traditional soft drink production.

Chinma, Anuonye, Ocheme, Abdullahi, Oni, Yakubu, & Azeez, (2016) examined bread quality, antinutritional and antioxidant properties by making bread trials with sourdough prepared with a mixture of fonio flour, Bambara nut flour, and wheat flour. Fonio flour and bambara nut flour were mixed in a 1:1 ratio and made into sourdough. Then, the mixture of wheat flour and sourdough powder obtained from fonio-bambara nut flour was mixed at ratios of 100:0, 90:10, 80:20, and 70:30 (wheat flour: sourdough powder) and used for bread production. According to the results of the content analysis of the bread samples, changes were observed in dietary fiber, in-vitro protein digestibility, mineral ratios; total amino acid and antioxidant (DPPH, FRAP) levels in parallel with the ratio of sourdough powder in the mixture. In addition, the amount of phytic acid (mg/100 g) varied as (1.63, 1.20, 1.25, 1.36). In terms of bread properties, the specific volume, color, and texture of the blended bread were not different from the control bread. At the end of the study, the addition of up to 10% sourdough flour was found to be feasible in terms of taste, flavor, and acceptability scores compared to wheat bread.

Ayo & Nkama (2004) conducted bread trials by mixing fonio flour and wheat flour in different ratios (100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 0:100) and evaluated the loaf volume, moisture content, and sensory qualities of the samples in comparison with the control sample. The

water-holding capacities of the prepared flours varied according to the mixing ratios (0.66, 0.95, 1.02, 1.17, 1.20, 1.26, 2.27), respectively. The specific volumes (cm^3/g) of the prepared bread samples were determined as (4.19, 3.89, 3.51, 3.30, 2.79, 2.50, 1.62), respectively. Average sensory scores for taste, smell, crust color, bread color, bread color, bread texture, and external appearance decreased from 7.8 to 2.95, 7.35 to 3.5, 6.60 to 2.70, 7.70 to 2.95, 7.65 to 2.90 and 7.95 to 2.2, respectively. In the criteria evaluated overall, a maximum of 30% fonio cereal flour was considered appropriate.

Olagunju, Omoba, Enujiugha, & Aluko, (2018) studied the production of crackers by mixing fonio flour (*Digitaria spp.*) and pigeon pea flour (*Cajanus cajan*) in certain ratios. Fonio flour: pigeon pea flour ratios were determined as 100:0, 80:20, and 70:30, respectively. The chemical composition, antioxidant, and antidiabetic properties of the cracker samples were evaluated. When the prepared samples were examined, crude fiber content, ash content, fat content, and protein content increased in parallel with the ratio of pigeon pea flour in the mixture. The carbohydrate content decreased with the decrease of fonio flour in the samples, which was found to be related to the proportional reduction of fonio, which is a good starch source, in the mixtures. When cracker samples were evaluated in terms of DPPH and FRAP; DPPH scavenging activity ranged between 28.96% and 59.34% according to the results. FRAP value varied between 0.07 and 0.79 mmol Fe^{2+}/mg depending on the sample concentration. Crackers with a 70:30 ratio showed the highest inhibition activity against digestive enzymes responsible for carbohydrate degradation and absorption in digestion and also gave the lowest glycemic index value (GI: 47.95).

Babarinde et al. (2020) investigated some quality parameters of flour produced by using fonio (*Digitaria iburua*) and pigeon pea (*Cajanus cajan*) in certain proportions. The mixture of fonio flour and pigeon pea flour was prepared at ratios of 100:0, 95:5, 90:10, 85:15, and 80:20 (fonio flour: pigeon pea flour) and analyzed for moisture, protein, fat, ash, crude fiber, energy, vitamins, minerals, and amino acids. For breakfast cereal production, 100:0 and 80:20 flour mixtures were prepared based on the results obtained from the first study and compared with cornflakes in terms of sensory properties. According to the results obtained, there was an increase in moisture, protein, fat, ash, and crude fiber ratios, but a decrease in carbohydrate and energy values. In terms of vitamin and mineral values, it was stated that especially the flour mixture prepared at 80:20 gave high results. In the sensory tests, no significant difference was found with standard breakfast cereal, but the addition of the fonio-pigeon pea mixture showed a significant increase in nutritional properties. At

the end of the study, it was stated that the fonio flour-pigeon pea flour mixture could be used in breakfast cereals at a ratio of 80:20.

CONCLUSION

Fonio (*Digitaria spp.)* is ancient African grain rich in carbohydrates, fats, fiber, vitamins, minerals, and amino acids. It is especially important as a staple food in times of food shortages. It grows easily in arid and poor soils without the need for fertilizers and other cultivation methods and can be harvested and stored in a short time. It is an important grain in many West African countries as it is often used in various religious or cultural rituals.

When consumed as a whole grain, it aids nutrition with its high dietary fiber content and therefore has nutraceutical properties. Despite its rich nutrient content and health benefits, fonio is under-researched compared to other types of cereals. This has started to change recently, especially as Fonio has started to gain ground in the production of foods rich in healthy and functional ingredients.

In general, as has been done for cereals such as rice, maize, and wheat, the structure, constituent properties, functionality, and use of fonio grain alone or as an additive in food products should be studied in detail. Also, the properties and functionality of Fonio and its products can be enhanced by using different processing techniques (high pressure, ozone, cold plasma, ultrasound processing, etc.).

It is possible to produce food products such as breakfast cereals, pasta, crackers, biscuits, cookies, crisps, etc., using fonio in formulations that are in line with the concept of healthy food with functional qualities, and the food sector, especially those looking for new products, can evaluate this potential. There is a great opportunity for the use of fonio as a content enrichment in new product formulations, given the recent scientific research and evaluations that promote the use of ancient and pseudo cereals.

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Chapter 6

APPLICATION OF NANOTECHNOLOGY IN FOOD SCIENCE

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1. INTRODUCTION

The Greek word "nano" means "dwarf". One billionth of a meter (10^{-9} m) is a nanometer. The diameter of DNA is between 2.5 nm and 60,000 times smaller than the diameter of a human hair or the size of a virus. A red blood cell is between 2,000 and 5,000 nm in size. A regular sheet of paper is approximately 100,000 nm thick. The term "nanoparticles" refers to particles with a particle size of fewer than 100 nanometers that offer novel applications and benefits through special phenomena. The majority of study has been done on nanomaterials, which are often powders made of nanoparticles that have different properties from powders with the same chemical makeup but much larger particles. Due to their distinctive functions and applications of nanomaterials, research is ongoing into their possibilities in the food nanotechnology sector, including food packaging, foods, and supplements. In a race around the world to use nanotechnologies in food production, processing, and packaging, tens of millions of dollars are being spent (Sekhon, 2010).

The widespread use of nanotechnology in daily life is revolutionizing society. Products made with nanotechnology are employed in many aspects of our lives today. The most popular of these goods are sunscreens made of ZnO and TiO_2. As they offer less skin bleaching and better UV reflection and absorption, TiO_2 and ZnO nanoparticles (NP) in these sunscreens are rapidly being included in photoprotective products (Chen & Wang, 2016). Some of the nanotechnology-based items we use every day include liquid crystal displays (LCD), deeply penetrating cosmetics, and wrinkle-resistant fabrics. Nanoliposome technology has been employed in the creation of mRNA-based vaccinations, which are medicines that slow the COVID-19 pandemic. Nowadays, the most popular industries for nanotechnological products are electronics, textiles, medicine, and pharmaceuticals. But the great application potential and unique features of nanomaterials have attracted the attention of the agricultural and food industries. Since the US Department of Agriculture published the first roadmap on September 9, 2003, it has been marching into the agricultural and food industries (He et al., 2019).

Due to their exceptional physiochemical nature and antibacterial characteristics, nanoparticles are frequently utilized in food science to combat a variety of pathogenic bacteria as well as in healthcare, crop protection, water treatment, food safety, and food preservation. Nanostructured materials are also used in the food industry as novel packaging material, encapsulated food components, and nanosensors. This chapter focuses on the use of nanotechnology in the food sector, including the utilization of nanoparticles and designed nanostructures.

2. THE USEFULNESS AND POTENTIAL OF FOOD NANOTECHNOLOGY

The global food and beverage market is worth several trillion dollars. The term "non-food" refers to a variety of substances that come into touch with food in addition to the basic materials used in food production and those found directly in food. As a result, the field of food science and technology finds nanotechnology to be appealing (Nile et al., 2020). In the food sector, 372 nanotechnological items have been marketed or are in the process of doing so, according to the StatNAno database. These goods fall into the following categories: 164 are supplements, 112 are packaging, 53 are foods, 31 are sports nutrition, and 12 are sensors (StatNano, 2022) However, it is clear from the listed products' ingredients that there aren't many nanotechnological items that are directly added to food.

Currently, food packaging, release (nanoencapsulation) systems, and food nanosensors are some possible applications of nanotechnology in the food business. Additionally, nanomaterials, which have a wide surface area and lower fat and sugar in foods while having the same effect when employed in smaller doses compared to their bulk equivalents, may be an option. In conclusion, there are four categories under which nanotechnology may be used in the field of food science. The prospective applications of nanomaterials in foods are discussed in this section.

2.1. Food Packaging and Coating

Food packaging is essential for maintaining food quality and extending product shelf life by protecting food from harmful external environmental variables such as light, moisture, microbes, and oxygen (D. Zhang et al., 2021). Food contact materials are designed to come into direct contact with food products during production, delivery, and storage (He et al., 2019). The main problem with food packaging materials is that they are porous. There is currently no packaging material that completely resists ambient gases, water vapor, food, and packaging material. In the food packaging sector, organic polymer materials such as polypropylene, polyethylene, polyethylene terephthalate, polystyrene, and polyvinyl chloride continue to be the top choice due to their low cost, ease of processing. Yet, their innate permeability to gases and other tiny molecules is the main issue (Sharma et al., 2017).

The food sector has been studying and developing nanotechnology as a revolutionary alternative to food packaging. When compared to traditional packaging materials, nanomaterials made for food packaging have various benefits. The functionality of packaging materials as well as the shelf life and quality of food products have all been improved by the

application of nanotechnology in food packaging. The following are a few examples of how nanotechnology is used in food packaging:

2.1.1.Barrier coatings

Nanoparticles such as clay and metallic nanoparticles can be used to create barrier coatings for packaging materials. These coatings can prevent the migration of gases and moisture into or out of the packaging, which can help to preserve the freshness of the food product. Also, these materials contribute superior barrier and mechanical properties compared to conventional packaging materials. Recent studies have shown that nanofillers offer superior mechanical and barrier qualities for packing as well as enhanced antibacterial and UV-blocking properties Swaroop & Shukla, 82018) sought to develop biofilm in one of the research carried out for this objective by adding polylactic acid (PLA) biopolymer with magnesium oxide (MgO) nanoparticles. The most significant improvements in tensile strength and oxygen barrier characteristics (up to 29% and 25%, respectively) over pure PLA films were seen in the 2% by weight-reinforced PLA films. This has been attributed to the polymer matrix's inclusion of nanoscale fillers, which led to a comparatively high level of surface interaction between the filler and the polymer chains. In contrast to pure PLA films, films containing 2% nanofillers saw an increase in water vapor permeability while their oxygen permeability fell by 25%. Additionally, PLA/MgO films displayed superior antibacterial activity.

According to Jo et al., (2018) polypropylene (PP), (Ag/PP) nanocomposite films containing AgNPs show better mechanical characteristics than regular PP. It was established that the increase in mechanical strength observed here was caused by the attraction between the polymer matrix and AgNP. According to S. W. Lee et al. (2021), the inclusion of ZnO nanoparticles considerably changed the characteristics of chicken skin gelatin/tapioca starch composite films. The study's findings were highlighted, highlighting how adding ZnO nanoparticles to the films as a nanofiller material enhanced their antibacterial and thermal properties while lowering their water vapor permeability.

Due to its mechanical, thermal, and barrier qualities as well as its inexpensive cost, nano clay is one of the many new nanomaterials that has been explored and used extensively for food packaging. For instance, a nanocomposite membrane with a 1 wt% bentonite clay/poly (vinyl alcohol) loading and a water permeance of 6500 gpu, and a selectivity value of 46, significantly increased permeance. In a study, Wu et al., (2019) developed a food packaging film by using sodium tripolyphosphate (TPP) as a cross-linking agent and a straightforward in situ self-assembly approach, a series

of chitosan/polylysine (-PL) bio-nanocomposite films were created. These films' mechanical, structural, physical, and antibacterial attributes were examined. By increasing the ratio of -PL, the bio-nanocomposite films demonstrated good antibacterial activity against *E. coli* and *S. aureus*. Moreover, a prolonged release from the films was provided by PL, which was closely associated with TPP concentration. The findings of this study indicated that chitosan/PL films have significant potential for usage in the food industry as an antibacterial biomaterial.

2.1.2. Antimicrobial nano-coating

Microbial deterioration is an important issue in the food sector as microbial growth affects directly public health. Hence, controlling bacterial decay is one of the most important issues in the preparation, processing, transportation, and storage of food. The use of novel nano antimicrobials has demonstrated excellent results in preventing food deterioration and prolonging food shelf life. The nanomaterial-encoded edible covering has also demonstrated its potential for food preservation and storage. Fruits and vegetables that have been coated with fresh food remain edible during storage and transit. As transportation and storage times increase, the active respiration processes may result in large postharvest losses as well as the poor nutritional and cosmetic quality of items. The prevention of such nutrition and weight loss is essential for extending the shelf life of fresh food goods. Temperature and relative humidity are the two main issues. Together, they affect the microbiological activities in the products as well as the respiration of fresh food. In addition to serving as a gas and moisture barrier, a thin layer of hybrid nano-edible films. The majority of edible coatings are made of organic compounds derived from natural extracts, as opposed to metal-based nanoparticles.

One of the most often employed nanomaterials as antimicrobials in the food sector is silver nanoparticles and nanocomposites. The FDA has authorized the use of 12 silver-containing zeolites or other compounds as food contact products for disinfection. Several recent research studies revealed that silver nanocomposites are safe for food packaging since they release and migrate silver nanoparticles in undetectable or negligible amounts into genuine food samples and food simulants (He & Hwang, 2016).

Silver nanoparticles (AgNPs) significantly hinder the permeability and respiratory processes of bacteria by adhering to the cell membrane's surface. AgNPs can also enter and harm bacterial cells, perhaps by interacting with substances like DNA that contain sulfur and phosphorus. A second mechanism for the bactericidal action of AgNPs involves the release of silver ions, which have the potential to be very reactive and

can interact with the negatively charged cell membrane (Morones et al., 2005). Another antibacterial mechanism, which was put forth by Sobye et al., (2015), proposes that AgNPs hinder or limit bacterial cell growth and metabolism, speeding up lysis. Additionally, it is claimed that AgNPs harm microbial cells through oxidative damage by promoting the production of reactive oxygen species (ROS) (Acharya et al., 2021). The most recent research on the use of AgNPs in food packaging was summarized by Simbine et al., (2019). Silver nanoparticles have shown potential as antibacterial agents in packaging materials, according to scientists, but they also emphasized the need for more research to determine their safety and efficacy.

In addition to AgNPs, other metallic nanoparticles such as zinc oxide, and titanium dioxide have been used to develop antimicrobial coatings for food packaging. These coatings can inhibit the growth of bacteria and other microorganisms, which can extend the shelf life of food products (Król et al., 2017; Kumar et al., 2020; S. W. Lee et al., 2021; Simbine et al., 2019).

Also, several bioactive components-loaded nanofibers have been used for food coating applications. In this regard, pomegranate seed oil (Kutlu et al., 2022), grape seed oil (Ceylan, Z., Kutlu, N., Meral, R., Ekin, M., M., Kose, 2021), black cumin oil (Ceylan et al., 2022) nisin (Oner et al., 2021), and curcumin (Meral et al., 2019) were applied to several food samples, and effective protection in terms of microbial growth limitation was obtained as a result of these applications.

2.1.3.Antioxidant nano-coating

Nanoparticles such as magnesium oxide (Swaroop & Shukla, 2018), titanium dioxide (J. Liu et al., 2021), and several plant-original materials can be incorporated into packaging materials to create oxygen scavengers. These scavengers can absorb oxygen that enters the packaging, which can help to prevent spoilage and extend the shelf life of food products. Ansarian et al., (2022) compared the effects of resveratrol (RES) and clove essential oil (CEO) as in vitro antioxidants in traditional and nanoemulsion-containing basil seed gum (BSG) films. Then, during 20 days of storage at 4 °C, the effects of the best nanoemulsion-based BSG film discovered through in vitro evaluations were assessed on the oxidative stability and sensory characteristics of minced camel meat. The findings revealed synergistic interactions between RES and CEO as well as stronger in vitro antioxidant activity for nanoemulsion containing BSG films than for conventional films. Additionally, when compared to the control group, minced camel meat wrapped in nanoemulsion-based BSG film that contained CEO 10 mg/kg and RES 4 g/mL had better results in

terms of thiobarbituric acid reactive substance, total carbonyls, sensory analysis, and peroxide value. The current study's findings can be used to develop a novel packaging strategy for meat and meat products.

Nanoemulsions containing additional active chemicals, such as cinnamaldehyde, garlic oil (Pérez-Córdoba et al., 2018), ginger essential oil (Cai & Wang, 2021), and oregano essential oil (J. Y. Lee et al., 2019), have also been employed to include antioxidant compounds in packaging materials.

2.1.4. Smart packaging

Packaging that can detect changes in temperature, humidity, or other environmental conditions that may have an impact on the quality of the food product can be made using nanotechnology. Supply chain managers or consumers can then be informed of this information, enabling more precise monitoring and management of the food product (Kuswandi, 2017). To make it easier to detect gases, odor changes, contaminating compounds (such as veterinary medications, poisons, pesticides, etc.), and pathogens, nanomaterials have been used for their chemical and electro-optical capabilities (Chausali et al., 2022).

Overall, applying nanotechnology to food packaging has the potential to increase food quality, decrease food waste, and improve food safety. To make sure these materials are risk-free for both the environment and human health, it is crucial to thoroughly assess their safety.

2.2. Nanoencapsulation

Nanoencapsulation is the process of encapsulating or trapping a distinct substance inside extremely small particles, often known as nanoparticles. These 1 to 100-nanometer-sized nanoparticles can contain a wide range of chemicals, including tastes or odors, medications, or even food or nourishment (Jafari, 2017).

To create nanoencapsulation, a variety of technologies can be used, including emulsion-based methods, coacervation, and electrostatic deposition, among others. In these processes, the material is enclosed and protected from deterioration and exposure to the environment by a shell or coating. Numerous advantages of nanoencapsulation include managing the release of active compounds over time, protecting delicate chemicals from oxidation or degradation, and improving the solubility and bioavailability of medications. Nanoencapsulation is a promising technology for a range of industries, including the pharmaceutical, food, and cosmetic industries, among others, as it can boost the stability,

sensory qualities, and shelf life of various commodities (Meral, Ceylan, et al., 2022).

Due to its numerous advantages for the encapsulation of food ingredients, nanoencapsulation has grown in favor. One application of nanoencapsulation in the food industry is the encapsulation of taste and fragrance molecules, which are often lost or damaged during food preparation or storage. By being contained within nanoparticles, these compounds can be protected from oxidation, volatilization, and other environmental impacts, extending their stability and shelf life. Another way to improve the bioavailability and potency of bioactive chemicals in the body is to encapsulate them, such as vitamins, minerals, and antioxidants. Nanoencapsulation permits these compounds to pass through the stomach and enter the small intestine, where they can be more efficiently absorbed, by protecting them from stomach acids and digestive enzymes. Additionally, the release of certain dietary elements, such as flavor- or nutrient-enhancers, can be gradually controlled via nanoencapsulation. This can enhance the sensory aspects of the food or prolong the release of nutrients in functional foods and supplements (Bazana et al., 2019; Meral, Kose, et al., 2022; Rostamabadi et al., 2020).

Nano-encapsulated substances come in a wide range. Here are a few examples:

2.2.1.Probiotic microorganisms

Live bacteria known as probiotics can help the host's health when given in sufficient doses. These probiotic formulations are anticipated to benefit the host by altering the gut microbiota, producing metabolic entities, neutralizing dietary carcinogens, triggering the manufacture of cytokines, and controlling infections. Probiotics must endure a variety of environmental threats, including low gastric pH, enzymatic breakdown, the antibacterial activity of bile salts, and competition with other bacteria, to achieve these health benefits. Probiotics must also successfully adhere to the gut epithelium to achieve persistent intestinal colonization. As a result, microbial encapsulation tactics are effective ways to safeguard probiotics and encourage their successful oral delivery to their intended site (Centurion et al., 2021). Several strains of this bacterial group's probiotic cells have been studied utilizing electrospun single or binary combinations of biopolymers to protect them in food and the digestive tract (Atraki & Azizkhani, 2021). For example, Ceylan et al., (2018) fabricated sodium alginate and PVA-based nanofibers that contained *Lactobacillus rhamnosus*. The analyses revealed that 83% of the *Lactobacillus rhamnosus* species maintained their viability

Atraki & Azizkhani, (2021) attempted to test the viability of some probiotics in a simulated gastrointestinal and gastric fluid environment by electrospinning nanofibers from corn starch and sodium alginate. 94.1% of *Lactobacilli* and 89.4% of *Bifidobacteria* were found to be viable after electrospinning. The results of the experiment showed that after two hours, *Lactobacilli* and *Bifidobacteria* decreased by 1.58 log and 1.03 log, respectively, in gastric and intestinal fluid, while non-encapsulated bacteria lost viability. There was a 3.02 log reduction in lactobacilli and a 2.55 log reduction in bifidobacteria after the 3 hours in this model of the gastrointestinal tract.

To produce functional fish fillets, Ceylan et al., (2019) nanoencapsulated *Lactobacillus reuteri* E81 strain with polyvinyl alcohol-based nanofibers. According to some reports, the bacteria in the nanofiber can retain 78% of their vitality. Additionally, it was found that probiotic bacteria could be kept alive for up to 4.38 log on the second day of storage and up to 3.39 log on the seventh day of storage when nanofiber was kept at 4°C.

With the help of sodium alginate and corn starch, Ghorbani & (Maryam, 2021) studied the viability stability of probiotic *Lactobacillus acidophilus* (LA5), *Lactobacillus rhamnosus* 23,527 LGG, *Bifidobacterium bifidum*, and *Bifidobacterium animalis* bacteria in yogurt for 20 days at 4°C and various pH levels (pH: 7.0-4.5-2.8). While 97.9% of the viability of the nanoencapsulated *Lactobacilli* and 96.9% of the viability of the *Bifidobacteria* were preserved in the yogurt after 20 days of storage, only 87.7% of the viability of the unencapsulated lactobacilli and 86.3% of the viability of the bifidobacteria were.

For simple gastrointestinal transit (GIT), Ragavan & Das, (2020) employed the probiotic yeast *Saccharomycopsis fibuligera* (S. fibuligera) VIT-MN04, wheat bran fiber (WBF), and exopolysaccharide together with 5% polyvinylpyrrolidone (PVP). The vitality of the yeast has been examined at 25°C, 4°C, and in vitro in the gastrointestinal environment. As a result, although the unencapsulated sample only managed to survive 82% in the gastrointestinal environment, the nanoencapsulated yeast cells demonstrated 97% vitality. The yeast cells that were encapsulated also remained alive for 56 days while being stored at 4°C.

2.2.2. Carotenoids

Today, the role of carotenoids-promising functional components in the human diet-is becoming increasingly important. Apart from their crucial role in photosynthetic organisms or as natural pigments, these substances are frequently referred to as health-promoting nutrients because of their many advantageous properties. However, the greatest

drawbacks preventing pharmaceutical and food carotenoid applications are their susceptibility to environmental and process stressors, limited water solubility, and low bioavailability. In this regard, lipid-based nano-delivery cargos, such as nano-liposomes, surfactant-based nano-carriers, nano-emulsions, nanostructured lipid carriers (NLCs), and solid lipid nanoparticles (SLNs), are proving to be an effective platform for protecting carotenoids against difficult environmental conditions as well as providing an effective controlled release (Rostamabadi et al., 2019).

Mehrad et al., (2018) aimed to increase the physicochemical stability of β-carotene by encapsulating it in whey protein isolate (WPI) stabilized solid lipid nanoparticles (SLNPs) containing palmitic acid and maize oil. WPI enhanced the system's colloidal stability and enhanced the oxidative stability of β-carotene.

2.2.3. Vitamins

Vitamin E is a potent antioxidant that is often added to foods and supplements to protect them from oxidation. Nanoencapsulation of vitamin E can improve its stability and bioavailability. For example, a study conducted by N. Liu and Park, (2009) reported that the stability of Vitamin E-loaded liposomes suspension during the 8 weeks of storage is over 90% under 4°C. Another study demonstrated that these nano complexes can be used to safeguard vitamin E to prolong shelf life, improve thermostability, and enhance feasibility for commercial use (Xia et al., 2014).

Vitamin D is an essential nutrient that plays an important role in bone health, immune function, and other physiological processes. However, it is often unstable and can be degraded by heat, light, and oxygen. Nanoencapsulation of vitamin D can protect it from degradation and improve its bioavailability. For example, Luo et al., (2012), reported that encapsulation of vitamin D3 in zein nanoparticles coated with carboxymethyl chitosan improved its chemical stability and controlled release property.

Vitamin A is essential for human health. Vitamin A was encapsulated in lipid nanoparticles to overcome its limited water solubility. Resende et al., (2020) created lipid nanoparticles loaded with vitamin A. For a month, the created nanoparticles remained stable in suspension. Moreover, in a simulated stomach, these nanoparticles did not disintegrate.

2.2.4. Minerals

A trace mineral called zinc is crucial for the growth and development of humans. Due to limited bioavailability and insufficient consumption

of foods containing zinc, there may be a zinc shortage in up to 40% of the world's population. Gülseren et al., (2012) fabricated whey protein isolate (WPI) nanoparticles, and their ability to incorporate $ZnCl_2$ were evaluated. After 30 days of storage at 22 °C, the nanoparticles still exhibited good integration efficiencies and were stable. The zinc content of the WPI particle suspensions was within the range of what healthy individuals need each day in terms of zinc.

2.2.5. Essential oils

Essential oils are natural oils extracted from plants that are commonly used in food, cosmetics, and aroma therapy. However, they are highly volatile and can be easily lost during processing and storage. Nanoencapsulation of essential oils can improve their stability and controlled release properties. Essential oils are often added to foods and beverages for their flavor and aroma properties, but they can be easily degraded or lost during processing and storage. Nanoencapsulation of essential oils can improve their stability and release properties. Also, the sharp odor of essential oils can be minimized with nanoemulsion systems (Ceylan et al., 2020; Ekin et al., 2021; Meral, Ceylan, et al., 2019).

2.2.6. Omega-3 fatty acids

Omega-3 fatty acids are essential nutrients that play a critical role in human health, including cardiovascular health, cognitive function, and inflammation. However, they are easily oxidized, leading to degradation and loss of activity. Nanoencapsulation of omega-3 fatty acids can protect them from oxidation and improve their stability(Kuznetcova et al., 2020; Vieira et al., 2022).

Current researchers have looked into the use of carrier systems including nanoparticles, nanofibers, and nanoemulsions to insert PUFA-rich oils into intricate food matrices (McClements et al., 2021).

For instance, coaxial electrospraying was used in the study done by Atay & Altan, (2021) to load black cumin oil into the zein nanoparticle. It was shown that after 55 days of storage at 60 °C, the peroxide number of the oil encapsulated with nanoparticles was three times lower than the unencapsulated form.

These are just a few examples of how nanoencapsulation can be used to improve the stability and functional properties of oils in food, cosmetic, and personal care products. There are many other studies and applications in this field, and ongoing research is exploring new ways to use this technology to enhance the efficacy and functionality of oils and other natural compounds.

2.3. Detection of Contaminants

The ongoing concern for human health and food safety has sparked innovation in the creation of technology for the quick assessment of toxicity risks owing to the presence of potentially dangerous compounds and toxins that can harm the environment and the quality of food products (Mustafa et al., 2017). It is possible to establish whether food is contaminated and whether products contain allergies by analyzing pollutants and allergens using detection technologies. The use of high-performance liquid chromatography-mass spectrometry (HPLC, LC-MS) to precisely quantify the concentration of a chemical analyte, for example, offers extremely high sensitivity and good selectivity (Hua et al., 2021). The exorbitant cost of these instruments and the prolonged sample preparation processes have, however, prompted researchers to look for efficient and trustworthy analytical techniques.

For quick field screening and sample quantification, quick and straightforward procedures with low reagent and power requirements are preferred. The use of these materials for pathogen detection and integration with biomolecules is made possible by the fundamental characteristics of the many types of nanostructures (Mustafa et al., 2017). Nanotechnology can be used to identify food contaminants in several ways:

2.3.1. Nanosensors

Nanosensors are instruments that use materials or structures at the nanoscale to identify and measure particular molecules or chemicals. They are made to be extremely sensitive and selective, enabling the detection of target molecules at extremely low concentrations in challenging samples like food or bodily fluids (Kuswandi, 2017).

Three primary parts are commonly found in nanosensors: a transducer, a recognition element, and a signal amplifier or output device. The transducer turns the interaction into a quantifiable signal while the recognition element is made to specifically engage with the target molecule. The signal is subsequently amplified or shown by the signal amplifier or output device, enabling the identification and measurement of the target molecule. Many nanomaterials, such as nanoparticles, nanotubes, and nanowires, can be used to create nanosensors. They can be functionalized with various recognition components, such as enzymes, aptamers, and antibodies, enabling the detection of a variety of compounds, including proteins, DNA, and tiny molecules (Srivastava et al., 2018).

Several industries, including biomedical diagnostics, environmental monitoring, and food safety, use nanosensors. Nanosensors can be used to identify and quantify pollutants in food samples, such as bacteria,

viruses, toxins, and allergies. This enables a quicker and more precise study and monitoring of food safety (Hua et al., 2021).

Nanosensors are devices that use nanoscale materials to detect and quantify specific molecules or substances. They can be used to detect food contaminants such as bacteria, viruses, toxins, and chemicals in food samples. With the help of so-called nanosensors, such as a collection of thousands of nanoparticles created to glow in various colors when in contact with food pathogens, it is possible to identify food deterioration. (Sozer & Kokini, 2009). For example, nanosensors based on gold and Zn nanoparticles have been developed to detect foodborne pathogens such as *Salmonella* and *Listeria monocytogenes* in food samples.

A low-cost biosensor was created by Davis et al., (2013) to identify *Listera monocytogenes* in food samples. Gold nanoparticles (AuNPs) and *Listera monocytogenes* -specific antibodies were added to screen-printed carbon electrode (SPCE) strips that were previously used by diabetic patients to measure their blood sugar levels. *Listera monocytogenes* was amplified by combining secondary enzyme-labeled antibodies with AuNPs to aid in detection. In samples of wild blueberries, this assay can identify *Listera monocytogenes* at 2 log CFU/g and distinguishes itself significantly from other enteric pathogens like *Escherichia coli* O157:H7 and *Salmonella typhimurium*. These findings suggest that the addition of AuNPs to the electrodes is essential for the design and manufacture of SPCE biosensors. This improved amperometric immuno-biosensing strip has commercial promise and is a cheap, quick way to find foodborne bacteria.

According to a study (Wang et al., 2015), a novel fluorescent probe combining carbon dots (CDs) and aptamers is suggested for the sensitive quantitative detection of *Salmonella typhimurium*. Hydrothermally, carboxyl-modified CDs with enhanced biocompatibility and monodispersity were produced using citric acid as the carbon source. Under the optimal conditions, the detection limit was reduced to 50 CFU mL^{-1} without sample enrichment, and a linear correlation between the concentration of *Salmonella typhimurium* and fluorescence intensity was found in the range of 103 to 105 CFU mL^{-1} using the equation I = 82.506 log C 203.17 with R^2 = 0.9903. Notably, tests for *Salmonella typhimurium* in tap water and egg samples verified the effectiveness of this strategy. If the right aptamers are chosen, the suggested method has promise for the quick and accurate identification of other bacteria.

Z. Zhang et al., (2018) used magnetic nanomaterials with photothermal effects to detect *Salmonella typhimurium*, which significantly decreased the steps of pre-enrichment and isolation of pathogenic bacteria and

shortened the operation time. Ultimately, they integrated the functions of capturing, detecting, and eliminating pathogenic bacteria into a single matrix.

Mahari et al., (2022) investigated the interaction of in-house produced antibodies with *Salmonella* serovars using a new electrochemical biosensor integrated with gold nanorods (GNRs). The suggested immunosensor showed a linear range of detection (1–10^5 CFU/mL) under ideal conditions, with detection limits of 105 and 23 colony-forming units (CFU) of S. ent and S. typhi, respectively. Under ideal conditions, the developed GNR/S. ent/S. typhi/Ab immunosensor was able to reliably identify S. ent/S. typhi in spiked meat and milk samples, respectively. It also had a long shelf life, good repeatability, and reproducibility. The created electrode was simple to make, produced a highly specific reaction, and showed barely any cross-reactivity with other Salmonella species.

In a study, *Listeria monocytogenes, Salmonella typhimurium*, and *Staphylococcus aureus* growth in culture medium were examined using a colorimetric nanosensor constructed from an aminated graphene oxide nanosheet and a bromophenol blue indicator based on Whatman paper. The findings revealed that while there was no L. monocytogenes colony present at the start of the experiment, it had grown to 189 0. 81 colonies after 24 hours ($p<0.05$). At the end of the incubation period, S. aureus growth had increased and had reached 196 4. 54 colonies ($p<0.05$). Additionally, from 0 to 203 + 5. 17 colonies of S. typhimurium were found in the culture medium ($p<0.05$). The findings also demonstrated that when bacterial colonies increased, the manufactured nanosensors' colors changed. Accordingly, all plates' nanosensor colors shifted from green to blue (Naghdi et al., 2021).

Ghazanfari et al., (2021) a highly sensitive and selective electrochemical sensor for the simultaneous measurement of Sudan I and bisphenol A created using a modified screen-printed electrode ($MoWS_2$/SPE) based on $MoWS_2$ nanoparticles. X-ray diffraction, scanning electron microscopy, and energy-dispersive X-ray spectroscopy were used to analyze the $MoWS_2$ nanoparticles produced by a hydrothermal process. In a phosphate buffer solution with a pH of 7.0, the $MoWS_2$/SPE demonstrated excellent electrocatalytic activity toward the oxidations of Sudan I and bisphenol A. The corresponding electrochemical signals appeared as two well-resolved oxidation peaks with significant peak potential differences of 120 mV. Sudan I had a linear response for selective determination in the concentration range of 0.05 to 700.0 M with a detection limit of 0.01 M. The suggested sensor has demonstrated that it can be used to identify the target analytes in samples of tap water and food.

These examples show the potential of nanosensors for the quick and accurate identification of a range of food pollutants, such as pesticides, antibiotics, heavy metals, and viruses. In comparison to conventional detection techniques, nanosensors have several advantages, including high sensitivity, selectivity, and specificity as well as the capacity to simultaneously detect many pollutants. Nanosensors are going to become more crucial tools for assuring the safety and caliber of our food supply as this field of study advances.

2.3.2. Nanoparticle-based assays

Nanoparticles can also be used in assays to detect food contaminants. For example, a study conducted by Zhou et al., (2011) reported the development of a gold nanoparticle-based colorimetric assay for the detection of melamine in milk samples.

2.3.3.Nanofiltration

Nanofiltration is a process that uses membranes with nanoscale pores to separate and remove contaminants from liquids. It can be used to remove contaminants such as bacteria, viruses, and toxins from food and water. For example, nano filtration membranes made of graphene oxide have been developed for the removal of heavy metals and organic pollutants from water. Membrane methods provide many advantages over traditional procedures, including the ability to avoid the use of toxic solvents or reagents and the ability to operate at moderate temperatures and pressures, which preserve the functional characteristics of food items. They also have low energy consumption, small machines, simple scalability, excellent removal efficiency, and fewer processing stages. Based on these characteristics, membrane methods represent sophisticated and affordable purification and fractionation approaches for solutions containing food. Numerous food processing applications, including the purification of water and sugar, the concentration of juice, the concentration of whey protein, and many more, have been researched using NF membranes, making the technology industrially (Yadav et al., 2022)

Further research and development are needed to fully realize the potential of nanotechnology in this field.

2.4. Fat and Sugar Reduction

Nanotechnology can reduce the amount of fat and sugar in food by changing the structure and content of food items at the nanoscale level. Nanoemulsions, which are very small drops of oil or water stabilized by surfactant molecules, are one method of achieving this. By substituting some or all of the fat in these foods with water or other low-calorie liquids, these

nanoemulsions can be employed to generate low-fat foods. The amount of fat in some foods can be decreased using these nanoemulsions. For instance, scientists have created a nanoemulsion-based method to cut the amount of fat in a cookie by up to 50%. The fat droplets in the cookie are reduced in size and distributed more uniformly when a nanoemulsion is used, giving the cookie a softer, cruncher, and less greasy appearance (Ekin et al., 2021). Nanoemulsions can also significantly alter the texture of food products. For instance, food companies like Unilever have used nanoemulsions to successfully reduce the fat level of ice cream from 16 to 1%. When compared to an emulsion, nanoemulsions can exhibit the gelation phenomenon at low-fat concentrations. In the food industry, this functional feature can help make goods with the desired texture and flavor, such as dressings and mayonnaise. On the use of nanoemulsions for various applications, such as the production of clear beverages, numerous patents have been generated (Saini et al., 2021). The dual system comprising the nanocrystalline cellulose and guar gum demonstrated more stability than market goods and improved the texture of the mayonnaise (Golchoobi et al., 2016).

Using nanoparticles, such as starch or cellulose nanoparticles, is another strategy. These particles can be used to alter the viscosity and texture of food, which can help recipes utilize less fat and sugar (Asghari et al., 2021; Velásquez-Cock et al., 2019). For instance, cellulose nanofibers can be added to salad dressings to enhance their viscosity and make them more filling, which can help individuals consume less dressing overall. It's crucial to keep in mind that the application of nanotechnology to food is still a developing topic, and more study is required to fully comprehend the advantages and disadvantages of these strategies. Regulatory bodies from all over the world are striving to create standards for the secure application of nanotechnology in food, and food producers are starting to investigate the use of these technologies in the creation of new products.

CONCLUSION

To create new food items, new food packaging, and new food storage, nanotechnology can enhance foods, making them tastier, healthier, and more nutrient-dense. However, the majority of the applications, at least in the short term, are focused on high-value products and are currently at an elementary level. There have been few successful uses of nanotechnology in food. Utilizing nanotechnology can improve food flavor and texture, lower fat content, or encapsulate elements like vitamins to prevent their deterioration throughout a product's shelf life. Additionally, packaging that keeps the goods inside fresher for longer can be made using nanomaterials. Nanosensor-equipped intelligent food packaging may even advise customers about the condition of the food within.

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Chapter 7

SUPERCRITICAL EXTRACTION TECHNOLOGY IN FOOD PROCESSING

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1. INTRODUCTION

The food world is evolving from using synthetic ingredients such as bioactive components, colorants, sweeteners, and flavorings to materials extracted from natural sources (Novais, Molina, Abreu, Santo-Buelga, Ferreira, Pereira, & Barros, 2022). Supercritical extraction technology is one of the various extraction techniques that derive these valuable ingredients from different foods and has applications in industries that include nanotechnology, the environment, and the food industry (Arumugham, Rambabu, Hasan, Show, Rinklebe, & Banat, 2021; Cansell, Aymonier, & Loppinet-Serani, 2003). The food industry extracts, purifies, and separates the components of chocolate, spices, oils, coffee extracts, and other foodstuffs to produce functional foods (Cavalcanti, Koshima, Forster-Carneiro, Gomes, Rostagno, Prado, & Meireles, 2022; Manan, Zahedi, & Mustapa, 2021). Supercritical extraction technology is innovative compared to other extraction techniques, is environmentally friendly, efficient, and cost-effective, and yields low-toxicity, low-residue, high-quality, and highly pure extract (dos Santos, Pinaffi-Langley, Ferreira, de Souza, Carvalho-Junior, & Teixeira-Costa, 2023; Capanoglu, Nemli, & Tomas-Barberan, 2022; Pavlova, Minakov, Platonov, Zhigarev, & Guzei, 2022).

2. EXTRACTION

Extraction, one of the separation techniques used (Çolak & Tülek, 2003), is the main process by which bioactive compounds can be obtained from biomass materials. The process is intended to maximize the number of aim compounds and obtain these extracts' highest biological activity (Çabas & İçier, 2021; Truong, Nguyen, Ta, Bui, Do, & Nguyen, 2019). Studies of alternative methods have recently increased to improve the less desirable aspects of extraction used to isolate valuable fat, essential oil, color substance, polyphenol, protein, and pectin in foods (Çabas & İçier, 2021).

3. SUPERCRITICAL EXTRACTION TECHNOLOGY

Supercritical extraction technology utilizes fluid movement at or near supercritical conditions to separate or extract (Okun & Shpigelman, 2022). A supercritical fluid is an element, material, or mixture heated over its critical temperature, pressurized above its critical pressure and exists in a single phase (neither gas nor liquid), and cannot be liquefied or evaporated by increasing pressure or temperature (Figure 1). Therefore, a supercritical fluid represents an intermediate form of matter between a gas and a liquid (Büyüktuncel, 2012). The critical point here refers to the maximum temperature and pressure at which the vapor and liquid phases

can be in equilibrium. In this technology, liquid CO_2 is used as a fluid and sent to the system at the desired working pressure through a high-pressure pump. Once in contact with the raw material in the extractor, it extracts components depending on the specific operating conditions applied. The mixture (supercritical carbon dioxide ($scCO_2$) and soluble components) then arrives at the separator, and when the pressure and temperature drop below the critical point, the fluid that leaves the solution is removed without leaving any residue, i.e., gas. Because the CO_2 gas removed from the environment is still pure, it can be fed back into the system after passing through a cooler. The extract remaining in the separator is pure and can be reused immediately, as it contains no solvent residue and is undegraded by exposure to high temperatures. This method can be easily combined with chromatographic and spectrophotometric techniques and utilized in online operations (Yıkar, Sahakyan, & Akgün, 2008). Solid and liquid samples are used in extraction.

The base extraction diagram consists of an extraction vessel filled with the raw material to be extracted. The solid sample starting material is dried and ground to facilitate the extraction. The most soluble compounds are extracted in the first step (e.g., essential oils), and the less soluble compounds (e.g., antioxidants) in the second step. Liquid co-solvent can be added to $scCO_2$ to raise the solvent strength against polar particles; although it is an excellent solvent for lipophilic compounds and has a low affinity for polar compounds (Reverchon & De Marco, 2006). Extraction is also carried out continuously or in batches in a high-pressure environment. In both cases, the supercritical solvent is introduced to the material from which a desired product will be extracted. The supercritical solvent, now saturated with the product to be extracted, is expanded to atmospheric conditions, and the dissolved product is recovered in the separation vessel, allowing the supercritical solvent to be recycled for further use (Mohamed & Mansoori, 2002).

The number of food applications has increased in recent years, as research and manufacturing for the extraction of plant materials has increased to produce 'natural' extracts for use as ingredients in functional foods (Ahmad, Masoodi, Rather, Wani, & Gull, 2019). Supercritical extraction has recently received attention for its use in extracting bioactive substances from plants at atmospheric temperatures; it prevents thermal denaturation and is considered an effective technique for separation studies due to its simplicity of design and construction (Sridhar, Ponnuchamy, Kumar, Kapoor, Vo, & Prabhakar, 2021).

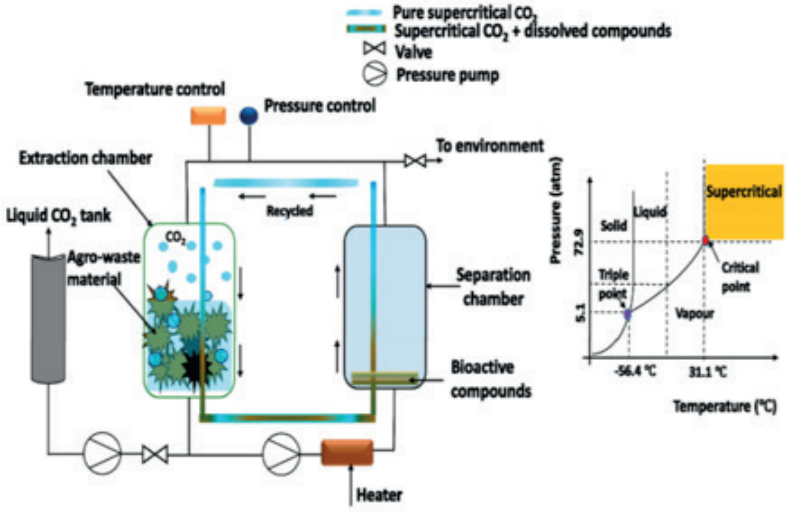

Figure 1. Supercritical extraction technology and principle of operation (Pattnaik, Pandey, Martin, Mishra, &Ashokkumar, 2021).

Supercritical fluids have been recognized as "green solvents for the future" due to their low energy consumption and ecological benefits (Knez, Pantić, Cör, Novak, & Hrnčič, 2019). There are a wide variety of supercritical fluid solvents, including carbon dioxide, ethane, propane, ethylene, methanol, ethanol, n-pentane, hexane, and water. Carbon dioxide and water are widely used. Carbon dioxide can easily reach supercritical conditions, and is nonflammable, harmless, non-corrosive, inexpensive, non-toxic, highly available, odorless, and tasteless (Okun Shpigelman, 2022; Zougagh & Valcárcel, Ríos, 2004), and is generally recognized as safe by the FDA. It is the most widely used supercritical fluid approved by EFSA to obtain a pure product in a short time (Difonzo et al., 2021). However, the use of carbon dioxide is limited by its insufficient solvency for highly polar analytes, though its solvency can be increased to some extent using a suitable modifier (Zougagh et al., 2004).

3.1. Parameters Affecting Supercritical Extraction Technology

3.1.1. Raw material

One challenge in implementing extraction techniques is determining experimental conditions, which strongly influence the effectiveness of the method (Weremfo, Abassah-Oppong, Adulley, & Dabie, Seidu-Larry, 2023).

One of the most important factors affecting the extraction result is the physical state of the sample, such as solid or liquid. Factors such as the particle size and shape of solid samples and material porosity directly affect the mass transfer rate of the process when working on the sample. To raise the extraction rate, the solid matrix should be chopped to maximize the mass transfer area. Very small particles that can compress the bed during comminution, increase the internal mass transfer resistance, and cause channels in the extraction bed that reduces the extraction rate should be avoided. However, in some applications (such as when working with samples with high water content) dispersants (e.g., diatomaceous earth) can be used to prevent the sample from clogging, along with a hydro matrix to absorb the liquid part of the sample. Generally, drying of the raw material is the best choice, and in some cases, the solvent must interact with the solute or the presence of water to facilitate solvent flow (Mendiola, Herrero, Castro-Puyana, & Ibáñez, 2013).

3.1.2. Solvent feed rate

After the extraction pressure and temperature, the solvent feed rate is the most important parameter for supercritical extraction. The flow rate of the solvent must be high enough to give an excellent extraction yield in a brief time and still provide sufficient contact time between the solvent and the solutes. A higher solvent flow rate encourages higher operating and capital costs that must be weighed carefully for industrial applications (Mendiola et al., 2013).

3.1.3. Solubility (pressure and temperature)

Density determines solvent strength and temperature and pressure control density. The temperature has an independent effect on both chromatographic retention and extraction efficiency and often has a profound effect on selectivity (Berger & Greibrokk, 2018).

3.1.4. Drying time

Drying time plays an important role in the $scCO_2$ process, as does extraction time. To improve extraction efficiency, the drying time must be optimized. The drying method should be energy efficient to preserve the natural physical and biochemical quality of the components in the food (Agrawal, Dubey, Khan, Siddique, Saraf, Saraf, & Alexander, 2020).

3.1.5. Using modifiers

Adding the modifiers to CO_2 can raise the solubility of the solutes, extraction efficiency, and extraction rate of non-polar solvents. The

quantity of modifiers in use is less than 10-15%. Common modifiers are ethyl alcohol, water, and methanol. A critical point of the mixture of CO_2:modifier differs from that of natural compounds, is important, and depends on the ratios of each. Two or three phases can coexist under the same conditions (Mendiola et al., 2013).

4. SUPERCRITICAL EXTRACTION TECHNOLOGY: ADVANTAGES AND DISADVANTAGES

In supercritical extraction, the desired component from the raw material can be extracted in a single step without the need for any further purification process and with no loss of solvent, and the solvent can be used repeatedly (Yıkar et al., 2008). Fluids have both gas and liquid properties; the liquid aspect behaves as a solvent, while the gas aspect has mass transfer properties, allowing high diffusion (Okun & Shpigelman, 2022). The produced liquid then serves as an extraction solvent with low toxicity and chemical inertness and is often offered as an alternative to organic solvents. Yet because it is not very polar, $scCO_2$ has a rather limited dissolution capacity. To overcome this, the CO_2 liquid can be combined with alcohol co-solvents such as methanol or ethanol (Fleurence, 2021).

Information on the use, advantages, and disadvantages of $scCO_2$ is shown in figure 2. As $scCO_2$ exhibits limited potential for the extraction of polar substances, either the temperature or pressure should be increased or 10% polar organic modifiers should be included when using it for extraction (Sunil & Kumar, 2020).

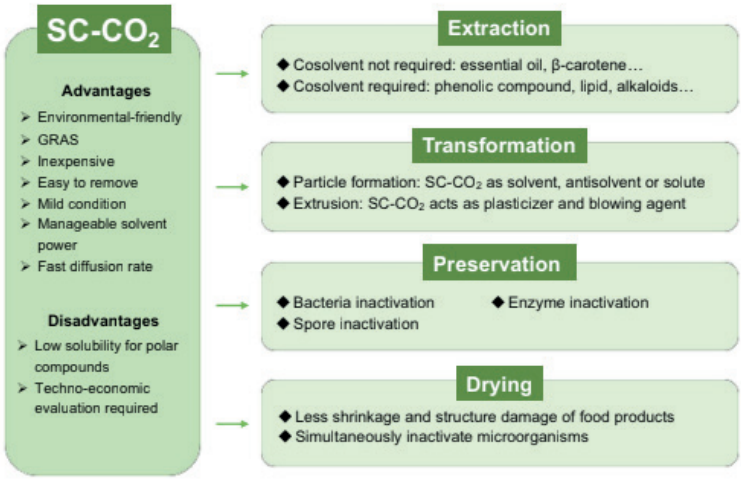

Figure 2. Implementation, advantages and disadvantages of $scCO_2$ application (Wang, Rao, Wu, Wang, Zhao, & Liao, 2021)

Experimental trials of this technology can sometimes be laborious, and accuracy and precision must be confirmed before experiments are performed in a given environment. For full experimental trials, room temperature, water content, particle size, and analyst collection often need to be constant. Day-to-day variability and repeatability on a given day should be considered. The data collection process should be meticulously monitored; for example, stopwatches and traditional clocks should be used to collect time-related information (Sharif, Rahman, Azmir, Mohamed, Jahurul, Sahena, & Zaidul, 2014).

5. AREAS OF USE IN THE FOOD SECTOR

Supercritical extraction is ideal for obtaining natural taste as extracts and is a wide field with applications in foods (Pekyardımcı, 1991). With supercritical extraction, compounds such as annatto, beta carotene, aroma substances, oils and essential oils, alkaloids, and volatile compounds in foods are harvested (Sridhar et al., 2021; Yılmaztekin, Erten, & Cabaroğlu, 2005). In addition, to determine the efficiency of the fluids used in supercritical extraction, the extraction is adapted and used with gas chromatography and gas chromatography-mass spectroscopy. Both qualitative and quantitative analyses of the extracted components can be performed (Yılmaztekin et al., 2005). Supercritical fluid environments provide the flexibility to obtain engineered products with a desired reaction composition (Yener, 2000).

CO_2 is the most common supercritical fluid used in the food industry. The color, composition, odor, and texture of the extract can be controlled, and extraction with supercritical fluid CO_2 preserves the aroma of the product (Benali & Boumghar, 2015). $ScCO_2$ is a highly developed method for extracting pigments and lipids, solubilizing nutritional valuable molecules beneficial to human health (Fleurence, 2021). It allows the extraction of these substances without toxic solvents such as chloroform or hexane. $scCO_2$ is also used to extract antioxidants, essential oils, green tea, vanilla, and ginger (Benali & Boumghar, 2015). For different propolis species, most studies have shown that extracts obtained with $scCO_2$ (using ethanol as a co-solvent) have higher biological potential (Machado, de Oliveira Reis, de Souza, Druzian & Pessoa, 2020).

5.1. Phenol Compounds

Supercritical extraction has been successfully used in food applications to recover such compounds as triglycerides, fatty acids, terpenoids, phytosterols, tocopherols, carotenoids, tocotrienols, and phenols (Pattnaik et al., 2021). It has been used to extract oleoresins from various plant samples such as tomatoes, turmeric, rosemary, spices,

herbs, vegetables, fruits, and others (Lee, Suleiman, Hadzir, & Chong, 2020). It has also been reported to have a positive effect on antioxidants; antioxidant extracts obtained by supercritical technology from apple pulp have higher antioxidant activity (Ferrentino, Morozova, Mosibo, Ramezani, & Scampicchio, 2018).

5.2. Decaffeination

The new commercial application is the decaffeination of coffee and tea, which is carried out before roasting (Machmudah, Kitada, Sasaki, Goto, Munemasa, & Yamagata, 2011). $ScCO_2$ extraction with water as a co-solvent is used to selectively extract caffeine from green tea (Kim, Kim, Kim, Oh & Lee, 2008). Using $scCO_2$, to extract caffeine from coffee beans reductions of 176% in terms of human health, 10.3% in terms of ecosystem diversity, and 16.1% in terms of resource availability can be realized (De Marco, Riemma, & Iannone, 2018).

5.3. Edible and Essential Oils

Supercritical extraction technology is among the new techniques recently developed for extracting oil from fruit kernels, such as almonds and apricot kernels (Pawar & Nema, 2023). $ScCO_2$ has replaced hexane in soybean oil extraction and has been tested in corn, sunflower, and peanut oil extraction. Applied research is being conducted on supercritical oil extraction from potato chips and other snack foods to meet consumer demand for lighter food (Benali & Boumghar, 2015). Supercritical extraction technology is promising for total fat analysis in some food products. It offers speed, full automation, reduced solvent consumption, low thermal damage, and the selective isolation of desirable components. Its disadvantages are high cost, incomplete recovery for some products, and complex equipment requirements (Dionisi & Hug, Aeschlimann, Houllemar, 1999). Advanced extraction methods that are faster, reduce solvent consumption, do not harm the environment, and increase the production of essential oils are necessary for the process to achieve a better market position (Yaman & Kuleaşan, 2016).

5.4. Drying

The $scCO_2$ drying technique offers an alternative to freezing drying; carrots dried in a supercritical fluid environment retain their shape better (Brown, Fryer, Norton, Bakalis, & Bridson, 2008) and direct $scCO_2$ drying without a pre-cooking step is seen as a promising alternative drying technology for the production of dried beet snacks (Tomic, 2020). In addition, $scCO_2$ pretreatment of foods shortens the dehydration process (Lee, Choi, & Lee, 2011). $ScCO_2$ technology is reportedly a promising

green technology in both dehydrating and deactivating microorganisms in a single step. It is cost-effective and safe and maintains product quality in industrial applications (Zambon et al., 2018).

5.5. Sterilization/Pasteurization

Food preservation includes physical, chemical, and $scCO_2$ methods (Buszewski et al., 2021). Food sterilization and pasteurization utilize supercritical extraction technology. Supercritical pasteurization is commanding more attention as an alternative technology for the pasteurization of foodstuffs (Gasperi, Aprea, Biasioli, Carlin, Endrizzi, Pirretti, & Spilimbergo, 2009). One study dried strawberry slices at 10 MPa and 40℃ for up to 6 hours and reported limited inactivation power against total mesophilic bacteria, yeasts, and molds below the detection limit (Zambon, Zulli, Boldrin, & Spilimbergo, 2022). Still, although $scCO_2$ can inactivate bacteria, spores, and enzymes, processing times are too long to make it commercially applicable (Wang et al., 2021). Though microbial inactivation by $scCO_2$ has great potential to improve food safety and quality, many technological and regulatory barriers need to be overcome (e.g., further process optimization, development on an industrial scale, obtaining complete data on organoleptic characteristics and storage time, quality certification). Further research on the efficacy of $scCO_2$ is needed (Buszewski et al., 2021).

5.6. Menu Design

An idea exists that foods processed with supercritical extraction technology will be selected on restaurant menus in the future. Some of the food names on menus include vitamin additives, de-alcoholized beverages, degreased potatoes, and encapsulated liquids (Figure 3) (Braga, Gaspar, & de Sousa, 2023; Brunner, 2005).

Figure 3. Examples of menus include mention of supercritical extraction technology (Braga et al., 2023; Brunner, 2005)

In general, supercritical extraction technology is a promising and green technology producing high-quality food products economically (Hasanov, Salikhov, &Oshchepkova, 2023). The food extracts obtained with supercritical technology are superior in quality, appearance, color, and consistency compared to extracts obtained with other technologies. They are sustainable in terms of waste management and are economical (Yıkar et al., 2008).

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Chapter 8

GENERAL PROPERTIES OF SESAME (*SESAMUM INDICUM L.*) AND ITS USAGE OPPORTUNITIES IN THE FOOD INDUSTRY

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1. INTRODUCTION

More animal foods are needed to meet the protein needs that are necessary for the physical and mental development of people. An adult needs roughly 70 g of protein per day, at least half of which should come from animal sources. Animal-source foods have a high biological value and are a good source of essential amino acids (Öztan, 2011; Nadathur, Wanasundara & Scanlin, 2016; Tayar & Yıldırım, 2020). People's needs for protein are rising along with the global population, yet non-animal protein sources are becoming more significant due to the high price and insufficient supply of animal products. Therefore, in addition to nourishment with proteins of animal origin, it is imperative to evaluate the proteins of vegetable origin. Thus, it has been reported that since the middle of the 20th, several studies have been conducted to identify new alternative protein sources due to the fear that protein sources can not satisfy the needs of human nutrition (Becker, 2007; Alexandratos & Bruinsma, 2012; Day, 2013; Nadathur et al., 2016; Sá, Moreno & Carciofi, 2020). Due to the growing global population, studies to intent the nutritional issue caused by protein deficiency have become more and more important, and new production methods have been created for the production of foods that are protein-enriched (Uğurluay, 2002; Dalatu, Mohammed & Umar, 2019). However, in addition to many processes applied in food production, studies have also been encountered in which some results have been published regarding the negative effects of some unnatural preservatives on human health (Goodman, McDonnel, Nelson, Vaughan & Weber, 1990; Knekt, Järvinen, Dich & Hakulinen, 1999; Çalışır & Çalışkan, 2003; Karatepe & Ekerbiçer, 2017). For this reason, food enterprises should prioritize the creation of useful products that might spark interest customer attention but won't be harmful to health.

While the food industry uses a variety of protein, gum, organic acid, dietary fiber, or natural preservatives that may improve functional properties and have positive health effects, a more recent strategy is to use flour, extract or liquid forms made from plant materials because they are more nutrient-dense and cost-effective (Elleuch, Bedigian, Besbes, Blecker & Attia, 2012; Kurt & Kılınççeker, 2012; Mete, 2014; Zouari, Besbes, Ellouze-Chaabouni & Ghribi-Aydi, 2016; Majdalawieh, Massri & Nasrallah, 2017; Olasunkanmi, Omolayo & Olusegun, 2017; Görgüç, Bircan & Yılmaz, 2019; Gençdağ, Görgüç & Yılmaz, 2020; Hallaç, Kılınççeker & Acar, 2022; Kılınççeker & Hallaç, 2022). Some by-products also referred to as waste, have the advantages of being affordable, simple to obtain, and typically unprocessed. It is indicated that peel, seed, and pulp can be used effectively in the prevention of important diseases, especially obesity, cardiovascular diseases, diabetes, celiac or cancer

(Çelik & Akbulut, 2013; Mirmiran, Bahadoran, Golzarand, Rajab & Azizi, 2013; Majdalawieh et al., 2017; Majdalawieh & Mansour, 2019). In this context, studies on grape, pomegranate, and pumpkin seed, thistle and pepper seed, pomegranate and watermelon peel, powdered onion peel, and various citrus wastes have been encountered and many superior properties of these products have been revealed (Guo, Yang, Wei, Li, Xu & Jiang, 2003; Hoye & Ross, 2011; Al-Sayed & Ahmed, 2013; Yılmaz, 2013; Kurt, Ceylan & Akkoç, 2019; Demir & Olcay, 2020; Hallaç et al., 2022; Hallaç & Kılınççeker, 2023). Additionally, it has been reported in various studies that sesame pulp, which has very valuable compounds in addition to its oil, is used in nutrition both animals and humans, used as a natural preservative in foods, and enhances the functional qualities of food (Atakişi, 1999; Uğurluay, 2002; Seena & Sridhar, 2005; Fıratlıgil-Durmuş, 2008; Zaki, Al-Oqaili & Tahreer, 2015; Akusu, Kiin-Kabari & Isah, 2019; Dalatu et al., 2019; Karakoç, 2021).

Millions of tons of agricultural food waste are occurring as a result of food production and consumption, and these wastes cause various environmental issues, primarily water pollution. Because of this, researches on waste brought into the economy are crucial (Fıratlıgil-Durmuş, 2008; Nayeri, Mirhosseini, Mafakheri & Zarrabi, 2018; Görgüç et al., 2019; Gençdağ et al., 2020). Studies have generally focused on the use of wastes as animal feed, conversion into single-cell protein by fermentation and production of biofuels, and obtaining natural preservatives with high value-added (Atakişi, 1999; Fıratlıgil-Durmuş, 2008; Yılmaz, 2013; Akca & Akpinar, 2021; Hallaç & Sancak, 2022; Uslu, Ertan, Kayacan & Tunalıoğlu, 2022). In Türkiye, biologically useful functional food ingredients are generally imported, resulting in serious economic losses. Companies producing food and food additives need to follow technological developments and review their infrastructure works to process industrial by-products and wastes into products with high value-added and to make this sustainable (Atakişi, 1999; Uğurluay, 2002; Ozdemir & Akinci, 2004; Seçer, 2016; Görgüç et al., 2019; Gençdağ et al., 2020; Akca & Akpinar, 2021).

From sesame (*Sesamum indicum* L.) and its by-products; oil, tahini, halva, Turkish bagel, cookie, bread, cake, croquant, paste, dried fruit pulp (pestil), ice cream, chocolate, kebab, and various soaps are produced (Figure 1). Increasing research on the use of sesame pulp, which is left over as a result of industrial processing and is classified as waste, in different industrial areas, especially in the food industry, comes across as an inevitable fact.

Figure 1. Products obtained from sesame (Sesamum indicum L.) and its by-products

2. GENERAL PROPERTIES OF SESAME

The flowers of the sesame plant, which belongs to the sesame family (*Pedaliaceae*), are white, purple, and pink, its seeds contain high levels of oil and protein and it is the first oilseed plant cultivated in the world (Baydar & Turgut, 2000; Ashri, 2007; Bedigian, 2010a; Bedigian, 2010b; Şahin, 2014).

There are forty species in the sesame genus, twenty-six of which are wild and fourteen of which are cultivated, and this plant is generally

grown in the tropical climate zone. Approximately 56% of the world's sesame production takes place in Asia and 44% in Africa (Dalatu et al., 2019). The country-based, sesame is grown primarily in Sudan, India, Myanmar, Tanzania, Nigeria, China, Africa, Ethiopia, Chad, and Pakistan (Anonymous, 2023). In Türkiye, sesame is grown as a second crop after cereals in the Aegean, Mediterranean, and Southeastern Anatolia regions (Şahin, 2014; Tan, 2015; Seçer, 2016). The lack of certified seeds in sesame cultivation, insufficient water supply during seed germination, insufficient incentives for producers, and the need for a lot of labor force during harvesting/threshing lead to a decrease in yield and an increase in imports (Uğurluay, 2002; Vasanthan, Geetha, Menaka, Vakeswaran & Parameswari, 2019). It is reported that Africa (58.9%) is ahead of Asia (37.4%) and America (3.7%) in sesame seed production with a significant difference, sesame cultivation areas are mostly located in Sudan (24%), India (18.9%) and Myanmar (10.2%), and whereas Türkiye has only 0.2% of the world sesame cultivation areas. Sesame yields in 2022 are reported to be 28.5, 40.6, and 122 kg/da in Sudan, India, and China, respectively, and 51.8 kg/da in the world (Anonymous, 2023).

Although sesame is a heat-loving plant, it is damaged by dry winds and rainfall during germination, and it is also adversely affected by the temperature difference between day and night and its development is delayed. However, the fact that sesame, which can generally grow in all kinds of soil, does not require much maintenance, can be planted in crop rotation with many agricultural products, is in constant need, its cultivation is effortless and it is resistant to drought are the prominent factors in cultivation (Bedigian, 2010b; Hegde, 2012; Seçer, 2016). In sesame harvesting, the bunches are shaken by hand or with the help of a stick, and the seeds are separated from the capsule. As a result of this process, the seeds are separated from the stalk/straw, and raw sesame grains are obtained. Shelled seeds appear yellowish-white, brown, or gray-black, while unshelled seeds appear cream or pearly white (Tunde-Akintunde & Akintunde, 2004; Şahin, 2014; Wilson, 2016).

3. ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF SESAME

Heidari Soureshjani, Obeidavi, Reisi Vanani, Ebrahimi Dehkordi, Fattahyan & Gholipour (2017) examined the effect of sesame oil, and olive oil and their synergy on *Staphylococcus aureus* and reported that olive oil and sesame oil had similar antimicrobial activity and that the inhibitory effect of the mixture of these oils was four times higher than when the oils were used alone. In addition, the researchers stated that these oils with antimicrobial effects can be used as burn ointment for medical purposes

since *S. aureus* causes infections mostly through skin and skin ulcers in humans. Zaki et al. (2015) reported in a study that sesame oil showed antimicrobial activity against *Acinetobacter* spp., *Staphylococcus* spp., *Streptococcus* spp., *Escherichia coli*, *Pseudomonas aeruginosa* and *Serratia marscense* tested except *Enterobacter* spp., *Klebsiella* spp., *Micrococcus* spp., and *Salmonella* spp., and that the antimicrobial effect of ginger and black pepper extracts with the addition of sesame oil was higher than the effect seen when used alone.

According to Shittu, Bankole, Ahmed, Bankole, Shittu, Saalu & Ashiru (2007) in a study in which they examined the antimicrobial and antifungal properties of sesame essential oils, they found that methanolic extract of sesame leaves obtained higher inhibition zone against *S. aureus* (39.3 mm) than cloxacillin (30 mm). Alshahrani, Alsreaya Al, Mashyakhi, Alqahtani, Moni Sivakumar, Alhazmi, Rehman & Alam (2020) reported that sesame seed essential oil had the highest antimicrobial effect on *E. coli* (27.3 mm), *S. aureus* (25 mm), *Streptococcus pyogenes* (22.3 mm), *Bacillus subtilis* (18.7 mm), *Klebsiella pneumoniae* (14.6 mm) and *P. aeruginosa* (12.7 mm). In a study investigating the antimicrobial and antifungal effects of silver nanoparticles synthesized with aqueous sesame extract (Nayeri et al., 2018), it was found that nanoparticles produced with aqueous sesame extract were effective against *E. coli*, *S. aureus*, *B. subtilis*, *Saccharomyces cerevisiae* and *Candida albicans* as a pathogenic fungus, and therefore, it was stated that unused parts of sesame can be used as a biological source for the synthesis of nanoparticles on an industrial scale at a very low cost.

Sallam, Abd-Elghany, Imre, Morar, Herman, Hussein & Mahros (2021) reported in a study that the addition of sesame oil or sesamol to meatballs significantly delayed lipid oxidation, sesamol showed stronger antioxidant activity than sesame oil, and sesame oil and sesamol are natural additives that can be used to improve the microbial quality of fresh meat products and extend their shelf life during cold storage.

Das, Datta, Mukherjee, Bose, Ghosh & Dhar (2015) reported that the polyphenolic content (28.9 mg GAE/100 g) of sesame honey containing lignans and phenolic compounds was positively correlated with color intensity, that this honey had a strong antimicrobial effect on *E. coli*, *Salmonella* Typhi and *Salmonella* Typhimurium, therefore had also significant effect on the growth of probiotic strains such as *Lactobacillus acidophilus* and *Bifidobacterium bifidum*.

Kurtkaya (2018) reported that the antioxidant substances contained in this product have an effect on the high resistance of sesame oil, which has a high unsaturated fat content, against oxidation, and Bopitiya &

Madhujith (2013) reported that sesame seed oil was found to have a strong antioxidant activity compared to α-tocopherol. Elleuch et al. (2012) also reported in a study that sesame paste (tahin) and methanol, ethanol, and acetone extracts of sesame seeds have high polyphenol content. Therefore, the researchers stated that sesame seed shells can be used in the preparation of low-calorie, high-fiber, and antioxidant-rich foods.

4. CONTRIBUTION OF SESAME AND ITS BY-PRODUCTS TO THE FOOD SECTOR

Sesame, which has been cultivated for about four thousand years, is one of the oldest oil crops produced in the world (Shewry, Beaudoin, Jenkins, Griffiths-Jones & Mills, 2002). Sesame has a very high fat and energy value and has an important role in nutrition in terms of protein, calcium, magnesium, potassium, and vitamin B in its structure (Namiki, 1995; Hall, 2003; Bozkurt, 2006; Elleuch, Bedigian & Zitoun, 2011). It is stated that sesame pulp, which is formed after sesame oil production, also contains high protein and minerals, but this pulp is only used as animal feed (Uğurluay, 2002; Hegde, 2012; Seçer 2016).

Sesame is generally used in bakery products such as Turkish bagel, cake, scone, and bread, in the confectionery sector, in the production of oil and margarine, and sesame seeds after being roasted and crushed the shell is removed and used in the production or as seeds in farming (Atakişi, 1999; Uğurluay, 2002; Arslan, Yener & Esin, 2005; Bozkurt, 2006; Hegde, 2012; Asghar, Majeed & Akhtar, 2014; Seçer 2016).

In one study, it was reported that a positive correlation was observed between the water retention capacity and oil retention capacity of sesame shells with decreasing particle size (Elleuch et al., 2012). In another study, it was reported that the use of defatted sesame seed shells (testae) and palm fiber concentrate in helva production was effective in improving the organoleptic qualities of the products. In addition, it was stated that these high-fiber compounds can improve the technological properties of foods by retaining water and fat together (Elleuch, Bedigian, Maazoun, Besbes, Blecker & Attia, 2014). Zouari et al. (2016) stated that the water and fat retention capacities and swelling ability of the flours increased as the sesame shell flour increased in the mixture of white wheat flour and sesame shell flour at different ratios, Seena & Sridhar (2005) stated that while protein additives enrich the textural quality as well as increasing the water retention capacity in foods. Olasunkanmi et al. (2017) stated that sesame oil and protein can be recovered simultaneously under optimum conditions due to their fatty acid profile and physicochemical properties and these products can be used as food additives in different areas.

Hazelnut and sesame protein concentrate absorb the oil better than water and this is advantageous for improving flavor retention time in some bakery products such as cakes and biscuits (Khalil, 2001). These concentrates are also effective in reducing water and fat loss when used in meat products (Chel-Guerrero, Perez-Flores, Betancur-Ancona & Davilla-Ortiz, 2002). In addition, it has been reported that biscuits made with different ratios of substituted unripe banana and defatted sesame flour have higher protein, fat, ash, and crude fiber content and lower carbohydrate content than biscuits made entirely from wheat flour (Chinma, Igbabul & Omotayo, 2012). In other studies, it was observed that the use of sesame seeds in cookie production had a positive effect on the nutritional composition (Akusu et al., 2019), and it was stated that biscuits with high nutritional value can be produced from wheat flour and defatted sesame flour mixtures (Gernah & Anyam, 2014). Karakoç (2021) reported that an increase in diameter values and a decrease in thickness values were observed in biscuits produced using terebinth, sesame, and flaxseed depending on the amount of oilseed used. In addition, flaxseed was found to have higher brittleness and hardness values compared to biscuits with sesame and terebinth seeds. It was reported that biscuits prepared by adding defatted sesame seed flour to millet flour received high scores in terms of flavor and crispness according to the results of sensory analysis (Alobo, 2001), and macaroni prepared by adding roasted sesame seeds to wheat flour (50%) gave more positive results in terms of water absorption capacity and solubility properties (Animashaun, Olorode, Sofunde & Idowu, 2017).

Arab, Freidja, Oomah, Benali, Madani & Boulekbache-Makhlouf (2020) reported that yogurts enriched with roasted sesame seeds had higher sensory acceptability than plain and probiotic yogurts. Kimani, Kunyanga & Ngugi (2022) also stated that sesame seeds, sorghum, and baobab fruits, which have superior properties, are underutilized crops in Africa but their use in foods can be effective in developing value-added products and ensuring food safety.

Tayar & Yıldırım (2020) stated that the main purpose of the meat industry should be to increase the quality and nutritional properties of the products by producing them under healthy conditions and to obtain new products with healthy and functional properties that can be preferred by consumers. In line with this scope, considering the functional properties that sesame, which is widely used in bakery products, can add to meat products such as meatballs, kebab, sausage, and salami, the use of sesame and its by-products in similar industries should be evaluated. In addition, Akca & Akpinar (2021) stated that the reuse of the wastes generated after the production of vegetable oils in the food industry is very important in

terms of waste utilization because they contain antioxidant substances/ phenolic compounds and have prebiotic properties.

According to Gençdağ et al. (2020), urbanization's impact on population growth makes it challenging to manage food waste and results in an unchecked buildup of trash. It should not be overlooked that using wastes rich in protein during the food manufacturing process can improve environmental hygiene and significant economic gains can also be obtained. According to a study by Gebremeskel, Ngoda, Kamau-Mbuthia & Mahungu (2021), although crude sesame oil has a significant economic value, these products can cause serious health problems due to the neglect in the hygiene of the enterprise together with the personnel working in the processing facilities. Gaining access to healthy and high-quality products will be considerably contributed by increasing the awareness-raising training on Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP) issues that are provided to the personnel working in food operations.

5. IMPORTANCE OF SESAME IN TERMS OF HEALTH AND NUTRITION

Sesame leaves are extensively employed in folk medicine in Africa and Asia to treat colds, eye pain, mouth sores, and bruised or deformed skin. Aqueous extracts obtained by boiling the leaves and roots that are antiviral against measles and chickenpox, while shampoos made with sesame oil extract have antifungal properties that are effective against *Tinea capitis*. Besides, sesame is said to contain anti-oxidant properties and health-improving effects. Sesame is typically grown for its culinary oil, and for thousands of years, people have utilized sesame seed oil for healing (Hammer, Carson & Riley, 1999; Shewry et al., 2002; Gandhi & Taimini, 2009; Sacco & Thompson, 2010; Hegde, 2012; Asghar et al., 2014; Görgüç et al., 2019).

Sesame, which has an important place in human and animal nutrition due to its high fat and protein values, has a very common use in industry. Sesame, which is widely used in pastries such as Turkish bagels, cakes, and scones, is also being evaluated in the production of chips, snacks, spice, confectionery, and tahin (Atakişi, 1999; Arslan et al., 2005; Hegde, 2012; Asghar et al., 2014). Namiki (1995) reported that sesame grains have 19.8% protein, 15.3% carbohydrate and 4.7% moisture content, Hall (2003) reported that sesame oil contains approximately 50% oleic acid, and linoleic acid, 10% palmitic acid, and 5% stearic acid, and Elleuch et al. (2011) reported that sesame is an important source of fat (44-58%), protein (18-25%), and carbohydrate (13-14%). It was stated that unroasted sesame oil was evaluated as superior to sunflower oil and inferior to olive oil and

that the antioxidants contained in sesame oil may be effective in the high resistance of sesame oil against oxidation, which is high in unsaturated fat (Kurtkaya, 2018). Sesame is also rich in sesamin, sesamolin, and sesamol, which play an important role in providing stability against the oxidation of oil and contribute to antioxidant activity. The presence of sesamin and sesamin in frying oils is preferred because sesamin and sesamol are converted to sesamol, which is a more potent antioxidant component at high temperatures, and sesamol provides a protective effect against oil autoxidation. It is stated that sesame oil contributes to the prevention of hypertension, hypercholesterolemia and cancer disorders, and aging, and also has the effects of reducing arachidonic acid levels and providing anti-inflammatory and estrogenic activities (Shahidi, Wanasundara & Wanasundara, 1997; Kanu, Bahsoon, Kanu & Kandeh, 2010).

The oil content of sesame seeds varies according to the species and these seeds containing approximately 40-60% oil are considered as high energy seeds (Hall, 2003; Elleuch et al., 2011; Kurtkaya, 2018; Dalatu et al., 2019). In addition, sesame seeds contain high levels of tryptophan and methionine compared to other oil seeds (Escamilla-Silva, Guzmán-Maldonado, Cano-Medinal & González-Alatorre, 2003).

Anilakumar, Pal, Khanum & Bawa (2010) reported that sesame should not only be used for culinary purposes and its seeds can be used for medicinal purposes due to its strong antimicrobial effect against *Staphylococci* and *Streptococci* and strong antifungal effect against skin fungi. In addition to these, the researchers stated that sesame seed can be evaluated in the pharmaceutical industry since sesamin is a component that increases the fat-burning process and reduces fat storage in the body by changing the gene expression of fatty acid oxidation enzymes, and there are also industrial usage possibilities of sesame seed in parallel with the recent studies on biodiesel production from sesame oil. Majdalawieh et al. (2017) reported that studies are showing strong anti-cancer properties since sesamin obtained from sesame seeds has anti-proliferative, pro-apoptotic, anti-inflammatory, anti-metastatic, pro-angiogenic and pro-autophagocytic activities. The researchers stated that sesamin could potentially be used as an effective adjunctive therapeutic agent in ameliorating tumor development and progression, and therefore could be used in the prevention or treatment of various types of cancer. Mirmiran et al. (2013) reported that the positive effects of unshelled milled sesame paste were observed positive effect in reducing cardiovascular disease risk factors in type-2 diabetics. Bhuvaneswari & Krishnakumari (2012) found that the ethanolic extract of sesame seeds was effective in controlling hyperglycemia in streptozotocin-induced diabetic rats and the treatment of nephropathy by significantly improving serum parameters due to its

therapeutic effect. However, Çelik & Akbulut (2013) stated that although oilseeds have a role in preventing cardiovascular diseases, there are not enough studies to reveal their effects on diabetes, and more studies are needed to investigate the effects of oilseeds on long-term blood sugar control.

In different studies, it has been reported that the pulp, which is the remaining part of sesame seeds after the oil is removed, contains an average of 40% crude protein and 24% mineral substance (phosphorus, potassium, calcium) (Uğurluay, 2002), and sesame flour after the oil is removed is a rich source of fiber, carbohydrate, and fat (Clerici, Oliveira & Nabeshima, 2013).

6. SOME NEGATIVE EFFECTS OF SESAME AND ITS BY-PRODUCTS

Although oilseeds contain nutritious and extremely important nutrients for humans, they may also contain some anti-nutritional factors. The fact that sesame seed is an allergenic food, the problems caused by the wastes, sesame seeds that are not produced or stored under hygienic conditions, and the reproduction of microorganisms that may pose a health risk in the products obtained from them are undesirable (Pekşen & Artık, 2005).

Pastorello, Varin, Farioli, Pravettoni, Ortolani, Trambaioli, Fortunato, Giuffrida, Rivolta, Robino, Calamari, Lacava & Conti (2001) reported that some patients experienced severe allergic reactions after consuming sesame-containing foods, with symptoms including laryngeal edema, urticaria, angioedema, shock, asthma, and gastrointestinal disorders. Patel & Bahna (2016) reported that sesame allergy is more common than cases caused by other seeds, with an estimated prevalence of 0.1-0.2%. Ma, Li, Zhang, Huang, Han, Ge, Sun & Chen (2020) also reported that sesame-related allergies are increasingly being reported worldwide with recent studies.

Arana, Peniche, Martinez & Iturriaga (2021) reported that the average total aerobic microorganism count in chia, amaranth, and sesame seeds was detected at 2.1 log CFU/g, 2.4 log CFU/g, and 3.8 log CFU/g, respectively, while coliform group microorganisms were between 0.48-0.56 log/g (MPN) and *E. coli* were found at very low levels in the three types of seeds.

Brockmann, Piechotowski & Kimmig (2004) reported that 8.33% of tahin, 11.27% of helva, and 12.5% of sesame seeds they examined were infected with *Salmonella* spp. Alaouie, Al-Khatib & Khachfe (2018) also reported that *E. coli* and *Salmonella* spp. were detected in 37% and 16%

of Lebanon tahin (sesame paste) samples, respectively, and *Salmonella* species were found only in traditionally produced products.

In their study on the microbiological quality of tahin produced in Saudi Arabia, Ayaz, Sawaya & Al-Sogair (1986) reported that the average total aerobic microorganism count was determined as $2x10^4$ CFU/g, *S. aureus* counts as 56 CFU/g and coliforms as 49 CFU/g.

According to Al-Bachir (2016), gamma irradiating sesame seeds at a dose of at least 3 kGy significantly decreased the count of microorganisms however, it was observed that the effect of this type of irradiation on the chemical composition and sensory properties of sesame seeds was not significant level. Osaili & Al-Nabulsi (2016), stated that an irradiation dose of 1 kGy reduced the inoculated *E. coli* O157:H7 and microbiota in tahin, did not affect ($p>0.05$) the quality (color and oxidative souring) of tahin, and that irradiation in tahin could be used as an effective application to inactivate *E. coli* O157:H7 as well as other foodborne pathogens. According to Al-Nabulsi, Olaimat, Osaili, Shaker, Elabedeen, Jaradat, Abushelaibi & Holley (2014), the presence of acetic acid and citric acid in meals made with tahin can greatly minimize the danger of this pathogen even though the *S.* Typhimurium they looked for in the tahin was effective in the development of salmonellosis outbreaks.

Sebaei, Refai, Elbadry & Armeya (2020) reported that the average AFB_1 concentration of the locally produced, unlabeled tahin samples (13.0 µg/kg) was higher than that of the branded tahin samples (0.1 µg/kg). To lower the danger of aflatoxin exposure, the researchers underlined the significance of using branded tahin rather than unlabeled tahin. AFB_1 was found in thirty-three, AFB_2 in eight, AFG_1 in one, and AFG_2 in one of the sesame offered for eating in Iran, according to Asadi, Beheshti & Feizy (2011).

CONCLUSIONS AND SUGGESTIONS

Sesame offers numerous advantages for nutrition and health in addition to making a significant economic contribution as a strategic product. Limited sesame cultivation in Türkiye, it restricts the expansion of the production of sesame and other products produced from sesame and its waste, especially sesame oil, and causes an increase in imports in this regard. Therefore, incentive programs for increasing sesame production in our country should be planned and implemented and continuity should be ensured in this regard. In addition, it has been observed that the remaining pulp after sesame processing is generally used as animal feed and this pulp, which has many functional properties, has not been fully evaluated. As a result, sesame and its wastes should be evaluated without

creating environmental pollution by following the latest developments in technology. In addition, these products with superior antioxidant and antimicrobial properties, which will contribute to the economy and add functional properties to the foods they are used of these products as natural preservatives and food additives should be expanded.

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Chapter 9

APPLICATIONS OF GREEN SYNTHESIZED NANOPARTICLES IN FOOD PACKAGING

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1. INTRODUCTION

Nanotechnology is a discipline that allows studies such as modeling, measurement, design, processing, and editing improving materials at 1-100 nanometer (nm) dimensions. It is a completely novel, fast-evolving branch of technology that aspires to give matter atomically and molecularly new or improved physical, chemical, and biological qualities. Studies on nanotechnology have significantly increased in the areas of food safety, food processing, agriculture, cosmetics, the environment, and medical science in recent years (Beykaya & Çağlar, 2016).

Nanoparticles (NP), a by-product of nanotechnology, are created using a variety of techniques. Usually top-down and bottom-up approaches are used when performing synthesis (Figure 1). In the top-down approach, the size of large structures is converted into smaller (nm scale) structures by applying various processes. Atoms are assembled into molecular forms in the nanoscale range using the bottom-up approach. The synthesis of NPs via chemical and biological processes frequently employs a bottom-up approach. NPs, which are the product of nanotechnology, cause physical and chemical differences in terms of their properties (Iravani, 2011). The shape, size, and crystals of NPs determine their applications in numerous fields. Therefore, the synthesis of NPs of different sizes and shapes is a problem in nanotechnology (Madhumitha & Roopan, 2013). The chemical and physical processes in the synthesis of NPs are hazardous to the environment because they require high-pressure temperatures and contain dangerous chemicals.

Additionally, due to their poor biocompatibility and instability, NPs created by chemical techniques are limited to application in biomedical applications. The "green synthesis" technique, also known as bioreduction, is another way to synthesize NPs. In this method, metal salts are reduced to nano size using plant extracts or microorganisms. The NP synthesized with this process has good stability and does not show toxic effects (Patil & Kim, 2018).

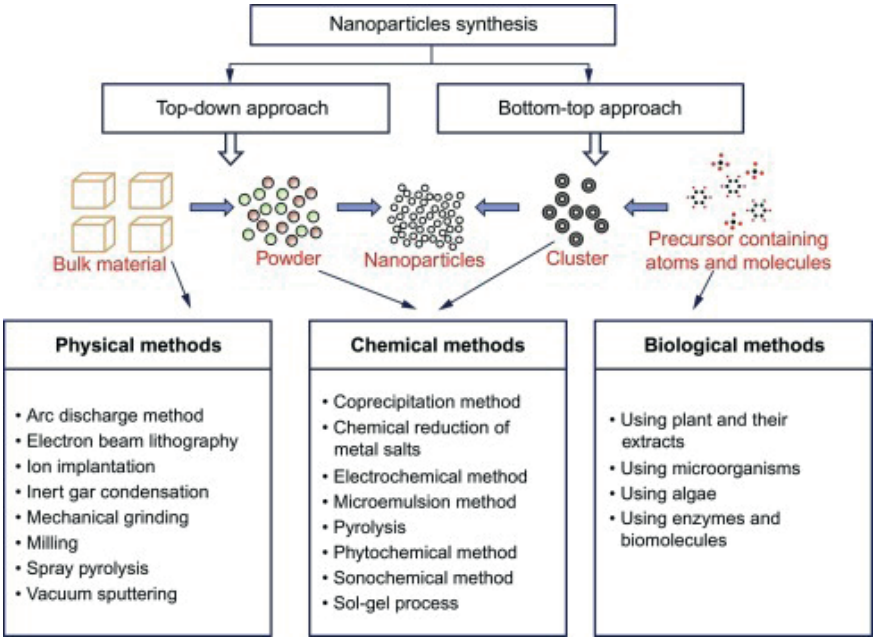

Figure 1. Top-down and bottom-up approaches for the synthesis of nanoparticles (Devatha & Thalla, 2018).

2. GREEN SYNTHESIS OF NANOPARTICLE

Green synthesis, commonly referred to as biological synthesis, is a useful technique for making NPs quickly and sustainably without the use of hazardous chemicals, high pressures, or high temperatures. For the green synthesis of NPs, biological resources such as yeast, bacteria, plants, fungi, algae, and biomolecules like enzymes can be utilized. Thus, inorganic metal ions are converted to metal NPs (Devatha & Thalla, 2018). In recent years, among the synthesis methods, biological synthesis (green synthesis) is known as the preferred method than other methods because it is cheaper, biocompatible, and environmentally friendly.

Biogenic NPs are those made from biological components, and the associated synthesis method is known as green synthesis. Biomolecules that have been obtained from prokaryotic or eukaryotic cells or that function as reducing agents are used in the green production of NPs (Jeevanandam & Chan, Danquah, 2016). By using green synthesis methods, metal NP, metal oxide NP, magnetic NP and quantum dot synthesis can be done (Luo & Shanmugam, Yeh, 2015). Recent studies have shown how to synthesize Rh, Cu, Pt, Cd, Pd, Ag, Ru, and Au utilizing various biological agents (Dikshit & Kumar, Das, Sadhu, Sharma, Singh, Gupta, Kim, 2021). It is expensive to synthesize NPs with the desired size

and morphological structure by synthetic procedures. A lot of research has been done recently to create synthesis processes that use natural reducing, coating, and stabilizing agents in the synthesis phase of NPs. Since plants or microorganisms act as reducing agents during the green synthesis process, no additional stabilizing agents are required in NP synthesis. For this reason, microorganisms and plants have an important place in NP synthesis (Hammamchi, 2019).

The synthesis of NPs, especially by microorganisms, includes the synthesis of various metal ions with a bottom-up approach as part of redox defense-based detoxification (Rónavári & Igaz, Adamecz, Szerencsés, Molnar, Kónya, Pfeiffer, Kiricsi, 2021). Compared to gram-positive bacteria, metallic NPs exhibit greater antibacterial efficacy against gram-negative bacteria. Therefore, NPs adhere to the bacterial cell wall and cell membrane, disrupting cell integrity and causing bacterial death. This situation is accepted as an antimicrobial activity indicator (Salem & Fouda 2021). In their study, Sudhasree et al. (2014) found that nickel NPs made using a green synthesis method had effective antibacterial activity, with the main effects being against *Klebsiella pneumoniae* and *Proteus vulgaris*.

The synthesizing of NPs using extracted plants is a fairly cheap and simple process. Plant extracts are used as reducers and stabilization agents in the synthesis of NPs. This is because different extracts include different proportions and compositions of biological reducing agents (Mukunthan & Balaji, 2012). The primary benefit of utilizing plant extracts and material in the green synthesis of metal NPs; the wastes resulting from the method are biologically compatible and do not require a special reaction medium (Kharissova & Dias, Kharisov, Pérez, Pérez, 2013; Benelli & Lukehart, 2017).

The reduction of metals is greatly supported by the presence of many different biomolecules in the plant, including proteins, vitamins, flavones, aldehydes, phenolics, alkaloids, polysaccharides, saponins, tannins, ketones, terpenoids and amino acids (Nath & Banerjee, 2013). These bioactive compounds exist in varying quantities, types, and compositions in various parts of the plant (Shah & Fawcett, Sharma, Tripathy, Poinern, 2015). Thus, their interaction with metal ions changes, contributing to the evolution of NPs of various sizes and shapes (Banerjee & Satapathy, Mukhopahayay, Das, 2014). Terpenoids are forms of terpenes in plants that consist of 5-carbon units of isoprene and have different functional groups. Shankar et al. (2004) stated that the terpenoids/sugars found in the leaf broth are involved in the synthesis in the formation of NPs they synthesized using Neem leaf broth.

Currently, there has been a lot of interest in the utilization of plants or plant extracts to turn metal salts into NPs (Madhumitha & Roopan, 2013). Researchers have recently created numerous metal oxide and metal nanoparticles using diverse plant extracts; such as zinc oxide nanoparticles (ZnONPs) (Chakraborty & Farida, Simon, Kasthuri, Mary, 2020; Zahiri Oghani & Tahvildari, Nozari, 2021; Suresh & Nethravathi, Rajanaika, Nagabhushana, Sharma, 2015; Kumar & Mudai, Roy, Basumatary, Mukherjee, Dutta, 2020), Silver nanoparticles (AgNPs) (Gudimalla & Jose, Varghese, Thomas, 2021; Jayaprakash & Vijaya, Kaviyarasu, Kombaiah, Kennedy, Ramalingam, Munusamy, Al-Lohedan, 2017), Iron oxide nanoparticles (FeNPs) (Kutuk & Çetinkaya, 2018), tin dioxide nanoparticles (SnO_2 NPs) (Garrafa-Galvez & Nava, Soto-Robles, Vilchis-Nestor, Castro-Beltrán, Luque, 2019), gold nanoparticles (AuNPs) (Donga & Bhadu, Chanda, 2020), lead nanoparticles (PbONPs) (Noukelag & Mohamed, Razanamahandry, Ntwampe, Arendse, 2021), palladium nanoparticles (PdNPs) (Yang & Li, Wang, Huang, Lin, Wang, Sun, Su, Opiyo, Hong, Wang, He, Jia, 2010), MnO_2 nanoparticles (MnNPs) (Prasad & Patra, 2017), sulfur nanoparticles (SNPs) (Salem & Albanna, Awwad, 2016). Studies of the literature have been reported on the antibacterial, antioxidant and antimicrobial properties of NPs made by green synthesis (Suresh et al., 2015; Jayaprakash et al., 2017).

3. CHARACTERIZATION OF NANOPARTICLES

Because of their unique composition, size, higher surface area to volume ratio, shape, and purity of components, NPs, the building blocks of nanotechnology, possess extraordinary properties (Jacob & Sharma, Balakrishnan, 2017; Khan & Saeed, Khan, 2019).

Before their use in diverse industries, physicochemical characterization is crucial due to the unique physical, mechanical, and chemical properties of the produced NPs. The analysis of various properties such as surface morphology, shape, stability, surface area, size, structure, elemental and mineral weathering, homogeneity, and density gives information about NPs and determines their usage applications. In the characterization of NPs, Condensation Particle Counter (CPC), High-Resolution Transmission Electron Microscopy (HRTEM), X-Ray Diffraction (XRD), Atomic Force Microscopy (AFM), Transmission Electron Microscopy (TEM), Field Emission Scattering Electron Microscopy (FESEM), Dynamic Light Scattering (DLS), Photon Correlation Spectroscopy (PCS), Scanning Electron Microscope (SEM), thermogravimetric ((TGA), energy dispersive X-ray (EDX) and Fourier transformed infrared spectroscopy (FTIR) are used. The most widely used of these methods are TEM, XRD and SEM (Mourdikoudis & Pallares,

Thanh, 2018; Punia & Bharti, Chalia, Dhar, Ravelo, Thakur, Thakur, 2021; Dikshit et al., 2021) (Figure 2). According to Iravani (2011), the synthesis of well-characterized and extremely stable nanoparticles depends on the choice of organism, enzyme activation, and ideal reaction conditions. With the characterization of the synthesized NPs, the applications, and areas where they can be used are determined.

4. APPLICATIONS OF GREEN SYNTHESIS IN FOOD PACKAGING

One of the most significant measures in ensuring food safety is food packaging. The adoption of proper packaging materials and processes to avoid food losses and offer nutritious and safe food products has long been the industry's top focus. Packaging systems are objects that can be constructed of any material and are used to maintain, store, manage, transmit, transport, and identify all products as well as the supply chain, which includes many crucial jobs and extends from the raw materials to the final consumers. Food packaging's main goals are to stop food from spoiling and becoming contaminated, to boost sensitivity by triggering the food's enzyme activity, and to stop weight loss. Research on the application of nanotechnology in food packaging technology to the food industry has been increasing in recent years (Ash & Raza, Roy, Golui, 2021). Nanotechnology in two main areas of food processing; it is known that it will provide specific properties in food packaging and food supplement/ingredients (Salem & Founda, 2021). For this purpose, various nanoparticles are added to the additives. Thus, the shelf life of the food is extended and the natural aroma, color, odor, and nutritional content of the food are preserved. In addition, studies continue to protect and improve the freshness of flavored products (Surengil & Kılınç, 2011). Nanotechnology is used in food packaging in different categories such as nanocomposite packaging materials, biodegradable nanocomposite packaging materials, and active and smart nano packaging (Saka & Terzi Gülel, 2015). Developments in food nano-packaging, such as bio-based, active, enhanced, and smart packaging, have recently received attention (Primožič & Knez, Leitgeb, 2021) (Figure 2).

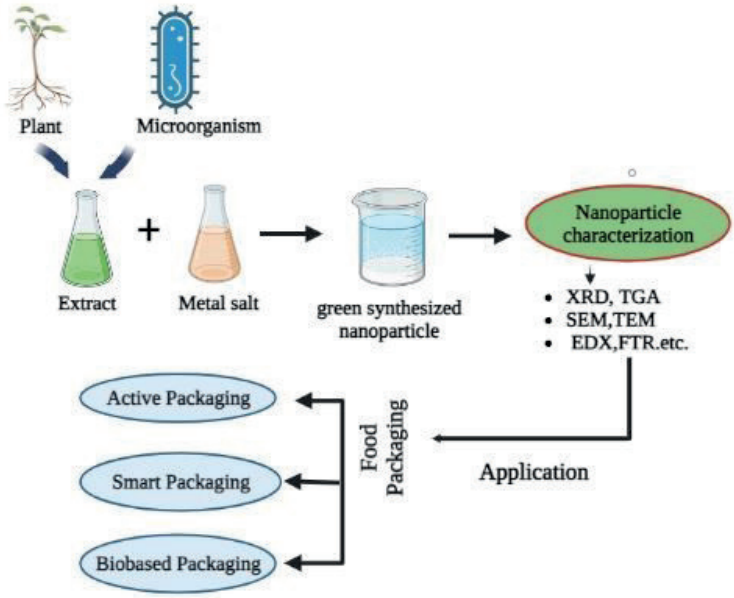

Figure 2. Applications of green synthesized nanoparticles in food packaging

Nanotechnology is used to generate small particles in a film of plastic to accelerate the flow of some gases by way of the material and pump out undesired carbon dioxide that would reduce the nutritional value of the food. These films are also in charge of barrier defense and stopping gases like oxygen/ethylene from causing food to degrade (Duncan, 2011; Silvestre & Duraccio, Cimmino, 2011). By incorporating nanotechnology into food packaging, new packaging materials are produced that perform better. Nanocomposite materials have a lot of potential to enhance the quality and safety of food packaging. NPs are utilized in food packaging on a more widespread basis, however, consumer views and willingness to accept them are impacted by worries about their toxicity and potential health risks. Numerous studies have shown that nanoparticles can migrate from packaging or containers into food In contrast to other migration rates, however, the number of instances of nanomaterial motion and migration has also been shown in numerous laboratory experiments to be quite low. The safety and toxicological consequences of NPs cannot be disregarded because of the possibility that NPs will migrate from packing material to food. In conclusion, Green synthesized NPs offer a viable solution to this issue (Jafarzadeh & Nooshkam, Zargar, Garavand, Ghosh, Hadidi, Forough, 2023).

Food safety is provided through active and intelligent packaging technologies, which also enhance product quality and extend product shelf life (Kuswandi & Wicaksono, Jayus, Abdullah, Heng, Ahmad, 2011). The development of active packaging technologies aims to guarantee the high quality of foods while extending their shelf life through longer storage periods. An active packaging system is one that not only shields the food from outside influences but also can manage and respond to the environment inside the box. Active packaging technology consists of carbon dioxide regulators, moisture absorbers, oxygen/ethylene scavengers, and antimicrobial packaging systems (Valdés & Valdés González, García Calzón, Díaz-García, 2009). New-generation active packaging frequently consists of sachets or coatings that also contain antibacterial, antioxidant, taste, and preservative ingredients to enhance food quality and safety (Primožič et al., 2021).

Smart packaging systems are those that monitor food conditions to give information on the packaged food's quality while it is being stored and transported. On the other hand, various indicators (freshness indicators, Time-temperature indicators (TTI)), microbial spoilage sensors, radio frequency identification (RFID), gas sensors, fluorescent-based oxygen sensors, and biosensors used in smart packaging technology provide information to the consumer about the quality of the food in the package (Robertson, 2006). The green synthesis of NPs in food packaging systems has brought a new perspective. In short, NPs synthesized by the green synthesis in the context of food packaging; it has various applications such as nano-bio trackers, nanocomposites, nanoencapsulation, nano biosensors, nanoemulsions, smart packaging, quality control and assurance (Kalpana & Rajeswari, 2017). Green synthesis of NPs is preferred over other physicochemical methods for smart packaging because of its easy, clean, cost-effective, safe, effective sources and environmentally friendly for high efficiency and purity.

Bio-based packaging refers to recyclable packaging sheets that are used to regulate moisture or transfer of gases in food products in order to enhance food safety, flavor profiles, and nutrition. Bio-based developments such as low-waste, biocompatibility, biodegradability, and eco-friendly packaging (Bio-based packaging) may also be accomplished with the use of bionanomaterials (Siracusa & Rocculi, Romani, Dalla Rosa, 2008). Bio-based packaging, like any other type of packaging, forms a barrier throughout the nutritional item and its surroundings, protecting it from the harmful effects of microorganisms, relative atmospheric moisture, and gas conditions. Biodegradable packaging films are distinguished from other packaging materials by their ability to be quickly dissolved by the activity of living organisms (Ash et al., 2021).

Studies on the green synthesis, production methods, performance, and characterization of NPs have shown positive effects on the potential and performance of bio-nanocomposite films and coatings in renewable food packaging materials. Despite considerable research on green synthesized nanomaterials and their use of active components in biopolymer films, there are no thorough evaluations that investigate the potential effectiveness of using produced by biological processes nanocomposites/nanomaterials in intelligent (smart) and active food packaging. Because of the impacts on preservation time, gas barrier qualities, and mechanical capabilities, there has been a lot of interest in packaging applications using various NPs in recent years (Paidari & Goli, Anari, Haghdoust, 2019; Hosseini & Ahari, Mahasti, Paidari, 2017; Eddin & Ibrahim, Tahergorabi, 2019). Nanomaterials having antimicrobial activity are used in active and intelligent packaging to safeguard food against hazardous bacteria, fungus, and viruses that can cause rotting, retain product freshness and lengthen shelf life (Nikolic & Vasiljevic, Auger, Vidic, 2021).

In recent years, there has been an increase in studies on the applications of green synthesis in food packaging. Green synthesis has attracted widespread attention as a dependable, sustainable, and environmentally friendly method of producing diverse nanomaterials such as hybrid materials, metal/metal oxide NPs, and biomaterials.

Jayaprakash et al. (2017) obtained silver nanoparticles (AgNPs) from Tamarind fruit extract by green synthesis in their study. The highly stable green synthesized AgNPs have been seen to remain stable for more than six months without the oxidation of oxygen. AgNPs obtained by green synthesis have suggested that it is environmentally friendly, have good antibacterial activity, and can be used in the field of medicine, the food industry, and bio-sensors.

Titanium dioxide (TiO_2), aluminum oxide (Al_2O_3), Silver (Ag), and zinc oxide (ZnO) NPs are among the metal oxide and metal NPs that are frequently used in food packaging applications; because they improve durability, gas and UV barrier characteristics, ethylene-scavenging and antimicrobial properties in food packaging (Garcia & Shin, Kim, 2018; Kumar & Bhattacharya, Singh, Halder, Mitra, 2017; Kumari & Brahma, Rajak, Singh, Kumar, 2016).

ZnO is a GRAS material with antibacterial characteristics, making it one of the best nanomaterials for food applications. The Food and Drug Administration has classified it as a material that is generally regarded as safe (GRAS). As a result, ZnONPs have been integrated into polymeric

matrices to enhance antimicrobial properties and improve packaging qualities (Espitia & Soares, Coimbra, Andrade, Cruz, Medeiros, 2012).

Plant extracts primarily operate as a stabilization and reducing agent in the green synthesis of zinc oxide nanoparticles (ZnONPs) (Chakraborty et al.,2020; Golmohammadi & Honarmand, Ghanbari, 2020). Green synthesis of ZnONPs is scalable, environmentally friendly, inexpensive, and easy. Kumar et al. (2020) used *Cassia fistula* fruit extract to create a nanocomposite film made from chitosan-gelatin and incorporating green synthesized ZnONPs. The produced hybrid film outperformed control films in terms of flexibility, thermal durability, and integrity of the structure. The formed chitosan-gelatin-ZnO nanocomposite films have reported that they have an effective antimicrobial defense against *E. coli*, and could be a promising alternative in active packaging that can extend the shelf duration of packaged foods.

Marrez et al. (2019) synthesized green silver nanoparticles (AgNPs) from flavonoids like pyrogallic and gallic acid, quercetin, and rutin. Green synthesized AgNPs were incorporated into cellulose acetate films, forming an environmentally safe nanocomposite film. It was determined that all AgNPs prepared and the produced nanocomposite film had high antimicrobial activity against pathogenic bacteria. They reported that this nanocomposite film is non-toxic and has the potential to be used in active food packaging systems.

Using plant extract from *Nymphae odorata*, researchers created sodium alginate films that were doped with green synthetic silver nanoparticles (AgNPs). The results showed that *Staphylococcus aureus* and *Escherichia coli* completely lost all of their bacterial activity at very low concentrations of AgNPs, and similar effects were also shown in movies. The outcomes supported the prepared AgNPs and films' effectiveness as antibacterial agents (Gudimalla et al., 2021).

Gao et al. (2020) compared the preservation impact of zinc oxide nanoparticles (ZnONPs) generated by *Citrus sinensis* peel extract to commercial ZnO nanoparticles before using them as nanocoatings on fresh strawberries. *It has been reported that ZnONPs synthesized with Citrus sinensis peel extract, when applied as a coating on strawberries, reduce weight loss and decay rate in strawberries. Therefore, ZnONPs synthesized with Citrus sinensis peel extract, which has low cost, harmless to the environment, low toxicity, and good performance, have shown great potential for practice in food packaging.*

In another study, banana peel extract was employed as an agent for capping and reducing to produce silver nanoparticles (AgNPs) that are favorable to the environment. Banana blocks covered with sodium

carboxymethyl cellulose (Na-CMC) and Na-CMC mixed with AgNPs (Na-CMC + AgNPs) were also kept for five days. Na-CMC + AgNPs-coated banana blocks showed better preservation because no mold development was seen during the storage period of five days. Finally, Na-CMC + AgNPs coated banana blocks did not develop mold during the five-day storage period, so the duration of storage of the product was extended (Goh & Cheok, Yeap, 2023).

CONCLUSIONS AND FUTURE VIEWPOINTS

Literature research shows that the green synthesis method has become popular in recent years. The cause for this may be that many chemicals used so far are toxic, carcinogenic, and environmental pollutants. It demonstrates that green synthesis of nanoparticles is basic to implement, low expenses, ecologically benign, and high yield. In recent years, the use of green synthesized metal oxide /metal nanoparticles in food industries has improved food packaging by creating "intelligent packaging systems" active and intelligent to preserve food goods from rotting and increase shelf living. To that end, "Green synthesis" is an alternative approach for the food packaging industry to develop NPs. Undoubtedly, more research is needed before green nano-based packaging systems can be integrated into the food industry. In the near future, green synthesis nanopackaging will be developed as food becomes more and more valuable as the world population and ecological responsibility increase.

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Chapter 10

GAMMA-AMINOBUTYRIC ACID (GABA) AS A BIOACTIVE COMPOUND IN FOOD ENRICHMENT

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1. INTRODUCTION

Gamma-aminobutyric acid (GABA) is an apoprotein and serves as a bioactive nitrogen-containing compound specifically classified as an amino acid. Originally discovered in connection with potato tuber tissue, it has a critical role as the brain's major inhibitory neurotransmitter. This neurotransmitter is produced through the α-decarboxylation process of L-glutamic acid catalyzed by glutamate decarboxylase. Its importance extends to a variety of microorganisms, plants, and animals, making it common in both prokaryotic and eukaryotic systems (Cataldo, Villegas, de Giori, Saavedra, & Hebert, 2020; Rayavarapu, Tallapragada, & Usha, 2021; Alizadeh Behbahani, Jooyandeh, Falah, & Vasiee, 2020; Sarasa, Mahendran, Muthusamy, Thankappan, Selta, & Angayarkanni, 2020).

Functionally, GABA works as a neurotransmitter that facilitates the transfer information between neurons. It is stored in vesicles within axon terminals and released into the synaptic space via exocytosis. From there it spreads to the postsynaptic neuron. In humans, the interaction of GABA with inhibitory synapses includes binding to GABA receptors, triggering the opening of ion channels that allow potassium ions to leave the cell and chloride ions to enter. This effect causes negative membrane potential changes, and hyperpolarization, and subsequently decreasing neuron excitability. GABA not bound to receptors can either undergo enzymatic degradation within the synaptic cleft or be actively transported back to the presynaptic axon terminal via a reuptake pump or transporter (Sarasa et al., 2020)

GABA acts as an important inhibitory neurotransmitter in the mammalian central nervous system, present in approximately 40% of brain synapses (Grewal, 2020). In particular, GABA and its receptors have been identified in non-neural tissues that contribute to functions within the peripheral system, endocrine processes, and oxidative metabolism (Sarasa et al., 2020). Its physiological effect is wide-ranging: it exerts hypotensive and relaxing effects, helps lower cholesterol levels, boosts immunity during stress, and exhibits anticancer properties by inhibiting cell proliferation (Ramos-Ruiz, Poirot, & Flores-Mosquera, 2018). In addition, GABA affects plasma concentration, brain protein synthesis, and growth-related hormones while regulating metabolic processes such as fat burning (Dhakal, Bajpai, & Baek, 2012).

In the field of neurological development and function, GABA plays a critical role in modulating synaptic transmissions and supporting the metabolism of brain cells. Hence, it has led to its investigation as a potential therapeutic agent for a variety of neurological disorders,

including Parkinson's, Alzheimer's, Huntington's disease, dementia, and bipolar disorder (Grewal, 2020).

There is a significant consumer demand for functional foods enriched with GABA. However, the levels of GABA found in natural foods tend to be relatively low, making them insufficient to achieve the desired health effects in individuals. In light of the growing public awareness regarding food security and natural processes, the use of enrichment technologies to increase GABA levels in foods, instead of external supplements, shows potential for making it more appealing to health-conscious consumers. This chapter aims to provide a comprehensive perspective on GABA production, fortification technologies, applications in the food industry, and the health effects.

2. GAMMA-AMINOBUTYRIC ACID (GABA) PRODUCTION

GABA can be produced in three different ways: chemical synthesis, enzymatic biocatalysis, and microbial fermentation. Various chemical routes have been proposed for the synthetic production of GABA. A complex approach involves a five-step process that includes nitrile reduction, ester hydrolysis, and deethoxycarbonylation. This method synthesizes GABA from a functionally modified intermediate obtained by the alkylation of ethyl bromoacetate with diethyl cyano malonate. An alternative technique uses microwave-assisted decarboxylation of L-glutamic acid with isophorone as an inducer. In addition, GABA was synthesized via the carboamination reaction of alkenes catalyzed by copper complexes (Grewal, 2020). The study by Lie, Farmer, & Macquarrie (2018) demonstrated the production of GABA from glutamic acid derived from waste gluten.

While chemically synthesized GABA has fast reaction rates and high yields, challenges such as corrosive reactants, toxic byproducts, and harsh conditions have limited its application due to health concerns. As a result, chemically synthesized GABA has been banned as a food additive because of these problems (Luo, Liu, Xie, Bilal, Liu, Yang, &Wang, 2021). GABA is present in trace amounts in biological tissues, making its extraction from natural sources difficult (Sarasa et al., 2020). By comparison, microbial production turns out to be an attractive and promising approach. This is primarily due to several factors:

a) Use of renewable resources such as L-glutamate, monosodium glutamate (MSG), glucose, xylose, lignocellulose, and glycerol as fermentation substrates or co-substrates.

b) Reduced environmental pollutants during production.

c) Mild fermentation/reaction conditions.

d) Alignment with the demand for environmentally friendly products in the food and pharmaceutical sectors (Luo et al., 2021).

Among microorganisms, bacteria, and molds are considered primary sources for GABA production. In particular, the main types of molds driving production are *Monascus, Neurospora, Rhizopus,* and *Aspergillus*. However, lactic acid bacteria (LAB) strains have been extensively investigated for GABA production among bacteria (Diana, Quílez, & Rafecas, 2014).

2.1 Plant-Derived GABA Production

GABA also exists in plants and microorganisms, where its production is induced as part of a stress-defense mechanism. This induction serves to restore impaired respiratory processes and stimulate energy production in stressed plants. (Shelp, Aghdam, & Flaherty, 2021). Metabolically, GABA is linked to the tricarboxylic acid (TCA) cycle in plants. Its role extends beyond metabolism, encompassing vital signaling functions within the plant life cycle. Notably, GABA contributes to key processes such as pollen tube growth, facilitation of programmed cell death, and modulation of genetic attributes associated with cell wall structure (Michaeli & Fromm, 2015; Li, Dou, Zhang, & Wu, 2021). It is significant to highlight that the potential for GABA production is not limited to animals and plants. Lactic acid bacteria and yeasts, frequently used in the production of commercially fermented foods, also possess the capacity to yield GABA (Diana et al., 2014). This highlights the wider applicability and importance of GABA synthesis in various biological contexts.

Two predominant pathways for GABA biosynthesis have garnered extensive scrutiny in diverse plant species. Foremost among these pathways is the GABA shunt, distinguished by its prominence. A secondary pathway is evident, involving the degradation of polyamine, primarily putrescine. These pathways, investigated extensively, have provided comprehensive insights into GABA synthesis across plants (Michaeli & Fromm, 2015; Li et al., 2021; Shelp, Bown, & McLean, 1999; Shelp, Bozzo, Trobacher, Chiu, & Bajwa, 2012a; Shelp, Bozzo, Zarei, Simpson, Trobacher, & Allan, 2012b; Shelp, Bozzo, Trobacher, Zarei, Deyman, & Brikis, 2012c). In addition, a different prospective pathway in GABA metabolism has been considered as an alternative pathway involving non-enzymatic progression. This pathway takes place with the participation of the amino acid proline, especially under conditions of oxidative stress (Signorelli, Dans, Coitiño, Borsani, & Monza, 2015).

The Synthesis of GABA from Glutamate

The central pathway that controls the metabolism and degradation of GABA is widely recognized as the GABA shunt, a shortened pathway that skips the two steps in the tricarboxylic acid (TCA) cycle (Michaeli & Fromm, 2015). This important pathway is mediated by a highly conserved triple enzyme, namely glutamate decarboxylase (GAD, EC 4.1.1.15), GABA transaminase (GABA-T, EC 2.6.1.19), and succinic semialdehyde dehydrogenase (SSADH; EC 1.2.1.16) (Shelp et al., 1999; Shelp et al., 2012a; Clark, Di Leo, Dhanoa, Van Cauwenberghe, Mullen, & Shelp, 2009). The GABA shunt begins with the irreversible α-decarboxylation of the amino acid glutamate to GABA, which is catalyzed by GAD in the cytosol. This enzymatic conversion requires the consumption of a proton and the release of carbon dioxide (Michaeli & Fromm, 2015; Li et al., 2021) (Figure 1).

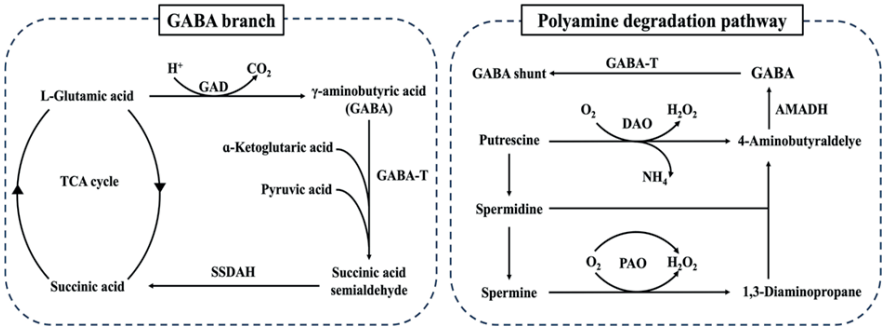

Figure 1. Two main anabolic pathways of GABA. GAD: glutamate decarboxylase; GABA-T: GABA transaminase; SSAD H: succinic semi-aldehyde dehydrogenase; DAOdao: diamine oxidase; Pao: polyamine oxidase; AMAD H: γ-amino butyraldehyde dehydrogenase (Hou, Tang, Feng, Niu, Shen, Wang, & Zhou, 2023).

GAD, a distinctive enzyme involved in GABA metabolism, exhibits specificity for L-glutamate, relies on pyridoxal 5'-phosphate as a cofactor, and has a calmodulin (CaM) binding domain that facilitates the formation of complex GAD complexes. In particular, GAD works optimally at pH 5.8 (Shelp et al., 1999; Shelp et al., 2012a; Xu, Wei, & Liu, 2017; Diez-Gutiérrez, San Vicente, Barron, Del Carmen Villaran, & Chávarri, 2020). Following GABA biosynthesis, its catabolic progression occurs within the confines of the mitochondrial matrix. The translocation of GABA into the mitochondria marks the beginning of this phase, which a reversible conversion occurs via GABA transaminases (GABA-T). In this context, GABA is converted to succinic semialdehyde (SSA) with pyruvate or glyoxylate acting as amino acceptors (Michaeli & Fromm, 2015; Clark

et al., 2009). In particular, the optimum pH range of 8 to 10 controls this enzymatic phase. The next step involves the irreversible oxidation of SSA to succinate, a reaction facilitated by succinic semialdehyde dehydrogenase (SSADH), an intrinsic mitochondrial enzyme (Shelp et al., 1999). The succinate produced takes on the role of an electron donor, which integrates back into the tricarboxylic acid (TCA) cycle. As such, the GABA shunt is accomplished with the participation of succinate, thus giving a cyclical structure within the metabolic pathway. This ultimately requires glutamate to recycle the carbon skeleton and channel it effectively into the TCA cycle throught the GABA shunt (Li et al., 2021). Alternatively, SSA exhibits the potential to be directed to the synthesis of γ-hydroxybutyric acid (GHB) via SSA reductase (Michaeli & Fromm, 2015; Shelp et al., 2012a). This conversion, characterized by its reversibility, emerges as a crucial link in GABA metabolism. GHB is, in turn, oxidized back to succinic semialdehyde by GHB dehydrogenase, an enzyme that facilitates the conversion of GHB to GABA via GABA transaminases (Shelp et al., 2012a).

The Synthesis of GABA from Putrescine

In addition to glutamate-induced GABA synthesis, the degradation of polyamines to GABA has been subject to extensive scrutiny (Shelp et al., 2012c; Diez-Gutiérrez et al., 2020). This particular metabolic pathway prominently involves the involvement of two key enzymes: diamine oxidase (DAO) and 4-aminobutyraldehyde dehydrogenase (ABALDH). The early stages of this process involve a different sequence of reactions that result in the conversion of the amino acid arginine into putrescine throught an alternative, multi-step pathway (Shelp et al., 2012c). After synthesis, putrescine undergoes oxidation catalyzed by oxygen-dependent DAO, yielding 4-aminobialdehyde or rarely Δ-1-pyrroline as metabolic intermediates (Shelp et al., 2012c). The ensuing conversion requires either of these forms to be converted to GABA mediated by NAD^+ dependent ABALDH action (Li et al., 2021; Shelp et al., 2012c). Notably, both DAO and ABALDH prefer a slightly acidic environment with an optimum pH range of 6.5 (Yang, Chen, Han, & Gu, 2012). Subsequently, GABA derived from putrescine can undergo degradation, and a compound similar to GABA, known as succinate, can be obtained as a component of the tricarboxylic acid (TCA) cycle (Diez-Gutiérrez et al., 2020). However, the functionality of this metabolic pathway is essentially dependent on the availability of oxygen, a vital substrate for diamine oxidase (DAO). According to this, stressors that disrupt oxygen availability and cellular redox balance emerge as effective determinants controlling both DAO and 4-aminobutyraldehyde dehydrogenase (ABALDH) activities.

Consequently, these disruptions impose limitations on GABA production via the putrescine pathway (Shelp et al., 2012c). This interaction further explains that GABA synthesis in plants in response to oxygen-deprived stimuli predominantly favors the glutamate pathway over putrescine-derived pathways. The initial concentration of GABA in plant tissues remains at low levels. However, their levels can undergo significant amplification, increasing severalfold in response to various stimuli. These stimuli encompass a range of environmental stressors as well as the challenges posed by insect infestation and pathogenic attack (Li et al., 2021; Shelp et al., 1999; Shelp et al., 2012a). According to the findings described by Shelp et al. (2012c), these stimulatory triggers encompass a wide variety of biotic and abiotic stress factors. Specifically, these factors include sudden temperature changes, drought conditions, hypoxia events, salinity stress, flooding, cytosolic acidification, increased reactive oxygen species (ROS) levels, and exposure to harmful heavy metals.

The Synthesis of GABA from Proline

The alternative pathway suggested by Signorelli et al. (2015) requires a non-enzymatic conversion of GABA from the amino acid proline under conditions of oxidative stress. This molecular transformation occurs primarily in the setting of plant exposure to stress factors that trigger cellular degradation due to the generation of reactive oxygen species (ROS). This process is characterized by the tendency of the amino acid proline to react with hydroxyl radicals, eventually leading to the formation of pyrrolidine-1-yl via a spontaneous decarboxylation process. Subsequently, the resulting pyrrolidine-1-yl species are converted to Δ1-pyrroline, which then acts as a substrate for enzymatic conversion to GABA via the catalytic action of the Δ1-pyrroline dehydrogenase enzyme (Signorelli et al., 2015).

2.2. Microbial GABA Production

There are two main pathways in the microbial synthesis of GABA: the glutamate decarboxylase (GAD) pathway and the putrescine (Puu) pathway. The Puu pathway, which is present in *Aspergillus oryzae* and *Escherichia coli* but not in common producers such as *Bifidobacterium* or *Lactobacillus*, consists of two distinct pathways. In the first pathway, Puu is converted to γ-glutamyl-Puu, which is then oxidized to give γ-Glu-GABA. This compound is then hydrolyzed to form GABA. In the second pathway, Puu is directly reduced to γ-amino butyraldehyde, which is further oxidized to produce GABA. The GAD pathway used by the majority of GABA-producing organisms involves the uptake of monosodium glutamate (MSG) or glutamate into the cell. Next, these

compounds undergo decarboxylation with pyridoxal-5-phosphate (PLP), which acts as a coenzyme. The Glu/GABA antiporter then transports the resulting GABA into the extracellular space (Diez-Gutiérrez et al., 2020).

As shown in Figure 2, the predominant metabolic pathway for GABA biosynthesis in most GABA-producing microorganisms involves α-decarboxylation of L-glutamate. This irreversible reaction is catalyzed by a pyridoxal 5'-phosphate (PLP) dependent GAD enzyme. Most of the available data on GABA production by microbial fermentation is based on this metabolic process. It has also been previously discussed that GABA can be synthesized from the degradation of putrescine, polyamines, or ornithine in various microorganisms (Luo et al., 2021).

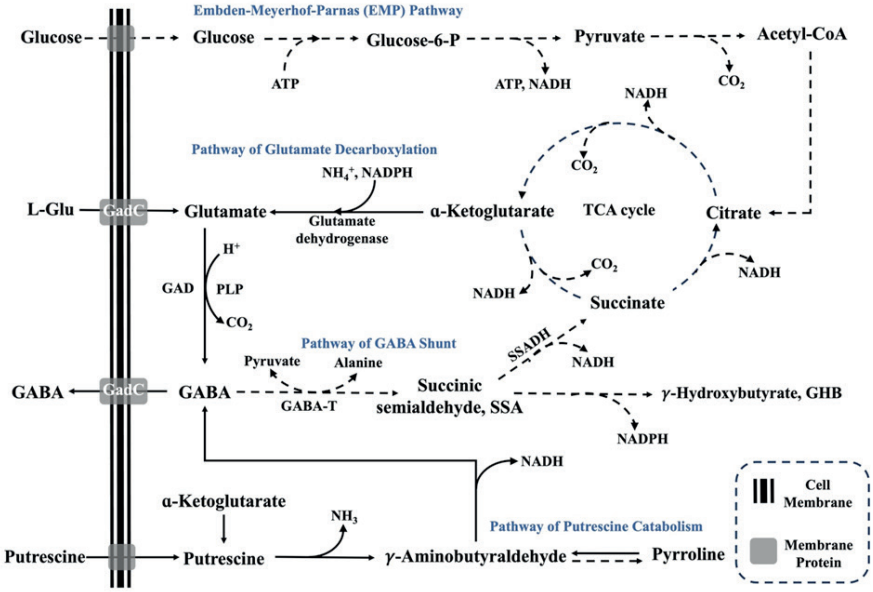

Figure 2. Major metabolic pathways of GABA biosynthesis and degradation in microorganisms (Dark arrows indicate metabolic pathways for biosynthesis of precursors for GABA production, dotted arrows indicate EMP pathway, TCA cycle, and GABA shunt) (Luo et al., 2021).

The central pathway of GABA synthesis involves the conversion of α-ketoglutarate, a product of the TCA cycle, to succinate via intermediates such as glutamate, GABA, and succinic semialdehyde. This sequence is conserved in prokaryotes and eukaryotes and is known as the GABA shunt. Various enzymes participate in this pathway. Initially, α-ketoglutarate is converted to glutamate via transamination and glutamate dehydrogenase reactions. Next, glutamate decarboxylation to form GABA occurs via the enzyme glutamate decarboxylase (GAD), an irreversible step. During this

reaction, the GAD uses a proton and releases CO_2. This GAD enzyme is critical in GABA synthesis. It functions as a rate-controlling enzyme and relies on pyridoxal phosphate (PLP) as a cofactor. Mammalian species express two isoforms of GAD, GAD67, and GAD65, with genes located on chromosomes 2 and 10, respectively, in humans. GAD67 primarily synthesizes GABA in the brain, while GAD65 is predominant in pancreatic cells (Luo et al., 2021).

The third enzyme involved in the GABA shunt is GABA transaminase (GABA-T). This step facilitates GABA catabolism leading to the production of succinic semialdehyde (SSA) via the GABA transaminase enzyme. SSA is then converted to succinate with the help of succinic semialdehyde dehydrogenase (SSADH) and the resulting succinate is allowed to integrate into the TCA cycle for further metabolic processing. In particular, succinate plays a critical role as an electron donor in the mitochondrial electron transport chain, an essential component of the tricarboxylic acid cycle. In a variety of organisms, including plants, animals, and *Escherichia coli*, succinic semialdehyde can be converted to γ-hydroxybutyric acid (GHBA) via the enzyme γ-hydroxybutyrate dehydrogenase (GHBDH) (Sarasa et al., 2020).

Beyond the metabolic pathways involving the GAD system, polyamine degradation, and GABA shunt, some acid-tolerant bacteria exhibit an additional layer of GABA biosynthesis containing the GadC protein located in the cell membrane. This protein is responsible for extracellular L-glutamate transport and intracellular excretion of GABA and contributes significantly to GABA production in these organisms (Luo et al., 2021).

GABA Production by Lactic Acid Bacteria

Lactic Acid Bacteria (LAB) are a group of gram-positive, acid-tolerant bacteria that exhibit various shapes such as cocci or sticks. They share common physiological and metabolic features. LAB plays a vital role in the fermentation of a wide variety of foods and finds wide use as starter cultures in both traditional and industrial food fermentation processes. One of the primary metabolic byproducts during carbohydrate fermentation is lactic acid, which significantly affects the physiological functions of LAB. In acidic environments, different LAB strains have evolved various acid resistance mechanisms to maintain cell viability (Sarasa et al., 2020).

These defense systems encompass a variety of strategies, including the F0F1-ATPase system, which is responsible for maintaining pH balance in the cytosol by transporting protons. Similarly, the cation/proton antiporter and symporter systems contribute to acid resistance.

Another group of systems relies on glutamate or arginine as substrates. The arginine-dependent system involves the production of an intracellular alkaline compound, while the glutamate-dependent system involves the incorporation of glutamate and GABA (gamma-aminobutyric acid) to deplete intracellular protons. This process then replaces the formed product with another glutamate substrate. This innovative mechanism converts an extracellular amino acid into an extracellular compound, simultaneously depleting intracellular protons and raising the intracellular pH (Sarasa et al., 2020).

GABA, a four-carbon compound, is common among bacterial species and serves a metabolic function within the Krebs cycle. Their role in bacteria can vary, showing strain-dependent variations. In particular, acid tolerance stands as a basic criterion for identifying probiotic strains. An important mechanism that assists bacteria in maintaining a neutral pH under acidic stress involves relying on glutamate-antiporter reactions. In this process, bacteria use special transporters to transport glutamate into the cell. Within the cytoplasm, decarboxylation of glutamate takes place leading to the consumption of intracellular protons. The resulting GABA is then excreted from the cell via an antiporter mechanism, causing an increase in intracellular pH due to hydrogen ion removal. In addition, the extracellular pH rises as extracellular glutamate is exchanged for the more alkaline GABA (Sarasa et al., 2020). Consequently, the primary role of glutamate decarboxylase is to regulate the pH of the bacterial environment by using the decarboxylation reaction to consume hydrogen ion. Lactic acid bacteria such as *E. coli, Shigella, Lactobacillus* and especially *L. lactis* harbor the glutamate decarboxylase gene. The expression of these genes is vital for bacterial survival under acidic pH conditions. Therefore, increased GAD activity becomes crucial for survival in an acidic environment. This high activity enables bacteria to overcome the low pH challenges of fermented foods, gastric juices, and volatile fatty acids in the gastrointestinal tract (Sarasa et., 2020; Diana et al., 2014).

3. THE NATURALLY OCCURRING GABA IN FOODS

GABA is present across a wide spectrum of food sources, predominantly encompassing cereals, legumes, pseudo-cereals, fruits, vegetables, tea, and edible mushrooms. Nevertheless, as shown in Table 1, the natural GABA content in most foods tends to be low, despite relatively high GABA levels in certain sample varieties.

Table 1. Dietary sources of GABA (Hou et al., 2023)

Foodstuffs	Varieties	Contents
Cereals	Brown rice (*Oryza sativa* L.)	5.28–27.00 mg/100 g
	Red rice (*Oryza sativa* L.)	1.18–2.91 mg/100 g
	Black rice (*Oryza sativa* L.)	0.67–7.46 mg/100 g
	Wheat (*Triticum aestivum* L.)	4.55–14.68 mg/100 g
	Barley (*Hordeum vulgare* L.)	1.96–54.00 mg/100 g
Legumes	Yellow soybean (*Glycine max* Merr.)	12.00–19.00 mg/100 g
	Black soybean (*Glycine max* Merr.)	4.38–61.00 mg/100 g
	Mung bean (*Vigna radiata* L)	0.63–13.25 mg/100 g
	Chickpea (*Cicer arietinum* L.)	6.42 mg/100 g
	Faba bean (*Vicia faba* L.)	5.00 mg/100 g
Pseudo-cereals	Quinoa (*Chenopodium quinoa* Willd.)	7.00–66.10 mg/100 g
	Common buckwheat (*Fagopyrum esculentum* Moench.)	10.34 mg/100 g
	Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.)	42.60–112.50 mg/100 g
	Amaranth (*Amaranthus* L.)	1.20 mg/100 g
Fruits	Grape (*Vitis vinifera* L.)	58.93–109.83 mg/L*
	Apple (*Malus pumila Mill.*)	942.00 nmol/g*
	Mulberry fruit (*Morus alba* L.)	17.10–33.60 mg/100 g
	Kiwifruit (*Actinidia chinensis Planch.*)	2.54–19.14 mg/100 g
	Lychee (*Litchi chinensis Sonn.*)	170.00–350.00 mg/100 g*
	Jujube fruit (*Ziziphus jujuba Mill.*)	15.03–33.34 mg/100 g
Vegetables	Tomato (*Solanum lycopersicum* L.)	219.86–404.89 mg/100 g
	Spinach (*Spinacia oleracea* L.)	232.10–381.00 mg/100 g
	Sweet potato (*Ipomoea batatas* (L.) Lam.)	0.13–0.50 μmol/g*
	Eggplant (*Solanum melongena* L.)	23.28–38.12 mg/100 g*
Tea (*Camellia sinensis* (L.) O. Ktze	White tea	3.49–207.00 mg/100 g
	Green tea	0.24–87.00 mg/100 g
	Black tea	2.65–55.50 mg/100 g
Edible mushrooms	White mushroom (*Agaricus bisporus*)	18.00–20.00 mg/100 g
	Shiitake mushroom (*Lentinula edodes*)	17.00–35.00 mg/100 g
	Oyster mushroom (*Pleurotus pulmonarius*)	32.15–57.73 mg/100 g

The asterisk (*) represents fresh weight.

Based on the above table, the levels of GABA exhibit substantial disparities across diverse categories of foodstuffs, often attributable to varietal distinctions, growth climates, and agronomic conditions. In particular, significant differences can occur even within the same food category. Moreover, the GABA content in various food products is markedly insufficient to adequately meet human health needs through traditional consumption patterns. In light of these circumstances, subsequent research has diligently endeavored to increase the GABA content in foods through various enrichment technologies (Hou et al., 2023).

4. GABA ENRICHMENT TECHNOLOGIES

Various strategies aimed at increasing their functionality have been used to increase the GABA content in foods. These methodologies encompass anaerobic treatment, cold treatment, germination, microbial fermentation, new processing techniques, additional abiotic stress inductions, and hybrid approaches combining different methodologies.

4.1. Germination

Germination emerges as a cost-effective and simple process with the potential to increase the nutrient profile and bioactive components in grains and seeds. The increase in GABA levels observed during germination is attributed to the creation of a low-oxygen environment that occurs when seeds are immersed in the growth medium. This oxygen-deficient environment contributes to intracellular acidification, thereby causing an increase in glutamate decarboxylase (GAD) activity. In addition, recent studies have revealed a remarkable aspect in the context of GABA enrichment during germination: a notable increase in the expression of the GAD gene in brown rice. This finding reveals an additional mechanism that promotes GABA accumulation throughout the germination process (Hussain, Jabeen, Naseer, & Shikari, 2020). In a study conducted by Kamjijam, Bednarz, Suwannaporn, Jom, & Niehaus, (2020), it was demonstrated that the GABA content was notably higher in germinated rice grains as compared to their non-germinated counterparts. Consistent findings have been documented across diverse germinated seed varieties, including but not limited to peanuts, barley, and brown rice (Munarko, Sitanggang, Kusnandar, & Budijanto, 2021).

4.2. Salt Treatment

Salt stress is a common method used to increase GABA levels. This is achieved by immersing the sample in a concentrated solution of NaCl or $CaCl_2$. This approach is often synergistically combined with the

germination process to increase the GABA content in seeds, grains, and sprouts. Ji, Shi, Xie, Zhang, Chen, Du, & Shi (2020) explained that wheat exhibited a significant increase in GABA content with an increase in glutamate decarboxylase (GAD) activity under NaCl stress. Yin, Yang, Guo, & Gu, (2014) showed that soybean seeds exposed to NaCl stress caused increased GAD, diamine oxidase (DAO), and γ-aminobutyraldehyde dehydrogenase (AMADH) activities with gene expressions. This complex interaction resulted in a remarkable increase in the GABA content in soybeans.

4.3. Anaerobic Treatment

Anaerobic treatment is one of the oldest used techniques for GABA enrichment, which is achieved by methods that involve the introduction of CO_2 or N_2, alternating cycles of anaerobic and aerobic conditions, and lowering the air pressure to create a low-oxygen environment. This approach finds its obvious application in GABA-tea production. In hypoxic conditions, carbohydrates undergo anaerobic fermentation leading to the accumulation of ethanol and lactic acid in the cellular environment, resulting in cytoplasmic acidification. In this context, the rate-limiting glutamate decarboxylase (GAD) enzyme responsible for GABA biosynthesis in the cytoplasm is activated. This enzyme then catalyzes the decarboxylation of glutamate to GABA, a process dependent on H^+ ion incorporation. Compared to other stress factors such as mechanical damage, dryness, and cold stimulation, anaerobic treatment emerges as the most effective way to increase GABA content in plants (Reggiani, Cantu, Brambilla, & Bertani, 1988).

4.4. Cold Treatment

Research pertaining to the augmentation of GABA content through cold treatment predominantly centers on fruits and vegetables. Exposure to low temperatures induces a cascade of physiological alterations within plant cells, encompassing the elevation of carbohydrate and amino acid concentrations and the facilitation of cytoplasmic Ca^{2+} release. This, in turn, triggers the activation of glutamate decarboxylase (GAD) activity, initiating the degradation of glutamate and its subsequent conversion into GABA (Li, Luo, Liao, Shen, Li, Liu, & Zou, 2018; Zhou, Hu, Zhao, Yu, & Zhou, 2016). Noteworthy findings by Zhou et al., (2016) highlight an increased GABA content in litchi fruit during both cold treatment and storage, correlating with heightened GAD activity and diminished GABA transaminase (GABA-T) activity. Li et al. (2018) underscore the superior enrichment efficacy of gradient freezing treatment compared

to conventional freezing methods, specifically in the context of GABA augmentation within mulberry leaves.

4.5. Microbial Fermentation

Dairy products alongside some fruit juices have emerged as carriers for the purpose of GABA enrichment. Recent investigations have unveiled the GABA-producing capabilities of a diverse array of microorganisms, spanning bacteria, fungi, and yeasts. Predominantly, GABA-producing strains are sourced from cheese, pickles, and starter cultures. The basic principle behind microbial fermentation-based enrichment is found in the microbial-specific high GAD activity that enables the conversion of glutamate to GABA within the branched metabolic pathway. Currently, fermentation-based enrichment technology finds predominant application in liquid media such as milk and certain juices and semi-solid foods such as cheese to increase their GABA content. Furthermore, the potential for improved GABA yield lies in strain selection and optimization of the fermentation process, which encompasses variables such as strains, substrates, active ingredients, temperature, and pH (Hou et al., 2023).

The field of biotechnology on microbial fermentation has made significant progress in recent years. Significant experimental research has been undertaken to explore its broad application in the food processing industry, including techniques such as bacterial mutagenesis, gene disruption, and gene overexpression. Common strains used for enriching of GABA in both food production and industrial contexts primarily belong to bacterial taxa, including *Escherichia coli (E. coli),* lactic acid bacteria*, Streptomyces,* and *Streptococcus*. Complementing this lineup are fungal genera such as *Aspergillus* and *Rhizopus*, as well as various yeast species (Azhari, Wan-Mohtar, Kadir, Abd Rahim, & Saari, 2018; Hun Jin, Hong, Lee, Yoon, Pawluk, Yun, & Mah, 2021; Yu, Ren, Wang, & Huang, 2018). An evolving body of evidence confirms the capacity of gut-derived strains, including *Lactobacillus, Bifidobacterium*, and *Bacteroides*, to competently synthesize GABA in vitro, thereby expanding potential pathways for GABA production (Barrett, Ross, O'Toole, Fitzgerald, & Stanton, 2012; Yunes, Poluektova, Dyachkova, Klimina, Kovtun, Averina, & Orlova, 2016).

4.6. Other GABA Enrichment Technologies

Several new methods to increase GABA content have been used to increase GABA accumulation in unprocessed food sources, including cold plasma, vacuum impregnation, ultrasound, and high-pressure treatments. In a recent study by Li, Li, Wu, & Tan (2023), it was reported that the GABA content in germinated brown rice (GBR) treated with

cold plasma pretreatment exhibited a noteworthy increase compared to untreated GBR, even when subjected to the same germination duration. Furthermore, the introduction of short-time vacuum impregnation prior to germination was demonstrated to significantly enhance GABA production in rice, as elucidated by Kamjijam, Suwannaporn, Bednarz, Na Jom, & Niehaus (2021). Additionally, the alteration of surface microstructure in coffee leaves through ultrasonic treatment was observed to facilitate the migration of glutamate into cells, subsequently leading to heightened GABA content (Sun, Ji, Ma, & Chen, 2022). This study manifested a substantial elevation of 12% and 124.1% in GAD and DAO activities, respectively. High hydrostatic pressure treatment (300 MPa, 10 min) has further been established as an effective means for GABA enrichment in soybean, achieving a GABA content of 1.87 μmol/g through the augmentation of apparent GAD activity, as shown by Ueno, Kawaguchi, Oshikiri, Liu, & Shimada (2019).

5. GABA- ENRICHED FOODS

Gamma-aminobutyric acid (GABA) is one of the preferred nutritional supplements due to its health-promoting properties (Cunha, Coelho, Ribeiro, & Silva, 2022). Because of the low GABA content in foods, there is significant interest in GABA-rich foods within the field of food and medicine (Koh, Lim, Teoh, Kobun, & Rasti, 2023).

The global market size of GABA-related foods is steadily growing and is estimated to reach 6.7 billion in 2023 (Hou et al., 2023). Some products enriched with GABA are shown in Table 2. GABA-enriched foods are recognized by the Japanese government as FOSHU (Foods for Specified Health Uses). Formulations of GABA, which are claimed to possess calming and soothing effects, are also commercially available as dietary supplements. Pharma-GABA™ is an FDA-approved commercial product as a food ingredient (Linares, O'Callaghan, O'Connor, Ross, & Stanton, 2016).

In a study, dark chocolate was enriched with pure GABA powder at concentrations of 0.05%, 0.10%, and 0.15%, and the effect of GABA enrichment on rheological, melting, shelf life, and sensory properties was investigated. Researchers determined that during the chocolate production process, the GABA content was significantly reduced and to solve this problem, enriching the product with 0.15% GABA after the cocoa butter melting step was quite effective. It was determined that enrichment with GABA in chocolate production did not have any significant effect on rheological, melting, shelf life, and sensory properties, but caused an increase in moisture, protein content, and hardness of dark chocolate. In addition, the significant increase in GABA content and ACE inhibitory

activity with the addition of 0.15% GABA was one of the important findings (Koh et al., 2023).

Cunha et al. (2022) determined that *Enterococcus malodorous* SJC25 strains isolated from cheese were good GABA producers and conducted a study on the use of these isolates in passion fruit and pineapple whey beverages. Both beverages were found to have a positive acceptability score of over 3 in the sensory analysis. They determined that the GABA content in whey-based beverages reached 250–300 mg/100 mL, which is equivalent to the amount found in GABA supplements.

In a study conducted on fermented Thai shrimp (Kung-Som), *Lactobacillus futsaii* CS3, a GABA-producing microorganism, was utilized as a starter culture. the GABA content in the experimental Kung-Som with the incorporated starter culture reached levels up to four times greater than that of the control (without the starter culture) or commercial Kung-Som products. Furthermore, the use of *Lactobacillus futsaii* CS3 as a starter culture reduced the fermentation time compared to the control sample. It was determined that this culture could enhance microbiological safety and sensory development when compared to the control sample (Sanchart, Rattanaporn, Haltrich, Phukpattaranont, & Maneerat, 2017).

Redruello, Saidi, Sampedro, Ladero, Del Rio, & Alvarez (2021) produced GABA-enriched cheese by using GABA-producing *L. lactis* strains as starting cultures for cheese production. The GABA concentrations of the cheeses were found to be between 350 mg/kg and 457 mg/kg. In this study, it was determined that when the average GABA accumulation was 384 mg/kg, consuming 50 g of cheese per day (providing about 19 mg of GABA) had a sufficient level to have positive effects on human blood pressure.

In a study, it was determined that some *Lactobacillus* strains isolated from traditional Pico cheese were able to produce high amounts of GABA. The researchers suggested the *Lactobacillus* strain (especially *Lb. plantarum* L2A21R1) *is* a starter culture for the production of GABA-rich fermented functional foods due to its high GABA production (936.8 mg/L) (Ribeiro, Domingos-Lopes, Stanton, Ross, & Silva, 2018).

It was observed that fermentation of faba bean flour with *Lactobacillus plantarum* VTT E-133328 (at 30 °C for 48 hours) caused a significant increase in GABA content (Coda, Melama, Rizzello, Curiel, Sibakov, Holopainen, & Sozer, 2015).

Table 2. GABA-enriched foods by different techniques

Product	Enrichment types	References
Dark chocolate	pure GABA powder (0.05%, 0.10%, 0.15%)	(Koh et al., 2023)
Whey-Based Beverage	Fermentation (5% *E. malodoratus* SJC25 culture)	(Cunha et al., 2022)
Honey syrup	Fermentation (*Lactobacillus* sp. Makhdzir Naser-1)	(Gharehyakheh et al., 2019)
Cherry-kefir beverage	Fermentation (*Lactobacillus* sp. Makhdzir Naser-1)	(Gharehyakheh, 2021)
Thai fermented shrimp	Fermentation (*Lactobacillus futsaii* CS3)	(Sanchart et al., 2017)
Adzuki bean	Germination	(Jiang, Xu, Zhang, Li, Tang, Cao, & Zhang, 2023)
Cheese	Fermentation (GABA-producing *Lactococcus lactis* strains isolated from camel's milk)	(Redruello et al., 2021)
Functional milk beverage	Fermentation (*Lactobacillus plantarum* C48)	(Servili, Rizzello, Taticchi, Esposto, Urbani, Mazzacane, ... & Di Cagno, 2011)
Functional grape must beverage	Fermantation (*Lactobacillus plantarum* DSM19463)	(Di Cagno, Mazzacane, Rizzello, De Angelis, Giuliani, Meloni., ... & Gobbetti, 2010)
Novel plant-based probiotic drink	Fermantation (GABA-producing *Lactobacillus pentosus* 9D3 isolated from Thai pickled weed)	(Kittibunchakul et al., 2021)
GABA-Enriched Bioactive Yogurt	Fermantation (*Streptococcus thermophilus* APC151 strain)	(Linares et al., 2016)
Functional bread enriched with GABA	Fermantaion *(Lactobacillus plantarum* C48 *and Lactococcus lactis subsp. lactis* PU1	(Coda, Rizzello, & Gobbetti, 2010)

6. HEALTH BENEFITS OF GABA-ENRICHED FOODS

GABA-enriched foods have various health benefits, including anti-hypertensive, anti-diabetic, and anti-inflammatory properties. neuroprotection, improving sleep quality, and relieving depression. Table 3 shows the health benefits of foods enriched with GABA.

High blood pressure, also known as hypertension, is a significant risk factor for stroke and heart disease. The majority of individuals diagnosed with hypertension take antihypertensive medications. While medication intake is effective in reducing blood pressure, changes in lifestyle and dietary habits can aid in preventing the development of hypertension. Some studies have indicated that GABA could have beneficial effects in

controlling blood pressure (Pouliot-Mathieu, Gardner-Fortier, Lemieux, St-Gelais, Champagne, & Vuillemard, 2013).

In a study focused on hypertension, an experimental Cheddar cheese was produced using a *Lactococcus lactis* ssp. *lactis* (a GABA-producing microorganism) as a starter culture. During the research, a group of 23 male participants (ranging from 23 to 63 years old) consumed 50 g of the experimental cheese daily for a duration of 12 weeks, resulting in an intake of 16 mg of GABA. This study determined a decrease in systolic and mean blood pressure in the male participants (Pouliot-Mathieu et al., 2013).

Inoue et al. (2003) demonstrated that consuming only 10-12 mg of GABA per day for 12 weeks via GABA-enriched fermented milk was effective in people with mild hypertension. In a conducted study, the researchers determined the antihypertensive effect of a GABA-rich tomato variety in hypertensive rats (Yoshimura, Toyoshi, Sano, Izumi, Fujii, Konishi, & Obata, 2010).

Obesity and diabetes are important diseases that affect the lives of millions of people each year. Diabetes is an increase in blood glucose (sugar) levels above normal, and this disease adversely affects the blood vessels, kidneys, and nervous system. Inhibition of α-amylase and α-glucosidase, which is enzymes that hydrolyze carbohydrates, are effective in controlling diabetes (Kittibunchakul, Yuthaworawit, Whanmek, Suttisansanee, & Santivarangkna, 2021).

Kittibunchakul et al. (2021) determined that a new plant-based probiotic beverage produced by fermentation of brown rice milk with GABA-producing *Lactobacillus pentosus* inhibited key enzymes related to obesity and diabetes. It has been determined that this beverage plays a role in increasing the inhibition of lipase, α-amylase, and α-glucosidase. In an in vitro study on mice, *Lactobacillus brevis* strains producing GABA were found to reduce glucose levels in diabetic mice (Abdelazez, Abdelmotaal, Evivie, Melak, Jia, Khoso, & Meng, 2018)

Today, many people experience mental anxiety, which can lead to insomnia as a result of stress and reduced tolerance. GABA has the effect of improving sleep quality by relieving anxiety or depression through the neuroendocrine pathway (Heli et al., 2022).

Anxiety and stress have adverse effects on the immune system. Moreover, these diseases can also lead to heart disease, hypertension, ulcers, depression, and other illnesses. The measurement of IgA in saliva is important for assessing both immune function and mood in humans. In a conducted study, the impact of GABA intake on brain waves was

investigated in 13 subjects. Researchers determined that GABA not only induces relaxation but also reduces anxiety (Abdou, Higashiguchi, Horie, Kim, Hatta, & Yokogoshi 2006).

Oh & Oh (2004) determined that brown rice extracts with high levels of GABA prevent the proliferation of leukemia cells and have a stimulating effect on cancer cell apoptosis. Song, Du, Xiong, Jiang, Zhang, Tian, & Yan (2016) determined that the GABA molecule was quite effective in preventing the proliferation of colon cancer cells. Researchers have recommended the use of GABA in colon cancer polychemotherapy.

Table 3. Health benefits of GABA-enriched foods

Source	Properties of source	Health benefits	References
Fermented milk	The preparation of *Lactobacillus casei* strain Shirota, and *Lactococcus lactis* YIT 2027)	Decrease in systolic and mean blood pressure	(Inoue et al., 2003)
Cheese	The preparation of GABA-producing *Lactococcus lactis* ssp. *lactis* strain	Decrease systolic and mean blood pressure in male	(Pouliot-Mathieu et al., 2013)
Grape must beverage	The preparation of *L. plantarum* DSM19463	Anti-hypertensive effect and dermatological protection	(Di Cagno et al., 2010)
Novel plant-based probiotic drink	The preparation of GABA-producing *Lactobacillus pentosus* isolated from Thai pickled weed	Antioxidant, anti-obesity, and anti-diabetic	(Kittibunchakul et al., 2021)
Brown Rice Extracts	Germination	Anticancer	(Oh & Oh, 2004)
Malaysian brown rice	Malaysian brown rice varieties	Modulation of blood cholesterol levels	(Roohinejad, Omidizadeh, Mirhosseini, Rasti,Saari, Mustafa, S., ...& Manap, 2009)
Commercial GABA	Pharma-GABA, (Pharma Foods International Co., Japan)	Relaxation and immunity	(Abdou et al., 2006)
Fermented *Laminaria japonica*	Preparation of fermented *Laminaria japonica* by *Lactobacillus brevi*s BJ20	Neuroprotective effect	(Reid, Ryu, Kim, & Jeon, 2018)
Diabetic model mice	Preparation of *Lactobacillus brevis* KLDS 1.0727 and KLDS 1.0373 strains	Effective on diabetes	(Abdelazez et al., 2018)
Commercial GABA	GABA (Sigma-Aldrich, Shanghai, China)	Effective on colon cancer	(Song et al., 2016)

The neurological disorder manifests itself with physical or psychological symptoms in a part of the brain or nervous system. Epilepsy, Alzheimer's disease, cerebrovascular diseases, multiple sclerosis, Parkinson's disease, neuro infections, and insomnia are examples of these disorders (Ngo & Vo, 2019). In a study examining the neurodegenerative effects of GABA-enriched fermented *Laminaria japonica* associated with aging, it was stated that this fermented *Laminaria japonica* was effective in improving on short-term memory and physical function. It could be recommended for protection against degenerative effects (Reid et al., 2018).

CONCLUSION

Gamma-aminobutyric acid (GABA) is a neurotransmitter found in the brain and other nerve tissues. Foods can be enriched with GABA, which is a naturally occurring component in foods. Fermented foods are rich sources of GABA because they contain bacteria involved in the production of GABA. In recent years, there has been exploration into the potential of enhancing the nutritional value and health benefits of foods by incorporating GABA into them. Enrichment of foods with GABA offers numerous many potential health benefits. GABA has a calming effect on the nervous system. GABA-enriched foods also exhibit additional health benefits, including anti-hypertension, anti-cancer, and anti-inflammatory.

The primary challenges for future GABA research involve exploring high GABA-producing strains, enhancing GABA stability during storage, and developing new enrichment technologies that do not compromise food quality or other active constituents. A deeper understanding of GABA has the potential to open up new possibilities for its incorporation in the development of functional foods.

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Chapter 11

FRACTIONATION OF WHEY PROTEINS

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1. INTRODUCTION

Whey is a dairy by-product that is formed during cheese making and contains almost 20% of milk proteins. Whey is also formed when making casein and yogurt (strained). Whey components are important for nutrition, but they also impose risks for the environment when they are unconsciously disposed of as waste. Therefore the products to be produced from whey can be used to support nutrition and reduce environmental wastes. Many different products, such as whey powder, whey concentrate, and whey protein isolate, are being produced recently from whey and various whey processing techniques are being used to produce these products. Fractionation is one of these processes. Methods, such as the chromatographic method and membrane method, can be used to fractionate whey. The method to be used for fractionation is determined according to the nature of the component intended to be fractionated. These methods can also be used in combination with others in some cases.

This section describes the properties of whey and the methods being used to fractionate whey.

2. WHEY

When the history of whey is researched, it is believed that it is based on the use of calf tripe to store and transport the milk, and whey is obtained as the milk is clotted by chymosin, which is available in tripe (Smithers, 2008). When historical sources are examined, it is seen that Hippocrates (BC 466-377), Avicenna (AD. 980-1037), Hermann Boerhaave (1668-1738), and Thomas Sydenham (1624-1689) made several researches on whey and they recommended whey to their patients for treatment purposes (Özdemir, 2018).

Whey is known as "Molke" in German and "Lactoserum" in French. The greenish or yellow-colored liquid remaining after the clot, which occurs by clotting the milk with rennet or organic acid, is separated, and is called whey (Evren, Apan, Tutkun Şıvgın, & Öztürk, 2011).

Whey differs, depending on its production type (Figure 1). Generally, it is divided into two, namely "sweet whey" or "sour whey". The by-product that is achieved by settling casein at pH 6.5 by using rennet for clotting the products, such as semi-hard and hard cheese, is called hard cheese whey" or "rennet whey" whereas the by-product that is achieved by clotting the milk at pH 4.5 with lactic acid bacteria or organic acids when producing the products, such as cream cheese, quark, paneeer, and strained yogurt, is called "sour whey" or "acid whey (Caessens, Daamen, Gruppen, Visser, & Voragen, 1999). Additionally, the whey that is achieved by boiling curd

when producing cheese is called boiling whey, and the whey that is formed as a result of production of casein is called technical whey (Dinçoğlu & Ardıç 2012).

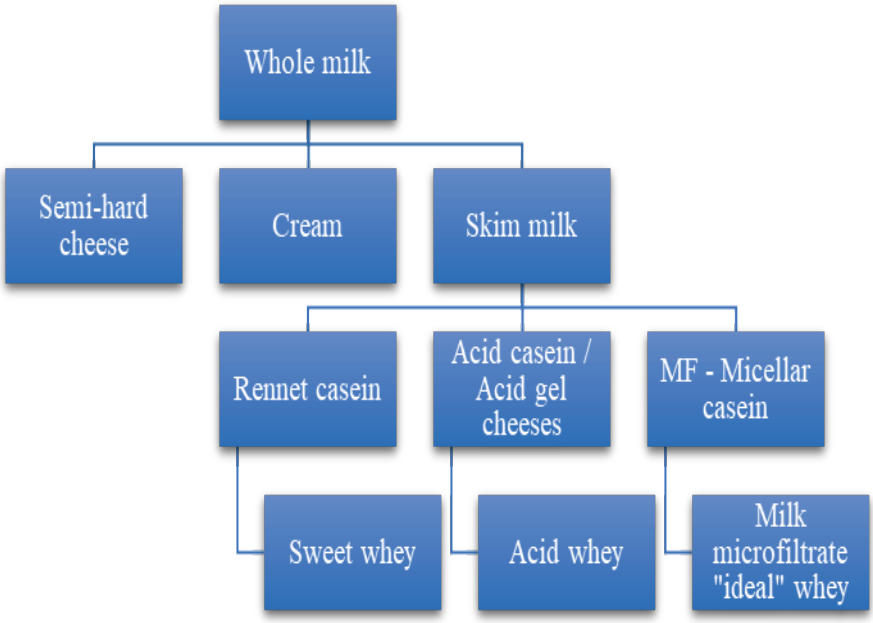

Figure 1. General classification of whey types (Foegeding, Davis, Doucet, & Mc Guffey, 2002; Gangurde, Chordiya, Patil, &Baste, 2011)

Approximately 85% of the milk used to produce cheese passes to the cheese's juice and whey contains approximately 20% of whey proteins (McIntosh, Royle, Le Leu, Regester, Johnson, Grinsted, R.L., Kenward, & Smithers, 1998; Mete, 2012). Whey is composed of 93% of water. Its dry matter part contains a substantial amount of protein (lactoglobulin, lactalbumin, and serum proteins), fat, lactose, vitamins, and minerals (Alpkent & Göncü, 2003; Jeličić, Božanić, & Tratnik, 2008). The composition of whey includes important vitamins, such as folic acid, cyanocobalamin (B12), and riboflavin (B2), which gives its color to whey (Metin, 2005, Jeličić et al., 2008). Additionally, the concentration of potassium, sodium, and chlorine, which is available in whey, varies depending on the origin of the product. For example, the concentrations of calcium and phosphor are much more in sour whey, compared to sweet whey (Jeličić et al., 2008). The composition of whey varies, depending on the production type of cheese. The composition of whey is given in Table 1 below.

Table 1. The composition of whey (g/L) (Yerlikaya, Kınık, & Akbulut, 2010).

Component	Acid whey	Sweet whey
Dry Matter	63.0-70.0	63.0-70.0
Lactose	46.0-52.0	44.0-46.0
Protein	6.0-10.0	6.0-8.0
Phosphate	1.0-3.0	2.0-4.5
Lactate	2.0	6.4
Calcium	0.4-0.6	1.2-1.6
Chlorine	1.1	1.1

About 22 million tons of cheese are produced worldwide every year, and most of the milk used during production turns into whey (National Milk Council Report, 2023). When whey is disposed to streams, canals, or the sea, the components in the structure of whey are broken down by microorganisms, and various organic acids are formed. Also, the amount of dissolved oxygen in the water, which is used during the breakdown of lactose in the structure of whey by anaerobic bacteria, is consumed more rapidly (Dedebaş, 2009; Özel Yavuz, 2017). When whey is unconsciously given to the soil, the mineral balance of the soil is disrupted (Özçelik, 2013). Therefore, unconscious disposal of whey can cause environmental problems and economic losses (Ertürk & Özgen, 2021).

The high nutritional value of whey caused this product to be used in different areas. Today, whey and whey products are used in bread, pastry and bakery products as well as meat products such as sausage and salami, dairy products such as yogurt and ice cream, production of whey drinks and baby foods, animal feeds, sports nutrition and cosmetics industry (Demir, Elgün, & Argun, 2009; Bekiş, 2019; Özdemir, 2018; Alpkent & Göncü, 2003; Dairy Council of California, 2013). Additionally, whey powder, whey concentrate, whey protein isolate, demineralized whey with reduced lactose, and various pure proteins are produced from whey (Basette & Acosta, 1988; De la Fuente, Hemar, Tamehana, Munro, & Singh, 2002; Smithers, 2008). The composition of some whey products is given in Table 2.

Table 2. Composition of some whey products (Dairy Council of California, 2013)

Product	Lactose (%)	Protein (%)	Fat (%)
Whey powder	63-75	11-14.5	1-1.5
Whey protein concentrate	4-52	28-89	1-9
Whey protein isolate	0.5-1	90-95	0.5-1
Hydrolyzed whey protein concentrate	<8	>80	>10
Hydrolyzed whey protein isolate	0.5-1	>90	0.5-1

2.1. Whey Proteins

Whey is a polymorphic and heterogeneous combination of proteins containing varying amounts of protein fractions (Pal & Radavelli-Bagatini, 2013). Whey proteins are composed of globular proteins consisting of basic, acidic, hydrophilic, and hydrophobic amino acids. The main protein of whey is β-lactoglobulin. In addition, whey contains α-lactalbumin (α-La) and glycomacropeptide and immunoglobulins, bovine serum albumin, lactoferrin, lactoperoxidase, and proteo-peptone (Kavaz Yüksel, Yüksel, & Ürüşan 2019; Chen, Qu, Gras, & Kentish, 2023) (Table 3).

Table 3. Properties of whey protein. (Korhonen, 1995; De Wit, 1998; Fee, & Chand, 2006; Madureira et al., 2007; Guo & Wang, 2016; Aguero et al., 2017; Ramos et al., 2017)

Protein	Content (%w/w)	Molecular mass (kDa)	Isoelectric point (pI)	Denaturation temperature, T_m (°C)
β-Laktoglobulin	40-50	18.4	5.35-5.49	75
α-Laktalbumin	12-15	14	4.5-4.8	26 (apo form)
Glycomacropeptide	12	6.8	4.3-4.6	64 (holo form)
Immunoglobulins	8	150-1000	5.5-8.3	
Bovine serum albumin	5	66.0	4.7-5.1	80
Lactoferrin	1	76.5	7.0-9.0	60-65 (apo form)
Lactoperoxidase	0.5	78	9.2-9.9	90 (holo form)
Proteose-peptone	0.19	4-22	3.3-3.7	

β-Lactoglobulin is the highest amount of protein among whey proteins. It is available in many different types of milk, including human milk. Known as retinol-binding protein, this protein is stable at pH 2 in a dimer structure (Kazmierski & Correding 2003; Ramos et al. 2016). The amino acid sequence of β-Lactoglobulin contains the amino acid, named cysteine. This protein plays a role in the regulation of phosphorus metabolism in the mammary gland, muscle development, glutathione (GSH) synthesis, formation of passive immunity in newborns, and as a fatty acid and lipid binding protein (Farrell, Behe, & Enyeart 1987; Perez & Calvo 1995; De Wit, 1998).

α-Lactalbumin is the second most abundant protein among whey proteins. It is a component of the 'lactose synthetase' enzyme complex that provides lactose synthesis (Ars, 2004) and α-Lactalbumin has three different genetic variants (Fox, 1989). It has a high amount of tryptophan in its structure. Furthermore, this protein is very rich in leucine, lysine, threonine, and cysteine (Sousa, Lira, Rosa, De Oliveira, Oyama, Santos, &

Pimente 2012). α-lactalbumin is used as a coenzyme for the biosynthesis of lactose (Fox, 1989). Additionally, this protein has the ability to bind to minerals such as Ca and Zn (Sousa et al., 2012). α-Lactalbumin is an important energy source for newborns. Especially pure α-lactalbumin is used in the production of baby foods (Fox, 1989).

Glycomacropeptide consists of a peptide chain with 64 amino acids. Glycomacropeptide is obtained from casein during the first enzymatic step in cheese production with yeast (Chen et al., 2023). This protein has a high content of essential amino acids (Sousa et al., 2012). Glycomacropeptide increases the release of cholecystokinin, promotes the absorption of minerals, protects against bacteria and viruses in the digestive tract, and reduces the secretion of gastric acid as well as the risk of heart disease (Sousa et al., 2012).

Immunoglobulins are one of the minor components of whey proteins. It is composed of a single component. It is composed of two light (20,000 Da) and two heavy (50,000-70,000 Da) chains linked by sulfide bonds (Ars, 2004). There are various varieties of immunoglobulins, such as IgG1, IgG2, IgA and IgM. They also have a role in providing passive immunity for infants and strengthening the immune system in adults (Walzem, Dillard, & German, 2002; Metin 2005).

Bovine serum albumin cannot be synthesized in the mammary cell and passes from serum to milk together with immunoglobulins through the paracellular route (Ars, 2004). This protein can bind lipids and free fatty acids (De Wit, 1998).

Lactoferrin has a single peptide chain of 25 amino acids. This is a protein with iron in its structure (Korhonen & Pihlanto Leppala, 2005). It prevents the development of hepatitis by inhibiting the production of proinflammatory cytokines (Sousa et al., 2012). Additionally, this protein plays a role in iron absorption from the intestines and acts as a protector against *Listeria monocytogenes, Escherichia coli, Shigella dysenteriae, Salmonella typhimurium*, and some *Bacillus* species in newborns (Shah, 2000; DeWit, 1998).

Lactoperoxidase is an oxidoreductase that has an antimicrobial effect on the mammary gland and the gastrointestinal tract of newborns during lactation (Çankaya, Şişecioğlu, Yörük, & Özdemir, 2006).

Protease peptone is defined as the proteins remaining in solution after milk is heated at 95°C for 20 minutes and then acidified to pH 4.7 with 12% trichloroacetic acid (Swaisgood, 1982). This fraction can be divided into four major components while recognizing other minor components. The protease peptone component is found only in three different wheys.

This fraction is also not associated with casein. Protease peptone contains more than 17% carbohydrates and has a molecular weight of 20,000 (Girardet & Linden, 1996).

3. FRACTIONATION OF WHEY PROTEINS

Proteins have different functions, such as mechanical support, enzymatic catalysis, transport, storage, transmission of nerve impulses, coordinated movement, immune protection, and control of growth and differentiation. Therefore, each protein is important in terms of nutrition. Fractionation and purification of proteins are also important in terms of determining the functions of the protein, determining the mechanism of each function, and producing components that will be used in the field of food and pharmaceuticals (Telefoncu, 1996).

The fractionation of whey proteins is based on the physical and chemical properties of the components. Whey proteins can be fractionated separately or as a group, depending on these properties.

3.1. Chromatographic Separation Method

Chromatography technique is based on the principle of variable dispersion of different compounds in different phases. There are two phases in this technique, namely stationary and mobile. The mobile phase containing the substance intended to be separated is passed through the stationary phase. Meanwhile, the component of the substance intended to be separated, which is in the mobile phase, interacts with the stationary phase in several degrees. Velocities of the components in the mobile phase change, depending on this interaction. While the speed of the component with more interaction decreases, the speed of the component with less interaction is more than the others. The interaction that occurs depends on the size of the molecule, polarity, specific bonding, or force of electrostatic attraction (Enginün, 1996).

Size exclusion chromatography, affinity, and ion exchange chromatography are generally used for the separation of whey proteins by chromatographic method.

3.1.1. Size exclusion chromatography

Size exclusion chromatography is also known as molecular elimination chromatography and gel filtration chromatography. Size exclusion chromatography is a separation technique based on the difference in size of proteins and other biological macromolecules. Size exclusion chromatography is used to isolate one or more components, desalt the buffer, remove non-protein contaminants, separate (protein aggregates,

and determine the molecular weight of a compound (Anonymous, 2002; Cutler, 2004). The gel structure is formed by establishing three-dimensional networks, which is achieved by cross-linking the natural or synthetic polymers contained in the size exclusion chromatography. The different pore sizes formed in the gel may vary according to the amount of cross-linking in the gel (Konak, Turhan, & Certel, 2014).

When we look at the studies on the separation of proteins from whey, it is seen that various gel filters are used. For example, immunoglobulins are isolated by using a Sephacryl S-300 gel filter, and β-lactoglobulin and α-lactalbumin are isolated using a Sephadex medium. However, size exclusion chromatography showed low efficiency and solubility in the isolation of whey proteins (Forsum, Hambraeus, & Siddiqi 1974; Al-Mashikhi & Nakai 1987; Rojas, dos Reis Coimbra, Minim, Zuniga, Saraiva, & Minim 2004). Therefore, this method is mostly used in combination with methods such as ion exchange and affinity.

3.1.2. Affinity chromatography

Affinity chromatography is a method for molecular recognition. This method ensures that proteins are separated according to their biological functions rather than their physical or chemical properties (Wilchek & Chaiken, 2000; Zou, Luo, & Zhou, 2001). Its easy use, high selectivity, speed, and capability to purify the target protein in one step make affinity chromatography more advantageous than other systems (Doonan & Cutler, 2004; Konak et al., 2014). The affinity chromatography method is based on specific and reversible adsorbing of the desired biomolecule by ligands (antibody, enzyme inhibitor, cofactor, metal chelates, etc.) that can be immobilized on an insoluble matrix and contain complementary binding ends of this molecule (Kumpalume & Ghose, 2003; Wilchek & Chaiken, 2000).

The ion chelation property was used in the studies carried out for the purification of whey proteins by affinity chromatography (Chen et al., 2023). For example, the surface of silica gel was purified by α-lactalbumin (66% yield) by modifying the copper ions with more absorbable β-diketoamine groups and allowing it to form complexes with some specific groups in the proteins (such as carboxylate, amine, and mercaptan) (Gambero & Kubota, Gushikem, Airoldi, Granjeiro, Taga, Alcantara, 1997). Copper (II)-chelating sepharose is isolated from a whey concentrate with α-lactalbumin, with a recovery of 80% and a very high purity of 90% in a study performed by using a fast flowing column (Blomkalns & Gomez, 1997).

3.1.3. Ion exchange chromatography

Ion exchange chromatography is a method that benefits from the difference in charge carried by proteins at certain pH values. This method can be used effectively in the separation of proteins up to 70 kDa. In this system, the separation process takes place, based on reversible adsorption between the charged molecules and the oppositely charged and immobilized ion exchange groups (Yamamoto & Miyagawa, 2000; Konak et al., 2014).

Cation exchange resins can be used in the industry in order to isolate lactoferrin and lactoperoxidase from whey or skim milk. Cation exchange resins in the column are adsorbed after being fully loaded with proteins in the fractionation process. Different concentrations of salt solutions were used to separate lactoferrin and lactoperoxidase from resin beads (Metin, 2005. Yerlikaya et al., 2010).

Various technologies were used to increase the separation efficiency of ion exchange chromatography and to reduce buffer material consumption. The use of "simulated moving bed" technology in ion exchange chromatography can result in an approximately 50% increase in efficiency and a 5-fold reduction in buffer consumption compared to conventional chromatographic processes. However, implementation of this technology in the industry has not been widespread due to increasing pressure drop and difficulties in modeling multi-component systems (Chen et al. 2023). For this purpose, a single-step expanded bed adsorption process was applied with cation-exchanging Fastline SP in ion exchange chromatography, which is thought to be suitable for industrial applications. As a result of this application, lactoferrin was separated at high purity (88.5%) and recovered (77.1%) from sweet whey (Du, Lin, Xiong, & Yao, 2013; Chen et al. 2023).

Anion-ion exchangers were used to separate α-lactalbumin and β-lactoglobulin. While α-lactalbumin and β-lactoglobulin were carrying a net negative charge in sweet whey (at pH 6.5), the resins showed strong affinity against β-lactoglobulin. Cation exchangers can also be used for the fractionation of α-lactalbumin and β-lactoglobulin (Chen et al. 2023). However, this approach has not become widespread due to the possible negative charge of these proteins at the natural pH of whey. It was reported in a study that all positively charged proteins could be captured using an SP sepharose cation exchanger after the whey was adjusted to pH 4 with 1 M sulfuric acid (Doultani, Turhan, & Etzel, 2004).

Various studies were also conducted on the combination of cation exchange and anion exchange chromatographic steps. However, it was reported that the overall recovery and purity of proteins in this application

is lower compared to other methods and its commercial application is difficult (Chen et al. 2023).

Another study to improve ion exchange chromatography is the use of cryogens. Cryogels are chromatographic media formed as a single monolith within the column. Super acro pores available in the structure of monolith provide better passage of colloidal liquids. An anion exchanging cryogel, based on poly (2-hydroxyethyl methacrylate), was used in a study to isolate immunoglobulin-G from casein whey. As a result of this process, approximately 95% purity and 94% recovery were obtained (Dong, Chen, Dai, Johnson, Ye, Shen, Yun, Yao, Lin, & Yao, 2013). Separation of lactoferrin from milk and acid whey using cationic polyacrylamide (carboxy-PAAm) cryogels was achieved with approximately 85% yield and 90% purity (Billakanti & Fee 2009).

3.2. Membrane Separation Method

Membrane filtration is generally used for concentration. Although the membrane filtration method is mostly based on differences in molecular weight, milk, and whey components can be differentiated with this method by utilizing their properties such as sizes, concentrations, and electrical charges. Fractionation of proteins by membrane filtration can occur in cases where the difference in molecular weights such as α-lactalbumin and immunoglobulin-G is too great, or the difference between their sizes and charges is too much (Chen et al. 2023).

The membrane filtration method is used as a cross-flow filtration system in industrial processing of whey. In this system, the whey moves parallel to the membrane and the component to be separated passes through the membrane thanks to the applied pressure (Chen et al. 2023).

3.2.1. Microfiltration

Microfiltration is applied in order to fractionate and concentrate solid particles in liquid and gas phases and molecules dissolved in a solution, based on their relative sizes. Microfiltration is a pressure-assisted (<2 bar) membrane separation technique used for the separation of molecules, the molecular weight of which changes between 50 and 500 kDa (Dedebaş, 2019). Microfiltration membranes are classified as symmetrical and asymmetrical. Symmetrical membranes contain pores of uniform size, while asymmetrical membranes consist of multiple layers of different structures and pore sizes (Strathmann, Giorno, & Drioli, 2011; Wang & Zhou, 2013; France, Kelly, Crowley, & O'Mahony, 2021). The pore size of the membranes used in this filtration is between 0.1-10 μm. Therefore, microfiltration is used to remove bacteria and fat in whey processing

(Tarhan, 2000). For example, after the removal of blood, somatic cells, fat globules, and casein micelles from colostrum by microfiltration (0.1 μm), immunoglobulins can be recovered by using ultrafiltration membranes containing 100 kDa MWCO (Molecular weight cut off) [Cowan & Ritchie, 2007).

3.2.2. Ultrafiltration

Membranes used in ultrafiltration are not defined according to their pore diameters, but according to the spherical molecular weight (MWCO) that cannot pass through the membrane. The NMWC of membranes used in ultrafiltration varies between 2,000-300,000. Additionally, ultrafiltration systems can be pressured between 1-15 bar. Membranes used in ultrafiltration are regenerated cellulose (RC), polysulfone (PS), polyacrylonitrile (PAN), polyvinylidene fluoride (PVDF), and polyethersulfone (PES) membranes (Henning, Baer, Hassan, & Dave, 2006). Regenerated cellulose polyethersulfone and polyvinylidene fluoride membranes exhibit low protein binding properties and are often used to process protein solutions (Ho, 2007). However, polyethersulfone membranes are most widely used in dairy processing due to their low cost and good thermal and mechanical stability.

Separation of fractions with similar dimensions, such as β-lactoglobulin and α-lactalbumin, cannot be achieved in a single step in filtration systems. In this case, the separation of proteins can be achieved by optimizing the model (batch, continuous, and diafiltration) and parameters (such as pressure and volume reduction ratio) in the filtration process using polyvinylidene fluoride and polyethersulfone membranes (Muller, Daufin, & Chaufer, 1999; Muller, Chaufer, Merin, & Daufin 2003; Espina, Jaffrin, & Ding, 2009; Marella, Muthukumarappan, & Metzger, 2011; Chen et al., 2023).

In some cases, some properties of proteins can be manipulated to improve the performance of the separation process. Proteins are either precipitated or dissolved with changes in temperature and pH values. These processes enable a more effective separation in the filtration process (Chen et al. 2023). For example, heating α-lactalbumin at pH 3.4-3.9 up to 50-55°C allows it to precipitate with bovine serum albumin and Immunoglobulins by losing its bound calcium. In this case, β-lactoglobulin is obtained by using microfiltration and ultrafiltration membranes and applying a diafiltration process (Pearce, 1983; Maubois & Pierre, Fauquant, Piot, 1987; Wu, 2003). After the precipitated α-lactalbumin is dissolved by re-adjusting the pH value, bovine serum albumin, and immunoglobulins -g can be removed using a 10 or 20 kDa ultrafiltration membrane (Chen et al. 2023).

Studies were carried out on the modification of polymeric membranes used in the ultrafiltration system. Functional groups are added to the surface of these membranes in order to increase electrostatic repulsion between the charged surface and proteins (Van Reis, Brake, Charkoudian, Burns, & Zydney, 1999; Cowan & Ritchie 2007). This method enables a more effective separation of α-lactalbumin, which has neutral properties at pH 4.3 and 8 mS/cm conductivity, from positively charged β-lactoglobulin molecules (Arunkumar & Etzel, 2013). The inclusion of a sulfonic acid group on the composite regenerated cellulose membrane (100 kDa) surface increased the rejection of negatively charged proteins. So, this system could be used to concentrate whey proteins (Arunkumar & Etzel, 2015).

3.2.3. Electrodialysis

Electrodialysis is designed to separate ionic species such as salts and organic acids using charged polymer membranes. Cations migrate to the cathode and anions to the anode, leaving the feed stream under an electrical potential driving force. Electrodialysis is used in the dairy industry to remove salt from skimmed milk and for the demineralization of whey (Chen et al. 2023). Electrodialysis cannot separate proteins and lactose because it is impermeable to molecules and ions larger than 500 Da (Dlask, & Vaclavikova, Dolezel, 2016). However, when this system is replaced with filtration membranes, large charged molecules such as whey proteins can be selectively transported by the electrical driving force. For this purpose, an electrodialysis system is used with an ultrafiltration membrane. These systems are more selective in separating whey than traditional filtration systems and the formation of an impermeable pollution layer is less seen. However, the purity of the product stream containing the target proteins is low due to the inability to separate small inorganic ions and organic acids (Lawrence, Kentish, O'Connor, Barber, & Stevens, 2008; Chen et al. 2023).

3.3 Other Methods

In addition to chromatographic and membrane filtration methods, studies on different techniques are also performed for the separation of whey proteins. Polyethylene glycol (PEG 1500) and potassium phosphate were used at pH 7 in a study to isolate α-lactalbumin from whey by using an aqueous two-phase systems technique. In this study, yield rates were reported to reach 99% for α-lactalbumin and 97% for α-lactalbumin, and purity rates up to 95% for α-lactalbumin and 91% for α-lactalbumin (Chen et al. 2023). It is also reported in a study on aqueous two-phase systems technique that 95% protein (bovine serum albumin and β-lactoglobulin

and α-lactalbumin) precipitates from the whey and 80% of lactose can be recovered in the lower phase in a system that contains ammonium sulfate and polyethylene glycol at pH 4 (Gonzalez-Amado, Tavares, Freire, Soto, & Rodriguez, 2021).

Magnetic fishing is a technique used for whey protein purification thanks to the development of magnetic nanotechnology. Magnetic nanoparticles that are bound to target proteins can be rapidly separated by magnetic decantation (Chen et al. 2023). Various studies were carried out on the separation of whey proteins using the magnetic fishing technique and magnetic particles, which are prepared by adding polyglutaraldehyde-coated iron oxide crystals to ion exchange groups, were used to absorb lactoferrin from bovine whey (Heeboll-Nielsen, Justesen, & Thomas, 2004; Meyer, Berensmeier, & Franzreb, 2007). Monodisperse magnetic particles coupled with heparin were used as a magnetic affinity adsorbent to isolate lactoferrin from casein whey (Chen, & Guo, Guan, Liu, 2007). Magnetite nanoparticles coated with citric acid and Cu2+ ions were used in a study for the isolation of β-lactoglobulin from whey (Nicolas, Ferreira, & Lassalle, 2019).

CONCLUSION

Due to the biological, nutritional, and functional properties of whey proteins, the demand to use their isolate forms in the food and pharmaceutical industry has increased. This situation has also required a more pure and efficient fractionation of proteins. Membrane filtration and chromatographic methods are generally used for separation of whey proteins. These studies show that using these methods in combination with each other or combining them with various technologies gives better results. However, these methods must certainly be developed by taking into account the problems that may arise when implementing them in the industry.

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Chapter 12

VEGAN MILKS AND ITS PRODUCTION

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1. INTRODUCTION

Milk is a unique food in the nutrition of infants and humans. World milk production in 2023 is forecast to reach 944 million tonnes, an increase of 0.9 percent from 2022, according to the Food and Agriculture Organization of the United Nations (FAO). High milk consumption is directly associated with the nutritional benefits of its ingestion, such as the increased availability of proteins, minerals, fats, and sugars. Despite this unique nutritional value of milk, people still searched for food/beverage that could be an alternative to animal milk.

Nowadays, people have turned to alternative diets due to their interest in functional foods, difficulty accessing animal milk sources (material or inadequacy), and dietary preferences (vegan/vegetarian diet, religious reasons, lactose intolerance, allergy).

Plant-based beverages are also among these alternative diets. While animal foods are a high protein source with their balanced amino acid distribution, herbal drinks are accepted as functional foods because they are rich in bioactive components necessary for humans such as minerals, vitamins, dietary fibers and antioxidants, phenolic acids, flavonoids, and betacyanins stimulate the growth of probiotic bacteria (Das, Raychaudhuri, & Chakraborty, 2012; Colombo Pimentel, Karoline Almeida da Costa, Eduardo Barão, Rosset, & Magnani, 2021). These plant-based beverages have been referred to as "plant milk", "milk substitute", "milk alternative", "imitation milk", "milk analog", "vegan milk" and "milk-like beverage" because the beverages derived from water extracts of grains, nuts, legumes and plant seeds and fruit pits resemble cow's milk. According to the grand view research, global dairy alternatives are expected to grow at a compound annual growth rate (CAGR) of 12.6% from 2023 to 2030 (Anonymous, 2023).

Plant-based milk is a colloidal system formed by a continuous phase of water. The dispersed phase of particles comprises protein fractions, starch granules, solid parts of plant matrices, and lipid droplets. (Briviba & Gräf, Walz, Guamis, Butz, 2016). Quality and preservation of plant-based milk are as important as the production process. This section will give general production methods and preservation practices of plant-based milk.

2. VEGAN MILKS and ITS PRODUCTION

Plant-based milk is the soluble extract of plant materials such as cereals, pseudo-cereals, seeds, vegetables, and nuts. Plant-based milk substitute presents a high content of fibers, isoflavonoids, antioxidants, and monounsaturated and polyunsaturated fats, besides being lactose, cholesterol,

and animal protein-free (Chalupa-Krebzdak, Long, & Bohrer, 2018). Despite all these advantages, Plant-based milk can only resemble animal milk in terms of organoleptic and rheology because it differs from animal milk in terms of protein and micronutrient composition (Vagadia, Vanga, Singh, Gariepy, & Raghavan, 2018). Plant-based milk has lower protein contents and lower variability of amino acids than animal milk. In addition to the low digestibility of plant-derived proteins, it also contains many anti-nutrient substances, such as trypsin inhibitors, phytic acid, and inositol phosphates that prevent the digestion of nutrients (Bocker & Silva, 2022).

The nutritional value of plant-based milk can be enhanced by adding minerals and vitamins, and amino acid variety can be increased by combining plant-based milk from different sources. The unit operations performed in the different plant raw materials processing to obtain plant-based milk are similar (Bocker & Silva, 2022).

Table 1. Plant raw materials used for manufacturing plant-based milk substitutes (Wijaya & Romulo, 2021; Ilyasoglu & Yilmaz, 2019)

Cereals	Pseudo-cereals	Oil seeds	Legumes	Vegetables	Nuts
Rice	Quinoa	Sunflower seed	Chickpea	Coconut	Almond
Oat	Buckwheat	Hemp	Soybean	Potato	Cashew
Maize	Amaranth	Sesame	Kidney Bean		Peanut
Barley	Chia	Flax	Lupin		Hazelnut
Sorghum	Teff		Pea		Walnut
Wheat			Cowpea		Pistachio
Rye			Peanut		
Millet					

According to the physiological structure of the plant source, the pre-treatments applied to the plant for plant-based milk production differ (Sethi, Tyagi, & Anurag, 2016);

a) Nuts and seeds are peeled,

b) Cereals, pseudo-cereals, and vegetables are commonly immersed in hot water and dried,

c) roasting or adding acids and bases (peeling) to enhance the emulsion stability, facilitating the removal of toxic compounds and increasing the process yield.

After the pre-processing, the grinding process is applied. The grinding process increases the extraction efficiency by increasing the contact surface. In addition, grinding the raw plant material by adding water facilitates extraction and provides less energy consumption. Fig 1 shows the production of plant-based milk substitutes.

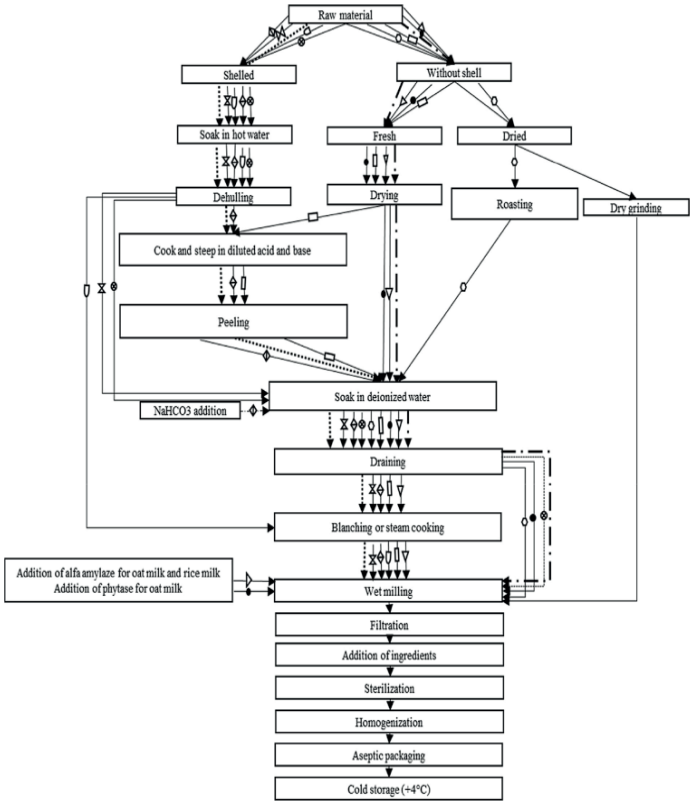

Figure 1. Proses flow chart for production of plant-based milk substitute (Aydar, Tutuncu, & Ozcelik, 2020)

Table 2. According to Fig 1, Each arrow mark indicates the type of raw material (Aydar, Tutuncu, & Ozcelik, 2020)

Raw Material	Flow Chart
Almond	
Cashew	
Coconut	
Hazelnut	
Peanut	
Sesame	
Soy	
Tiger nut	
Oat	

Coconut, peanut, soy, walnut, cashew, almond, and hazelnut have hard shells. After removing the hard shell, soak the raw material in hot water to dehull the fruit's inner skin. After dehulling, The fresh raw material requires a drying process if the raw material is groatless and fresh or is dehulled before processing. The following process is the roasting or dry grinding process (Aydar &Tutuncu, Ozcelik, 2020).

1.1. Roasting

The roasting process is used for hazelnut, peanut, almond, sesame, and cereal milk substitute production. Roasting increases the final product's emulsion stability index and protein isolate solubility (Zaaboul & Raza, Cao, Yuanfa, 2019). Callewaert & Festjens, Neirynck (2012), reduced the benzaldehyde and pyrazine concentration in almond milk to less than 0.5 ppm by applying the roasting process and obtained a neutral taste in their patent study published in 2012. According to the study of Ahmadian-Kouchaksaraei & Varidi, Varidi, Pourazarang (2014) on the production of sesame milk, the roasting process decreases acidity, total solids content, protein, fat, and improved flavor and aroma by preventing bitterness and a chalky taste. Chavan & Gat, Harmalkar, Waghmare (2018) applied a 5-sec roasting process at 130°C after drying for germinated and ungerminated barley, finger millet, and moth bean seeds to make a non-dairy fermented probiotic beverage. Germinated probiotic drinks had higher values of TEAC and TPC.

1.2. Dry Grinding

Dry grinding may not be used in plant-based milk production. Dry grinding can be applied as an alternative to wet grinding. For example, in the almond milk patent obtained in 1997, the production starts with dry grinding. and the final product, its aspect, and color are close to those of the semi-skimmed cow's milk (Berger, Bravay, & Berger, 1997).

1.3. Peeling

The peeling process uses acid or base to peel the inner shells of fruits such as hazelnut, sesame, walnut, tiger nuts, and Brazilian nuts. Peeling can also be done using only water instead of acid and base. However, this method increases the processing time. The length of the peeling process also depends on the raw material. For example, peeling the inner shell of

walnuts and almonds by soaking them in water overnight, for a period of 18–20 h respectively; however, the walnuts can be peeled in two to three minutes by applying 2% citric acid at 90 °C (Cui, Chen, Wang, & Han, 2013; Maghsoudlou, Alami, Mashkour, & Shahraki, 2015). With the peeling process, the bitter taste problem caused by the toxic substances in the shell is also eliminated (Quasem, Mazahreh, & Abu-Alruz, 2009; Junliang & Jinliang, 2008).

1.4. Soaking in Water

Soaking in water is applied to soybeans, hazelnuts, rice, almonds, tiger nuts, grains, sesame seeds, and peanuts (Bernat, Chafer, Chiralt, & Gonzalez-Martınez, 2014; Alozie Yetunde & Udofia, 2015; Bernat, Chafer, Chiralt, Laparra, & Gonzalez-Martinez, 2015; Aboulfazli, Shori, & Baba, 2016; Kizzie-Hayford, Jaros, Zahn, & Rohm, 2016; Sethi et al., 2016; Kohli, Kumar, Upadhyay, & Mishra, 2017; Padma, Jagannadarao, Edukondalu, Ravibabu, & Aparna, 2018; Chavan et al., 2018).

The swelling and softening are achieved by soaking cereals and nuts in water. Thus decreasing the time needed for blanching and increasing the extraction yield of nut milk (Aydar et al., 2020).

According to Sethi et al. (2016), Alkaline can be applied to the soaking water of sesame seeds, soybeans, and peanuts. It has been observed that adding $NaHCO_3$ increases the stability and reduces the off-flavor of the sesame milk (Ahmadian-Kouchaksaraei et al., 2014).

1.5. Blanching

Blanching is applied for the reduction of microbial load and enzyme inactivation. The blanching conditions applied in the studies in the literature are as follows: Soybean 1.25% $NaHCO_3$ for 30 min (Kohli et al., 2017); 0.5% NaHCO3 for 30 min (Kundu, Dhankhar, & Sharma, 2018); Almond water at 90°C for 3 min (Maghsoudlou et al., 2015); Steam bath at 85°C for 5–30 min (Makinde & Adebile, 2018); Coconut Water at 80°C for 10 min (Seow & Gwee, 1997); Sesame Water at 100°C, 30 min. (Karshenas, Goli, & Zamindar, 2018); Steaming at 95 °C, 15–30 min (Ahmadian-Kouchaksaraei et al., 2014); Rice at 80°C, cooked for 15 mins (Padma et al., 2018); Water at 103°C, 3 h (Ismail, Abou-Dobara, & Nawal, 2018); Quinoa 1:7 (quinoa: water), 112°C for 30 min (Pineli, Botelho, Zandonadi, Solorzano, de Oliveira, Reis, & Teixeira, 2015).

1.6. Wet Milling

Wet milling is a process in which grinding is applied by adding a certain amount of water to the raw material. Wet milling conditions

applied in the studies in the literature are as follows: Sesame is mixed with water 5 times their weight and ground for 20 min (Ahmadian-Kouchaksaraei et al., 2014); Peanut 1:9 water, 18,000 rpm, 2 min, 4 cycles (Zaaboul et al., 2019); Tiger nut 1:1 water, 800 W (Costa Neto, Gomes, Justo, Pereira, Amaral, Rocha Leão, & Fontes Sant'Ana, 2019); 200 g water for 50 g, 13,000 rpm, 20 min (Kizzie-Hayford, Jaros, Schneider, & Rohm, 2015); Hazelnut 10,000 rpm, 10 min (Gul, Atalar, & Saricaoglu, Yazici, 2018); 10 g of soybeans are ground for 10 min using 250 mL boiling water. (Aboulfazli et al., 2016); Almond 1:9 water at 18,000 rpm for 2 min (Dhakal, Giusti, & Balasubramaniam, 2016); Walnuts are mixed with water 4.5 times their weight at 50°C and ground for 5 min. (Cui et al., 2013); Cashew 1:3 water (Manzoor & Manzoor, Siddique, Ahmad, 2017); Groundnut 2000 rpm for 10 min (Adeiye & Gbadamosi, Taiwo, 2013); Pine nut and rice mixture 1:8 water, 1 min at 15,000 rpm, 2 min at 20,000 rpm (Lee & Rhee, 2003).

1.7. Filtration

Filtration is a process using different filter materials such as double-layered cheesecloth (muslin cloth), and filtering paper with different sizes (150 mesh sieve, filter paper, 180 μm sieve, four μm-pore-size filter, and 100 μm filter) to separate the cake and milk (Aydar et al., 2020). Also, ultrafiltration, centrifugation, and decantation could be used.

1.8. Ingredients

The main ingredient used in plant-based milk is water. The hardness and pH of the water used in production affect the quality of the final product. The substances that determine the hardness and pH of the water can interact with the emulsifiers and stabilizers to be added to the milk and reduce their effectiveness (McClements et al., 2019).

Food additives such as vitamins, minerals, stabilizers, emulsifiers, flavoring agents, coloring agents, salts, and preservatives are usually added during the formulation process. Adding food additives improves plant-based milk's nutritional quality, bioavailability, and shelf life. Some insoluble particles such as protein, starch, fiber, and minerals decrease the stability of plant-based milk (Reyes-Jurado et al., 2021; Mäkinen et al. 2016).

1.9. Homogenization

After adding stabilizers and emulsifiers, the homogenization process ensures that the particles remaining in the milk are smaller and dispersed, preventing aggregate formation and lipid droplets formation (Mäkinen et al. 2016).

1.10. Sterilization

Heat treatment applications are made in order to reduce the microbial load and inactivate enzymes in the product. This improves the shelf life of the product. For this purpose, pasteurization ultra-high temperature (UHT) and ultra-high-pressure homogenization (UHPH) treatment is applied. In addition, high heat treatment application changes the nutritional quality by affecting the water-soluble proteins and vitamins. Optionally, fortification of the product with food additives could be applied aseptically after heat treatment (Romulo, 2022). After the plant-based milk product is packaged and stored, it is ready for distribution. The storage temperature must be +4 °C. Many types of packaging could be applied, such as carton systems (the most common, e.g., Tetra Pak), plastics, and glass. Plant-based milk could also be processed into reconstituted powder form using spray or drum drying (Abdullah, Taip, Kamal, & Rahman, 2018).

CONCLUSION

Plant-based nutrition has become a lifestyle. In order to meet consumption demands, plant-based milk sterilization and emulsion stability should be provided, and shelf life should be increased. For consumers who eat only plant-origin and prefer plant-based milk, it must be fortified with protein, vitamin, and mineral supplements. In Plant-based milk production, optimum production processes should be preferred for the best end product in terms of sensory and composition. The amount of micronutrients that need to be fortified externally can be reduced by combining different plant sources to produce milk with the best nutritional value.

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Chapter 13

MINIMALLY PROCESSED FOODS: OVERVIEW

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1. INTRODUCTION

Over the past decades, with the advancement of technology, the seeds of this idea were planted with the desire of consumers to consume all products as fresh as the first day. The reason is to keep those fresh foods' natural nutritive values and sensory attributes, such as flavor, odor, and taste texture, in other respect wanting no additive use in foods.

From this point of view, Convenient foods, fresh fruits, and vegetables are good examples. This growing consumer demand for less processed foods became more critical with no or lesser additives challenges to food technologists (Siddiqui et al., 2011).

In addition, the demand for functional foods to prevent or control diseases is growing. Organizations like WHO, USDA, FAO, and EFSA recommend increasing fruit and vegetable consumption to decrease cardiovascular diseases and cancer risk (Allende et al., 2006). With this requirement, the Minimally processed food term came to approve oneself, and there is no curtain owner/year for this idea. Minimal processing techniques and all these demands force us to enhance safer foods (Gilbert, 2000).

In minimally processed foods, the main aim is reducing the negative influence of processing, like heat treatment, by unitarily of various hindrances to guarantee an acceptable safety level with a good shelf life. It is impossible to have perfect knowledge of all factors of a food product, micro-organism, and production and storage conditions directly or indirectly influencing safety and quality. Since the safety of food products is a presupposition, there is a need to have more knowledge on better locating the risks, allowing less over-processing for making safe foods (Zwietering, 2002).

2. UNDERSTANDING MINIMALLY PROCESSED FOOD

Minimally processed food refers to whole, natural, or minimally altered ingredients that retain nutritional integrity. Unlike heavily processed foods, which often contain additives, preservatives, and artificial ingredients, minimally processed foods are closer to their original state. They undergo limited processing methods, such as cleaning, cutting, or drying, without compromising their inherent goodness According to a study published in the Journal of the Academy of Nutrition and Dietetics, minimally processed foods provide higher nutrient density than their heavily processed counterparts (Reedy et al., 2010).

2.1. Purposes of Minimal Processing

The purposes of minimal processing rank are;

Making food chemically and microbiologically safe,

Preserving the taste, color, and safety of food to facilitate their owners

Extending shelf life and increasing consumption

General-purpose meals prepare easily and quickly.

• Before the boiling step of traditional spreads (for example, sorting, washing, peeling, slicing)

• Add added value such as chopping, husking, pitting, low-level irradiation, and packaging by product

• Maintain quality characteristics similar to those of their fresh state (e.g., fruits and vegetables) or identical in consumption (i.e., piece-bread, cooking-cooling, cooking-freezing, sous-vide, ready meals)

• Achieving sufficient shelf life to transport food from the production area to the consumer, including procedures that cause slight alterations in the quality of nutrition (keeping their fresh appearance) (Alzamora, et al., 2016).

2.2. Pros and Cons of Minimally Processed Foods

2.2.1. The benefits of minimally processed food:

1. Enhanced Nutritional Value: Minimally processed foods retain more nutrients than heavily processed alternatives. Fruits, vegetables, whole grains, and legumes naturally provide essential vitamins, minerals, and fiber that contribute to optimal health. A review published in Nutrients Journal highlights the higher content of antioxidants and phytochemicals in minimally processed plant-based foods (Weaver et al., 2014).

2. Reduced Chemical Exposure: By choosing minimally processed foods, we can minimize our exposure to artificial additives, preservatives, and synthetic chemicals commonly found in heavily processed foods. This promotes a more natural and wholesome diet. A study published in Food and Chemical Toxicology emphasizes the potential health risks of consuming certain food additives (Suez et al., 2014).

3. Improved Digestive Health: The fiber content in minimally processed foods aids digestion and supports a healthy gut microbiome. These foods are often easier to digest and can help prevent digestive issues such as constipation, bloating, and discomfort. According to research

published in Nutrients, dietary fiber from minimally processed plant-based foods is crucial in promoting gut health (Slavin, 2013).

4. Sustained Energy Levels: Minimally processed foods, particularly whole grains, provide a steady release of energy due to their complex carbohydrate content. They can help stabilize blood sugar levels and prevent energy crashes, providing sustained daily vitality. A study published in the Journal of the American College of Nutrition suggests that whole grains provide long-lasting energy and contribute to overall metabolic health (Reynolds et al.,2019).

2.2.2. The drawbacks of minimally processed food:

1. Limited Convenience and Shelf Life: Minimally processed foods typically have a shorter shelf life than heavily processed alternatives. Without the use of preservatives or additives, they are more susceptible to spoilage. This may require more frequent grocery shopping and meal preparation, which can be less convenient for individuals with busy lifestyles. A study published in the Food Science & Nutrition journal found that consumers often cited convenience influencing their food choices (Martin-Belloso, 2007).

2. Food Safety Concerns: Minimally processed foods may carry a higher risk of foodborne illnesses if not handled or prepared correctly. Since they undergo minimal processing, they retain more natural bacteria, which can pose a risk if not cooked or stored correctly. It is essential to follow safe food handling practices and adhere to recommended cooking temperatures to reduce the risk of contamination. The Centers for Disease Control and Prevention provide guidelines for safe food handling and storage (U.S. Centers for Disease Control and Prevention, 2021).

3. Limited Availability and Accessibility: Access to a wide variety of minimally processed foods may be limited in some areas. Specific communities, particularly in food deserts or low-income areas, may have limited access to fresh fruits, vegetables, and whole grains. This can make it challenging for individuals in such areas to incorporate minimally processed foods into their diets. A study published in the journal Public Health Nutrition highlights the disparities in access to healthy foods and the impact on dietary patterns (Moore& Diez Roux 2006).

4. Increased Preparation Time: Preparing meals with minimally processed ingredients often requires more time and effort than relying on pre-packaged or heavily processed foods. This can be a barrier for individuals with busy schedules or limited culinary skills. A study published in the journal Appetite found that lack of time and cooking

skills were perceived as barriers to consuming minimally processed foods (Lavelle et al., 2019).

5. Cost Considerations: Minimally processed foods, mainly organic or locally sourced, may be more expensive than heavily processed or convenience foods. The higher cost can be a barrier for individuals on a tight budget, limiting their ability to prioritize minimally processed options. A systematic review published in the International Journal of the Academy of Nutrition and Dietetics emphasizes that cost constraints influence dietary choices (Leung et al., 2013).

2.3. Minimal Processing Methods

2.3.1. Minimal processing of foods with thermal methods

Thermal techniques are widely employed in the preparation and preservation of food. Using heat leads to desirable (formation of aroma components protein coagulation, starch swelling, and textural softening) and undesirable changes (loss of fresh appearance, flavor, texture vitamins and minerals, thermal degradation of biopolymers) (Ohlsson& Bengtsson 2002).

1. Pasteurization: Pasteurization is a standard thermal method used to reduce the microbial load in food while maintaining its nutritional integrity. It involves heating food to a specific temperature for a certain period to destroy harmful bacteria, yeasts, and molds—this process is employed widely for milk, juices, and other beverages.

An investigation conducted and published in the Journal of Food Science and Technology examined the impacts of various pasteurization techniques on the quality attributes of apple juice. It concluded that pasteurization helped reduce microbial contamination without significant losses in nutritional quality (Canumir, et al., 2002).

2. Aseptic Processing: Aseptic processing involves sterilizing the food product and the packaging separately and then combining them under sterile conditions. The food product is heated to a high temperature using heat exchangers, direct steam injection, or microwave heating, followed by rapid cooling. The sterile food is then aseptically filled into pre-sterilized containers, ensuring the absence of microorganisms. Aseptic processing offers several advantages, including extended shelf life, preservation of sensory attributes, and reduced reliance on preservatives. A study published in the Journal of Food Engineering demonstrated the effectiveness of aseptic processing in maintaining the quality of liquid and particulate food products during storage (Aurina&Sari, 2022).

3. Blanching: Blanching is a short thermal treatment that involves immersing food in boiling water or steam for a brief period, followed by rapid cooling. It commonly uses to inactivate enzymes, improve texture, and enhance color retention in fruits, vegetables, and nuts.

A research article published in the Journal of Food Science examined the effects of blanching on the quality of frozen garlic. (Zhang et al., 2021).

4. Steam Cooking: Steam cooking involves using hot steam to cook food. It is a gentle and effective thermal method that helps retain food's natural flavors, colors, and nutrients. Steaming is commonly used for vegetables, seafood, and grains.

A study published in the Journal of Agricultural and Food Chemistry investigated the impact of different cooking methods, including steam cooking, on the antioxidant capacity of broccoli. It revealed that steaming preserved the highest levels of antioxidants compared to boiling or microwaving (Vallejo et al., 2003)

5. Sous Vide Cooking: Sous vide cooking involves vacuum-sealing food in a bag and cooking it at a precisely controlled low temperature in a water bath. This method helps retain the ingredients' natural flavors, moisture, and nutritional content.

A scientific review published in the Journal of Food Science examined the effects of sous vide cooking on the nutritional quality of various foods. It concluded that this method resulted in minimal losses of nutrients, particularly water-soluble vitamins, compared to traditional cooking methods (Kathuria et al., 2022).

6. Infrared Heating Technology: Infrared (IR) heating involves using electromagnetic radiation with wavelengths between visible light and microwaves to transfer heat directly to the surface of the food. This method has gained attention recently due to its ability to provide rapid and uniform heating while minimizing undesirable effects on food quality. A study published in the Journal of Food Engineering investigated the effect of infrared heating on apple slices' drying kinetics and quality attributes. It demonstrated that infrared drying resulted in shorter drying times and superior color retention compared to conventional hot air drying (Kornacki& Johnson 2001).

7. Electric Volume Heating Methods/ Ohmic Heating: Ohmic heating, or Joule heating or electrical resistance heating, involves passing an electric current directly through the food to generate heat. This method relies on the food matrix's electrical resistance to uniformly generate heat. Ohmic heating offers several advantages, such as rapid and uniform heating, reduced processing time, and minimal nutrient

loss. A study published in the Journal of Food Engineering demonstrated the effectiveness of ohmic heating in preserving the quality attributes of various food products, including fruits, vegetables, and dairy products (Bansal, et al., 2014, Nanjegowda, et al., 2022).

8. Radio Frequency Heating: Radiofrequency (RF) heating involves the application of electromagnetic energy at radio frequencies to heat food products. The RF energy causes polar molecules within the food to rotate, generating heat throughout the product. RF heating offers faster heating rates, enhanced microbial safety, and improved sensory quality. Research published in the Journal of Food Science demonstrated the potential of RF heating for the rapid and uniform heating of liquid and semi-solid foods while maintaining their quality attributes (Awuah et al., 2014).

9. Microwaves: Microwave heating utilizes electromagnetic waves at microwave frequencies to heat food products. Microwaves generate heat by causing water molecules to vibrate, producing friction and heating the food. This method offers rapid and efficient heating, reduced processing time, and improved energy efficiency. A study published in the Journal of Food Engineering evaluated the application of microwave heating for minimal processing of fruits, vegetables, and other food products, highlighting its potential to preserve the quality attributes of these foods (Maskan, 2000).

10. Induction Heating: Induction heating involves using high-frequency electromagnetic fields to generate heat within conductive materials. This method is particularly suitable for heating metallic or conductive food products. Induction heating offers advantages such as precise and controlled heating, energy efficiency, and reduced processing time. Research published in the Journal of Food Science and Technology demonstrated the effectiveness of induction heating for minimal processing of metallic food products, including seafood and meat, while maintaining their sensory and nutritional properties (Rudnev et al., 2017, Aaliya, et al., 2021).

Progress in pasteurization and sterilization will involve a day-by-day increase in the importance of process control and product safety. Integrated production information systems with online sensors will allow surveillance of the process. Nowadays, these systems are conflictual trends for providing increased safety than using preservation methods like acid, sugar, and salt content lowering in food products. This usage needs more research and more development about the engineering aspects of food semi-aseptic (pasteurization) and aseptic (sterilization) processes and combining thermal and non-thermal methods for optimal results (Ohlsson & Bengtsson 2002).

2.3.2. Minimal processing of foods with nonthermal methods

Non-thermal methods have emerged as alternatives to traditional thermal processing techniques for foods, offering minimal processing solutions that preserve the sensory and nutritional attributes of the products. These methods utilize physical or chemical processes to achieve microbial safety and extend shelf life without subjecting the food to high temperatures. This chapter explores some of the vital non-thermal methods used in minimally processed foods, discussing their principles and advantages supported by relevant references.

1. High-Pressure Processing (HPP): High-pressure processing involves subjecting food products to elevated pressures, typically between 100 and 1000 MPa, for a specific period. This non-thermal method inactivates microorganisms, including pathogens, by disrupting their cellular structure. HPP offers advantages such as minimal nutrient loss, improved food safety, extended shelf life, and preservation of sensory attributes. A study published in the Journal of Food Science evaluated the effectiveness of HPP in preserving the quality and safety of various food products, including meat, seafood, fruits, and vegetables (Tao, et al., 2014).

Pulsed Electric Field (PEF) Processing: Pulsed electric field processing involves applying short pulses of high-intensity electric fields to food products. The electric field disrupts cell membranes, leading to microbial inactivation and enzyme inactivation. PEF processing offers advantages such as minimal nutrient loss, improved food safety, and extended shelf life. Research published in the Journal of Food Engineering demonstrated the efficacy of PEF processing in preserving the quality and safety of various food products, such as fruit juices, dairy products, and liquid foods (Syed, et al., 2017).

2. Ultraviolet (UV) Light Processing: Ultraviolet light processing utilizes UV radiation to inactivate microorganisms in food products. UV light, particularly in the UV-C range (200-280 nm), damages microbial DNA, rendering them unable to reproduce. UV light processing offers advantages such as rapid microbial inactivation, minimal impact on sensory attributes, and reduced reliance on chemical sanitizers. A study published in the Journal of Food Protection investigated the effectiveness of UV light processing in reducing microbial contamination in various food products, highlighting its potential as a non-thermal microbial control method (Baysal, 2018).

3. Pulsed Light Processing: Pulsed light processing involves the application of intense, short-duration pulses of broad-spectrum light to food surfaces—the pulses of light cause microbial inactivation

by damaging their DNA and proteins. Pulsed light processing offers advantages such as rapid microbial inactivation, minimal nutrient loss, and reduced reliance on chemical sanitizers. Research published in the Journal of Food Protection demonstrated the effectiveness of pulsed light processing in reducing microbial contamination on different food surfaces, including fruits, vegetables, and meat products (Oms-Oliu, et al., 2010).

4. Irradiation: Irradiation involves exposing food products to ionizing radiation such as gamma rays, X-rays, or electron beams. These radiation sources penetrate the food, affecting microorganisms by damaging their DNA and cellular structures. Irradiation can also inhibit the growth of spoilage microorganisms and delay ripening, offering benefits in terms of food safety, shelf-life extension, and quality preservation. The dose of radiation applied depends on the specific food product and the desired outcome. A study published in the Journal of Food Science discussed the principles and mechanisms of action of irradiation in controlling foodborne pathogens and spoilage microorganisms (Badr, 2004).

5. Ultrasound: Ultrasound involves high-frequency sound waves (>20 kHz) to create mechanical vibrations and acoustic cavitation in food products. These physical effects can disrupt cell membranes, enhance mass transfer, and promote microbial inactivation. Ultrasound can be applied through different modes, including direct contact, immersion, or sonotrode. The intensity and frequency of ultrasound can be adjusted to achieve desired processing outcomes. A study published in the journal Ultrasonics Sonochemistry discussed the principles and mechanisms of ultrasound in food processing (Mason& Lorimer, 2002).

6. Cold Plasma Technology: Plasma, the fourth state of matter following solid, liquid, and gas, can be harnessed through two types of treatments: thermal and cold plasma processes, the latter being nonthermal. Thermal plasma generates vast amounts of energy through high temperatures, while cold plasma technology operates at temperatures ranging from 25 to 65 degrees Celsius, without relying on heat. When gas is ionized, it forms free radicals such as ions and electrons. Plasma is typically created by subjecting gases like air oxygen, argon, helium, and nitrogen to various forms of energy, including electrical, thermal, or magnetic fields. This process results in the formation of plasma, which contains positive and negative ions, as well as reactive species like ozone and singlet oxygen (O) (Jadhav et al., 2021).

7. Supercritical Technology: Supercritical technology revolves around the use of supercritical fluids as a viable alternative to organic solvents. These fluids exhibit properties that partially resemble gases and liquids. They possess gas-like diffusivity and viscosity while maintaining liquid-like density. A wide range of fluids can be employed as supercritical fluids, but carbon dioxide stands out as an exceptional choice for food processing. This is because it can attain a supercritical state at relatively mild temperatures and pressures (31.1°C and 7.4 MPa, respectively). Supercritical fluids have found extensive application in the food industry, including extraction, microbial inactivation, and enhancing mass transfer during synthesis processes. Recent research conducted by Lefebvre et al. (2021) demonstrated that supercritical carbon dioxide is an effective solvent for extracting antioxidants from rosemary. The temperature and pressure of CO_2 used were 25°C and 20 MPa, respectively, and had no detrimental impact on the purity of the extracted compounds. In another study, Bertolini et al. (2020) examined the efficacy of supercritical carbon dioxide compared to traditional pasteurization and high-pressure processing in reducing microbial contamination in pomegranate juice. The authors found that pomegranate juice treated with supercritical carbon dioxide exhibited bacterial growth below the detectable limit even after 28 days of storage (Jadhav et al., 2021).

8. Ozone: Ozone, denoted chemically as O_3, consists of three oxygen molecules. It forms when molecular oxygen (O_2) combines with singlet oxygen (O). Due to its high reactivity and instability, ozone cannot be stored and must be generated as required. Ozone serves as a potent antibacterial disinfectant against various foodborne bacteria. It can be utilized in gaseous form or as ozonated water. Ozone exhibits multiple mechanisms by which it induces cell death in microorganisms. Notably, it damages protein structures, leading to the dysfunction of microbial enzymes and impacting their metabolic activity. Gimenez et al. (2021) conducted a study involving the treatment of meat with 280 mg O_3/m3 for five hours, with intermittent ozone pulses of 30 minutes every 10 minutes, targeting L. monocytogenes. The treatment demonstrated effectiveness; however, prolonged exposure caused undesirable effects such as color changes and lipid oxidation (Jadhav et al., 2021). In recent years, ozone treatments have gained significant attention as a postharvest method for fruit and vegetable preservation, as it leaves no residues (Dilmaçünal& Kuleaşan, 2018).

2.4. Hurdle Technology in Food Preservation

Hurdle technology's name sounds new, but it has been used to start with human life. Foodborne diseases are mainly caused by microorganisms such as bacteria, yeasts, molds, and protozoa in food, their growth, spread,

and metabolic activity (Bigi et al., 2022). The name of the hurdle means that it's an application for protecting foods from microbial load. Hurdle technology finds different application areas depending on the type of food like fruits and vegetables, salads, juices, milk and dairy products, meat and meat products, fish and other seafood, bakery products, pasta, canned products, and spices for increasing the shelf life and by the way ensuring microbial safety. Hence, hurdle technology aims are eliminating undesirable microorganisms without losing sensory and nutritional attributes. Because of this reason, hurdle technology is not using one; on the contrary, using multiple technologies together to have the best product.

For Hurdle technology, several mechanisms became crucial, four of which affect the growth of microbes in foods. These are;

- Homeostasis
- Metabolic exhaustion
- Stress relaxation
- Multi-target preservation of food (Pal, et al., 2021)

Homeostasis is the tendency of organisms to protect themselves against extracellular events. For the preservation of foods, homeostasis is a critical matter. The homeostasis mechanism is a hurdle, disrupting the microorganisms, stopping multiplying, and inactivating or dying. Some samples in Table 1 show that microorganisms endure many critical homeostatic reactions.

Table 1. Homeostatic responses by microorganisms to various stress factors

Reduced nutrients	Nutrient scavenging; oligotrophy; 'stationary- phase response'; generation of 'viable nonculturable' forms
Low-pH level	Extrusion of protons across the cell membrane; maintenance of cytoplasmic pH; maintenance of transmembrane pH gradient
Lowered water activity	Osmoregulation; accumulation of 'compatible solutes';avoidance of water loss; maintenance of membrane turgor
Low temperature for growth	'Cold shock' response; changes in membrane lipids to maintain satisfactory fluidity
The high temperature for growth	Heat shock' response; membrane lipid changes
Raised levels of oxygen	Enzymic protection (catalase, peroxidase, superoxide dismutase) from H2O2 and oxygen-derived free radicals
Presence of weak organic acid preservatives	As lowered pH, and sometimes extrusion of the organic acid
Presence of biocides	Phenotypic adaptation; reduction in cell wall–membrane permeability
Ionizing radiation	Repair of single-strand breaks in DNA
High voltage electric discharge	Lower electrical conductivity of the spore protoplast
Competition from other microorganisms	Formation of interacting communities; aggregates of cells showing some degree of symbiosis; biofilms

Alakomi, H., Skytta, E., Helander, I., Ahvenainen, R., 2002. The hurdle concept. Chapter 7. In: Ohlsson, T., Bengtsson, N. (Eds.), Minimal Processing Technologies in the Food Industry. CRC Press LLC, New York, pp. 175–195.

Metabolic Exhaustion: The microorganism in hurdle-treated foods uses its energy to maintain homeostasis, thus becoming metabolically exhausted and is an auto-sterilization of food products.

Stress Reaction: Some microorganisms become more resistant or aggressive under stress by generating stress shock proteins. Several factors, such as water activity, pH, heat, ethanol, oxidative compounds, and starvation, induce the synthesis of protective stress shock proteins. Exposure to multiple stresses can weaken the organism metabolically. Therefore, multi-target preservation of foods can be the key to avoiding the synthesis of stress shock proteins.

Multi-target preservation of food Multi-target preservation refers to the simultaneous application of multiple preservation techniques or hurdles to enhance the effectiveness of food preservation, rather than relying on a single preservation method, combining multiple barriers to create a synergistic effect that inhibits the growth of microorganisms and extends the shelf life of food products.

The concept of multi-target preservation recognizes that microorganisms have different sensitivities and adaptive mechanisms to various barriers. Employing multiple hurdles makes it more challenging for microorganisms to develop resistance or overcome the combined stress. This approach helps ensure food products' safety and quality over an extended period.

2.4.1. Hurdle application

The hurdles in hurdle technology can categorize into three main types: physical hurdles, physicochemical hurdles, and microbial hurdles. These hurdles work together to create a challenging environment for microorganisms, inhibiting their growth and ensuring food safety.

The primary hurdles in hurdle technology, whether applied as a process or additive hurdles, include high temperature (F value), low temperature (t value), water activity (aw), acidity (pH), redox potential (Eh), competitive microorganisms (such as lactic acid bacteria), and preservatives (such as nitrite, sorbate, and sulfite) (Akritidou, et al., 2023). Additionally, more than 50 hurdles with potential applications in animal- or plant-based foods have been identified to enhance product stability and/or quality. Table 2 provides examples of some of these hurdles (Alakomi, et al, 2002).

Table 2. Examples of hurdles used to preserve foods

Type of hurdle	Examples
Physical hurdles	Aseptic packaging
	Electromagnetic energy (microwave, radio frequency, pulsed magnetic fields, high electric fields)
	High temperatures (blanching, pasteurization, sterilization, evaporation, extrusion, baking, frying)
	Ionizing radiation
	Low temperatures (chilling, freezing)
	Modified atmospheres
	Packaging films (including active packaging, and edible coatings)
	Photodynamic inactivation
Physico-chemical hurdles	Carbon dioxide
	Ethanol
	Lactic acid
	Lactoperoxidase
	Low pH
	Low redox potential
	Low water activity
	Maillard reaction products
	Organic acids
	Oxygen
	Ozone
	Phenols
	Phosphates
	Salt
	Smoking
	Sodium nitrite/nitrate
	Sodium or potassium sulfite
	Spices and herbs
	Surface treatment agents
	Carbon dioxide
Microbially derived hurdles	Antibiotics
	Bacteriocins
	Competitive flora
	Protective culture

Hurdles can be applied sequentially or synchronously to achieve the desired preservation effects. In sequential application, hurdles are implemented as a series of processing steps, where each step contributes to the overall preservation process. For example, a food product may undergo steps such as blanching, packaging, and pasteurization in a specific order to achieve the desired microbial control and extend shelf life. In synchronous applications, hurdles are combined and formulated to create a specific environment or condition during processing simultaneously. These applications can involve controlling factors like water activity, acidity, or

temperature to inhibit microbial growth and enhance product stability. For instance, in formulating a product, specific ingredients or additives may be used to achieve a targeted water activity or acidity level that inhibits microbial growth and preserves the quality of the product. Both sequential and synchronous application of hurdles offers flexibility in designing preservation strategies for different food products, considering their specific requirements and desired outcomes.

- heating (F)
- chilling (t)
- water activity (aw)
- acidity (pH)
- redox potential (Eh)
- preservatives (pres.)

Temperature control:

Heating is a commonly used hurdle in hurdle technology to inhibit the growth of microorganisms and extend the shelf life of food products (Li, et al., 2014, Khan, et al., 2017). The heating process uses a heat source applied at high temperatures. The process duration and temperature are optimized to render microorganisms inactive and halt enzymatic activity.

Heating reduces food spoilage and minimizes the risk of foodborne illnesses by killing microorganisms or halting their growth. Thermal treatment can be applied through different methods, such as pasteurization and sterilization.

- **Pasteurization** is a short-duration heating process carried out at low temperatures (typically between 60-85 °C). This process significantly reduces the population of pathogenic microorganisms while allowing some thermotolerant microorganisms to survive. Pasteurization is widely used to preserve sensitive foods like dairy products, fruit juices, and other heat-sensitive foods.

- **Sterilization** is a long-duration heating process conducted at high temperatures (typically between 110-135 °C). Sterilization is more effective in extending the shelf life of foods since it is a process where all microorganisms are killed. This method is commonly employed for preserving durable foods such as canned goods and ready-to-eat meals.

Heating provides food safety by killing or inactivating microorganisms. However, certain nutrients such as vitamins, antioxidants, and flavor

compounds can be heat-sensitive, so the process time and temperature need to be carefully controlled.

Maintaining low temperatures through refrigeration or freezing slows down microbial growth and enzymatic activity

- **Cooling:** Cooling is storing or processing food products at low temperatures. Cooling slows down the growth rate of microorganisms and reduces enzymatic activities. Refrigerators, cold storage facilities, and cooling systems use to control the temperature of food products. The cooling process effectively enhances the stability of many food products, such as fresh fruits and vegetables, dairy products, meat products, and seafood.

- **Freezing:** Freezing involves exposing food products to low temperatures (typically -18°C and below). Freezing stops the activities of microorganisms and prevents their growth. Frozen foods achieve through freezing storage, freezers, and freezing technologies. Freezing provides a means to preserve a wide range of food items, such as fruits, vegetables, meats, seafood, and frozen desserts, allowing for their extended storage and freshness retention.

Cooling and freezing limit the growth rate of microorganisms and halt enzymatic activities, thereby preventing the spoilage of food products. These hurdles enhance the stability of food products while maintaining nutritional values and ensuring food safety through proper temperature control and storage durations.

Moisture control:

Controlling the available water content in food by reducing it through techniques like drying, salting, or the addition of osmotic agents can inhibit microbial growth.

Food products with low water activity restrict the growth and metabolic activities of microorganisms, thereby extending the shelf life of the product. On the other hand, high water activity in food allows for easier microbial growth, leading to quicker spoilage.

Various methods are employed in hurdle technology to reduce water activity and enhance the stability of food products. These methods include:

- **Drying:** The drying process reduces the water content of food, thereby lowering its water activity. As a result, microbial growth and enzymatic activities are limited.

- **Water Binding:** Water binding is used to increase the water activity in food products. For example, methods such as salting or sugaring help bind water molecules.

- **Packaging:** Appropriate packaging materials can be used to control the water activity of food products. Packaging with oxygen barriers or moisture barriers can be employed.

- **Water Activity Reducing Agents:** Adding water activity-reducing agents to food products helps decrease water activity. Desiccants or water binders can be used for this purpose.

Water activity is a crucial parameter for preserving and stabilizing food products. Hurdle technology reduces or controls water activity, limiting the growth of microorganisms and extending the shelf life of food products.

Acidity

Adjusting the acidity level of a food product can create an unfavorable environment for microbial growth. Acidic conditions (low pH) can inhibit the growth of many pathogens and spoilage microorganisms. Various methods can be used to reduce the acidity level in hurdle technology. Here are some commonly used methods for lowering acidity:

- **Adding Acids:** Adding acids is the simplest way to reduce the pH level of food products. Natural acids such as citric acid, lemon juice, acetic acid, or acids obtained through fermentation like lactic acid can be used.

- **Fermentation:** Acidic conditions in fermented products are achieved through producing organic acids by microorganisms, such as lactic acid. This process helps naturally lower the acidity level in some fermented foods.

- **Using Fermentation Brine:** The brine of fermented products increases the acidity level and provides an acidic environment. Fermented brines like pickle juice or kimchi brine can be used to increase the acidity level.

- **Acidity Regulators:** Some food additives can reduce or balance acidity levels. For example, acidity regulators like citrates or ascorbic acid can help adjust the pH level.

- **Bacterial Fermentation:** Certain bacterial strains, primarily used in dairy products, lower the acidity level by producing lactic acid. These strains produce acidic foods like yogurt, cheese, or fermented dairy products.

These methods are among the hurdles used in hurdle technology to reduce acidity levels and improve the stability of food products. However, when adjusting acidity, attention should be given to food safety and quality standards.

Redox potential (Eh)

Redox potential represents the ability of a substance to undergo electron exchange and can influence the metabolic activities of microorganisms.

Proper control of redox potential can limit the growth and activity of microorganisms. Oxidative environments can strain the energy production and nutrient breakdown capabilities of microorganisms, reducing their survival and reproductive abilities.

Redox potential can be controlled through various methods for food preservation:

- **Packaging:** Redox potential can be controlled by using appropriate packaging materials that provide an oxygen barrier, preventing oxidation.
- **Antioxidants:** Antioxidants can regulate redox potential by inhibiting oxidative reactions. This helps reduce oxidative spoilage in food products.
- **Modified Atmosphere Packaging (MAP):** By reducing the oxygen content in the packaging of food products through a controlled atmosphere, redox potential can be regulated.

Redox potential is an important factor in controlling unwanted oxidative reactions and microbial growth in food products. Therefore, in food preservation processes that utilize hurdle technology, redox potential should be considered and appropriate control should be ensured.

Bio preservatives:

Using natural antimicrobial compounds produced by microorganisms, such as bacteriocins or organic acids, helps inhibit the growth of spoilage and pathogenic microorganisms. *Bio preservatives* are a strategy used in hurdle technology to control the growth of microorganisms and extend the shelf life of food products. *Bio preservatives* are compounds that can be naturally or artificially derived and possess antimicrobial properties. These compounds inhibit the activities of microorganisms, reducing food spoilage and the risk of foodborne illnesses.

The use of preservatives can be implemented in various ways to enhance the stability and safety of food products:

Natural Biopreservatives: Natural compounds obtained from plants or microorganisms can use to achieve a preservative effect. For example, phenolic compounds found in certain plants may exhibit antimicrobial properties.

Fermentation Products: During the production of fermented foods, compounds produced by microorganisms can be utilized to achieve a preservative effect. For instance, lactic acid produced by lactic acid bacteria has antimicrobial properties.

Enzymatic Biopreservatives: Certain enzymes can inhibit the growth of microorganisms in food products. The use of these enzymes can provide biological protection.

Antimicrobial Extracts: Extracts derived from plants or microorganisms may contain antimicrobial components and can effectively extend the shelf life of foods.

Bio preservatives are an effective method for controlling microbial growth within hurdle technology. However, their use should consider legal regulations and food safety standards. Additionally, ensuring the effectiveness of preservatives requires proper determination of application methods and concentrations.

2.4.2. Hurdle technologies future

Hurdle technology holds a significant position in the food industry and would expect to gain even more importance (Xue, et al., 2023). It involves using various combinations of barriers to extend the shelf life of food products, control the growth of microorganisms, and ensure food safety (Akritidou, et al., 2023). The future of hurdle technology can shape in the following ways:

- **Optimization of Multiple Hurdles:** In the future, there will be progress in developing more effective and optimized combinations of barriers. The research will continue to enhance our understanding of their effects on microbial growth, leading to the identification of more efficient hurdle combinations.

- **New Barrier Components:** The discovery and utilization of new ingredients and natural antimicrobial agents in the food industry will increase. Newly investigated compounds and extracts with known effects on microorganisms will create more effective and natural hurdles.

- **Application of Advanced Technologies:** Advanced technologies will offer new opportunities for hurdle technology. Advancements

in nanotechnology, molecular biology, and genetic engineering will contribute to developing more effective and precise barriers.

- **Active and Intelligent Packaging:** Active and intelligent packaging will use more effectively to extend the shelf life of food products and control microbial growth. Such packaging will possess features like antimicrobial components, emission control, oxygen and moisture barriers, and actively interact to preserve food quality.

- **Sustainability-Focused Innovations:** In the future, there will be an increased emphasis on the sustainability of hurdle technology. Innovations focusing on energy efficiency, waste reduction, and minimizing environmental impacts will make hurdle technology more environmentally friendly.

With these advancements, hurdle technology is expected to find more applications in the food industry and play a significant role in food safety, shelf-life extension, and sustainability (Khan, et al., 2017, Mohammad, et al., 2023).

2.5. Packaging

Processed foods undergo various treatments to become ready for consumption, while minimally processed foods are subjected to fewer processes and are closer to their natural state. These types of foods preserve their nutritional value while minimizing the use of additives and chemicals in the production processes.

Packaging plays a significant role in the safe and hygienic delivery of minimally processed foods to consumers. Good packaging protects the product's quality, extends its shelf life, keeps it fresh, and prevents physical damage during transportation.

In the packaging of minimally processed foods, preference is given to using natural and environmentally friendly materials. Such packaging materials often have recyclable or biodegradable properties, minimizing environmental impacts and promoting sustainable production and consumption cycles.

Packaging of minimally processed foods also includes transparent packaging that reflects the natural characteristics of the product. Transparent packaging allows customers to see the product's contents and increases their trust in its quality. Labels provide clear information about processing methods, ingredients, and nutritional values.

Modified Atmosphere Packaging (MAP) is the most used packaging method to enhance the quality and shelf life of minimally processed

foods. In this method, the atmosphere inside the package is controlled and adjusted in terms of gas composition to reduce the effects that cause spoilage in the food.

MAP is commonly used for minimal processing and extending the shelf life of delicate and perishable foods such as fresh fruits, vegetables, seafood, and meat. This method slows down the growth of spoilage-causing microorganisms by reducing the oxygen level inside the package and adding inert gases such as carbon dioxide or nitrogen (Almenar, et al., 2023)

MAP can be achieved through vacuum packaging or gas injection methods. Vacuum packaging removes the air inside the package, reducing the oxygen level. Gas injection, on the other hand, adds inert gases (typically carbon dioxide, nitrogen, or mixtures) in specific proportions to create the desired atmospheric conditions.

The advantages of this method include preserving quality criteria such as color, aroma, texture, and nutritional values, slowing down the spoilage process, extending shelf life, and maintaining a fresh appearance and edibility. Additionally, MAP contributes to the physical durability of foods during transportation and storage.

However, the use of MAP alone is not sufficient. While this method slows down microbial growth, it does not eliminate all microorganisms. Therefore, ensuring appropriate hygienic conditions and maintaining proper storage temperatures are essential for product shelf life and food safety. Additionally, MAP may cause a departure from natural atmospheric conditions and lead to a decrease in certain nutritional values.

The growing need for fresh, nutritious, and durable food products necessitates advancements in packaging creation. Consequently, novel packaging designs have emerged, which possess the capability to perceive and transmit data about the enclosed food items. However, to proceed further, it is essential to clarify the distinctions between three commonly interchanged terms: active, intelligent, and smart packaging. Active, intelligent, and smart food packaging are innovative solutions that have revolutionized the way food is packaged, preserved, and communicated with consumers (Versino, et al., 2023).

Active packaging refers to packaging materials that actively interact with the food product to extend its shelf life and maintain its quality. These packages often contain substances or systems that can release antimicrobial agents, antioxidants, or moisture regulators. By actively controlling the food environment, active packaging helps to slow down

spoilage processes and preserve freshness (Amin, et al., 2022, Pandey, et al., 2022).

Intelligent packaging takes the concept further by incorporating sensors, indicators, or monitoring devices that can provide information about the condition of the food inside the package. These sensors can measure parameters such as temperature, humidity, or gas levels, allowing for real-time monitoring of the food's freshness and safety. Intelligent packaging enables producers, retailers, and consumers to make informed decisions regarding the quality and suitability of the product (Almasi, et al., 2022, Versino, et al., 2023).

Smart packaging represents the most advanced form of packaging technology. It combines active and intelligent features with the ability to communicate information to users. Smart packaging often utilizes technologies such as RFID (Radio Frequency Identification), NFC (Near Field Communication), or QR codes to provide consumers with detailed product information, including origin, production methods, nutritional content, allergens, and even recipes. Smart packaging enhances transparency and consumer engagement, facilitating informed choices and improving the overall food experience (Zuo, et al., 2022, Bibi, et al., 2017).

In conclusion, modified atmosphere packaging is an effective method for preserving the quality and extending the shelf life of minimally processed foods, providing consumers with fresher and more nutritious options while reducing food waste. Therefore, the packaging of minimally processed foods ensures their safe, healthy, and environmentally friendly delivery to consumers while preserving their quality and offering natural and nutritious choices. Active, intelligent, and smart food packaging offers various benefits, including extended shelf life, enhanced safety, real-time monitoring, and increased consumer engagement. These advancements in packaging design align with the growing demand for fresh, healthy, and convenient food products in today's market (Versino, et al., 2023).

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Chapter 14

USES OF PROTEASES OBTAINED FROM MICROORGANISMS IN THE FOOD INDUSTRY

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1. INTRODUCTION

Enzymes are ecological biocatalysts that are biodegradable and have little or no harmful effects on the environment. Enzymes are very specialized and catalyze biological processes. Depending on their natural circumstances, such as solvent characteristics, temperature, pH, and they often operate in temperate climates. Enzymes known as proteases function as catalysts in the breakdown of peptide bonds in proteins. It is essential in a variety of industries, including protein hydrolysis, food processing, cleaning supplies, and the extraction of pharmaceutical compounds. Microbial proteases are advantageous over enzymes derived from animals or plants and have also become a priority source for the development of new enzymes. Microbial proteases are preferred because of their rapid proliferation, diversity, and long shelf life. In addition, new proteases can be obtained by genetically modifying microorganisms.

Important physiological functions for proteases include both production and decomposition. Microbial proteases have been employed in food fermentation since the earliest days of humanity. Proteases are effectively used in several sectors nowadays because of advancements in protein engineering and genetic engineering techniques. Proteases are also used in areas other than the food sector, including detergents, textiles, leather, agriculture, waste management, cosmetics, animal husbandry, and medicine. Researchers are investigating different methods to find, redesign, or artificially create enzymes that are more suitable for industrial processes in response to novel uses and rising demand. In addition to having commercial economic value, these enzymes are sustainable and environmentally safer. Various fungal and bacterial proteases have very important commercial places in the industry.

Proteases act as catalysts for the hydrolysis of proteins. They are commonly referred to as peptidases or proteinases (Ramesh & Harani Devi, Chattopadhyay, Kavitha, 2020). These hydrolytic enzymes originated in animals and are widely distributed in nature. (Kumar & Grover, Sharma, Batish, 2010), plants (Duarte & Duarte, Moreira, Cavalcanti, Lima-Filho, Porto, 2009), and microorganisms (Rai & Mukherjee, 2010; De Souza & Bittencourt, Caprara, Freitas, Almeida, Silveira, Fonseca, Filho, Junior, Magalhães, 2015). Proteases are found in all living forms, including protists, eukaryotes such as fungi, and prokaryotic organisms such as bacteria and archaea. Several viruses encode their proteases (Rodamilans & Shan, Pasin, García, 2018). Furthermore, 75% of all commercial proteases in the world come from microorganisms (Razzaq & Shamsi, Ali, Ali, Sajjad, Malik, Ashraf, 2019). Microorganisms are novel sources of proteases and have several industrial uses. This variety results

from the diversity and specialized nature of the metabolic processes of microorganisms (Tavano & Berenguer-Murcia, Secundo, Fernandez-Lafuente, 2018; De Souza et al., 2015).

2. CLASSIFICATION OF PROTEASES

Proteases are categorized as class 3 enzymes, hydrolases, and subclass 3.4, peptide hydrolases or peptidases, by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology. Any enzyme that hydrolyses peptide bonds will be referred to as "peptidase" instead of "peptide hydrolase" by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (Sandhya, Nampoothiri, & Pandey, 2005; Dunn, 2010; Yegin & Dekker, 2013).

These enzymes can be generally categorized into exopeptidase and endopeptidase, depending on their domain of activity. Exopeptidases work with the bonds of peptides at the substrate's ends, whereas peptide bonds that are not at the ends are hydrolyzed by endopeptidases, resulting in the creation of shorter peptides. In addition, exopeptidases are also classified as aminopeptidases at the N-terminus and carboxypeptidases at the C-terminus, depending on the amino acid ends at which a particular exopeptidase act (Naveed, Nadeem, Mehmood, Bilal, Anwar, & Amjad, 2021).

Another method of categorizing proteases is based on their mechanism of action as well as the presence of any number of specific residues of amino acids where the action takes place. This method identifies cysteine, serine, glutamic, threonine, asparagine, mixed, aspartic, and other proteases as the major classes of proteases (Contesini, Melo, & Sato, 2018). According to the ideal pH ranges of proteolytic enzymes, the MEROPS database (https://www.ebi.ac.uk/merops/) is divided into neutral proteases, acidic proteases, and alkaline proteases (Tavano et al., 2018).

3. PROTEASE RESOURCES

Interest in proteases has increased due to high demands in different industry areas. Innovative research into proteases, which are found everywhere in nature, i.e., in microorganisms, animals, and plants, is also increasing for this reason. Because they grow more quickly than plants and animals and take up less area to grow, one of the most widely used enzymes in the industry in worldwide use is microbial proteases (Ray & Ghosh, Ringø, 2012; Bhatia & Ullah, Hoque, Ahmad, Yang, Bhatt, Bhatia, 2021). In addition, microbial proteases generally have a longer shelf life, and there is no significant loss of activity even if they are stored in suboptimal conditions for weeks (Gupta, Beg, Khan, & Chauhan, 2002; De Souza et al., 2015).

The production of microorganisms is cheap and fast, and due to the different environments in which they are produced, it is quite easy to select microorganisms that produce enzymes with targeted properties (Putatunda & Kundu, Bhatia, 2019). Additionally, it is well known that proteases derived from microorganisms are more readily adjusted and have the capacity to change the way a process works than proteases derived from animals or plants (Gurung, Ray, Bose, & Rai, 2013).

In addition, due to the fact that they reproduce in a short time and have a simpler genetic structure, it is much easier to genetically modify microorganisms (Ali, Ullah, Qasim, Rahman, Khan, Sadiq, & Adnan, 2016). Two-thirds of all proteases used in industry are derived from microorganisms. The most significant proteases are found in bacteria and fungi, and although viral proteases are also significant, they are not nearly as important as fungal acidic proteases. For the purpose of making proteases for the market, the genus *Bacillus* is especially significant in bacteria. In fungi, on the other hand, *Trichoderma, Aspergillus, Penicillium*, etc. genera prevail (Gurumallesh, Alagu, Ramakrishnan, & Muthusamy, 2019).

Although several proteases have been found and described in various viruses, their economic significance has not increased significantly, and they are mostly utilized to increase the potency of various antiviral drugs. Serine proteases have been linked to the Hepatitis C virus, cysteine proteases to the Adenovirus and Alphavirus, and aspartic proteases to HIV, etc.; these are some of the proteases of viral origin (Sharma & Gupta, 2017).

Microbial proteases are frequently produced as proteins outside the cell in nature. The proteases that microorganisms in the fermentation broth create help the fermentation process (Tavano et al., 2018). Microbial protease synthesis has considerably increased. Additionally, research efforts are ongoing to find novel sources of enzymes, sophisticated methods of producing enzymes, and creative uses for enzymes by using extremely prolific fungal-bacterial strains and genetically altered microorganisms. Additionally, research activities are ongoing to find novel sources of enzymes, sophisticated methods of producing enzymes, and creative uses for enzymes.

4. MICROBIAL PROTEASES AND APPLICATIONS IN THE FOOD SECTOR

Food scientists throughout the world are looking at natural alternatives as chemical usage in the food industry is thought to be dangerously growing. Environmentally friendly microbial proteases show significant potential for replacing chemical substances. Microbial

proteases have been utilized as biocatalysts in the fermentation of foods since earlier times. (Singh, Kumar, Mittal, & Mehta, 2016).

Proteases are used in many kinds of food-related procedures. The manufacturing of infant food, protein supplement production and the protein hydrolysates' debittering, the creation of a variety of fermented foods and beverages, including beer and cheese, getting rid of turbidity, etc. are the major uses of proteases in the food business (Banerjee, & Ray, 2017; Dos Santos Aguilar & Sato, 2018).

Psychrophilic and psychrotolerant bacteria (which grow best between 20 and 35 °C) generate proteases that are suited to the cold (Białkowska, Gromek, Florczak, Krysiak, Szulczewska, & Turkiewicz, 2016). According to Kuddus and Ramteke (2012), these enzymes are employed to manufacture functional food components by hydrolyzing proteins, softening cold meat and enhancing flavor, speeding up cheese maturation, and processing haze-producing proteins in beer (Kuddus & Ramteke, 2012). Table 1. lists the uses for proteases derived from microbial sources in the food industry. In this section, proteases are classified according to the food areas in which they are used.

Table 1. Application of proteases obtained from microorganisms in the food sector (Mehta & Sehgal; 2019).

Microorganism	**Enzyme**	**Industry**	**Function/benefits**
Penicillium citrinum	Acid proteinase	Dairy	Milk coagulation
Rhizopus oryzae			
Aspergillus sp.			
Pleoticusmuelleri			
Pseudozyma hubeiensis	Aminopeptidase		Faster cheese ripening, debittering
Lactobacillus sp.			
B. licheniformis			
B. subtilis	Neutral proteinase		
A. oryzae			
A. niger	Serine-protease		Helps cheese to age by destroying proteins
A. niger	Endo- and exopeptidases	Baking	Dough conditioner, enhance dough extensibility
Lactobacillus brevis	Aminopeptidases	Beverage	Protein breakdown during mashing
L. plantarum			
A. niger	Prolyl-endoproteinase		Limits the production of haze
Aspergillus sp.	Acid protease	Meat and fish processing	Enhances the nutritional value and functional qualities of proteins
A. niger,	Neutral and alkaline proteases		Removing skin and scales from fish and making fish sauce
B. licheniformis			

4.1. Application in Dairy Industry

Proteases are mostly used in the dairy industry to make cheese. Molecular weights of microbial milk coagulant proteases range from 30,000 to 40,000, and include an acid aspartic protease class (Rao, Tanksale, Ghatge, & Deshpande, 1998). Chymosin, or rennin, is the main enzyme traditionally used to cause milk to coagulate while making cheese. Acid proteases' primary function in the manufacture of cheese is to break down peptide bonds to create para-K-casein and macro peptides. For making cheese, chymosin is chosen because of its strong casein specificity. Chymosin is being rapidly replaced in the production of cheese by aspartic proteases generated by GRAS (considered genetically safe) microorganisms such as *Endothia parasitica, B. subtilis,* and *Mucor miehei* (Rao et al., 1998).

Aspartic microbial proteases employed today as microbial rennet are obtained from *Penicillium oxalicum, Aspergillus oryzae, Mucor circinelloides, M. bacilliformis, M. miehei, M. pusillus, Rhizomucor pusillus, Rhizopus oligosporus,* and *Endothia parasitica,* (Vermelho, Cardoso, Nascimento, Pinheiro, & Rodrigues, 2015; Mamo & Asefa, 2018). These proteases are capable of severe proteolysis and are very resistant to varying pH levels and temperature variations. Releasing hydrophobic amino acid peptides at the C-terminal during ripening may result in a bitter flavor, a change in cheese texture, and reduced yield (Raksakulthai & Haard, 2003). Similarly, microbial proteases that act on casein in a similar way to chymosin are commercially available. These proteases, which produce around 30% of the cheese consumed worldwide, have been used in the USA since 1970. (Raksakulthai & Haard, 2003; Zaheer & Gupta, 2019).

Cloning and expressing the gene in a selective microbe is another method of obtaining calf yeast (Mold, yeast, or bacteria). Today, successful cloning and expression of the calf chymosin gene have been performed using *Kluyveromyces lactis, Saccharomyces cerevisiae,* and *Escherichia coli* (Zaheer & Gupta, 2019). *Arsukibacterium ikkense*'s cold-active protease enzymes were applied to break down caseins at low temperatures. As a result, it was observed that antioxidant milk protein hydrolysates and Acetylcholine esterase (ACE) inhibitors were produced. These enzymes are recommended to be used in dairy products and to enhance the nutrient content of functional foods. (De Gobba & Tompa, Otte, 2014).

Silva et al. claim that they discovered distinct characteristics in the aspartic protease they isolated from *Aspergillus clavatus*. They have shown that the enzyme can be used in the production of aroma enhancers and cheese in dietary supplements (Sampaio e Silva, Knob, Tremacoldi, Brochetto-Braga, & Carmona, 2011). In *Geomyces pannorum* and

Aspergillus oryzae, a novel aspartic protease gene has been identified, as reported by Gao et al. They stated that it would be more profitable to use this protease in cheesemaking because of its substrate specificity (Gao, He, Wei, & Zhang, 2018; De Souza, De Andrade, Koblitz, & Fai, 2023).

4.2. Use in The Processing of Meat

Myofibrils and connective tissue proteins found in muscle are broken down by a variety of microbial proteolytic enzymes. Meat is tenderized using aspartic and neutral proteases from *Aspergillus oryzae* and *Bacillus subtilis,* respectively. *B. subtilis, B. alcalophilus, B. licheniformis,* and *B. lentus* produce neutral proteases, and bacterial enzymes that soften meat, such as subtilisin are the most well-known (Mehta & Sehgal, 2019). For beef tenderization (myofibril breakdown), Mageswari et al. (2017) used freeze-activated peptidases isolated from *Chryseobacterium soli*. According to their findings, the rate of myofibril disintegration by the proteolytic enzyme increased rapidly and reached 221%. The taste of cold meat is increased by a protease from *Pseudoaltermonas* sp. because it releases more necessary and flavourful amino acids than mesophilic proteases (Mageswari, Subramanian, Chandrasekaran, Karthikeyan, & Gothandam, 2017; He, Chen, Li, Zhang, & Gao, 2004).

Alkalise (Novozymes) treatment of soybean proteins results in hydrolysates with minimal bitterness, excellent protein yield, and great solubility (Rao & Huan, Chen, Xiao, Li, He, 2023). Chitin is also produced by proteases. Chitin is used in by-products of the meat and fish industries, the waste of crustaceans, and the recovery of proteins (Białkowska et al., 2016).

4.3. Application in Bakery Products

Thermophilic enzymes are used in detergents, baking, and fermentation (De Souza et al., 2015). Serine proteases from *Thermoactinomyces vulgaris, Bacillus licheniformis,* and *Thermus mediterraneanus* are evaluated in bakery products. It is stated that with the addition of a sufficient amount of thermostable serine proteases, baked goods have a significant effect on delaying staling and crumb softness. (De Souza et al., 2023).

Gluten is used in the bread sector, it is partially hydrolyzed by a heat-sensitive fungal protease to leave the dough faster. The FDA (Federal Register, FDA, Federal Security Agency, Part 17, Bakers Products) got the first microbial protease from *Aspergillus oryzae* that was acknowledged and approved for use in cooking in 1952 (Lyons, 1982). The elasticity of the dough can be changed by using microbial proteases to increase the dough's strength and flexibility (Deniz, 2019; Mehta & Sehgal, 2019).

The wheat flour batter contained three times more water-soluble peptides than the artificially fermented dough used as a control after being exposed to *Rhizopus oryzae* and *Enterococci* proteases for 48 hours (M'hir, Rizzello, Di Cagno, Cassone, & Hamdi, 2009). Wheat flour contains gluten; this protein creates the properties of bakery dough. The elasticity of the dough depends on two essential components, glutenin, and gliadin, which give the dough viscosity. Because of their capacity to create a viscoelastic network that may trap carbon dioxide, these proteins enable the dough to expand during baking procedures (Wang, Lu, Li, Zhao, & Han, 2017). Proteases are applied in the bakery industry to provide dough uniformity, change the expansion properties of gluten, reduce dough consistency, increase loaf volumes, increase the spread rate of cookies, and improve flavor (Deniz, 2019; Okafor & Ofoedu, Nwakaudu, Daramola, 2019). It is also necessary to enhance color and flavor (Dong & Karboune, 2018). After hydrolyzed using an acid protease from *Aspergillus usamii*, wheat gluten has been shown to have increased water solubility and the ability to store water and fat (Deng & Wang, Yang, Song, Que, Zhang, Feng, 2016).

4.4.Processed Fruit-Based Beverages

Proteases prevent protein-phenol interaction and do not remove phenolic compounds, lowering the turbidity during cold storage (Pinelo, Zeuner, & Meyer, 2010). Protease and pectinase enzymes work together synergistically and produce a lower level of haze and turbidity in juices (Cerreti, Liburdi, Benucci, & Esti, 2016; Pinelo et al., 2010).

It has been shown that adding fungal acid protease to kiwi juice helps to stop the haze from forming and lessen turbidity while the fruit is kept chilled (Meyer, Köser, &Adler-Nissen, 2001). Protease, pectinase, and peptidase derivated from *Aspergillus* spp. have been reported to significantly reduce the instant turbidity of sour cherry juice (Pinelo et al., 2010).

4.5.Use as a Reducing Bitterness and Flavour Enhancer

Wheat protein-derived vegetable hydrolysates are mostly utilized in soups and seasonings as flavor enhancers. For instance, cheese is made using hydrolysates made from milk proteins. Soups, meat products, sauces, and bouillons are often flavored using hydrolysates made from animal protein. (Nielsen, 2010). They have a bitter taste depending on the number and type of hydrophobic groups in long-chain and aromatic amino acids. Exopeptidases are the enzymes most commonly used to break down bitter peptides (Raksakulthai & Haard, 2003). *Bacillus amylolichefaciens* for producing cheese flavor in the cheese industry. In addition, *A. oryzae*

and *L. helveticus* are preferred as flavor enhancers and bitterness relievers (Moschopoulou, 2018).

L. casei, L. sake, and *L. curvatus* are employed to produce aminopeptidases that are used to enhance the sensory quality of fermented sausages (Nandan & Nampoothiri, 2020). Proteases, which are used as color enhancers, cheese flavor enhancers, and cookie flavor enhancers because they are active and stable at alkaline pH levels, are produced by *B. licheniformis* (Tavano et al., 2018). Exopeptidases and endopeptidases are used together to remove the bitterness of hydrolysates containing highly hydrophobic proteins, such as cheese, corn, soy, and casein proteins, and to improve the flavor of cured meat products. (Raksakulthai & Haard, 2003).

4.6. Beer Production

An endo-proteinase produced from *Aspergillus niger* is used to prevent cold-haze formation and to continue fermentation without reducing foaming (Lopez & Edens, 2005; Bamforth, 2023). Papain has been used in the purification process to break down the turbidity-forming polypeptides. However, nowadays, proteases obtained from *Bacillus subtilis* are preferred as haze reducers. These enzymes have a pH of around 5.6 and an ideal temperature between 45 and 50 °C (Serna-Saldivar & Rubio-Flores, 2017). When beer was incubated with protease from *Aspergillus niger*, it hydrolyzed proline proteins and produced a haze-free peptide. This acidic enzyme has been confirmed to inhibit the formation of cold haze in bottled beer when added, even at low levels. It has been shown the addition of enzymes does not affect beer foam (Lopez & Edens, 2005). Additionally, the thermophilic protease produced from *Bacillus licheniformis* may be utilized in the brewing of beer. (Liu & Guo, Ma, 2023).

5. PROTEASE ENGINEERING

Although naturally occurring proteases may not be ideal for industrial applications, bioengineering advancements have made it possible to synthesize customized proteases for desired procedures.

Because of the vast biodiversity that exists, the search for data is an invaluable resource from which proteases can be created specifically for industrial use. For instance, the genetic information of "extremozymes" or extremophile-isolated enzymes may contain the appropriate special activity required for commercial applications. (Bilal & Iqbal, 2020). Mutations can be used for the production and isolation of proteases

through the development of recombinant proteins from genetically modified *E. coli* (Kotb, 2013).

CONCLUSION AND FUTURE VIEWPOINTS

Since proteases are of biological origin, they are superior in nature to chemical processes. It is a unique class of enzymes with degrading and synthesis potential. Therefore, as part of green and clean technology, proteases have several uses that are beneficial to the environment, and they have an excellent future ahead of them.

While 60% of the market for industrial enzymes worldwide are proteases, 40% of this amount is microbial proteases (Majumder & Kanekar, 2017). Traditionally, proteolytic enzymes have been utilized to improve the flavor and texture of high-protein diets. In the future, protein engineering will take priority in the design of proteases with novel properties. Advanced fields of science such as genetic and protein engineering, computational biology, and molecular biology are applied by researchers to obtain strains that produce engineered proteases.

The newly designed enzymes are produced quickly and cost-effectively by microorganisms. Efforts to produce new enzymes will have a profound effect on the quality of the food produced and will be pioneering in the design of new and different enzymes.

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Chapter 15

THREE-DIMENSIONAL (3D) PRINTING APPLICATIONS IN FOOD TECHNOLOGY

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1. INTRODUCTION

Three-dimensional (3D) printing is a technique for creating computer-aided designed things that use a layer-by-layer deposition procedure. The application of 3D printing technology has made significant advancements in various fields, including the food industry (Joshi, Sahu, Bareen, Prakash, Bhandari, Sharma, & Naik, 2021). 3D printing in the food industry offers unique possibilities for customization, complexity, and creativity, changing the way, we think about food production and consumption (Le Tohic, O'Sullivan, Drapala, Chartrin, Chan, Morrison, Ker ry, &Kelly, 2018; Taneja, Sharma, Ayush, Sharma, Mousavi Khaneghah, Regenstein, Barba, Phimolsiripol, & Sharma, 2022).

The first study on 3D printing technology was conducted and published in the 1980s. 3D printing, also known as "additive manufacturing", "rapid prototyping" and "3D printing technology" were mentioned together first time in the same years. Charles W. Hull conducted the initial research on 3D printing technology, which produces 3D objects from digital data (Bogue, 2013). The 3D printer functions similarly to a normal inkjet printer. 3D printing is the method of creating three-dimensional items using additive processes in which layers are placed down in succession to build a full object (Berman, 2012).

Recent advances in 3D food printing, understanding of structural components of foods such as shape/color, and advancements in material components and model design have all contributed to the development of four-dimensional (4D) food printing. 4D printing occurs when the shape and color modifications of 3D printed structures are arranged after printing (Momeni, Hassani, Liu, & Ni, 2017; Liu & He, Guo, Chen, Bhandari, Zhang, 2021). 4D printing technology is a more advanced form of 3D printing. The use of a specific formula printing ink in the preparation stage, water, pH, temperature, etc., in the qualities of the printed product in the presence of stimuli causes changes in color, taste, texture, shape, etc. 4D food printing allows the color, texture, taste, and other properties of the printed object to be self-transformed, resulting in a delightful and appetizing product (Rastogi & Kandasubramanian, 2019; Ma, Zhang, Wang, Liang, Ren, &Ren, 2020).

Food 3D printers are specialized machines designed to handle edible materials and create food items with precision (Sun, Peng, Zhou, Fuh, Hong, & Chiu, 2015). These printers use different techniques such as extrusion, powder binding, or sintering to deposit food materials layer by layer, gradually building up the desired object. The printers are equipped with special food-grade cartridges, syringes, or extruders that control the flow and deposition of food materials. 3D printing in the food industry

utilizes various food materials, including dough, chocolate, sugar paste, purees, and gels (Godoi, Prakash, & Bhandari, 2016; Wang, Zhang, Bhandari, & Yang, 2018; Pérez, Nykvist, Brøgger, Larsen, & Falkeborg, 2019; Zhang, Pandya, McClements, Lu, & Kinchla, 2022). In this regard, the ability to alter food textures and nutritional content is a significant benefit of 3D printing. These substances have been precisely designed to have the optimum texture and consistency for printing (Derossi, Caporizzi, Azzollini, & Severini, 2018).

3D printing allows for the creation of complex and intricate food designs that are challenging to achieve using traditional methods. Pastry chefs, chocolatiers, and culinary artists can use 3D printers to produce intricate and visually stunning dessert decorations, chocolate sculptures, or sugar confections. This opens up new possibilities for artistic expression and gastronomic experiences, captivating consumers with unique food designs (Varvara, Szabo, & Vodnar, 2021).

One of the most significant advantages of 3D printing in the food industry is the ability to customize food production to meet individual needs and preferences. By utilizing 3D printers, it becomes possible to create personalized meals and food items tailored to specific dietary requirements, allergies, or nutritional needs. This customization enables a higher level of precision and individualized nutrition, catering to a diverse range of consumers (Sher & Tutó, 2015; Rodgers, 2016). 3D printing technology allows for the customization of food items to meet specific dietary needs and restrictions. For individuals with chewing and swallowing problems and allergies, intolerances, or dietary restrictions such as gluten-free or vegan diets, 3D printing provides a means to create safe and suitable food options. By controlling the ingredients and printing parameters, 3D printers can produce food items tailored to meet specific dietary requirements, ensuring food safety and consumer satisfaction (Derossi, Husain, Caporizzi, & Severini, 2020; Le-Bail, Maniglia, & Le-Bail, 2020).

With 3D printing, precise control over the composition and distribution of ingredients in food items can be achieved. This enables the incorporation of functional ingredients such as vitamins, minerals, probiotics, or dietary supplements into specific areas of a food product. Ingredients also like proteins, carbohydrates, fats, and fibers can be incorporated to achieve desired nutritional profiles and functionality. The ability to target nutrient delivery and enhance the nutritional profile of food items offers opportunities for personalized nutrition and improved health benefits (Vieira, Oliveira, Amado, Fasolin, Vicente, Pastrana, & Fuciños, 2020; Picciotti, Massaro, Galiano, & Garganese, 2021; Zhao, Zhang, Chitrakar, & Adhikari, 2021).

With 3D printing technology, food is produced by using shelf-stable materials, minimizing waste, and optimizing resources. This technology has the potential to address food shortages, reduce logistical challenges and provide nutritional solutions in harsh environments (Lee, 2021). Additionally, 3D printing can contribute to sustainable food production by utilizing alternative ingredients and reducing waste. It allows for the utilization of unconventional food sources such as plant-based proteins, algae, or insect-derived ingredients, promoting resource efficiency and reducing environmental impact. By harnessing the versatility of 3D printing, food companies can explore innovative and sustainable food alternatives to address global food challenges (Peppel, 2015; Sher & Tutó, 2015).

The application of 3D printing technology in the food industry has seen substantial expansion and improvement over the years. Technology, initially started as an experimental concept has now evolved into a promising field with significant potential for innovation, customization, and sustainability in food production. In this chapter, information is given about 3D printer technology, its use in food technology, material properties suitable for printing, functional components, and food safety in the 3D printing process.

2. FUNDAMENTALS OF FOOD 3D PRINTING

The basic principle behind 3D printing is to design the product using CAD software, transform the file so that it can be read by the printer in stereolithography (STL) format, and then print the object in layer format (Attaran & Attaran, 2020). 3D printing can be classified into several types based on the type of material used and the process format. Food printing is a new concept that can be used to produce new products using many different food inks. It provides the added benefit of individualized nutrition and tailored design, which is unconventional and exciting in terms of food's general perception. Therefore, 3D printing has gotten an enormous response in the food industry. Technology enables distinctive food structure design and raises the food's flavor profile. To understand the fundamentals of food 3D printing, it is essential to explore the working principles, food materials, and printing techniques involved. Additionally, there are four windows for printing 3D foods. Printability, productivity, material characteristics, and process mechanism are those. The desired rheological characteristics for the printing process are taken into account during the formulation of food ink (Nachal, Moses, Karthik, & Anandharamakrishnan, 2019; Sandhu & Singh, 2022).

2.1. Working Principles of Food 3D Printers

Food 3D printers are specialized machines designed to handle edible materials and create food items with precision. To create 3D modeled product designs, 3D printers use SLT files, a CAD format (Yang, Zhang, & Bhandari, 2017). The printing process is initiated by sending the platform and headers the proper signals using the data collected by the printer cartridge. This cycle repeats for each printed layer (Sun et al., 2015; Kewuyemi, Kesa, & Adebo, 2022) (Figure 1).

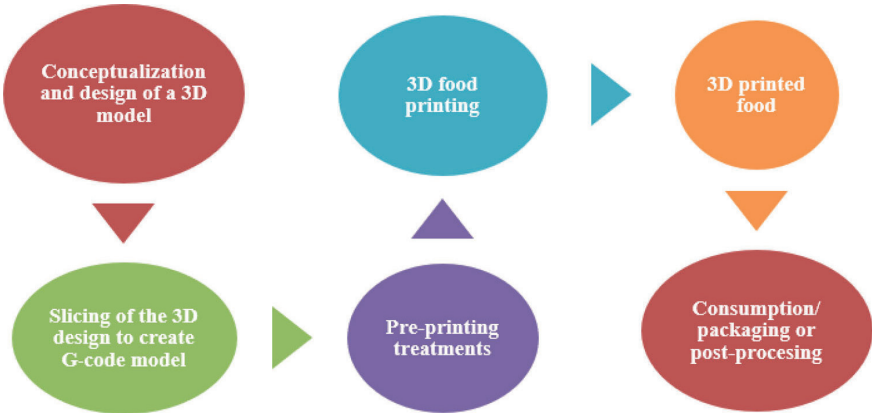

Figure 1. 3D food printing process overview (Kewuyemi et al., 2022).

The working principles of these printers involve the following steps:

Digital Design: Typically, a food printer is made up of three blocks: Through the use of software, a computer enables user and printer interaction. The program also enables communication between the computer and the motor control box (Baiano, 2020).

Slicing: The digital design is sliced into thin cross-sectional layers, each representing a layer of the final food item. The slicing is the critical parameter process that converts the 3D model into a series of 2D layers that the printer can understand (Guo, Zhang, & Bhandari, 2019).

Material Preparation: Edible materials suitable for 3D printing, such as dough, chocolate, or gels, are prepared. Starch and/or proteins can be added to create a plasticizing effect that makes it possible to print fiber-rich materials. They also optimized printing parameters loaded into specialized food-grade cartridges, nozzle diameters, syringes, or extruders. These cartridges control the flow and deposition of the food materials during the printing process. The rheological, thermal, and chemical properties of food ingredients can be utilized to assess the

structural accuracy of printed food products (Liu, Zhang, Bhandari, & Wang, 2017).

Printing Process: The printer follows the instructions from the sliced digital design to deposit the edible material layer by layer, gradually building up the final food item. The G-code regulates the movement of the printer head, which is in charge of the 3D printer's material release. The movement takes place in three axes; X, Y, and Z as the object is being printed depending on the information embedded in the G-code, precisely depositing the material according to the design (Leontiou, Georgopoulos, Karabagias, Kehayias, Karakassides, Salmas, & Giannakas, 2023).

Solidification: The printed layers may solidify or set naturally through cooling or drying, depending on the food material utilized. To speed up the solidification process, extra techniques like heat or UV radiation may occasionally be used. Extrusion is a procedure that includes depositing a powder- or liquid-based material, heating it, and then cooling it to produce solidification or hydrogel formation. As a result, using computerized design modeling and path planning, the 3D-printed food is created in real-time by deposition or sintering, layer by layer (Uhlmann, Kersting, Klein, Cruz, & Borille, 2015).

2.2. Types of Food Materials Used in 3D Printing

Food materials used in 3D printing are specially formulated to have the right consistency and texture for the printing process. The choice of food material depends on the desired properties of the final food item. There are three types of ingredients for food printing. Materials that can be printed natively; conventional food ingredients that cannot be printed; substitute ingredients. The materials that are naturally printed can either be extruded straight from a syringe, as is the case with bread, cheese, and chocolate or they can be found in powder form (such as sugar, starch, etc.) (Agunbiade, Song, Agunbiade, Ofoedu, Chacha, Duguma, Hossaini, Rasaq, Shorstkii, Osuji, Owuamanam, Okpala, Korzeniowska, & Guine, 2022) (Figure 2). Traditional food items such as meat, rice, vegetables, and fruits that cannot be printed must be given flow and viscosity enhancers in order to be appropriate for the extrusion process. The substances starch, pectin, xanthan gum, agar, gelatin, and alginate are frequently utilized. Novel sources of bioactive and functional substances, such as carbohydrates, proteins, lipids, vitamins, minerals, and fibers derived from insects, algae, probiotic organisms, and leftovers from agricultural and food operations are examples of substitute ingredients (Zhao et al., 2021).

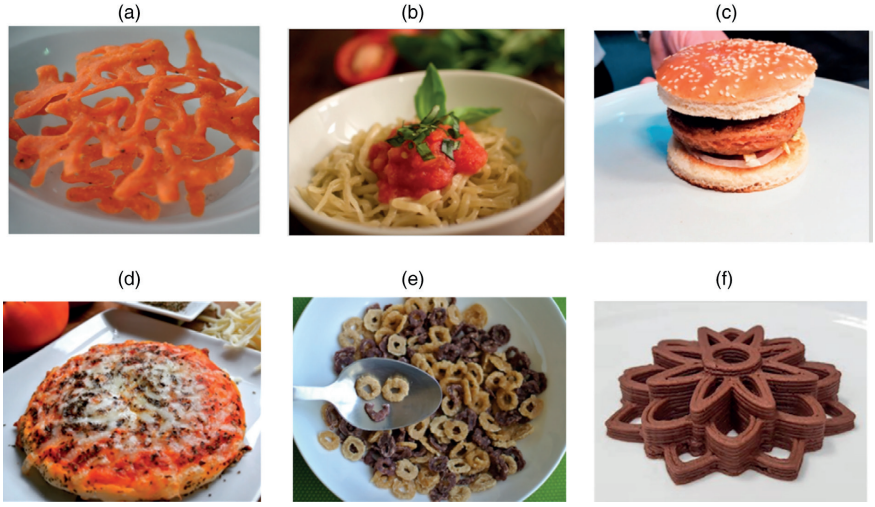

Figure 2. Food samples printed by 3D Printer (a) thin crackers (b) pasta (c) bread rolls (d) pizza (e) oat cereal (f) chocolate (Agunbiade et al., 2022).

Some common food materials used in 3D printing include:

Dough: Dough-based materials are commonly used in 3D printing to create bakery products like bread, cookies, pastries, and pasta. These materials are typically made from a combination of flour, water, yeast, and other ingredients. The dough is carefully prepared to have the appropriate viscosity and elasticity for extrusion-based printing, allowing it to be deposited layer by layer to form the desired shape (Derossi, Caporizzi, Paolillo, & Severini, 2021; Pulatsu, Su, Lin, &Lin, 2020).

Chocolate: Chocolate is the most suitable and popular material to be used in 3D food printing, thanks to the unique melting and solidification properties of the cocoa butter it contains. Chocolate-based materials consist of cocoa solids, cocoa butter, sugar, and other ingredients. They need to be melted and tempered to the right consistency before being loaded into the 3D printer (Godoi, Bhandari, Prakash, & Zhang, 2018).

Gels and purees: Gels and purees made from fruits, vegetables, or other ingredients are used in 3D inkjet printing to add color, flavor, and texture to food items. These materials can be customized to achieve different viscosities and consistencies, allowing for the creation of unique shapes and textures. Gels and purees are often used to enhance the visual appeal of dishes, add bursts of flavor, or incorporate specific nutritional components (Attaran & Attaran, 2020).

Dairy products: Dairy-based food materials, including cheese, cream, butter, milk-based materials, yogurt, and whey protein, can be

utilized in 3D printing to create a wide range of customized and delicious food items. The use of dairy-based food materials in 3D printing offers unique flavor profiles, nutritional benefits, and culinary possibilities. Additionally, in the production of food, proteins, or hydrocolloids polymers that may form gels or viscous solutions are frequently utilized to modify the rheological and sensory qualities. Gelatine, along with pectin, carrageenan, agar, and alginate are commonly used due to their gelling ability (Saha & Bhattacharya, 2010). Whey proteins have good protein bioavailability and also the ability to modulate gel properties (Cao & Mezzenga, 2020) and enhance printability. However, it is crucial to take into account aspects like processing techniques, temperature control, and post-printing considerations to maintain the desired texture and taste of the final printed food items (Riantiningtyas, Sager, Chow, Thybo, Bredie, & Ahrné, 2021).

Alternative protein sources: The microstructural and textural characteristics of printed food are influenced by protein. Proteins make up the majority of the material supply, which makes pH and isoelectric points the most important variables. As the demand for alternative protein sources grows, researchers and food companies are exploring the use of plant-based proteins, algae, fungi, seaweeds, and even insects as materials for 3D printing. These ingredients are also rich sources of protein, dietary fiber, and/or a range of valuable bioactive. These alternative protein sources are often processed and transformed into protein-rich pastes or powders that can be extruded or used in combination (Nachal et al., 2019).

2.3. Printing Techniques and Processes

Different printing techniques and processes are employed in food 3D printing, depending on the desired outcome and the properties of the food materials. The most common technique is extrusion-based printing, where a material is pushed through a nozzle or syringe and deposited layer by layer (Tejada-Ortigoza & Cuan-Urquizo, 2022; Leontiou et al., 2023). Four types of 3D printing are frequently used in the food industry: binder jetting, extrusion-based printing, inkjet printing, and selective laser sintering printing (SLS) (Feng & Zhang, Bhandari, 2019; Jiang, Zheng, Zou, Tong, Han, & Wang, 2019). These types are presented in Figure 3.

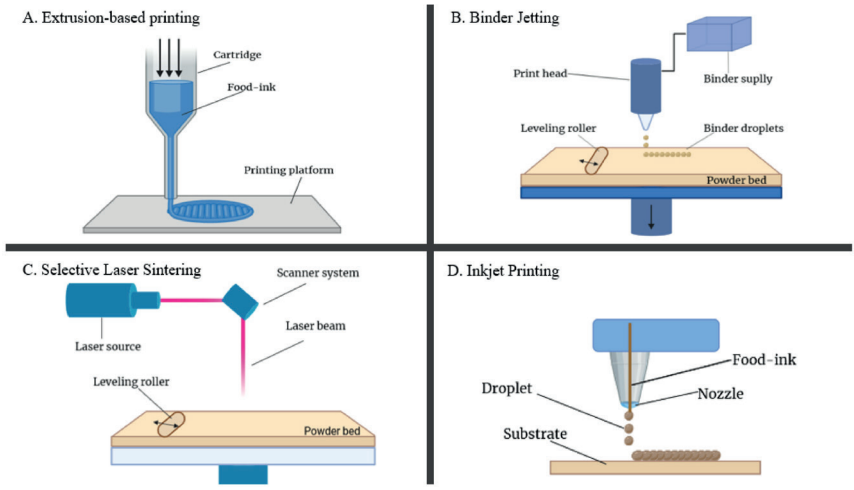

Figure 3. Concept figures for 3D food printed Technologies (Varvara et al., 2021).

Binder jetting: In this technique, layers of powdered food materials are selectively bound together using a liquid (glue) binder. The binder acts as an adhesive, creating a solid structure as the layers are stacked (Nachal et al., 2019). To increase the mechanical strength and enable the deposition of the subsequent layer, the surface is typically heated by irradiation after the binder has been applied. For each layer, repeat these instructions. Thereby, through this advanced concept, it is easy to achieve unique and complex products in a shorter time compared to conventional methods (Dankar & Haddarah, Omar, Sepulcre, Pujolà, 2018). Binder jet printing has several advantages, including a large number of printable materials, a fast printing speed, and the capacity to produce complicated shapes. Some disadvantages include obtaining foods with a rough appearance and the need to dehydrate the finished items or further prepare the structure in order to improve its strength (Pitayachaval, Sanklong, & Thongrak, 2018).

Extrusion-based printing: In this approach, is also known as fused deposition modeling (FDM). Semi-solid molten thermoplastic materials are extruded via a moving head and subsequently collected in thin layers. By heating the material just beyond its melting point, it is ensured that it hardens quickly after extrusion and that each layer fuses with the underlying layer (Sun & Zhou, Yan, Huang, Lin, 2018). Extrusion-based printing is utilized in the manufacturing of soft products such as dough, chocolate, meat paste, mashed potatoes, and cheese. Among the advantages of this technology is the ease of use of the printing equipment and the large variety of materials that may be printed. Its drawback is that it cannot be used to produce intricate structures (Yang et. al., 2017;

Baiano, 2020). Print quality is affected by elements such as processing conditions, printing materials, and post-processing. This approach requires food components with high viscosity and mechanical strength (Liu et al., 2017).

Inkjet printing: Inkjet-based 3D printing involves the precise deposition of small droplets of edible ink onto a substrate layer by layer. In order to produce surface fills or food decorative items, inkjet printing uses a spray of droplets that are delivered by a heating or electromechanical head (Kuruth & Levy, Klocke, Childs, 2007). This technique allows for the creation of colorful and intricate designs on food surfaces. There are two different inkjet printing techniques used: continuous printing and drop-on-demand printing. In the first technique, ink is continuously ejected from a piezoelectric crystal that vibrates at a certain frequency. Although drop-on-demand printing in the second method is slower than continuous printing, it exhibits superior resolution and precision (Liu et al., 2017). The rheological properties of the ink, surface characteristics, and processing variables like temperature, printing height and rate, and nozzle diameter affect the printing precision. Low-viscosity materials including chocolate, liquid dough, icing, meat paste, jams, and gels are suitable applications for this technique. One advantage of this method is that it offers the possibility to design ink droplets, personalized food images, and also fast production. this method is only suitable for surface design (Godoi et al., 2016; Voon, An, Wong, Zhang, & Chua, 2019).

Selective laser sintering printing: In this technology, the energy provided by a laser or a sintering source represented by hot air causes certain sections of the particle bed to fuse. The method is done layer by layer until the food is complete (Shirazi & Gharehkhani, Mehrali, Yarmand, Metselaar, Kadri, Osman, 2015; Nachal et al., 2019). Each layer of the food matrix can contain different food material components, indicating that this technology can be applied to multiple materials (Lille & Nurmela, Nordlund, Metsä-Kortelainen, Sozer, 2018). The beginning materials are powdered foods such as starch granules, sugars, and fats A significant reduction in nutrient concentration occurs, especially as this technique uses heat to join the layers. So, it is not suitable for producing healthy food (Liu et al., 2017).

Each printing technique requires specific equipment, settings, and considerations to ensure the successful creation of food items with the desired characteristics (Leontiou et al., 2023).

Moreover, an innovative use for 3D printing is also 'bioprinting,' which was initially used to construct living cells and is currently utilized to manufacture meat analog. The most often utilized techniques for this

process are microprinted and laser-assisted printing it is concerned with the creation of biological materials and living cell cultures (Murphy & Atala, 2014). Research is being conducted to generate plant cell-based foods and a variety of foods with textural features similar to the native product, as well as diverse packaging materials. 3D bioprinting of meat has the potential to reduce animal slaughter and so replace conventional meat production while avoiding environmental and health concerns (Handral, Hua Tay, Wan Chan, & Choudhury, 2022). The technique is still being developed for food applications and must overcome obstacles like economics, nutrition and organoleptic qualities, industrial scale-up, nutrient inputs for cell culture, food safety, and ethical concerns (Portanguen & Tournayre, Sicard, Astruc, Mirade, 2019).

3. NUTRITIONAL CONTROL AND FUNCTIONAL 3D-PRINTED FOODS

One of the significant advantages of 3D printing in the food industry is the ability to have precise control over the nutritional composition of printed food items. This technology allows for the creation of functional foods with customized nutritional profiles to meet specific dietary needs and preferences. With the help of 3D printing, a variety of totally customized foods are now available to suit the demands, tastes, and dietary requirements of people of all ages, genders, jobs, and healthy lifestyles. This is done by modifying the composition, density, or texture to the user's preferences and needs (Rodgers, 2016).

3.1. Incorporating functional ingredients in 3D-printed food

3D printing enables the creation of functional foods that go beyond basic nutrition. Functional foods are designed to provide additional health benefits beyond their basic nutritional value. Through 3D printing, functional ingredients such as probiotics, omega-3 fatty acids, antioxidants, or dietary fibers can be incorporated into food items. This opens up possibilities for creating food products with targeted health benefits, such as improved digestion, enhanced immune function, or cardiovascular support (Baiano, 2020).

A variety of foods can be produced using 3D food printing technology to satisfy the needs of both those who choose just particular foods (such as vegetarians) and people who have certain conditions (such as obesity, hypertension, and diabetes) related to nutrient components. Gluten-free, lactose-free, and allergic diets can now be accommodated by creating specialty food options using 3D printing. Individual nutritional requirements can be satisfied by modifying the content of the printed food or by utilizing alternative ingredients (Aguilera & Park, 2016).

Moreover, 3D food printing can help with chewing and swallowing problems. Since about 25% of older people have chewing and swallowing problems, they get the nutrients they need from appetizing and unappetizing pureed foods. 3D food printing can ease chewing and swallowing difficulties, also known as dysphagia. Dysphagic patients can sometimes be fed nutritionally deficient purees (Baiano, 2020; Portanguen et al., 2019). In reality, foods that have been pureed or strained can be employed as printable materials in 3D printing to restore their original appearance or provide original, delicious textures (de Roos, 2013).

3D nutrition technology can be used to adjust nutrient concentrations, reduce undesirable ingredients, or add the required amount of nutrients to develop a new food structure and correct deficiencies. 3D food printing can be used to create nutrients for special groups such as the elderly, athletes, pregnant women, and youth by incorporating nutritious nutrients such as vitamins, fiber, and phytochemicals into conventional meals (Aguilera & Park, 2016).

Another purpose of 3D food printing is to create customized food, both in terms of sensory qualities and nutritional content. Implementation of 3D printing in personalized or targeted nutrition, for instance, could add active components (Varvara et al., 2021). Some food products contain supplements or other additives that are intended to benefit health, such as vitamins, minerals, probiotics, fiber, and others (Vieira et al., 2020). These foods, which are characterized by the proper nutrient intake, are simply created for each individual's needs with the aid of 3D food printing technology (Varvara et al., 2021). 3DFP can be used to create a customized diet for those with particular dietary needs and circumstances. This means that only certain ingredients are introduced into the 3D printer. The dish is made using specific ingredients, and this enables each person to prepare it according to their nutritional requirements. For instance, a person with chronic kidney disease who has low potassium levels has to eat food. 3D food printing enables ingredients to be added to the dish and delivered in a customized manner (Burke-Shyne, Gallegos, & Williams, 2021). Furthermore, given the demands of children and adolescents, deficits in nutrients such as protein, vitamins, and minerals (particularly iron and calcium) when 3D food printing is used are possible. It can be utilized to obtain food products with a better nutritional profile (Varvara et al., 2021).

Probiotic microorganisms are also used in the production of functional food produced by 3D printing. It is important to preserve cell viability in functional food production. In the literature, some studies on the use of 3D printing to create nutritious/healthy foods; Probiotic bacteria provide the health balance of intestinal microorganisms and

provide health benefits to the host when taken in appropriate doses (Hill, Guarner, Reid, Gibson, Merenstein, Pot, Morelli, Canani, Flint, Salminen, Calder, & Sanders, 2014). Probiotics can also produce some antimicrobial agents and play an immunomodulatory role. However, it is also beneficial for people suffering from certain diseases such as inflammatory bowel diseases, periodontal diseases, and metabolic diseases (de Almada, Nunes de Almada, Martinez, & Sant'Ana Ade, 2015). Zhang & Lou, Schutyser, (2018) investigated the feasibility of probiotics (*Lactobacillus plantarum* WCFS1) and dough-based 3D-printed foods and the survival of related strains during cooking. They found that increasing the structure's surface-to-volume ratio allowed them to bake the food for only six minutes at 145°C, producing food that complied with the criterion of probiotic foods (viable counts of bacteria > 10^6 cfu/g). Another application of 3D printing is the creation of functional foods by adding probiotics to printed materials. Liu et al. (2017) optimized the printing temperature and nozzle diameter for the inclusion of *Bifidobacterium animalis* subsp. *lactis* into 3D-printed mashed potatoes. They determined that the probiotic viability could only be decreased by using a small nozzle diameter (0.6 mm) and keeping the mashed potatoes at 55°C for 45 minutes. Probiotic viability was unaffected by the 3D-printed functional food's 12-day storage at 5°C.

Due to their abundance of vitamins and minerals, fruits and vegetables must be consumed in sufficient quantities each day in order to prevent numerous illnesses (Derossi et al., 2018; Azam, Zhang, Bhandari, & Yang, 2018), produced vitamin D-enriched 3D printed orange concentrate/ wheat starch blends using Arabic, guar, and k-carrageenan gums. The fruit-based snack designed by Derossi et al.(2018) was nutrient-tailored to provide the 5–10% of energy, calcium, iron, and vitamin D needed by kids between the ages of 3 and 10.

Another application of 3D printing is the manufacturing of functional foods by adding protein to printed materials. According to Lille et al.(2018), utilized 3D printing technology to produce healthy structures rich in fiber and protein but low in fat or sugar. In another study, the rennet-induced gelation of milk proteins was evaluated as a potential method for the formation of 3D-printed food structures (Uribe- Alvarez, O'Shea, Murphy, Coleman-Vaughan, & Guinee, 2021). Researchers are investigating the suitability of edible insect dust as a component of 3D-printed foods. This is because insect dust is rich in protein, which varies between 40-70% depending on the species and their life cycle (Verkerk, Tramper, Trijp, & Martens, 2007). A novel approach to promoting this sustainable food source and a creative means of easing people's aversion to eating insects is to use insect protein as a component in 3D printers (Severini, Azzollini, Albenzio, & Derossi, 2018a).

In conclusion, 3D printing technology in the food industry enables precise control over the nutritional composition of printed food items, leading to the creation of functional foods that can meet specific dietary needs and preferences. Through customized nutritional profiles, controlled portion sizes, enhanced nutrient delivery, personalized dietary options, and functional food design, 3D printing promotes improved nutrition, supports individual health goals, and expands the possibilities of culinary innovation.

4. FOOD SAFETY AND QUALITY ASSURANCE

4.1. Ensuring food safety 3D printing process

As 3D printing technology continues to advance in the food industry, it is important to ensure food safety and maintain high-quality standards. As with other food production methods, it is necessary to make the consumption of 3D-printed foodstuffs safe and to pay attention to quality assurance measures. It is related to the contact with 3D-printed components and the manufacturing of food ingredients, and it might have to do with microbiological issues or leachable material migration (Baiano, 2020).

The choice of materials used in 3D printing plays a crucial role in food safety. It is important to select food-grade materials that are safe for contact with food and comply with relevant regulatory standards. This includes using food-safe plastics, metals, or other materials that have been approved for use in food applications. It's essential to ensure that the materials used do not leach harmful substances into the food during the printing process or when consumed (Lipson & Kurman, 2013).

It is very important to provide a clean and hygienic printing environment and packaging material to avoid contamination. Proper sanitation practices, including regular cleaning of the 3D printer components, surfaces, and utensils, are vital to ensure that the printed food items are free from any harmful bacteria or pathogens. Adhering to good manufacturing practices (GMP) and following hygiene protocols will help minimize the risk of foodborne illnesses. Therefore, printed food safety depends on the type of 3D printer and the implementation of effective cleaning protocols (Versino, Ortega, Monroy, Rivero, López, & García, 2023).

3D printers designed particularly for food consumption meet all of these standards, although they can be quite expensive. Instead, 3D printers that can also print food are very inexpensive, but the majority

of their parts are made of plastic, which can produce ultrafine poisonous particles that are harmful to human health (Sun et al., 2018).

4.2. Quality control measures and hygiene considerations

The ingredients used in 3D printing must meet quality and safety standards. It is important to source ingredients from reputable suppliers and to make sure they are fresh, uncontaminated, and fit for consumption. Clear labeling of ingredients and potential allergens should be provided to inform consumers about the contents of printed foodstuffs.

Proper temperature control is essential during the 3D printing process to ensure food safety. Depending on the material and food item being printed, specific temperature ranges may need to be maintained to prevent the growth of harmful bacteria and ensure proper heating or cooling of the food. Careful monitoring and control of temperatures, both during printing and storage, are necessary to minimize the risk of foodborne pathogens. In a previous study on printed smoothies, Severini et al.(2018), found a microbial load of 4.28 Log cfu/g, thus showing that every single part of 3D printers that come into contact with food, both in home/food service uses and on an industrial scale, must be sterilized before printing (Severini, Derossi, Ricci, Caporizzi, & Fiore, 2018b).

Implementing quality assurance testing is crucial to ensure the safety and quality of 3D-printed food items. This can involve microbiological testing to detect any potential pathogens, as well as sensory analysis to assess the taste, texture, and overall quality of the printed food. Regular testing and analysis help identify any issues or deviations from quality standards and allow for necessary adjustments or improvements in the printing process (Yang et al., 2017).

Food safety and quality assurance should be top priorities when incorporating 3D printing technology into the food industry. By following hygiene practices, using food-grade materials, ensuring ingredient safety, controlling temperatures, conducting quality assurance testing, and complying with regulatory standards, food professionals can confidently produce 3D-printed food items that meet the highest safety and quality standards.

5. EMERGING AND FUTURE INSIGHTS OF 3D PRINTING OF FOODS

3D printing technology is making significant advances in the food industry by providing innovative product profiles and personalized nutrition. As technology continues to advance, some key developments and new trends are emerging that are shaping the future of 3D printing in

the food industry (Sun et al., 2018). 3D food printers, though advantageous, are still an emerging technology that must overcome several challenges, including cost, speed, and ease of use. The costs of 3D food printing are becoming competitive for smaller productions such as customized foods and personalized meals. It is necessary to provide product price balances depending on the supply of various 3D printable components and the volume of the printer. While the speed in 3D food printing is sufficient for home use, it is time-consuming for mass production in the industry. For this reason, it is necessary to provide adaptation to the transition to industrial production (Baiano, 2020).

The technology enables the creation of complex geometries, intricate patterns, and artistic designs that are difficult to achieve using traditional culinary techniques. Chefs and food designers can explore new dimensions of creativity, resulting in visually appealing and aesthetically pleasing food presentations. It is foreseen that this technology will also offer an alternative to traditional dishes in the future and can provide an edible decoration limited only by the imagination of the users (Zhang et al., 2022).

Many researchers believe that 3D food printing can eventually solve global food shortage problems and different nutritional needs. Through 3D printing, functional ingredients, such as vitamins, minerals, probiotics, or nutraceuticals, can be incorporated into food items. This allows for the development of food products that promote specific health benefits, such as improved digestion, enhanced immune function, or increased energy levels. In addition, 3D food printing is also applied in food areas such as military food, space food, and elderly food. In order to improve the duration of life support systems during space missions, NASA (National Aeronautics and Space Administration) is working on the possibility of developing 3D-printed food systems. With these systems, it is aimed to ensure safety, acceptability, diversity, nutritional stability, a shelf life of several years, minimal spacecraft resources, and waste minimization (Lupton & Turner, 2016).

As 3D printing technology evolves, there is an increasing integration of robotics and automation. Automated systems can handle various tasks in the 3D printing process, such as ingredient dispensing, layering, and cleaning. This integration streamlines the printing process, improves efficiency, and enables the production of food items on a larger scale. In addition to this technology, it has started to pioneer the potential of 4-dimensional (4D) food printing, which offers an extra dimension by producing the shape, sensory properties (color, taste, and aroma), and functionality of printed materials as desired (Teng, Zhang, & Mujumdar,

2021; Zhao et al., 2021; Taneja et al., 2022; Tejada-Ortigoza & Cuan-Urquizo, 2022).

CONCLUSION

In conclusion, 3D printing technology is revolutionizing the food industry, bringing food products, customization, and innovation. With the advantages of creating complex designs, tailoring food products, and combining alternative ingredients, 3D printing contributes to food preparation and presentation. The development of personalized food products that fully meet each person's preferences, dietary restrictions, and nutritional needs is the result of advances in 3D printing.

Moreover, the emerging trends in 3D printing, such as the exploration of novel food designs, the development of functional foods, and the integration of robotics and automation, are further shaping the future of the industry. These trends not only offer exciting possibilities for food technology but also have the potential to promote sustainability and healthier food choices.

However, as the adoption of 3D printing in the food industry progresses, it is important to prioritize food safety, quality assurance, and regulatory compliance. Hygiene practices, careful selection of materials, and adherence to food safety standards are essential to ensuring that 3D-printed foodstuffs are safe for consumption.

Overall, 3D printing in the food industry holds tremendous promise, allowing for culinary innovation, personalized nutrition, and enhanced dining experiences. As technology continues to advance, it will undoubtedly shape the way we think about food, enabling us to explore new flavors, designs, and textures, while also addressing the evolving needs of consumers. The future of 3D printing in the food industry is filled with endless possibilities, and its impact will continue to unfold as researchers, chefs, and food professionals push the boundaries of what can be achieved with this transformative technology.

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Chapter 16

OZONE APPLICATIONS IN FOOD PROCESSING

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1. INTRODUCTION

Ozone (O_3) is considered an oxidizing agent that has many uses in the food and agriculture sector. The application of O_3 in foods is a promising environmentally friendly, practical technology, and economical that has attracted industry interest in increasing the shelf-life and maintaining food safety (Pandiselvam et al., 2018). The food business is currently undergoing an active transformation process to address the challenges of meeting the growing food requirements for a rapidly increasing global population. This involves implementing solutions such as reformulating products with healthy and sustainable ingredient options, incorporating proteins, vitamins, and antioxidants into foods, and labeling products according to their organic, gluten-free, antibiotic-free, allergen-free, non-GMO, and non-GMO properties. Additionally, measures are being taken to gradually reduce the calorie and carbohydrate content of foods, prolong the shelf-life, and prevent loss throughout the supply chain of the food business. It is important to note that harvested products undergo changes due to physical, chemical, and biological factors, which ultimately lead to spoilage. (Chiozzi, Agriopoulou, & Varzakas, 2022; Guine, Florenca, Barroca, & Anjos, 2020).

Heat treatment is a well-known technique for preserving food, but it has drawbacks that should be considered. However, in recent times, emerging processing novel technologies have been developed and offer an alternative means of food preservation. With the increasing demand for sustainable and eco-friendly food production practices, there are now many advanced techniques available to consumers. This trend responds to the growing need for minimally processed foods and represents a shift towards clean and green production. O_3 technology is a widely known processing method for food that is often considered "emerging" or "innovative". The discovery of O_3 dates back to 1840, when Christian Schonbein, a German chemist, first identified it. Fewson, a US inventor, later developed an O_3 generator in 1888. In 1893, O_3 was initially used for water treatment in the Netherlands, while in 1909 Germany implemented its usage for preserving meat. Since 1906, Europe has been using O_3 to purify water, while ozonated air was introduced in 1955 for sterilizing purposes (Prabha, Barma, Singh, & Madan, 2015).

New technologies are emerging for food preservation, reducing or eliminating pathogenic microorganisms in food, as well as inhibiting enzymes or extracting bioactive compounds useful in the food sector (Aguilar et al., 2019). Food has traditionally been processed using conventional thermal processing techniques, which are based on temperature-time to kill food-borne pathogens and reduce spoilage

microorganisms (bacteria, viruses, and parasites) or destroy enzyme activity to make food safe while increasing the shelf life of foods. Traditional methods used in food treatment are the most energy-intensive technology and can be divided into pasteurization, sterilization, evaporation, and ultra-high temperature according to heat intensity.

For many foods, thermal food processing is an effective food treatment method. However, these thermal processes can adversely affect the physico-chemical, organoleptic, and nutritional characteristics of the food products. Heat processing can also lead to lipid oxidation, water loss, and modification in fatty acid contents. Today's consumers demand safe and clean food without loss in organoleptic and nutritional properties (Jadhav, Annapure, & Deshmukh, 2021). The food business is looking for new processing methods to prevent the adverse effect of traditional methods on food characteristics (Rocha et al., 2022; Valoppi et al., 2021). A number of technologies are emerging to address the shortcomings of traditional thermal food processing (such as pasteurization) and to meet the growing consumer demand for natural, more nutritious, and "fresh-like" food products (Islam et al., 2022). Emerging technologies are increasingly used in food and have attracted the attention of the food industry. Therefore, it was mainly used as a substitute for the pasteurization process to achieve quality optimization and shelf-life extension. The most successful commercial applications, particularly high-pressure processing, have replaced traditional thermal pasteurization.

New technologies also contribute to environmental sustainability by using biomolecules and natural bioactive compounds from sustainable sources to delay oxidation, degradation, and decay. It aims to ensure high quality and adequate shelf life in fresh-like (natural) products that are minimally processed, additive-free, and microbially safe.

Today's consumers demand that food is not only safe but also easy to use, has a longer shelf life with fewer additives, is low in calories, and is inexpensive and environmentally friendly without the need for additional preparation (Pandiselvam et al., 2022). Several new emerging treatment methods have been designed as alternatives to thermal treatments, and the methods have begun to be implemented that offer the opportunity to produce higher quality food, increase consumer preference, increase food safety, and improve process efficiencies.

To inactivate the microorganisms that are harmful to the safety of the product with traditional methods, temperature is used at different degrees and periods. Traditional thermal food processing technologies, such as pasteurization, sterilization, canning, baking, roasting, and frying, apply heat to a food for a specified period to reduce microbial activity,

destroy enzyme activity, and induce physical or chemical changes to meet a specified quality standard. However, heat treatment technologies are energy intensive, processing times can be long, and negatively impact the nutritional and sensory properties of food (Galanakis, 2021; Z.-H. Zhang et al., 2019). Despite this, heat treatment can deteriorate the structure of the product with the formation of by-products or undesirable reactions in the food, leading to loss of aroma, structure, and nutrients and generally loss of quality of the food product. Therefore, the ideal processing method is to achieve microorganism inactivation and degradation reactions other than by heating or freezing by a method that is essentially a non-thermal technique (Amit, Uddin, Rahman, Islam, & Khan, 2017). A range of non-thermal food preservation technologies is emerging to address the shortcomings of traditional processes, improve processing efficiencies, and create value-added products for manufacturers and consumers. These technologies use different mechanisms to inactivate microorganisms in the food product (Chacha et al., 2021).

New technologies include cold atmospheric plasma, high hydrostatic pressure processing, pulsed UV light, irradiation, pulsed light, microwave, oscillating magnetic fields, ultrasound, membrane processes ozone, chlorine dioxide, ohmic heating, pulsed electric-fields, supercritical carbon dioxide removal. This minimal food processing technologies alternative not only shelf-life demand but also provide acceptable quality and sensory quality of food: In particular, nonthermal methods, considered value-added treatments, have gained prominence over the past years as a viable replacement to traditional methods by directly reducing energy and water consumption during treatments (Picart-Palmade, Cunault, Chevalier-Lucia, Belleville, & Marchesseau, 2018).

Better nutritional and sensory quality were key factors in the companies' use of nonthermal methods. Over the past decade, some of the non-thermal novel methods have become commercially available, however, most have limited uses and are still in the development phase at the laboratory or pilot scale, resulting in higher investment costs than full-scale industrial thermal equipment and broad industrial applications requiring more scientific studies and industrial practices (Rocha et al., 2022). However, many technological developments have brought to the fore different approaches to food preservation by consumers.

2. OZONE CHEMISTRY

O_3 is a colorless inorganic molecule formed by the combination of three oxygen atoms (O) and is used as the most powerful oxidizing agent known in nature. Due to its powerful antimicrobial properties, it can be a good alternative to traditional food preservation. It can inactivate

microorganisms such as viruses, bacteria, and molds in a short contact time. In addition, the rapid decomposition of oxygen and the absence of toxic residues in food is clean and acceptable, an environmentally friendly green technology. In general, ozone is a triatomic molecule (O_3) formed by the breakdown of an oxygen molecule by radiation (below 240 nm) of short-wavelength ultraviolet rays from the sun. Large quantities of this gas were produced by generators for industrial use. O_3 is a light blue gas and partially soluble in water. It has an oxidation-reduction potential (2.07 V) (Muthukumarappan, Halaweish, & Naidu, 2000). In 1888, Fewson invented a generator to make O_3 in the USA. O_3 has been used in Europe since 1906 to purify water and in 1955 ozonated air was used to sterilize it. Generally approved as safe for food application (GRAS) (Cullen, Tiwari, O'Donnell, & Muthukumarappan, 2009; Khadre, Yousef, & Kim, 2001).

Ozone is a triatomic molecule (O_3) formed by the breakdown of an oxygen molecule by radiation (below 240 nm) of short-wavelength ultraviolet rays from the sun. O_3 is a very reactive gas and is used as the most powerful oxidizing agent known in nature. O_3 is one of the strongest oxidizing agents. Due to its strong oxidation-reduction potential, O_3 is particularly effective as a microbiome. O_3 breaks down into hydroperoxy, superoxide, and hydroxyl radicals. Because of its antimicrobial properties, it can be a good alternative to traditional food preservation (Premjit, Sruthi, Pandiselvam, & Kothakota, 2022). O_3 can inactivate microorganisms such as viruses, bacteria, and molds in a short contact time. O_3 can be delegated to be either 100% natural or natural depending on O_3 usage. In addition, the rapid decomposition of oxygen and the absence of toxic residues in food is clean and acceptable, an environmentally friendly green technology (Gonçalves, 2009).

O_3 is a bioactive oxidizing disinfectant that breaks down into O_2 and O_1. The latter molecule is highly reactive and leads to the breakdown of bacterial cell walls and changes the function of proteins and carbohydrates. Due to this property, it shows high effectiveness in eliminating bacteria, fungi, and mold, as well as in inactivating viruses. The antibacterial effect of O_3 primarily affects the integrity of the bacteria by destroying their membrane structure (Rangel et al., 2021).

O_3 has a very high oxidizing power and produces no by-products. O_3 gas occurs naturally because ultraviolet rays from the sun break down O_2 in the atmosphere and convert it into O_3 molecules. O_3 has an unstable triatomic O_2 molecule formed by one O_2 atom and two atomic O_2 molecules (21% in air). This third oxygen atom tends to detach from the molecule because it is very weakly bound. This oxygen atom, left alone when separated from the molecule, is extremely active and oxidizes any substance around it. O_3 is a powerful antioxidant and antimicrobial

agent. Its antimicrobial activity comes from being a powerful oxidizing agent like hydrogen peroxide. In particular, in the decomposition, the formed hydroxyl radical is not stable, although it has a very large oxidative activity. It attaches to microorganisms, causing their cell walls to break down and die. After fluorine, O_3 is the strongest oxidizing agent in the world. It kills bacteria and viruses 3,000 times more effectively and faster than chlorine. Thanks to its high reactivity, O_3 reacts with and oxidizes various inorganic and organic molecules and atoms. As a result of these reactions, it neutralizes bacteria and microbes in the air (reduces the growth rate by 3 to 4 times or inhibits them completely) and provides sterilization, and destroys organic molecules. It attacks and destroys all microorganisms. O_3 has been shown to provide sterilization when applied to food and water. It is an alternative method to surface decontamination of viruses and bacteria in a very short time. The disinfecting effect begins at an O_3 level of 0.01 ppm; It destroys 90% of bacteria at a concentration of 0.02 ppm. Due to its microorganism-killing effect, intensive research is being carried out into its use for food preservation and sterilization. Foods such as high-fat fresh meat require more O_3 than low-fat, high-carb fruits and vegetables. O_3 can be applied directly to unprocessed foods during pre-treatment or storage, or to processed products during storage.

The inactivation of the microbial load in food by O_3 is highly dependent on the type of surface composition, the microorganism, the type of food product, the O_3 concentration applied, and the duration of application. The low concentration and short contact time of ozonation are sufficient to extend the shelf life of vegetables and fruits without affecting their organoleptic properties. The O_3 concentration applied to refrigerated stored food is 2-7 ppm. A 4-10 minute contact of water with O_3 provides disinfection. About 0.1-0.5 mg/l O_3 kills almost all bacteria. O_3 disinfection occurs when O_3 ruptures or melts the cell membranes of bacteria (Dubey, Singh, & Yousuf, 2022). Various factors such as water quality, ambient temperature, pH, product composition, and contact time influence the bactericidal effectiveness of O_3. High doses of O_3 impair sensory properties such as the color and taste of food. It has been observed that very low O_3 concentrations are very effective in inhibiting microorganisms during cold storage of fruit and vegetables. It is an effective disinfectant to decrease the microbial load on the food surface and an effective fumigant to protect against insects (grain) during storage.

The antimicrobial activity of O_3 is related to its high-oxidation potential, which leads to the degradation of microbial cell components. It was believed that the main target of ozonation is the bacterial cell. Two main mechanisms have been discovered in the destruction of target microorganisms by O_3: the first mechanism is that O_3 oxidizes the amino

acids and sulfhydryl groups of proteins and enzymes to shorter peptides. The second mechanism is O_3 also oxidized polyunsaturated fatty acids to acid peroxides. The cell disruption and the subsequent escape of cell contents result from the ozone-induced oxidation of unsaturated lipids in the cell membrane. Severe destruction and damage to nucleic acids can potentially lead to cell death. O_3 has been shown to inactivate a wide variety of organisms including parasites, yeast, fungi, viruses, viruses, and bacteria, and also oxidizes organic compounds and synthetic chemicals such as pesticides, herbicides, and detergents. O_3, both in gaseous form and dissolved in water, is used in the food industry to eliminate microbes from foods and food-contact surfaces (Hellwig, 2019).

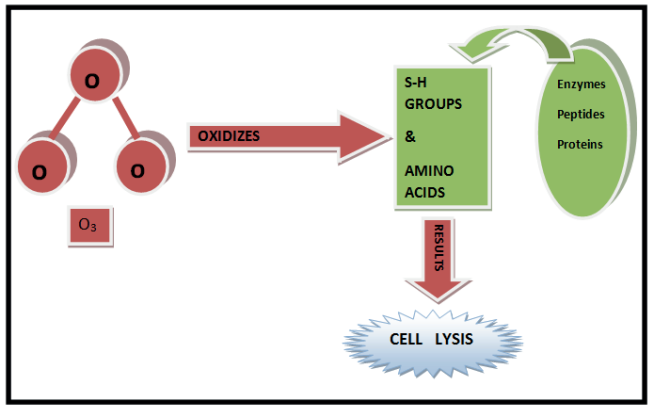

Figure 1. Schematic diagram showing the antimicrobial mechanism of ozone (Anonymous, 2023)

O_3 was recognized as generally safe (GRAS) by the FDA in 1982. O_3 molecules are destroyed within 30 minutes. eventually, it begins to convert to oxygen. O_3 does not generate waste and by-products, it reacts very quickly and is quickly consumed (it cannot be stored, is unstable at high concentrations, and turns into ordinary oxygen). Therefore, it should be manufactured where and when it is used. O_3 is used in two ways (as a gas or as ozonated water): a) release of ozone gas into the air; b) Add ozone gas to the water (Neale et al., 2021). O_3 is used in the food industry to remove aflatoxin and decrease the antimicrobial flora of meat, fish and chicken, fruits, vegetables, and spices. O_3, which is a good alternative to traditional disinfectants, is an effective disinfectant that can potentially be used in the food industry due to the advancement of generator production technology due to its small footprint, residue-free, non-mutagenic and non-carcinogenic (Xue, Macleod, & Blaxland, 2023). Since O_3 is rapidly decomposed, it must be prepared immediately before use. The two

known methods for generating O_3 are (1) corona discharge and/or (2) UV photochemical production. O_3 is generated by controlled high-energy electric charge/field corona discharge technology by passing dry air - or an O_2-containing gas mixture - through a temperature. O_3 is generated by UV photochemical generation of UV light (140–190 nm) shining on O_2 molecules. In both methods, the O_2 molecules are split into individual oxygen atoms, which combine with some other O_2. Once ozone-forming molecules are formed, O_3 can be dissolved in water as part of an aqueous solution or used directly as a gas form. O_3 is more effective in gaseous form than when dissolved in water. In water, O_3 quickly decomposes into oxygen. Generating O_3 using corona discharge technology has its advantages: it can generate a lot of O_3, it can be used in water and many other applications, it removes odorous compounds, and it is excellent for long-term use of devices. Although it is cheaper to generate O_3 with UV technology, less O_3 is generated in limited applications (Anonymous, 2020).

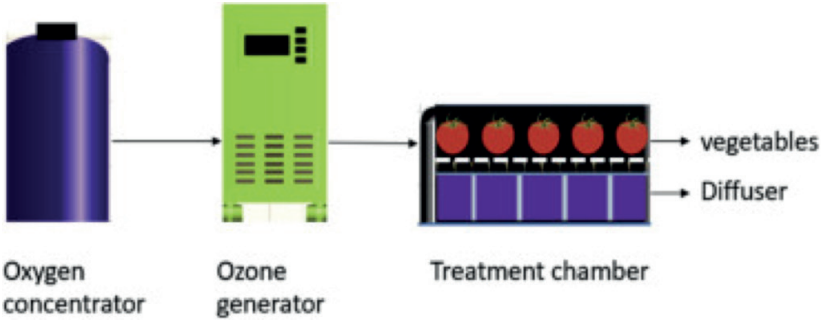

Figure 2. Generating ozone in aqueous form (Button, 2020).

The gaseous form is preferred because of the higher stability of O_3 compared to the aqueous form. Due to the gas stability of O_3, an O_3 generator is necessary in practice. Because O_3 is dispersed in the water, the oxygen is concentrated early in the process before entering the generation unit. Due to the instability, immediate use of ozonated water is essential. When applying gaseous ozone to food, air or pure oxygen flows into the O_3 generating unit and then enters the process chamber for gas treatment of the food. Although O_3 turns into oxygen gas after use, it is very important to know that O_3 is a harmful gas that can be fatal in low or high concentrations and irritate the respiratory tract. Therefore, it is necessary to monitor the amount of residual O_3 remaining after treatment and then pass this exhaust gas through an O_3 destructor to ensure that no O_3 remains and is not released into the environment (Epelle et al., 2022).

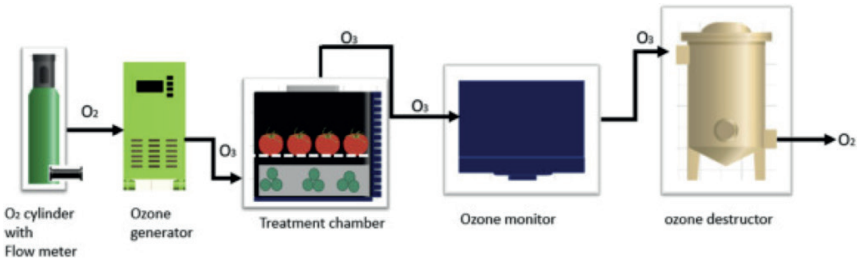

Figure 3. How ozone is produced, monitored, and applied in gaseous form (Button, 2020).

O_3 is 50% stronger than chlorine without using such a high concentration. Residual O_3 quickly decomposes into oxygen and leaves no residue on food. O_3 is effective in increasing the shelf life and microbial safety of meat and poultry, fish, dairy, beverages, and condiments. Its effectiveness against pathogens and spoilage microorganisms depends on several factors (e.g. food product, targeted microorganisms, level of contamination, gas or water, concentration, use, contact time, humidity [as gas application], ambient or product temperature, etc.). Today, O_3 is replacing traditional methods of sanitation such as steam, hot water, and chlorine-based chemicals. It is gaining popularity in food processing plants as the least expensive and chemical-free way to manage the safety of foods (Katherine & Ali, 2008).

3. APPLICATION OF OZONE IN FOOD INDUSTRIES

Food can be applied both as aqueous sprays and as gaseous ozone. Fresh produce sanitation is one of the common applications of non-thermal food processing techniques. O_3 is relatively sensitive to the product. High levels of O_3 can be used before the taste or appearance of the product changes. Another benefit of O_3 is that it extends product shelf life by decreasing the microbial flora in the wash water and on the product surface, keeping the wash water clean and using less water.

The effect of O_3 is not only limited to fresh carcasses but also to processed meat. The shelf life of meat products depends on the number and diversity of the microbial population. Contamination of meat with microorganisms occurs at various stages of slaughter, storage, and processing. O_3 also reduces bacterial contamination of beef and prevents the growth of various microorganisms on the meat's surface. The microbial effectiveness is positively influenced by low temperatures and a long exposure time. It is used in the spray wash water. A major problem with eating meat diseases is contamination of the meat with *Listeria*, *E. coli*, *Salmonella,* and *Campylobacter.* Therefore, the meat processing plant is

researching new methods to combat pathogens. The use of 10-20 g/l O_3 in warehouses increases the storage time of carcasses by 30-40%. In certain cases, O_3 affects lipid oxidation and leads to unfavorable conditions that affect product quality. Because O_3 is a strong oxidizing agent, O_3-treated products can cause meat quality problems if care is not taken. It has been found that lipid oxidation is affected by O_3 treatment. The change in the parameter is possibly related to the high oxidizing power of O_3 and the characteristic lipid profile of chickens, which is rich in unsaturated fatty acids. The oxidation of lipids and the formation of by-products can lead to changes in the aroma and flavor profile of meat (Khanashyam, Shanker, Kothakota, Mahanti, & Pandiselvam, 2022).

Poultry meat treated with ozonated water reduced the overall bacterial load. There were no adverse effects on the color or lipid peroxidation or off-flavor of O_3 poultry meat, and surface microflora growth was also retarded and shelf life extended. O_3 treatment of poultry appeared to be an effective method in reducing gram-negative microorganisms. These results showed that O_3 is a good disinfectant for meat. O_3 increases lipid oxidation in duck and chicken meat while destroying essential fatty acids. The O_3 treatment did not cause a significant increment in lipid oxidation or sensorially rated off-flavor production in meat. Therefore, with proper care, it is possible to use O_3 as an antimicrobial agent for poultry without adversely affecting lipid stability and appearance (Muhlisin, Utama, Lee, Choi, & Lee, 2016).

O_3 is a potential fumigant for deworming stored produce and is an effective antimicrobial agent with little or no effect on grain quality. The use of ozone for grain products reduces bacterial and fungal growth, storage pest control, and mycotoxin breakdown. O_3 treatment has minimal impact on the nutritional quality of the flour. O_3 has a significant impact on dough properties (Sivaranjani, Prasath, Pandiselvam, Kothakota, Mousavi, & Khaneghah, 2021).

The exposure of grain to O_3 is usually carried out in hermetic storage halls with a certain moisture content of the grain and a minimum layer thickness. The operations of the process include pre-wetting and ozonation of the grains in a reactor.

O_3 exposure is a viable alternative for increasing the shelf life of a variety of food products such as pickles, juices, jam, jelly, sorbet, ice cream, and nutraceutical applications (Sarron, Gadonna-Widehem, & Aussenac, 2021). Post-harvest processing, poor handling and storage, inadequate transportation facilities, use of contaminated wash water and cross-contamination from other produce, and microbial hazards of fresh fruit and vegetables (Murray, Wu, Shi, Jun Xue, & Warriner, 2017). Even low

O_3 concentrations extend the shelf-life of fruits and vegetables. The trend is due to ethylene. Removal of oxidation and other metabolic products by O_3. The effectiveness of O_3 was compared to chlorine solutions and peracetic acid (Korany et al., 2018). The highest reduction in microbial load population was achieved with O_3 in strawberries, apples, lettuce, and melons contaminated with *L. monocytogenes* and *E. coli O157:H7* (Linares-Morales, Gutiérrez-Méndez, Rivera-Chavira, Pérez-Vega, & Nevárez-Moorillón, 2018; Nayak et al., 2020; Tzortzakis & Chrysargyris, 2016).

O_3 is commonly used in fruit and vegetable processing to decompose mycotoxins, inactivate pathogenic and spoilage microorganisms, and destroy chemical residues such as pesticides. O_3 is widely used in food processing plants, including surface decontamination of foods and water disinfection applications. It is regularly used in the washing of fresh produce. The O_3 application on grapes and blackberries significantly reduces decay caused by fungal infections, thus increasing their peel (Botondi, Barone, & Grasso, 2021; Horvitz, Arancibia, Arroqui, Chonata, & Virseda, 2021).

The effectiveness of respiratory rate inhibition increased with increasing aquatic O_3 concentration, showing that aqueous ozone could slow tissue metabolism (Miller, Silva, & Brandão, 2013). O_3 treatment on vegetables has similar benefits to fruit storage and processing.

Bacterial counts were greatly reduced when O_3 was applied to fruits and vegetables. In addition, pathogenic microorganisms in fruit and vegetable products were reduced by the O_3 treatment. A quick rinse with 1.4 mg/L of ozonated water for 15 minutes of exposure time can remove more than ¼ of the residual pesticides on the vegetable. However, it has been suggested that higher concentrations of O_3 could drive off even greater amounts of pesticide fires. It indicates that for every product there is a critical limit of O_3 exposure time and concentration, exceeding which can damage the product. Ozone application had no significant effect on the color change of tomatoes, apples, and papayas during storage. Conversely, the application of O_3 on tomatoes at 38 to 95 g/L for 10 minutes delayed the red color occurring during storage. O_3 at 10 to 115 g/L carrot orange they noticed the bleaching effect on the red color. O_3 has extended the shelf-life of cabbage, carrots, and potatoes. Vegetables such as lettuce, parsley, and spinach resulted in an average 2-log decrease in *E. coli* and *L. innocua*. In addition, onions were treated with O_3 during storage. The number of bacteria is greatly reduced without changing the sensory quality and chemical composition (Silva Neto et al., 2019; Sarron et al., 2021; Sun, Wu, Zhang, & Wang, 2022).

Several researchers have examined the hygiene application of O_3 in freshly cut products with the aim of microbial inactivation while preserving sensorial quality and nutritional characteristics (Botondi et al., 2021; (Di Gioia et al., 2020). Detrimental changes in functional ingredients, flavor, and color typically occur with pasteurized juices (Aghajanzadeh, Ziaiifar, & Verkerk, 2021). Great demand for safe, preservative-free, and healthy juices with "fresh-like" properties (Aneja, Dhiman, Aggarwal, & Aneja, 2014). O_3 application resulted in color changes in orange juice, cider, and strawberry juice (Sung, Song, Kim, Ryu, & Kang, 2014). When water is ozonated, hydroxyl radicals are formed in the environment, which open up aromatic rings and can lead to the oxidation of organic acids, ketones, and aldehydes. Jaramillo Sánchez, Garcia Loredo, Contigiani, Gómez, and Alzamora (2018) observed a slight increase in browning of ozonated peach juices. The development of browning can be dependent on non-enzymatic and enzymatic reactions in non-ozonated peach juice, which can be explained by phenolic compound oxidation. In addition, O_3 treatment does not affect the pH, Brix, titratable acidity, turbidity, and cider, tomato, and orange juice.

The seafood industry uses O_3 for cleaning processing surfaces. Freezing is the most important method to overcome seafood perishability. O_3 cleaning also helps preserve the quality of seafood. The use of O_3 during production results in a cleaner facility. The use of dipping ozone-rich water technique or washing fish and crustaceans resulted in an effective decrease in microbial load while not affecting product quality. O_3 suppresses the initial count of all E. coli bacteria, increasing their durability by almost 25%. It is shown that the addition of 0.6 ppm O_3 to 3% NaCl reduced the total microbial flora in the skin of the evacuated fish (Luiz et al., 2017). Combining O_3 with cold storage is an attractive method to extend the shelf-life of fish. The use of aqueous ozonation to wash fish reduced microflora. Aqueous ozonation minimizes wash time and improves color. O_3 therapy was used for washing fish surimi. The researchers found that dried squid exposed to ozone reduced *micrococci* and did not cause any discoloration.

Water is an important part of the food processing industry. Since extensive water is used in the food plants, great emphasis is placed on economic considerations. O_3 purification is of great interest because ozone's antimicrobial activity is approximately 3,000 times that of chlorine and it also efficiently dissolves in water. O_3 reacts directly at low pH, while the non-selective, indirect reaction of O_3 with water at high pH produces reactive oxygen compounds (Epelle et al., 2023).

O_3 can also be used to control stored grain and other foods from mycotoxins, fungi, and insects as an alternative to chemical treatment. O_3

is widely used also in disinfection, clean-in-place (CIP), sterilization, and fumigation of production areas, factories, and surfaces.

CONCLUSION

O_3 has been an effective surface decontamination method for improving the safety/quality/functionality of foods. It is an energy-saving method since the use of O_3 does not contain very high temperatures. More research efforts are required to examine the use of O_3 in the preservation of foods where infestations from molds, bacteria, and insects are common. Despite ozone's proven benefits in producing safe-to-eat foods with high quality, specific treatment conditions need to be standardized for each food. Pasteurization, high-pressure treatment, UV, membrane treatment, and some combination applications of freezing and ozonation can be very effective in microbial inactivation and increase the shelf-life of foods.

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Chapter 17

POSSIBILITIES OF USING MILK THISTLE (*SILYBUM MARIANUM L.*) PLANTS IN THE FOOD INDUSTRY

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1. INTRODUCTION

The use of wild plants by humans as a food source or for therapeutic purposes has been known since ancient times. Especially they were widely used after the 1900s (Baytop, 1984; Faydaoğlu & Sürücüoğlu, 2011).

It is understood from the literature that different plants have been used in the treatments of many diseases such as cancer, which has been a great issue for humanity for centuries. The development of modern medicine has also supported applications of plant-based treatment from a technical point of view, increasing the possibilities of its use in the treatment of different diseases (Baytop, 1984; Kähkönen, Hopia, Vuorela, Rauha, Pihlaja, Kujala, & Heinonen, 1999).

Although there are many synthetic antioxidants and antimicrobial substances such as butylated hydroxytoluene (BHT), butylated hydroxy anisole (BHA), and ter-butyl hydroquinone (TBHQ) in food and the pharmaceutical industry, studies have shown that these compounds can have toxic and carcinogenic effects on humans. The emergence of findings on the subject has directed both the industry and scientists to use natural resources instead of chemical ones. This major change in the industry increasingly popularized the use of vegetable sources, which are cheap and easily accessible (Çalışır & Çalışkan, 2003; Elzaawely, Xuan, Tawata, 2005; Rajeshwar Kumar, Gupta, & Mazumder, 2005; Karatepe & Ekerbiçer, 2017).

Furthermore, the accessibility and affordable prices for healthier options increased the use of such natural resources and raised the quality of the products in the mentioned sectors. The belief that dietary fiber, protein, and carbohydrates might have positive effects on the texture of the products, as well as the protective compounds in the structures of natural plants, thus created positive effects on consumer preferences and has guided further studies (Kılınççeker, 2014; Kılınççeker, 2015; Mardar & Znachek, 2019).

The compounds that provide the antioxidant properties of these plants are called the 'phenolic substances' which are found in the structure of plants. Substances such as flavonoids and phenolic acids, which are polyphenolic compounds, are materials with strong antioxidant properties and can be found in different parts of plants at different rates (Pietta, 2000; Kılınççeker & Kurt, 2008; Heim, Taigliaferro, & Bobilya, 2002).

When the literature is examined, different studies can be found on the use of many plants in various sectors due to their antioxidant and antimicrobial properties. One such plant is the milk thistle plant

(*Silybum marianum*), which has many good properties if used right, but the applications of this plant in the food industry are quite inadequate, unfortunately. In Türkiye, this plant is also known as ala kenger or milk kengel, and it is a one-year plant that can grow naturally and can be cultivated (Figure 1). Due to the phytochemicals in its structure, it is widely used in the treatment of liver diseases in many countries around the world. It is also used as a diuretic, appetite stimulant, and pain reliever. It is important to evaluate it as a safe plant since it has no known side effects (Baytop, 1984; Montvale, 2000).

Figure 1. Milk thistle plant

This plant is a common species in Southern Europe, North Africa, Türkiye, and Russia. It widely grows in Türkiye, especially in the Mediterranean region, Aegean region, and South Eastern Anatolia, on the edges of fields and in vacant lands. While many parts of this plant such as flowers, stems and leaves can be used for consumption, the most commonly preferred part is the seed. This plant is consumed in salads, boiled, or fried in many countries, and it is a rich source of fiber, Ca and K. There are recommendations regarding the use of the seed part in the composition of bakery products by turning it into flour (Figure 2). Especially the high ratio of unsaturated fatty acids and the presence of flavonolignans as structural content are among the other advantages (Eren & Şar, 2020; Doğan, Kara, Gur, & Bagci, 2022; Marceddu et al., 2022).

Figure 2. Milk thistle plant seed

Although the results vary in many studies, in general, the milk thistle seed has 19-30% protein, 20-30% fat, and 24.2-26.3% carbohydrates, and can also contain approximately 29-30% crude fiber, as well as essential amino acids and fat. It has also been emphasized as a good herbal source in terms of acids and minerals (Khalil, 2008; El-Haak et al., 2015; Apostol, Iorga, Mosoiu, Racovita, & Niculae, 2017). The most common substances in the composition of seed oil are oleic acid at 36.7% and linoleic acid at 39.7%, approximately. The remainders are palmitic acid and others. (Dogan et al., 2022). In addition, it has been stated that it is advantageous for the food industry because it is a gluten-free plant source (Bedrnícek et al., 2022).

In a study, the proportional values of some components of the seeds of milk thistle plant obtained from different regions were determined as shown in Table 1. Similar to other studies, it was emphasized in this study that the seed of the plant is rich in proteins, oils, and fiber contents. It has been emphasized that according to these components, it can be beneficial for many industries (Aziz et al., 2021).

Table 1. Some components of thistle plant seed (Aziz et al., 2021).

	Varieties	**Proteins (%)**	**Ash**	**Fat**	**Fibre**	**Moisture**
			(%)	**(%)**	**(%)**	**(%)**
Location 1	Blue	30.09	2.37	19.74	7.4	6.27
	White	27.60	2.10	20.79	7.0	6.90
Location 2	Blue	25.65	2.07	21.51	6.40	5.13
	White	23.01	1.83	22.73	5.60	5.60
Location 3	Blue	21.45	1.98	22.90	4.73	4.87
	White	20.74	1.25	23.19	4.39	5.01

In addition to antioxidant activity in many studies, thistle seeds are said to have anti-inflammatory, anti-carcinogenic, anti-atherosclerotic, anti-hypertensive, anti-diabetic, and anti-obesity properties. For this reason, especially results that reveal their importance for health, as well as nutrition, are mentioned (Montvale, 2000; Khalil, 2008; Aziz et al., 2021; Krystyjan et al., 2022).

This plant contains structurally flavonolignans derivative compounds (silymarin: silybin, iso silybin A and B, silychristine, silydianin, 2,3-dihydro silybin, 2,3-dihydrosilychristine, 3-deoxysilycristine, 3-deoxysilydianin, isosilychristin, silandrine, silyhermin, and neosilyhermin). It also contains some flavonoids (taxifolin, quercetin, dihydrochemferol, kaempferol, apigenin, naringin, eriodicthiol, chrysoeriol) and other compounds (fixed fats, sterols, dihydroconiferyl alcohol, proteins, and mucilage). The active compound is a flavonolignan mixture called silymarin. The major compound in silymarin is silybin. Thistle seeds generally contain 1-5% silymarin. The extracts obtained from the seeds contain 70-80% silymarin. On the other hand, other parts (leaf, flower, and root) of the thistle do not contain silymarin. The aromatic hydroxyl groups in the structure of some components of silymarin shown in Figure 3 are highly radical quenchers, that is, antioxidant elements (Quaigle et al., 1999; Çelik & Kan, 2013; Eren & Şar, 2020; Aziz et al., 2021).

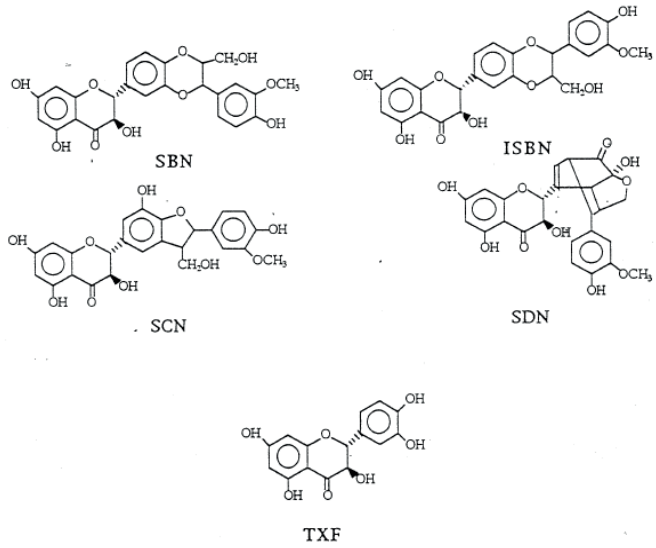

Figure 3. The basic structure of the important components of silymarin, SBN (silybin), ISBN (isosilybin), SCN (silychristine), SDN (silydianin), TXF (taxipholine) (Quaglia et al., 1999).

Most of the substances that make up silymarin are effective components of the milk thistle plant, are flavolignan derivatives mentioned above, and also shown structurally (Figure 3). However, considering both the amount and the benefits it provides, the most major and popular one among them is highlighted as silybin. In a study carried out to emphasize the importance of this component, as a result of further analyses applied to different parts of milk thistle, the part with the highest silybin was determined as the seed (Table 2). After, it has been stated that the use of this part in the applications to be made in future processes may produce more effective results (Rodriguez et al., 2018).

Table 2. The silybin contents of different parts of the milk thistle plant (Rodriguez et al., 2018)

Part of plant	**Content (mg/g DW)**
Leaves	0.113±0.004
Petioles	0.043±0.002
Flowers	0.083±0.001
Stems	0.064±0.001
Seeds	7.434±0.041
Germinated seeds	0.852±0.002

For example, in a study, it was revealed that the addition of milk thistle extracts and derivatives as well as pure silymarin to food slows down the oxidative process, helps maintain the nutritional quality of the foods they are applied to, and extends the shelf life of the product. It has also been specifically stated that these plant derivatives can exhibit a protective effect against cytotoxicity caused by arsenic, which is a serious problem in heavily polluted areas (Marceddu et al., 2022).

In another study, the free radical scavenging capacities of silymarin, which is added to sunflower oil at different concentrations, with a synthetic antioxidant, BHT, were compared (Table 3). As a result of the experimental studies, it was understood that the radical scavenging capacity increased significantly in parallel with the increase in the concentration of silymarin in the medium. In the aforementioned study, it was emphasized that silymarin can be a good antioxidant for such products and the importance of studies on this component for future periods (Abdelazim, 2017).

Table 3. DPPH scavenging activity of silymarin (Abdelazim, 2017).

Treatment	**DPPH scavenging activity (%)**
BHT 200 ppm	72.78±0.25
Silymarin 100 ppm	70.27±0.19
Silymarin 150 ppm	73.71±0.11
Silymarin 200 ppm	77.89±0.20
Silymarin 250 ppm	82.80±0.01
Silymarin 300 ppm	86.77±0.02
Silymarin 350 ppm	86.77±0.02
Silymarin 400 ppm	86.77±0.02
Silymarin 450 ppm	86.77±0.02
Silymarin 500 ppm	83.78±0.15

In the same study, the disc diffusion method was used in the trials to determine the antimicrobial effect of silymarin against some standard microorganisms, and the disc diameters that increased linearly with the antimicrobial effect were measured (Table 4). As a result of the analyses carried out; it was determined that silymarin at different concentrations showed significant antimicrobial effects against gram-positive bacteria, gram-negative bacteria, and molds used in the study, whereas it was significantly effective against only *G. candidium* from yeasts. In the study, it was observed that the antimicrobial effect of silymarin increased with the increase in concentration and it was more effective, especially on molds. In line with all the results found in the aforementioned study, it was stated that silymarin could be a natural antimicrobial agent for the food industry (Abdelazim, 2017).

Table 4. Antimicrobial effect of silymarin (Abdelazim, 2017)

Microorganisms	**Diameters (mm) of inhibition zones at different silymarin concentrations (100-500 ppm).**								
	100	**150**	**200**	**250**	**300**	**350**	**400**	**450**	**500**
Gr (+) bacteria									
B. subtilis ATCC 33221	23	23.5	24.5	25	26	26.2	26.5	27.8	28
B. cereus ATCC 33018	22	23	23.5	24.5	25	26	26.2	26.5	27.5
B. cereus NRRLB 3711	22	23.2	23.7	24.7	25.2	26.3	26.5	26.8	27.6
S. aureus ATCC 20231	21	22	23	23.5	24	24.7	25.6	26	27
Gr (-) bacteria									
E. coli ATCC69337	25	25.4	26	26.5	27.1	27.3	28	28.4	29
E. coli 0157:H7	25.1	25.5	26.2	26.5	27.2	27.5	28	28.4	29
P. aeruginosa ATCC 9027	24.8	25.3	26	26.3	26.5	27	27.5	28.4	29.1
Yeast									
S. cerevisiae NRRLY2034	0	0	0	0	0	0	0	0	0
C. lipolytica NRRLY1095	0	0	0	0	0	0	0	0	0

G. candidum NRRLY552	21	21	21.5	22	23	23	24	24.5	26
Mold									
A. niger NRRL326	35	37	40	42	44	45.3	46	46.5	47
A. flavus MERCN 101	37	41	43	44	45.5	46	46.5	47	48
A. parasiticus	32	33	35	34	34.6	34.7	44.4	45.2	46.2
Penicillium sp.	31	32	33	33.5	34.1	35.5	36.5	37	37.9
Total antimicrobial spectra	318.9	331.9	313.9	352.5	362.2	369.5	385.7	392.2	402.3

While many studies mention the advantages of the milk thistle plant, some studies caution about the nitrate accumulation in the edible parts of this plant is an issue that needs further study. In these studies, it was stated that the use of this plant in human nutrition should be expanded by controlling nitrate accumulation if necessary (Marceddu et al., 2022).

As it can be understood from the studies highlighted above, although there are some studies on the use of thistle plants in different industries, it has been observed that research on its use in the food industry is insufficient and the necessity of new research on this subject has been particularly emphasized (Mardar & Znachek, 2019; Bojňanská et al., 2020; Krystyjan et al., 2022). From this point of view, this study is aimed to emphasize that there may be more useful areas of use in the food industry depending on the functional properties of the structural elements of the thistle plant and that this issue should be investigated in detail.

2. STUDIES ON THE USE OF MILK THISTLE PLANTS IN THE FOOD INDUSTRY

Due to the above-mentioned properties of the thistle plant, it is understood that it can both improve product structure and increase shelf life in the food industry. Since it is a natural resource, it is thought that it may have positive effects on consumer health as well as its use in product development. Some of the studies based on this idea are summarized below as examples.

In a study conducted by Apostol et al. (2017), 5%, 10%, and 15% of oil-separated milk thistle seed flour was added to bread wheat flour and the effect of this flour on some quality properties of the dough was investigated. At the end of the study, it was determined that thistle seed flour used significantly increased the amount of calcium, magnesium, iron, and potassium in the dough, and rheological properties were formed within the desired limit values.

Bortlíková et al. (2019) determined that the quality values of the baked biscuits produced with thistle seed flour were similar to the samples

made with wheat flour, in their study by mixing 5-30% milk thistle seed flour, whose functional feature was emphasized, depending on its components. They found also that some physical and chemical properties could be improved in the biscuits they produced. As a result, they stated that this seed can be used in the production of biscuits rich in functional components.

Mardar & Znachek (2019) researched the effects of these herbal elements in developing healthy bread in their study using thistle, ash, rosehip, and green tea extract powders. They determined the optimum composition of the bread they produced by mathematical modeling. They made some analyses about the chemical quality, sensory quality, and biological value of the bread produced in this way. In the analysis made at the end of cooking, while the protein and fiber ratios in the bread increased, they found a decrease in the starch content. They also found that breads produced with herbal powders contain biologically active substances that are beneficial for health. They found that bread especially contains compounds with antioxidant activity and hepatoprotective activity and can be recommended for healthy nutrition. In addition, they gave a 6-month shelf life guarantee for bread at 18 °C and 70-75% relative humidity.

In the research conducted by Bojňanská et al. (2020), the effects of dough prepared by adding reduced fat milk thistle seed flour to wheat flour and rye flour in different proportions on the fermentation process and the quality of the baked products were investigated. It was determined that milk thistle seed flour added to the composition changed the viscoelastic properties of the dough, increased the development time and stability of the dough, and as a result, all the bread obtained as the final product had high volume and pore structure. At the end of the study, it was stated that adding 5%, 10%, or 15% milk thistle seed flour to a mixture of wheat flour + rye flour could give suitable results for dough rheology, and according to the baked product results, it was emphasized that milk thistle seed flour could be a suitable plant source for bakery products with functional properties.

In another study by Krystyjan et al. (2022), ground milk thistle seed was used at rates of 5-20% in biscuit production and the effects of this seed on some quality criteria were determined. As a result, although the samples containing ground seeds were of lower volume and lower hardness, it was observed that the spreading rate increased in these samples. Especially, it was determined that using this seed increased the amount of phenolic substance in the biscuit content and accordingly the antioxidant properties. Also, while it was determined that the biscuits

containing 5% ground seeds were more preferred in sensory scores, the amount of 20% negatively affected the product quality.

Similarly, thistle seed pulp whose oil was separated by pressing by Bedrnícek et al. (2022), was obtained in various sizes and added to bread compositions made with different gluten-free flours. In the results obtained in this study; it was observed that adding 10% milk thistle pulp fraction significantly increased the protein, fiber, fat, ash, and silymarin content in gluten-free bread. In addition, it was determined that the breads obtained by adding this material were superior in terms of sensory properties and texture. Based on all the findings, it was also stated that the thistle seed fraction, whose oil was separated, would increase the quality of bread in terms of nutrition and increase the rate of functional components beneficial for human health in the product.

In the last work, Hallaç and Kılınççeker (2023) investigated the effect of adding 7.5% of thistle seed flour and wheat flour mixtures prepared in different proportions to chicken meatballs on some properties of meatballs after frying. They observed that milk thistle seed flour was added to wheat flour at the rate of 50% and 70%, which increased the yields after frying. They found that the *a* value increased in all samples with milk thistle flour. In addition, they found that the use of 50% and 70% increased the amount of moisture retained in the fried meatballs. They determined that the sensory scores of the meatballs prepared with this flour were close to those of the control. As a result, they stated that it can be recommended thistle seed flour be mixed with wheat flour at the rate of 50% and 70% and used in making meatballs for consumers.

CONCLUSIONS

As it can be understood from all the aforementioned, while the thistle plant contains important elements in terms of nutrition, some parts such as leaves and seeds obtained from this plant are sources that should be evaluated due to their components. Especially in the food industry, with the use of these plant parts, it is thought that while the deterioration of foods can be delayed, some diseases in consumers can be prevented. In addition, it is understood that they can be used as alternative natural and cheap materials instead of synthetic antibiotics in application and thus they can make a significant contribution to the economy. In addition to nutritionally important elements such as dietary fiber, protein, and fat, it is thought that they can increase the quality and extend the shelf life of the foods they are added to, especially due to their antioxidant and antimicrobial bioactive compounds. Also, it can be said that they can provide protective properties against diseases such as celiac disease, obesity, and cancer, which are important issues of recent years. In the

development of new products, they will contribute to both the protection of consumer health and the development of their technological properties in foods by adding them to the composition in a way that does not impair their sensory qualities and by the production of new functional foods. In addition to human consumption, it is thought that the use of these plant parts in animal nutrition may have important benefits in preventing zoonotic diseases, protecting animal health and environmental health, and reducing feed costs. Considering these, it is foreseen that new business areas can be developed within the scope of the evaluation of natural plants. In this direction, as can be understood from the experimental results mentioned, it was concluded that further research on thistle plants and their components in food production practices would be beneficial for both food producers and consumers.

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The rapid increase in the world's population and developments in the food sector lead both producers and consumers to seek alternative food. In addition, the development of awareness of healthy nutrition accelerates this search. As a result of the aforementioned developments, products that can meet the demands of consumers in terms of economy and health are being put forward. Especially in food raw materials or production processes, new products and methods are tried to be developed by going beyond the traditional methods. In this way, while protecting the nutrition and health of the consumers, an economic balance is tried to be achieved. It is aimed to develop maximum quality products by using new methods. When we look at the literature in line with the aforementioned, it is understood that while many studies stand out, it is understood that the researches on some subjects are insufficient and these subjects should be examined. In this book, some of these topics are discussed and their applications in the food industry are emphasized. In this way, it is desired to give an idea about the mentioned applications to both producers and consumers. In addition, it is aimed to present an alternative reference book to academics who conduct research on these subjects.

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