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Doç. Dr. Fatma HEPSAĞ

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Chapter 1

THE IMPORTANCE OF HERBS AND SPICES IN REDUCING THE GLYCEMIC INDEX OF RICA

Assoc. Prof. Dr. Ali YILDIRIM¹

¹ Department of Food Engineering, Faculty of Engineering, Harran University,
Şanlıurfa, TÜRKİYE

*ayildirim10@gmail.com, ORCID: <https://orcid.org/0000-0001-7226-1902>

1. RICE AS A COMPONENT OF HUMAN NUTRITION

Rice (*Oryza sativa* L.) belongs to the Poaceae family, which is a category of monocotyledonous herbs and plants. Together with wheat and barley, rice constitutes the dietary staple for over half of the global population. It plays a crucial role in the diets and livelihoods of more than 3.5 billion individuals. Annually, it is estimated that over 850 million tons of rice are cultivated worldwide, with rice plants occupying approximately 256 hectares of land. Asia stands as the foremost rice producer, accounting for about 90% of the global rice output. Of this production, roughly 75% is consumed by Asians, making rice a vital component of their diet (Kurniasih et al., 2019).

In addition to its role in human nutrition, rice is also utilized in the production of various other products and possesses medicinal properties. As a staple food in numerous nations, rice holds significant importance. Guyer et al. (1998) indicate that approximately three billion individuals in Asia derive nearly half of their caloric intake from rice. This grain is exclusively consumed by humans, with over 20% of the global population relying on rice for their caloric needs (Garris et al., 2005). According to Yusof et al. (2005), rice serves as a primary source of energy, nutrients, and carbohydrates. It contributes to 20% of the global dietary energy supply and is rich in thiamine, riboflavin, niacin, and dietary fibre. Unmilled rice retains more nutrients compared to its milled or polished white counterpart. Furthermore, rice is a fundamental component of the culinary practices of diverse cultures, each exhibiting unique preferences for the texture, flavour, colour, and stickiness of the rice they consume. The nutritional profile of rice can be enhanced through both traditional selective breeding methods and modern technologies, including genetic modification of the plant's genome.

Numerous elements influence the structure, chemical makeup, and physical characteristics of rice. One of the primary elements is amylose content. This component is utilized in medical applications to assess allergies and bodily reactions to insulin and glucose, as well as to predict the digestion rate of cornstarch. Foods high in amylose content is linked to lower blood glucose levels and a healthier gastrointestinal system when compared to those with minimal amylose levels (Frei et al., 2003). Therefore, the constituents of rice exert beneficial effects on human health.

Irtwange (2000) concludes that each grain, particularly rice, is evaluated according to its nutritional components, physical characteristics, cooking methods, flavour, and aroma. The glycemic index of rice (GI), which indicates the rate at which rice elevates blood glucose levels, is in-

fluenced by the duration and quantity of water utilized during cooking. The volume of water employed in the preparation of rice impacts its digestibility, which refers to the speed at which it is processed by the body (Daomukda et al., 2011).

Whole grain rice serves as a chemical repository, primarily containing 80% starch, which consists of varying proportions of amylose and amylopectin, along with 6–8% proteins. In contrast, fats, dietary fiber, minerals, and secondary metabolites are present only in trace amounts, as they are lost in the rice bran during the milling process (Butardo Jr & Sreenivasulu, 2016). Starch is typically broken down by humans into glucose, which cells utilize to generate energy for metabolic activities, while any surplus energy is stored as glycogen or fats for future use (Reynolds et al., 2019).

In contemporary society, individuals seek the highest quality rice available for consumption globally. For rice to gain acceptance among consumers, its quality must be satisfactory (Asghar et al., 2012). During the cooking process, moisture interacts with the rice's components, causing the grains to swell due to gelatinization. Additionally, certain constituents and components of the rice may dissolve into the cooking water. The absorption of moisture by the rice kernels is crucial for effective rice preparation and cooking (Kasai et al., 2005).

The impact of soaking temperature on water absorption and solubility is significant. The heated kernels, following the soaking process, lead to a combination of starch granules and irreversible swelling. Consequently, the starch granules transform into gelatin and undergo degradation, resulting in various changes in the rice. This transformation is crucial for subsequent processing operations, including eating quality, cooking, milling, and storage (Patindol et al., 2008)..

2. STRUCTURE OF THE RICE GRAIN

The morphological structure of rice grain is illustrated in Figure 1. Approximately 70% of the rice grain comprises endosperm, 21% hull, 8% rice bran, and 1% embryo (Chakuton et al., 2012). The starchy endosperm represents the predominant component in rice caryopsis, accounting for 89-94%, while the remaining components, including aleurone, seed coat, and nucellus (4-6%), embryo (2-3%), and pericarp (12%), are classified as minor constituents. The rice grain, also known as rough rice or paddy, features a protective outer layer termed hull, surrounding the rice fruit or caryopsis, which can be referred to as brown, cargo, dehulled, or dehusked rice. The rice caryopsis is composed of three primary components: the seed coat and the embryo, known as the germ, the nucellus, referred to as the pericarp, and the endosperm. The pigment is localized within

the pericarp. The endosperm includes the aleurone layer that encases the embryo, safeguarding the germ, along with the sub aleurone layer and the starchy inner endosperm (Champagne et al., 2004) (Figure 1). Both the embryo and aleurone cells are abundant in protein bodies, which contain lipid bodies and globoids or phytate bodies (Juliano & Tuano, 2019). The sub aleurone layer is characterized by a high lipid and protein content, featuring smaller amyloplasts and starch granules compared to the starchy endosperm. The endosperm constitutes approximately 90-91% of the total weight of rice. The pericarp layer and the aleurone layer collectively are known as the bran of rice, which is significant in discussions regarding nutritional composition and the milling process (Juliano, 1972).

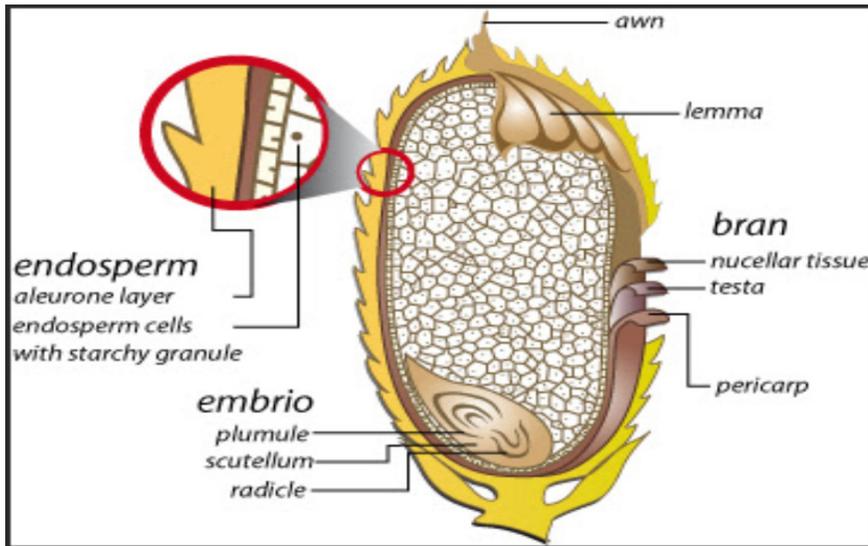


Figure 1. *The structure of rice grain (Shakri et al, 2021).*

3. CHEMICAL COMPOSITION OF RICE

The removal of the pericarp results in a decrease in the levels of lipids, proteins, fibre, and ash, which in turn reduces carbohydrates and total sugars, along with minor constituents such as vitamins, free amino acids, and free fatty acids (Zhou et al., 2002). Phenolic compounds are classified as secondary metabolites in plants. Flavonoids, which represent the predominant category of phenolic compounds in plants, consist of 15 carbon atoms arranged into two aromatic rings connected by a three-carbon chain. These compounds can be categorized into various groups, with anthocyanidins being the most prevalent, responsible for the red, pink, and purple hues of plants that attract animals for pollination and seed dispersal. The colour of the pericarp is linked to the phenolic content in the

grain, typically being more abundant in red and black pericarp grains. Research indicates that the dark purple colour group exhibits a greater concentration of polyphenols and anthocyanins compared to the red-brown colour group (Singh, 2002; Goffman & Bergman, 2004). The analysis focused on three primary chemical compositions: proximate analysis, anthocyanin content, and antioxidant activity.

The proximate composition pertains to the quantitative examination of macromolecules present in food items. These measurements are commonly referred to as nutritional facts, which are typically displayed on food product labels (Shen et al., 2009). The proximate analysis results for selected rice samples are presented in Table 1. Generally, the carbohydrate content is highest in white rice, exceeding 80%, as carbohydrates constitute the primary component of rice. The moisture content ranks as the second highest in rice, varying between 10.04% and 12.88%. This moisture content is crucial for regulating the shelf-life of rice. The ash content is found to be highest in black rice and lowest in white rice. Additionally, white rice has the lowest protein percentage. The fat content ranges from 0.07% to 1.74%, with black rice exhibiting the lowest fat content, while it is recognized as a significant source of fibre, recording the highest percentage at 8.47%.

Table 1. Proximate composition of white, brown, pigmented and glutinous rice (Shen et al., 2009)

Rice samples	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Total dietary fibre (%)	Energy (kJ/100 g)
White rice	12.08	0.39	5.96	1.24	80.14	7.07	1523.57
Brown rice	12.88	0.55	6.48	1.74	78.21	8.37	1487.90
Pigmented (Black) rice	11.07	0.90	6.35	0.07	78.26	8.47	1457.72
Glutinous rice	10.04	0.82	8.14	1.12	78.89	7.47	1502.65

4. IMPORTANCE OF CARBOHYDRATES AND STARCH FOR GLYCEMIC INDEX

The human body requires energy to maintain its functions. Carbohydrates serve as a vital energy nutrient, existing in various forms. Recent studies have indicated that hyperglycaemia and obesity contribute to health issues that affect numerous individuals (Deepa et al., 2008). Carbohydrates are not merely a source of calories; rather, specific types of carbohydrates are incorporated into the diet based on physiological

conditions (Marquart et al., 2002). Contrary to previous popular beliefs, carbohydrates have a beneficial effect on the body. They are present in fruits, breads, vegetables, cereals, and dairy products, making them a crucial component of a balanced diet. Upon consumption of any carbohydrate variety, the digestive system converts it into simple sugars that are absorbed into the bloodstream. It is important to note that not all carbohydrates are equivalent, as different types exert distinct influences on blood sugar levels (Jenkins et al., 1981). Rice, for instance, is abundant in carbohydrates and serves as a primary energy source. Nevertheless, excessive consumption should be avoided, as it can lead to diabetes and weight gain (Shi & Gao, 2011). When consumed in moderation, rice can be an invaluable source of energy.

The primary constituent of rice is starch, which comprises amylose and amylopectin. Rice is typically regarded as a food that ranks high on the glycemic index (GI), which is linked to various risks including diabetes, obesity, and cardiovascular diseases. Different varieties can yield rice with differing amounts of amylose, consequently leading to a range of glycemic index values (Gunaratne et al., 2020).

Cereal grains are among the primary sources of starch, which significantly influences the technical characteristics and nutritional value of starchy products. Starch is composed of polymeric chains of glucose residues, primarily in the forms of amylose and amylopectin. Amylopectin features a branched structure made up of D-glucose units that are interconnected by α -1 \rightarrow 4 bonds, with branches linked through α -1 \rightarrow 6 glycosidic bonds. In contrast, amylose has a linear arrangement of D-glucose residues that are linked solely by α -1 \rightarrow 4 bonds (Lee & Lee, 2012).

Starch granules consist of a coagulation of numerous starch molecules, which can be separated into primarily linear chain amylose and highly branched amylopectin. The primary distinction in the composition of rice starch arises from the relative proportions of these two factions within the starch granules. This variation, in conjunction with the diffusion chain length and the frequency and spacing of branching points in the amylopectin molecule (Lu et al., 1997), contributes to the overall characteristics of the starch. Furthermore, the outer layer of the grain kernel serves to protect the starch from enzymatic degradation (Juntunen et al., 2002).

5. IMPACT OF MILLING ON THE NUTRITIONAL VALUE OF RICE

Due to a variety of natural and anthropogenic factors, products are often lost, especially during the harvesting phase. Additional waste occurs

in subsequent stages such as storage, transportation, and handling. The primary causes of these losses are typically linked to inadequate handling practices during the drying and milling processes. Statistically, the losses incurred during milling and polishing are significant. The extent of nutrient loss during these phases is contingent upon the degree of processing. Furthermore, the drying methods employed for rice differ across regions; they may be optimized in affluent areas but remain underdeveloped in rural settings. The improper operation of machinery during milling significantly exacerbates these losses. Collectively, these issues result in the production of substandard rice, which adversely impacts market prices (Abbas et al., 2011).

Farmers find themselves in a dilemma between producing high-quality rice that commands a premium price and opting for lower-quality rice that is sold at a reduced price. The outer layer of the grains is abundant in vitamins, fats, proteins, and minerals, while the starchy endosperm is comparatively less nutritious. Washing and removing the outer layer prior to cooking diminishes the nutritional benefits of the grains. White rice and milled rice are the predominant varieties, accounting for 40 to 70% of total production.

The bran layer and germ are particularly rich in vitamin B and minerals, including iron; however, these components are eliminated during the milling process. It is estimated that approximately 80% of thiamine is lost when brown rice is milled into white rice. Other nutrients, such as niacin, riboflavin, and iron, are also sacrificed with the removal of the bran layer (Abbas et al., 2011).

6. DIABETES

One of the most prevalent diseases globally is Diabetes mellitus (DM). In 2010, the Center for Disease Prevention and Management reported that approximately 19 million individuals in the United States were diagnosed with diabetes, and around 2 million people over the age of 20 were newly diagnosed with the condition that same year (Post et al., 2012). Diabetes mellitus is a complex metabolic disorder that arises from defects in insulin secretion and function (Bhathena & Velasquez, 2002; Willett et al., 2002). It has been proposed that the consumption of foods with a high glycemic index can elevate the risk of developing diabetes through two primary mechanisms: 1- prolonged intake of high glycemic index foods necessitates increased insulin production, which places stress on the pancreas and ultimately leads to glucose intolerance; 2- products with a high glycemic index may promote insulin resistance (Brand-Miller et al., 2003; Willett et al., 2002).

Individuals with diabetes are particularly affected by excessive car-

bohydrate intake, notably from simple and easily metabolized carbohydrates such as starch and sugar. In contemporary society, consumers are increasingly health-conscious and seek to regulate carbohydrate consumption within their bodies. This includes opting for carbohydrates that are digested slowly, which helps maintain blood sugar levels at a moderate range, thereby preventing diabetes and metabolic syndrome (Shi & Gao, 2011). Consequently, to prevent and manage type 2 diabetes and associated issues within rice-consuming populations, it is highly beneficial to consume rice with lower glycemic index values (Azam et al., 2020).

In its most basic form, pancreatic beta cells fail to sustain sufficient insulin secretion, resulting in hyperglycaemia that contributes to diabetes. The factors leading to beta-cell dysfunction are a mix of genetic predispositions and environmental influences. In the case of type 1 diabetes, the autoimmune response mediated by T-cells appears to target beta cells as the primary mechanism of the disease; conversely, insulin resistance is the predominant metabolic disturbance observed in type 2 diabetes. The terminology has evolved from referring to diabetes in youth and adulthood as insulin-dependent and non-insulin-dependent diabetes, ultimately leading to the classifications of type 1 and type 2 diabetes (Rother, 2007). Globally, the prevalence of type 2 diabetes is rapidly rising. Modifying dietary habits can aid in preventing the onset of type 2 diabetes and in managing blood glucose levels.

The condition is marked by a reduction in β cell populations within the pancreas, resulting from an autoimmune assault that leads to diminished insulin secretion (Atkinson et al., 2015). A distinctive feature of type 1 diabetes (T1D) is the rapid destruction of all β cells, which occurs in the absence of islet-associated autoantibodies and is accompanied by the swift onset of hyperglycaemia (Hanafusa & Imagawa, 2007). In contrast, type 2 diabetes (T2D) involves a pathophysiological process of hyperglycaemia characterized by two primary dysfunctions: insulin resistance in tissues that respond to insulin and β cell impairment (Kahn et al., 2009). Modifications in lifestyle, including increased caloric intake and reduced physical activity, contribute to weight gain and obesity, thereby exacerbating the prevalence of type 2 diabetes. Furthermore, adherence to a low glycemic index (GI) diet has been linked to a lower risk of developing type 2 diabetes and cardiovascular diseases (Barclay et al., 2008).

7. THE GLYCEMIC INDEX (GI) AND RICE

The glycemic index (GI) refers to the extent of blood glucose elevation following the consumption of 50 grams of carbohydrates from a specific food, in comparison to the blood glucose response after ingesting 50

grams of carbohydrates from a reference food, typically glucose (Willett et al., 2002; Alminger & Eklund-Jonsson, 2008). Numerous factors influence the GI level and its corresponding value in the bloodstream after food consumption. A significant factor is the type of sugar present in the food (Voss et al., 2008). It is important to clarify that not all sugars possess a high GI. In reality, the GI values for different types of sugar can vary significantly, ranging from 23 to 105, contingent upon the specific type of sugar.

Foods that are rich in carbohydrates which decompose swiftly during digestion and subsequently release glucose rapidly into the bloodstream are generally characterized by a high glycemic index. Conversely, foods that consist of carbohydrates that decompose more slowly and release glucose at a gradual pace into the bloodstream are typically associated with a low glycemic index. A lower glycemic index signifies slower digestion and absorption rates of carbohydrates in foods, and it may also reflect an enhanced extraction of carbohydrate-digesting products from the liver and peripheral tissues (Wolever, 1990). The glycemic index of a particular food or meal is primarily influenced by various dietary factors that impact nutrient digestibility or insulin secretion, as well as the specific type of carbohydrate consumed.

The glycaemic index of a food quantifies the rate at which blood glucose levels increase and the speed at which the body normalizes these levels following digestion. The glycaemic index (GI) is assigned a value between 0 and 100, with pure glucose designated as 100, indicating the relative rise in blood glucose two hours post-consumption of that food. Foods characterized by a low glycaemic index are believed to offer protective benefits against serious health conditions such as diabetes, obesity, and cardiovascular diseases. The glycaemic index (GI) of any given food is primarily influenced by its carbohydrate content and type (El, 1999).

White bread and glucose have predominantly served as reference foods in educational contexts. In contrast, rice is generally more palatable and readily accessible. When compared to white bread, white rice is particularly suitable in these regions. As a result, the majority of Asians consider white rice a staple food. Furthermore, research has indicated that the Japanese population, which adheres to the globally recognized glycemic index assessment method, has not been extensively reported. Thus, the limited publications available in the literature can be regarded as representative of common Japanese foods. Prior to the application of the glycemic index (GI) concept in Japanese research and clinical practice, it was essential to evaluate the glycemic index of frequently consumed Japanese foods, primarily using white rice as a reference (Sugiyama et al., 2003).

Ranawana et al. (2009) investigated the various methods of cooking rice and the appropriate preparation techniques. They proposed that improved glycemic responses could be achieved if rice is allowed to cook for a longer duration. For instance, when Basmati rice is cooked for 10 minutes, it exhibits a lower glycemic index (GI) compared to when it is cooked for a longer period, such as 20 minutes (Al-Mssalleem et al., 2011). It is well established that the GI of rice varies with cooking time. According to research conducted by Mirhashemi & Seyyedi (2011), rice cooked for 35 minutes and subsequently boiled for 10 minutes had a GI of approximately 55. Conversely, if the same rice variety was boiled for 5 to 8 minutes and then allowed to boil for an additional thirty minutes, its GI increased to 66. Rice is prepared for human consumption, and the gelatinization of starch along with its GI is influenced by the temperature and the boiling water used (Collings et al., 1981). There are several reasons why rice may be soaked prior to cooking. Typically, rice is cooked before soaking, which helps to expand the grains and results in a more visually appealing product (Juliano, 2003). Therefore, the existing literature indicates that soaking and the conditions under which rice is cooked can elevate its glycemic index. Skipping this soaking step may be advantageous for those seeking rice with a lower glycemic index (Lin et al., 2010).

7.1. Types of Glycemic Index

Foods are categorized into three groups according to their glycemic index (GI) levels, which are classified as low, medium, and high GI (Figure 2). The glycemic index serves as a tool to assist individuals with diabetes in selecting foods that are low in GI (Venn and Green, 2007). In addition to diabetes management, low GI foods are beneficial in combating serious health issues such as heart attacks, complications, and obesity (Galali, 2014). Health-conscious individuals tend to favor the consumption of low GI foods due to their ease of digestion and minimal complications; these foods can also help reduce blood sugar levels. Similarly, individuals often limit their intake of high GI foods because of their detrimental effects on health. Conversely, it is advisable to restrict the consumption of foods with high GI values. According to Voss et al. (2008), high GI foods are detrimental to health as they are rapidly absorbed and digested, causing swift fluctuations in blood sugar levels. In contrast, low-GI foods are digested and absorbed at a slower rate compared to their high-GI counterparts. Consequently, low-GI foods result in a gradual increase in blood glucose that persists over a longer duration. On the other hand, high-GI foods are quickly digested and absorbed, leading to a rapid and significant spike in blood glucose levels, followed by insulin release that can often result in transient hypoglycaemia (Figure 2) (Galgani et al., 2006; Venn & Green, 2007).

Rice products possess a high glycemic index (GI), and excessive consumption of rice can lead to health issues. This is particularly detrimental as it elevates blood sugar levels, making it especially harmful for individuals with diabetes. One of the key components of rice is amylose; a higher amylose content correlates with better quality rice. In other words, rice with a high amylose content has a lower GI. Conversely, rice that is low or medium in amylose tends to have a higher GI (Sajilata et al., 2006). Foods that are high in starch are beneficial for human health. The glycemic index of white rice can vary significantly, typically ranging from 54 to 121 (Miller et al., 1992). However, it is important to note that high GI foods can reduce blood glucose levels within two hours post-meal, potentially leading to a hypoglycaemic state (Ludwig, 2002). Therefore, it is advisable to limit the intake of high glycemic index (GI) meals.

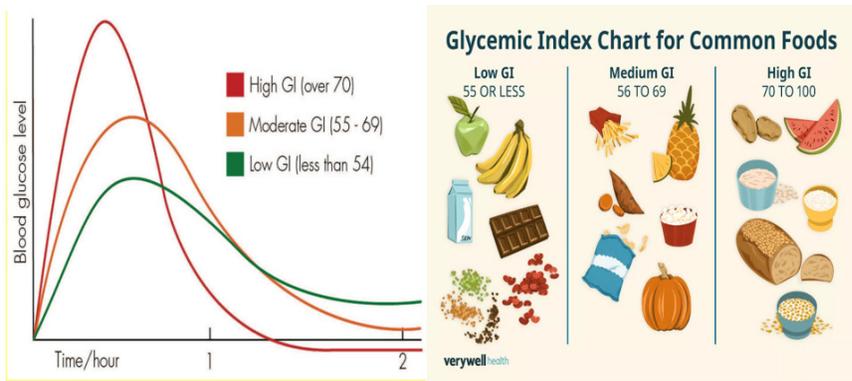


Figure 2. Glycemic index types graph and Glycemic index chart (Atkinson et al., 2021)

8. HERBS AND SPICES

In general, the leaf of a plant utilized in culinary practices is commonly known as culinary herbs, while other parts of the plant, which are typically dried, are referred to as spices. Both herbs and spices possess a rich history of contributing to health benefits and culinary applications (Turner, 1992). The advantages associated with culinary herbs and spices are largely attributed to their antioxidant properties. Antioxidants, which act as free-radical scavengers, have the potential to prevent, delay, or alleviate numerous chronic and age-related diseases in humans, including cancer, diabetes, and heart disease (Banini et al., 2006). It is crucial to diversify the foods we consume. In other words, it is essential to continuously alter our diet. One significant benefit of this practice is the reduction of the risk of developing diabetes, particularly type II diabetes. Additionally, it aids

in managing blood glucose levels (Broadhurst et al., 2000). The herbs and spices that are frequently utilized and associated with improved health outcomes include cinnamon, turmeric, red pepper, black seed, parsley, and dill. Certainly, there are numerous other herbs and spices that can positively influence our health. The use of spices to enhance the flavour of foods is an age-old tradition.

It is evident that spices and herbs have been utilized for medicinal purposes, as well as for flavouring and preserving food, since the Middle Ages. Meiselman (2020) notes that the discovery of new roads and routes to explore distant locations facilitated the use of herbs and spices in Europe, particularly during the 1800s. As previously mentioned, spices and herbs possess medicinal properties that can prevent and manage chronic diseases; they also enhance the aroma, flavour, and colour of food and beverages. According to Jiang (2019), Americans tend to use herbs and spices for therapeutic purposes and medicinal applications. In addition to their medical uses, herbs and spices can positively influence and enhance individuals' psychological well-being. They may also contribute to improved brain function. While research exists regarding the impact of herbs and spices on human psychology, there is limited evidence concerning diabetes, apart from the effects of ginseng on glycemia, which relates to the body's ability to process glucose (Tapsell et al., 2006).

Limited research neither supports nor contradicts the hypothesis that spices and herbs mitigate diabetes. While many studies have indicated that spices and herbs positively influence health, they are not uniform in every regard. For instance, herbs are derived from the leaves of plants, whereas spices can originate from any part of the plant (Embuscado, 2015). This distinction can be considered the primary difference between the two. It is widely accepted that a culinary herb refers to the leaves of plants utilized in cooking. Conversely, spices can be sourced from any part of a plant, including cloves, bark, roots, berries, and aromatic seeds. An example of this is cinnamon, which is derived from the bark. Typically, spices are harvested when the plant has reached full maturity and the flowering phase has concluded, followed by a drying process. For instance, the dried seed of coriander is a well-known spice, while its leaves serve as a source of herb. In terms of the roots and stems of coriander, they can be utilized as herbs or incorporated directly into cooking, similar to garlic and onion (Turner, 1992). In summary, the same plant can provide both herbs and spices simultaneously.

A report from the U.S. Department of Agriculture indicates that the consumption of spices and herbs by Americans has increased significantly in recent years compared to five decades ago. Among the most frequently

utilized spices and herbs are Ginger and Chili pepper (Barnes et al., 2008). Additionally, there has been a remarkable rise in the use of herbs and spices across the United States. Between ten to twenty percent of American adults employ herbs and spices for medicinal purposes (Clarke et al., 2015; Leng & Gany, 2014). This suggests that Americans have recognized the potential health benefits of herbs and spices, which can yield positive effects without adverse side effects. Spices are known to offer numerous advantages. Nevertheless, the benefits derived from spices depend on their method of consumption and the presence of bioactive components within them. The health benefits of spices and herbs are extensive, as they help to prevent inflammation, micronutrient deficiencies, and oxidative stress. Furthermore, they are effective in reducing the risk of cancer, cardiovascular diseases, diabetes, and neurodegenerative disorders. Many herbal practitioners advocate for the use of spices as a means to manage diabetes and its associated health complications (Akolade et al., 2019)..

Spices positively influence human health; however, each spice possesses unique characteristics. The impact of each spice is contingent upon its specific array of phenolic compounds. Additional research is necessary to confirm the effects of spices on diabetes (Sanlier & Gencer, 2020). Given that spices are abundant in phenolic constituents, they facilitate the antioxidant processes within the body (Jiang, 2019). In spite of these advantageous properties, the majority of polyphenols are not sufficiently absorbed, and their plasma concentrations are fleeting due to the rapid metabolism of these compounds. This variability in phenolic compounds across different spices, along with their interactive effects, renders the influence of spices on diabetes quite intricate (Cazzola & Cestaro, 2014).

8.1. Herbs & Spices for Diabetes

Metformin, a widely used first-line therapy for diabetes, is derived from the French lilac (*Galega officinalis*). To date, it remains the sole plant-based treatment for diabetes. At present, cinnamon provides the most substantial clinical evidence regarding the role of culinary herbs and spices in diabetes management, although this evidence is not wholly persuasive. Preliminary *in vitro* investigations of spices including turmeric, cloves, bay leaf, and cinnamon have demonstrated their potential to promote insulin activity (Khan et al., 1990; Khan et al., 2003). These spices were anticipated to be utilized in the nutritional management of diabetics at standard serving sizes. Nevertheless, the impact of spices on the glycemic index (GI) varies among individuals. The influence of spices on GI appears to be significantly contingent upon personal differences among individuals. It has been extensively documented that the glycemic index is affected by interstitial motility, the chemical makeup of carbohy-

drates, cooking techniques, the presence of dietary fibres, and the concurrent digestion of other nutrients such as proteins and fats (Matsui et al., 2001; Jenkins et al., 1988).

Conventional herbs and spices may also play a role in regulating blood glucose absorption (Broadhurst et al., 2000). Spices serve as culinary components that have historically been utilized for flavour enhancement, colouring, preservation, and are recognized for their therapeutic benefits. Their application in traditional medicinal practices is well-documented for various human ailments, including diabetes (Asif, 2011; Neff, 2003). It is evident that a growing number of Americans are exploring the use of herbs and spices for medicinal and therapeutic purposes, particularly in the treatment of various chronic ailments, alleviation of disease symptoms, and management of common health issues (Isbill et al., 2018).

Herbs and spices such as clove, rosemary, sage, oregano, and cinnamon are recognized as excellent sources of antioxidants due to their elevated levels of phenolic compounds (Opara & Chohan, 2014; Neveu et al., 2010). Numerous medicinal herbs and plants have been studied, with cinnamon emerging as one of the most effective agents for regulating blood glucose levels (Broadhurst et al., 2000). Spices have served as food enhancers, utilized for colouring, flavouring, and preservation for millennia. Additionally, these spices have long been acknowledged for their medicinal properties, with their applications documented in traditional medicine systems over extensive periods. The beneficial physiological effects of these spices also suggest potential therapeutic applications across a range of pathological conditions. Recommendations for healthy eating, alongside strategies to manage diabetes, promote physical activities such as walking and exercise. In recent times, the significant rise in physical inactivity has led to an increase in obesity and type-2 diabetes, highlighting the notion that obesity and a sedentary lifestyle may be primary contributors to the escalating diabetes crisis (Qi et al., 2008; Yang et al., 2010).

Historically, plants served as the primary element in the treatment of diseases, particularly in the context of therapeutic insulin and medical nutrition therapy. Dietary interventions included the application of traditional medicines sourced from plants. These traditional remedies are composed of herbal materials such as herbs, spices, and vegetables, which do not always qualify as natural food components. In recent times, spices have also played a role in addressing the presence of diabetic versus protective compounds. Additionally, they are utilized as food additives to enhance the flavour and colour of everyday meals (Srinivasan, 2005).

Herbs utilized in the management of diabetes are not a recent develop-

ment. Historically, various plants and their extracts have been employed to combat diabetes. Numerous traditional medicines are derived from medicinal plants, minerals, and organic compounds. The World Health Organization (WHO) has catalogued 21,000 plants that serve medicinal purposes globally. Among these, 150 spices are commercially utilized on a large scale (Zohary & Hopf, 2000). Various extracts from traditional medicinal herbs have been applied in the management of diabetes. However, many have demonstrated minimal or no impact on glycemic control in experimental studies, whereas certain herbs exhibit hypoglycaemic properties (Bailey & Day, 1989). In summary, previous research indicates that “enhancing our lives with spices” could serve as a flavourful and practical approach to maintaining bodily health. Spices are abundant in antioxidant substances and may possess pharmacological properties beneficial for managing diabetes and its associated complications. The recognized anti-diabetic properties seem to arise from the stimulation of the pancreas to release insulin, modulation of glucose absorption, or the insulin-sparing effects of bioactive compounds such as phenolic compounds.

8.2. Turmeric for Diabetes

Turmeric is a significant herb utilized in numerous herbal remedies for diabetes due to its ability to reduce blood sugar levels, enhance glucose metabolism, and amplify insulin function. This effect may partly stem from its chromium content. Curcumin is particularly noteworthy as it prevents galactose-induced cataract formation even at minimal doses (Biswas et al., 2005). Research indicates that turmeric and curcumin effectively lower blood sugar levels in rats with alloxan-induced diabetes (Arun & Nalini, 2002). Turmeric belongs to the ginger family, scientifically classified as Zingiberaceae. As noted by Deepa (2007), it has a long-standing history of medicinal use, particularly in India, where it is referred to as the golden spice of India. Beyond its role in flavoring and coloring food, turmeric serves various other purposes. Similar to cinnamon, it possesses antioxidant and anti-inflammatory properties (Hsu & Cheng, 2007). Thus, turmeric is not solely employed for enhancing flavor and taste but also has a multitude of additional applications.

Turmeric is particularly esteemed for its curcumin content, which serves as the primary pigment of the spice. Curcumin, in conjunction with other related pigments, imparts a yellow hue to the rhizome of the plant. From a chemical perspective, the pigments are classified as polyphenols, specifically referred to as curcuminoids. In recent years, both curcumin and turmeric have been extensively studied for their antioxidant and anti-inflammatory properties, revealing that curcumin acts as a potent antioxidant and immunomodulatory agent (Gupta et al., 2013).

The findings from these investigations suggest that turmeric exerts an antidiabetic effect, primarily attributed to curcumin. Consequently, research has been conducted administering curcumin alone, in addition to turmeric. Experts have suggested that a daily intake of 500-1000 mg of curcumin may yield beneficial effects on damage inflicted by diabetes or other metabolic disorders within the body (Sanlier & Gencer, 2020).

The primary active constituents of turmeric are curcuminoids, which include curcumin (diferuloylmethane), bisdemethoxycurcumin, tetrahydrocurcumin, and demethoxycurcumin. Curcumin is the most prevalent active compound utilized in both *in vivo* and *in vitro* studies involving animals and humans. Recently, traditional Indian medicine has employed turmeric powder for the treatment of various conditions, including hepatic disorders, wounds, cough, diabetes, coryza, anorexia, biliary disorders, sinusitis, and rheumatism (Syu et al., 1998). The yellow pigment found in this spice is derived from the rhizomes (roots) (Bagchi & Preuss, 2004). The impact of curcumin on diabetes, particularly its blood-glucose-lowering effect in individuals with diabetes, was investigated by Srinivasan (1972).

8.3. Cinnamon for Diabetes

Cinnamon, a tree from the Lauraceae family, is naturally cultivated in countries including Vietnam, China, India, and Bangladesh. The primary active compound in cinnamon is Cinnamaldehyde, which has been shown to exert antihyperlipidemic and antihyperglycemic effects in animal studies (Li et al., 2012). It is hypothesized that the preventive properties of cinnamon against diabetes may be attributed to phenolic compounds that inhibit the interaction of carbonyl species (Ballali & Lanciai, 2012). A prior study investigated the hypothesis that cinnamon could enhance glucose tolerance in individuals with type 2 diabetes (Khan et al., 2003). Cinnamon is recognized as a food ingredient that exhibits insulin-mimetic properties alongside various biologically active substances (Qin et al., 2003). Nearly all culinary recipes incorporate some form of cinnamon. Cinnamaldehyde, a key component of cinnamon, contributes to its medicinal applications (Abd El-Baky et al., 2009). Additionally, cinnamon supports antioxidant processes that mitigate inflammation and has been demonstrated to assist in lowering blood cholesterol levels (Huang et al., 2007). Furthermore, cinnamon can combat diabetes by reducing blood sugar levels. The mechanisms through which cinnamon lowers sugar levels include slowing food digestion and enhancing insulin sensitivity in the body (Jarvill-Taylor et al., 2001). When used appropriately, cinnamon offers numerous health benefits.

The Chinese Cinnamon is recognized as the most suitable option for managing hyperglycaemia in individuals with type 2 diabetes (Ríos et al., 2015). Research has been conducted on the anti-diabetic properties of cinnamon both in vitro and in vivo. The studies utilized bark extracts from both species of cinnamon and measured blood glucose and plasma insulin levels in rats (Verspohl et al., 2005). Consequently, cinnamon supplementation may play a significant role in enhancing insulin sensitivity in humans and regulating glycaemic levels in vivo, not only providing an immediate effect but also sustaining it for up to 12 hours (Ismail et al., 2010).

It was proposed that incorporating cinnamon into the diets of individuals with type 2 diabetes could reduce the risk factors associated with cardiovascular diseases and diabetes (Khan et al., 2003). Research was conducted on the impact of cassia (Chinese cinnamon) on healthy male participants. The researchers concluded that the inclusion of cassia may play a significant role in enhancing insulin sensitivity and regulating the glycemic index in humans. They noted that the benefits of cassia diminish once the treatment is discontinued (Solomon & Blannin, 2007). This document demonstrates that diabetes is a rapidly increasing condition associated with hypertension and cardiovascular diseases. The findings of this study will significantly contribute to the understanding of diabetes, particularly highlighting the importance of incorporating cinnamon into the diet and recognizing its associated risk factors, as cinnamon has been shown to enhance blood glucose levels in individuals with diabetes (Altschuler et al., 2007). Clinical researchers and essential scientists have investigated the potential of cinnamon to improve glycemic control in individuals suffering from diabetes (Anderson et al., 2004).

8.4. Parsley for Diabetes

Parsley (*Petroselinum crispum*) is grown globally, including in European, Asian, and Mediterranean regions, and is utilized as a spice, in salads, and as a herbal remedy (Vora et al., 2009). This herb is abundant in antioxidants, such as luteolin and flavonoids, which are known to identify and eliminate free radicals in the body, thereby contributing to oxidative stress in cells (Rashwan, 2012). Additionally, parsley is a significant source of vitamin A and tocopherol (Ozsoy-Sacan et al., 2006; Soliman et al., 2015). It also provides starch, as well as vitamins C, B, zinc, and β -carotene (Caunii et al., 2010).

In contemporary medicine, parsley is employed to address a range of ailments, including thrombosis, Alzheimer's disease, and strokes. It is extensively recognized for its role in combating cardiovascular diseases

(Al-Daraji et al., 2012). Furthermore, parsley possesses considerable nutritional benefits and demonstrates antioxidant and neutralizing properties (Mahmood et al., 2014). This herb contains a flavonoid known as myricetin, which has the potential to reduce glucose levels and mitigate insulin resistance. Historically, it has been used as a treatment for diabetes in Turkey (Ajmera et al., 2019).

In the battle against diabetes and the reduction of blood glucose levels, parsley is recognized as one of the most potent herbs, particularly in the Middle East, with Turkey being a notable example. Parsley (*Petroselinum crispum*) is among the medicinal herbs utilized by individuals with diabetes in Turkey, and it has been documented to effectively lower blood sugar levels. Kooti et al. (2016) conducted a study examining the effects of parsley on diabetic-induced rat skin. Its efficacy in humans appears to be promising.

8.5. Dill for Diabetes

Dill, scientifically referred to as *Anethum graveolens* L., thrives in warm and tropical climates for optimal growth. This herb can be cultivated throughout the year (Amanpour et al., 2017). One of the essential criteria for diagnosing and managing diabetes involves the measurement of fasting blood glucose levels. The use of traditional herbs for blood sugar regulation has gained traction in recent years due to their minimal side effects. Sargolzaei et al. (2017) examined the impact of dill tablets on fasting blood sugar levels in individuals with type 2 diabetes. Dill (*Anethum graveolens*) is an herb prevalent in European and Asian cuisines; in addition to its culinary applications, it is abundant in various nutrients and has historically been utilized to address several health issues, such as digestive problems, halitosis, and colic in infants (Jana & Shekhawat, 2010). Chronic elevation of blood sugar levels is associated with an increased risk of conditions such as insulin resistance, metabolic syndrome, and type 2 diabetes (DeFina et al., 2012). Dill has been suggested to possess blood-sugar-lowering properties (Goodarzi et al., 2016). The leaves of dill are a source of essential nutrients, including carotenoids, vitamin C, beta-carotene, polyphenols, and chlorophylls (Lisiewska et al., 2006). Furthermore, dill is recognized as a potent antioxidant, exhibiting antispasmodic and antimicrobial characteristics (Chahal et al., 2017). Recent preclinical studies have also indicated its hypoglycaemic, anti-gastric irritation, anticancer, and anti-inflammatory properties (Oshaghi et al., 2016).

8.6. Red Pepper for Diabetes

Red Pepper belongs to the Solanaceae family. All herbs derived from *Capsicum* species are edible and can also be utilized as spices (Luning et al., 1995). This pepper is abundant in antioxidant compounds, including vitamins A and C (Howard et al., 1994; Lee et al., 1995). According to a study by Abak & Pakyurek (1995), red pepper is a favored product among the Turkish population. The drying process involves exposing the pepper to sunlight in open air, which typically lasts between 8 to 10 days (Öztekin et al., 1999). In numerous countries, particularly Turkey and India, pepper serves as a primary spice.

Capsicum annum serves as a significant natural colouring and flavouring agent in various nations, particularly in Turkey, where it is regarded as a crucial spice. Turkey ranks among the largest producers of red peppers globally, following Mexico and China (Kabak & Dobson, 2017). In 2014, a total of 200,000,000 kg of this variety of red peppers was harvested for spice production. The majority of this yield, approximately 75%, is cultivated in the Şanlıurfa, Gaziantep and Kahramanmaraş province, situated in the southeastern region of Turkey (Korkmaz et al., 2017).

Red pepper is created by drying and grinding the hot variety, which serves as a spice and flavouring agent in food processing (Isidoro et al., 1995). Red pepper contains approximately 0.2–2% capsaicin, the compound responsible for the flavor in capsicums (Chopan & Littenberg, 2017). It is suggested that red pepper may assist in weight management by enhancing feelings of satiety and fullness, while also reducing energy and fat intake (Janssens et al., 2014). This research indicates that the inhibition of α -amylase by red pepper in the human gastrointestinal tract could be effective in managing diabetes by decreasing the absorption of hydrolysed glucose from starch. Additionally, the study aimed to investigate the pericarp, stalk, and placenta of red pepper for their phenolic content, free-radical scavenging activity, and inhibition of key enzymes associated with type 2 diabetes, specifically α -amylase and α -glucosidase, as well as their role in hypertension management (Chen & Kang, 2014). Red pepper may be a viable option for dietary management of type 2 diabetes (Kwon et al., 2007).

8.7. Black Seed for Diabetes

Black seed, scientifically known as *Nigella sativa*, is widely recognized for its medicinal applications and is also consumable as food (Kooti et al., 2016). This plant is primarily cultivated in the Southwest regions of Asia throughout the year and belongs to the Ranunculaceae family. It is an annual herbaceous species that thrives in Mediterranean and Asian nations,

including Italy, Indonesia, Pakistan, India, and Afghanistan. For centuries, its seeds have been utilized for medicinal purposes. Black seed is acknowledged for its medicinal properties, particularly its anti-hyperglycaemic and anti-hyperlipidaemic effects (Mathur et al., 2011). It has been employed in diabetes models for the regulation of glucose and lipid levels (Grover et al., 2002; Maobe et al., 2013; Patel et al., 2012). Furthermore, numerous studies have documented the anti-diabetic effects of black seed (Kaatabi et al., 2012; Sultan et al., 2014). The powder derived from black seed has been shown to improve lipid levels, blood glucose, and insulin profiles in individuals with Type 2 diabetes, demonstrating reasonable safety (Bilal et al., 2008).

Black seed oil may assist in the prevention of both type-1 and type-2 diabetes by promoting incomplete regeneration of beta-cells in the pancreas, enhancing insulin levels in the bloodstream, and lowering elevated glucose levels. Additionally, it has been demonstrated to improve digestion and glucose tolerance comparably to metformin, a common medication for type-2 diabetes, without exhibiting any adverse effects. Numerous research studies have corroborated that black seed oil aids in stabilizing blood sugar levels in diabetic patients and also stimulates pancreatic function. The recommended dosage for type-2 diabetes is two grams of black seed oil per day (Hussain & Hussain, 2016).

9. THE RELATIONSHIP BETWEEN SATIETY AND CARBOHYDRATES

The influence of sugars on feelings of fullness has been previously examined by numerous researchers. It has been determined that the engagement of a nutrient with receptors located in the gastrointestinal tract significantly contributes to the triggering of satiety by carbohydrates, as evidenced by the finding that the intraduodenal delivery of glucose reduces subsequent food consumption more effectively than glucose administered intravenously (Lavin et al., 1996). Furthermore, there is supporting evidence that the reduction of subsequent food intake due to carbohydrates is more pronounced when they are introduced into the stomach rather than directly into the small intestine, and this effect is even more significant following oral intake compared to intragastric delivery (Cecil et al., 1998). These latter findings suggest a hierarchy within the gastrointestinal processes that facilitate the impact of carbohydrates on satiety and imply that the interaction among orosensory, gastric, and small intestinal elements enhances these effects (Feinle et al., 2002).

In relation to the sucrose disaccharide composed of glucose and fructose, review studies indicate that 25 g of sugars appears to be the mini-

mum threshold for triggering satiety (Anderson & Woodend, 2003). An examination of the influence of refined carbohydrates on satiety was conducted (Hammersley et al., 2007). Various factors affecting the glycemic index, including fat, fiber, and protein content, may exert independent effects on satiety. Notably, foods with a low glycemic index are often high in fiber, which could enhance satiety (Benelam, 2009). The role of capsaicin in enhancing satiety, along with its comparative oral and gastrointestinal effects and its influence on food intake or macronutrient selection, was evaluated (Westerterp-Plantenga et al., 2005). Carbohydrates that digest slowly, such as starches and soluble fiber, may promote satiety due to the delayed gastric emptying and subsequent digestion caused by the formation of viscous gel-like substances upon consumption (Howarth et al., 2001). The effect of cooking on spices diminishes their efficacy; when spices undergo heat treatment during cooking, there can be a significant loss of biochemical activity. According to Suresh et al. (2007), heat treatment resulted in a curcumin loss from turmeric ranging from 27 to 53%; the degradation of piperine from black pepper varied between 16 and 34%, while the loss of capsaicin from red pepper ranged from 18 to 36%.

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Chapter 2

**BLACK CHOKEBERRIES (ARONIA
MELANOCARPA)**

Assoc. Prof. Pembe EVCİ ÇÜRÜK¹

¹ Food Technology Department, Kadirli Applied Sciences Faculty,
Osmaniye Korkut Ata University, Türkiye.

ORCID: 0000-0003-4764-7242.

The increasing global interest in natural resources in the health and nutrition sector is increasing the importance of medicinal and aromatic plants rich in polyphenols, which have antioxidant and antimicrobial properties (Yilmaz and Ciftci, 2021). As a result of the rapid increase in the world population and the constantly developing and expanding global economy, the pressure on natural resources is increasing daily. Hence, the optimal and most efficient use of available resources is of paramount importance. Establishing orchards and adhering to technical principles in fruit growing is crucial for profitable production. Among fruits, berries, in particular, are known to be rich in natural antioxidants and possess high antioxidant capacity and anthocyanin levels. The increase in global consumption in recent years has increased the importance of berries in domestic and foreign trade. Black chokeberry is a berry-like fruit species that has become widely recognized worldwide in recent years and may be cultivated in temperate climates with moderate pH levels.

Black chokeberry (*Aronia melanocarpa*) is among the most preferred fruits today As a result of its high antioxidant activity and rich polyphenol content (Denev et al. 2019). This species, recommended as a remedy for digestive system diseases, cardiovascular diseases, and some cancers, has recently gained popularity both nationally and globally.

A member of the Rosaceae family, black chokeberry is a species with berry-like fruits. Introduced to Europe in the early twentieth century, aronia has been cultivated over time, giving rise to standard varieties such as “Albigowa”, “Ahonnen”, “Aron” (Denmark), “Autum Magic”, “Dabrowice”, “Egerta”, “Galicjanka” (Poland), “Hakkija”, “Fertödi” (Hungary), “Hugin” (Sweden), Kurkumachki” (Finland), “Kutno”, “McKenzie”, “Morton”, “N”, “Nero” (Czech Republic), “Nova”, “Rubin” (Russia), “Serina”, “Viking” (Finland), and “Wies” (Šnebergrová et al., 2014; Fidanci, 2015; Sidor and Gramza-Michałowska, 2019). In 2017, for the first time in Turkey, two aronia varieties were registered by the Atatürk Horticulture Central Research Institute, affiliated with our ministry, and certified saplings began to be produced. Additionally, its resistance to mechanical harvesting, transportation, and storage makes this species a widely recognized choice among more farmers.

Botanical Characteristics and Distribution

Black chokeberry is a perennial species originating in North America that grows in a bush form, up to 80-240 cm tall (Fig. 1/A, B), and bears purple-black berries approximately 6 mm in diameter. The leaves are 3-7 cm long and glossy and hairless (Fig. 1/C). Its white-pink flowers bloom in May (Hardin, 1973; Scott and Skirvin, 2007). Black chokeberry flowers

are hermaphroditic. A cluster contains 20-25 flowers (Fig.1/D), each of which produces 8-14 fruits (Fig. 1/E). The flowers have five sepals, five petals, and 10-30 stamens, characteristic of the Rosaceae family. The flowering period lasts 20-25 days. Black chokeberry is a self-fertile species and does not require a pollinator (Poyraz Engin and Mete, 2018).



Fig. 1. Black chokeberry plant: A) Black chokeberry sapling, B) Black chokeberry tree, C) Black chokeberry leaf, D) Black chokeberry flower, E) Black chokeberry fruits (Anonymous, 2025).

The natural range of *A. melanocarpa* is mountain marshes and foothills extending from the northeastern part of North America (Rossell and Kesgen, 2003). Additionally, the countries with the highest aronia cultivation in the world are the United States, Canada, Germany, Russia, Poland, Japan, Bulgaria, the Czech Republic, Slovakia, Ukraine, Denmark, Estonia, Sweden, Latvia, Finland, and Lithuania. According to 2019 data, aronia is cultivated in Turkey on a total area of 78 hectares. The top five provinces in aronia cultivation are Kırklareli (240 decares), Bursa (141 decares), Manisa (90 decares), Kırşehir (48 decares), and Yalova (48 decares) (Poyraz Engin and Yılmaz, 2019). Aronia cultivation has not yet become widespread in our country. The Ministry of Agriculture and Forestry is taking the necessary steps to encourage aronia cultivation. It is anticipated that these incentives will increase the interest of farmers and entrepreneurs in aronia cultivation.

Uses

Black chokeberry is considered the healthiest fruit in the world by many experts, is called a super fruit, and is considered a kind of vitamin bomb (Michalak, 2015). Black chokeberry fruits are used fresh, in

the fruit juice industry, alone or in cocktails with other fruit juices; in the pharmaceutical industry; in milk and dairy product technology; in aronia ice cream; in aronia milk; in dried fruit technology; in fruit bread, buns, cakes, puddings, and pastries; in the spice industry; in fruit salads; in jam, marmalade, and canning; and in the dried leaves and fruit as tea and in diet menus. Aronia juice is a good option for increasing the flavonoid and anthocyanin levels of other fruit juices (Kokotkiewicz et al., 2010). It is used as an ornamental plant due to its white flowers in the spring and its red-black fruits and red leaves in the autumn. Aronia, with its showy white clusters of flowers, is an ornamental shrub, and its berries is utilized in preserves, juice, and wine production (Hao et al., 2015). Aronia berries, with their high anthocyanin content, are applied in the production of functional (especially antioxidant-rich) juices and teas (McKay, 2001; Balcerek and Szopa, 2005; Gonzalez Molina et al., 2008). Aronia berries may be consumed directly, and this species is utilized as a remedy for colds and as a tea. However, because they can have a sour and astringent taste, processing may be necessary to eliminate the negative effects of these qualities (Scott and Skirvin 2007).

Aronia, which can also be enjoyed at breakfast, is utilized in sauces or salsas, garnishes, and adds a distinct flavor and aroma. Both the fruit and fresh leaves is utilized as tea. Aronia is a fruit that is utilized in creams and soaps, skin care, and the cosmetics industry as a raw material for fragrances. Its extract is applied in the pharmaceutical industry in the production of syrups and dietary supplements (Wolski et al., 2007). An essential part of Mediterranean diets, aronia is extremely rich in vitamins, minerals, and fiber. In addition to being consumed as a food rich in nutrients, the fruits of the plant are also used as traditional herbal medicine. Aronia, recommended and used as a hypertensive and anti-atherosclerotic medication in Russia and Eastern European countries, is among the most significant plants that have gained popularity in this region to meet the need for herbal medicine (Kokotkiewicz et al., 2010). As a result of its high pectin content, it is utilized in mixed jams with low-pectin fruits (Scott and Skirvin, 2007). It can also be used to improve the flavor, color, and antioxidant properties of jams (Wojdyło et al., 2008).

Cultivation and Advantages

Aronia is a shrubby species that sheds its leaves in winter. The species's height can range from 90-240 cm and its width can range from 60-180 cm. Hence, the habitus of the variety to be grown determines the planting density. For Viking and Nero varieties, a planting density of 1.5 m within the row and 3.5 m between the rows is recommended. Because Aronia is relatively tolerant of shade, the distance between rows may be reduced

even further. However, it is reported to yield better in open, sunny areas (Brand, 2010).

The species may be grown in a variety of soil conditions, from moist and swampy soils to dry and sandy soils. Soil amendments with compost promote healthier and more productive plants. The optimum soil pH for cultivation is 6-6.5. However, it can tolerate pHs up to 5-8.5 (Everhart, 2013).

Combined NPK fertilization is reported to increase the growth and yield of *A. melanocarpa*, but this application should be kept at moderate levels. Excessive NPK application causes a significant decrease in anthocyanin levels (Jeppsson and Johansson, 2000). The recommended fertilizer doses for achieving high anthocyanin yields in aronia cultivation are 5 kg/da N, 4.4 kg/da P, and 10 kg/da K (Jeppsson and Johansson, 2000). Alkaline (N, K, and Si) fertilizer application increases fruit size and firmness (Skupień et al., 2008). Chlorocholine chloride application increases polysaccharide and anthocyanin concentrations in fruits (Stroev and Martynov, 1979). Ethanolamine phosphate application also significantly increases anthocyanin content and promotes the conversion of saccharides to phenolics. However, although polyamine biosynthesis catabolites cause a slight decrease in anthocyanin levels, they significantly increase flavonoid concentrations (Hudec et al., 2006).

Irrigation is a critical factor in cultivation, especially in the first years. In arid regions with insufficient rainfall, seedlings should be supported by irrigation once or twice a week during the first years. As the species matures, the need for irrigation will decrease (Brand, 2010). In areas with sufficient rainfall, cultivation may be carried out without the need for irrigation. This makes cultivation more economical (Yılmaz et al. 2021). Furthermore, fruit cracking As a result of high water intake during cultivation causes a noticeable decrease in anthocyanin levels and fruit browning, so the irrigation regime and seasonal rainfall must be carefully monitored (Jeppsson and Johansson, 2000). Extending the harvest period can lead to a longer harvest, which can lead to oxidation of anthocyanins and undesirable browning of the fruit, similar to drying at high temperatures (Bober and Oszmianski, 2004). hence, although the harvest period is extended over a period of two months, fruits that have reached harvest maturity should be collected without delay.

Black chokeberry is unique in that it may be harvested for approximately two months. This leads to significant variation in the composition of the fruits (Kulling and Rawel 2008; Poyraz Engin and Mert 2020). Harvesting takes place during the period when the fruit color characteristic

of the variety develops. In addition to being harvested by hand, aronia berries can also be harvested using the mechanized method used for harvesting. While some fungi, particularly within the genus *Alternaria*, are known to exhibit symptoms on aronia (Wee et al., 2006), experiments have reported that crop quality is not affected (Kokotkiewicz et al., 2010).

The dormancy period for aronia plants begins in November or December, depending on the variety and ecological conditions. Plants that have reached reproductive maturity require chilling to bloom. Dormancy period is estimated to be around 800-1000 hours. As a result of its late flowering, the flowers and small fruits are not damaged by late spring frosts (Figure 2) (Yurtkulu, 2022).



Figure 2. Aronia plants during dormancy period. (Anonymous, 2025)

Composition and Nutritional Value of Aronia

Fresh black chokeberry fruits have a higher antioxidant capacity than any other fruit based on ORAC (Oxygen Radical Absorbance Capacity) values. Aronia juice also has the highest antioxidant capacity among polyphenol-rich beverages, with TEAC values four times higher than blueberry juice, cranberry juice, or red wine. The ORAC value (Oxygen Radical Absorbance Capacity) is a measure used for foods to determine the absorption capacity of substances called free radicals in our bodies that cause various diseases. A higher ORAC value indicates a higher antioxidant content. Foods with high ORAC values have cancer-fighting properties and may also have a retarding effect on aging. Aronia, known as a super fruit and with the highest antioxidant properties, has been documented to have an ORAC value as high as 16.06. Studies on the effects

of aronia (*Aronia melanocarpa*), a type of berry, on human health have revealed that its fruits have the highest antioxidant capacity and anthocyanin content compared to other berries. Regular consumption of this fruit has been found to protect against cardiovascular diseases, digestive system diseases, and some cancers. Aronia berries are considered medicinal plants in Russia because of their biochemical properties. They are applied in the treatment of various illnesses, including colds, stomach ailments, intestinal, liver, and gallbladder diseases, as well as radiation poisoning. They increase good cholesterol levels and help fight heart disease and other cardiovascular problems. They are also highly effective in controlling blood pressure, maintaining healthy blood sugar levels, and strengthening the immune system, thus strengthening the metabolism against colds and flu-like infections. It nourishes the brain and nervous system and has a high antioxidant capacity that helps combat aging.

The total phenolic content of aronia varies between 2,000 and 8,000 mg/100g of DW. The amount of phenolic content varies depending on the variety, growing conditions, and harvest date (Benvenuti et al., 2004; Oszmianski and Wojdyło, 2005; Sueiro et al., 2006; Hudec et al., 2006). Polyphenols, particularly anthocyanins and procyanidins, constitute the main group of bioactive compounds in aronia fruits, providing a large portion of the species's antioxidant properties. Other phenolics include chlorogenic and neochlorogenic acids, as well as small amounts of tannins (Oszmianski and Wojdyło, 2005; Matilla et al., 2006).

In addition to polyphenols, the fruits are a good source of sugar (10-18%), pectin (0.6-0.7%), sorbitol, and parasorboside (Niedworok and Brzozowski, 2001; Wolski et al., 2007; Kulling and Rawel, 2008). A low oil content (0.14% fresh weight) consisting of linoleic acid glycerides and phosphatidylinositol has also been reported in aronia berries (Zlatanov, 1999). Aronia berries contain anthocyanins and procyanidins, which are well known for their high antioxidant capacity, leading to anticancer, antiviral, antibacterial, antidiabetic, anti-inflammatory, antimutagenic, cardioprotective, gastroprotective, hepatoprotective, immunomodulatory, and radioprotective activities that have been identified in both in vitro and in vivo studies (Jurikova et al., 2017).

Black chokeberry fruits contain vitamins B1, B2, B6, and C, niacin, pantothenic acid, folic acid, α - and β -tocopherol, and carotenoids (including β -carotene and β -cryptoxanthin), along with minerals (Razungles et al., 1989; Stralsjo et al., 2003). The contents of β -sitosterol and campesterol, among the triterpenes, have been identified (Zlatanov, 1999). More than 40 in total, dominated by cyanohydrin, benzaldehyde, hydrocyanic acid, and its contents contains high amounts of volatile com-

pounds (Kokotkiewicz et al., 2010). Amygdalin, characteristic of the seeds of many plants in the Rosaceae family, has also been detected in the pulp of Aronia fruit (Weinges et al., 1998).

Among the compounds contained by Aronia, the most extensively studied pharmacologically are flavonoids, represented by anthocyanins and procyanidins (Kokotkiewicz et al., 2010). The flavanols contained in Aronia are the main procyanidins. The amount of procyanidins, out of a total of 40, is dominated by cyanohydrin, benzaldehyde, and hydrocyanic acid. The amount varies between 0.66% and 5.18% by dry weight (Wu et al., 2004). In aronia berries, anthocyanins are the second largest group of phenolic compounds, with a concentration range between 0.60% and 2.00% by dry weight (Kokotkiewicz et al., 2010). The presence of anthocyanins is one of the most significant factors determining the species's antioxidant capacity. Aronia possesses higher antioxidant activity than many other plants due to its substantial anthocyanin content.

Black chokeberry fruit juice has been documented to inhibit phosphatidylcholine oxidation and is approximately twice as effective as blackcurrant (*Ribes nigrum* L.) juice in this regard. Furthermore, it exhibited a synergistic effect with α -tocopherol, which is not observed in blackcurrants. Hence, aronia is utilized not only as a coloring agent but also as an effective antioxidant that protects α -tocopherol and unsaturated lipids in food products (Graversen et al., 2008).

It has been determined that aronia extract may affect the growth of some breast and colon cancers through sulfotransferase inhibition, thereby altering the availability of estrogen receptors (Kokotkiewicz et al., 2010). These results are quite remarkable, as exposure to estrogen has been shown to reduce the risk of colon cancer in women (Grodstein et al., 1999).

Various researchers have reported that aronia extract has a neuroprotective effect, improves cognitive and locomotor activities, exhibits anti-neuroinflammatory effects, and accelerates learning (Valcheva-Kuzmanova et al., 2013; Lee et al., 2017; Lee et al., 2018; Daslakova et al., 2019; Kim and Shin, 2020; Wen et al., 2021). The polyphenols in aronia species extracts and juice have a high ability to suppress oxidative stress. This property of the aronia species makes it valuable in cancer research. Many studies have demonstrated the therapeutic and anticancer properties of aronia against various types of cancer, including pancreatic cancer (Thani et al., 2014), breast cancer (Kedzierska et al., 2010), and colon cancer (Bermúdez-Soto et al., 2007; Yu et al., 2021). Aronia juice has also been reported to kill embryonal carcinoma stem cells (Sharif et al., 2013).

The generally understood cardioprotective activity of aronia may be attributed to the lipid-lowering, antiaggregatory, and direct vasoactive effects of its anthocyanin-rich extracts (Ganji et al., 2003). The fruits contain relatively high amounts of K and Zn, as well as varying amounts of Na, Ca, Mg, and Fe (Ognik et al., 2006).

Black chokeberry extracts have been shown to provide in vitro bacteriostatic activity against *Staphylococcus aureus* and *Escherichia coli* and to possess antiviral activity against influenza A virus (Valcheva-Kuzmanova and Belcheva, 2006). Inhibition of Staphylococcus and Salmonella pathogens has also been observed (Puupponen-Pimia et al., 2005a, b, c). As a result of their high phenolic content, it has also been reported that aronia extracts have an antimicrobial effect in the small intestine (Jaroniewski, 1998).

CONCLUSION

Black chokeberry's high adaptability to soil and climate conditions allows it to be easily cultivated in diverse regions. Its long flowering period ensures less damage from late spring frosts. The absence of any known diseases or pests that cause economic losses reduces the risk of cultivation. Its long harvest period allows farmers to spread their labor over time and cultivate with lower labor costs. Furthermore, its suitability for mechanized harvesting makes it a suitable species for growing on large plots of land. Its fruits are used industrially for processing into many different products, as well as for enriching the ingredients of other foods. Due to their high antioxidant content, the fruits exhibit protective effects against various diseases, particularly cancer. Furthermore, as a result of the increasing interest in alternative medicine and natural resources, this species's popularity has increased day by day, earning it the reputation of being a "super"/"miracle" species. As a result of its wide range of uses and people's inclination towards healthy products, marketing is expected to be a non-issue. With all these superior properties, aronia cultivation is expected to expand rapidly in Turkey and around the world.

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Chapter 3

THE USE OF FRUITS AS FUNCTIONAL ADDITIVES IN ICE CREAM PRODUCTION

Kurban YAŞAR¹

¹ Osmaniye Korkut Ata University, Faculty of Engineering and Natural Sciences,
Department of Food Engineering, 80000 Osmaniye, Türkiye. E-Mail: kurbanyasar@osmaniye.edu.tr, <https://orcid.org/0000-0002-6514-2101>

1. Introduction

Ice cream is a widely consumed dairy-based dessert valued for its pleasant sensory characteristics and high nutritional content. It appeals to all age groups, particularly children, as a source of energy and essential nutrients. In addition to macronutrients such as fat, protein, and carbohydrates, ice cream provides micronutrients including calcium and vitamins A, D, and E. However, conventional ice cream formulations typically lack dietary fiber and natural antioxidants (Güven et al., 2010; Ismail et al., 2020). In response to increasing consumer awareness of health and wellness, there has been growing interest in the development of functional ice creams enriched with bioactive compounds to enhance both their nutritional and physiological benefits (Patil & Banerjee, 2017).

Recent advances in functional food technology have encouraged the incorporation of probiotic cultures, natural antioxidants, and alternative sweeteners into frozen dairy products (Januário et al., 2018). Ice cream is a complex multiphase system consisting of uniformly dispersed air bubbles, fat globules, and ice crystals, with fat playing a key role in determining its body and texture (Karaca et al., 2009). The increasing demand for reduced-fat products has led to the use of protein-, carbohydrate-, and lipid-based fat replacers that maintain desirable structural and sensory characteristics (Akbari et al., 2019). Moreover, frozen dairy matrices provide an excellent vehicle for bioactive compounds due to their structural stability and low-temperature storage, positioning ice cream as a convenient carrier of health-promoting ingredients (Goraya & Bajwa, 2015).

Among functional ingredients, red fruits such as strawberries, raspberries, blackberries, cherries, blueberries, and cranberries are of particular interest. These fruits contribute to product appeal through their vibrant colors, pleasant flavors, and abundance of bioactive compounds (Cosme et al., 2022). They are rich sources of vitamins A, C, and E, as well as minerals such as calcium, potassium, and magnesium. In addition, they provide dietary fiber and phenolic compounds with potent antioxidant activity. Naturally occurring pigments like carotenoids, flavonoids, and anthocyanidins not only enhance visual quality but also help protect cells from oxidative stress (Lu et al., 2021). These compounds have been associated with multiple physiological effects, including antioxidant, anti-inflammatory, and anticancer activities, as well as benefits for visual health.

According to the World Health Organization (WHO), adults should consume at least 400 g of fruits and vegetables daily to maintain optimal health, yet data from 2015 reveal that only 55% of the global population

meets this target (Rodríguez et al., 2021). Natural antioxidants such as thiols, ascorbic acid, and polyphenols have been shown to be more effective than synthetic antioxidants in reducing oxidative stress and the risk of chronic diseases (Rahaman et al., 2023). In addition, fruit-derived bioactive peptides have recently gained attention as sustainable, culturally acceptable functional ingredients (Sosalagere et al., 2022). However, fruits and vegetables are highly perishable, leading to significant postharvest losses. To overcome these challenges, physical preservation techniques (e.g., ultraviolet light, high pressure) and chemical treatments (e.g., ozone, organic acids) are employed to maintain freshness and nutritional quality (Panahirad et al., 2021).

Dried fruits offer a practical alternative to fresh fruits due to their extended shelf life, concentrated nutrients, and ease of use in food formulations. Epidemiological studies have demonstrated an inverse relationship between dried fruit consumption and the prevalence of cardiovascular and metabolic disorders. For example, the European Food Safety Authority (EFSA) recognizes prunes as beneficial for digestive health and recommends a daily consumption of 100 g (Alasalvar et al., 2023). The inclusion of fruits in ice cream not only enriches its nutritional composition but also improves its sensory attributes, such as color, aroma, and taste, enhancing overall consumer acceptance. Due to their anticancer, antimutagenic, and anti-inflammatory effects, fruits are considered promising natural sources for the production of functional ice creams (Tarakçı & Durak, 2020).

Furthermore, the utilization of fruit-processing by-products such as peels, seeds, and pulp represents a sustainable approach to food production. These by-products are rich in polyphenols, flavonoids, carotenoids, unsaturated fatty acids, and tocopherols, making them valuable for the development of functional foods (Munekata et al., 2023). Increasingly, such by-products are incorporated into ice cream, baked goods, and meat products as functional additives. Concurrently, innovative processing methods are being explored to reduce undesirable compounds, including pesticides, aflatoxins, and antinutritional factors, thereby improving the safety and quality of fruit-derived functional ingredients (Munekata et al., 2023).

2.1. Chemical and Functional Effects of Fruit Components on Ice Cream

Fruits and their derivatives—such as purees, powders, extracts, and by-products—serve not only as natural colorants and flavoring agents in ice cream formulations but also as valuable sources of bioactive compounds that enhance the nutritional and functional quality of the prod-

uct. Their incorporation contributes to increased antioxidant activity, improved mineral and vitamin content, and enhanced technological properties. For instance, the addition of *Fortunella margarita* (kumquat) puree was shown to significantly elevate the vitamin C concentration, b* color values, and potassium levels of ice cream samples (Çakmakçı et al., 2016).

Betacyanins extracted from the pulp of *Hylocereus polyrhizus* (dragon fruit) have been explored as natural alternatives to synthetic colorants such as E-162, owing to their pigmenting potential and antioxidant-enhancing effects (Gengatharan et al., 2021). Similarly, *Basella rubra* L. fruit extract retained 86.6% of its color stability under low-temperature conditions, demonstrating its feasibility as a stable natural pigment in frozen desserts (Kumar et al., 2015). The use of kiwi juice also enhanced the nutritional quality of ice cream as red-fleshed kiwi variants yielded the highest antioxidant activity and phenolic content. The study further revealed that formulations containing 49% of either green, golden, or red kiwi exhibited shear-thinning rheological behavior, with green kiwi producing the lowest overrun and fastest melting rate (Sun-Waterhouse et al., 2013).

Alternative ingredients such as aquafaba (chickpea cooking water), have gained attention for their ability to improve melting resistance and microstructure through superior foaming capacity (Erem et al., 2024). In another study, soy whey-based ice cream enriched with dragon fruit peel exhibited a total phenolic content of 108 mg GAE/100 g and 51.84% antioxidant activity, confirming the functional contribution of fruit by-products (Putra et al., 2024). Similarly, ice cream containing 1.5% lucuma fruit powder achieved the most desirable sensory properties; however, higher inclusion levels negatively affected acceptability, highlighting the importance of optimizing additive concentration (Babu et al., 2025).

Rheological properties are particularly critical in formulations containing concentrated fruit extracts. Bozdogan et al. (2020) demonstrated that sumac extract exhibited Newtonian flow behavior across different concentrations and temperatures, with viscosity directly influenced by these parameters. Such findings emphasize the necessity of understanding temperature–viscosity relationships when integrating fruit-derived components into ice cream systems to ensure optimal texture and processability.

Eco-friendly extraction of pectin from *Passiflora edulis* (passion fruit) peel was reported to increase overrun, reduce melting rate, and enhance technological stability in synbiotic ice cream, while simultaneously improving total phenolic content and antioxidant capacity (Pimisa et al.,

2024). Fruit-derived prebiotics such as inulin, oligofructose, and polydextrose have also been shown to enhance the functional potential of ice cream by increasing antioxidant, antidiabetic, and antihypertensive effects. Particularly, polydextrose and oligofructose were found to improve the fatty acid profile, melting resistance, and overall functional performance (da Silva et al., 2020).

Anthocyanins, though valuable for their color and antioxidant properties, are thermolabile compounds that degrade rapidly at elevated temperatures. Studies by Bozdoğan and Yaşar (2019, 2021), on red meat radish and black carrot-based beverages reported first-order degradation kinetics of anthocyanins, with shorter half-lives under thermal stress. These findings underline the necessity of minimizing heat exposure during ice cream production to preserve pigment stability and bioactivity. Furthermore, cryogenic shock freezing and nanoscale fragmentation techniques have been applied to fruit and vegetable-based sorbets, significantly enhancing the release and bioavailability of soluble pectin, vitamins, and antioxidants by up to 2.5–3.5 times compared with untreated samples (Pavlyuk et al., 2018).

The inclusion of *Physalis peruviana* L. (golden berry) in ice cream formulations (5–15%) improved viscosity, melting time, and mineral contents (K, Fe, Zn), whereas excessive addition reduced protein, calcium, and phosphorus levels. Thus, a 10% supplementation level was identified as optimal for balancing sensory and nutritional properties (Erkaya et al., 2012).

2.2. Effects of Fruit Additions on Sensory Quality and Consumer Preference

Sensory properties—including color, aroma, taste, and texture—play a decisive role in determining consumer preference for ice cream. The integration of fruits can substantially enhance these attributes, improving both the appeal and functional value of the product.

In vegan ice creams formulated with tropical fruits such as pitanga, umbu, and mangaba, the mangaba-enriched variant achieved the highest sensory scores, particularly in flavor and aroma (Candido & de Castro Neto, 2025). Similarly, ice creams containing 10–15% avocado or papaya puree exhibited superior sensory attributes in taste and texture (Hassan et al., 2024). Moolwong et al. (2023) also found that formulations with 20% avocado pulp achieved the highest consumer acceptance.

In goat milk-based ice cream, banana- and blackberry-flavored variants were most preferred by panelists (Tarakçı & Durak, 2020). Green tea

powder, when added at 1%, improved flavor and color; however, higher inclusion levels resulted in bitterness and decreased probiotic viability (Gündoğdu et al., 2022). In plant-based formulations, the incorporation of banana and mango provided sensory qualities comparable to traditional dairy-based products, making such formulations attractive to health-conscious consumers (Ogo et al., 2021).

In formulations combining dragon fruit and pumpkin, a 50:50 ratio yielded the most desirable color, taste, and texture scores, with the natural pigments of dragon fruit enhancing visual appeal (Sutrisno & Amalia, 2020). Similarly, in *Prunus spinosa* L. (blackthorn) ice creams, increasing fruit content elevated titratable acidity, viscosity, and a^* color values, while reducing pH, total solids, and fat content. Although the phenolic richness improved antioxidant activity, sensory differences were primarily associated with color (Kavaz Yüksel, 2015).

The addition of persimmon (*Diospyros kaki*) puree also increased total phenolic content, antioxidant capacity, and textural stability, with 24% identified as the optimal concentration for both functional and sensory attributes (Karaman et al., 2014). Complementary stabilizers such as salep and exopolysaccharide-producing cultures have been shown to further enhance texture, pH stability, and overall acceptability, particularly in buffalo milk yogurt ice cream (Yeydem & Yaşar, 2023).

Incorporating dietary fibers from fruit by-products such as peach peel and pulp also improved the mineral composition: specifically, Ca, K, Mg, and P levels. While peel fiber slightly reduced acceptability, 1% pulp fiber addition received the highest preference scores, confirming its dual benefit for nutrition and sensory appeal (Yangılar, 2016).

2.3. Contributions of Fruit Additions to Microbiological and Functional Stability

Fruit-derived components enhance not only the chemical and sensory qualities of ice cream but also its microbiological safety and probiotic viability. The natural acidity, phenolic compounds, and prebiotic fibers in fruits contribute to maintaining a stable microbial environment while supporting the survival of beneficial bacteria.

Pectin extracted from *Passiflora edulis* peel significantly increased the viability of *Lactobacillus acidophilus* and improved structural characteristics such as overrun, in synbiotic ice cream (Pimisa et al., 2024). Similarly, formulations containing jujube and blueberry displayed extended first drip times and preserved probiotic stability for up to 60 days, correlating with increased phenolic content (Şentürk et al., 2024). The inclusion of

Malus floribunda fruit sauce enhanced both total phenolic content and sugar levels while prolonging the survival of *L. acidophilus* La-5® (Arslaner & Salik, 2022).

In ice creams containing up to 15% cornelian cherry (*Cornus mas*) puree, vitamin C content and overrun values increased, while viscosity decreased due to the fruit's high phenolic concentration (Topdaş et al., 2017). Environmentally extracted passion fruit peel pectin (PFPEP) maintained *L. acidophilus* viability at 7 log CFU/g for 120 days while achieving sensory scores above 7, demonstrating both microbiological safety and consumer acceptance (Pimisa et al., 2024).

Moreover, in goat milk ice cream enriched with white fruits (WM) and dark-colored fruits (BM) of *Myrtus communis*, *Lactobacillus casei* 431 exhibited a marked prebiotic response. The BM variant showed higher phenolic and antioxidant levels, while WM achieved superior sensory acceptance, indicating that both types of myrtle fruit contribute distinct functional and sensory benefits (Öztürk et al., 2018).

Direct incorporation of *Lactobacillus acidophilus* LA-5 into fruit-based ice cream formulations was found to effectively maintain probiotic viability at 10⁷ CFU/g even after 10 weeks of storage, confirming the potential of fruit-enriched probiotic ice creams as functional dairy desserts (Senanayake et al., 2013). Similarly, in formulations enriched with dietary fibers derived from fruit and cereal by-products, the populations of *L. acidophilus* and *Bifidobacterium animalis* subsp. *lactis* remained above 6.0 log CFU/g throughout storage. Notably, the inclusion of fiber did not negatively influence sensory attributes while contributing to probiotic stability and overall functional value (Ayar et al., 2018).

In another study, the development of functional ice cream containing 5% rowanberry (*Sorbus aucuparia*) and *Lactobacillus rhamnosus* demonstrated high antioxidant potential and probiotic viability (8.24 log CFU/g). Although the addition of rowanberry slightly reduced pH and brightness, it enhanced color intensity and improved the functional quality of the final product (Goktas, 2023). Similarly, in probiotic ice creams enriched with grape seed powder, pomegranate seed powder, sesame seed powder, grape seed powder was identified as the most effective additive due to its high phenolic and antioxidant content. This ingredient supported the survival of *L. rhamnosus* and *B. animalis* BB-12 strains during storage, while maintaining stable pH and acidity levels and markedly improving the functional profile of the samples (Akca & Akpinar, 2021).

The incorporation of phenolic compounds such as ellagic acid, gallic acid, pomegranate peel extract (PPE), and grape seed extract (GSE), also

enhanced the total phenolic content and DPPH radical scavenging activity in ice cream formulations. These compounds promoted the survival of *Lactobacillus casei* Shirota at ≥ 7.21 log CFU/mL over 60 days of storage, with PPE demonstrating the highest efficacy in terms of both functional performance and sensory quality (Sagdic et al., 2012).

Likewise, probiotic ice creams containing Butiá fruit (*Butia eriopatha*, South American palm fruit) and *Bifidobacterium lactis* maintained probiotic viability at 8.51 log CFU/g for 90 days, while achieving strong consumer acceptance. The formulation exhibited increased levels of flavonoids and carotenoids with minimal loss of total phenolics and ascorbic acid, indicating enhanced antioxidant potential and extended shelf-life stability (dos Santos Cruxen et al., 2016).

3. Conclusion and Recommendations

The incorporation of fruits and their derivatives into ice cream formulations represents a promising approach to developing nutritionally enhanced and functionally superior frozen desserts. Fruits contribute essential nutrients, including vitamins, minerals, and dietary fiber, while providing natural color, aroma, and flavor. More importantly, they are rich in bioactive compounds such as phenolics, flavonoids, carotenoids, and anthocyanins, which collectively enhance antioxidant activity and confer multiple health benefits. The inclusion of fruit purees, powders, or extracts improves technological and structural parameters—such as viscosity, melting resistance, and overrun—while also enriching sensory appeal.

Beyond compositional improvements, fruit-based additives play a vital role in promoting probiotic viability and microbiological stability. Studies have consistently shown that the natural acidity, phenolic content, and prebiotic fibers found in fruits create a favorable microenvironment for beneficial bacteria such as *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium lactis*, helping maintain viable counts above the therapeutic threshold during storage. Furthermore, fruit-derived components such as pectin and polyphenols not only enhance the texture and mouth-feel of ice cream but also extend its shelf life by reducing oxidative degradation.

From a sensory perspective, fruit additions enhance visual quality, color intensity, and flavor complexity, increasing consumer preference and market potential. However, the effectiveness of fruit incorporation depends largely on the type of fruit, its processing method, and the concentration used. Over-fortification can lead to undesirable effects such as texture hardening, acidity imbalance, or bitterness. Therefore, optimization

of fruit concentration and processing parameters is critical to achieving an ideal balance between functional benefits and sensory acceptability.

The sustainability dimension of fruit use should also be emphasized. The valorization of fruit-processing by-products such as peels, seeds, and pulp provides an environmentally friendly and economically viable strategy for reducing food waste and promoting circular food production. These by-products are rich in polyphenols, fibers, and unsaturated fatty acids, making them suitable for incorporation into ice cream as functional and natural stabilizing agents.

In conclusion, fruit-enriched ice creams have the potential to serve as functional foods that combine nutritional enhancement with consumer appeal. Future research should focus on:

1. Optimizing formulation variables such as fruit concentration, particle size, and incorporation stage to preserve bioactive compounds and achieve consistent quality.
2. Exploring synergistic combinations of fruits with probiotics, prebiotics, and natural stabilizers to further enhance health benefits and product stability.
3. Applying advanced processing techniques such as cryogenic freezing, microencapsulation, and high-pressure homogenization to minimize bioactive degradation and improve textural integrity.
4. Evaluating long-term stability and sensory performance during storage under different temperature conditions to ensure commercial feasibility.

Integrating fruits into ice cream production not only supports the creation of healthier and more sustainable food products but also aligns with current consumer demand for natural, functional, and clean-label alternatives in the frozen dessert market.

Declaration of Use of AI Technologies

During the preparation of this book chapter, the author used ChatGPT (OpenAI) to correct grammar and spelling. After using this tool, the authors reviewed and edited the content and took full responsibility for the final version of the publication.

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Chapter 4

THE ROLE OF FRUIT ADDITIVES IN FUNCTIONAL YOGURT PRODUCTION AND TECHNOLOGICAL IMPROVEMENTS

Kurban YAŞAR¹

¹ Osmaniye Korkut Ata University, Faculty of Engineering and Natural Sciences,
Department of Food Engineering, 80000 Osmaniye, Türkiye. E-Mail: kurbanyasar@osmaniye.edu.tr, <https://orcid.org/0000-0002-6514-2101>

1. Introduction

Yogurt is a traditional fermented dairy product produced through lactic acid fermentation, renowned for its high nutritional value and numerous health-promoting effects (Sahan et al., 2008; Saritaş et al., 2024). Originating from the Turks of Central Asia, yogurt has evolved into a globally consumed food product (Güven et al., 2005). Compared to fresh milk, yogurt is digested two to three times more rapidly, making it a favorable option for individuals with digestive problems, lactose intolerance, or those recovering from illness or antibiotic therapy. Additionally, yogurt has been associated with beneficial effects on the immune system, improved iron absorption, and cholesterol reduction (Król et al., 2025). Its naturally low-fat content contributes to cardiovascular health, positioning yogurt as an essential component of balanced dietary habits (Zhuang et al., 2025).

In recent years, heightened consumer awareness regarding nutrition and wellness has substantially increased the demand for functional foods. Within this framework, probiotic yogurts have gained remarkable popularity due to their ability to enhance gut health, inhibit pathogenic microorganisms, lower serum cholesterol levels, and strengthen immune function (Karaca et al., 2019). To further enhance these benefits, antioxidants and bioactive compounds such as prebiotic substrates and fruit fibers are frequently incorporated into yogurt formulations.

Starter cultures have been fundamental to industrial yogurt production for over a century. Among them, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* remain the most widely employed species. These bacteria produce exopolysaccharides (EPS) that contribute to yogurt's texture, flavor, and shelf-life by improving water-holding capacity and structural stability, often reducing or eliminating the need for additional stabilizers (Şahan et al., 2006; Yeydem & Yaşar, 2023; Bulca & Büyükgümüş, 2024).

Functional foods are designed not only to meet basic nutritional requirements but also to provide additional health benefits (Sun-Waterhouse, 2011). Incorporating fruits into yogurt formulations enhances phenolic content, antioxidant potential, and sensory attributes (Oliveira et al., 2018; Sukhikh et al., 2019; Prestes et al., 2021). Fruits rich in antioxidants—such as cranberry, blueberry, pomegranate, and rosehip—are particularly effective in improving yogurt's color, flavor, texture, and bioavailability (Sahingil et al., 2022; Mutlu et al., 2025).

Fruits are valuable sources of vitamins, antioxidants, phenolics, and dietary fiber (Oliveira et al., 2018). When used as yogurt ingredients, they

not only impart desirable flavor and aroma but also enhance product stability by increasing antioxidant capacity (Prestes et al., 2021). For example, fruit additions such as cranberry, blueberry, pomegranate, apple, and blackcurrant significantly elevate phenolic content and antioxidant activity (Mutlu et al., 2025). Likewise, prebiotic components such as apricot fiber promote the viability of probiotic microorganisms and improve the microbial quality of yogurt (Karaca et al., 2019).

Overall, the incorporation of fruits and prebiotics in yogurt formulations contributes to the development of nutritionally enhanced, sensorially appealing, and functionally superior products. In synbiotic mango yogurt, for instance, the combination of mango pulp and inulin substantially improved both phenolic composition and antioxidant potential (Minj & Vij, 2025). Such functional fortifications not only enhance the bioavailability of nutrients but also support immune and metabolic health, underscoring the multifaceted benefits of fruit-enriched yogurt.

2. Effects of Fruit Supplementation on the Chemical Properties of Yogurt

The incorporation of fruits into yogurt formulations significantly enhances the product's chemical and nutritional characteristics by enriching it with phenolic compounds, vitamins, minerals, and antioxidants. Fruits are rich sources of polyphenols and essential micronutrients that not only increase the antioxidant potential of yogurt but also improve the bioavailability of these compounds (Oliveira et al., 2018). For example, yogurt fortified with sour cherry puree exhibited a substantial increase in total phenolic content while maintaining its antioxidant capacity (Şengül et al., 2012).

Numerous studies have consistently confirmed that fruit supplementation enhances the chemical attributes of yogurt. The addition of pineapple (15%) and orange (5%) has been shown to elevate ash content and iron concentration, thereby improving the overall mineral and nutritional profile (Boev et al., 2025). Furthermore, the incorporation of fruit pulps enriches the yogurt matrix with bioactive compounds and dietary fibers, which enhances antioxidant activity, probiotic stability, and overall functionality. These components also interact with milk proteins, promoting gelation and emulsification processes that contribute to improved texture and sensory appeal (Priyashantha et al., 2025).

Synbiotic yogurts enriched with monk fruit (*Siraitia grosvenorii*) extract have demonstrated high levels of total phenolics, flavonoids, and mogrosides, resulting in a more nutritious product (Ban et al., 2022). Regular consumption of these yogurts has been linked to beneficial effects on

phenylalanine metabolism, bile secretion, and lipid metabolism, particularly through favorable modulation of hepatic phospholipid biomarkers such as phosphatidylcholines and phosphatidylethanolamines.

Similarly, yogurts supplemented with white sapote (*Casimiroa edulis*) extract and orange blossom honey showed significant improvements in total phenolic and flavonoid contents as well as antioxidant activity. These additions also positively affected total solids, acidity, pH, viscosity, and water-holding capacity, contributing to improved product stability and texture (Khateeb et al., 2025).

In camel milk yogurts enriched with persimmon (*Diospyros kaki*) pulp, increases in total phenolic and flavonoid contents were observed alongside enhanced antioxidant activity. At supplementation levels of 3%, 5%, and 10%, total solids and viscosity values rose, whereas pH decreased, indicating better structural stability (Ait El Alia et al., 2023).

The use of black chokeberry (*Aronia melanocarpa*) pomace as a supplement has also been reported to significantly enhance yogurt's composition. Formulations containing 1%, 2%, and 3% pomace showed an increase in total solids up to 13.71%, primarily due to the pomace's richness in fiber and phenolic compounds (Pădureț et al., 2024). Likewise, the addition of Cornelian cherry (*Cornus mas L.*) concentrate, which is abundant in vitamin C, anthocyanins, and organic acids, provided strong antioxidant, antimicrobial, and anti-inflammatory effects while improving both nutritional quality and rheological stability (Bozdoğan, 2017).

Anthocyanins—natural pigments responsible for the red, purple, and blue hues of many fruits—also play a key role in enhancing yogurt's antioxidant properties and visual appeal. Previous studies on functional beverages such as fermented turnip and red radish juices have demonstrated that anthocyanins degrade following first-order kinetics with increasing temperature (Bozdoğan & Yaşar, 2019; Bozdoğan & Yaşar, 2021). Therefore, using anthocyanin-rich fruits in yogurt not only improves antioxidant potential but also enhances consumer acceptance due to the appealing color.

Yogurt enriched with *Euryale ferox* fruit peel extract exhibited a remarkable improvement in total phenolic content and water-holding capacity (up to 63.64%), attributed to the peel's abundance of polyphenols and dietary fibers (Qian et al., 2024). Similarly, the addition of *Melastoma dodecandrum Lour* fruit powder at a 1% level significantly increased mineral concentrations, including phosphorus (2%), calcium (8%), potassium (24%), copper (46.15%), and manganese (87.55%) (Rashwan et al., 2022).

In frozen yogurt fortified with 30% red dragon fruit (*Hylocereus polyrhizus*) puree, significant increases were observed in phenolic content, dietary fiber, and antioxidant activity. Although total solids decreased, protein content rose substantially, indicating improved nutritional value (Susanti et al., 2022). In addition, inoculating citrus juices with *Lactiplantibacillus plantarum* strains was found to alter the phenolic composition, decreasing naringin levels by 61.5% while increasing quinic acid and other bioactive phenolics (Ímece et al., 2024).

Collectively, these findings demonstrate that fruit supplementation markedly improves the chemical and functional properties of yogurt. The enrichment of yogurt with fruit-based bioactive compounds enhances antioxidant capacity, mineral content, and nutrient bioavailability, further reinforcing yogurt's role as a valuable functional food.

3. Effects of Fruit Supplementation on the Viscosity and Textural Properties of Yogurt

The beneficial influence of fruit supplementation on the viscosity and textural characteristics of yogurt has been extensively reported in the literature. Fruit-derived components, particularly fibers and pectins, improve the water-holding capacity (WHC) of yogurt, thereby minimizing syneresis and enhancing overall consistency. In yogurts enriched with reconstituted elderberry juice, WHC reached as high as 96.4% at a 25% supplementation level, effectively preventing spontaneous syneresis. Gel strength also increased to 5.626 g, an effect attributed to the pectin and fiber content of elderberry juice, which promote structural reinforcement within the gel matrix (Cais-Sokolińska & Walkowiak-Tomczak, 2021).

The addition of Cornelian cherry (*Cornus mas L.*) concentrate has been shown to significantly enhance both viscosity and flow behavior. This fruit concentrate increased the apparent viscosity of yogurt and induced a transition toward more Newtonian flow characteristics, with these rheological changes closely linked to concentration levels (Bozdogan, 2017). Similarly, the inclusion of apricot fiber and Godapara fruit mucilage improved WHC, reduced syneresis, and strengthened the structural integrity of yogurt. The mucilage, rich in soluble fiber, also enhanced spoonability and consumer acceptability (Samanmali & Kananke, 2025).

Fruit-based additives not only increase viscosity but also contribute to a more cohesive and uniform microstructure. The incorporation of pomegranate peel, passion fruit, and açai pulp has been reported to improve the gelation of milk proteins, thereby enhancing the mechanical stability of the yogurt matrix (Priyashantha et al., 2025). In yogurts fortified with mango pulp and inulin, WHC increased up to 93.67%, while syneresis

was reduced. These effects were attributed to the stabilizing functions of pectin and dietary fiber naturally present in mango pulp, which aid in water binding and matrix stabilization (Minj & Vij, 2025).

In formulations containing black chokeberry (*Aronia melanocarpa*) juice and pomace, notable textural differences were observed. Yogurts supplemented with pomace exhibited significantly higher viscosity and hardness compared to juice-fortified samples, which showed lower values for these parameters. Furthermore, at a 3% pomace level, adhesiveness and elasticity were substantially increased, indicating an improvement in the yogurt's structural cohesion (Pădureț et al., 2024).

Supplementation with *Euryale ferox* fruit peel extract also enhanced both viscosity and textural stability. Scanning Electron Microscopy (SEM) analysis revealed a more compact and homogeneous protein network within the yogurt matrix, correlating with improved water retention and reduced syneresis (Qian et al., 2024). Likewise, the addition of *Berberis aristata* fruit extract increased WHC up to 62% and minimized syneresis, effects linked to the extract's high levels of phenolic compounds and dietary fibers (Mueed et al., 2024).

Frozen yogurt enriched with 30% red dragon fruit (*Hylocereus polyrhizus*) puree demonstrated significant textural improvements and strong consumer appeal. The fortified product exhibited a smooth and uniform texture, vivid purple coloration, and a soft mouthfeel, characteristics attributed to the presence of betacyanin pigments in the fruit (Susanti et al., 2022). Similarly, in camel milk yogurt supplemented with 10% persimmon (*Diospyros kaki*) puree, viscosity values increased to 27,040 cP, accompanied by enhanced structural integrity and reduced syneresis. These improvements were associated with the natural thickening and fiber content of persimmon (Ait El Alia et al., 2023).

Du et al. (2023) also demonstrated that the incorporation of mulberry pomace polysaccharides (MPP) significantly improved yogurt texture. MPP supplementation enhanced WHC, reduced syneresis, and strengthened the gel network through interactions between natural pectin structures and milk proteins. At a concentration of 0.5%, syneresis decreased to 12.59%, confirming improved structural stability and durability.

Overall, fruit supplementation markedly enhances the viscosity and textural quality of yogurt. Fruits rich in pectin and dietary fiber promote the formation of a denser and more homogeneous gel network, resulting in improved consistency, higher WHC, and reduced syneresis. These enhancements not only increase sensory acceptance but also contribute to the development of structurally stable yogurt formulations suitable for

industrial-scale production.

4. Effects of Fruit Supplementation on the Sensory Properties of Yogurt

Fruit supplementation markedly enhances the sensory profile of yogurt by improving its flavor, aroma, color, and overall acceptability. Numerous sensory evaluations have demonstrated that fruit-enriched yogurts receive higher consumer preference scores than plain formulations (Mutlu et al., 2025). The incorporation of fruit extracts, purees, and powders not only improves sensory appeal but also enriches the product with bioactive compounds that contribute to color and flavor stability.

Anthocyanin-rich fruits such as mulberry significantly enhance the visual and antioxidant qualities of yogurt. In yogurts enriched with mulberry fruit extract (MFE), total phenolic content (TPC) and total antioxidant capacity (T-AOC) increased by 27.34% and 97.73%, respectively, accompanied by higher DPPH and ABTS radical scavenging activity (Cai et al., 2025). Similarly, yogurts fortified with fruit powders—including mango, papaya, beetroot, and apple—showed high sensory acceptance, with mango-enriched samples rated the best due to their smooth texture and pleasant sweetness (Cao et al., 2025). The addition of pineapple (15%) and orange (5%) also enhanced taste, texture, and aroma, with citrus notes contributing to increased consumer satisfaction (Boev et al., 2025).

Yogurts supplemented with black chokeberry (*Aronia melanocarpa*) received high scores for appearance and color, although the inclusion of pomace imparted a slightly astringent taste that reduced flavor preference (Pădureț et al., 2024). In contrast, *Euryale ferox* fruit peel extract improved aroma, mouthfeel, and creaminess, yielding a smoother and more homogeneous product (Qian et al., 2024). Likewise, yogurts enriched with *Berberis aristata* extract were rated highly for color, taste, and texture—attributes associated with increased L^* (lightness) and b^* (yellowness) values due to the fruit's high phenolic content (Mueed et al., 2024).

Among frozen yogurt samples, those fortified with 30% red dragon fruit (*Hylocereus polyrhizus*) puree achieved the highest sensory scores. Their vivid purple color, mild sweetness, and fruity aroma were linked to the presence of betacyanin pigments (Susanti et al., 2022). Similarly, camel milk yogurts containing 5% persimmon (*Diospyros kaki*) puree were preferred by panelists for their creamy texture and well-balanced flavor profile (Ait El Alia et al., 2023). Yogurts with 5% bael pulp were also positively evaluated, though higher addition levels (10%) led to an overpowering flavor that reduced acceptability (Yapa et al., 2023).

The addition of *Dovyalis caffra* pulp imparted a bright yellow color that was well appreciated, although higher acidity negatively affected taste scores at 15% addition (Waweru et al., 2024). Strawberry- and blackberry-enriched yogurts were rated highly for their vibrant color, fruity aroma, and balanced sweetness (Kaptan & Kayışoğlu, 2024). In freeze-dried yogurts, the inclusion of mulberry syrup further improved taste, aroma, and color intensity, while the high pectin and fiber content contributed to a firmer, more stable texture (Huynh et al., 2023).

Synbiotic yogurts containing monk fruit extract (*Siraitia grosvenorii*) also received excellent sensory evaluations, achieving sweetness and flavor intensity comparable to sugar-added products but without additional sweeteners (Ban et al., 2022).

Overall, fruit fortification substantially improves yogurt's sensory appeal by enhancing its visual, aromatic, and textural qualities. Fruit-derived pigments and phenolic compounds contribute to more attractive coloration and richer flavor, while fibers and pectins improve mouthfeel and consistency. Collectively, these attributes increase consumer satisfaction and expand the commercial potential of fruit-enriched yogurt products.

5. Effects of Fruit Supplementation on the Antioxidant Capacity and Health-Related Properties of Yogurt

Environmental factors such as altitude and climate can influence the phenolic composition and stress response of plant-based ingredients, which in turn affect the functional and nutritional quality of derived food products (Özoğul & Küpe, 2025). In this context, fruit supplementation has proven to be an effective strategy for enhancing the antioxidant potential and health-promoting properties of yogurt. Fruits are natural reservoirs of phenolic compounds, flavonoids, and anthocyanins, all of which contribute to increased radical scavenging activity and improved bioavailability.

Yogurts fortified with restructured elderberry juice demonstrated a significant rise in antioxidant capacity due to the high anthocyanin and polyphenol content of the fruit. Samples containing 25% elderberry juice achieved ABTS values of 36.6 $\mu\text{M TE/g}$, and in vitro gastrointestinal simulation indicated 62–66% bioavailability of antioxidant compounds (Cais-Sokolińska & Walkowiak-Tomczak, 2021). Similarly, freeze-dried yogurts supplemented with white mulberry (*Morus alba L.*) syrup exhibited 12.22 mg AAE/100 g DPPH and 22.55 mg AAE/100 g ABTS activity, corresponding to a 15.99% improvement over control samples (Huynh et al., 2023).

Yogurts containing blackcurrant and elderberry jams also showed elevated antioxidant capacity, attributed to their high phenolic and anthocyanin content. Among them, elderberry jam-enriched yogurts exhibited the highest anthocyanin levels (Janoušek Honesová et al., 2024). Similarly, *Dovyalis caffra* pulp increased total polyphenol and ascorbic acid contents, resulting in greater radical scavenging activity, particularly at 15% addition (Waweru et al., 2024).

Camel milk yogurts enriched with persimmon (*Diospyros kaki*) puree demonstrated similar enhancements, reaching maximum phenolic content and antioxidant capacity at 10% supplementation (Ait El Alia et al., 2023). Yogurts fortified with black chokeberry (*Aronia melanocarpa*) juice and pomace achieved DPPH scavenging activity up to 75.38%, with the highest total phenolic content observed in pomace-enriched samples (Pădureț et al., 2024).

Fruits such as pomegranate, passion fruit, and açai pulp significantly enhanced the antioxidant potential of yogurt, owing to their rich composition of phenolic acids, carotenoids, and fibers that effectively scavenge free radicals (Priyashantha et al., 2025). Yogurts enriched with sapote extract and honey also maintained high phenolic levels during storage, with chlorogenic and p-hydroxybenzoic acids contributing to long-term antioxidant stability (Khateeb et al., 2025).

In synbiotic mango yogurts containing 6.56% mango pulp and 1% inulin, ABTS activity reached 1213.59 $\mu\text{mol/L}$ TEAC, confirming the role of phenolics and carotenoids in enhancing bioactivity (Minj & Vij, 2025). Likewise, *Euryale ferox* fruit peel extract supplementation resulted in strong antioxidant performance, with ABTS and DPPH activities of 80.54% and 72.58%, respectively (Qian et al., 2024). Yogurts containing pomegranate peel extract also exhibited elevated phenolic, flavonoid, and anthocyanin contents, leading to increased antioxidant activity. Moreover, ethanolic extracts improved the retention of phenolic compounds within the yogurt matrix while reducing pH and syneresis, thus improving both functionality and stability (Jany et al., 2024).

In summary, the incorporation of fruit-derived ingredients substantially enhances the antioxidant and health-related properties of yogurt. Polyphenols, flavonoids, and anthocyanins act synergistically to increase radical scavenging activity, support cardiovascular and immune health, and exhibit anti-inflammatory effects. By enriching yogurt with these bioactive compounds, fruit supplementation not only boosts functional value but also contributes meaningfully to consumer well-being

6. Effects of Fruit Additions on the Microbiological Stability of Yogurt

Fruit supplementation has been shown to play a significant role in maintaining the microbiological stability and probiotic viability of yogurt during storage. The incorporation of fruit-based ingredients supports the growth and persistence of beneficial lactic acid bacteria by providing natural sugars, fibers, and bioactive compounds that act as prebiotic substrates.

Turgut and Çakmakçı (2018) reported that probiotic yogurts fortified with strawberry marmalade successfully maintained viable counts of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* above the recommended threshold ($\geq 10^7$ CFU/mL) throughout refrigerated storage, while achieving high sensory acceptability. Similarly, the addition of fruit powders—such as apple, papaya, mango, and beetroot—enhanced the survival of *Lactocaseibacillus rhamnosus* GR-1 during 30 days of storage. Among these, papaya powder yielded the highest probiotic viability, with counts consistently exceeding 10^7 CFU/mL (Cao et al., 2025).

The inclusion of plant-derived phenolic compounds, flavonoids, and anthocyanins in yogurt formulations has also been linked to improved probiotic survival. Rashwan et al. (2023) demonstrated that these bioactive molecules enhance the resistance of lactic acid bacteria during storage, increasing water-holding capacity by 20–25% while reducing syneresis by 15–32%. Çakmakçı et al. (2012) similarly observed that probiotic yogurts enriched with banana marmalade supported the survival of *L. acidophilus* and *B. bifidum* up to seven days, with *B. bifidum*-containing samples achieving the highest sensory scores.

Several studies have confirmed that fruit pulps and purees sustain probiotic counts throughout storage. Priyashantha et al. (2025) reported that fruit supplementation preserved the viability of *L. acidophilus* and *Bifidobacterium* spp. during refrigerated storage. In camel milk yogurts enriched with 5% persimmon (*Diospyros kaki*) pulp, microbial stability was markedly improved compared to other formulations, likely due to the prebiotic fiber content of persimmon (Ait El Alia et al., 2023). Similarly, the combination of mango pulp and inulin in synbiotic yogurt enhanced both microbial stability and probiotic survival, with *Lactobacillus* and *Bifidobacterium* species maintaining viable counts throughout storage. The prebiotic effect of inulin further prolonged probiotic viability under simulated gastrointestinal conditions (Minj & Vij, 2025).

Garofalo et al. (2024) found that the inclusion of fruit purees in sheep milk yogurt did not negatively influence microbial populations. All sam-

ples maintained viable counts of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* above 8.0 log CFU/g, indicating that fruit additions do not interfere with starter culture activity. Similarly, yogurts supplemented with 5% bael pulp sustained *Lacticaseibacillus rhamnosus* GG counts above 10⁷ CFU/mL for 21 days, accompanied by reduced syneresis compared to the control (Yapa et al., 2023).

Microbiological safety is another key aspect influenced by fruit addition. In yogurts enriched with *Dovyalis caffra* pulp, no growth of coliforms, yeasts, or molds was detected during 21 days of storage, demonstrating effective post-pasteurization control and good hygienic quality (Waweru et al., 2024). Probiotic yogurts enriched with strawberry and blackberry purees also maintained high viability levels of *L. acidophilus* and *B. bifidum* throughout storage, with counts of 8.11 and 7.58 log CFU/g, respectively—an effect attributed to the prebiotic fiber content of these fruits (Kaptan & Kayışoğlu, 2024).

In freeze-dried yogurt formulations, the addition of mulberry syrup did not negatively affect microbial stability. Populations of *L. bulgaricus* and *S. thermophilus* remained stable during storage, while freeze-drying extended shelf life without compromising probiotic viability or nutritional quality (Huynh et al., 2023). Similarly, synbiotic yogurts enriched with monk fruit extract (*Siraitia grosvenorii*) exhibited high survival rates of *L. acidophilus* and *B. bifidum*, supported by the natural prebiotic activity of mogrosides in the extract (Ban et al., 2022).

Moreover, *Lactiplantibacillus plantarum* strains have shown strong resilience in low-pH environments such as grapefruit juice, remaining viable for up to 14 days—highlighting their potential as robust probiotic candidates in fruit-supplemented yogurt formulations (İmece et al., 2024).

In conclusion, fruit supplementation enhances both the microbial and probiotic stability of yogurt by supplying fermentable substrates and prebiotic fibers that sustain beneficial bacterial growth. The integration of fruit pomaces, purees, and powders not only maintains probiotic populations above the functional threshold throughout storage but also improves product texture and safety. These findings underscore the synergistic relationship between fruit-derived components and probiotic cultures, thereby reinforcing the nutritional and functional value of fruit-enriched yogurts.

7. Conclusion and Recommendations

The incorporation of fruit-derived ingredients into yogurt formulations has emerged as an effective strategy for enhancing nutritional,

functional, and sensory qualities while maintaining microbiological stability. Fruits are rich sources of polyphenols, flavonoids, anthocyanins, vitamins, and dietary fibers, which synergistically improve antioxidant capacity, mineral composition, and probiotic viability. Numerous studies have demonstrated that fruit supplementation not only elevates the total phenolic content and radical scavenging activity but also contributes to improved texture, viscosity, and water-holding capacity through the interaction of fruit fibers and pectins with milk proteins. Additionally, fruit-enriched yogurts consistently receive higher consumer preference scores due to their appealing color, enhanced aroma, and improved mouthfeel.

From a technological perspective, fruit additions support structural reinforcement of the yogurt matrix, reduce syneresis, and increase product stability during storage. The use of prebiotic-rich fruits such as persimmon, mango, and monk fruit further promotes probiotic survival and extends shelf life, confirming their functional and technological relevance in modern yogurt production. These attributes align with growing consumer demand for health-promoting, clean-label, and naturally fortified dairy products.

Future research should focus on optimizing processing parameters to preserve the stability of heat-sensitive bioactive compounds such as anthocyanins and phenolic acids during pasteurization and fermentation. Moreover, exploring underutilized native fruits rich in polyphenols and fibers may contribute to the development of region-specific functional yogurts with distinctive sensory and nutritional profiles. Integrating advanced analytical techniques—such as metabolomics, rheology, and microstructural analysis—will further elucidate the interactions between fruit components and milk proteins, enabling targeted formulation strategies for superior quality and functionality.

Declaration of use of AI Technologies

During the preparation of this book chapter, the authors used ChatGPT (OpenAI) to correct grammar and spelling. After using this tool, the authors reviewed and edited the content and take full responsibility for the final version of the publication.

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Chapter 5

RECENT DEVELOPMENTS IN METHODS FOR DETECTING AFLATOXIN IN EDIBLE OILS

Adnan BOZDOĞAN¹

¹ Department of Food Engineering, Osmaniye Korkut Ata University, Osmaniye/
Turkey

Phone: +90-328 827 10 00/3666; Fax: +90-328-825 00 97.

ORCID: 0000-0002-3612-5898

e-mail: bozdogan@osmaniye.edu.tr

1. Introduction

In recent years, food contamination has become one of the most significant threats to food safety. Contaminated foods not only cause adverse effects on human health, such as acute poisoning and chronic diseases, but also cause economic losses, negatively impacting production and healthcare systems. Consuming contaminated foods requires individuals to spend significant resources, both financial and time, on treatment. Food contamination can be caused by many biological, chemical, and physical factors. Among these, microbial contamination is the most common, typically caused by bacteria, fungi, molds, viruses, and their toxins or metabolic byproducts (Shaber et al., 2022).

Mycotoxins are common food contaminants that can deteriorate the appearance, taste, odor, and overall quality of foods. These toxins are secondary metabolites produced by fungi such as *Fusarium*, *Aspergillus*, and *Penicillium*, and can have various toxic effects on both humans and animals. To date, over 400 different mycotoxins have been identified (Holban and Grumezescu, 2018; Wang et al., 2018; Lee and Ryu, 2017).

The main mycotoxins found as natural contaminants in food include aflatoxins (AF), ochratoxin A (OTA), zearalenone (ZEN), fumonisins (FB), trichothecene derivatives (TRT) such as nivalenol (NIV), and deoxynivalenol (DON) (Kebede et al., 2020). Among these toxins, aflatoxins, in particular, are considered one of the most critical public health problems due to their high toxicity and potent carcinogenic properties. Aflatoxins have been reported to be responsible for approximately 25% of animal deaths (Azam et al., 2021; Ünüsan, 2019).

Aflatoxins; They are toxic metabolites synthesized by mold species such as *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius*. This group constitutes the most researched mycotoxin class due to both toxic and carcinogenic effects. The most common aflatoxins are aflatoxin B₁ (AFB₁), aflatoxin B₂ (AFB₂), aflatoxin G₁ (AFG₁), and aflatoxin G₂ (AFG₂). AFB₁, in particular, has been identified by the World Health Organization-International Agency for Research on Cancer (WHO-IARC) as the only mycotoxin with scientifically confirmed carcinogenic activity. Epidemiological studies conducted by IARC in Asia and Africa have shown a positive association between the consumption of AFB₁-contaminated foods and the incidence of liver cancer (Rustom, 1997; Binder et al., 2007; Koray et al., 2022). AFB₁ can contaminate many agricultural products, particularly peanuts, pistachios, hazelnuts, corn, red peppers, and figs. Therefore, identifying food products at risk for aflatoxin is crucial for food safety and protecting public health (Kabak, 2007).

Edible vegetable oils play an indispensable role in human nutrition as a source of basic energy and essential fatty acids. However, every stage from the cultivation of oil crops to the production of the final product can be exposed to various external factors (e.g., mycotoxins) that can affect the quality and safety of the product. Because many oil crops, such as corn, peanuts, soybeans, rapeseed, sunflower seeds, olives, and hazelnuts, exhibit seasonal characteristics, they are easily affected by factors such as climatic conditions, pests, and environmental factors. This situation, in particular, increases susceptibility to *Aspergillus flavus* infection (Mao et al., 2019).

After harvest, storage conditions (e.g., temperature and humidity levels) and storage methods used can cause the oil to spoil or become rancid. Furthermore, when raw materials used in the edible oil production process are contaminated with *Aspergillus* species, the risk of aflatoxin B₁ (AFB₁) contamination in freshly pressed oils arises (Shavakhi et al., 2022; He et al., 2022). Therefore, AFB₁ contamination in edible vegetable oils is considered a significant food safety problem on a global scale (Bordin et al., 2014; Shephard et al., 2018).

Numerous studies conducted in different countries have revealed that mycotoxin contamination in edible vegetable oils is widespread (Bordin et al., 2014; Cavaliere et al., 2007). This has led international organizations such as the World Health Organization (WHO), Food and Agriculture Organization (FAO), European Union Commission, and Codex Alimentarius Commission (CODEX) to set standards for maximum residue limits and detection methods for mycotoxins (Li et al., 2019; Nabizadeh et al., 2018). Mycotoxin detection can be achieved using various analytical methods, including high-performance liquid chromatography (HPLC), liquid chromatography (LC), thin-layer chromatography (TLC), and immunoassay-based approaches (Zheng et al., 2006). Although numerous studies have been conducted on mycotoxin analysis in foods, further research is needed to improve the accuracy and reliability of detection methods, particularly those used in edible vegetable oils. Therefore, this review comprehensively examines methods developed and reported by researchers in recent years for the detection of mycotoxins in edible oils.

2. Mycotoxin Detection Methods in Edible Oils

The fact that mycotoxins pose serious health risks even at low levels and the existence of numerous edible oil types with different physical and chemical properties have led researchers to develop various analytical methods for the reliable detection of mycotoxins in these products. Sensitivity, speed, accuracy, cost-effectiveness, and ease of application are

among the fundamental criteria for evaluating methods used for mycotoxin detection. Furthermore, modern approaches aim to develop versatile methods that allow the detection of not only targeted mycotoxins but also non-target compounds (Khaneghah et al., 2018).

Generally, mycotoxin detection has relied on two fundamental approaches:

(i) Reference methods for quantitative analysis, i.e., analytical techniques with high accuracy and reproducibility,

(ii) Rapid methods for preliminary screening of large sample groups, i.e., practical tests that allow for the analysis of high-volume samples in a short time (Khaneghah et al., 2018).

This review examines in detail the rapid mycotoxin detection methods developed specifically for use with large sample sets. The main techniques among these methods are:

2.1. Chromatography-Based Methods

High-Performance Liquid Chromatography (HPLC)

Among chromatographic analysis techniques, High-Performance Liquid Chromatography (HPLC) is one of the most widely used methods for the detection of mycotoxins. This technique has gained widespread acceptance in the food safety field due to its high sensitivity and ability to analyze multiple compounds simultaneously. Indeed, many national and international organizations, such as the Chinese National Standard, the European Committee for Standardization (CEN), and the Association of Analytical Chemists (AOAC), have adopted HPLC as a reference standard (Turner et al., 2015).

One of the most important advantages of HPLC is its ability to measure multiple target analytes with high accuracy and reproducibility. In recent years, researchers have developed hybrid detection strategies that combine the method with detection systems such as fluorescence detectors (FLD), ultraviolet (UV), diode array detectors (DAD), and mass spectrometry (MS) to enhance HPLC's performance (Zhu et al., 2020). These combinations provide higher reliability, sensitivity, and selectivity in the determination of target compounds compared to conventional HPLC.

In particular, the HPLC-FLD combination is used as the standard method for the determination of aflatoxin B₁ (AFB₁) in edible vegetable oils. This technique can successfully detect low AFB₁ concentrations between 0.01–0.04 $\mu\text{g kg}^{-1}$ and 0.005–0.03 $\mu\text{g L}^{-1}$ (Wang et al., 2020).

Mass spectrometry (MS) significantly improves analytical performance when integrated with HPLC. This method offers the advantages of high sensitivity, selectivity, accuracy, and efficiency (Capriotti et al., 2014). Tandem mass spectrometry (MS/MS), in particular, simplifies the analysis process by eliminating the need for sample purification during the extraction phase (Malachová et al., 2014). Additionally, high-resolution mass spectrometry (HRMS) provides measurement precision at approximately 0.001 atomic mass units, enabling the identification of both targeted mycotoxins and unknown compounds such as heavy metals (Nathanail et al., 2015).

One of the most widely used methods for mycotoxin detection in edible oils is the LC-MS/MS approach (Tsiplakou et al., 2014). Afzali et al. (2012) noted that MS/MS techniques are increasingly being used in mycotoxin analysis and that these methods offer advantages such as high resolution, sensitivity, and suitability for a wide variety of oil matrices. Similarly, studies by Zhang et al. (2019), Eom et al. (2017), and Pereira et al. (2015) have shown that these methods can be effectively applied to different types of edible oils.

In contrast, gas chromatography (GC), because it is generally used for the analysis of volatile compounds, has limited application in the detection of low-volatility mycotoxins. To increase mycotoxin resolution, column conditions similar to HPLC, UHPLC, or UPLC can be used in GC analysis. A new method developed by Hidalgo-Ruiz et al. (2019) combined an Ultra-High-Performance Liquid Chromatography (UHPLC) system with a triple quadrupole mass analyzer (QQ-MS/MS), and this technique was successfully applied to the analysis of various mycotoxins, including AFB₁, in 194 different edible vegetable oil samples.

Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) is an adsorption-based chromatographic method suitable for the separation of complex mixtures (Qian et al., 2015). Developed in the 1950s, this technique is widely used today in many fields such as biology, medicine, chemistry, and especially food science. Its simplicity, low cost, and rapid results have made TLC a traditional and reliable analysis method in laboratories.

The use of TLC in food analysis is quite broad. Various studies demonstrate that this technique can be effectively applied at all stages of food production at the levels of raw materials, ingredients, additives, and contaminants (Santos et al., 2019; Gauthier et al., 2011). TLC exploits the varying adsorption affinities of different components against the same adsorbent surface. As the mobile phase (solvent) passes over the station-

ary phase (adsorbent), the components continuously undergo cycles of adsorption, desorption, and re-adsorption, thus effectively separating the components of the mixture (Sun et al., 2018).

Although the TLC method has been used for many years, it also has some limitations. Among the method's significant disadvantages are its low detection accuracy, the potentially harmful effects of volatile solvents on experimental operators and the environment, and complex sample preparation processes (Villiani et al., 2015).

However, in recent years, the integration of TLC with modern analytical technologies has significantly expanded the method's application area. Combining it with techniques such as image analysis, surface-enhanced Raman spectroscopy (SERS), mass spectrometry (MS), and nuclear magnetic resonance (NMR) increases both the sensitivity and selectivity of TLC, enabling more comprehensive analyses (Lin et al., 1998; Sereshti et al., 2018).

Thanks to this multidisciplinary approach, TLC is now effectively used not only for qualitative analysis but also for the detection of harmful substances such as aflatoxin (AF) in complex food matrices such as edible oils. Therefore, thin-layer chromatography maintains its importance as a rapid, economical, and practical analytical tool in food science (Lin et al., 1998).

2.2. Spectroscopic Technology

Fluorescence Spectrophotometry

Spectrum-based detection technologies have been developed and widely used in recent years for the determination of aflatoxin (AF) contamination in foods (Tao et al., 2018). Among these technologies, fluorescence spectrometry, in particular, stands out as a method that exhibits high potential for the detection of AFs in various agricultural products and foods (Wu et al., 2018; Yadav et al., 2021). Fluorescence spectrometry characterizes the molecular structure of compounds by measuring the fluorescence emission generated by the absorption of ultraviolet (UV) or visible light by target molecules in the sample. This method is notable for providing high sensitivity and selectivity in the analysis of AFs and other chemical compounds (Ma et al., 2018).

The fluorescence phenomenon provides a highly convenient principle for the identification and monitoring of target compounds. For example, AFB1 emits a characteristic fluorescence in the 425–500 nm range when excited with UV light in the 340–400 nm range; this property allows the

analysis of AFB₁ in different foods by fluorescence spectroscopy (Ma et al., 2018).

In recent years, advances in laser-induced fluorescence (LIF) technology have further increased the method's potential for application in food analysis. The use of laser light sources at specific wavelengths provides the method with both higher specificity and superior detection sensitivity. The most important advantage of LIF technology is that it offers online, rapid, non-destructive, and direct detection of AFB₁ based on its characteristic fluorescence peak. Studies have demonstrated the development of LIF-based models that enable the rapid and accurate determination of AFB₁ in different edible oil types. The findings demonstrate that LIF technology can be used as an effective tool for the rapid and non-destructive detection of AFB₁ in edible oils (Chen et al., 2021).

Infrared (IR) Spectroscopy

Infrared (IR) spectroscopy has gained widespread acceptance as an effective analysis method in food safety due to its rapid detection capacity, simple sample preparation process, and high adaptability. Because this technique operates over a broad electromagnetic spectrum spanning 780–2500 nm, it has been successfully used in the analysis of a wide variety of foods, including edible oils, meat products, seafood, fruits, and vegetables (Rohman et al., 2016; Nicolaï et al., 2007; Kurz et al., 2010).

When IR radiation contacts a sample, it is reflected, absorbed, or transmitted by molecular bonds. These interactions, coupled with changes in the light's energy and molecular vibrations, produce characteristic signals specific to specific chemical bonds. Thus, the obtained spectral data provide information about the chemical composition and structural properties of the sample (Ravikanth et al., 2016; Morsy and Sun, 2013).

IR spectroscopy, specifically for edible oils, is effective in numerous applications, such as classifying different oil types, assessing oil quality, and identifying harmful components (Luo et al., 2011; Mignani et al., 2011). In recent years, near-infrared (NIR) spectroscopy has become a particularly attractive research area for determining mold contamination and the presence of aflatoxin (AFB₁) in edible oils. In this context, researchers have developed NIR-based models for the qualitative and quantitative analysis of AFB₁ contamination, significantly increasing the applicability and accuracy of infrared spectroscopy in food safety monitoring systems (Yao et al., 2022; Song et al., 2021).

Terahertz (THz) Spectroscopy

With rapid advances in optical and electronic technologies, terahertz (THz) spectroscopy has emerged as a groundbreaking method for non-destructive food analysis and has become an important research area (Rawson and Sunil, 2021; Feng and Otani, 2021). Operating in a frequency range between microwave and infrared spectroscopy, THz technology combines the features of both methods, offering widespread use in both basic scientific research and industrial applications (Kawano and Ishibashi, 2008).

Advances in chemometric analysis techniques have enabled THz spectroscopy to perform highly sensitive detection and quantification in complex food matrices, making it a powerful analytical tool in the food industry (Kawano and Ishibashi, 2008; Wang et al., 2017). By combining THz spectroscopy with chemometric approaches, researchers have developed models capable of rapidly and non-destructively determining the presence of aflatoxin B₁ (AFB₁) in edible oils. Although the accuracy of this method is somewhat lower than that of conventional chromatographic techniques, it is attracting attention as an alternative detection platform in food safety monitoring systems (Chen and Lijuan, 2014).

Recent studies have shown that the accuracy of THz-based systems can be improved through the integration of preprocessing algorithms and multidisciplinary analysis approaches. Liu et al. (2019) lowered the limit of detection (LOD) of AFB₁ to 1 µg kg⁻¹ in their model and increased the overall accuracy to over 90%. These advancements strengthen the applicability of THz technology for the detection of mycotoxins in edible oils.

However, THz spectroscopy also has some limitations. These limitations include low detection sensitivity, limited sample penetration depth, the presence of scattering effects, high equipment cost, and insufficient reference databases (Wang et al., 2021). Therefore, future studies are expected to improve these weaknesses of THz technology, making it more widely applicable in food safety applications.

Surface-Enhanced Raman Spectroscopy (SERS)

Raman spectroscopy (RS), considered a complement to infrared (IR) spectroscopy, is an analysis technique that is particularly sensitive to symmetric vibrations of non-polar functional groups (e.g., C=O, C–C, and S–S bonds) (Wu et al., 2021; He et al., 2019). Thanks to these features, RS stands out as a fast, accurate, and minimal sample preparation method for food analysis (Zhu et al., 2022).

However, traditional Raman spectroscopy suffers from some sensitivity limitations due to the weak signal intensity of Raman scattering. To address these shortcomings, researchers have developed surface-enhanced Raman spectroscopy (SERS) technology, which is based on the principles of electromagnetic field amplification and chemical signal enhancement. SERS allows for a million-fold amplification of the Raman signal, offering a powerful alternative for the detection of trace compounds.

Currently, the use of SERS technology for aflatoxin (AF) detection is still in its infancy; however, the integration of materials science, biotechnology, and chemical analysis techniques is accelerating advances in this field (Wu et al., 2021). Recent studies have demonstrated the development of innovative SERS-based methods for the detection of aflatoxin B₁ (AFB₁) in edible oils. These methods include antibody and aptamer-labeled SERS sensors, SERS-based sandwich immunoassay systems, and nanomaterial-supported surface designs (Yang et al., 2017; Chen et al., 2018).

The findings demonstrate that SERS technology has become a promising tool for the sensitive detection of aflatoxins, particularly in food safety inspections. However, several significant challenges remain before this technology can be widely implemented. Among these, the design of new target-specific nanomaterials, standardization of measurement protocols, increased signal reproducibility, and translation of laboratory findings to the industrial scale are prominent (Toh et al., 2015; Jiang et al., 2020).

In summary, Raman and SERS technologies offer a powerful analytical platform for the rapid, sensitive, and non-destructive detection of toxic compounds such as AFB₁ in edible oils and have the potential to become a key component of future food safety monitoring systems.

2.3. Immunoassay-based methods

These methods are widely used for the initial screening and assessment of mycotoxin contamination in edible oils due to their simplicity, low cost, high sensitivity, and selectivity. In this context, enzyme-linked immunosorbent assay (ELISA) is one of the most frequently preferred methods for mycotoxin detection (Bordin et al., 2014).

In quality control applications, ELISA-based kits are often used to rapidly and reliably determine the presence of mycotoxins (Qi et al., 2019). The method's operating principle is based on antibody-antigen interaction, and although shortening the incubation time provides the advantage of reducing analysis time, it can lead to a decrease in diagnostic sensitivity. During analysis, mycotoxins are extracted with a suitable solvent and then conjugated with the enzyme to react. The resulting color change

(blue dye) is measured with an ELISA reader at a wavelength of 450 nm (Trucksess, 2001).

One of the limitations of the ELISA method is that, despite the high specificity and sensitivity of the antibodies used, they can sometimes interact with the target mycotoxins, leading to biases in actual concentration measurements (Trucksess, 1995). Therefore, other immunoassay techniques have been developed alongside ELISA, including membrane-based immunoassays such as flow cell-based assays and lateral flow tests.

Membrane-Based Immunoassay

These methods can be divided into two main groups: flow-based analysis and lateral flow tests (strip tests). In the flow-based analysis method, antibodies are immobilized on the membrane surface, and the extracted mycotoxin sample is applied to the membrane. If the mycotoxin level is below the specified cutoff point, colored spots appear on the membrane; if levels exceed the cutoff point, these colored spots are not observed. This approach is rapid, user-friendly, and applicable in the field, and does not require additional equipment. However, the main limitation of the method is that it is semi-quantitative, making it difficult to accurately interpret concentrations in samples close to the cutoff point (Paepens, 2004).

In recent years, lateral flow immunoassays (LFIA) have been widely used for the rapid detection of mycotoxins in foods. The advantages of these tests include high analysis speed, user-friendly design, and high stability to various environmental conditions. On the other hand, because they provide semi-quantitative results, HPLC or other reference methods are often required to confirm the detected values (MZ et al., 2007). In general, immunochemical-based methods are designed to develop rapid, practical, and easily applicable detection systems in edible oil and other food matrices (Beloglazova and Eremin, 2015).

Fluorometric Analysis Method

In this method, the enzyme reaction is measured based on the fluorescence spectra of the resulting substrate. For the method to operate with high sensitivity, fluorescence interferences that may be caused by other substances co-extracted with the mycotoxin must be eliminated beforehand. Two cleanup methods are generally used for this purpose: immunoaffinity column cleanup and solid-phase extraction (SPE) column cleanup (Zheng et al., 2006). These procedures increase the accuracy of the measurement, allowing for the sensitive and reliable determination of mycotoxins.

Fluorescence polarization (FP) method

This method is based on the principle of fluorescence anisotropy (also known as fluorescence polarization, FP) and is based on the competition between mycotoxin and mycotoxin fluorescence for a specific antibody. While fluorescence polarization is not a direct measurement of fluorophore concentration, it does involve measuring the orientation of fluorescence emission in horizontal and vertical directions. In this method, polarization increases when an antibody binds to the tracer, while in the presence of free mycotoxin, less antibody binds to the tracer, thus decreasing polarization; this value is inversely proportional to the mycotoxin concentration (Maragos and Kim, 2004).

Compared to ELISA, fluorescence polarization analysis has two significant advantages: i) the measurement is not dependent on enzymatic reactions; ii) there is no need to separate bound and free compounds. Therefore, FP analyses do not require an elution step and avoid time-consuming procedures such as waiting for enzyme reactions to produce color (Maragos and Plattner, 2002).

Immunochromatographic Testing (ICT) Method

Another common mycotoxin detection method is immunochromatographic testing (ICT), which is typically used to provide visual detection in a yes/no format (Majdinasab et al., 2015).

However, immunoassay-based methods have some limitations. In particular, the highly selective molecular recognition mechanisms for mycotoxins can limit the detection of unknown or emerging toxins. Such emerging risks can be overcome by using array-based platforms that simultaneously identify different analytes in multi-target, spatially separated regions (Urusov et al., 2015). In this context, biosensor-based array assays (IT) have been developed and are capable of detecting 10 different mycotoxins simultaneously (Zagheri et al., 2015).

2.4. Other Newly Developed Methods

Other rapid mycotoxin detection methods include microarray technology, wavelet analysis, the Luminex xMAP® platform, and molecularly imprinted polymers (MIPs) (Zhao et al., 2017). These approaches offer advantages such as high parallelism, speed, and multiple analyte detection over classical immunoassay and chromatographic methods, enabling the efficient assessment of mycotoxin contamination in edible oils and other food matrices.

Microarray technology

This method was developed to identify a large number of microorganisms, particularly by detecting DNA fragments such as PCR products. Prominent advantages of the method include high-throughput analysis and the ability to work with small sample sizes.

However, the method also has some limitations: i) potential uncertainty in the results obtained; ii) statistical errors arising from RNA extraction, amplification, and hybridization steps; iii) The method can only detect fungal mycotoxins, not all mycotoxins, in various food matrices such as edible oil (Call et al., 2003). Therefore, PCR-based mycotoxin detection is often used in conjunction with other analysis methods.

Evanescence Wave Technology

Evanescence Wave Technology (EWT) is an analysis method based on evanescent wave excitations that produce a fluorescent signal. In recent years, EWT has become increasingly popular for the rapid and sensitive detection of mycotoxins in food (Taitt et al., 2016).

There are two main formats of EWT for mycotoxin detection: i) surface plasmon resonance (SPR) biosensor, which measures changes in the refractive index between two transparent media; ii) fiber optic immunosensor, which can be implemented in two formats: competitive and non-competitive (Maragos and Thompson, 1999). In one application of fiber optic immunosensors, fumonisin B1 (FB1) was detected in edible oil samples at concentrations ranging from 10–1000 ng/L, with a detection limit of 10 ng/L (Thompson and Maragos, 1996). This method is considered a valuable alternative for the detection of mycotoxins in edible oils due to its advantages of high sensitivity, rapid analysis, and field applicability.

Luminex xMAP® technology

This method offers a multi-analysis platform designed for the detection of mycotoxins in various food matrices, such as edible oil. The approach combines flow cytometry techniques, optical systems, and digital signal processing with microsphere-based technologies and has been developed as a patented design. The main advantages of the method are: i) High speed and short analysis times; ii) High throughput, allowing for the processing of large numbers of samples; iii) Multi-analyte detection capability, allowing for the simultaneous monitoring of multiple mycotoxins; and iv) Versatility and reproducibility, meaning reliable and repeatable results can be obtained in different matrices (Zheng et al., 2006).

Molecularly imprinted polymers (MIPs)

This method is based on the creation of specific identification points within the material using a template. The template ensures the correct position and orientation of the material's structural components through self-assembly mechanisms (Ulbricht et al., 1996).

Molecularly Imprinted Polymers (MIPs) are generally applied to the detection of mycotoxins using the following steps:

- i) Solid-phase extraction,
- ii) Use of biosensor devices,
- iii) Isolation via chromatographic matrix (Ulbricht et al., 1996).

Advantages of the MIP method include:

- i) Low-cost preparation of synthetic receptors,
- ii) Flexibility to apply to any specific compound,
- iii) Affinity similar to natural biomolecules,
- iv) Ability to function as a solvent,
- v) Stability under different pH, temperature, and pressure conditions,
- vi) Compatibility with microfabrication techniques,
- vii) Overall low cost (Zheng et al., 2006).

However, MIPs also have some disadvantages:

- i) Variability of template costs depending on the component,
- ii) Incomplete definition of stereochemical structure,
- iii) Inappropriate mass transfer isotherms in the polymer matrix,
- iv) Variability of MIPs in the solvent environment, which can lead to a decrease in selectivity and sensitivity (Zheng et al., 2006).

3. Conclusion and Recommendations

Traditional, modern, and biosensor-based technologies are being effectively used to identify toxins in contaminated foods. The most commonly applied methods for the detection of edible toxins are spectroscopic and chromatographic techniques. In recent years, significant progress has been made in developing and refining these traditional methods based on

the integration of different disciplines.

These advances in mycotoxin detection techniques provide rapid and reliable screening tools for edible oils and, in particular, enable the application of immunoassay-based methods with high diagnostic sensitivity. However, the number of mycotoxins and their modified compounds detected by these methods is steadily increasing, although most techniques generally target a single mycotoxin. Therefore, the development of methods capable of combined and multiplex mycotoxin detection is critical for effective contamination monitoring and prevention and requires cost-effective commercialization.

Kaynaklar

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Chapter 6

USE OF ENZYMES IN FRUIT JUICE PRODUCTION

Adnan BOZDOĞAN¹

¹ Department of Food Engineering, Osmaniye Korkut Ata University, Osmaniye/
Turkey

Phone: +90-328 827 10 00/3666; Fax: +90-328-825 00 97.

ORCID: 0000-0002-3612-5898

e-mail: bozdogan@osmaniye.edu.tr

1. Introduction

Enzymes play a key role in food processing by catalyzing many chemical reactions in the production of different food products. The use of enzymes has gained significant importance in the modern food industry because they simplify production steps and make processes more efficient (Oslen, 2000). Today, enzymatic processes are widely used, especially in the extraction and clarification stages of fruit juices. Enzymatic breakdown of cell walls increases the extraction efficiency of fruit juice and increases the amounts of reducing sugars, soluble solids, galacturonic acid, titratable acid, etc. (Joshi et al., 1991; Bhat, 2000; Chauhan et al., 2001). The pulp resulting from this process has a lower viscosity, reducing waste (Dorreich, 1996).

The level of enzymatic degradation of biomaterials; It depends on many parameters such as the type of enzyme used, incubation time and temperature, enzyme amount, mixing speed, pH value, and the presence of different enzyme combinations (Baumann, 1981; Raveendran et al., 2018). Today, pectinase, cellulase, and hemicellulase enzymes are called “maceration enzymes” and are widely used to improve the quality of fruit juices in the pressing, extraction, and clarification processes (Michele, 2020). In addition, enzymes such as alpha-amylase and amyloglucosidase are preferred to prevent turbidity, especially in starchy fruits such as apples (Uhlig, 1998). Consequently, the use of enzymes contributes significantly to both improving product quality and helping producers achieve the high production capacity demanded by the market (Michele, 2020).

2.1. Pectins and Pectolytic Enzymes

2.1.1. Pectin

Pectin is a high-molecular-weight polysaccharide with a linear chain structure composed of α -1,4-linked D-galacturonic acid units located in the middle lamella of plant cell walls (Torres-Fanela et al., 2003). Pectic substances found in fruit juices are one of the primary factors in the formation of turbidity (Borrego, 1989). The increase in viscosity caused by these substances negatively affects filtration efficiency during fruit juice processing (Rombouts, 1980). Therefore, pectic enzymes play an important role in eliminating these problems by degrading these pectins.

The use of pectinases not only increases fruit juice yield but also ensures a clearer and more fluid product. As a result of pectin hydrolysis by pectinase, the formation of pectin-protein complexes increases, leading to greater flocculation and thus reducing the viscosity and turbidity of the juice. Pectic enzymes are generally classified into three main groups:

pectin methyl esterase (PME), polygalacturonase (PG), and pectate lyase (PAL) (Haidar Abbasi, 2010; Ranveer Singh, 2005).

2.1.2 Pectolytic Enzymes

Pectolytic enzymes are widely used in the fruit processing industry to increase production yield, increase the fluidity and clarity of fruit juices, and improve filtration properties. These enzymes also facilitate the breakdown of plant tissues (maceration) and the extraction of intracellular components, releasing aroma compounds, proteins, polysaccharides, and starch (Dorreich, 1996; van den Broek et al., 1997). Industrial-scale production of pectolytic enzymes is generally achieved through fungal species such as *Aspergillus niger* or *Aspergillus aculeatus* (Naidu and Panda, 1999).

Pectic enzymes such as pectin lyase, pectin methylesterase, endo- and exo-polygalacturonase, pectin acetylerase, rhamnogalacturonase, and endo- and exo-arabinase; It is effectively used in the extraction and clarification of fruit and vegetable juices (Galante et al., 1998). Currently, acidic pectinases obtained from *Aspergillus* species are mostly preferred in the fruit juice industry. Commercial pectinase preparations generally contain mixtures of polygalacturonase, pectate lyase, and pectin esterases. Pectate lyases act on esterified pectin, while polygalacturonases act on unesterified pectin; therefore, in some cases, prior action of pectin esterases is necessary. The type of pectate enzymes used in practice and the processing conditions may vary depending on the type of fruit juice to be produced (Sieiro et al., 2012).

The main industrial uses of pectolytic enzymes are summarized in Table 1. In general, the primary purpose of these enzymes is to facilitate the extraction of fruit juices and increase their clarity. A study conducted in India reported the effectiveness of pectinase in the production of kendu juice, a seasonal fruit known for its medicinal and nutritional properties (Panda et al., 2021). Pectinases have also yielded successful results in the clarification of fruits such as apple, kiwi, orange, tangerine, pineapple, sapodilla, and carambola (Abdullah et al., 2007). Furthermore, these enzymes are used in processes such as liquefaction, maceration, and cloud stabilization (Saxena, 2015). Pectolytic liquefaction processes, particularly in tropical fruits, have led to both qualitative and quantitative changes in their composition, increasing the amount of carotenoids released into the juice (Essa and Salama, 2002).

Table 1. Enzyme Applications Used in Fruit Juices

Sample	Enzyme	Enzyme concentration	Temperature, °C	Time	Responses	References
Grape Juice	Pectinase	1.5-3 ml 100kg	50 - 60	1 hour	Increase in juice yield, titratable acidity, color intensity, and organic acids	Li m a ve ark. 2015)
Lemon Juice	Pectinase	0-1200 U/L	25-50	0 - 9 0 min	Increase in total phenolic content, color, clarification, sugar, dry matter and proteins	Mak- t o u f ve ark. (2014)
Peach Juice	A mixture of pectinases, hemicellulases, and beta-glucanases	2 4 0 - 1 2 0 0 ppm	18-45	30 - 150 min	Decrease in viscosity and pulp	Sant in ve ark. (2008)
G u a v a mash	Cellulase	0 . 0 4 8 - 0.132%	50	1 1 . 7 - 68.3 min	Increase in extraction yield, total sugar, ascorbic acid, and total phenol compounds	Nguyen (2013)
Pineapple Juice	Cellulase, pectinase, hemicellulase	2 5 - 1 5 0 mg/100 mL	35-55	2 1 0 - 540 min	Increase in juice yield, viscosity, and clarification	K u - mar ve Sharma (2012)
Pomegra- nate Juice	Pectolytic enzyme	0-15 µL/L	25	120 min	Increase in juice yield, color, total soluble solids and antioxidant amounts	Rinaldi ve ark. (2013)

Watermelon Juice	Masazyme	0.01–0.1%	30-50	20–120 min	Increase in juice yield and clarity. Decrease in viscosity	(Saxena ve ark. 2012)
Red pitaya Juice	A mixture of pectinases, hemicellulases, and beta-glucanases	0.01–0.1%	30-50	20-100 min	Increase in vitamin C and total phenol compounds	(Nur'Aliaa ve ark. 2011)
White pitaya suyu	A mixture of pectinases, hemicellulases, and beta-glucanases	0.01–0.1%	30-50	20-100 min	Increase in juice yield and clarification	(Nur'Aliaa ve ark. 2010)
Apple Juice	A mixture of pectinases, hemicellulases, and beta-glucanases	10 U	40-50	60 min	Increase in total phenol compounds, antioxidant capacity and clarification	(Sandrive ark., 2013)
Apricot Pulp	Pectinase	0–1.2%	30-50	5 h	Increase in juice yield	Chahave ark. (2001)
Banana Juice	Pectinase	5–10%	25-40	50-80 h	Increase in clarification, acidity and reducing sugar	Barman ve ark., 2015)
Blueberries Juice	A mixture of pectinases, hemicellulases, and beta-glucanases	10 U	4-50 h	60 min	Increase in total phenol compounds, antioxidant capacity and clarification	(Sandrive ark., 2013)
Cherry juice	Pectinase	0–0.5%	50	1 h	Increase in clarification	Pineve ark. (2010)

2.1.2.1 Pectin methylesterase

Pectin methylesterase (pectin pectinhydrolase, EC 3.1.1.11) is also known in the literature by various names, such as pectinesterase, pectase, pectin methoxylase, pectin demethoxylase, and pectolipase. This enzyme acts by removing methoxyl groups from methylated pectin molecules. PME, a hydrolase belonging to the carboxylic acid esterase class, deesterifies the methyl groups on the galacturonic acid chain of the pectin

molecule. This process creates charged regions that can interact with calcium ions (Ca^{2+}). These regions form Ca^{2+} pectate gels, causing them to precipitate, thus clarifying the fruit juice (Baker and Bruemmer, 1972). Pectin methylesterase activity is most easily observed in the pH range 4–7.5, resulting from the release of protons resulting from the ionization of carboxyl groups. Pectin methylesterase, particularly that obtained from the white peel layer (albedo) of oranges, is one of the most extensively studied enzymes (MacDonnell et al., 1950). In the absence of divalent cations, pectin methylesterase has no significant effect on the viscosity of pectin-containing solutions. However, when Ca^{2+} ions are present, calcium bridges form between the pectic acid chains, and this cross-linking increases the solution's viscosity (Whitaker, 1984).

2.1.2.2 Polygalacturonases

Polygalacturonases [poly(1,4- α -D-galacturonide) glucanohydrolase, EC 3.2.1.15] act by cleaving the α -(1 \rightarrow 4) bonds between D-galacturonic acid units. These enzymes have distinct preferences for poly[α (1–4)-D-methylgalacturonic acid] (pectin-like substrates) or poly[α (1–4)-D-galacturonic acid] (pectic acid-like substrates) and can attack the polymer chain either from the end (exo-cleavage) or from the interior of the chain (endo-cleavage). Based on these properties, four different types of polygalacturonases have been identified. These types can be distinguished based on their substrate requirements, rate of reducing group formation, rate of viscosity reduction, and the nature of the products formed early in the reaction.

Polygalacturonase activity is usually assessed by measuring two key parameters during the reaction: (a) the rate of increase in the number of reducing groups and (b) the rate of decrease in the viscosity of the substrate solution (Rexova-Benkova and Markovic, 1976).

2.1.2.3 Pectate lyases

Lyases facilitate the non-hydrolytic degradation of pectic or pectinates by cleaving pectic polymers via a trans-eliminative mechanism (Sakai et al., 1993). These enzymes cleave glycosidic bonds at C-4 while simultaneously removing a hydrogen atom from C-5, forming D4,5-unsaturated products (Codner, 2001; Albersheim et al., 1960). Lyases are classified into four main groups based on their mechanism of action and target substrates: (I) endopolygalacturonate lyase (EndoPGL, EC 4.2.2.2), (II) exopolygalacturonate lyase (ExoPGL, EC 4.2.2.9), (III) endopolymethylgalacturonate lyase (EndoPMGL, EC 4.2.2.10), and (IV) exopolymethylgalacturonate lyase (ExoPMGL) (Jayani et al., 2005).

Pectate lyase activity is assessed by measuring the rate of increase in

absorbance at 235 nm due to double bond formation. All pectate lyases require the Ca^{2+} ion; however, this requirement is not always valid for polygalacturonases. Additionally, ethylenediaminetetraacetic acid (EDTA) generally inhibits pectate lyase activity by chelating Ca^{2+} ions (Whitaker, 1984).

2.2. Cellulose and Cellulase Enzyme

2.2.1 Cellulose

Cellulose is the most abundant structural component in plants (Saha, 2002). While it can sometimes be found in nearly pure form in nature, it is most often found within a matrix of other structural biopolymers such as cellulose fibers, hemicelluloses, and lignin (Marchesseault and Sundararajan, 1993; Lynd et al., 1999). Cellulose is formed by D-glucose units linked by β -1,4 glycosidic bonds, forming a highly ordered crystalline structure (Ghose, 1984). This orderliness makes cellulose difficult to break down and requires additional effort.

Cellulases are enzymes involved in the hydrolysis of cellulose and can generally be produced on cellulosic materials (Lee and Koo, 2001). For cellulose to be completely degraded, the different components of cellulases must work synergistically. The cellulase complex consists primarily of three main components: cellobiohydrolase (exo-glucanase or exo- β -glucanase), endoglucanase (carboxymethylcellulase, CMCCase or endo- β -glucanase), and β -glucosidase (Bhat, 2000).

2.2.2. Cellulase Enzyme

Cellulases are a group of biological enzymes that convert cellulose into soluble sugars and glucose. They are widely distributed in nature and are produced primarily by bacteria and fungi. Symbiotic bacteria living in the multi-chambered stomachs of ruminants, particularly in the rumen, also produce cellulase. In contrast, humans and most animals cannot synthesize cellulase and therefore cannot utilize all the energy in plant material (Kuhls and Lieckfeldt, 1996; Wilkins et al., 2007).

Cellulases convert cellulose to glucose by hydrolyzing cell wall polysaccharides and derived celluloses. These enzymes are effectively used in the extraction and clarification of fruit and vegetable juices, oil extraction from oilseeds, animal feed preparation, increasing wetting and water absorption efficiency, enhancing the nutritional value of fermented foods, improving the rehydrability of dried vegetables and soups, oligosaccharide production, and biomass conversion (Beguin and Aubert, 1994; MK Bhat and S. Bhat, 1997). Cellulases also play a role in carotenoid extraction

and the production of food coloring agents. Fungi such as *Aspergillus niger*, *Aspergillus nidulans*, and *Aspergillus oryzae* are preferred for microbial cellulase production (Sukumaran et al., 2005). The term cellulase encompasses three main enzymes that convert cellulose to glucose: endo- β -1,4-glucanases (EG; EC 3.2.1.4), exo- β -1,4-cellobiohydrolases (CBH; EC 3.2.1.91), and β -glucosidases (BG; EC 3.2.1.21) (Clarke, 1996; Schulein, 1988). The complete cellulase complex, consisting of these three components, works synergistically to convert crystalline cellulose to glucose. Endo- β -1,4-glucanases and exo- β -1,4-cellobiohydrolases break down cellulose into small cellobiose and other cellulo-oligosaccharides, which are then converted to glucose by β -glucosidase (Sukumaran et al., 2005).

2.3 Other Enzymes

2.3.1 Hemicellulases

Hemicellulases, a large group of enzymes encompassing endo- and exo-xylanases, galactanases, xyloglucanases, and mannanases, are responsible for degrading hemicelluloses, which are widely found in nature. These enzymes hydrolyze hemicelluloses, resulting in the formation of a variety of short oligomers. For example, xylanases (EC 3.2.1.8) cleave β -1,4 bonds in the xylan backbone, producing short xylooligomers. Similarly, β -mannanases (EC 3.2.1.78) degrade mannan-containing hemicelluloses, forming short β -1,4-mannooligomers; These oligomers can then be converted to mannose by β -mannosidases (EC 3.2.1.25) (Shallom and Shoham, 2003).

2.3.2 Amylase

Amylases are historically one of the oldest and most widespread commercial biocatalysts, accounting for over 30% of the global enzyme market. They are widely used, particularly in the starch processing sector, facilitating the liquefaction of starch and its conversion into sugars. They also have important applications in areas such as baking, pulp and paper production, fruit juice clarification, detergent production, textile desizing, and distillation (Soy et al., 2021).

Amylase is an enzyme that breaks down starch into sugars and, found in human saliva, initiates the chemical process of digestion. Additionally, bacterial and fungal amylases are available for industrial use. All amylases are glycoside hydrolases and target α -1,4-glycosidic bonds. Thanks to these properties, amylases are widely used in baking, brewing, cake and starch syrup production, digestive food ingredients, and fruit juice production; they also play an important role in fruit juice clarification (Couto and Sanroman, 2006).

Maceration enzymes are generally applied in a two-stage process: In the first stage, after the fruit is crushed, the pulp is partially or completely liquefied. This process not only increases juice yield and shortens processing time but also improves the extraction of valuable fruit components. In the second stage, after the juice is obtained, pectinases are used for clarification; this reduces the viscosity of the juice, increases filtration rate, and improves the stability of the final product (Bhat, 2000).

3. Conclusion and Recommendations

The use of enzymes in the food industry has increased significantly in recent years, enabling both cost reductions in production processes and higher added value for products. The fruit juice sector, in particular, which historically produced low yields, has significantly increased production volumes and product quality thanks to enzyme applications. Today, enzymes are indispensable components for the beverage industry. The use of enzymes improves the sensory properties of fruit juices, resulting in higher quality products in terms of flavor, color, and clarity. The primary factors determining the effectiveness of enzyme applications are enzyme concentration, incubation time, and temperature. Applications carried out under appropriate conditions increased fruit juice yield, reduced viscosity and turbidity, and increased color brightness and total soluble solids. Furthermore, it has been observed that incubation time positively affects pigment content, while increasing enzyme concentration significantly improves juice clarity.

In the future, it is possible to further increase production efficiency and product quality through more effective and controlled use of enzyme technologies in the fruit juice industry. Therefore, further comprehensive research into optimizing parameters such as enzyme concentration, incubation time, and temperature is crucial. Furthermore, the development of enzyme blends specifically formulated for different fruit types could offer innovative solutions to the industry. Enzyme use should be integrated into sustainable production strategies, not only for increased yield but also to meet the demand for natural, additive-free, and healthy products. Collaborative work with industry will contribute to the development of more efficient production processes, both economically and environmentally.

Kaynaklar

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