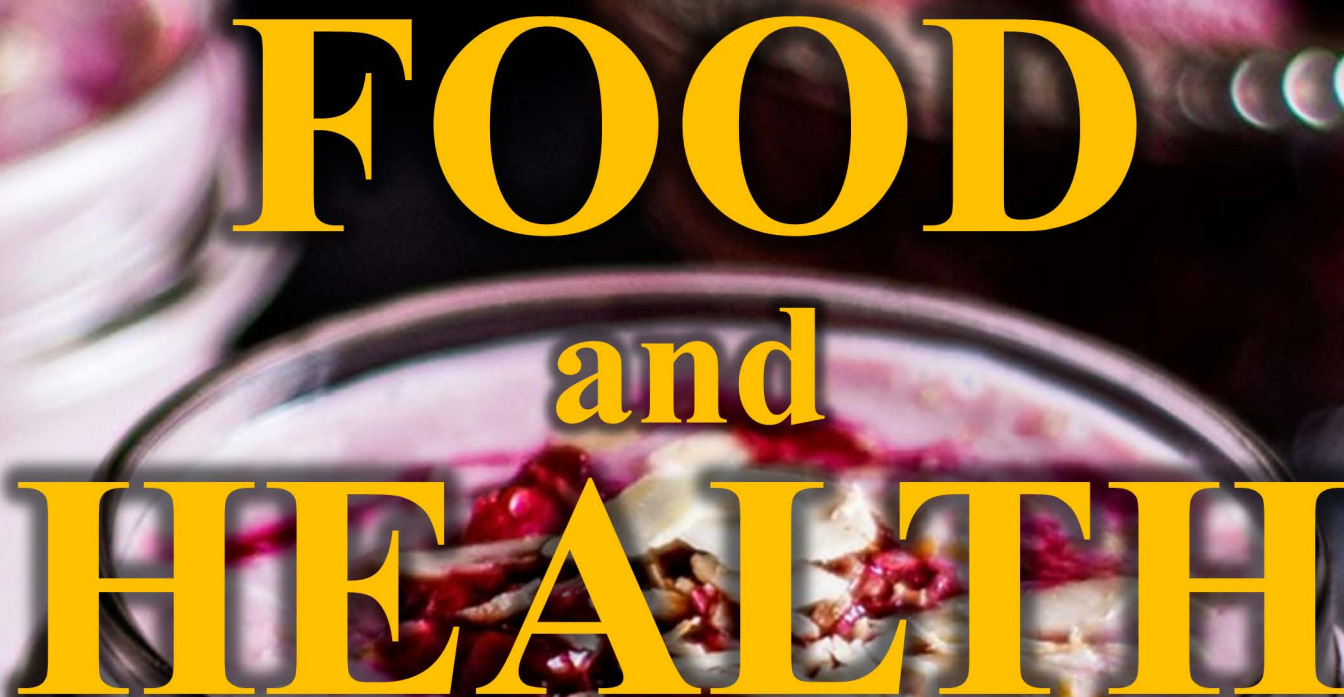


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FOOD and HEALTH

A glass jar filled with a thick, pink smoothie. The smoothie is topped with fresh raspberries, sliced banana, and almond flakes. The jar is set against a dark background with bokeh light effects.

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The global fight against *trans*-fat: Policy insights and labeling information based on alternative substitutes in Türkiye

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ABSTRACT

Trans-fat consumption is an important risk factor for cardiovascular diseases, which is one of the leading causes of death worldwide. With the 'REPLACE' action plan of the World Health Organization, which aims to zero global *trans*-fat consumption by 2023, steps are taken by the countries of the world to zero *trans*-fat. In this article, *trans*-fat content information of packaged food products was researched in Türkiye. *Trans*-fat content was classified into 4 categories and a total of 1411 packaged food products divided into 11 categories were examined and it was found that 71.2% of them contained *trans*-fat-free content. According to the label information, the usage rate of 'no *trans*-fat or *trans*-fat free' statements in all categories is 25%. In addition, it was determined that 7.1% of these products used vague expressions that did not provide clear information about *trans*-fat content. When evaluated in general, the use of alternative substitutes should be encouraged and effective policies should be developed in order to eliminate *trans* fat in Türkiye as well as in the rest of the world.

Keywords: *Trans*-fat, *Trans*-fatty acids, 'REPLACE' action plan, Food labeling, Food security

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Introduction

Issues such as difficulties in ensuring food safety, barriers to access to healthy food, population growth, and the effects of climate change, slow improvement of food processing conditions, and limits on the use of alternative healthy substitutes increase the importance of food safety issues. In this context, unhealthy eating habits, one of the most important problems of today, should not be seen as an individual lifestyle choice. Health problems that occur as a result of unhealthy nutrition are a reflection of international legal regulations and institutional strategies (Parziale & Ooms, 2019). Fat substitutes with *trans*-fat content are used to meet the expectations of many foods, to gain desired textural, rheological, and sensory properties, and to improve shelf life. For this reason, international roadmaps have been created for the classification and zeroing of foods with *trans*-fat content. The World Health Organization (WHO) has published the 'REPLACE' action package to remove industrially produced TFA from the global food supply by 2023. The REPLACE action package has 6 modules and provides a strategic roadmap for removing industrially produced TFA from national food sources (Ghebreyesus & Frieden, 2018; Huang et al., 2020; Spajić, 2020).

Trans-fatty acids (TFA) can be defined as monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA) containing at least one carbon-carbon *trans*-double bond in fatty acids. Although the chemical composition of a *cis* and *trans*-fatty acid is the same, this change in bond structure causes changes in many functional properties (Lichtenstein, 2015; Pase et al., 2021). Research so far has shown that frequent consumption of TFA-containing foods causes cardiovascular risks, neuropsychiatric sensitivity, and hyperactive behavior. In addition, it has been reported that oxidative damage in the brain regions of the offspring increases in the case of frequent consumption of foods containing TFA during pregnancy and lactation (Mouratidou et al., 2014; Oteng & Kersten, 2020; Pase et al., 2015; Spajić, 2020; Trevizol et al., 2015; Verneque et al., 2020). *Trans*-fat is one of the major causes of more than 5.7 million deaths per year worldwide, according to 2020 data, due to occlusion of the heart vessels and causing heart attacks (WHO, 2021). In Türkiye (80 million population), approximately one hundred thousand people (23% of all deaths) died due to cardiovascular diseases in 2020. One of the important factors causing death from cardiovascular diseases is the consumption of foods containing *trans* fatty acids (World Health Rankings, 2021). Due to the very high industrial TFA content of some fast-food foods, 10 to 25 g of TFA per day may be consumed in many countries.

It is stated that taking about 5 g of *trans*-fat in daily consumption is associated with a 25 percent increase in the risk of heart disease (Stender & Jorn, 2006).

With the publication of a study in 1993 that TFA consumption increases the risk of coronary heart disease, it attracted media attention, and efforts were made to limit the intake of industrially produced TFA (Willett et al., 1993). In 2003, Denmark became the first country to take legal action to restrict TFA by enacting a law limiting industrially produced TFA to a maximum of 2% in food products (Stender, 2020; Tarar et al., 2020). To date, 62 countries around the world have been involved in the TFA restriction process and have implemented a national policy, strategy and action plan. For 64 countries, it is known that no steps have been taken yet in terms of combating TFA. More than forty percent of the world's population currently lives in countries with mandatory TFA restrictions (WHO, 2020b). Examining the policies to limit TFA across Europe, the EU has agreed to limit industrially produced TFA in all food products to 2 g TFA per 100 g total fat. These regulations will directly apply in all member States of the EU as well as in the European Economic Area (EEA) (Temkov & Mureşan, 2021). Due to its harmonization process with the EU, Türkiye made legal regulations in May 2020 to limit TFA to 2 g per 100 g of total oil. The regulations entered into force in 2021 (WHO, 2020a). In this context, the roadmap created by the WHO specific to *trans*-fat directly affects food packaging, consumer preferences and policies to be created (Hartmann et al., 2018).

REPLACE Action Plan to Limit TFA Consumption

The REPLACE action package created to reset the industrially produced TFA consists of a strategic roadmap consisting of 6 modules. The first module in this roadmap is defined as 'Review'. This module includes reviewing the available information on industrially produced TFA at the country level, examining the production-consumption processes, determining the policies applied and to be implemented, and gathering information about potential *trans*-fat sources in the market. The ultimate goal is to identify and implement best-practice legislative measures to remove industrially produced TFA from the national food supply (WHO, 2019b).

In general, legislation and regulations do not specify which type of oil should be used instead of industrially produced TFA. Using healthier oils instead of oils containing TFA will meet the health expectations of consumers at the highest level. The second module, 'Promote', provides a roadmap for the development and implementation of strategies for identifying industrially produced oils with the lowest risk of TFA

and promoting the use of these healthier oils. These strategies generally include agricultural policies and industry incentives for healthy fats, covering the whole process from agriculture to industry to government policies in order to facilitate and accelerate the *transition* to healthy fats (WHO, 2019c).

In order to eliminate industrially produced TFA, the selection of policies to be carried out and the framework of these policy arrangements should be well defined. The third module, *Legislate*, provides guidance on designing, enacting and implementing legislation appropriate to each country's structure, or selecting policies so that existing legislation can be updated to reduce industrially produced TFA (WHO, 2019d).

In order to remove TFA from the global food supply in accordance with the REPLACE action plan, it is very important that how many TFA source foods are consumed in countries and that the policies implemented to eliminate TFA be legally followed. The fourth module, *Assess*, was created to guide the necessary practices in order to reset a country according to its TFA consumption rates (WHO, 2019e).

While countries are implementing many practices to eliminate TFA in foods with their policies, they should also develop a communication strategy very carefully. In determining the policies to be implemented, industry representatives should be in communication with regard to cost, management of changes in product formulation. The fifth module, *Create*, addresses the necessity of conducting the communication channels with the most effective methods in policy determination and implementation processes and offers suggestions (WHO, 2019f).

The last module, *Enforce*, includes a series of activities such as informing stakeholders about the new legislation in the implementation of policies for resetting TFA, collecting complaints from consumers, inspections of products and facilities, analysis, control of labeling, and sanctions in case of violation. In general terms, it is stated that the process of resetting the TFA amount will be maintained quite easily when the issues such as establishing a good communication environment in the policy development processes, conducting the audits within a certain plan, and balancing the sanctions in case of violations are taken into account (WHO, 2019g).

Trans-Fat Source PHO and Alternative Fat Replacements

Unsaturated fatty acids found in vegetable oils naturally have only *cis*-double bonds. The primary source of TFA, which is the focus of the 'REPLACE' action package, is partially hydrogenated oils (PHOs), formed as a result of the partial hydrogenation process (Parziale & Ooms, 2019; WHO, 2019a). Although naturally and industrially occurring TFA is in the

range of 3-6% to oils obtained from ruminant milk and meat sources, it can occur at a rate of about 2% during the refining of vegetable oils, about 3% when exposed to high temperature applications for a long time, and about 25-45% in PHO production (Martin et al., 2007; Temkov & Mureşan, 2021; Van Duijn, 2005). The United States Food and Drug Administration (FDA) defines PHO as 'oils and fats that are hydrogenated but incomplete or not fully saturated and have an iodine value (IV) greater than 4' (Kotoski & Srigley, 2018). It can be listed as an alternative to partially hydrogenated oils; fully hydrogenated oils, natural oils at room temperature (oils rich in oleic acid, oils containing antioxidants and emulsifiers), natural fats at room temperature (animal fats, palm, palm kernel, coconut oils), fractionated oils (liquid palm-olein, solid palm, palm-stearin), interesterified oils (enzymatic, chemically *transformed* oils with fatty acid profile), mixed oils (viscous liquid-solid oil mixture), combination mixtures (a mixture of fractionated oils and interesterified oils) (Dunford, 2017; Van Duijn, 2005).

In the early uses of the partial hydrogenation technique applied to fats, it was assumed that all saturated fatty acids were healthier than unsaturated fatty acids and that replacing saturated fatty acids with unsaturated fats containing *trans*-fatty acids would result in lower cardiovascular disease. Today, it is also clear that reducing the consumption of saturated fatty acids and replacing them with healthier alternatives is beneficial. Therefore, WHO reports (Downs et al. 2018) from time to time state that it is necessary to exclude partially hydrogenated fats from the diet, to limit TFA intake to less than 1% of total energy intake, and to limit saturated fatty acid intake to less than 10% of the total. PHO in products should be replaced with formulations containing as little saturated fatty acid as possible and as much unsaturated fatty acid as possible. Saturated fatty acids should be used in a limited number of applications for which there is no alternative. There is a significant difference between using palm kernel oil high in saturated fat as an alternative to oil high in TFA, and replacing it with a mixture of oils high in unsaturated fat and some saturated fat. The second approach is much healthier (Dunford, 2017; Van Duijn, 2005).

Health Evaluation of PHO Alternatives

For a fat substitute to be considered healthy, it is generally accepted that Saturated Fatty Acid (SFA) should be as low as possible and PUFA as high as possible, preferably containing both omega-6 and omega-3 fatty acids. In addition, these oil substitutes are expected to have high oxidation resistance (rich in oleic acid). Each step of processing oils and fats for the production of PHO-free oil substitutes (refining, fractionation, hydrogenation, interesterification, blending) incurs

some costs. This makes palm oil, the cheapest oil in the world, indispensable. This oil is about one-third cheaper than even common soybean oil (Spajić, 2020; WHO, 2019c).

For liquid oils, palm oil liquid fractions, animal-derived liquid oils, tropical liquid oils are not recommended for health reasons. Liquid oils with low PUFA and high MUFA content are usable alternatives to oil substitutes, which are not recommended for health reasons. Antioxidant-rich oils containing high PUFA, oils containing high MUFA-medium PUFA are recommended because of their positive health effects. For semi-solid oils, palm oil semi-solid fractions, animal origin oils, tropical oils are not recommended for health reasons. Semi-solid oils obtained by producing hardstocks by interesterification using oils containing low PUFA and blending hardstocks with oils containing low PUFA can also be used as an alternative to fat substitutes, which are not recommended for health reasons (Abramovič et al., 2018; Menaa et al., 2013; WHO, 2019c). Hardstocks produced by interesterification using oils containing high PUFA and semi-solid oils obtained by blending hardstocks with oils containing high levels of PUFA are recommended because of their positive health effects. For solid oils; palm oil solid fractions, fully hydrogenated vegetable oils, coconut oil, palm kernel oil are not recommended for health reasons. Solid fats obtained by producing hardstocks by interesterification using oils containing high PUFA, solid fats obtained by mixing hardstocks with oils containing high PUFA can be used as an alternative to fat substitutes that are not recommended for health reasons. In addition, solid fats obtained by producing hardstocks by interesterification using oils containing MUFA are not highly recommended. Solid fats are generally not recommended due to limited positive health effects (Albuquerque et al., 2011; WHO, 2019c).

The most effective way to completely remove industrially produced TFA from the global food supply, ban or strictly limit the use of TFA-containing foods is to establish and enforce regulations with frequent inspections. A growing number of countries, such as Türkiye, are complying with the WHO's call to remove industrially produced *trans*-fats from their food sources by 2023 and are developing policies to take the necessary measures. As a requirement of the policy of zeroing *trans*-fats worldwide until 2023, this study summarizes the current situation by dividing packaged foods in Türkiye into 4 classes according to their *trans*-fat content.

Materials and Methods

Data Collection

Market research on the *trans*-fat content of packaged foods consumed in Türkiye was carried out through five national supermarket chains determined by the preliminary study. In the selection of the five nationwide supermarket chains, the markets with considerably high market share and product variety, reflecting the consumer preferences across the country in the products they sell were preferred. Official permission and ethics committee approval were obtained from all supermarket chains and administrative authorities included in this study. In order to examine the *trans*-fat label information and nutritional value panels of food products in supermarkets, 11 food categories were determined. The categories were determined as bakery products, convenience foods, oils, powdered beverages, spreads-sauces, pastry products, breakfast cereals-baby foods, processed meats, ice creams, chocolates and chips-snacks (Ricardo et al., 2019). It was not necessary to examine packaged food products that do not contain oil and do not have the potential for industrial *trans*-fats (such as beverages, honey products, dairy products).

At least 3 photographs were taken, including the front of each package, the *trans*-fat information panel, if any, the ingredient list, weight and the nutritional value panels. In order to ensure the healthy progress of the data collection process, the photographs taken were quickly saved in the data pool. In order to avoid duplication, product information was entered in detail and codes were entered for each product, taking into account the category it was linked to. Generally, it is preferred to photograph large-sized products in similar products where the package size differs. For this study, data collection, classification and recording were carried out by 2 field workers. The first of the field workers has expertise in the field of dietetics, the second in the field of food engineering. All field workers have training in food composition and labeling. In addition, field workers photographed the products using smartphones with high-resolution cameras. The data collection process was carried out during the summer holidays of 2021 so as not to disrupt the operation of the supermarkets, and photographs were taken on Tuesday, Wednesday and Thursday when the customer density was relatively low. Photographs of the products were taken during the daytime hours and for a maximum of 30 minutes in a way that would not disturb the customers. All photographing processes were completed within 2 months. Field workers kept ID cards during data collection. Field workers tried not to communicate with customers and emphasized that it was an 'ordinary labeling exercise' when necessary. As a result of all this work, 1411 samples were examined one by one and approximately

5049 photographs were taken. All the methods used in the collection and classification of labeling data were developed by Kanter et al. carried out by adapting their working model (Kanter et al., 2017).

Classification of Trans-Fat Content According to Label Information

Keywords used to identify products with *trans*-fat content are listed in Table 1 (Modified from Ricardo et al., 2019). The term 'specific' is used for products with keywords known to be sources of *trans*-fat. Partially hydrogenated oil, hydrogenated vegetable oil and hydrogenated are selected for this category. Classifications of 'probably' for products likely to be a source of *trans*-fats, 'low possibility' for products that are unlikely to be a source of *trans* fat, and 'uncertain' for products with ambiguous keywords were used. Shortenings and margarines were determined for products probably to be sources of *trans*-fat. Although advanced techniques are applied today to eliminate these products from being a source of *trans*-fat, they are still considered a source of *trans*-fat (Abramovič et al., 2018; Stender, 2020; Tarar et al., 2020).

Table 1. Classification of *trans*-fat content according to label information

Classifications of <i>trans</i> -fat	Keywords
Specific	Partially hydrogenated oil, hydrogenated vegetable oil, hydrogenated
Probable	Shortenings, margarine
Low possibility	Fully hydrogenated oil, refined oil, non-hydrogenated oil, palm olein, frying oils, vegetable cream, shea
Uncertain	Organic vegetable oil, oil powder, palm oil fractions, butter flavored vegetable oil, vegetable oil

Results and Discussion

For the bakery products (biscuits, cakes, crackers and bakery products) category, 312 samples were examined (Table 2). The term 'vegetable oil' is used in the contents of these products at a rate of 92%. The remaining 8% use the terms vegetable oil powder, butter flavored vegetable oil, margarine, vegetable anhydrous oil, Fully Hydrogenated Oil (FHO), PHO and Hydrogenated Oil (HO). Next to these statements on the packages, the current oil content was listed in parentheses. PHOs, which were indicative of the presence of *trans*-fat, were found at an average rate of 3.2% among 312 samples, and contents probable to contain 1.9% of *trans*-fat were

found. Fat content with a low possibility of *trans*-fats was detected in 18% of the products in this category, and ambiguous expressions were used in 3.2%. According to the list of ingredients, 74% of the 312 samples had fat contents without the potential to contain *trans*-fats (Figure 1). In the label information, the statements 'no *trans*-fat or *trans*-fat free' were used in 34.9% and 65.1% of them did not find any information about *trans*-fat (Figure 2).

127 samples were examined for the category of ready-to-eat foods (instant frozen products, instant soups, ready-made desserts, canned foods). The term 'vegetable oil' is used in the contents of these products at a rate of 90% (Table 1). The remaining 10% use the terms frying oil, vegetable refined oil, margarine, vegetable anhydrous oil, FHO and HO. PHO, which was a clear indicator of *trans* fat content, was found at a rate of 0.8% in the content information and contents that were probable to contain 5.5% *trans*-fat have been identified. Low possibility was determined in 15.7% of the products in this category, and uncertain expressions were used in 5.5% of the products. Based on the ingredient list, 72.4% of the 127 samples had a fat content without the potential to contain *trans*-fats (Figure 1). In the label information, the statements 'no *trans*-fat or *trans*-fat free' were used at a rate of 13.4%. No information on *trans*-fat was found in 86.6% (Figure 2). It was useful to underline that packaged foods in this category have the least *trans*-fat content information.

83 products were reviewed for the spreads and sauces category. The term 'vegetable oil' is used in the contents of these products at a rate of 88% (Table 1). The remaining 12% did not use a term related to fat content. PHO, which was a clear indicator of *trans*-fat content, and probable *trans*-fat source content information were not found. Low possibility was determined in 11% of the products in this category, and uncertain expressions were used in 14.5% of the products. According to the ingredient list, 74.7% of the 83 samples examined had a fat content without the potential to contain *trans*-fats (Figure 1). In the label information, the statements 'no *trans*-fat or *trans*-fat free' were used at a rate of 26.5%. No information on *trans*-fat was found in 73.5% (Figure 2).

For the fat category (refined oils, unrefined oils, margarines, shortenings), 52 samples were examined. The term 'vegetable oil' is used in the contents of these products at a rate of 65.4%. The remaining 34.6% were termed margarine and shortening. PHO and HO, which were clear indicators of *trans*-fat con-

tent, were not included in the content information. The content information that there was a probable source of trans-fat is 40.6%. The high value of this value was due to the fact that although margarine production processes have recently been improved with different techniques, they were still considered as a potential source of trans-fat. Low possibility was determined in 4.8% of the products in this category, and uncertain expressions were used in 3.8% of the products. According to the ingredient list, 48.1% of the 52 samples examined had a fat content without the potential to contain trans-fats (Figure 1). In the label information, the statements 'no trans-fat or trans-fat free' were used at a rate of 38.5%. No information on trans-fat was found in 61.5% (Figure 2). These results were low, as expected, since there was no need to use the expression for trans-fat in liquid oils.

53 samples were examined for pastry materials. The term 'vegetable oil' is used in the contents of these products at a rate of 96.2%. The remaining part does not contain a term related to fat content. While PHO and HO content, which were clear indicators of trans-fat content, are 2%, no fat content that could be a probable source of trans-fat was found. Low possibility was determined in 43.4% of the products in this category, and uncertain expressions were used in 3.8% of the products. According to the ingredient list, 50.9% of the 53 samples examined had a fat content without the potential to contain trans-fats (Figure 1). In the label information, the statements 'no trans-fat or trans-fat free' were used in pastry products (Figure 2). According to these results, it was seen that more than half of the pastry products, according to the content information, use fat content with more saturated fatty acids.

117 samples were examined for the snacks category (chips, snacks, corn products). The term 'vegetable oil' is used in the contents of these products at a rate of 94.9%. The remaining part does not contain a term related to fat content. PHO was found at a rate of 4% in the content information no fat content that could be a probable source of trans-fat was found. Low possibility was determined in 46.2% of the products in this category, and uncertain expressions were used in 3.4% of the products. According to the ingredient list, 47% of the 117 samples examined had a fat content without the potential to contain trans-fats (Figure 1). In the label information, the statements 'no trans-fat or trans-fat free' in snack products were found in 52% of all products. No expression of trans-fat was found in the remaining 44% (Figure 2). According to the

results of this category, it can be said that almost half of the products examined consist of products that may contain trans-fat.

For the chocolate and wafer category, 221 samples were examined (Table 2). All of these products use the term 'vegetable oil' in their ingredients. PHO and its derivatives were not found in this category. In addition, there were no ingredients that could be a probable source of trans-fats. In the samples in this category, the rate of low-probability trans fat source was found to be 20.4%. There was no expression that was described as uncertain according to the content information. According to the 221 samples examined, there was a fat content of 79.6% without the potential to contain trans-fat (Figure 1). While it was determined that the expressions 'no trans-fat or trans-fat free' were used for this category at a rate of 40.7%, no information about trans-fat was found at a rate of 59.3% (Figure 2). According to these data, it can be said that three quarters of the products in this category were safe in terms of trans fat content, and the remaining quarter were produced from fat substitutes with a low probability of trans fat content. Despite this, it was noteworthy that 40.7% stated 'no trans-fat or trans-fat free'.

For the ice cream category, 87 samples were examined. The term 'vegetable oil' is used in the contents of these products at a rate of 88.5% (Table 1). In the remaining part, no expression was used regarding the oil content. PHO was not seen in the content information and contents that could probable trans-fats were not detected. In 3.4% of the products in this category, ingredients with a low possibility of trans-fat content were determined, and uncertain expressions were used in 4.6% of the products. According to the ingredient list, 92% of the 87 samples examined had a fat content without the potential to contain trans-fats (Figure 1). In the label information, the statements 'no trans-fat or trans-fat free' were not used in the entire ice cream category (Figure 2). It can be said that the packaged foods in this category were the least likely to contain trans-fat according to the label information.

The number of samples examined for the processed meat category (sausage, salami, sausage, ham, etc.) is 127. The term 'vegetable oil' is used in the contents of these products at a rate of 24.4% (Table 1). This value was low as expected. Also in the content information, the term "animal fat" was used at a rate of 89% and there was no statement about the fat content of 7.9%. Specific ingredients, probable ingredients, low possibility ingredients that were a clear indication of trans-fat

content were not found in the label information. In 9.4% of the products in this category, uncertain expressions were used regarding the oil content. According to the ingredient list, 90.6% of the 127 samples examined had a fat content without the potential to contain trans-fats (Figure 1). In the label information, the statements 'no trans-fat or trans-fat free' were not used in the whole processed meat category (Figure 2). Since the use of industrially produced oils in packaged products produced in this category was limited, the probability of trans-fat content was very low.

The number of samples examined for the breakfast cereals, bars, baby food category is 189. The term 'vegetable oil' is used in the contents of these products at a rate of 75.1% (Table 1). In the content information, in 24.9%, a term related to oil content was not used. Specific ingredients, probable ingredients, low possibility ingredients that were a clear indication of trans-fat content were not found in the label information. In the products in this category, uncertain expressions were used in 24.9% of the products regarding the oil content. According to the ingredient list, 75.1% of the 189 samples examined had a fat content without the potential to contain

trans-fats (Figure 1). When the label information was examined, the expressions 'no trans-fat or trans-fat free' were used in this category at a rate of 15.9%, while no expression related to trans-fat content was found in 84.1% (Figure 2). The rate of ambiguous statements about the use of industrially produced oils in packaged products produced in this category was quite high compared to other categories. It has been observed that the use of the expressions "no trans-fat or trans-fat free" in the label information is also limited.

The number of samples examined for instant products such as powdered beverages and coffee is 43. All of these products use the term 'vegetable oil' in their ingredients (Table 1). Specific ingredients, probable ingredients, and uncertain statements that were a clear indication of trans-fat content were not found in the label information. It was observed that FHO, which was in the low possibility category related to trans-fat content, was used in all of the products in this category (Figure 1). When the label information was examined, it was determined that the expressions 'no trans-fat or no trans-fat' were never used in this category (Figure 2).

Table 2. Number and proportions of packaged foods that contain possible sources of *trans*-fat in the ingredient list and that contain *trans*-fat information (yes/no) on the packaging (n = number of products)

Categories	Number of Products Examined	List of Ingredients															
		Use of the term 'vegetable oil'		Specific (%)		Probable		Low Possibility		Uncertain		Without <i>trans</i> fat		<i>Trans</i> -fat content information (Yes)		<i>Trans</i> -fat content information (No)	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
1	312	286	92	10	3.2	6	1.9	55	18	10	3.2	231	74	109	34.9	203	65.1
2	127	114	90	1	0.8	7	5.5	20	15.7	7	5.5	92	72.4	17	13.4	110	86.6
3	83	73	88	0	0	0	0	9	11	12	14.5	62	74.7	22	26.5	61	73.5
4	52	34	65.4	0	0	21	40.4	4	4.8	2	3.8	25	48.1	20	38.5	32	61.5
5	53	51	96.2	1	2	0	0	23	43.4	2	3.8	27	50.9	0	0	53	100
6	117	111	94.9	4	3	0	0	54	46.2	4	3.4	55	47.0	65	55.6	52	44.4
7	221	221	100	0	0	0	0	45	20.4	0	0	176	79.6	90	40.7	131	59.3
8	87	77	88.5	0	0	0	0	3	3.4	4	4.6	80	92.0	0	0	87	100
9	127	31	24.4	0	0	0	0	0	0	12	9.4	115	90.6	0	0	127	100
10	189	142	75.1	0	0	0	0	0	0	47	24.9	142	75.1	30	15.9	159	84.1
11	43	43	100	0	0	0	0	43	100	0	0	0	0	0	0	43	100
TOTAL	1411	1183	83.8	16	1	34	2.4	256	18.1	100	7.1	1005	71.2	353	25	1058	75

- | | | | |
|---|--|----|--|
| 1 | Bakery products (biscuits, cake, crackers, and bakery products) category | 6 | Snacks category (chips, snacks, corn products) |
| 2 | Ready-to-eat foods (ready frozen products, ready soups, ready desserts, canned foods) category | 7 | Chocolate and wafer category |
| | | 8 | Ice cream category |
| 3 | Spreads and sauces category | 9 | Emulsified meat products category |
| 4 | Edible oils category | 10 | Breakfast cereals, bars, baby food category |
| 5 | Pastry ingredients category | 11 | Powdered drinks category |

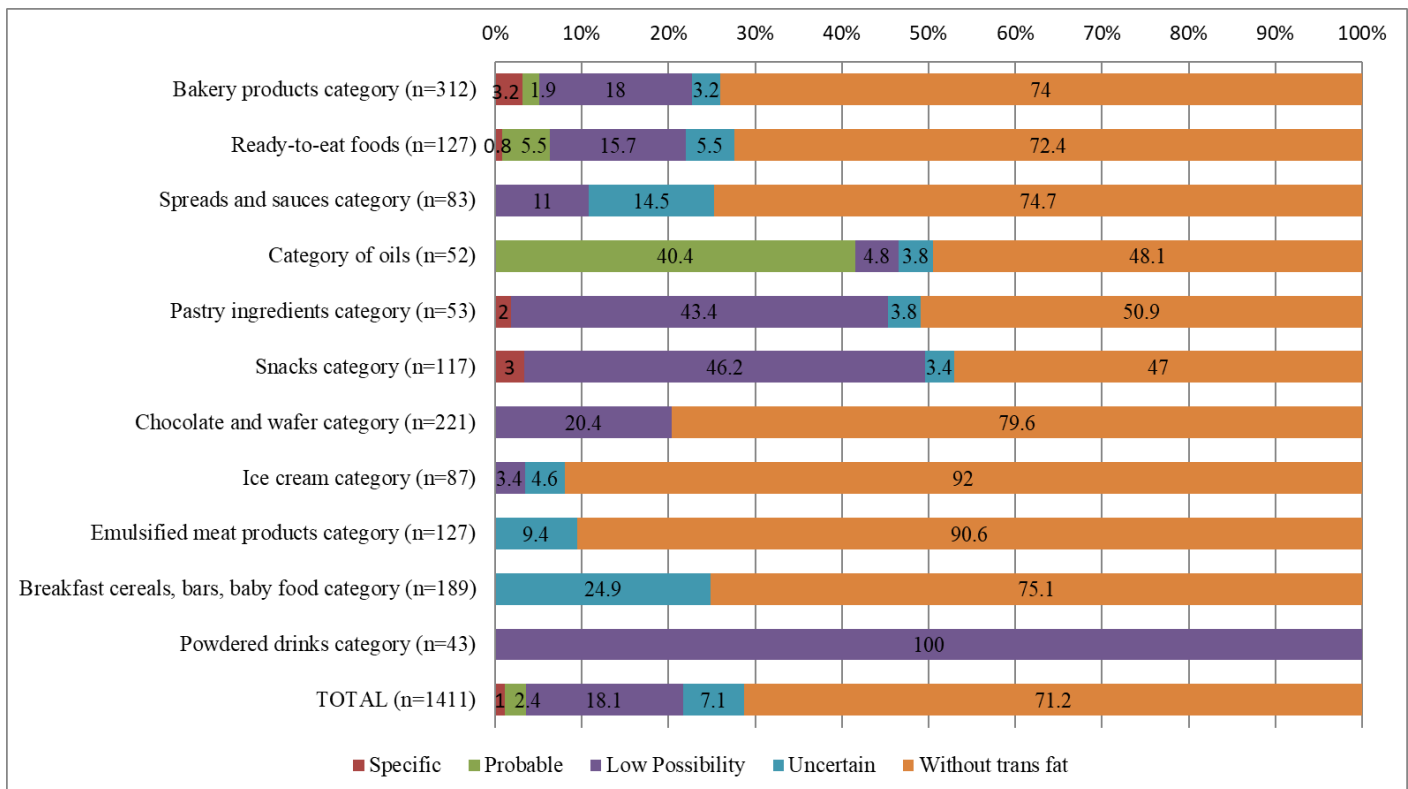


Figure 1. The rate of products in five categories regarding the presence of trans-fat content according to label information

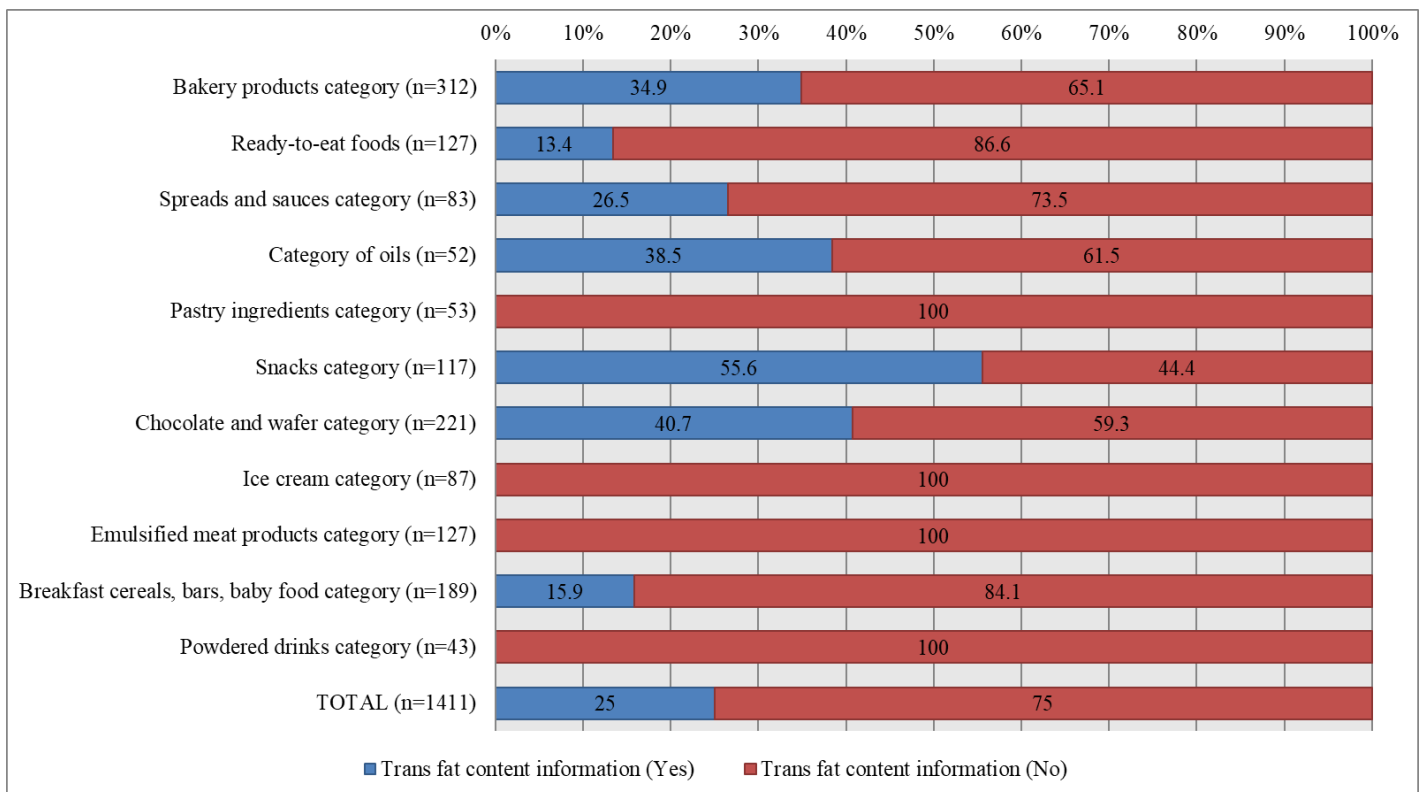


Figure 2. The yes/no ratios regarding the trans-fat content declared in the information panel of packaged foods

When all these categories were examined in general, a total of 1411 products were examined. 83.8% of these products use the term 'vegetable oil' in their ingredients (Table 1). When examined in general, the use of the term 'vegetable oil' at a high rate of 83.8% is important in terms of minimizing the confusion of concepts. While specific ingredients were found at a rate of 1%, probable ingredients at a rate of 2.4%, and low possibility ingredients at a rate of 18.1%, uncertain statements were found in the label information at a rate of 7.1%. When all categories were examined, 71.2% of all products were not found to contain trans-fat (Figure 1). According to the label information, the use of the expressions 'no trans-fat or trans-fat free' is 25% in all categories. The remaining 75% did not include this information (Figure 2). When all these data were evaluated in general, it is seen that 71.2% of 1411 products do not use industrial oils that can be a source of trans-fat. The low probability content of 18.1% is mostly due to the FHA content. According to the packaging information, more than half of 18.1% FHA usage is palm oil-based FHO usage, which can be considered as an ambiguous figure. This is because many packages only report palm oil content, but do not specify which palm oil derivative is used. It is also noteworthy that ambiguous expressions were used in 7.1% of all these products. Similarly, studies have been carried out in countries such as Portugal (Costa et al., 2016), Slovenia (Kušar et al., 2021), Pakistan (Tara et al., 2020), USA (Albers et al., 2008; Garsetti et al., 2016), Canada (Franco-Arellano et al., 2020), European Union (Mourati-dou et al., 2014), Thailand (Chavasit et al., 2020), Brazil (Ricardo et al., 2019), and Australia (Huang et al., 2020) to determine the trans-fat content of packaged foods available in the market. Many of these sources have investigated trans-fat content by performing analytical analysis with a small number of samples. There are two sources for comparing the data obtained in this study. In the study conducted by Ricardo et al., (2019), specific trans-fat-containing components were found at a rate of 20% in bakery products, 6% in prepared foods, 2% in spreads and sauces, 2% in edible oil, 12% in snacks, and 0.6% in breakfast cereals category. The data obtained were found to be quite higher than the results obtained in this study. Franco-Arellano et al., (2020) scanned industrial trans-fats in their study in 2013-2017 and examined a total of 32875 samples. When each category is examined, it can be said that similar results in trans-fat content of packaged foods are also found in our country.

In general, it is reported that trans-fat content is higher in pastry products, bakery products and ready-made foods compared to other categories. When these studies were examined, it can be said that they were compatible with the data obtained. There are various ways around the world to reset trans fatty acids and meet consumer health expectations. Unsaturated oils containing high levels of both omega-3 and omega-6 polyunsaturated fatty acids and oils with the lowest saturated fat ratio should be preferred as the healthiest oils. In cases where the oil is used only for frying, liquid oils such as canola oil that do not oxidize quickly and contain high oleic acid should be used. For food products where the use of saturated fat is

inevitable, solid fats such as animal fats, fractional oils and fully hydrogenated oils may be more cost-effective. However, it is not recommended to use these oils with high saturated fat content as an alternative to partially hydrogenated oils. For the production of some food products, mixing oils with natural fats with a high saturated fat content is one of the most economical ways to change the proportion of partially hydrogenated oils. For example, mixing 1% or 2% fully hydrogenated oil with oil produces viscous oil that is easy to use. As a result of interesterification processes, in which the fatty acids in the oil molecule are mixed and the fatty acid profile of the oil is rearranged, more solid fat structuring capacity can be formed at lower total saturated fat levels. For example, a baking shortening made by interesterification 25% of palm-stearin with soybean oil will have about 30% saturated fat. Combination blends (a mixture of fractionated oils and interesterified oils) are a very suitable method for formulating the fatty acid profile of products.

Conclusion

In terms of food security, many countries are taking steps in the global fight against trans-fat and developing policies towards zeroing it. The policies to be developed are the most necessary steps for food safety, and it is imperative to carry out scans related to the current food content in order to develop these policies. Thanks to the policies to be developed, the producer will be directed to healthier alternative substitutes and processing methods and will be supervised. Labeling regulations, on the other hand, will guide the consumer and meet the healthy food expectations they desired healthy food expectations. Labeling information, which directly determines the consumer's choice and perception of food, depends on the contents of packaged food products. While the roadmap created by WHO specifically for trans-fat aims to provide consumers with healthier foods, consumer health also depends on the working performance of those who have the legal authority of the states. The effort and time spent by conscious consumers for food selection can only be facilitated by legal regulations. However, the processing of vegetable oils also needs to be improved to benefit the consumer. Coordinated policies need to be developed on issues such as the selection of oilseeds that can be used to produce healthier substitute oils, improvement of cultivation, harvesting process, production supports, storage, transportation and processing conditions. It is necessary to determine the oil seeds that are currently being produced but with insufficient yield, to carry out Research and Development (R&D) studies to increase their yield, to identify new oilseed varieties that can be used as oil instead, and to adapt to the production conditions. In addition, R&D investment may be needed to develop new oilseed varieties with more attractive health benefits and to improve processing such as refining. In this respect, the success of R&D studies for the vegetable oil industry to reduce the erucic acid content of canola oil from past to present constitute an important example.

When evaluated in general, 25% of packaged food products in Türkiye contain information that can guide the consumer correctly regarding the trans-fat content. It would be expected that the trans-fat content information would be higher especially in bakery products and ready-made foods that contain or may contain trans-fat. The fact that 71.2% of the 1411 packaged food products examined have food ingredients that are not likely to contain trans fats, which is an optimistic rate for consumers. Of course, this ratio needs to be improved in terms of consumer benefit as stated in the PHO substitution alternatives section. In order to improve the consumer's food choice and perception, it would be beneficial to have more information about the trans-fat content of the products, especially in the snacks, chocolate products and bakery products category. Finally, it can be said that packaged foods in Türkiye are in encouraging condition in terms of trans-fat content. However, it should not be forgotten that effective policies should continue to be developed by government institutions in terms of continuity and the supervision mechanism should work effectively in order to lower the current level.

Compliance with Ethical Standard

Conflict of interests: The author declares that for this article they have no actual, potential, or perceived conflict of interests.

Ethics committee approval: The author declares that this study does not include any experiments with human or animal subjects; therefore, no ethics committee approval is needed.

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Food choices among Portuguese teenagers: a case study

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ABSTRACT

The Mediterranean Dietary Pattern (MDP) is currently accepted as being protective against some of the most worrisome chronic non-communicable diseases in today's society. It is important to instil this nutritional pattern in young people as it is at this point in life that healthy eating habits are created and later maintained. Assessing the adherence of students to the Mediterranean Dietary Pattern (MDP). A case study was carried out with 463 students aged between 14 and 20 years old. Parents of the youngsters were also surveyed to find out about the accuracy of the youth's answers and to determine the type of food preparation practiced in the family. Those who participated in this study had an informed consent, previously signed by their parents and voluntarily expressed their willingness to participate, although they could drop out at any time if they so wished. The results showed a good adherence to the Mediterranean Dietary Pattern (MDP) by the respondents (46.47%) and their knowledge about the concept of the Mediterranean Diet can be classified as "good" (39.7%) and "reasonable" (36.6%). The main conclusions of the study are that the students participating in the study have a good knowledge about the Mediterranean Food Pattern and show a high level of adherence to it.

Keywords: Mediterranean diet, KIDMED, Health

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Introduction

A food pattern is generally understood as a system of social and cultural norms and rules, socially built in accordance with the variety of objective conditions of existence and the psycho-affective economy of individuals that guide, regulate, shape, and give meaning to the act of eating. These food patterns (or models) are the result of a long and deep interaction between biotopes, the biopsychic and the sociocultural environments. These norms and rules are imposed from the outside, throughout the primary socialization process the shaping of tastes, foods, and meals (Lopes, 2006).

Mediterranean food can be defined as being an efficient management of a set of foods and culinary techniques that allowed the survival of the various southern communities and, at the same time, being the result of a social and cultural adaptive construction to a certain type of environmental conditions (Graça 2014; Sikalidis et al, 2021).

The Mediterranean Dietary Pattern (MDP) was first studied in Crete in the 1940s. Upon arriving at the island of Crete, researchers from the Rockefeller Foundation discovered levels of health and longevity that were not expected owing to the apparent poverty and reduced access to health care by local populations. They found levels of mortality from cardiovascular diseases lower than those recorded in the United States of America, and the different eating habits of that population began to make them suspect that there was a relationship between the Mediterranean Dietary Pattern and the non-emergence and development of chronic non-communicable diseases. In the following decades, Ancel Keys and his team would confirm this. Since then, health has been linked to the intake of foods characteristic from regions with climatic similarities to those found in Mediterranean-influenced countries (Graça 2014; Ntanasi et al, 2018; Caradonna et al, 2020).

According to Serra-Majem, (2014) the Mediterranean Diet Pattern (MDP), defined in 1993 at the International Conference on Mediterranean Diets, is defined as a lifestyle marked by diversity in life style habits, combined with characteristics such as: (1) high consumption of foods of vegetable origin (poorly refined cereals, vegetables, fruit, dried and fresh pulses and nuts and oilseeds); (2) consumption of fresh, less processed and local products, respecting their seasonality; (3) use of olive oil as the main fat for cooking or seasoning food; (4) low to moderate consumption of dairy products; (5) frequent consumption of fish and low/infrequent consumption of red meat; (6) consumption of water as the beverage of choice and low to moderate consumption of wine with the main meals; (7) simple culinary procedures with ingredients in the right proportions; (8) practice of daily physical activity

and (9) the habit of having meals with family and friends, promoting coexistence among people at the table.

The teaching and dissemination of concepts associated with healthy eating requires clear pedagogical models that can be understood by the general population. Therefore, food guides have emerged, sometimes as graphic representations that allow simplified dissemination of food and nutritional recommendations. The Mediterranean Food Pattern Pyramid is a food guide, which was initially developed in 1994, and later updated in light of the tradition and culture inherent to the Mediterranean region. The most current representation, created by the Mediterranean Diet Foundation, expresses an example of a sustainable food pattern, where nutrition, local production, biodiversity, and culture are strongly linked, with a reduced impact on the environment (Pinho et al, 2016).

The traditional Mediterranean Diet (MD) pyramid was updated in 2010 to adapt to the modern lifestyle. It incorporates both qualitative and quantitative elements in food selection. The new pyramid follows the design of the previous one: it places foods that should support the diet at the bottom and relegates foods that should be consumed in moderation to the graphically narrower upper strata. But, in addition, there are signs of a cultural and social order, closely linked to the Mediterranean lifestyle, based on the concept of food (understood in a broad sense). It is not just a matter of prioritizing a particular type of food, but the way in which it is selected, cooked, and consumed. It also reflects the composition and number of servings of main meals (FDM, 2021).

The present work is a case study focused on a group of young people between 14 to 20 years of age from a school located at the city of Vida do Conde (Portugal). The study took the form of an observational epidemiological study, with an analytical and cross-sectional nature, whose general objective was to relate food consumption of these young people with their degree of adherence to the Mediterranean Diet. More specific objectives included (i) the characterization of eating and consumption habits; (ii) the analysis of the adherence to the Mediterranean Dietary Pattern and (iii) the analysis of food consumption habits of the household of the young people surveyed.

Materials and Methods

From a total of 587 students who attended the thirty-two classes surveyed in the present study (secondary education level), 463 students accepted to participate in the study, with 147 (31.7%) attending the 10th grade, 154 (33.3%) the 11th grade and 166 (35.9 %) the 12th grade, as shown in figure 1. The

total number of students who accepted to answer the survey corresponds to 79.0 % of the total population of secondary education level at the chosen school, which is within the expected range and is considered representative of the group determined with the OpenEpi calculator, Version 3, using the equation $n = [EDFF * Np(1-p)] / [(d2/Z21-\alpha/2*(N-1)+p*(1-p)]$

For the present study two questionnaires were developed: questionnaire A, which was applied to students, and, questionnaire B to be sent by email, addressed to the parent (co)responsible for the purchase and /or preparation of household food. The questionnaires were prepared based on the foods listed in the Mediterranean Diet Pyramid, created by the Mediterranean Diet Foundation, to which some beverages (wine and soft drinks) were added (FDM, 2021).

Data collection took place over a period of seven months, between October 1st, 2020, and April 30th, 2021.

Considering the target population, the objectives of the work, and the type of study, the two surveys were applied in stages. As shown before, the size of the sample was considered to be representative of the total population of secondary education in the school cluster.

Stage 1 - Application of questionnaire A to 463 students regarding their eating habits. This survey was answered at school.

Stage 2 - Application of questionnaire B to parents regarding the eating habits practiced at home. This survey was sent to the parents of the participating students through a link created for this purpose and sent through the institutional email of the school.

For ethical reasons, the study was preceded by an informal meeting with the Director of the School to present the project and obtain authorization for the application of the questionnaires. The young people who participated in this study had an informed consent, previously signed by their parents, and voluntarily expressed their willingness to participate, although they could drop out at any time if they so wished.

Questionnaire A

Questionnaire A was designed to be applied in the school context. It consisted of eight parts with a total of 18 questions.

Part A - Identification of the school year and sociodemographic data;

Part B - Anthropometric data;

Part C - Eating habits;

Part D - Frequency of meals;

Part E - Place of meals from Monday to Friday;

Part I - Knowledge about healthy food products;

Part J - Main sources of information for food knowledge;

Part K- Self-assessment of knowledge about the Mediterranean Diet;

This questionnaire aimed at obtaining data on food consumption habits, namely the adoption of some type of special diet, the consumption of foods associated with the Mediterranean Diet, the number of daily meals, and the place of meals.

Questionnaire B

Questionnaire B, electronically completed, was developed in the Google Forms application to complement the information obtained on the consumption and eating habits of the household of the young respondents. Addressed to the person responsible for purchasing and/or preparing the household's food, the aim was to confirm the information provided by the young people and to understand whether adherence to the Mediterranean Diet is a consequence of habits transmitted by adults at home.

This questionnaire consisted of two parts, with a total of thirty-two questions.

The first part of the questionnaire intended to collect socio-demographic data and anthropometric characterization of the respondent; the second part intended to collect information on the household's food consumption habits.

The frequency scale of the second part of this questionnaire presented the options “never or only once a month”, “twice a month”, “once a week”, “2 to 4 times a week”, “5 to 6 times a week” and “daily or twice or more times a day”.

The statistical analysis of the data was carried out using the data collected in the Google Forms application. The data collected in questionnaire A was inserted in the application after having the answers from the students, while questionnaire B was electronically completed, by one parent of the students.

The characterization of the eating habits of the students and their households in terms of food frequency and consumption habits was carried out through the analysis of the consumption frequencies of different foods associated with the Mediterranean Dietary Pattern (MDP).

Results and Discussion

Characterization of Sample A

The 467 students that responded the survey, had a good distribution among the three last years of school (secondary level of education), as shown in figure 1.

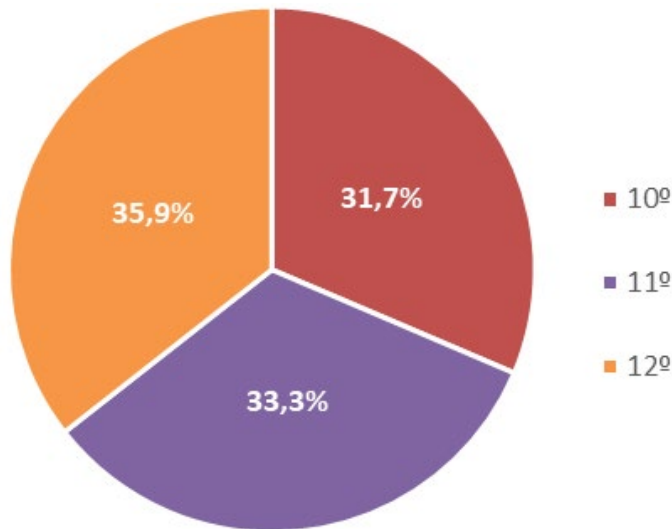


Figure 1. Year of schooling of students in the present study

From the total number of questionnaires, it was found that 248 students were male and 215 were female, corresponding, respectively, to 53.6% and 46.4%, with the vast majority (95.7%) being Portuguese (Fig.2).

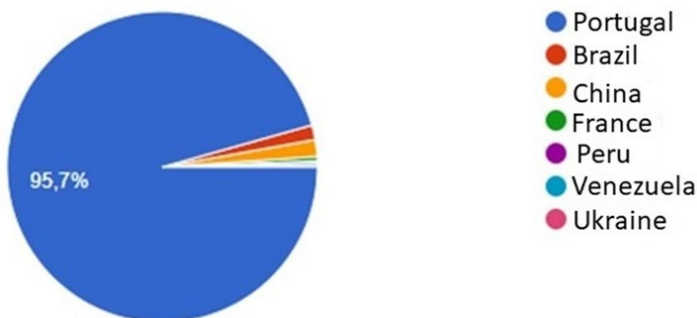


Figure 2. Nationality of students

Regarding the fact of following any special types of diet, 84.1% of the participants reported not to practice any special type of diet. The remaining 15.9% of students reported following specific diets as follows:

- 13 students (2.8%) reported being vegetarian,
- 8 students (1.7%) followed a gluten-free diet,
- 17 students (3.7%) followed a lactose-free diet,

- 28 students (6.0%) followed a weight loss diet and
- 8 students (1.7%) indicated that they practiced another type of diet.

Once this 15.9% of students reported to following specific diets they were not included in the statistical analysis although they were allowed to participate in the study. Our sample was then reduced to 431 students but remained representative of the group (OpenEpi calculator, Version 3).

Before presenting our results regarding the eating habits of the study population, it is important to stress that the Mediterranean Food Pattern refers to not only the type of food that is consumed but also to the way it is cooked, the number of daily meals as well as the conviviality with family or friends. That is why it is important to analyse the daily eating habits of the study population to assess their adherence to the Mediterranean Dietary Pattern (MDP) (FDM, 2021).

Regarding daily meals, 9% of students reported the habit of not eating breakfast; 20.5% said they did not consume food in the middle of the morning; 7% reported not having a mid-afternoon snack and 51.8% reported not having supper.

While an average of 84% of students only consume breakfast from Monday to Friday and an average of 72.4% consume the morning snack only from Monday to Friday, practically all students (98.5%) consume the meal “lunch” every day of the week. Figure 3 summarizes these eating habits by meal and by day of the week with regard to Breakfast, Morning Snack, and Lunch, and Figure 4 summarizes the same eating habits, but in relation to Afternoon Snack, Dinner, and Supper.

It was found that:

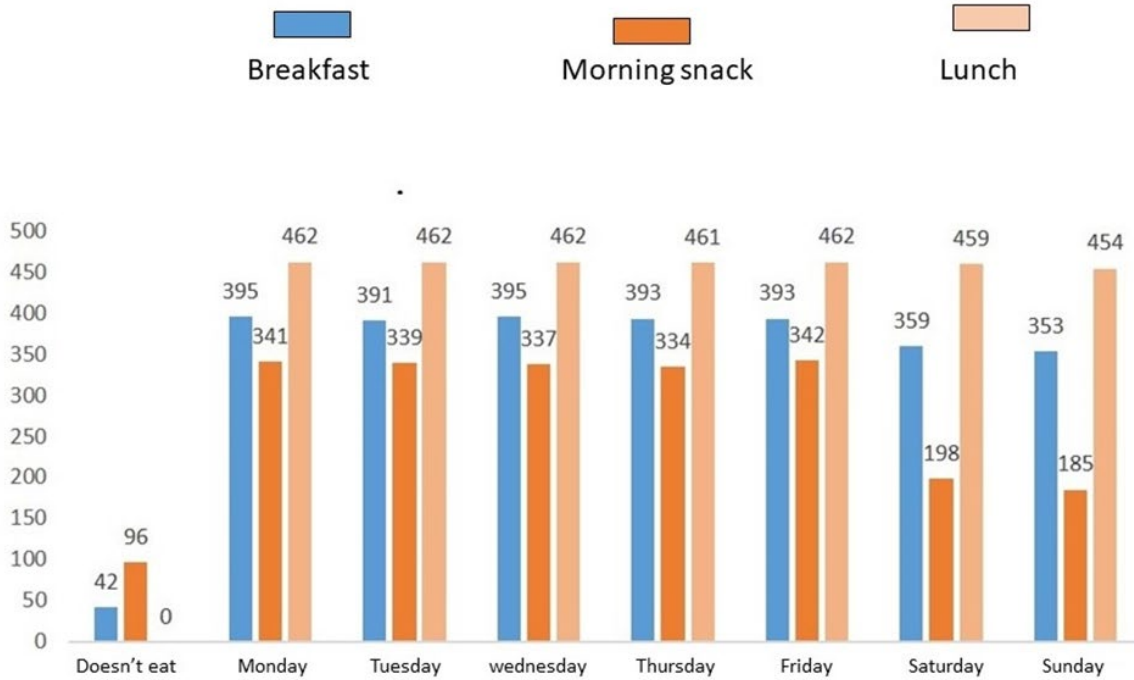
- the afternoon snack is consumed daily by an average of 84.6% of students,
- dinner is consumed daily by an average of 97%
- supper is consumed daily by an average of only 30.6% of students, with an increase to 38.1% during weekends (Figure 4).

Regarding the place where meals are consumed from Monday to Friday, it was observed that:

- Breakfast at home — 395 (84.5%) students;
- Breakfast at the school bar — 13 (2.8%);
- Breakfast brought from home — 12 (2.6%).

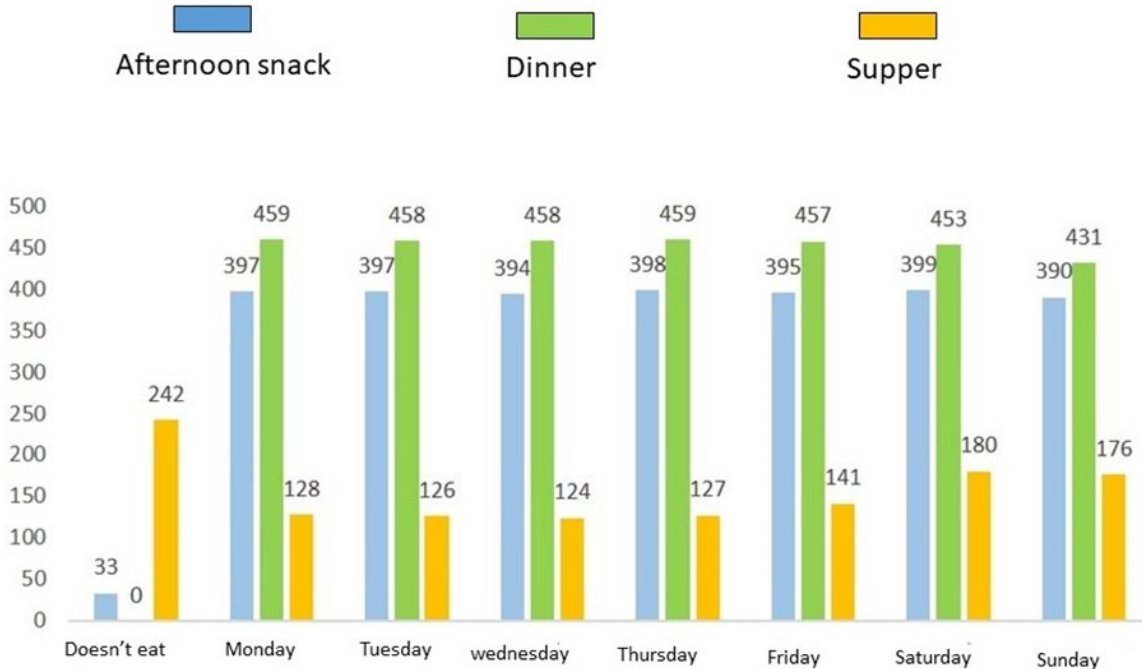
Of the students who consume the morning snack we found that:

- 276 (69.8%) bring it from home;
- 98 (24.8%) purchase products from the school bar and
- 18 (4.5%) eat at home



Numbers in bars show the actual number of students in the study have has the habit of consuming the referred meal in each day of the week.

Figure 3. Eating habits by meal and by day of the week (Breakfast, Morning Snack and Lunch)



Numbers in bars show the actual number of students in the study that have the habit of consuming the referred meal in each day of the week.

Figure 4. Eating habits by meal and by day of the week (Snack, Dinner and Supper)

Lunch is mostly taken in the school canteen together with school friends (286 students - 61.2%), however, 170 (36.4%) students eat this meal at home, 98 (20.9%) students have lunch at a coffee shop or restaurant and 46 (68.6%) bring it from home. In all cases, this meal is never eaten without the company of friends or family.

Regarding the afternoon snack, most students (245 students – 52.5%) reported that they brought it from home, although 113 (24.1%) made this meal at home and 110 (23.5%) purchased products at the school bar. Dinner is also mostly made at home (97.2%) but 13 (2.8%) students reported having dinner at a coffee shop or restaurant. 175 students (37.5%) made supper at home.

In answer to the question “do you know what healthy food products are?”, 458 students (99.3%) said “yes” and only 3 (0.7%) said they did not know.

Regarding the main sources of information about food knowledge, it was found that parents were pointed as the main source of information (71%), followed by health professionals (doctors, nutritionists, dieticians (53%)), the Internet (47.2%), teachers (24.7%) and finally television (19.1%).

Evaluating their own knowledge about the Mediterranean Diet, given the options “Very good”, “Good”, “Fair” and “Poor” it was found that 11% of the students consider that they have a “very good” knowledge; 39.7% consider having “good” knowledge on the subject; 36.6% classify their knowledge as “reasonable” and 12.7% recognize having a “poor” knowledge of the topic.

The adherence of these young people to the Mediterranean dietary pattern was also analysed using the Mediterranean Diet Quality Index for children and adolescents (KIDMED Index as shown in Table 1) (Serra-Majem et al, 2004; Altavilla and Caballero-Pérez, 2019). This questionnaire was also answered at school. The results obtained are shown in Table 1.

From the analysis of the answers obtained, the consumption of a fruit or fruit juice every day (73.2%) stands out positively; 71.7% consumed fresh or cooked vegetables at least once a day; 70.8% consumed fish at least 2 to 3 times a week; consumption of legumes more than once a week represented 74.5%; consumption of pasta or rice 5 or more times a week represented 86.9%; 81.5% consumed cereals or products derived from cereals (bread, etc.) for breakfast; the use of olive oil at home is a habit for 94.4%; 83.5% have the habit of having breakfast; and the consumption of dairy products (milk, yogurt, etc.) for breakfast represented 82%.

As negative aspects, we found that the low consumption of a second fruit daily (29%) stands out as well as the consumption of fresh or cooked vegetables more than once a day (37.4%). The consumption of nuts at least 2 to 3 times a week (18.6%) and the consumption of yogurt and cheese other than at breakfast (36.6%) is also poor.

From the previous analysis, it is possible to assess the levels of adherence to the Mediterranean Dietary Pattern (MDP) (Table 2).

It stands out that the students participating in this study have good adherence to the Mediterranean Diet.

Characterization of Sample B

89 parents answered questionnaire B. The answers obtained were mostly given by female participants (86.5%) and they were mostly in the age group between 42 to 45 years of age.

Regarding the level of schooling, it was found that all parents had more than basic school, as shown in Figure 5.

With regard to employability, 74.1% of the respondents reported being active, and 12.9% were unemployed.

To the question about the habit of cooking meals at home, a percentage of 90.9% were affirmative answers; 8% of the respondents answered that they cooked sometimes and only 1 participant (1.1%) said that did not cook.

Food consumption habits of the households of the young people surveyed showed that the reasons that most influenced the choice/purchase of food on a daily basis were

- Freshness of the food (89.8%)
- Family preference (68.2%)
- The price (65.9%)
- The fact that food was produced locally (23.9%)
- The easiness/speed in food preparation and confec-tion (19.3%)
- The availability of points of purchase (18.2%)
- The fact that foods were typical from the region (10.2%)
- Packaging or presentation (8%)
- The fact of food being related to cultural or ethnic roots and finally (4.5%)
- Easiness of food transporting (3.4%) (fig.6).

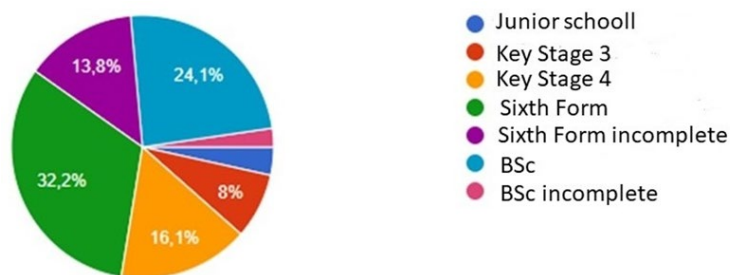
As for the use of aromatic herbs such as celery, rosemary, chives, coriander, mint, bay leaf, basil, oregano, parsley, sage, and thyme to season their cooking, the majority of participants (82%) reported having this habit while 10.1% mentioned their sporadic use and 7.9% said they did not have the habit of using aromatic herbs.

Table 1. KIDMED survey result

	Number	Yes	No
Do you consume a fruit or fruit juice every day	467	42	125
Do you consume a second fruit every day	467	138	329
Do you eat fresh or cooked vegetables regularly, at least once a day	467	336	131
Do you eat fresh or cooked vegetables regularly, more than once a day	467	177	290
Do you consume fish regularly (at least 2-3 times a week)	467	331	135
Do you go, more than once a week, to “fast-food” (hamburger) restaurants	467	58	409
Do you eat pulses more than once a week	467	349	118
Do you eat pasta or rice almost every day (5 or more times a week)	467	409	58
Do you eat cereal or cereal products (bread, etc.) for breakfast	467	382	85
Do you eat nuts (walnuts, almonds, etc.) regularly (at least 2 to 3 times a week)	467	88	379
Do you use olive oil at home	467	443	24
Do you usually have breakfast	467	392	75
Do you have dairy products (milk, yogurt, etc.) for breakfast	467	384	83
Do you eat prepared food or pastries for breakfast	467	117	350
Do you eat 2 yogurts and/or cheese (40g) daily	467	171	296
Do you eat sweets or treats several times a day	467	73	394

Table 2. Levels of adherence to Mediterranean Dietary Pattern (MDP) by KIDMED

Level of adherence	Frequency	Relative frequency (%)
High adherence (>8)	244	52,25%
Intermediate adherence (4/7)	217	46,47%
Low adherence (<=3)	6	1,28%
Total	467	100%

**Figure 5.** Level of education of parents

It should be noted that 93.3% of participants consider that the preparation of meals at home, with family and friends, provides them pleasure and well-being.

With regard to the dietary habits of the adults surveyed, and regarding the use of olive oil as the main cooking fat, it was found that this fat is used daily by all the adults that cook at home with a minimum consumption of 2 tablespoons per day which can easily rise up to 6 or 7 tablespoons daily.

Regarding the frequency of consumption of pre-prepared meals, the majority (65.5%) answered that they consume these products once a week while 21.8% said they did not consume them. In addition, 11.5% said that they consumed pre-prepared meals twice a week and 1.1% admitted to consuming them 4 times a week.

As for the consumption of certain products of animal origin and cereals and their frequency of its consumption in the last 30 days, the results are shown in Table 3.

From the analysis of the table 3, it can be concluded that white meat, pork, fish, and eggs are structuring foods in the participants' diet, as are yogurts and bread. Soups, vegetables, and fruit are also present with a high frequency and with regard to the consumption of beverages, tea and/or coffee stand out as being consumed every day or even more than once a day.

We also asked about cooking methods, namely boiled, grilled, fried, roasted, stewed, and stir-fried, as well as the frequency of the consumption of each type of cuisine (table 4). From the analysis of the table, one may conclude that there is a good variety of cooking methods that are used regularly.

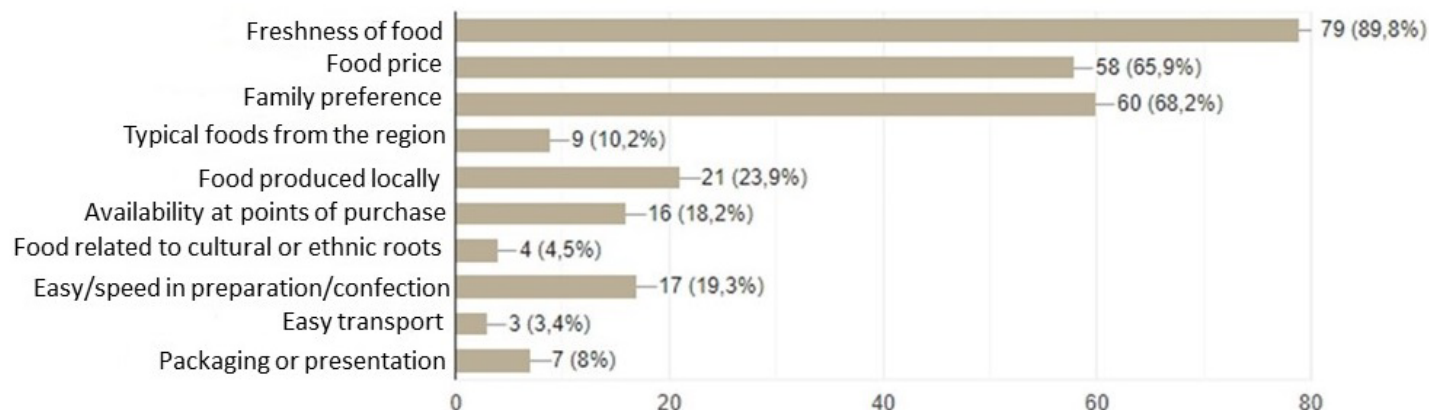


Figure 6. Reasons that influence food purchase

Table 3. Frequency of consumption of food products

Products	Frequency	Never or less than once a month	Twice a month	Once a week	2 to 4 times a week	5 to 6 times a week	Once a day	2 or more times a day
Processed meats		32	21	34	2	-----	-----	-----
Red meat		29	19	76	49	3	2	-----
white meats		2	4	19	46	11	4	3
Fish		2	12	32	33	8	2	-----
Eggs		1	15	29	35	8	1	-----
Milk		13	1	19	12	15	24	5
Yogurts		11	4	16	24	11	16	7
Cheese		8	7	23	15	20	13	3
Breakfast cereals		32	6	20	11	7	10	3
Bread		4	5	19	16	15	21	9
Soup		11	4	20	18	14	17	5
Potatos		6	4	26	37	14	2	-----
Various raw or cooked vegetables		3	2	21	18	16	16	13
Pulses		5	4	32	27	12	5	4
Rice, spaghetti or pasta		1	4	19	33	14	10	8
Fresh fruit		1	1	18	14	13	16	26
Natural fruit juices		29	13	24	12	6	2	3
Bottled fruit juices		77	26	47	16	-----	3	6
Coffee and/ot tea		6	-----	21	6	5	15	36
Herbal teas		55	6	8	5	5	6	4
Wine		53	7	17	2	-----	9	1
Other alcoholic beverages		70	6	5	2	2	4	-----

Table 4. Cooking methods and frequency of consumption

Frequency Cooking method	Never or less than once a month	Twice a month	Once a week	2 to 4 times a week	5 to 6 times a week	Once a day	2 or more times a day
Boiled (includes fish stews)	15	14	31	21	4	4	-----
Grilled	4	8	26	35	11	4	1
Fried	14	19	35	20	-----	1	-----
Oven baked	5	8	42	26	6	1	1
Stews	5	8	38	29	7	2	-----
Stir-fried	8	11	42	24	1	1	2

Regarding the eating habits of the young people surveyed, it was found that most of them do not practice any special type of diet with those who report doing so, the most representative being the slimming diet (6%). An identical result was presented in the results of the National Food and Physical Activity Survey (IAN-AF) 2015-2016, in which only 6.8% of the individuals reported following a special diet (INE, 2017).

The fact the majority of students that participated in the study consume regularly the main meals (breakfast, lunch and dinner) as well as the morning and afternoon snack, as shown before in the analysis of results, is well in accordance with the Mediterranean Dietary Pattern (MDP) which advises the consumption of several meals during the day, dividing the amount of energy between them instead of concentrating all the energy necessary for body function in just one or two main meals (D’Innocenzo et al, 2019). Our results about the study of food consumption habits and adherence to the Mediterranean Dietary Pattern are reinforced by the results obtained with the KIDMED survey. We feel confident with the results shown in relation to the KIDMED questionnaire since it has already been proven the reproducibility and validity of this index with Portuguese adolescents (Rei et al, 2021). In addition, the results of the present study are in accordance with those found in another study regarding Portuguese children (Rito et al, 2018). We observed that some students have their meals at home even during weekdays. This means that either they live within walking distance of school or their parents are able to fetch them from school at mealtime. Whichever the case, this is important since it reinforces family bonds but even the students who eat at school facilities or at local restaurants mentioned the company of friends during meals. This is also an important and strong characteristic of the Mediterranean Dietary Pattern (MDP). In addition, the fact that these students’ parents have a good level of education (as shown in figure 5) also influences the food choices made by families and taught to youngsters (Almeida et al, 2021; Hanemaayer et al, 2022).

In this study, participants showed to have a good habit of eating breakfast, which is in accordance with the Mediterranean traditions, although we found a slight drop between consumption on weekdays *versus* weekends. The 2018 HBSC (Health Behaviour in School-aged Children), a collaborative study of the World Health Organization (WHO) that aimed at studying the lifestyles of adolescents and their behavior in various scenarios of their lives, pointed in the same direction (Inchley et al, 2020). In that study participated 6997 young students and it was reported a difference of 5.5 pp lower in the consumption of breakfast on weekends than on weekdays (Inchley et al, 2020). However, an inverse result was found in an earlier study carried out in 2012 in which a sample of 276 young people, between eleven and sixteen years old, showed that between 63.8% and 64.5% ate breakfast during the weekdays, increasing that frequency during the weekends to 81% on Saturday and 84.4% on Sunday (Matos, 2018). When analysing, breakfast consumption among adolescents, the IAN-AF 2015-2016 results indicate that the prevalence is 91.6% (INE, 2017).

As for the mid-morning meal, our results show inverse values from those observed in the study by Mateus (2012) in which during the weekdays only 2.0% to 2.9% ate this meal with the frequency increases during the weekend to 37.1% on Saturday and 38.4% on Sunday. This may be due to a geographical difference being the study by Mateus (2012) done in the south of the country (Algarve) while our study relates to students in the northern rural part of Portugal. In the 2015-2016 IAN-AF (national inquiry to families) results, the prevalence was found to be 53.2% (INE, 2017) leveling the results of both studies.

These two meals (breakfast and mid-morning meal) are particularly important in the diet of young people since they represent the first source in the day of water and infusions, dairy products, fruit and fruit juices, as well as bread and other poorly refined cereals in accordance with the principles and

recommendations of the MD Pyramid (FDM, 2021). Compared to other studies, the present one shows results more in line with the MD indications regarding these meals, although it may be concluded that a considerable number of young people deviate from these guidelines, especially during weekends, which indicates that they spend too many hours without eating. Although without certainties, we may think of small changes in the habits of the young Portuguese students in the sense of skipping breakfast during the weekends. This may be due to the atypical year in which the present study was developed (during the COVID-19 pandemic period) in which most students spent more time at home than usual. This factor may have led to this change in eating habits owing to the changes in the school schedules.

Regarding the remaining daily meals, we found that lunch was eaten by all the students every day of the week, with no significant difference in its frequency during weekends. This is well in accordance with the new Mediterranean Diet Foundation Expert Group's pyramid (Lacatusu et al, 2019) although different values were found by Mateus (2012) that reported differences for this meal for weekdays (between 29.0% and 34.5%) and weekends (92.5% on Saturday and 94.4% on Sunday). A similar result to the present study was reported in the 2015-2016 IAN-AF results (INE, 2017), where the prevalence is 97.9%.

As for the afternoon snack, it seems that the majority of the surveyed young people consume it daily, although with a value relatively lower than that found in the 2015-2016 IAN-AF results (INE, 2017) with a prevalence of 91.9%, but much higher than that found by Mateus (2012) in which there was a consumption between 19.9% and 22.1% from Monday to Friday and 68.7% on Saturday and 71.2% on Sunday.

Dinner is one of the most frequently eaten meals in this study, with an average of 97% of students having it every day of the week, similar to what Mateus and the IAN-AF 2015-2016 found (Mateus, 2012; INE, 2017).

Supper is the least frequent meal among the young people surveyed in this study, being eaten daily on average by only 30.6%, with an increase to 38.1% during weekends. An identical trend was found in other studies (Mateus, 2012; INE, 2017). This is not surprising since supper isn't strongly associated with Portuguese food habits (Graça, 2020; Ferreira et al, 2021).

From the analysis of the data obtained in this work, it can be concluded that the main meals (lunch and dinner) are the ones that are consumed more regularly, especially during weekdays and, compared with data from other studies mentioned before, the students surveyed have habits of more frequent

consumption, namely in relation to the intermediate meals. Bibliography points out as beneficial the daily consumption of meals at regular times and eaten in a quiet environment, as they favor the digestion of food and the regulation of the amount ingested (Esaulenko et al, 2016, Kanjilal et al, 2021; Agathão et al, 2021). The importance of breakfast, which should never be missed, is highlighted, as its omission can cause metabolic changes, resulting from prolonged fastings, such as a decrease in the ability to be attentive, concentrate, and logical reasoning, that is, causes difficulties in thinking, understanding, and learning, reasons that are often enough to affect school results (Sousa, 2013). It is also mentioned that in addition to food, there are other important factors that also influence health status and longevity, such as naps, the number of meals per day, and the presence or absence of snacks (Pinho et al, 2016; Bellisle, 2021).

Regarding the place where meals are taken from Monday to Friday, it was found that the majority of the surveyed students have lunch in the school canteen. This habit is confirmed by other studies (Mateus, 2012; Matos, 2021). As for the knowledge about healthy food products, it was found that practically all students claim to have this knowledge and mention parents as being the main source of information. Teachers appear in the fourth position of the five possible response options as a source of transmission of this type of knowledge as also happens in other studies (Mateus, 2012).

Analysing the Portuguese school curricula, published by the General Directorate for Education (DGE), it was found that although the theme of food and nutrition is present in several study cycles, only in the 9th grade the topic "Mediterranean Diet" is introduced (DGS, 2012).

For educators, it is fundamental to have food models they can rely on, whether they are health professionals, education professionals, or parents. The promotion and defense of the Mediterranean Diet can (and should) be used as a resource for more comprehensive education, aiming at preparing future citizens who are more aware of their roots and able to make healthy food choices.

The integration of the Mediterranean dietary pattern concept in the curricular goals of basic education allows the dissemination of concepts and behaviors associated with a healthy lifestyle, not only because it is important but also because levels of interest and satisfaction with school worsen with age and with the pressure of schoolwork (Pinho, et. al, 2016; Graça, 2016; Lima, 2018; Inchley, et. al, 2020; Matos, 2021).

Thus, the fact that students consider that their nutritional learning is provided by teachers in less than 25% of the participants may be seen as positive because, as the present study

shows, students follow a diet that we may consider healthy which may be due to their parents' knowledge and also because the way teachers approach food topics (integrated and planned in other subjects) allowing students to learn new concepts without being imposed on them.

Still regarding questionnaire A, 52.25% of respondents showed a high adherence to the Mediterranean Dietary Pattern (MDP), 46.47% were at the intermediate level of adherence, and only 1.28% had a low level of adherence. Compared with other studies carried out at national level, the same situation was found with only a difference of 4.12pp compared to the present study (Mateus, 2012). More recently, in the study published by Bôto et al (2021) with the objective of characterizing the eating habits, health, and adherence to the Mediterranean Dietary Pattern of young people at the secondary education level, with a sample of 325 young people aged between 15 and 19 years, the results indicated a low adherence to the Mediterranean Dietary Pattern in 9.0% of the participants, an intermediate adherence in 45.5% and a high adherence in 45.5%. In this case, there is a deviation of 7.72 pp, in the low adherence to the Mediterranean Dietary Pattern (MDP) compared with the present study. These three studies have in common the fact that they have used a similar methodology regarding the way for assessing eating habits, since they were all carried out using the same questionnaire to measure the adherence to the Mediterranean Dietary Pattern (MDP).

In relation to questionnaire B, which aimed at assessing the eating and consumption habits of the families of young students, we found that the participants have the habit of cooking at home and that they consider that the preparation of meals with family and friends provides them with pleasure and well-being, as mentioned before. Participants were, in general, female which seems to show that these activities are still carried out mostly by women. By analysing the 89 valid responses, a considerable number of participants (always over 25 years old) were found to have their own food production, namely vegetables, fruits, eggs or herbs, which is presumed to be due to the rural environment where the study was conducted, and which reveals consumption habits of local, fresh, seasonal and sustainable foods. In the MD Pyramid, these foods are located in the group of foods that should be consumed daily (Abreu, 2014; Ferreira, 2014; Pinho et al, 2016).

Comparing the consumption observed in this study with the recommendations of the MD pyramid, the daily/weekly consumption of olive oil, dairy products, aromatic herbs, white meats, eggs, legumes, processed meats, red meats, and sweets stands out positively. Negatively, we found a low daily/weekly consumption of water or infusions, fresh fruit,

vegetables, rice, pasta, bread, nuts, and fish (fresh or frozen). Potato consumption stands out for its excessive consumption.

The Mediterranean Diet is essentially characterized by the predominance of vegetable products, including fruit, vegetables, cereals, oilseeds, and legumes, and by the consumption of olive oil as the main source of fat. Olive oil is recognized for its cardioprotective properties, being pointed out as the most beneficial fat for daily use. Its consumption is advisable, either to season or to cook (Pinho et al, 2016; Sikalidis et al, 2021). Dairy products and fish are present in this dietary pattern in a moderate amount, as well as wine, particularly at meal times. The consumption of white meat and eggs balances the low consumption in frequency and quantity of red meat and sausages, as well as sugar. The richness of fruit and vegetables assumes a relevant presence in the Mediterranean Food Pattern, either in the form of salads, soups or in other food preparations. Sharing meals and traditions together with moderate physical exercise, favored by the mild climate, completes the model of healthy living (Sousa, 2013; Davis et al, 2015; Pinho et al, 2016; INE, 2017). Stews correspond to the main cooking techniques used, a traditional component associated with the various soups and broths, where the addition of vegetables and legumes is preferred, with a small amount of meat or fish, seasoned essentially with olive oil and aromatic herbs (Sousa, 2013; Valagão, 2014; Pinho et al, 2016).

The results obtained in the present study, through survey B, stand out positively in what concerns the simplicity and variety of cooking methods. However, it was found that pre-prepared and ready-to-eat meals are consumed by 65.5% of participants once a week and that fried foods are consumed once a week by 42.7% of households.

A factor to be highlighted here is that the “freshness of the food” and the “family preference” are the reasons that most influence the choice/purchase of food on a daily basis. However, the family budget is mentioned by 32.5% of the participants as a limiting factor to the purchase of certain foods, namely fish, meat, fruits, and vegetables. Similarly, data from the DGS report (DGS, 2016; DGE, 2021) indicates that in 2014, despite the majority of people inquired (71.4%) said that “there were no changes in the consumption of any food considered essential in the last 3 months owing to economic difficulties for its acquisition”, 26.6% answered affirmatively to this question. Truninger (2019) verified an identical result to that of the present study where the biggest drops in food consumption of families in a situation of food insecurity were verified in the consumption of fish (49.1%) and meat (44.2%). There was also a considerable decrease in the con-

sumption of vegetables (33.3%) and fruit (33.2%). In addition, the DGS and DGE reports (DGS, 2016; DGE, 2021) referred to 2014 and since then with the pandemic situation all over the world, food insecurity became higher.

In the present study, it was also found that the purchase of food products is mostly done once a week, preferably in dedicated stores (butcher, fishmonger, fruit store, etc.) and in supermarkets close to home. Traditionally, the city where this study took place offers residents a wide range of fairs and markets, which include a variety of fresh and seasonal food products. The results obtained about this issue may have been influenced by the limitations on trade, imposed by the DGS due to the Covid-19 pandemic, namely with the end of fairs and municipal markets.

Some limitations were imposed in the course of this study. The main ones being related to the fact that it was carried out during the pandemic period of Covid 19, which may have impaired the characterization of eating and consumption habits and lifestyles of students and their families with higher accuracy. The size of the sample in survey B was also a constraint, given that only 19.6% of parents from the total number of participating students gave their contribution. Low adherence may be related to the fact that the questionnaire was carried out online, and it was not possible to make a follow-up as it happened for questionnaire A. Another plausible reason may be related to the possible lack of access or familiarity with the internet.

Conclusion

Considering that eating habits play a central role in maintaining good levels of health, it is at school age that special attention should be paid, not only because there is a close relationship between a balanced diet and school performance, but also because it is in this group that it is worth to introduce eating habits that promote healthy lifestyles. Considering also that the health problems related to unhealthy eating habits and the lack of physical activity are extensive and costly to society, it will be more effective to promote healthy eating models, such as the Mediterranean Diet, preventing some diseases related to lifestyles, instead of overloading the National Health Service and the family budget with treatments that sometimes are expensive also from a psychological point of view. This study reinforced the results of previous investigations, reiterating the extreme importance of a set of systematic and long-term concerted actions by various national, local, and regional actors, in order to create conditions for healthy diets beginning at school age.

The main conclusion of the present study is that the students participating in this study showed good adherence to the

Mediterranean Dietary Pattern with 52.25% having a high adherence and 46.47% having an intermediate level of adherence. We also concluded that the students consider having a good knowledge about this theme, which is gratifying and reflects the efforts that schools and other agents have made over the years to promote the knowledge about the importance of this type of dietary pattern in health promotion.

Compliance with Ethical Standards

Conflict of interests: The author declares that for this article they have no actual, potential, or perceived conflict of interest.

Ethics committee approval: Verbal consent was witnessed and formally recorded. The study was preceded by an informal meeting with the Director of the School to present the project and obtain authorization for the application of the questionnaires. The young people who participated in this study had informed consent, previously signed by their parents, and voluntarily expressed their willingness to participate, although they could drop out at any time if they so wished.

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Toz bebek mamalarının mikrobiyolojik kalitelerinin ve mama hazırlama önerilerine uygunluğunun araştırılması

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ÖZ

Bu çalışmada Türkiye piyasasında satışa sunulan toz bebek mamalarının mikrobiyolojik kalitelerinin incelenmesi ve mama firmaları tarafından önerilen hazırlama talimatlarının Dünya Sağlık Örgütü (DSÖ) tavsiyelerine uygunluğunun değerlendirilmesi amaçlanmıştır. Çalışmada 10 farklı firmaya ait 36 adet toz bebek maması *Cronobacter sakazakii*, *Bacillus cereus*, *Staphylococcus aureus*, koliform ve fekal koliform bakteri, küf-maya, toplam mezofilik aerobik canlı bakteri sayıları açısından incelenmiştir. Mama firmaları tarafından önerilen hazırlama talimatlarının DSÖ önerilerine uygunluğu değerlendirilmiştir. İncelenen mama örneklerinin iki tanesinde *C. sakazakii* tespit edilmiştir. Mama örneklerinin dokuz tanesinde *B. cereus*, dört tanesinde *S. aureus*, 10 tanesinde koliform ve sekiz tanesinde fekal koliform bakteri çeşitli düzeylerde saptanmış olup belirlenen bu değerler yasal limitlerin üzerinde bulunmuştur. Mama firmaları tarafından önerilen hazırlama talimatlarının DSÖ önerilerine uygunluğu değerlendirildiğinde, *C. sakazakii*'nin kontrolünde kritik nokta olan su sıcaklığının >70°C olması gerektiğine dair bir talimat hiçbir mama firması tarafından belirtilmemiştir. Mamalarda mikrobiyolojik kalitenin yasal sınırlar ile belirlenmiş olmasına karşın incelenen mama örneklerinin bazılarında hedeflenen mikrobiyolojik kaliteye ulaşılamadığı ve firmalar tarafından belirtilen mama hazırlama talimatlarının gıda güvenliği açısından yeterli olmayabileceği gözlenmiştir.

Anahtar Kelimeler: Toz Bebek Maması, Mikrobiyolojik Kalite, Mama Hazırlama Talimatlar

ABSTRACT

Investigation of microbiological quality of powdered infant formulas and their suitability for formula preparation recommendations

The present study aimed to investigate the microbiological quality of powdered infant formulas on sale in Turkey and to assess the compliance of the preparation instructions recommended by the formula companies with the guidelines of the World Health Organization (WHO). In the study, 36 powdered infant formulas belonging to 10 different companies were analysed regarding the presence of *Cronobacter sakazakii*, *Bacillus cereus*, *Staphylococcus aureus*, coliform and faecal coliform, mould-yeast, total mesophilic aerobic bacteria counts. *C. sakazakii* was detected in two of the examined formula samples. At various levels, *B. cereus* in nine, *S. aureus* in four, coliform bacteria in 10 and faecal coliform bacteria in eight of the formula samples were found and these identified values were discovered above the legal limits. When the compliance of the preparation instructions recommended by the formula companies with the WHO guidelines was evaluated, the instruction requiring the water temperature to be >70°C, which is critical in the control of *C. sakazakii*, was not included in the preparation instructions of any formula companies. Although the microbiological quality of the formulas was determined by the legal limits, it was observed that the targeted microbiological quality could not be reached in some of the examined formula samples and the instructions prepared by the companies may not be adequate for the food safety.

Keywords: Powdered Infant Formula, Microbiological Quality, Formula Preparation Instructions

Giriş

Anne sütü bebeğin sağlıklı büyümesi ve gelişmesi için gerekli tüm sıvı, enerji ve besin öğelerini içeren, biyolojik yararlılığı yüksek ideal bir besindir. Anne sütü sadece bir besin olmayıp bebeği bulaşıcı ve kronik hastalıklara karşı korumakta ve bebeğin duyuşsal ve bilişsel gelişimini desteklemektedir (World Health Organization, 2022; Centers for Disease Control and Prevention, 2022). Anne sütü üstün özellikleri ve faydaları sebebiyle bebekler için ilk tercih olmasına rağmen, emzirmenin yeterli ve mümkün olmadığı zamanlarda bebek mamaları kullanılmaktadır (Martin vd., 2016).

Bebek mamalarının içeriği anne sütüne benzetilmiş olup toz veya sıvı formda üretilmektedir (Martin vd., 2016). Toz mamalar ekonomik olmaları ve kolay kullanımları nedeniyle sıklıkla tercih edilmektedir. Ancak toz mamalar mevcut üretim süreçleri nedeniyle steril ürünler değildir ve patojen mikroorganizmaları içerebilmektedirler (Kent vd., 2015). Bebekler olgunlaşmamış bağışıklık ve gastrointestinal sistemleri nedeniyle gıda kaynaklı enfeksiyonlara karşı son derece hassas olup mamalardaki muhtemel bir mikrobiyel kontaminasyon bebeklerde ciddi hastalıklara ve hatta ölümlere sebep olabilmektedir (Cho vd., 2019; Zheng vd., 2021). Bebek sağlığı ve sağ kalımı açısından bebek mamalarının ulusal ve uluslararası kriterlere uygun yüksek mikrobiyolojik kaliteye sahip olması son derece önemlidir (Kent vd., 2015).

Cronobacter sakazakii (*Enterobacter sakazakii*), bebek mamalarında en sık bildirilen patojen olmanın yanı sıra mikrobiyolojik kaliteyi gösteren en önemli bakterilerden biridir (Xie ve Liu, 2021). *C.sakazakii*, bebeklerde hayati risk oluşturan menenjit, sepsis ve nekrotizan enterokolit enfeksiyonlarına neden olmaktadır ve vaka ölüm oranı %40'a kadar yükselmektedir (Xie ve Liu, 2021; Stryzko vd., 2020). *C.sakazakii*'nin yüksek sıcaklığa direncinin olması, kuru ortamlarda iki yıldan fazla yaşayabilmesi ve çeşitli materyallere tutunup antimikrobiyel müdahalelere dirençli biyofilm oluşturabilmesi, toz bebek maması üretim tesislerinde, hastanelerde ve ev ortamında yok edilmesini ve önlenmesini karmaşık ve zor hale getirmektedir (Henry ve Fouladkhah, 2019).

C.sakazakii'nin yanı sıra *Bacillus cereus* (*B.cereus*) ve *Staphylococcus aerus* (*S.aureus*) bebek mamalarında sıklıkla bildirilen diğer patojen bakterilerdir (Sezer vd., 2015). *B.cereus* sporlu bir bakteri olup sporları kurutma, vakumlama, dondurma, ısıtma, dezenfektan ajanlar, iyonizasyon, radyasyon ve ultraviyole ışık gibi birçok işleme dayanıklıdır (Pei vd., 2018). *S.aureus* ise ısıya dayanıklı enterotoksinler üretmesi nedeniyle gıda kaynaklı önemli bir patojendir (Wang vd., 2012). *S.aureus* ve *B.cereus* Birleşmiş Milletler Gıda Tarım Örgütü (Food and Agriculture Organization, FAO) ve

Dünya Sağlık Örgütü (DSÖ) tarafından belirlenen bebek maması kontaminasyonu ile ilişkili düşük riskli bakteriler arasında yer almaktadır (World Health Organization, 2004).

Literatürde bebek mamalarının mikrobiyolojik kaliteleri farklı kapsam ve yöntemler ile incelenmiştir. Yapılan araştırma sonuçlarına göre bebek mamalarında *C.sakazakii* sıklığı Latin Amerika'da %3.9 (5/128) (Parra-Flores vd., 2020), Çin'de %2.8 (56/2020) (Fei vd., 2017), İran'da %7.2 (9/125) (Mardaneh ve Dallal, 2017) ve Türkiye'de %4.8 (3/62) (Tokatlı-Demirok ve Arıcı, 2019) saptanmıştır. Bu sonuçlardan farklı olarak Kuzey Kıbrıs Türk Cumhuriyeti'nde yapılan çalışmada tahıl bazlı bebek mamalarında ve tamamlayıcı besinlerde *C.sakazakii* saptanmamıştır (Ziver vd., 2020). Benzer şekilde Türkiye'de bebek mamaları, devam formülleri ve bazı tamamlayıcı besinlerin hijyenik kalitelerini değerlendiren çalışmada da hiçbir örnekte *C.sakazakii*, *Salmonella* spp., ve *Escherichia coli* bulunmamıştır. Bunun yanı sıra incelenen örneklerde koliform, *B.cereus*, *S.aureus*, küf ve maya kontaminasyon seviyeleri sırasıyla %0.74, %2.22, %0.74, %23.70 ve %2.96 olarak bulunmuştur (Vural ve Genç, 2022).

Bebek mamalarındaki endojen patojen ve bozulma yapıcı mikroorganizmaların yanı sıra biberonların yetersiz temizlenmesi, uygun olmayan mama hazırlama ve yeniden ısıtma süreçleri bu mikroorganizmaların çoğalmasına neden olabilmektedir (Sani vd., 2013). Mamaların hazırlanması, kullanılması ve depolanması süreçlerindeki potansiyel kontaminasyonları önlemek için DSÖ tarafından bir kılavuz yayımlanmıştır (World Health Organization, 2007). Ancak mama kutularının ve/veya paketlerinin üzerinde DSÖ tavsiyelerine uygun hazırlama aşamalarına yer verilip verilmediği belirsiz olup DSÖ tavsiyelerine uygunluğunun kontrol edilmesi potansiyel kontaminasyonları önlemek açısından önem taşımaktadır (Sani vd., 2013). Literatürde mama etiketlerinde yer alan hazırlama talimatlarının DSÖ tavsiyelerine uygunluğunu araştıran sadece bir çalışma (Sani vd., 2013) olup ülkemizde ise böyle bir çalışmaya rastlanılmamıştır. Bu nedenle, bu çalışmada ülkemizde satışa sunulan toz bebek mamalarının mikrobiyolojik kalitelerinin incelenmesi ve mama firmaları tarafından önerilen hazırlama talimatlarının DSÖ tavsiyelerine uygunluğunun değerlendirilmesi amaçlanmıştır.

Materyal ve Metot

Bu çalışmada İstanbul'daki çeşitli eczane ve marketlerde satılan 10 farklı mama firmasına ait 24 adet toz mama (% 66.6) ve 12 adet toz sütlü tahıllı ek gıda (% 33.3) olmak üzere toplam 36 adet toz mama örneği incelenmiştir. Mamaların 7 tanesi 0-6 ay yenidoğanlar için kullanılan toz mamalar, 8 tanesi

6-9 ay bebekler için kullanılan toz mamalar, 5 tanesi 9-12 ay bebekler için kullanılan toz mamalar, 4 tanesi prematüre bebekler için kullanılan toz mamalar ve 12 tanesi toz sütlü tahıllı ek gıdadan oluşmaktadır. Toz sütlü tahıllı ek gıdalar ticari olarak “kaşık maması” olarak satılmakta olup bu çalışmada bu örnekler için “sütlü tahıllı ek gıda” terimi kullanılmıştır. Çalışmadaki toz mamalar DSÖ tarafından hazırlanan “Toz bebek mamalarının güvenli hazırlanması, depolanması ve kullanımı yönergeleri (World Health Organization, 2007)” mama hazırlama talimatlarına uygunluğu açısından değerlendirilmiştir. Yönerge 12 aya kadar kullanılan toz bebek mamalarını kapsadığı için 24 adet toz bebek maması değerlendirilmeye alınırken 12 adet sütlü tahıllı ek gıda incelenmemiştir.

Satın alınan toz bebek mamaları mikrobiyolojik açıdan değerlendirilinceye kadar orijinal ambalajlarında ve saklama koşullarına uygun olarak oda sıcaklığında, ışık almayan ortamda bekletilmiştir. Analiz öncesi toz mamalar ilk kez açılarak mama kutusunun üzerinde belirtilen mama hazırlama talimatlarına uygun şekilde hazırlanmıştır. Mama hazırlama suyu için serum fizyolojik kullanılmış olup önce kaynatılıp sonra da her bir mama kutusunun üzerinde belirtilen sıcaklığa kadar soğutulmuştur. Bulaş olmaması için örnekler alınırken işlemler uygun koşullar ve ortamlarda gerçekleştirilmiştir. Kullanılacak bütün malzemeler ve besiyerleri steril olarak hazırlanmış ve mikrobiyolojik analizler aseptik koşullarda yapılmıştır. Mamaların mikrobiyolojik kalitelerini değerlendirmek için mama örnekleri *C. sakazakii* varlığı yönüyle incelenmiş ayrıca *B. Cereus*, *S. aureus*, koliform ve fekal koliform bakteri, küf-maya, toplam aerobik mezofilik canlı bakteri (TAMCB) sayımı yapılmıştır.

Coronabacter sakazakii analizinde 25'er gram mama örneği tartılarak üzerine 225 mL tamponlanmış peptonlu su eklenecek homojenize edilmiş ve 37°C'de 24 saat ön zenginleştirilmeye tabi tutulmuştur. Elde edilen kültürden 10 mL miktarda 90 mL Enterobacteriaceae Enrichment (EE) broth içine aktarılmış ve 37°C'de 24 saat inkübe edilmiştir. İnkübasyonun sonunda EE broth içinde gelişen kültürlerden *Enterobacter sakazakii* selektif Chromagar ve VRBD (Violet Red Bile Dekstroz) agar besiyerlerine ekimler gerçekleştirilmiş, 37°C'de 24 saat inkübe edilmiştir. İnkübasyonun ardından besiyerlerinde gelişen şüpheli koloniler (Chromagar için mavi-yeşil renkte, VRBD agar için kırmızı renkte) Trypticase Soy Agar besiyerine aktararak 25°C'de 48-72 saat inkübe edilmiştir. İnkübasyonun sonunda sarı pigment oluşturan koloniler *C. sakazakii* olarak değerlendirilmiştir (Muytjens, 1988).

Diğer mikrobiyolojik analizler için ise 10'ar gram mama örneği tartılıp 90 mL steril serum fizyolojik (% 0.85 NaCl) içinde homojenize edilerek ileri dilüsyonları hazırlanmıştır. Elde edilen dilüsyonlardan *S. aureus* ve *B. cereus* sayımı için de yüzeye yayma, koliform ve fekal koliform bakteri sayımı için çift katlı dökme plak, küf-maya ve TAMCB sayımı için dökme plak yöntemi ile Tablo 1'de yer alan besiyerleri kullanılarak ekimler gerçekleştirilmiştir (Anonim, 2005; Temiz, 2010).

Analiz edilen mamaların mikrobiyolojik uygunluğunun değerlendirilmesi Türk Gıda Kodeksi Bebek Formülleri Tebliği (2008) ve Mikrobiyolojik Kriterler Tebliği'ne (2011) göre yapılmıştır.

Tablo 1. Mikroorganizmalar için kullanılan besiyerleri ve inkübasyon koşulları

Table 1. Media Used for Microorganisms and Incubation Conditions

Mikroorganizma	Besiyeri/İnkübasyon koşulları
<i>C. sakazakii</i>	Buffered Peptone Water, 37°C'de 24 saat
	Enterobacteriaceae Enrichment broth, 37°C'de 24 saat
	Chrom Agar, 37°C'de 24 saat
	Violet Red Bile Dekstroz Agar, 37°C'de 24 saat
	Trypticase Soy Agar, 25°C'de 48 saat
<i>B. cereus</i>	<i>Bacillus cereus</i> Medium 30°C'de 48 saat
<i>S. aureus</i>	Baird Parker Agar 37°C'de 24-48 saat
Fekal koliform	Violet Red Bile Agar 44.5°C'de 24-48 saat
Koliform	Violet Red Bile Agar 37°C'de 24-48 saat
Küf-maya	Yeast Extract Glucose Chloramphenicol Agar / 28°C'de 3-5 gün
TAMCB*	Plate Count Agar / 37°C'de 48 saat

* Toplam Aerobik Mezofilik Canlı Bakteri

Bulgular ve Tartışma

Bebeklerin gelişmemiş bağışıklık sistemleri nedeniyle bebek mamalarında gıda güvenliğinin sağlanması önem arz etmektedir. Toz bebek mamalarındaki potansiyel enfeksiyon riski mamaların yanlış koşullarda hazırlanması, kullanılması veya saklanması gibi uygulamalar sonucu artabilmektedir (Martin vd., 2016). Bebek mamalarındaki bu riski en aza indirmek için DSÖ tarafından “Toz bebek mamalarının güvenli hazırlanması, depolanması ve kullanımı yönergeleri” geliştirilmiştir (World Health Organization, 2007). Bu çalışmada 24 adet toz bebek mamasının etiketlerindeki mama hazırlama beyanları, DSÖ yönergesi önerilerine göre değerlendirilmiş ve sonuçlar Tablo 2’de verilmiştir. Mama firmaları tarafından önerilen hazırlama talimatlarının DSÖ tavsiyelerine uygunluğuna bakıldığında incelenen örneklerin hiçbirinde DSÖ tavsiyelerinin tamamına yer verilmediği saptanmıştır. Mamaların yarısında (%50) mama hazırlamadan önce ellerin su ve sabunla yıkanması önerilirken, tamamında (%100) suyun kaynatılması gerektiği belirtilmiştir. Mama hazırlama suyunun soğutulma sıcaklığı genellikle 35-50 °C arasında ifade edilmiş olup >70 °C’ye kadar soğutulmasını belirten hiçbir mama firması olmamıştır. *C. sakazakii*’nin kontrolünde su sı-

caklığının >70°C olması kritik noktalardan biri olup bu araştırmada incelenen mama firmalarının belirttiği mama suyunu soğutma sıcaklığı (35-50°C) *C. sakazakii* riskini hiçbir şekilde azaltmamaktadır (World Health Organization, 2007). Benzer şekilde Sani vd. (2013) tarafından yapılan çalışmada da mamaların hazırlama talimatları DSÖ yönergesine uygunluğu açısından incelenmiş olup mamaların kapsamlı hazırlama önerileri olmasına rağmen hiçbirinde su hazırlama sıcaklığının >70°C olması gerektiği önerisinin bulunmadığı bildirilmiştir. Bu çalışmada incelenen mamaların hepsinde “etikette bildirilen miktarda mama ekleyin ve karıştırın” önerisine rastlanırken hiçbirinde “hazırlamış olduğunuz mamayı akan suyun altında tutarak veya buz dolu bir kaba koyarak hızlı bir şekilde besleme sıcaklığına soğutun” önerisine yer verilmemiştir. Ayrıca mama etiketlerinin hepsinde mamaların her seferinde taze hazırlanması ve artan formülün kullanılmaması gerektiği belirtilmiştir. Bebek mamalarında mama hazırlama, kullanma ve saklama önerilerine doğru, basit, anlaşılır ve uygulaması kolay şekilde yer verilmesi patojen mikroorganizmaların çoğalmasını önlemek açısından önemlidir (Crawley, 2020). Bebeklerde mama kaynaklı olası enfeksiyonları önlemek ve azaltmak için yasalar tarafından toz bebek mamalarının ürün etiketlerinin düzenlenmesi ve kontrol edilmesi gerekmektedir (Parra-Flores vd., 2015).

Tablo 2. Toz Bebek Mamalarının DSÖ Mama Hazırlama Önerilerine Uygunluğunun Değerlendirilmesi

Table 2. Evaluation of the Compliance of the Powdered Infant Formulas with the WHO Formula Preparation Guidelines

DSÖ toz mama hazırlama önerileri	Uygun		Uygun Değil		Bilgisi Yok	
	Sayı	%	Sayı	%	Sayı	%
Mamanın hazırlanacağı yüzeyi temizleyin ve dezenfekte edin.	2	8.3	0	0	22	91.7
Ellerinizi su ve sabunla yıkayıp tek kullanımlık peçete ile kurulayın.	12	50	0	0	12	50
Yeterli miktarda su kaynatın.	24	100	0	0	0	0
Uygun miktarda kaynatılmış suyu soğutun (>70°C) sterilize edilmiş besleme kabına veya biberona alın.	0	0	24	100	0	0
Etikette bildirilen miktarda mama ekleyin ve iyice karıştırın.	24	100	0	0	0	0
Hazırlamış olduğunuz mamayı akan suyun altında tutarak veya buz dolu bir kaba koyarak hızlı bir şekilde besleme sıcaklığına soğutun.	0	0	0	0	24	100
Besleme kabının veya biberonun dışını tek kullanımlık peçete ile kurulayın.	2	8.3	0	0	22	91.7
Mama, sıcak su ile hazırlandığından bebeğin ağzının yanmaması için bebeğinizi beslemeden önce mama sıcaklığını kontrol edin, gerekirse soğutmaya devam edin.	19	79.2	0	0	5	20.8
İki saat içinde tüketilmeyen mamayı kullanmayın.	24	100	0	0	0	0

DSÖ: Dünya Sağlık Örgütü

Toz bebek mamaları, *C. sakazakii* enfeksiyonları nedeniyle bebeklerde ciddi hastalıklar ve ölüm ile ilişkilendirilmektedir (Parra-Flores vd., 2015). Amerika Birleşik Devletleri Hastalık Kontrol ve Korunma Merkezleri tarafından yayımlanan “Besin güvenliği ve erken bebeklik döneminde *Cronobacter* enfeksiyonları” başlıklı raporda 1961-2018 yılları arasındaki *Cronobacter* enfeksiyonları incelenmiştir. Rapora göre enfeksiyon geçiren bebeklerin çoğunun yenidoğan (%67) olduğu, enfeksiyon geçirenlerin %38’inin öldüğü ve bu vakaların %79’unun yakın zamanda toz bebek maması tükettiği bildirilmiştir. Rapora göre çalışmanın son çeyreğinde bildirilen vaka oranlarında ciddi artışların olduğu, önceki yıllarda bildirilen vakaların genellikle hastanede yatan ve prematüre bebeklerde görüldüğü ancak son yıllarda hastanede yatmayan ve zamanında doğan bebeklerde arasında da prevelansın artış gösterdiği saptanmıştır (Strysko vd., 2020).

Birleşmiş Milletler Gıda ve Tarım Örgütü ile DSÖ tarafından toz bebek mamalarında bulunabilen ve bebeklerde enfeksiyona neden olabilen mikroorganizmalar, oluşturdukları risklere göre sınıflandırılmış ve *C. sakazakii* en yüksek risk grubu olan A sınıfında gösterilmiştir (World Health Organization, 2004). Türk Gıda Kodeksi Mikrobiyolojik Kriterler Yönetmeliği (2011)’ne göre bebek maması formülleri ve devam formüllerinde (özel tıbbi amaçlı diyet gıdalar dahil) 25 g örnekte *Cronobacter* spp. bulunmamalıdır. Türkiye’de yapılan meta analizde bebek mamalarında *C. sakazaki* prevelansı 0.01 (0.00-0.01) olarak saptanmıştır (Al vd., 2020). Bu çalışmada incelenen sütlü tahıllı ek gıdaların 2’sinde *C. sakazakii* saptanmıştır (Tablo 3). Çalışmada mama paketleri ilk defa açılıp analiz edildiği için bu durumun bakterilerin pastörizasyona dirençli olmasından, pastörize edilmeden ilave edilen gıda katkılarından veya hazırlama aşamasındaki kontaminasyondan kaynaklanabileceği düşünülmüştür (Kalyantanda vd., 2015).

B. cereus, FAO/DSÖ tarafından bebek maması kontaminasyonu ile ilişkili düşük riskli bakteri sınıflandırmasında (C grubu) yer almasına rağmen (World Health Organization, 2004) bebek mamalarındaki prevelansı gıda kaynaklı enfeksiyon salgınlarına neden olacak kadar yüksektir (Pei vd., 2018). Çin’de 6656 toz mamanın incelendiği araştırmada örneklerin %7.53’ünün *B. cereus* ile kontamine (≥ 10 kob/g) ve %1.1’inde bu düzeyin 100 kob/g üzerinde olduğu saptanmıştır (Pei vd., 2018). Mamalarda *B. cereus* kontaminasyon sıklığı Kore’de %20.9 (Kim vd., 2011), Libya’da %64.3 (Shadlia-Matug vd., 2008) olarak bildirilmiştir. Türkiye’de yapılan çalışmalarda ise bebek mamalarının *B. cereus* ile kontaminasyon oranı %10 (Sezer vd., 2015), %7 (Bahçeci vd., 2018) ve %6.3 (Var vd., 2021) olarak bulunmuştur. Bu çalışmada 5

adet toz bebek maması ve 4 adet sütlü tahıllı ek gıda olmak üzere örneklerin 9 tanesinde (%25) $1.0 \times 10^3 - 3.0 \times 10^3$ kob/g düzeyleri arasında *B. cereus* tespit edilmiştir (Tablo 3). Bu düzey Türk Gıda Kodeksi Mikrobiyolojik Kriterler Yönetmeliği (2011) tarafından belirlenen bebek mamalarında tolere edilebilir *B. cereus* miktarlarının ($5 \times 10^1 - 5 \times 10^2$ kob/g) üzerindedir. Toz mamalarda *B. cereus* sporları sıklıkla bulunabilmekte ve uygun koşullarda gelişebilmektedir. Mamanın uygun olmayan koşullarda hazırlanması, soğutulması ve saklanması bu sporlarının çoğalmasına ve toksin oluşumuna neden olabilmektedir (Sadek vd., 2018).

Bebek mamaları sahip oldukları pH, su aktivitesi, yapıları ve besin bileşimleri (protein ve nişasta) nedeniyle *S. aureus*’un gelişmesi ve stafilokok enterotoksinlerinin oluşması için potansiyel risk içermektedir (Genç vd., 2021). Bebek mamaları ve devam sütleri ile yapılan çalışmaların bazılarında (Var vd., 2021; Ergün vd., 2002) Türk Gıda Kodeksi Bebek Formülleri Tebliği (2008)’ne uygun şekilde *S. aureus* tespit edilmemiştir. Ancak bu çalışmada toz mamaların 1’inde ve sütlü tahıllı ek gıdaların 3’ünde olmak üzere toplam 4 mamada (%11.1) $2.0 \times 10^2 - 7.0 \times 10^2$ kob/g düzeyinde *S. aureus* saptanmıştır (Tablo 3). Benzer şekilde Wang vd. (2012) inceledikleri toz bebek mamalarının %11.2’sinde *S. aureus* tespit etmişlerdir. *S. aureus* genellikle insanlardan gıdalara bulaşmakta, gıda üretimindeki hammadde ve/veya üretim, hazırlama aşamasındaki kontaminasyonlar mamalarda *S. aureus* gelişimine neden olabilmektedir (Genç vd., 2021).

Bebek mamalarının mikrobiyolojik kalitesini gösteren en önemli parametrelerden biri koliform grubu bakterilerdir (Buchanan ve Oni, 2012). Bebek mamalarında yapılan araştırmalarda %12.9 (Tokatli-Demirok ve Arıcı, 2019), %22 (Sezer vd., 2015) ve %25 (Ergün vd., 2002) oranında koliform bakteri tespit edilmiştir. Benzer şekilde bu çalışmada da 36 örneğinin 10 tanesinde (% 27.7) $1.0 \times 10^1 - 3.0 \times 10^3$ kob/g düzeyinde koliform bakteri, 8 tanesinde (%22.2) $2.0 \times 10^1 - 2.04 \times 10^3$ kob/g düzeyinde fekal koliform bakteri varlığına rastlanılmıştır (Tablo 3). Türk Gıda Kodeksi Mikrobiyolojik Kriterler Yönetmeliği (2011)’ne göre Enterobacteriaceae familyası içindeki bakteriler mamalarda hiç bulunmamalıdır. Bu familyanın içerisinde koliform, fekal koliform ve *Enterobacter sakazakii* (*C. sakazakii*) yer almaktadır. Mamalarda koliform ve fekal koliform bakterilerin varlığı fekal kökenli kontaminasyon, mamanın elde edildiği hammadde-nin özellikle de sütlerin hijyenik kalitelerinin düşük olması, pastörizasyon uygulamalarının yetersizliği ve pişirme ve pastörizasyon sonrası kontaminasyon ile ilişkilendirilmektedir (Buchanan ve Oni, 2012).

Tablo 3. Mama Örneklerinin Mikrobiyolojik Analiz Sonuçları (kob/g)**Table 3.** Results of Formula Samples' Microbiological Analysis (kob/g)

Örnek No ve Çeşidi	<i>C. sakazakii</i>	<i>B. cereus</i>	<i>S. aureus</i>	Koliform bakteri	Fekal koliform bakteri	Küf-maya	TAMC
1-Toz	-	<10 ²	<10 ²	<10 ¹	<10 ¹	<10 ¹	<10 ¹
2-Toz	-	<10 ²	<10 ²	<10 ¹	<10 ¹	<10 ¹	<10 ¹
3-Toz	-	<10 ²	<10 ²	<10 ¹	<10 ¹	<10 ¹	<10 ¹
4-Toz	-	<10 ²	<10 ²	<10 ¹	<10 ¹	<10 ¹	<10 ¹
5-Toz	-	1.0 x 10 ³	<10 ²	<10 ¹	<10 ¹	<10 ¹	<10 ¹
6-Toz	-	<10 ²	<10 ²	<10 ¹	<10 ¹	2.0 x 10 ¹	3.0 x 10 ¹
7-Toz	-	<10 ²	<10 ²	<10 ¹	<10 ¹	2.0 x 10 ¹	1.0 x 10 ²
8-Toz	-	<10 ²	<10 ²	<10 ¹	<10 ¹	3.0 x 10 ¹	5.0 x 10 ¹
9-Toz	-	<10 ²	<10 ²	<10 ¹	<10 ¹	2.3 x 10 ¹	1.1 x 10 ²
10-Toz	-	<10 ²	<10 ²	<10 ¹	<10 ¹	<10 ¹	<10 ¹
11-Toz	-	<10 ²	<10 ²	<10 ¹	<10 ¹	3.0 x 10 ¹	<10 ¹
12-Toz	-	1.0 x 10 ³	<10 ²	<10 ¹	<10 ¹	<10 ¹	<10 ¹
13-Toz	-	<10 ²	<10 ²	<10 ¹	<10 ¹	3.0 x 10 ¹	3.0 x 10 ¹
14-Toz	-	1.0 x 10 ³	<10 ²	<10 ¹	<10 ¹	<10 ¹	3.6 x 10 ²
15-Toz	-	3.0 x 10 ³	<10 ²	2.0 x 10 ¹	2.0 x 10 ¹	<10 ¹	3.8 x 10 ²
16-Toz	-	<10 ²	<10 ²	<10 ¹	<10 ¹	<10 ¹	3.7 x 10 ²
17-Toz	-	<10 ²	<10 ²	<10 ¹	<10 ¹	<10 ¹	1.13 x 10 ³
18-Toz	-	1.0 x 10 ³	<10 ²	<10 ¹	<10 ¹	<10 ¹	5.0 x 10 ¹
19-Toz	-	<10 ²	<10 ²	<10 ¹	<10 ¹	<10 ¹	4.5 x 10 ²
20-Toz	-	<10 ²	<10 ²	1.0 x 10 ¹	<10 ¹	<10 ¹	
21-Toz	-	<10 ²	<10 ²	<10 ¹	<10 ¹	<10 ¹	4.1 x 10 ²
22-Toz	-	<10 ²	<10 ²	3.0 x 10 ³	2.04 x 10 ³	<10 ¹	9.72 x 10 ³
23-Toz	-	<10 ²	3.3 x 10 ³	4.8 x 10 ²	9.0 x 10 ¹	<10 ¹	6.0 x 10 ²
24-Toz	-	<10 ²	<10 ²	5.0 x 10 ¹	3.0 x 10 ¹	<10 ¹	7.0 x 10 ¹
1-STEĞ*	-	2.0 x 10 ³	<10 ²	<10 ¹	<10 ¹	<10 ¹	7.6 x 10 ²
2-STEĞ	-	<10 ²	<10 ²	<10 ¹	<10 ¹	<10 ¹	<10 ¹
3-STEĞ	-	<10 ²	<10 ²	<10 ¹	<10 ¹	<10 ¹	9.0 x 10 ¹
4-STEĞ	-	<10 ²	<10 ²	<10 ¹	<10 ¹	<10 ¹	2.0 x 10 ¹
5-STEĞ	-	<10 ²	<10 ²	<10 ¹	<10 ¹	<10 ¹	<10 ¹
6-STEĞ	-	<10 ²	<10 ²	1.69 x 10 ³	1.38 x 10 ³	<10 ¹	1.74 x 10 ³
7-STEĞ	-	<10 ²	<10 ²	1.6 x 10 ²	1.3 x 10 ²	4.0 x 10 ¹	4.7 x 10 ²
8-STEĞ	+	<10 ²	<10 ²	1.43 x 10 ³	1.36 x 10 ³	2.0 x 10 ¹	1.5 x 10 ³
9-STEĞ	+	<10 ²	<10 ²	1.52 x 10 ³	1.26 x 10 ³	1.0 x 10 ¹	1.56 x 10 ³
10-STEĞ	-	1.0 x 10 ³	7.0 x 10 ²	<10 ¹	<10 ¹	<10 ¹	1.0 x 10 ¹
11-STEĞ	-	1.0 x 10 ³	5.0 x 10 ²	2.0 x 10 ¹	<10 ¹	<10 ¹	5.0 x 10 ¹
12-STEĞ	-	1.0 x 10 ³	2.0 x 10 ²	<10 ¹	<10 ¹	<10 ¹	5.0 x 10 ¹

*STEĞ: Sütlü Tahıllı Ek Gıda

Mamalarda bulunan küf-maya yükü yetersiz hijyen koşullarını göstermekte olup zararlı alerjenlere neden olarak sağlıklı olumsuz etkileyebilmektedir (Abdelreda ve Ajmi, 2016). Vural ve Genç (2022) bebek maması, devam formülleri ve bazı tamamlayıcı besinleri mikrobiyolojik kalite açısından değerlendirdikleri çalışmalarında örneklerin % 23.7'sinde küf ve %2.96'sında maya saptamışlardır. Heperkan vd. (2017) ise toz bebek maması ve devam formüllerinde küf ve mayaya rastlamamışlardır. Bu çalışmada toz mamaların 6'sı, sütlü tahıllı ek gıdaların 3'ü olmak üzere örneklerin 9 tanesinde (% 25) $1.0 \times 10^1 - 4.0 \times 10^1$ kob/g düzeyinde küf-maya tespit edilmiştir (Tablo 3). Tespit edilen bu düzey Türk Gıda Kodeksi Bebek Formülleri Tebliği (2008) tarafından belirlenmiş limit değerin (1.0×10^2 kob/g) altındadır. Mamalarda tespit edilen küf ve maya yükünün üretim aşamasında hammaddenin uygun koşullarda depolanmaması veya hazırlanma aşamasındaki kontaminasyon ile gelişmiş olabileceği düşünülmektedir. Bu nedenle üretimde ve mamaların hazırlanmasında hijyen kurallarına ve uygun koşullarda depolanmasına dikkat edilmelidir.

Toplam aerobik mezofilik canlı bakteri sayısı gıda maddelerinin mikrobiyolojik kalitesini gösteren önemli bir parametre olup bu değerin yüksek olması patojen bakteri varlığı veya gıdanın hijyenik durumu hakkında bilgi verebilmektedir (Buchanan ve Oni, 2012). Parra-Flores vd. (2020), 128 bebek mamasını 61'inde (%47.6) $>10^4$ kob/g aerob bakteri saptamıştır. Sezer vd. (2015), 50 adet bebek maması ve devam sütünde TAMCB sayısını ortalama 1.0×10^3 kob/g bulmuş ve 1 örnekte (%2) Türk Gıda Kodeksi kriterlerinin üzerinde TAMCB saptamıştır. Bu çalışmada ise toz mamaların 10'unda, sütlü tahıllı ek gıdaların 15'inde olmak üzere 25 mamada (%69.4) $2.0 \times 10^1 - 9.72 \times 10^3$ kob/g düzeyinde TAMCB saptanmış olup (Tablo 3) tespit edilen bu düzey Türk Gıda Kodeksi Bebek Formülleri Tebliği (2008) limit düzeyinin (1.0×10^4 kob/g) altındadır. Benzer şekilde Tokatlı-Demirok ve Arıcı (2019) çalışmasında da incelenen 62 bebek mamasının 54'ünde TAMCB saptamışlardır. Bebek mamaları ve devam formüllerinde toplam organizma sayısındaki en önemli değişiklikler, hazırlama ve tüketim arasındaki aşamada meydana gelmektedir. Bu nedenle annelere ve hastane çalışanlarına özel eğitim verilerek hazırlanan bebek mamalarının uygun koşullarda en kısa sürede tüketilmesinin sağlanması önemlidir (Sezer vd., 2015).

Bu çalışmada sınırlı sayıda toz bebek mamasının incelenmesi çalışmanın sınırlılığıdır. Gelecek çalışmalarda Türkiye piyasasında yer alan toz bebek mamaları ve ek gıdalar mikrobiyolojik ve DSÖ mama hazırlama talimatları açısından kapsamlı bir şekilde değerlendirilebilir. Bu kısıtlılığına rağmen bu çalışma, Türkiye piyasasında yer alan bebek maması

firmalarının neredeyse tamamına ait belirli örnekleri mikrobiyolojik kalite açısından incelemesi nedeniyle önem taşımaktadır. Ayrıca Türkiye piyasasındaki mama firmaları tarafından önerilen hazırlama talimatlarının DSÖ tavsiyelerine uygunluğunu değerlendiren herhangi bir çalışmaya literatürde rastlanmamış olup bu çalışmanın konu hakkında literatüre katkı sağlayacağı düşünülmektedir.

Sonuç

Bu çalışmada Türkiye'de satışa sunulan 10 farklı firmaya ait toplam 36 adet toz bebek maması ve toz sütlü tahıllı ek gıda mikrobiyolojik açıdan incelenmiş ve mama firmaları tarafından önerilen hazırlama talimatlarının DSÖ tavsiyelerine uygunluğu değerlendirilmiştir. Toplam 36 mama örneğinin iki tanesinde *C. sakazakii* tespit edilmiş olup dokuz tanesinde *B. cereus*, dört tanesinde *S. auerus*, 10 tanesinde koliform, sekiz tanesinde fekal koliform, dokuz tanesinde küf-maya ve 25 tanesinde TAMCB çeşitli düzeylerde tespit edilmiştir. Mama firmaları tarafından önerilen hazırlama talimatlarının DSÖ tavsiyelerine uygunluğuna bakıldığında ise incelenen örneklerin hiçbirinde DSÖ tavsiyelerinin tamamına yer verilmediği ve özellikle de *C. sakazakii* açısından kontrol edici kritik nokta olan suyun hazırlama sıcaklığının $>70^\circ\text{C}$ olması önerisinin belirtilmediği görülmüştür.

Toz bebek mamalarındaki olası patojenlerin üretimdeki kontrolünün yanı sıra hazırlama, kullanma ve depolama sırasındaki kontaminasyonları azaltmak için uluslararası kuruluşların mama hazırlama, kullanma ve depolama önerilerine uyulması son derece önemlidir. Toz bebek mamalarının ürün etiketlerinin düzenlenme kurallarının ülkelere özgü mevcut çalışmalar göz önüne alınarak güncellenmesi ve sürecin takip etmesinin toz bebek maması kaynaklı enfeksiyonların azaltılmasında etkili olabileceği düşünülmektedir.

Etik Standartlar ile Uyumluluk

Çıkar çatışması: Yazarlar bu yazı için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan etmişlerdir.

Etik izin: Araştırma niteliği bakımından etik izne tabii değildir.

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Sous vide ve geleneksel pişirme yöntemlerinin kabak ve yeşil fasulyenin renk özellikleri ve antioksidan kapasitesine etkisinin karşılaştırılması

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ÖZ

Pişirme işleminin sebzelerin fiziksel ve kimyasal özelliklerinde değişimlere yol açtığı bilinmektedir. Yeşil fasulye ve kabak Türk mutfağında yaygın bir kullanım alanına sahiptir. Bu çalışmanın amacı Sous vide (SV) ile geleneksel pişirme (haşlama ve buharda) yöntemlerinin yeşil fasulye ve kabağın renk özellikleri ve antioksidan kapasitesine etkilerinin karşılaştırılmasıdır. Taze ve 3 farklı pişirme yöntemi uygulanmış sebze örneklerinin renk özellikleri (L*, a* ve b*), toplam fenolik madde miktarı (TFM), 2,2-difenil-1-pikrilhidrazil (DPPH) radikal temizleme aktivitesi ve demir (III) iyonu indirgeme antioksidan gücü belirlenmiştir. Pişirme işlemi sonrası sebzelerin parlaklık değerlerinde azalma ve kırmızılık-yeşillik değerlerinde artışlar gözlenmiştir. Sebzelerin TFM içeriğinin en iyi korunduğu pişirme yönteminin SV yöntemi olduğu belirlenmiştir. Pişirme yöntemlerinin antioksidan kapasiteye etkisinin sebze çeşidine göre farklılıklar gösterdiği de belirlenmiştir. Kabak örnekleri için haşlama yönteminde yeşil fasulye örnekleri için buharda pişirme yönteminde en yüksek antioksidan kapasite değeri elde edilmiştir.

Anahtar Kelimeler: Antioksidanlar, Pişirme yöntemleri, Sous vide, Sebzeler

ABSTRACT

Comparison of the effect of sous vide with traditional cooking methods on color properties and antioxidant capacity of zucchini and green bean

Cooking process is known to cause changes in the physical and chemical properties of vegetables. Green beans and zucchini are widely used in Turkish cuisine. The aim of this study was to compare the effects of Sous vide (SV) with traditional cooking (boiling and steaming) methods on the color properties and antioxidant capacity of green beans and zucchini. Color characteristics (L*, a* and b*), total phenolics content (TPC), 2,2-diphenyl-1-picrylhydrazil (DPPH) radical scavenging activity and iron (III) ion reduction antioxidant power of fresh and cooked vegetables by 3 different cooking methods were determined. After the cooking process, a decrease in the lightness values and an increase in the redness-greenness values were observed. It was determined that the TPC of the vegetables was best preserved in the SV method. The effects of cooking methods on the antioxidant capacity were determined to differ according to the vegetable variety. The highest antioxidant capacity value was obtained for the zucchini samples in the boiling method and for the green bean samples in the steaming method.

Keywords: Antioxidants, Cooking methods, Sous vide, Vegetables

Giriş

Epidemiyolojik çalışmalar, meyve ve sebze tüketimi ile kardiyovasküler rahatsızlıklar, kanser ve yaşlanma arasında negatif bir ilişki olduğunu göstermiştir. Meyve ve sebze tüketiminin sağlık üzerine olumlu etkileri bu ürünlerin C vitamini, E vitamini, karotenoidler ve fenolik maddeler gibi antioksidan özellik gösteren bileşenler içermesi ile ilişkilendirilmektedir (Kaur ve Kapoor, 2001). Sebzelerin antioksidan madde içeriği, sebzelerin genetik özelliklerine, yetiştirilme koşullarına, hasat sonrası dağıtım ve depolanma koşullarına ve sebzelerin işlenmesi esnasında uygulanan işlemlere bağlı olarak değişmektedir (Danesi ve Bordoni, 2008). Sebzeler genellikle pişirilerek tüketilmektedir. Pişirme işleminin sebzelerin kimyasal bileşiminde değişimlere yol açarak biyoaktif bileşenlerin miktarını ve biyoyararını etkilediği bilinmektedir (Miglio vd., 2008).

Haşlama ve buharda pişirme en yaygın kullanılan geleneksel pişirme yöntemleridir. Geleneksel pişirme yöntemleri besin öğeleri ve lezzet öğelerinde kayba yol açabilmektedir (dos Reis vd., 2015). Bu kayıpları en aza indirmek için yeni pişirme yöntemleri üzerinde çalışılmaktadır. Sous vide (SV) (vakum altında pişirme) yeni pişirme yöntemlerinden birisidir. Bu yöntemde gıdalar vakum altında paketlenir, vakumlanmış paketteki gıdalar kontrollü koşullarda (sıcaklık ve süre) pişirilir (Baltalı ve Akoğlan Kozak, 2017). Yüksek sıcaklık nedeni ile yapısı bozulan veya oksidasyonla kayba uğrayan besin öğelerinin kontrollü ısı işlem uygulanan SV pişirme tekniğinde daha az kayba uğradığı düşünülmektedir (Coşansu ve Kıymetli, 2016).

Literatürde yer alan çalışmaların bir kısmında pişirme işlemi sonrası antioksidan kapasitede artış bir kısmında ise antioksidan kapasitede azalma meydana geldiği rapor edilmiştir (Ferracane vd., 2008; Jiménez-Monreal vd., 2009; Lafarga, vd., 2018; Miglio vd., 2008; Wachtel-Galor vd., 2008). Pişirme işleminin sebzelerinin antioksidan kapasitesine etkisinin sebzelerin morfolojik özellikleri ve besinsel kompozisyonu ve pişirme yöntemi koşullarına bağlı olarak değişiklik gösterdiği düşünülmektedir.

Yeşil fasulye ve kabak Türk mutfak kültüründe yaygın kullanım alanına sahiptir. Antioksidan maddeler açısından zengin olan bu sebzelerin pişirilmesinde yaygın olarak haşlama ve buharda pişirme yöntemleri kullanılmaktadır. Literatürde pişirme yöntemlerinin sebzelerinin antioksidan kapasitesine etkisinin incelendiği çok sayıda çalışma bulunmakla birlikte SV pişirme yönteminin etkisini inceleyen özellikle yeşil fasulye ve kabak için sınırlı sayıda çalışmaya ulaşılmaktadır. Bu çalışmada SV pişirme yöntemi ile geleneksel pişirme yön-

temlerinden haşlama ve buharda pişirme işlemlerinin sebzelerin toplam fenolik madde içeriği ve antioksidan kapasitesine etkilerini kıyaslamak için sebzelerin toplam fenolik madde miktarı ve antioksidan kapasitesi belirlenmiştir. Ayrıca sebzelerin renk özellikleri de belirlenmiştir.

Materyal ve Metot

Sebzeler

Sebzeler Trabzon'da yerel bir pazardan temin edilmiştir. Sebzeler 4°C'de muhafaza edilmiş ve 24 saat içinde hazırlama işlemine başlanmıştır.

Sebzelerin Pişirme İşlemi için Hazırlanması

Sebzeler bol su ile yıkamanın ardından, ayıklanmış ve kurulanmıştır. Homojen iriliğe sahip olması için kabaklar yaklaşık 1 cm kalınlığında ve yeşil fasulyeler ise 4-5 cm uzunluğunda doğranarak 100 g olarak hassas terazide tartılmıştır. Pişirme işlemleri 3 tekrarlı uygulanmıştır.

Pişirme Yöntemleri

Çalışmada haşlama, buharda pişirme ve Sous vide yöntemleri uygulanmıştır.

Yapılan çalışmada; haşlama ve buharda pişirme yöntemlerinde pişirme süresinin belirlenmesi, Sous vide pişirme yönteminde ise sıcaklık ve sürenin belirlenmesi amacıyla ön denemeler yapılmıştır.

Haşlama yönteminde; hassas terazide 100 g örnek tartılmış ve içerisinde 500 mL kaynamış su bulunan tencereye alınan sebzeler pişirilmiştir. Kabaklar için 15 dakika ve fasulyeler için 20 dakika pişirme süresi uygulanmıştır.

Buharda pişirme yönteminde; hassas terazide 100 g örnek tartılmış ve sebzeler 'Fakir Hausgerate' buharlı pişirme cihazına yerleştirilmiştir. Kabaklar için 25 dakika ve fasulyeler için 20 dakika pişirme süresi uygulanmıştır.

Sous vide pişirme yönteminde; hassas terazide 100 g örnek tartılmış ve sebzelere vakum altında ambalajlama işlemi uygulanmıştır. Sıcak su banyosunda kabaklar 90°C - 45 dk ve fasulyeler 90°C - 60 dk pişirilmiştir.

Fenolik Madde Ekstraksiyonu

Fenolik madde ekstraksiyonunda % 80'lik metanol kullanılmıştır (Kosewski vd., 2018). Homojenize edilmiş örnekten 0.5 gram tartılmış, 5 mL çözücü eklenmiş ve 30 dakika ultra-

sonik banyoda ekstraksiyon işlemi uygulanmıştır. Ekstraksiyon işlemi sonrası numune 5 dakika 4000 rpm'de santrifüj edilmiş ve üst faz alma işlemi gerçekleştirilmiştir. Üst faz alındıktan sonra kalıntıya 5 mL çözücü eklenip ekstraksiyon ve santrifüj işlemleri tekrar edilmiş ve süzme işlemi sonrası süzöntü hacmi 10 mL'ye tamamlanmıştır. Ekstraktlar - 18°C'de muhafaza edilmiştir.

Toplam Fenolik Madde Analizi

Toplam fenolik madde analizi Folin Ciocalteu yöntemine göre gerçekleştirilmiştir. 0.1 mL ekstrakt üzerine 0.5 mL folin reaktifi (1:10 suyla seyreltilmiş) eklenerek 5 dakika karanlık ortamda bekletildikten sonra 0.4 mL sodyum karbonat (1 M) çözelti üzerine eklenmiştir. Ardından çözeltiliye 3.6 mL su ilave edilerek karıştırıldıktan sonra bir saat karanlık ortamda bekletilmiştir ve UV-VIS spektrofotometre ile (Shimadzu UV-1800, Japonya) 760 nm'de absorbans okuması gerçekleştirilmiştir. Kalibrasyon grafiği gallik asit (10-20-40-60-80-100-125 mg/L) ile hazırlanmıştır.

DPPH Radikal Temizleme Aktivitesi

Antioksidan aktivenin belirlenmesinde DPPH radikal temizleme yöntemi kullanılmıştır. Ekstrakt (1 mL) deney tüpüne alınmış ve üzerine DPPH reaktifi (60 µM, 1 mL) ilave edilmiştir. Deney tüpleri karıştırılmış ve karanlıkta 1 saat bekletilmiştir. UV-VIS spektrofotometre ile (Shimadzu UV-1800, Japonya) 515 nm'de absorbans ölçülmüştür. Kalibrasyon grafiği Troloks (6.25-12.5-25-50-100-150 µM) ile hazırlanmıştır.

FRAP Demir (III) İyonu İndirgeme Antioksidan Gücü

FRAP demir (III) iyonu indirgeme antioksidan gücü yöntemi kullanılmıştır. FRAP reaktifi sodyum asetat tamponu (pH=3.6), TPTZ (10 mM) ve demir-3-klorür (20 mM) 10:1:1 oranında karıştırılmasıyla hazırlanmıştır. Ekstrakt (0.5 mL) deney tüpüne alınmış ve üzerine FRAP reaktifi (1.5 mL) ilave edilmiştir. Tüpler tüp karıştırıcıda karıştırılmıştır. Tüpler 20 dakika bekletilerek UV-VIS spektrofotometre ile (Shimadzu UV-1800, Japonya) absorbansları 593 nm'de okutulmuştur. Kalibrasyon grafiği Troloks (3.125-6.25-12.5-25-50-100 µM) ile hazırlanmıştır.

Renk Tayini

Kabak ve yeşil fasulye örneklerinde renk ölçümü, her tekrardan alınan örneklerin üzerindeki üç farklı noktadan ölçüm yapılarak gerçekleştirilmiştir. Renk tayin cihazı (Konica Minolta CR-400, Japonya) ile L*, a* ve b* değerleri tespit edilmiştir.

İstatistiksel Analiz

Araştırma sonuçlarının değerlendirilmesinde SPSS 22.0 (IBM) programı kullanılmıştır. Sonuçlar ortalama ± standart sapma olarak hesaplanmıştır. Elde edilen bulgulara tek yöllü varyans analizi uygulanmıştır. Pişirme işlemleri arasındaki farklılıkları belirlemek için Duncan testi uygulanmıştır. Örnekler arasındaki farklılıklar % 95 güven aralığında verilmiştir.

Bulgular ve Tartışma

Üç farklı pişirme yönteminin kabak ve yeşil fasulyenin renk özellikleri, toplam fenolik madde miktarı ve antioksidan kapasitesi üzerine etkileri incelenmiştir.

Renk Analizi

Renk analizi sonuçları Tablo 1'de verilmiştir. Pişirme yöntemlerinin L* değeri üzerine istatistiksel olarak önemli ve negatif bir etkisinin olduğu tespit edilmiştir (p<0.05). Pişirme yöntemlerine göre fasulye örneklerinin L* değerleri arasında istatistiksel olarak önemli bir fark bulunmamıştır (p>0.05). Kabak örneklerinde ise haşlama yönteminin L* değerinde en fazla azalmaya neden olan yöntem olduğu belirlenmiştir. Kabak örneklerinin a* değeri üzerine pişirme yöntemlerinin istatistiksel olarak önemli bir etkisinin olmadığı tespit edilirken (p>0.05) fasulye örneklerinin a* değeri üzerine istatistiksel olarak önemli ve pozitif bir etkisinin olduğu tespit edilmiştir (p<0.05). Pişirme yöntemlerinin sebzelerin b* değeri üzerine istatistiksel olarak önemli bir etkisinin olmadığı belirlenmiştir.

Tüketici tercihini etkileyen önemli kalite parametrelerinden birisi gıdaların renk özellikleridir. Pişirme işlemi sonrası sebzelerin renk özelliklerinde değişimler meydana geldiği belirlenmiştir. Sebzelerin parlaklık değerinde azalma gözlenirken kırmızılık-yeşillik değerinde artış gözlenmiştir. Literatürdeki çalışmalarda da benzer sonuçlar elde edilmiştir. Yeşil fasulye ile yapılan bir çalışmada haşlama ve SV pişirme sonrası (Guillén vd., 2017) ve kabak ile yapılan bir çalışmada ise haşlama ve buharda pişirme işlemi sonrası (Miglio vd., 2008) L* değerinde azalma ve a* değerinde artış tespit edilmiştir. a* değerinde meydana gelen değişim sebzelere yeşil rengini veren klorofil pigmentinin parçalanması ile ilişkilendirilmiştir.

SV pişirme yöntemi geleneksel yöntemlerle kıyaslandığında kabak örneği için rengin haşlama yöntemine göre daha iyi korunduğu fasulye örneği için ise önemli bir farklılık görüldüğü yorumu yapılabilir.

Tablo 1. Renk analizi sonuçları**Table 1.** Results of color analysis

L*		
Yöntem	Kabak	Fasulye
Taze	75.86±2.14 ^a	59.27±0.78 ^a
Haşlama yöntemi	52.20±1.04 ^d	47.57±1.53 ^b
Buharda pişirme yöntemi	61.38±0.42 ^b	47.20±2.60 ^b
Sous vide yöntemi	56.00±1.01 ^c	46.14±2.52 ^b
a*		
Yöntem	Kabak	Fasulye
Taze	-8.04±0.43 ^a	-13.09±0.29 ^d
Haşlama yöntemi	-7.02±0.93 ^a	-7.92±1.12 ^c
Buharda pişirme yöntemi	-7.09±0.67 ^a	-4.82±0.96 ^b
Sous vide yöntemi	-6.94±0.39 ^a	-2.66±0.49 ^a
b*		
Yöntem	Kabak	Fasulye
Taze	27.61±1.76 ^{ab}	21.96±0.99 ^a
Haşlama yöntemi	24.30±3.10 ^b	22.45±2.78 ^a
Buharda pişirme yöntemi	29.90±1.61 ^a	21.82±1.48 ^a
Sous vide yöntemi	27.02±0.94 ^{ab}	20.34±1.38 ^a

Aynı sütün içinde farklı harflerle gösterilen değerler arasında Duncan çoklu karşılaştırma test sonuçlarına göre istatistiksel olarak önemli fark vardır ($p<0.05$)

Toplam Fenolik Madde Analizi

Toplam fenolik madde analiz sonuçları Tablo 2’de verilmiştir. Haşlama ve SV pişirme yöntemlerinin kabak örneklerinin toplam fenolik madde miktarı üzerine etkisi istatistiksel olarak önemsiz bulunurken ($p>0.05$) buharda pişirme yönteminin istatistiksel olarak önemli ve negatif etkisinin bulunduğu tespit edilmiştir ($p<0.05$). Fasulye örneklerinde ise haşlama yönteminin istatistiksel olarak önemli ve negatif etkisi tespit edilirken ($p>0.05$) diğer iki yöntemin etkisinin istatistiksel olarak önemsiz olduğu belirlenmiştir ($p<0.05$).

Bu çalışmada pişirme işlemi uygulaması sonrasında sebze örneklerinin toplam fenolik madde miktarında azalma meydana geldiği gözlenmiştir. Turkmen vd. (2005) tarafından yapılan bir çalışmada biber, kabak, yeşil fasulye, bezelye, brokoli ve pırasa örneklerine haşlama ve buharda pişirme yöntemleri uygulanmış olup biber ve yeşil fasulye örneklerinde haşlama işlemi sonrası artış tespit edilirken bezelye, kabak, brokoli ve pırasa örneklerinde azalma tespit edilmiştir. Buharda pişirme yönteminde ise yeşil fasulye ve brokoli örneklerinde artış gözlenirken kabak, bezelye ve pırasa örneklerinde azalma olduğu saptanmıştır. Fenolik madde miktarında azalma ısı işlem etkisi ile fenolik maddelerinin parçalanmasına ve fenolik

madde miktarında artış ise ısı işlem ile bağlı formdaki fenolik maddelerin serbest hale geçmesi ile ilişkilendirilmektedir. Bu çalışmada elde edilen sonuçlar fenolik maddelerin en iyi korunduğu pişirme yönteminin SV yöntemi olduğunu göstermiştir. SV yönteminde diğer pişirme yöntemlerine göre daha düşük sıcaklık (<100 °C) uygulanmaktadır.

Tablo 2. Toplam fenolik madde analiz sonuçları (mg/100g km).**Table 2.** Results of total phenolic content analysis (mg/100 g dm)

Yöntem	Kabak	Fasulye
Taze	762.71±194.21 ^a	369.34±11.40 ^a
Haşlama yöntemi	618.07±10.64 ^{ab}	321.58±27.26 ^b
Buharda pişirme yöntemi	533.56±59.90 ^b	327.99±21.39 ^{ab}
Sous vide yöntemi	668.32±71.15 ^{ab}	356.27±22.64 ^{ab}

Aynı sütündeki farklı harflerle gösterilen değerler arasında Duncan çoklu karşılaştırma test sonuçlarına göre istatistiksel olarak önemli fark vardır ($p<0.05$). km: kuru madde

Antioksidan Kapasite

DPPH radikal temizleme aktivitesi analizi sonuçları Tablo 3’de verilmiştir. Kabak örneklerinde haşlama ve SV pişirme yöntemlerinin etkisinin istatistiksel olarak önemli ve pozitif olduğu tespit edilirken ($p<0.05$) buharda pişirme yönteminin etkisinin istatistiksel olarak önemsiz olduğu tespit edilmiştir ($p>0.05$). Fasulye örneklerinde haşlama ve SV pişirme yöntemlerinin etkisinin istatistiksel olarak önemli ve negatif olduğu tespit edilirken ($p<0.05$) buharda pişirme yönteminin etkisinin istatistiksel olarak önemsiz olduğu tespit edilmiştir ($p>0.05$).

FRAP analiz sonuçları Tablo 4’de verilmiştir. Kabak örneklerinde haşlama ve SV pişirme yöntemlerinin etkisinin istatistiksel olarak önemli ve pozitif olduğu tespit edilirken ($p<0.05$) buharda pişirme yönteminin etkisinin istatistiksel olarak önemsiz olduğu tespit edilmiştir ($p>0.05$). Fasulye örneklerinde üç yönteminin de istatistiksel olarak önemli ve negatif bir etkisinin olduğu tespit edilmiştir ($p<0.05$).

Bu çalışmada pişirme işlemleri sonrası kabak örneklerinin antioksidan kapasitesinde çoğunlukla artış gözlenirken fasulye örneklerinin antioksidan kapasitesinde çoğunlukla azalma gözlenmiştir. Pişirme yöntemlerinin sebzelerin antioksidan kapasitesine etkisini inceleyen çalışmaların bir bölümünde antioksidan kapasitede artış bir bölümünde ise azalma olduğu rapor edilmiştir. Turkmen vd. (2005) tarafından yapılan bir çalışmada haşlama ve buharda pişirme işlemleri sonrası biber, kabak, yeşil fasulye, brokoli ve ıspanak örneklerinde DPPH radikal temizleme aktivitesinde artış ve bezelye örneğinde ise azalma meydana geldiği rapor edilmiştir. Antioksidan aktivitedeki artış sebzelerde bağlı formdaki antioksi-

dan maddelerin ısı etkisi ile serbest forma geçmesiyle ilişkilendirilmiştir. Isı etkisiyle meydana gelen nem kaybının da bu artışta etkisi olabileceği ifade edilmiştir (Lafarga vd., 2018b). Antioksidan aktivitedeki azalma sebzelerdeki antioksidan özellik gösteren maddelerin ısı etkisi ile parçalanması ile ilişkilendirilmektedir.

Tablo 3. DPPH analiz sonuçları ($\mu\text{mol}/100\text{g km}$)

Table 3. Results of DPPH analysis ($\mu\text{mol}/100\text{g dm}$)

Yöntem	Kabak	Fasulye
Taze	524.51 \pm 116.32 ^b	915.20 \pm 123.54 ^a
Haşlama yöntemi	1115.30 \pm 179.75 ^a	613.92 \pm 54.23 ^b
Buharda pişirme yöntemi	368.53 \pm 58.27 ^b	822.17 \pm 116.11 ^a
Sous vide yöntemi	893.36 \pm 219.71 ^a	450.63 \pm 40.17 ^b

Aynı sütündeki farklı harflerle gösterilen değerler arasında Duncan çoklu karşılaştırma test sonuçlarına göre istatistiksel olarak önemli fark vardır ($p<0.05$). km: kuru madde

Tablo 4. FRAP analiz sonuçları ($\mu\text{mol}/100\text{g km}$)

Table 4. Results of FRAP analysis ($\mu\text{mol}/100\text{g dm}$)

Yöntem	Kabak	Fasulye
Taze	417.79 \pm 127.02 ^b	1117.40 \pm 107.81 ^a
Haşlama yöntemi	1223.07 \pm 139.56 ^a	745.46 \pm 71.85 ^b
Buharda pişirme yöntemi	648.30 \pm 75.85 ^b	783.09 \pm 133.15 ^b
Sous vide yöntemi	952.64 \pm 215.55 ^a	688.02 \pm 117.15 ^b

Aynı sütündeki farklı harflerle gösterilen değerler arasında Duncan çoklu karşılaştırma test sonuçlarına göre istatistiksel olarak önemli fark vardır ($p<0.05$). km: kuru madde

Pişirme işlemlerinin sebzelerin antioksidan kapasitesi üzerine etkisi inceleyen çalışmalarda alınan farklı sonuçlar sebze çeşidi, pişirme yöntemi koşulları ve antioksidan madde ekstraksiyon koşullarının farklı olması ile açıklanabilir. Sebze çeşidine göre pişirme süresi değişmektedir. Pişirme süresi arttıkça ısıdan etkilenen bileşiklerin kaybı artmaktadır. Antioksidan maddelerin çözünürlük özellikleri farklıdır. Kullanılan çözücü çeşidine göre antioksidan madde ekstraksiyon verimi değişmektedir. Ekstraksiyon verimine bağlı olarak antioksidan aktivite değeri değişebilmektedir.

Bu çalışmada pişirme işlemi sonrası kabak örneklerinde antioksidan aktivitede artış gözlenirken fasulye örneklerinde azalma gözlenmiştir. Fasulye örneklerine uygulanan işlem süresi (20-60 dakika) kabak örneklerine uygulanan işlem süresinden (15-45 dakika) daha uzundur. İşlem süresi arttıkça ısı etkisiyle parçalanmış antioksidan madde miktarı artmış olabilir.

SV pişirme yöntemi geleneksel yöntemlerle kıyaslandığında kabak örneğinde buharda pişirme yöntemine göre daha üstün bir yöntem olduğu ve fasulye örneği için yöntemler arasında önemli bir farklılık bulunmadığı yorumu yapılabilir.

Sonuç

Pişirme sonrası sebzelerin parlaklık değerinde azalma ve kırmızılık-yeşillik değerinde artış gözlenmiştir. Toplam fenolik madde miktarında ve antioksidan aktivitede çoğunlukla azalma meydana geldiği belirlenmiştir. Toplam fenolik madde miktarında en az kaybın SV yönteminde olduğu belirlenmiştir. Kabak örneklerinde haşlama yönteminde ve fasulye örneklerinde buharda pişirme yönteminde antioksidan kapasitenin diğer yöntemlere kıyasla yüksek olduğu tespit edilmiştir. Tüm analiz sonuçları birlikte değerlendirildiğinde kabak için SV yöntemi ve yeşil fasulye için buharda pişirme yöntemi önerilebilir. Sağlıklı pişirme yöntemlerinin geliştirilmesi sebzelerin içerdiği sağlık üzerine olumlu etkileri bulunan bileşenlerden azami ölçüde faydalanmayı sağlayacaktır.

Etik Standartlar ile Uyumluluk

Çıkar çatışması: Yazarlar bu yazı için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan etmişlerdir.

Etik izin: Araştırma niteliği bakımından etik izne tabii değildir.

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Teşekkür: -

Açıklama: Bu yayın “Sous vide yönteminin kabak ve yeşil fasulyenin antioksidan kapasitesine etkisinin belirlenmesi” başlıklı yüksek lisans tezinden hazırlanmıştır.

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The application of blends of bambaranut and millet vegetable milk in the development of plant-based yoghurt analogues: Proximate composition, physiochemical properties, microbial safety and consumer's acceptability

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ABSTRACT

This study aimed to produce plant-based yoghurt analogues from the blends of Bambaranut and millet milk. Yoghurt samples were produced from blends of Bambaranut milk and millet milk using *Streptococcus thermophilus* and *Lactobacillus bulgaricus* as starter cultures. Yoghurt samples were subjected to chemical, microbiological and organoleptic assessment. The results of the chemical analysis revealed moisture, protein, ash, fat, fibre, carbohydrates and energy contents ranged from 87.61-78.26%, 6.85-3.68%, 0.76-0.59%, 2.70-1.81%, 0.34-0.26%, 12.88- 5.60 and 92.94-57.50% respectively Total solids of between 12.39 and 21.74% were obtained with titratable acidity of 0.21, 0.65, 0.21, 0.23, 0.23, 0.25, 0.30 and 0.90%, respectively. The syneresis of the samples ranged from 40.28 to 18.90% while all the samples showed fairly acidic levels. A viscosity of between 250 and 784cp was obtained. The microbiological examination revealed an acceptable level for all the samples. There were observable significant differences in terms of overall acceptability, taste, and flavour between cow milk yoghurt and the yoghurt analogues.

Keywords: Vegetable milk, Plant-based, Yoghurt analogues, Sustainable diets

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Introduction

One of the largest problems facing the world food system in the twenty-first century is meeting the need for protein while maintaining environmental standards. Food systems today are responsible for nearly a quarter of all anthropogenic greenhouse gas (GHG) emissions, as well as deforestation, biodiversity loss, freshwater consumption, and water pollution. They are also ineffective in appropriately feeding people, resulting in a rise in food safety and health issues. Malnutrition affects around a third of the world's population in various ways (hunger, obesity, and micronutrient deficiencies) (Röös *et al.*, 2018; Elechi *et al.*, 2022). Diet-related hazards are now the world's third biggest cause of mortality, and malnutrition is the major cause of lost years of healthy life (Elechi *et al.*, 2022, GBD, 2020). According to Froggatt and Wellesley (2019), meat and dairy production is a major contributor to environmental change and the depletion of natural resources as the livestock industry is responsible for an estimated 40% of the world's arable land, 36% of crop calories produced, 29% of agricultural freshwater (Mottet *et al.*, 2017), and 14.5% of all human greenhouse gas (GHG) emissions. (Gerber *et al.*, 2013). Individuals who consume too much meat or dairy are more likely to be overweight, obese, and develop non-communicable illnesses connected to their diets, such as cardiovascular disease, type 2 diabetes, and certain cancers (Popkin *et al.*, 2012; Rouhani *et al.*, 2014; Bouvard *et al.*, 2015). Additionally, the UN has identified improper animal antibiotic usage as a major contributor to the rise in antimicrobial resistance (Van Boeckel *et al.*, 2017), while the expansion of livestock production raises significant concerns about animal welfare (Mason and Lang, 2017).

Dairy products continue to be important and main agricultural products, with global milk production expected to reach 861 Mt in 2020 and expand at a rate of 1.7% per year to 1020 Mt by 2030 (FAO, 2022). Particularly dairy products have long played a significant and durable role in the diet of the general public, where they are ingested for a variety of reasons, including enjoyment, dietary demands, and special health advantages, including probiotic ingestion. These dairy products range from kefir to yoghurt and other fermented meals to cheese (Tamang *et al.*, 2016; Pua *et al.*, 2022). Consumers are starting to actively look for alternatives to conventional bovine dairy products despite their current popularity because of their possible long-term detrimental effects on human health and the environment as well as other ethical concerns

(Mendly-Zambo *et al.*, 2021) which violates sustainable diet principles. A sustainable diet is seen as one that has a minimal environmental effect and contributes to food and nutrition security as well as a healthy life for current and future generations (FAO and BI 2010). Sustainable diets are culturally acceptable, accessible, economically fair, and inexpensive, as well as nutritionally adequate, safe, and healthy, while also maximising natural and human resources. As a result, greenhouse gas emissions and land usage are the most often employed measures to assess diet sustainability (Jones *et al.*, 2016). Therefore, there is growing agreement in the scientific, environmental, and public health sectors that a fundamental change in eating habits away from excessive meat and dairy consumption is required immediately to address the unsustainable nature of the livestock industry (Froggatt and Wellesley 2019). As a result, there is now more interest in plant-based dairy alternatives, which are thought to have advantages over conventional dairy products.

Due to their additional health advantages, plant-based dairy alternatives are of great interest, and their use and market share have been expanding (Cichonska and Ziarno, 2022). By 2028, the market for plant-based dairy products is projected to grow at a CAGR of 12.5% and total USD 52.58 billion (Grand View, 2022). Vegan-friendly labels (depending on additives) (Mendly-Zambo *et al.*, 2021; Yadav *et al.*, 2017; Roselló-Soto *et al.*, 2019); "free-from" properties (Hartmann *et al.*, 2018) for lactose, cholesterol, and dairy allergens like casein; reductions in consumer concerns about hormone and antibiotic residues; typically high content in vitamins, minerals, other bioactives, phytochemicals, and added functionalities, such as dietary fibre or pre-/probiotic activity (Cichonska and Ziarno, 2022; Roselló-Soto *et al.*, 2019) are some of the attractive features of plant-based dairy alternatives compared to conventional dairy products. Plant-based milk alternatives are liquids made from the breakdown (crushing) of water-extracted plant material (cereals, pseudo-cereals, legumes, oilseeds, nuts), and homogenization of these liquids produces a particle size distribution in the range of 5-20 μm , which resembles cow's milk in appearance and consistency (Sethi *et al.*, 2016). In terms of nutritional content, plant-based milk differs from one another. As a result, mixing two or more types of plant-based milk to produce a product with a high nutritional content equivalent to cow's milk is a crucial processing step (Sethi *et al.*, 2016). Recent research has established the critical role of these plant-based beverages in

enhancing or managing the immune system, reducing the risk of cardiovascular and gastrointestinal diseases with improved physiological functions, reducing the risk of low bone mass, and very high levels of antioxidants with free radical scavenging properties (Paul *et al.*, 2019). Furthermore, the addition of some beneficial and nutritionally significant components increases the product's quality, primarily by improving protein quality, mineral bioavailability, and the availability of a few key elements (Akin and Ozcan, 2017).

Yoghurt is arguably the most popular and diverse fermented dairy analogue. Yogurt is manufactured by fermenting milk with lactic acid bacteria - *St. thermophilus* and *L. bulgaricus*, which are responsible for the characteristic yoghurt flavour. Set, stirred, drinkable, flavoured-the seemingly endless variations of yoghurt products with different fat levels (normal, low-fat, and fat-free) on the market belie the humble makeup of its microbiota (Kayanush *et al.*, 2017). Lactose is converted during the fermentation process into lactic acid, which gives yoghurt its distinct tangy flavour and causes the acid gelation of casein, a significant milk protein (Pua *et al.*, 2022). This creates the cohesive gel that gives dairy yoghurts their distinctive hard, thick texture (Boeck *et al.*, 2021; Ar yana and Olson, 2017). The creation of a stable, pleasant, and dairy-like yoghurt alternative has significant challenges due to the absence of casein in plant matrices and dairy-incompatible natural plant flavours (Boeck *et al.*, 2021; Hickisch *et al.*, 2016; Łopusiewicz *et al.*, 2020). The addition of a fermentation process could enhance the organoleptic properties of such plant-based products and create a clean-label fermented dairy analogue with sufficient consumer acceptability, as many plant-based matrices struggle to achieve a natural dairy-like profile without the extensive use of processing aids and flavourings (Pua *et al.*, 2022). The majority of commercially available fermented plant-based yoghurt substitutes are created from nuts, drupes, and seeds (cashew, almond, and coconut). None has been used from the blend of bambaranut and millet.

The Bambaranut (*Vigna subterraenea (L.) verdc*) is a legume with an African origin. It is the third most significant legume, but it is also one of the most neglected (Murevanhema & Jideani, 2013). It has been dubbed a "whole food" because of its high nutritional value. In comparison to fresh whole milk, which contains 88% moisture, 4.8% carbohydrates, 3.2% proteins, 3.4% fat, 0.7% ash, and 0.01% cholesterol, its seeds contain 49-63.5% carbohydrates, 15-25% protein, 4.5-7.4% fat, 5.2-6.4% dietary fibre, 3.2-4.4% ash and 2% minerals. Its

chemical makeup is similar to that of soybeans. Furthermore, due to its nutritional content, functional characteristics, antioxidant potential, and drought-resistant cultivation, BGN has been identified as a promising crop. The acceptability of Bambaranut milk was reported to be higher than that of milk derived from other legumes such as soybeans and cowpeas (Murevanhema & Jideani, 2013). Bambaranut milk has a strong enough nutritional profile to support probiotic development. As a result, Bambaranut milk may be fermented with lactic acid bacteria to generate a probiotic drink that not only boosts the nutritional content of the bean but also aids in addressing malnutrition (Murevanhema & Jideani, 2013).

Millions of the poorest people in the places where millet is produced rely on it for energy, protein, vitamins, and minerals. Millet, like sorghum, has 9-13 percent protein on average, however there have been considerable fluctuations in protein content ranging from 6 to 21%. Millet is a popular weaning food since it is one of the least allergic and easily digested grains. It is good for making non-sticky gruel and porridge because it has less water-extractable dietary fibre than wheat and oats.

Diversified dairy products are in short supply in the dairy industries, but demand for them is growing. Although it has been demonstrated that livestock produces around 14.5% of all human-induced greenhouse gas (GHG) emissions, both developing and developed countries continue to consume large amounts of energy-rich meals, which include meat, milk, and other dairy products (Weinrich, 2019). By 2050, it is predicted that 9 billion people will be eating plant-based protein, which has the potential to not only significantly lessen the severely negative environmental effects of meat consumption but also provide a source of good and nutritious meals for those individuals (Chaudhary & Tremorin, 2020; Monnet *et al.*, 2019; Willett *et al.*, 2019). Few legumes and oilseeds have been widely used in this area to produce non-dairy, healthy, affordable plant-based milk alternatives (Sethi *et al.*, 2016). Soy is the most popular plant-based ingredient for yoghurt (Yang *et al.*, 2021) with other plant components like coconut and almonds gaining popularity (Gupta *et al.*, 2022). Novel ingredients, such as lupins (Hickisch *et al.*, 2016), African Yam Bean (Amakoromo *et al.*, 2012); oats (Brückner-Gühmann *et al.*, 2019), sesame seed (Ibrahim, 2013), peas (Ben-Harb *et al.*, 2018), Baobab (Eke *et al.*, 2013), quinoa (Lorusso *et al.*, 2018), Bambara Groundnut (Eke *et al.*, 2012) and flaxseed (Mousavi *et al.*, 2019) are also

being assessed. Yoghurt can also be made by making blends from different sources as reported by Olakunle (2012) for soy milk and corn milk and Kolapo and Olubamiwa (2012) for coconut milk and soy milk. Lower protein concentrations and variable gelation characteristics of these proteins compared to casein might be the cause of the various textural qualities of commercial plant-based yoghurts, necessitating the use of gelling agents (Gupta *et al.*, 2022). This study aimed to undertake product development and quality assessment of yoghurt analogues made using Bambaranut and millet milk blends, with implications for nutrient-dense yoghurt product diversification and commercial potential.

Materials and Methods

Source of Material and Preliminary Treatments

About 2.0 kg each of Bambaranut and Millet was purchased from a small market in the Lafia metropolitan region of Nassrawa state, Nigeria. Whole milk powder (Peak), sucrose (Golden Penny brand), and commercially available yoghurt starter cultures (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) were acquired at a local shop in Makurdi, Benue state, Nigeria. Tito, a local and prominent dairy yoghurt manufacturer in Nigeria's north-central area, provided the cow's milk yoghurt utilised as a reference sample in this study. The majority of the chemicals and equipment used for sample preparation (NaHCO_3) and analysis are analytical grade and were bought from a local Nigerian chemical store and the Department of Food Science and Technology, University of Agriculture, Makurdi-Nigeria. After hand sorting and screening, stones, dirt, and faulty seeds that can affect the beverage's taste and shelf life were removed. Each 5litre plastic bucket was filled with clean Bambaranut and millets. All components are kept in a household refrigerator and employed in product development within seven days.

Preparation of Bambaranut Milk

With minor adjustments, Bambaranut milk was made according to the technique of Eke *et al.* (2012) as shown in figure 1. One kilogramme of bambaranut was divided into three kilogrammes, i.e. (1:3) warm drinking water with 50 g of sodium bicarbonate, steeped for 18 hours overnight. The Bambaranuts were then drained, washed three times with drinking water, and blanched for 25 minutes in boiling water with baking soda (0.5 percent NaHCO_3) to kill the lipoxygenase enzyme. The blanched seeds are rinsed, skinned, and crushed into a paste in the blender (model: Qlink; QBL-15L40) for seven

minutes with a little warm water. The paste was prepared up to a 1:3 weight-to-volume ratio. The resultant slurry was filtered and pressed through a double-layered muslin cloth, and the resulting extract (milk) was heated in a pot at 95-100°C for 15 minutes with constant stirring. After heating, the bambaranut milk was then manually homogenised with a glass rod and sieved again. It was then cooled to room temperature of 22°C, kept in a domestic refrigerator at 5°C, and utilised for product formulation within 24 hours.

Preparation of Millet Milk

As described, the Amal *et al.*, (2012) method was adopted and adapted for millet milk manufacturing as shown in figure 1. By soaking and removing the seeds floating on top of one kilogramme of millet, it was cleaned and sorted. The excellent seeds were collected and drained in a plastic basket. After that, the good seeds were steeped for 8 hours. The soaked seeds were drained and crushed in a blender (model: Qlink; QBL-15L40). To extract the milk, the slurry is pressed using cheesecloth. The extract was pasteurised for 5 minutes at 95-100°C. It was homogenised, sieved again, and cooled to room temperature of 22°C before being kept in a household refrigerator at 5°C and utilised within 24 hours for product development.

Cow Milk Yoghurt

The plain cow's milk yoghurt utilised in this study came from a manufacturer in Benue State, Nigeria. Ramtito Dairy produces Tito yoghurt, which comprises a 2% yoghurt starter made from *S. thermophilus* and *L. delbrueckii subsp bulgaricus*. The product was chosen as a research reference because it is a popular, affordable, and widely accessible cow's milk-based yoghurt formulation in Benue state and its environs.

Food Products Formulation

Five different food formulations are made by blending the Bambaranut milk (BM) produced with the Millet milk (MM) to obtain various yoghurt analogues as shown in table 1. The resulting blends are homogenized and pasteurized at 72°C for 5 min in a water bath and cooled immediately to room temperature ($28 \pm 2^\circ\text{C}$). These were then packaged in 500mL plastic bottles with air-tight lids as shown in figure 3, and stored in a household refrigerator from where samples are taken for different yoghurt analogues analysis.

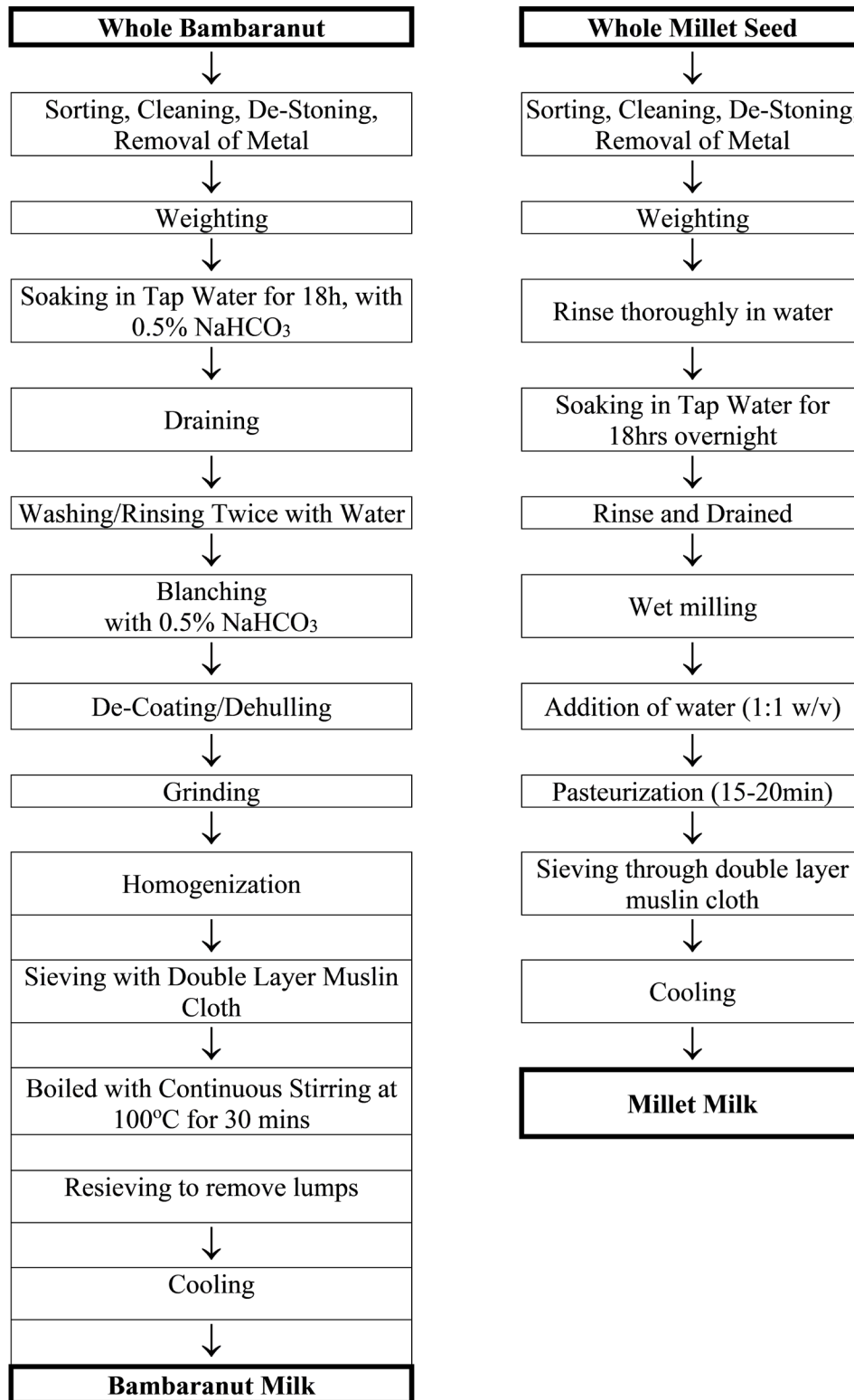


Figure 1. Preparation of bambaranut and millet plant based milk

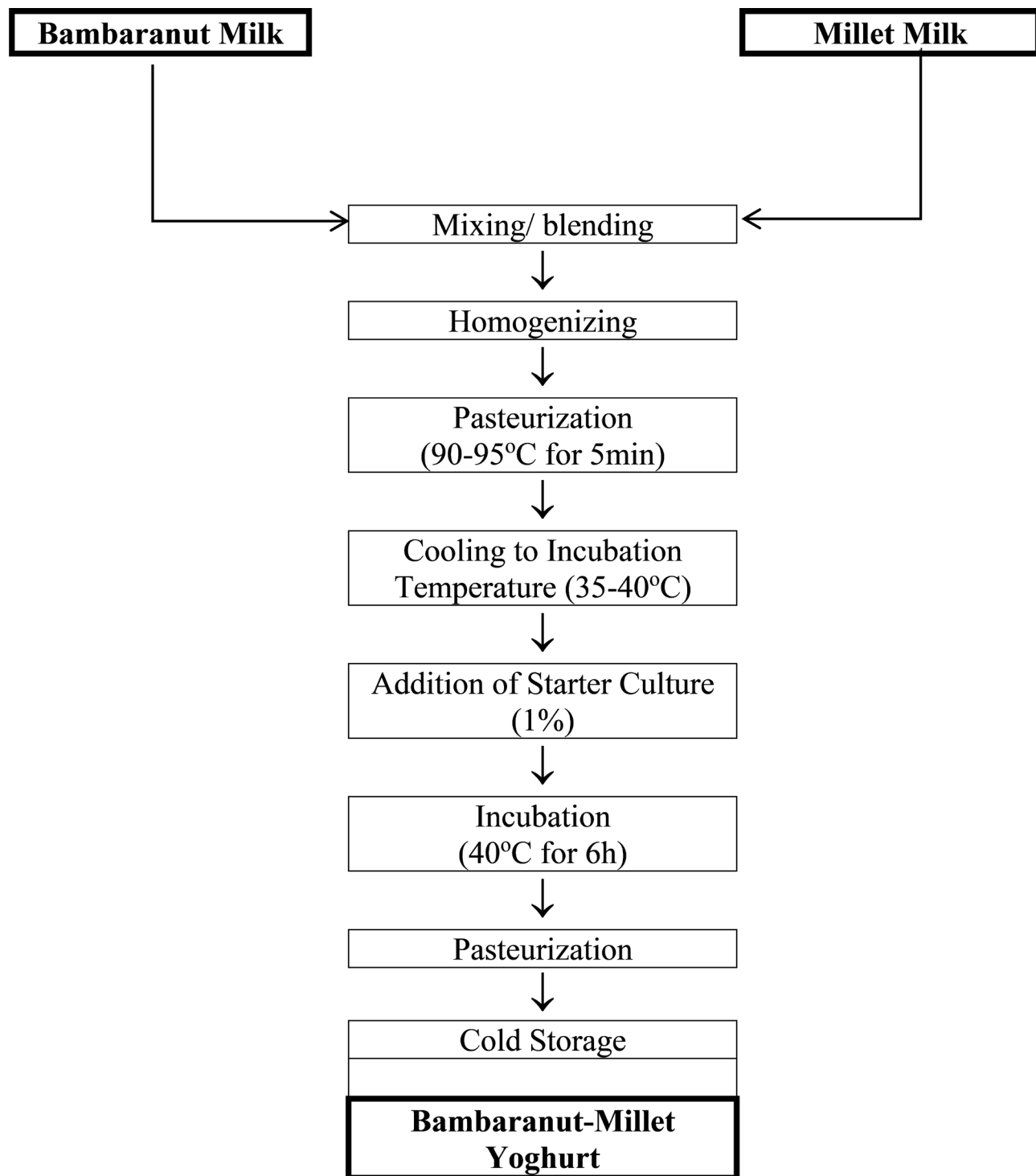


Figure 2. Flow chart for the production of bambaranut – Millet yoghurt analogues



- 347 = 100% bambara nut without cow milk.
 411 = 100% bambara nut with cow milk.
 459 = 90% bambara:10% millet.
 505 = 80% bambara : 20% millet.
 619 = 70% bambara:30% millet
 677 = 50% bambara:50% millet.
 702= commercial yoghurt cow milk yoghurt

Figure 3. Display of yoghurt samples

Preparation of Yoghurt Samples

Yoghurt analogues were made from the food formulations as shown in Figure 2. Five hundred millilitres of prepared milk containing various proportions of Bambaranut and Millet milk were each enriched with 6% sucrose, 5% banana flavouring, and 4% premium whole milk powder and thoroughly blended in a blender. The resulting mixture was sterilised in an autoclave for 15 minutes at 121°C. The sterilised milk was chilled to roughly 40-45°C using a fan. A sterile spoon was used to add 1% of the mixed starter culture (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*), which was then incubated at 40°C for 12 hours. At the completion of the incubation, the mixture was placed in the refrigerator for 3 hours to cease fermentation. The resultant yoghurt analogues products were pasteurized and filled into clean PET bottles, corked and stored from where samples are taken for subsequent analyses.

Analyses

Proximate Composition Analysis of Samples

The ash, crude protein, crude fat, and moisture content of the samples were determined by using standard methods of AOAC (2012). Carbohydrate was determined by difference:

$$\text{Carbohydrate} = 100 - (\% \text{ Moisture, Protein, Fibre, Fat, and Ash})$$

The caloric values of the samples will be determined by summing the multiplied values for crude protein, crude fat, and carbohydrate by 4kcal, 9kcal, and 4kcal respectively.

Physio-Chemical Analysis

Syneresis: To determine susceptibility to syneresis (STS), the yoghurt sample (20 mL) was placed on a Whatman No 1 filter paper on top of a funnel and the yogurt was filtered under vacuum for 10 min. The liquid that passed through the filter paper was collected and recorded. The index of syneresis was calculated from the formula:

$$\text{STS} (\%) = \frac{V_1}{V_2} \times 100$$

V_1 = Volume of whey collected after drainage

V_2 = Volume of yoghurt sample

Viscosity: A Brookfield viscometer [Brookfield Model RVD E230, USA] with spindle number 6 was used to measure the apparent viscosity at 50 rpm at 25°C.

pH: The pH was determined by the method described by AOAC, (2012) using pH meter. About 2.0 mL of the sample was homogenized in 20 mL of de-ionized water in a beaker. The pH meter (Hanna HI-98128) was standardized using a buffer solution of pH 4.01 and 9.20. The electrode was rinsed with de-ionized water and dipped into the homogenate allowing sufficient time for stabilization before taking the reading.

Total Titratable Acidity (TTA): Ten grams of sample is dissolved in 100mL distilled water contained in a beaker. Then 10mL aliquots are titrated against 0.1M NaOH in the presence of phenolphthalein indicator to a clear endpoint. Titratable acidity is then calculated as given below

$$\text{Lactic Acid} (\%) = \text{ml NaOH} \times 0.9$$

Table 1: Recipe Used in Preparing Yoghurt Analogue

Ingredients	Quantity
Bambaranut/Millet Milk	300 mL
Sucrose	18 g
Flavorant (Banana Flavour)	15 mL
Powdered Cow Milk	12 mL
Starter Culture	3g

Table 2: Blend Formulation (%)

Samples	Bambaranut Milk	Millet Milk
A	100	-
B*	100	-
C*	90	10
D*	80	20
E*	70	30
F*	50	50
R	Commercial cow milk	Yoghurt

* Inclusive of 4% (w/v) of powdered cow milk

Total Solids: The yoghurt slurry contains some solid particles that tend to separate on standing based on their density. Three gram of the yogurt was weighed in a round-bottomed metal dish with its weight noted. The dish with the sample was placed in a boiling water bath for 30 min. The dish with the sample was placed in an oven at 100°C for 2.5 hrs. The dish is cooled in a desiccator for 30 min and weighed. The total solids are calculated as shown:

$$\%TS = 100 - \% \text{Moisture content}$$

Microbiological Analyses

The total viable count of the yoghurt analogues was determined using the pour plate technique. (0.1 mL) of the appropriate dilution was placed on nutrient agar plates. The plates were incubated at 35°C for 48 h and colony-forming units per g sample (cfu/g) were estimated. For mold and yeast count; the above procedure was repeated using potato dextrose agar and incubation was done at 25°C for 72 h.

Consumer Acceptability Test

The manufactured yoghurt samples were delivered to 35-member untrained consumers made up of personnel and students from the University of Agriculture, Makurdi, Benue State, who are experienced with yoghurt consumption. On a nine-point hedonic scale, samples were scored for colour/appearance, flavour/aroma, viscosity/consistency, taste/mouth-feel, sourness, and overall acceptability, with 9 indicating extreme acceptance and 1 indicating severe dissatisfaction as

shown in the table below. To unify the conditions of the evaluation, all samples were prepared in disposable plastic cups coded with a three-digit number, evaluated by each panelist in a monadic order, following a balanced-incomplete box design (Stone *et al.*, 2020). The samples were served in three sessions consisting of 3–4 samples for each round and served in random order to each panelist. The samples' identities were kept hidden. During the test, the panelists were asked to pause between the sample and cleanse their palates with prepared tap water at room temperature (Samakradhamrongthai *et al.*, 2021). The evaluation was performed in individual booths under white light at the Sensory Evaluation and Consumer Testing Unit (Department of Food Science and Technology, College of Food Technology and Human Ecology, University of Agriculture, Makurdi, Nigeria).

9-Point Hedonic Scale	
Dislike Extremely	1.0
Dislike Very Much	2.0
Dislike Moderately	3.0
Dislike Slightly	4.0
Neither Like nor Dislike	5.0
Like Slightly	6.0
Like Moderately	7.0
Like Very Much	8.0
Like Extremely	9.0

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) by using SPSS Statistics for Windows,

Version 23.0 (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp). Where the ANOVA test indicated significant differences and treatment means were separated using Duncan's multiple range test at a 5% probability level.

Results and Discussion

As shown in table 3, the moisture content of 87.61% obtained for sample A (100% BM with no cow milk) is higher than other samples (83.99% for 100% BM with 4% cow milk, 83.19% for 90:10, 82.63% for 80:20, 81.84% for 70:30 and 78.26% for 50:50). This might be because millet starch in form of milk slurry thickened Bambaranut yoghurt (Olanunle, 2012). All of the samples showed a progressive decline in protein content with a significant difference. The augmenting effect of milk powder might explain the greater protein content of the yoghurt samples compared to sample A. The steady loss of protein with increased millet supple-

mentation might be owing to the dilution impact of millet protein on Bambaranut protein, which is richer in protein. However, it is worth noting that the Bambaranut-Millet Yogurt's protein level is of good quality since it includes all of the essential amino acids. It has been demonstrated that by supplementing grain proteins with legume proteins, protein quality equivalent to or greater than that of animal origin may be produced. As a result, yoghurt analogues' protein compares favourably to the control (R) and is high in important amino acids because careful grain and legume selection and combination results in an essential amino acid pattern that is equivalent to or greater than the reference protein. Therefore, the comparative levels of protein in the samples are nutritionally significant in terms of the potential of these beverages to contribute to the increased protein intake of consumers. The protein obtained for sample A agreed with the findings of Kale *et al.* (2011) who reported protein content of date yoghurt was in the range of 3.45% to 3.47% and Gupta *et al.*, (2022) for commercially available dairy and plant-based yogurts.

The values obtained for ash content of the yoghurt samples had a range between 0.59-0.76% which is within the range of market yoghurt sold in Nigeria as reported by Olugbuyiro and Oseh (2011). There was a gradual increase ($p < 0.05$) in the ash contents of the samples. The rise in ash content detected in the samples is attributable to the mineral levels generated by the inclusion of millet. The percentage fat content changed sufficiently ($p < 0.05$). The average fat content is 2.70 percent for the highest and 1.81 percent for the lowest. Yoghurt with a fat concentration of less than 0.5 percent is labelled as non-fat yoghurt, while yoghurt with a fat content of 0.5-2.0 percent before adding bulking ingredients is labelled as "low-fat yoghurt" whereas yoghurt with a fat level greater than 3.25 percent is labelled as fat yoghurt according USDA (2001) specifications. As a result, the samples' fat level was within the range of low-fat yoghurt. Gupta *et al.*, (2022) obtained similar results for commercially available dairy and plant-based yogurts. Samples C, D, E, F, and R had low-fat content, which may contribute to a longer shelf life by minimising the risk of rancidity. Higher fat levels found in samples A and B, which might be attributable to the samples being made wholly from Bambaranut-an oilseed, enhanced lipolytic enzyme activity that degraded fat to glycerol and fatty acids, and the release of certain non-lipid ether extractable materials generated by fermenting bacteria.

The fibre content of the yoghurt samples was measured in the range of 0.26-0.34. The role of fibre in human nutrition has been established, fibre containing food are known to stretch the inner walls of the colon, allowing the transit of waste, making them an efficient therapy for constipation; it lowers blood cholesterol levels and reduces the risk of several forms

of cancer (Elinge *et al.*, 2012). As a result, the fibre content of Bambaranut-millet yoghurt analogues renders them nutritionally more important than the non-fibre reference sample R. As millet was added to samples C, D, E, and F, the carbohydrate content of these samples increased when compared to sample A. When compared to the content of certain traditional sources, the samples could not be regarded a prospective carbohydrate source (Elinge *et al.*, 2012). However, the yoghurt samples' low carbohydrate and fat content, along with their high fibre content, make them ideal for weight loss and preventing cardiovascular disease. As a result, making yoghurt analogues from grain blends can enhance the nutritional quality of Africa's young population, boost farm gate prices for small farmers, and encourage the cultivation of more grains and legumes in the Global South that can be utilised as yoghurt inputs (Shimelis, 2019).

The result of the physiochemical properties of the various yoghurt samples is presented in Table 4. Milk's nutritional and economic value is directly proportional to its soluble solids concentration. The higher the soluble solids concentration, the higher the nutritious value and the more milk products may be produced. Higher levels of suspended particles caused by particles in bambaranut-millet milk than in bambaranut milk might explain the higher solid values obtained for samples B, C, D, E, and F. The result of this study is in agreement with that of Olakunle (2012), however, the higher value recorded here could be attributed to the effect of powdered milk addition and processing method. All of the designed yoghurt samples had a total solid content that was comparable to commercial yoghurt with samples D, E, and F being higher. These results are in agreement with Eke *et al.* (2013) who observed that the total solid of yoghurt-like product produced from baobab fruit pulp emulsion and powdered milk ranged from 9.24 to 16.58. In another study by [20], the probiotic yogurt from soymilk was shown to be comparable to the standard cow milk yoghurt in terms of physico-chemical attributes.

The pH values in all samples reduced significantly ($P \leq 0.05$), according to statistical analysis. The greatest average pH was 4.64% in Sample A, while the lowest average pH was 3.85% in Sample F. The Bambaranut-millet yoghurts, except for A and R, were more acidic, with a mean pH range of 4.01-3.85%. Because A (4.63) does contain powdered milk, the pH difference between it and other samples might be attributed to the composition and production process and the acidifying action of lactic acid generated during fermentation. This suggests that the rate of acid generation by the starter culture was affected by the addition of millet milk. Despite this, according to FDA standards for yoghurt pH (4.6 or below), the pH findings for yoghurt are within specifications and within the

range of yoghurt marketed in Nigeria (Olugbuyiro and Oseh, 2011). The result of this study compared favourably with that of Kolapo and Olubamiwa (2012) Soy-coconut milk-based yoghurt. Due to the predicted effects of poor storage conditions, such as high temperatures in some tropical zones, which might impact the acidity of yoghurt, the pH values of the samples were adequately justified and appropriate for yoghurt marketed in the tropics (Olugbuyiro and Oseh, 2011). The findings regarding the pH of sample A are also in agreement with the findings of Sengupta *et al.* (2013) who found the average pH of whole soy-yoghurt was 4.5. The pH obtained for bambaranut and bambaranut-millet yoghurt is similar to values reported for soy and soy-corn yoghurt (Olakunle, 2012) which range between 4.50 and 4.00. The formation of lactic acid by *Lactobacillus* and other lactic acid bacteria causes the variation in the rate of pH reduction during yoghurt manufacture. Amakoromo *et al.* (2012) reported that the decrease in pH from 5.1 to 4.2 for African Yam Beans yoghurt from whole seeds is a reflection of the souring activity of lactic acid produced during fermentation. In addition to having a fatal and destructive impact on bacteria and inhibiting bacterial multiplication, the formation of lactic acid following fermentation lowers pH, halting any further development of pathogens and other hazardous microbes (Jayeola *et al.*, 2010). Also, each lactic acid starter culture has its own lactose absorption properties and acidification capacities, the pH of yoghurt varies. Furthermore, the addition of 4 percent milk powder to the yoghurt matrix may have buffered the yoghurt matrix, delaying the pH reduction in the samples. Also, Proteins in milk powder are known to act as buffers in food systems because of their ability to release or absorb free hydrogen atoms.

Statistical analysis revealed that during fermentation, titratable acidity increased considerably ($P \leq 0.05$) in all samples (Table 4). The acidity of milk rises with temperature, partially due to changes in the buffering capacity of milk salts and carbon dioxide release during heating. The heat breakdown of lactose into organic acids might be the cause of this rise in acidity. Furthermore, overall acidity increased ($p \leq 0.05$) as the pH decreased ($p \leq 0.05$) as a function of fermentation duration. This may be attributed to the development of lactic acid in the yoghurt (Sengupta *et al.*, 2013). The starter culture utilised and the composition of the premix used for fermentation influenced the development of acidity in the various yoghurt analogues produced. The higher titratable acids obtained for Samples D, E, and F (0.23, 0.25, and 0.34 percent, respectively) compared to Sample A (0.21 percent) might be due to the more acidic composition of the millet protein (Olakunle, 2012). However as titratable acidity increased, the pH decreased as a function of fermentation time. The capacity

of the microbes to ferment the available carbohydrates determines how much acid is produced in the medium. Furthermore, the total acidity significantly increased ($p < 0.05$) and pH significantly decreased ($p < 0.05$) which may be attributed to the formation of lactic acid in the Yogurt (Sengupta *et al.*, 2013). There was no significant difference ($p \geq 0.05$) in the titratable acidity (TA) for most of the yoghurts prepared. This could be attributed to the Millet and powdered milk being rich protein sources, they exert some buffering action stabilizing the acidity of yoghurt systems.

At 25 °C, the viscosity of experimental yoghurts ranged from 250 to 784 cP (Table 4) which compared favourably with that of Zanzi and Jideani (2012). There was a constant pattern of increased viscosity with increasing millet addition rate except for Sample A, which had no milk powder or millet milk. This related to the finding in Total Soluble Solids that TSS increased as the rate of millet milk addition increased. Sengupta *et al.* (2013) reported that Soy Yogurt is non-Newtonian and its viscosity is affected by the presence of solids in the suspension of the fat phase. The higher the solids concentration in the yoghurt mixture, the higher the viscosity and consistency of the finished product. The consistency indices of the non-cow's milk yoghurt formulations (Sample A) were typically low (250 cp). Because cow's milk has a larger total solids content than plant milk, increasing the cow's milk content should improve the consistency and viscosity of dairy products (Kpodo *et al.*, 2013). Yogurt manufacturers that wanted to generate yoghurt with higher viscosities and better consistency frequently added milk powder to the raw material to boost total solids and produce yoghurt with greater consistency and viscosity (Zanzi and Jideani, 2012). In comparison to the commercial yoghurt R, the Bambaranut-millet yoghurts analogues, fared better. Similar consistency and viscosity were obtained by Gupta *et al.*, (2022) for commercially available dairy and plant-based yogurts.

The texture of yoghurts or fresh fermented goods is described and measured by rheology, which includes terminology like viscosity and gel hardness, as well as syneresis, which refers to the yogurt's tendency to whey-off during storage. Syneresis is a common quality problem in yoghurt production. The rheology and sensitivity to syneresis of fermented milk products are important because they both have a significant influence on customer perceptions of the finished product's quality. Indeed, variations in yoghurt quality can result in a product with a too thin watery or too thick gooey texture, as well as a product with a high free whey content. Importantly, milk composition, processing techniques, and the inclusion of hydrocolloids all have an impact on yoghurt rheology and syneresis. As a result, syneresis percentages ranged from 40.28 to 18.90 percent. This is within the range reported by Oyeniyi *et al.*

(2014) for flavoured soy-yoghurt and Kale *et al.* (2011) for date yoghurt. Chacko *et al.* (2010) reported an average of 52.6% syneresis in soy-yoghurt fortified with 1.104% calcium sulphate. Therefore, in this study, the percentage synereses reported are considered satisfactory. The lower values obtained in the current experiment is attributed to the use of millet starch and full-cream powdered milk. Millet starch used in this current study caused the free water molecules within the yoghurt matrix to be better absorbed. Commercial yoghurt (sample R) exhibited the lowest levels of syneresis, whereas yoghurt sample A had the greatest amounts (18.90% and 40.28%), respectively. Commercial yogurt's (Sample R) low degree of syneresis may be related to the use of stabilisers and standardised milk in its production compared to other yoghurt counterparts. The microstructure of yogurt consists of chains and clusters of casein molecules and the susceptibility of syneresis is closely related to the space between casein clusters. In yoghurt produced with the same amount of Bambaranut-millet milk (Sample F) syneresis was reduced. Therefore, adding millet milk to the substrate bridged the gaps between casein clusters of Bambaranut-milk gel and reinforced the gel network. Similar reduction of syneresis was reported by Aydar *et al.*, (2021) for plant-based yoghurt produced from Jerusalem artichoke and almond milk.

Table 5 shows the microbial communities of the yoghurt samples. The completed product was microbiologically evaluated for the survival of starting microbes as well as the presence of undesirable spoiling and pathogenic organisms. For coliforms, there was no count. A maximum of 10.0 CFU/g coliforms is permitted in yoghurt, according to Turkish Standard Institute (TSI330) and USDA (2001). Coliforms in yoghurt are commonly thought to be a result of direct faecal contamination. The lack of coliforms indicates that all samples were free of faecal contamination; hence, the yoghurt samples' microbiological status corresponded to the accepted standard. The quantity of aerobic mesophilic bacteria was minor, with counts ranging from 6.62 to 6.20 log CFU/g, which is within an acceptable yoghurt level. Yeast and mould were found in modest amounts in all of the samples. Toxic metabolites (mycotoxin, e.g. aflatoxin) can be produced by yeast and moulds at concentrations more than 10.0 CFU/g, which can cause food poisoning and liver cancer in humans. The observed fungal count might be attributed to microorganisms present in the inoculums as a result of irresponsible handling during

manufacture and analysis. The USDA (2001) advised that the amount of yeast and mould in yoghurt be limited to 50 per gramme. Microbial requirements are regulated differently in different countries (Eke and Elechi, 2020). TVC in the range of $0-10^3$ CFU/g, 10^4-10^5 CFU/g, and $>10^6$ CFU/g is considered acceptable, slightly acceptable (tolerable), and unsatisfactory by the ICMSF. Based on the microbiological standards used, this study's findings are deemed good.

Sensory quality evaluations of yoghurt products were conducted to better understand the product qualities that influence customer preferences, allowing processors to alter product attributes to appeal to specific target consumers and to closely monitor product quality (Shimelis, 2019). The yoghurts' observable sensory characteristics are listed in Table 6. The USDA (2001) specifies a firm, pudding-like consistency with a smooth, uniform texture and a natural colour range of light white to off-white with a smooth, velvety appearance for all samples of yoghurts. The panellists deemed the appearance and consistency of all samples to be satisfactory because the mean values of the samples differed insignificantly. This is in agreement with Gupta *et al.*, (2022) who reported that sensory acceptability is affected by compositional and quality factors, such as protein source, texture, fat, sugar, or the form of the yogurt. Consumer liking, in general, positively correlates with the viscosity and smoothness of the product (Janiaski *et al.*, 2016). The results revealed that the flavour, taste, and sourness of the designed yoghurt samples differed significantly ($p<0.05$) from the control samples (i.e. commercial yoghurt R). This might be because commercial yoghurt has a lot of flavours and sweeteners. Overall acceptance of the formulated yoghurt samples was not substantially different ($p>0.05$), while overall acceptance of the commercial yoghurt was significantly greater ($p<0.05$) than that of the formulated yoghurt samples. This is contrary to the previous research that showed that some plant-based alternatives can be similarly liked to dairy yogurts in their mouthfeel profile (Greis *et al.*, 2020). For example, a recent study of commercial plant-based yogurts (soy, coconut, cashew, almond, and hemp) by Grasso *et al.*, (2020) found that soy and coconut yogurts were identical to dairy yogurts in terms of sensory acceptability and texture.

Table 3. Proximate Composition of Babaranut-Millet Yoghurt Analogues (%)

Parameter	A	B	C	D	E	F	R
Moisture	87.61±0.22 ^a	83.99±0.03 ^b	83.19±0.02 ^{bc}	82.63±0.03 ^{cd}	81.84±0.09 ^d	78.26±0.16 ^c	83.00±0.01 ^{bc}
Crude protein	3.68±0.06 ^e	6.65±0.02 ^a	6.85±0.02 ^a	6.65±0.04 ^a	6.35±0.17 ^b	5.67±0.03 ^d	6.65±0.01 ^a
Ash	0.59±0.02 ^f	0.64±0.03 ^e	0.66±0.01 ^d	0.68±0.01 ^c	0.70±0.03 ^b	0.76±0.03 ^a	0.71±0.03 ^b
Crude Fat	2.26±0.06 ^e	2.70±0.10 ^a	2.56±0.03 ^b	2.43±0.05 ^c	2.33±0.05 ^d	2.09±0.02 ^f	1.81±0.02 ^g
Crude Fibre	0.26±0.01 ^d	0.26±0.01 ^d	0.27±0.00 ^{cd}	0.28±0.01 ^{bc}	0.29±0.01 ^b	0.34±0.00 ^a	-
Carbohydrates	5.60±0.26 ^e	5.87±0.05 ^e	6.46±0.05 ^{de}	7.32±0.12 ^{cd}	8.49±0.12 ^b	12.88±0.11 ^a	7.83±0.04 ^{bc}
Calories (Kcal)	57.50±0.89 ^f	73.48±0.24 ^c	76.31±0.26 ^d	77.73±0.13 ^c	80.29±0.30 ^b	92.94±0.25 ^a	74.21±0.09 ^c

Values are means of triplicate determinations. Mean values with same superscript in a row are not significantly different ($p \geq 0.05$) Mean values having different superscript letter in a row are significantly different ($p \leq 0.05$).

KEY:

A = 100% Bambaranut milk (without Milk);

B = 100% Bambaranut milk (with Milk);

Bambaranut Milk: Millet Milk. C = 90:10; D = 80:20 E = 70:30; F = 50:50

R = Commercial Yoghurt

LSD = Least Significant Difference

Table 4. Physiochemical Properties of Babaranut-Millet Yoghurt Analogues (%)

Parameter	A	B	C	D	E	F	R
PH	4.64±0.10 ^a	4.10±0.20 ^{bc}	4.06±0.06 ^{bc}	4.05±0.03 ^{bc}	4.01±0.61 ^{bc}	3.85±0.09 ^c	4.37±0.12 ^{ab}
TTA	0.21±0.02 ^d	0.65±0.09 ^a	0.21±0.08 ^d	0.23±0.02 ^d	0.25±0.06 ^d	0.30±0.01 ^c	0.90±0.10 ^a
TSS	12.39±0.22 ^e	16.28±0.82 ^d	16.81±0.02 ^{cd}	17.37±0.03 ^c	18.16±0.09 ^b	21.74±0.16 ^a	17.00±0.02 ^c
Syneresis	40.28±1.16 ^a	38.22±1.54 ^b	28.77±1.30 ^{bc}	26.69±1.40 ^d	24.69±0.60 ^c	20.63±0.70 ^f	18.90±0.70 ^g
Viscosity (c_p)	250±29 ^f	344±26 ^e	385±12 ^d	480±33 ^{bc}	595±30 ^b	784±32 ^a	467±23 ^c

Values are means of triplicate determinations. Mean values with same superscript in a row are not significantly different ($p \geq 0.05$) Mean values having different superscript letter in a row are significantly different ($p \leq 0.05$).

KEY:

A = 100% Bambaranut milk (without Milk);

B = 100% Bambaranut milk (with Milk); R = commercial yoghurt.

Bambaranut Milk: Millet Milk. C = 90:10; D = 80:20 E = 70:30; F = 50:50

LSD = Least Significant Difference.

TTA = Total Titrable Acidity

TSS = Total Soluble Solid

Table 5. Microbiological Qualities of Babaranut-Millet Yoghurt Analogues (\log_{10} Cfu mL⁻¹)

Types of Microbes	A	B	C	D	E	F	R
Total Plate Count	6.32±0.04 ^c	6.39±0.02 ^d	6.43±0.03 ^c	6.46±0.02 ^c	6.55±0.05 ^b	6.62±0.06 ^a	6.20±0.02 ^f
Total Yeast and Mold count	1.94±0.03 ^d	2.03±0.12 ^c	2.23±0.02 ^a	2.21±0.03 ^a	2.17±0.02 ^b	2.09±0.03 ^c	1.30±0.02 ^e
Coliform	ND	ND	ND	ND	ND	ND	ND

Values with same superscript in a row are not significantly different ($p \geq 0.05$) while values having different superscript letter in a row are significantly different ($p \leq 0.05$).

KEY:

A = 100% Bambaranut milk (without Milk);

B = 100% Bambaranut milk (with Milk);

Bambaranut Milk : Millet Milk. C = 90:10; D = 80:20 E = 70:30; F = 50:50

R = Commercial Yoghurt

ND = Not detected

Table 6. Mean Scores of Sensory for Babaranut-Millet Yoghurt Analogues

Parameter	A	B	C	D	E	F	R
Appearance	6.90 ^b	7.37 ^{ab}	7.37 ^{ab}	7.35 ^{ab}	6.90 ^b	6.95 ^b	8.15 ^a
Mouth Feel	6.20 ^b	6.40 ^b	6.20 ^b	6.40 ^b	6.35 ^b	6.70 ^b	8.45 ^a
Sourness	5.40 ^b	6.30 ^b	5.75 ^b	6.40 ^b	6.30 ^b	6.40 ^b	7.90 ^a
Flavour	6.10 ^c	6.20 ^c	6.85 ^{bc}	7.05 ^b	6.85 ^b	7.05 ^b	8.30 ^a
Consistency	6.30 ^c	6.60 ^c	6.65 ^{bc}	6.75 ^{abc}	6.85 ^{abc}	7.30 ^a	7.20 ^{ab}
Overall Acceptability	6.60 ^b	6.65 ^b	6.55 ^b	7.13 ^b	7.05 ^b	7.25 ^b	8.55 ^a

Values are means of triplicate determinations. Mean values with same superscript in a row are not significantly different ($p \geq 0.05$) Mean values having different superscript letter in a row are significantly different ($p \leq 0.05$).

KEY:

A = 100% Bambaranut milk (without Milk);

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R = Commercial Yoghurt

LSD = Least Significant Difference

The disparity between the overall acceptance of formulated yoghurt samples and that of commercial yoghurt samples in this study could be due to the panellists' familiarity with cow's milk yoghurt versus the reformulated products (Ijarotimi and Keshinro, 2012); additionally, the lower consumption of plant-based yogurts may be due to their different textural properties, compared to dairy yogurts (Gupta *et al.*, 2022). The cross-linking of casein proteins is an intrinsic process in the formation of dairy yogurt gels, caused by heating and acidification by bacterial cultures, which contributes to the texture of these products (Yang *et al.*, 2021). Also, astringency in plant-based milk has been scientifically reported as the most important limiting factor in consumer acceptance of plant-based milk products. The native 'grassy' or 'beany' flavour in soy caused by hexanal and other aldehydic lipid oxidation (lipoxygenase, or LOX-catalysed) products, however, faces significant acceptability issues in some countries and is perceived as an off-flavour in dairy analogues (Pua *et al.*, 2022; Zhu *et al.*, 2020; Short *et al.*, 2021). However, the overall acceptability scores of the yoghurt's analogues were in the range of 6.55 to 7.25 on the 9-point hedonic scale indicating general acceptance of the samples by the consumers. Therefore, from this study, it is possible that the yoghurt analogues improved sensory quality may be attributable to the blending of raw materials and processing procedure, namely the heating and sterilising of the products and fermentation. Heating tends to deactivate the leptoxygenase enzyme that gives vegetable milk its beany taste. While studies showed that blending raw materials could result in improved hedonistic ratings, the mechanism behind this could be the masking of off-flavours, e.g., the beany note in legumes (Short *et al.*,

2021). Blending raw materials is also of great nutritional interest, especially for the amino acid profile of the ingredients as plant materials, unlike bovine dairy, have lesser amino acids and comparatively poorer digestibility (Cichonska *et al.*, 2022). Hence, the purposeful blending of plant materials could help to achieve a more complete amino acid profile without fortification (Pua *et al.*, 2022).

Conclusion

The possibility of making suitable yoghurt analogues from Bambaraunt and millet mixes was studied. To determine the qualitative qualities of the yoghurts and to investigate the market potential, nutritional, physico-chemical, microbiological, and sensory analyses were performed. The chemical composition of the bambaranut-millet yoghurt analogue samples and the commercial yoghurt manufactured from cow's milk was found to be almost identical in this investigation. The samples were clear of faecal contamination microbiologically. The sensory evaluation of the samples revealed that yoghurts made from bambaranut-millet milk differed significantly ($p < 0.05$) in some of the sensory attributes from those made from cow's milk, especially for overall acceptability, taste and flavour. However, all the yoghurt analogues received overall mean likeable and acceptable score of > 6.00 . Thus, suitable yoghurt analogues may be created with bambaranut and millet mixes with varied replacements up to 50% to make acceptable and economical yoghurt analogues. Diversification of yoghurt products should be prioritised for continued business growth and to meet unmet customer demand for safe and healthy goods. In summary, blending dif-

ferent raw materials could result in an ingredient with improved properties for subsequent formulation or fermentation into a plant-based dairy analogue with improved sensorial and nutritional properties. Therefore, the findings of this study demonstrated that yoghurt including grains and legumes can be processed using existing technologies, has a high nutritional content, and the processing technique produces microbiologically safe end products. More research is needed to discover remedies to prevent the gelatinization problem in millet milk production. Furthermore, research into utilising Bambaranut-millet milk mixes in the same way as cow and buffalo milk for cheese, curd manufacturing, and ice cream should be conducted. Shelf life, storage stability, and protein digestibility of yoghurt analogue products should all be researched.

Compliance with Ethical Standards

Conflict of interests: The author declares that for this article they have no actual, potential, or perceived conflict of interest.

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Afetlerde toplum beslenmesi ve hassas gruplara yönelik beslenme planı

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ÖZ

Afetler, bireylerin psikolojik ve fizyolojik savunmasızlığını arttırmaktadır. Gıda kaynaklarının kısıtlanması, silahlı güçlerle gıda kaynaklarına erişimin baskılanması, ulaşım ve pazarlama sisteminin bozulması ve artan ekonomik hasar afet bölgelerinde ciddi gıda açığına neden olmaktadır. Afetlerde bireylerin ekin ekme veya mahsul toplama işlemleri oldukça zor olmaktadır. Ayrıca afet alanında tohum, zirai ilaç, gübre temini ve sulama sistemleri üzerinde baskı oluşmaktadır. Afetlerde bireylerin önerilen enerji miktarı altında yetersiz beslenmesi enfeksiyon duyarlılığını arttırmaktadır. Afetlerde toplumda hem enfeksiyon hastalıklarının sıklığı ve şiddeti artmakta hem de bireylerin iyileşme süresi uzamaktadır. Afetlerde toplumun ve toplum içerisindeki hassas gruplarda yer alan afetzedelerin sağlığının korunması ve sürdürülmesi amacıyla afet beslenme planının hazırlanması oldukça önemlidir. Bu çalışmada afetlerde toplumun beslenmesi ve hassas gruplara yönelik beslenme planı irdelenmiştir.

Anahtar Kelimeler: Afet, Toplum beslenmesi, Hassas gruplar, Beslenme planı

ABSTRACT

Community nutrition in disasters and nutrition plan for vulnerable groups

Disasters increase the psychological and physiological vulnerability of individuals. Restriction of food resources, restriction of access to food resources by armed forces, deterioration of transportation and marketing systems, and increasing economic damage cause serious food shortages in disaster areas. In disasters, it is very difficult for individuals to plant or collect crops. In addition, there is serious pressure on the supply of seeds, pesticides, fertilizers, and irrigation systems in the disaster area. In disasters, inadequate nutrition of the individual under the recommended amount of energy increases the susceptibility to infection. As a result of this situation, both the frequency and severity of infectious diseases increase in society and the recovery period of individuals is prolonged. It is very important to prepare a disaster nutrition plan in order to protect and maintain the health of the disaster survivor society and the vulnerable groups within the society. In this study, the nutrition of the population in disasters and the nutrition plan for vulnerable groups were examined.

Keywords: Disaster, Community nutrition, Vulnerable groups, Nutrition plan

Giriş

Afet, toplumun tamamında veya belirli bir kısmında fiziksel, ekonomik ve sosyal kayıplara yol açan, olağan yaşamı etkileyerek insan faaliyetlerini kesintiye uğratan veya tamamen durduran, yerel kaynakların yetersiz kaldığı, toplumun mücadele etme kapasitesini aşan doğa, insan veya teknolojik kaynaklı olaylardır (AFAD,2022). Şiddetli soğuk, kıtlık ve kuraklık yavaş gelişen; deprem, sel, çığ, volkan ve heyelan ani gelişen afetlere örnektir. Savaş, göç, ulaşım kazaları, endüstriyel, nükleer, biyolojik ve kimyasal kazalar ise teknolojik veya insan kaynaklı afetler kategorisinde yer almaktadır (Bahadır ve Uçku, 2018). Afetlerin etkileri, insani krizlerin karakterinin insani krizlerin karakterlerini oluşturmaktadır. Afetler; siyasi istikrarsızlık, silahlı çatışmalar, göç, gıda kıtlığı, demografik yapıda bozulma ve halk sağlığı sistemlerinin çökmesine neden olmaktadır (Gasseer ve ark., 2004).

Afetlerde Besin ve Beslenme Sorunu

Afet maruziyeti, bireylerin psikolojik ve fizyolojik savunmasızlığını arttırmaktadır. Afetlerin olumsuz etkileriyle artan savunmasızlık, toplumun farklı katmanlarında akut ve geç dönem sağlık sorunlarının ortaya çıkmasına neden olmaktadır. Afetlerin meydana geldiği andan itibaren veya sonrasında gelişen veya gelişebilecek sağlık sorunlarının temel nedenleri üç kategori altında incelenmektedir (Etiler, 2012).

Bunlar:

- Çadır veya barınak gibi geçici yerleşim alanlarında yaşama (kalabalık yaşam ve kötü hijyen koşullarının neden olduğu bulaşıcı hastalıkların gelişimi/yayılımı),
- Gıda yetersizliği/kıtlığı (akut dönemde gıda kıtlığı, geç dönemde yetersiz ve dengesiz beslenme),
- Doğanın etkilenmesi veya bozulmasıdır (kimyasallarla veya cesetlerle suyunun kirlenmesi, zehirlenmeler, sivrisineklerin üremesi).

Gıda kaynaklarının kısıtlanması, silahlı güçlerle gıda kaynaklarına erişimin baskılanması, ulaşım ve pazarlama sistemlerinin bozulması ve artan ekonomik hasar afet bölgelerinde ciddi bir gıda açığına neden olmaktadır. Afetlerde bireylerin ekin ekme veya mahsul toplama işlemleri oldukça zor olmaktadır. Ayrıca afet bölgesinde veya yakınında tohum, zirai ilaç, gübre temini ve sulama sistemleri üzerinde baskı oluşmaktadır (Toole ve Waldman, 1997).

Doğa ve teknolojik (insan) afetlerde halk sağlığı çalışmalarının özellikle ishal, sıtma, kızamık, akut solunum yolu enfek-

siyonları ve beslenme yetersizliği üzerine odaklanması gerekmektedir (Toole ve Waldman, 1997). Afetlerde yüksek prevelanslı akut malnütrisyon ve mikro besin eksikliği sorunları görülmektedir (Young ve ark., 2004). Afet bölgesindeki akut beslenme yetersizliği toplum sağlığı için acil bir sorundur (Woodruff ve Duffield, 2002).

Açlık, kuraklık ve silahlı çatışmalar malnütrisyon insidansını arttırmaktadır (Amagai ve ark., 2014). Dünya nüfusunun dörtte birinden fazlası silahlı çatışmalara maruz kalan ülkelerde yaşamını sürdürmektedir (Corley, 2021). Çocukların yaklaşık %60'ının silahlı çatışma bölgelerinde veya çatışmadan etkilenen bölgelerde yaşadığı tahmin edilmektedir (Howell ve ark., 2020). Afet bölgesindeki 6 aylık bebek ve 5 yaş altı çocukların vücut ağırlığının ölçülmesi afetten etkilenen popülasyonun beslenme durumu hakkında bilgi vermektedir (Woodruff ve Duffield, 2002). Çocuklarda beslenme yetersizliği, boy-ağırlık Z skoru ve orta-üst kol çevresi ölçülerek saptanmaktadır. Boy-ağırlık Z skoru, 6-59 ay arası çocuklarda 2'den az veya orta-üst kol çevresi 11,5-12,5 cm arasında ise orta derecede malnütrisyon, boy- ağırlık Z skoru 3'ten az veya orta-üst kol çevresi 11,5 cm az ise ciddi malnütrisyon mevcuttur (Corley, 2021). Kosova'da 1999 yılında meydana gelen hava saldırısı sonrasında çocukların boy-ağırlık Z skorunun 2'nin altında olduğu saptanmıştır. Çocukların büyük bir bölümünde ise ödem gözlemlenmiştir.

Bu durum akut malnütrisyon prevelansının yüksek olduğunu göstermektedir. Yoğun iç çatışmaların hakim olduğu Afganistan'da 2000 yılında akut malnütrisyon prevelansının %5 ile %8 arasında olduğu tespit edilmiştir (Young ve ark., 2004). Silahlı çatışmaların ve kuraklığın hakim olduğu Yemen'de nüfusun üçte ikisi gıda güvencesizliği sorunu yaşamaktadır. Bu duruma ek olarak yaşanan kuraklık, kıtlık riskini doğurmaktadır. Somali toplumu da aynı gerekçeler ile gıda güvencesizliği yaşamaktadır. Japonya'da 2011 yılında 9.0 büyüklüğünde gerçekleşen tsunami ve nükleer felakete neden olan deprem sonrasında geçici sığınakta kalan bireylerde sodyum ve vitamin eksikliği düzeyinin arttığı saptanmıştır (Amagai ve ark., 2014). Teknolojik veya insan kaynaklı afetler kategorisinde yer alan göç sırasında ve sonrasında göçmenler beslenme sorunu yaşamaktadır. İstanbul'da yaşayan geçici koruma statüsündeki Suriyeli bireylerin karbonhidrat ağırlıklı tek öğün ile beslendiği, yeterli vitamin, mineral ve protein almadığı, yemek pişirmek için gerekli aletlerinin ve gıda malzemelerinin yetersiz olduğu saptanmıştır (Bahadır ve ark., 2016).

Afetlerde Toplum Beslenme Planı

Afetlere karşı ulusal ve yerel düzeyde müdahale organizasyonun sistematik olarak planlandığı Türkiye Afet Müdahale Planı'na göre afetlerde beslenme hizmetlerinin sorumluluğu Türk Kızılayı'na verilmiştir. Türk Kızılayı'na beslenme hizmetleri konusunda ulusal düzeyde İçişleri Bakanlığı, Sağlık Bakanlığı, Aile ve Sosyal Hizmetler Bakanlığı, Tarım ve Orman Bakanlığı destek sağlamaktadır. Bu kurumların taşra teşkilatları, Sivil Toplum Kuruluşları, özel sektör ve belediyeler Türk Kızılayı'nın yerel düzeyde iş birliği yaptığı birimlerdir (Usluer, 2021).

Afetlerde beslenme planı, altın saatler olarak adlandırılan afet sonrasındaki ilk 72 saat ve 72 saat sonrasına göre planlanmaktadır. Afet bölgesinde 0-72 saatlik süreç içerisinde gerçekleştirilen beslenme faaliyetleri erken dönem beslenme hizmetleridir (Türk Kızılayı, 2021).

Altın saatler (0-72 saat) içerisinde tedariki kolay, işlem gerektirmeyen, tüketimi kolay ve dayanıklılığı yüksek gıdaların sunulması önemlidir. Altın saatler içerisinde ekmek, simit, kek, meyve suyu bisküvi ve sıcak içeceklerin dağıtımı uygundur. Öncelikle ikram araçları ile gerçekleştirilen beslenme hizmetleri olayın büyüklüğüne göre mobil mutfak, mobil fırın, seyyar mutfak ve mutfak kitleri ile sürdürülmektedir. Altın saatler içerisinde sıcak yemek hazırlanana kadar bireylere acil beslenme kiti dağıtılmaktadır. Acil beslenme kiti, 1 adet 0,25 ml su, 1 adet tatlı bisküvi ve 1 adet 200 ml meyve suyundan oluşmaktadır. İlk 72 saat içerisinde afetzedelere kumanya dağıtılmalıdır. Kumanya paketleri içerisinde ekmek, peynir, meyve, helva ve içme suyu bulunmaktadır. Kumanyalar ihtiyaç halinde ara öğün olarak da dağıtılabilir. Afetlerde beslenme hizmetlerinin amacı bireylere besleyici değeri yüksek ve yüksek enerjili gıdaların sunulmasıdır. Yüksek kalori değerine sahip gıdalar (hamburger, pizza vb.) kan şekeri düzeyinde ani değişikliklere neden olup bireyleri yorgun bırakabileceği için tercih edilmemelidir (Oral ve ark., 2017).

Altın saatler sonrasında gerçekleştirilen beslenme hizmetleri uzun dönem beslenme hizmetlerini kapsamaktadır. Erken dönem beslenme hizmetleri sonrasında sıcak yemek hizmeti, afetzede ailelere yemek setinin dağıtılması ve kuru gıda yardımının yapılması planlanmalıdır. Uzun dönem beslenme hizmetleri kapsamında 5 kişilik bir ailenin ortalama 7 günlük beslenme ihtiyacını karşılayabilecek gıda kolisi hazırlanmalıdır. Gıda kolileri, Tip 1 ve Tip 2 olmak üzere iki çeşittir (Doğru ve Ede, 2020). Tip 1, içerisinde barbunya bulunan, Avrupa ve Balkan ülkeleri tarafından hazırlanan gıda kolileridir. Tip 2 ise içerisinde nohut bulunan Arap ve Ortadoğu ülkeleri tarafından hazırlanan gıda kolileridir. Her iki tip gıda kolisi ortalama 20,75 kg ağırlığa sahiptir.

Her iki gıda kolisi içinde 2,5 kg pirinç, 2 kg kuru fasulye, 5 kg un, 5 kg şeker, 0,75 kg tuz, 2 kg makarna ve 2,5 kg bulgur bulunmaktadır (Oral ve ark., 2017). Sphere Afete Müdahalede Asgari Standartlar ve İnsani Yardım Sözleşmesi'ne göre dağıtımı yapılacak her gıdanın en az 6 ay raf ömrünün olması gereklidir. Sphere Afete Müdahalede Asgari Standartlar ve İnsani Yardım Sözleşmesi, acil durum ve afet bölgesine süt tozu dağıtımının yapılmaması üzerinde durmaktadır (Sphere Projesi, 1998).

Bireylerin yemeklerini pişirebilmesi ve muhafaza edebilmeleri için mutfak setinin dağıtımı oldukça önemlidir. Beş kişilik bir ailenin beslenme ihtiyacını karşılamak üzere mutfak seti içerisinde; 5 adet sahan, 5 adet tabak, 5 adet çatal, 5 adet kaşık, 5 adet bardak, 1 adet sürahi, 1 adet çorba kepçesi, 1 adet yemek kepçesi, 1 adet tava, 1 adet büyük tencere, 1 adet küçük tencere, 1 adet kova, 1 adet leğen, 1 adet tuzluk, 4 adet sabun, 4 adet bulaşık süngeri ve 1 adet 20'li orta boy çöp torbası bulunmaktadır (Oral ve ark., 2017).

Afetlerde bireyler için günlük 2100 kcal enerji önerilmektedir (Yılmaz ve Arslan, 2018).

Önerilen bu enerji miktarı nüfusun %20'sinin adolesan (10-19) ve %56'sının yetişkin olduğu varsayılarak hesaplanmaktadır (Woodruff ve Duffield, 2002). Ayrıca önerilen enerji miktarı etkilenen toplumun sosyodemografik özelliklerine ve ortam şartlarına göre değişiklik göstermektedir. Hava sıcaklığının 20 °C altında olduğu bir afet bölgesinde afetzedeler için gerekli enerji miktarı her 5° C'de 100 kcal arttırılmalıdır. Afetlerde bireyler için önerilen 2100 kcal enerji için 400 g tahıl, 60 g baklagil, 25 g yağ ve 15 g şeker ihtiyacı duyulmaktadır (Yılmaz ve Arslan, 2018). Afetlerde ihtiyaç duyulan enerji gereksiniminin %10-12'si proteinlerden %17'si ise yağlardan karşılanmalıdır (Oral ve ark., 2017).

Afetzede bireyin önerilen enerji miktarı altında yetersiz beslenmesi enfeksiyon duyarlılığını arttırmaktadır. Afet bölgesinde enfeksiyon hastalıklarının sıklığı, şiddeti ve süresi uzamaktadır (Etiler, 2012). Yeterli ve dengeli beslenme, afetzede bireylerin ortam şartlarına uyumunu kolaylaştırarak fiziksel direncini arttıracaktır. Böylece bireylerin hastalık yükü azalacaktır (Güler ve Çobanoğlu, 1994).

Afetzede her birey için en az 15 litre temiz içme suyunun bulunması önerilmektedir (Etiler, 2012). Sphere Afete Müdahalede Asgari Standartlar ve İnsani Yardım Sözleşmesi her 250 kişiye en az bir su noktası bulunması gerekliliğini belirtmektedir (Sphere Projesi, 1998). Acil durum ve afetten etkilenen bölgede bireylere dağıtılan temiz içme suyu dehidratasyon gelişimini önleme amacıyla oldukça önemlidir (Etiler, 2012). Van-Erciş merkezli 23 Ekim 2011 tarihinde gerçekleşen dep-

rem sonrasında kurulan çadır kentlerde suyun donması sonucunda su kesintilerinin olduğu bildirilmiştir (Hassoy ve Durgun, 2020). Nepal’de 2015 yılında 7,8 büyüklüğünde gerçekleşen deprem sonrasında şehir su şebekeleri hasar görmüştür (Araç Özcan ve Ateş Duru, 2021).

İçme ve kullanma sularında ve gıdalarda meydana gelen kirleşme, ishaller hastalıkların artmasına ve ishal nedenli ölümlerin görülmesine neden olmaktadır. İshal nedenli bildirilen çocuk ölümlerinin %54’ünün malnütrisyon kaynaklı olduğu tahmin edilmektedir. İshalin neden olduğu dehidratasyon ile mücadele amaçlı afetzedelere oral dehidratasyon sıvısı verilmelidir (Etiler, 2012).

Afet bölgesinde ortam şartları göz önüne alınarak enerji değeri yüksek, besleyici, hızlı doku onarımı sağlayan, ishali önleyici ve tedavi edici özelliği bulunan, dayanıklı, depolaması, pişirmesi ve tüketimi kolay gıdaların temin edilmesi oldukça önemlidir. Afettede bireylere temin edilen tahıllı gıdalar (ekmek, bulgur, pirinç, bisküvi çorba vb.), bireyin acil enerji açlığını karşılamakla birlikte bireye protein, B grubu vitaminleri ve posa desteğinde sağlamaktadır. Afettede bireylere ulaştırılan kuru baklagiller (nohut, fasulye, mercimek vb.) dayanıklı, ucuz ve içerdiği demir, çinko, B grubu vitaminler ve protein ile besleyici değeri yüksektir. Afet bölgesine dağıtılan süt ve süt ürünleri (peynir, yoğurt vb.) içerdiği folik asit, riboflavin, kalsiyum ve protein ile sindirim sistemini destekleyerek vücut direncinin artmasını, ishali önlenmesini ve tedavi edilmesini sağlamaktadır.

Afettede bireylere tedarik edilen yumurtanın pişirilebilmesi kolay olduğu gibi A, D ve E vitamini, yüksek protein, çinko ve demir içeriği ile yüksek besleyiciliğe sahiptir. Afet bölgesine ulaştırılan yağlı tohumların (ceviz, badem, fındık vb.), tahin, pekmez gibi gıdaların besin değeri yüksek, yoğun enerji verici özellikte ve tüketimi kolaydır (Çiftçi, 2016).

Hassas Gruplarda Beslenme Planı

Afet sırasındaki ve afet sonrasındaki etkilere en fazla maruz kalan grup hassas gruplardır. Yalnız çocuklar, 0-5 yaş arasındaki çocuklar, adölesanlar, lezbiyen, gey, biseksüel ve transseksüel ve interseks (LGBTİ) bireyler, etnik azınlık gruplar, mülteciler, yaşlılar, gebe ve emzikli kadınlar, fiziksel ve psikolojik engeli bulunan bireyler hassas gruplar olarak tanımlanmaktadır (Soner ve Avcı Aydın, 2019). Afet bölgesinde bu grupta malnütrisyon, ishal, hepatik ve tüberküloz gibi enfeksiyon hastalıklarının gelişimini önlemek amacıyla beslenme planlaması yapılmalıdır (Aycan ve ark., 2002). Mevcut besin kaynakları belirlenerek elzem besinlerin hassas gruplara dağıtımı oldukça önemlidir (Türk Kızılayı, 2021b).

Bebek ve Çocuklarda Beslenme Planı

Afetlerde değişen şartlar bebeklerin ve çocukların beslenmesinde değişikliklere neden olmaktadır. Bebek ve çocuklarda beslenmenin negatif yönde değişimi ilk olarak kilo alımının durması veya kilo kaybı şeklinde ortaya çıkmaktadır. Yetersiz beslenme sonucu çocuğun boy uzaması yavaşlamakta veya tamamen durmaktadır. Marmara depreminden iki ay sonra yapılan çalışma ile afettede çocukların %3,8’inin boylarına göre ağırlık puanlarının zayıflık tehlikesi grubunda yer aldığı ve her 6 çocuktan birinin boyunun normal ölçülerin altında seyrettiği saptanmıştır. Beslenme sorunları ile mücadele etmek amacıyla çocuklar dört dönemde incelenmelidir. Bunlar (Etiler, 2012):

- 0-6 aylık bebekler
- 6-12 aylık bebekler
- 1-6 yaş okul öncesi dönem
- 7 yaş ve üzeri çocuklar

Bebeklerin hem besin kaynağı hem de bebek bağışıklık sisteminin temel ögesi olan anne sütünün önemi afetlerde daha önemli olmaktadır. Anne sütü içeriğindeki antibakteriyel ve antiviral ögeler afet bölgesindeki bebekler için su ve besinlerle bulaşan hastalıklara karşı koruma kalkanı oluşturacaktır (Etiler, 2012). Afet bölgesinde emziren kadının psikolojik ve fizyolojik nedenlerle süt salgısının azalması veya emziren kadının yaşamını kaybetmesi sonucu 0-6 aylık bebek sağlığı risk altındadır. Bu dönemde bebeklerin ihtiyacı olan besin değerleri süt, yoğurt, meyve püreleri ve sebzelerle desteklenmelidir (Yılmaz ve Arslan, 2018). Risk kategorisinde yer alan 0-6 aylık afettede bebekler muhallebi, bisküvi, tahıl veya sebze çorbaları ile doyurulmalıdır (Aycan ve ark., 2002).

Bu grupta yer alan afettede bebeklere mısır-soya veya buğday-soya şeklinde kombinlenmiş gıdalar verilebilir. Protein içeriği artırılmış çinko, demir, vitamin ve mineralden zengin bileşene sahip gıdalar afettede bebekler için planlanmalıdır (Oral ve ark., 2017). Anne sütüne ulaşamayan bebeklerin beslenmesi mama veya formüle ile desteklenmelidir. İran’da gerçekleşen deprem sonrasında 2 yaş altı çocuklara besin takviyesi olarak A ve D vitaminleri kalsiyum, demir ve multivitamin desteği verilmiştir (Doğru ve Ede, 2020). 6-12 ay arasındaki bebeklere ise ek olarak kuru baklagiller ve yumurta verilmelidir (Yılmaz ve Arslan, 2018). Demir eksikliğinin ek gıda döneminde yaygın olarak görülmesi afet bölgesindeki bebeklere demir desteğinin önemini ortaya koymaktadır (Etiler, 2012). Kızamık enfeksiyonu ve ishali bulunan çocukların A vitamini rezervi hızla azalmaktadır.

Azalan A vitamini rezervi antijenlere karşı antikor gelişimini azaltmaktadır (Besler ve Aksoy, 1990). Böylece afetzedenin bedeni enfeksiyonlara karşı duyarlı hale gelmektedir (Toole ve Waldman, 1997).

Bebek beslenmesinde bebeğin mevcut besin alerjisi olup olmadığı bilgisi alınmalıdır veya akut gelişen bir alerji durumu olup olmadığı saptanmalıdır (Yılmaz ve Arslan, 2018). Acil durum ve afetin yarattığı koşullar nedeniyle 1-6 yaş ve üzeri yaşlardaki çocukların beslenme sorunları çocuk beslenmesinin ihmali şeklinde karşımıza çıkmaktadır (Etiler, 2012). Özellikle aile bireylerinin tamamı vefat eden veya ailesi bulunamayan çocukların sağlık taraması en kısa sürede yapılmalıdır. Çocuğun psikososyal çevresi geliştirilmelidir. Gerekli psikolojik destek sağlanarak geliştirilen güven duygusu ile çocuk beslenmesi planlanmalıdır. Ebeveynlerini kaybetmiş bir çocuğa bakan gönüllü, komşu veya akrabalara ek gıda yardımı yapılmalıdır (Kaya ve Özcebe, 2013).

Gebe ve Emziren Kadınların Beslenme Planı

Emziren kadınların ihtiyaç duyduğu enerji miktarı diğer kadınlardan 500 kcal daha fazladır (Yılmaz ve Arslan, 2018). Hamile kadınların ise ihtiyaç duyduğu enerji miktarı 285 kcal artmaktadır (Oral ve ark., 2017). Hem anne hem de bebek sağlığı için A vitamini, demir, folat ve iyot içerikli besinlerin tüketilmesi önemlidir (Yılmaz ve Arslan, 2018). Yeni doğum yapmış bir kadının 6 hafta A vitamini alması gerekmektedir. İhtiyaç durumunda gebe afetzedelere 60 mg demir ve 400 g folik asit verilmelidir (Oral ve ark., 2017). Sıtma enfeksiyonu demir depolarını azaltmaktadır. Bu durum sonucunda anemi gelişimi hızlanmaktadır. Özellikle gebelik durumunda gelişen anemi bebeğin demir rezervini tehdit etmektedir (Gasseer ve ark., 2004). Afettede gebelerde anemi temelli ölü doğum, prematür doğum ve immün yetmezlik komplikasyonları ortaya çıkmaktadır. Afet temelli beslenme yaklaşımı ile gebeler nütrisyonel anemi riskinden korunmalıdır (Yurdakök ve İnce, 2009). Taze meyveler temiz su ile iyice yıkanarak fetüsün pestisitlere maruz kalması önlenmelidir.

Afet bölgesindeki gebelerin günlük 2 su bardağı süt veya yoğurt tüketmesi gereklidir (Oral ve ark., 2017). Hamile veya emziren kadınların orta-üst kol çevresinin 18,5-22,9 cm arasında olması akut orta malnütrisyonu göstermekteyken orta-üst kol çevresinin 18,5 cm'den az olması ciddi derecede malnütrisyonu göstermektedir (Corley, 2021). Afet sonrasında ergen gebe kadının beslenme programının düzenlenmesi ve takibi anne-bebek ölümü, erken doğum ve doğum komplikasyonlarının önüne geçilmesi konusunda önem taşımaktadır (Woodruff ve Duffield, 2002). Gebe ve emzikli kadınların zenginleştirilmiş gıda ürünlerini alması desteklenmelidir. Zenginleştirilmiş gıda ürünlerinin %10-12'si protein ve %20-

25'i yağlardan oluşmaktadır. Bu içerik gebe ve emziren kadının besin ihtiyacının üçte ikisini karşılamaktadır (Oral ve ark., 2017).

Yaşlılarda ve Kronik Hastalığı Olan Bireylerin Beslenme Planı

Afetlerin neden olduğu değişen koşullara yaşlı bireylerin hızlı adaptasyonu diğer bireylere kıyasla daha düşüktür. ABD'de 2005 yılında gerçekleşen Katrina kasırgasının etkilendiği Luisiana ve New Orleans popülasyonunun ancak %16'sı 60 yaş ve üzeri yaşta bireylerden oluşmaktayken ölenlerin %70'inin 60 yaş ve üzeri bireyler olduğu belirtilmiştir. Japonya'da 2011 yılında gerçekleşen Thoku depremi ve tsunami felaketi sırasında ölen bireylerin %56'sının ve afet sonrasında ölenlerin %89'unun 65 yaş ve üzeri yaşta bireylerden oluştuğu saptanmıştır (Çakır ve Atalay Aydoğmuş, 2020). Yaşla birlikte gelişen kas kaybı güçsüzlüğe neden olmaktadır. Yaşlı bireylerin bazal metabolizma hızı, günlük enerji ihtiyacı azalmaktadır. Yaşlı bireylerde iştahsızlık, sindirim sistemi bozuklukları ve besine ulaşma zorluğu görülmektedir (Doğru ve Ede, 2020). Afet bölgesinde yaşlı bireylerin beslenmesi fiziksel bağımlılığı dikkate alınarak planlanmalıdır. Yaşlı bireyler besinleri çiğneme ve yutmada zorluk yaşamaktadır.

Bu nedenle afettede yaşlıların protein ve mikro besin ihtiyacının karşılanması amacıyla püre kıvamında gıdalar sunulmalıdır (Doğru ve Ede, 2020). Özellikle parkinson hastaları besinleri ağızına götürmekte zorluk çekmektedir. Beslenememe sorunu yaşlı bireylerde konstipasyon (kabızlık) sorununun sık yaşanmasına sebep olmaktadır. Bu nedenle afettede yaşlı bireylerin sıvı ve posadan zengin gıdaları alması planlanmalıdır. Yaşlı afetzedelerin laktasif etkili kuru meyveleri tüketmeleri sağlanmalıdır. Ayrıca demans ve alzheimer hastası yaşlı afetzedelerin takip edilmesi oldukça önemlidir. Çünkü yemek yediğini unutan veya yemediği halde yediğini sanan yaşlı afetzedelerin beslenme sorunları yaşamaları kaçınılmazdır (Çakır ve Atalay Aydoğmuş, 2020).

Afetlerde yaşlı ve kronik hastalığı olan bireylerin beslenmesinin bozulması kronik hastalıklarının alevlenmesine neden olabilir. Kronik hastalığı bulunan afetzedelerin öğün sayısı, besin miktarı hastalık ve birey özelinde planlanmalıdır. Özellikle diyabet tanılı afetzedelerin kan şekeri düzeyi takip edilmelidir. Diyabet hastası bireylerin kan şekeri düzeylerinin ani düşüş ve yükselişini önlemek amacıyla pirinç yerine bulgur pilavı, beyaz ekmek yerine kepekli ekmek, meyve suyu yerine taze meyveleri tüketmeleri sağlanmalıdır. Hipertansiyon tanılı afetzedelerin magnezyum, potasyum ve kalsiyum içeren besinleri alması tansiyon düzeyi üzerinde etkili olmakta-

dır. Hipertansiyona sahip afetzedenin öğününde muz, portakal, şeftali, kayısı, mandalina, patates, ceviz ve fındık olacak şekilde planlanmalıdır (Yılmaz ve Arslan, 2018).

Hipertansiyon tanılı afetzedelerin öğünlerinden turşu, konserve gıdalar ve yüksek kalorili gıdaların çıkarılması gerekmektedir (Oral ve ark., 2017). Besinlerin emilimi için gerekli enzimlerin salınmaması ile karakterize olan kistik fibroz hastalık tanılı afetzedelerin besin emilimi oldukça güçtür. Bu afetzedelerin kalori ihtiyacı hastalık özelinde planlanmalıdır (Unicef, 2013). Bağışıklık sisteminin baskı altında olduğu afetzedelerin vücut bağışıklığının A, E, C ve B vitamini, çinko, demir, karoten ve selenyum içeren gıdalarla desteklenmesi oldukça önemlidir. Bu afetzedelerin besin planı tekrarlayan enfeksiyon riskini azaltacaktır (Oral ve ark., 2017).

Engelli Bireylerin Beslenmesi

Engelli bireyler, azalmış hareket kabiliyetine ek olarak afetlerde yakınlarını kaybettiği için yetersiz besin alımı ile karşı karşıya kalmaktadır. Yarı damaklı bir bebeğin afet bölgesinde beslenmesi zor olabilir. Serebral palsi hastası bir afetzede çocuğun çiğneme ve yutma engeli bulunmaktadır (Unicef, 2013). Engelli afetzedelerin uygun pozisyon veya duruşta beslenmesine özen gösterilmelidir. Bu durum engelli afetzedenin boğulma riskini azaltacaktır. Engelli bireylerin hareket kısıtlılığı güneş ışığından yararlanımı azaltmakta veya tamamen durdurmaktadır. Bu nedenle engelli afetzedelerin öğünleri D vitamini içeren gıdalar ile desteklenmelidir. Engelli afetzedelere magnezyum, demir ve çinkodan zengin gıdalar temin edilmelidir (Oral ve ark., 2017). Yutma güçlüğü bulunan engelli afetzedelere püre kıvamlı gıdalar sunulmalıdır. Engelli bireylerin afet bölgesinde toplumla sosyal olarak bütünleşmesi amacıyla toplumsal etkinliklere katılımları sağlanmalıdır (Altun, 2016).

Sonuç

Afetzede toplumun ve toplum içerisindeki hassas gruplarda yer alan afetzedelerin sağlığının korunması ve sürdürülmesi amacıyla afet beslenme planının hazırlanması oldukça önemlidir. Hassas gruplarda yer alan afetzedelerin besin ve kalori ihtiyaçları özenle ele alınmalıdır. Afet bölgesinde toplumun pişirme ve beslenme alışkanlıkları ile beslenme geleneği göz önüne alınarak adil bir planlama ve dağıtım faaliyetleri yürütülmelidir. Gereksiz ve afetzede toplumun kültürüne uymayan gıdalar topluma ulaştırılmamalıdır. Mevcut besin kaynakları belirlenerek elzem besinlerin hassas gruplara dağıtımı oldukça önemlidir. Hassas gruplara yönelik beslenme kartları oluşturulmalıdır. Böylece birey özelinde gıda temini, kalori gereksinimi ve beslenme planı geliştirilmektedir. Hassas gruplara yönelik beslenme ve tedavi amaçlı terapötik gıdalar kullanılabilir.

Özellikle hassas grupların yoğun olduğu afet bölgesinde gıda payı hesaplanırken oluşabilecek kalori kaybı göz önünde bulundurulmalıdır.

Etik Standartlar ile Uyumluluk

Çıkar çatışması: Yazarlar bu yazı için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan etmişlerdir.

Etik izin: Araştırma niteliği bakımından etik izne tabii değildir.

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Dut meyvesinin fitokimyasal profili ve farmakolojik özellikleri

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ÖZ

Dutgiller (*Moraceae*) familyasından *Morus* türlerinin meyveleri geleneksel olarak sağlık üzerine olumlu etkileri dolayısıyla yaygın olarak tüketilmektedirler. İçerdiği biyoaktif bileşenler (flavonol, antosiyanin, flavanonol, fenolik asitler, alkaloitler), mineraller, (potasyum, kalsiyum, sodyum ve magnezyum), vitaminler (B1 vitamini ve folik asit), yağlar (linoleik asit, palmitik asit, oleik asit) ve melatoninlerden dolayı birçok araştırmaya konu olmuştur. Yapılan *in vitro* ve *in vivo* araştırmalarda meyvelerindeki biyoaktif bileşenlerden dolayı antidiyabetik, antioksidan, nöroprotektif, hipolipidemik, anti-obezite, anti-inflamatuar, hepatoprotektif, antiparkinson, anti-tümör/ anti-kanser, antimikrobiyal ve bağışıklığı artırıcı etki gibi sağlık üzerine olumlu etkilerinin bulunduğu bildirilmiştir. Ayrıca, geleneksel bitkisel ilaç olarak tüketilen dut; diyabet, hipertansiyon, karaciğer, böbrek hasarı ve göz hastalığı tedavisinde yaygın olarak kullanılmaktadır. Ancak dut meyvelerinin farmakolojik özelliklerinin incelendiği *in vitro* çalışmalar daha yaygınken *in vivo* (hayvan ve klinik) çalışmalar ise sınırlı sayıdadır. Bu derlemede, *Morus alba*, *Morus rubra* ve *Morus nigra* meyvelerinin fitokimyasal profili ile *in vitro* ve *in vivo* farmakolojik özellikleri incelenmiştir.

Anahtar Kelimeler: *Morus alba*, *Morus rubra*, *Morus nigra*, Fitokimyasal bileşenler, Farmasötik özellikler

ABSTRACT

Phytochemical profile and pharmaceutical properties of mulberry fruits

The fruits of *Morus* species from the Mulberry family (*Moraceae*) are traditionally consumed widely due to their positive effects on health. The interest about its health effect are increasing due to its rich content of bioactive components (flavonol, anthocyanin, flavanonol, phenolic acids, alkaloids), minerals (potassium, calcium, sodium, and magnesium), vitamins (vitamin B1 and folic acid), oils (linoleic acid, palmitic acid, oleic acid) and melatonin. *In vitro* and *in vivo* studies indicated that the fruits have positive health effects such as anti-diabetic, antioxidant, neuroprotective, hypolipidemic, anti-obesity, anti-inflammatory, hepatoprotective, antiparkinsonian, anti-tumor/anti-cancer, antimicrobial and immune-enhancing effects due to the bioactive components. In addition, mulberry fruit are traditionally consumed for their diabetes, hypertension, liver, kidney damage, and eye improvement effects. However, there is only limited clinical studies about the pharmacological properties of mulberry fruits whereas more *in vitro* research are present. In this review, the phytochemical profile, *in vitro* and *in vivo* pharmacological properties of *Morus alba*, *Morus rubra*, and *Morus nigra* fruits were investigated.

Keywords: *Morus alba*; *Morus rubra*, *Morus nigra*, Phytochemical compounds, Pharmacological properties

Giriş

Son yıllarda, doğal ürünlere ve özellikle biyolojik olarak aktif bileşiklerce zengin olan meyve ve sebzelere antioksidan özellikleri ve bazı metabolik sendrom risklerini azaltma potansiyelleri nedeniyle artan bir ilgi vardır (Cao ve ark., 2018; Ma ve Zhang 2017; Veeresham, 2012). Onaylanan ilaçların yaklaşık %50'si doğal ürünlerden oluşmaktadır (Veeresham, 2012). Birçok ülkede yaşayan popülasyonların yaklaşık %80'i bitkisel ilaçlara güvenmektedir. Bitki kaynaklı ilaç pazarının ise 2020'de yaklaşık 35 milyar dolara ulaşacağı tahmin edilmektedir (Gryn-Rynko ve ark., 2016; Veeresham, 2012).

Üzüm meyveleri, düşük kalorileri ve sağlık üzerine olumlu etkileri olduğu bilinen polifenoller, mineral maddeler ve vitaminlerce zengin fonksiyonel gıdalardır (Zhang ve ark., 2018). Üzüm meyveleri denildiği zaman daha çok üzüm (*Vitis*), çilek (*Fragaria*), ahududu ve böğürtlen (*Rubus*), frenk üzümü ve bektaşi üzümü (*Ribes*), maviyemiş (*Vaccinium*), kuşburnu (*Rosa*), dut (*Morus*), mürver yemişi (*Sambucus*) gibi cinsler ve bunlara bağlı türler akla gelmektedir.

Dut, Moraceae familyasının *Morus* cinsine aittir ve bu cinsin 24 türü ve 100 çeşidi bilinmektedir (Ercisli ve Orhan, 2007). Asya, Afrika, Amerika, Avrupa ve Hindistan dahil birçok bölgede genellikle, ipekböceklerini beslemek için yetiştirilmektedir (Khan ve ark., 2013; Ning ve ark., 2005; Yang ve ark., 2010). Geleneksel doğu tıbbında ise dut; diyabet, hipertansiyon, karaciğer, böbrek hasarı, göz iyileştirme tedavisinde ve saçların erken beyazlamasını engellemek için de kullanılmaktadır (Wei ve ark., 2018; Yang ve ark., 2010).

Meyvesinden faydalanılan ve yaygın olarak yetiştiriciliği yapılan *M. alba* L. (beyaz dut), *M. nigra* L. (karadut), ve *M. rubra* L. (kırmızı veya mor dut) dut türleri diğer dut türlerine göre daha fazla biyoaktif bileşene ve terapötik potansiyele sahip oldukları için öne çıkmıştır (Can ve ark., 2021). Ülkemizde dut ağaçları potansiyelinin %95'i *M. alba*, %3'ü *M. rubra* ve %2'si *M. nigra* türlerine ait olduğunu belirtmektedir (Ercisli, 2004).

Dut iştah açıcı bir meyvedir ve kalorisi düşüktür (Wang ve ark., 2013). Dut meyvelerinin pH'sı < 3.5 olduğu için ekşi bir tada sahiptir (Can ve ark., 2021). Dut meyvelerinin su oranı yüksek (yaklaşık %80) olduğu için muhafazası zordur (Yang ve ark., 2010). Meyveleri taze, dondurulmuş ve kurutulmuş olarak tüketilmesinin yanında şaraba, şerbete, konserveye, marmelada, sirkeye, meyve suyuna, liköre ve pekmeze dönüştürülür, aynı zamanda doğal boya olarak kozmetikte de kullanılır (Hojjatpanah ve ark., 2011; Jan ve ark., 2021; Sengül ve ark., 2005).

Dut meyveleri anti-inflamatuar, anti-kolesterol, anti-diyabetik, antioksidan ve anti-obezite etkileri gibi potansiyel farmakolojik özelliklere sahiptir (Inanc ve ark., 2020; Kang ve ark., 2006; Kim ve Park 2006; Ye ve ark., 2002; Yu ve ark., 2021; Zhang ve Shi 2010). Bu farmakolojik özellikler, antosiyaninler de dahil olmak üzere polifenol bileşiklerinin varlığına atfedilmiştir ve aynı türe ait olsa bile dut meyvelerinin farklı renklerinden dolayı farklı miktarlarda antosiyaninlere sahip olabileceği bildirilmiştir (Ramappa ve ark., 2020; Wang ve ark., 2013). Duttan izole edilen başlıca antosiyaninler ise siyanidin-3-rutinozit ve siyanidin-3-glukozittir (Liu ve ark., 2004; Suh ve ark., 2003). Dut meyvelerinden elde edilen antosiyaninlerin, düşük yoğunluklu lipoprotein (LDL) oksidasyonunu engelleyebileceği ve serbest radikalleri temizleyebileceği bildirilmiştir (Chen ve ark., 2005; Du ve ark., 2008).

M. nigra meyvesinin etanolik ekstraktlarının düzenli olarak Sprague-Dawley sıçanlarına verildiği bir çalışmada bu ekstraktın malondialdehit içeriğini azaltarak karaciğer yağlanmasını zayıflattığı ve lipid metabolizması anormalliklerini düzenlediği bildirilmiş ve bu sıçanlarda kara dut ekstraktının arteriyel ateroskleroz gelişimini baskıladığı tespit edilmiştir (Jiang ve ark., 2017).

Fitokimyasal Bileşikler

Dut meyvelerinden fitokimyasal bileşiklerin izole edilmesi ve kompozisyonunun detaylı bir şekilde belirlenmesi ile ilgili yapılan çalışmalar son yıllarda artış göstermiştir. Dut yaprakları ve kabukları ile karşılaştırıldığında, dut meyvelerinin geleneksel tıpta kullanımına daha az rastlanmaktadır (Kusano ve ark., 2002). Morbus türleri flavanoidler başta olmak üzere flavonoller, antosiyaninler ve fenolik asitlerden hidroksi-benzoik asitleri yüksek miktarda içerirler (Yang ve ark., 2016). Dut meyvelerinde bulunan başlıca fitokimyasal bileşenler Şekil 1'de kısaca özetlenmiştir.

Dut meyvelerinin (*Morus alba*, *Morus nigra* ve *Morus rubra*) fitokimyasal bileşikleri çeşitli çalışmalarda bildirilmiştir (Arfan ve ark., 2012; Chan ve ark., 2016; Imran ve ark., 2010). Chen ve ark., (2017) dut meyvelerindeki fenolik bileşik düzeylerinin böğürtlen, yaban mersini, ahududu ve çilekten daha yüksek olduğunu tespit etmiş ve dut meyvelerinin iyi bir fenolik kaynağı olarak kullanılabilirliğini bildirmiştir.

Morus alba L. dut türü meyvelerinin kırmızı, mor ve mor-kırmızı gibi farklı renkleri vardır. Aramwit ve ark., (2010) mor dut meyve özütünün, kırmızı ve mor-kırmızı dut meyve ekstraktlarından daha yüksek toplam şeker ve antosiyanin içeriğine sahip olduğunu bildirmiştir. Bu durumun antosiyaninleri sentezlemek için öncü madde olarak şekerlere ihtiyaç

duyulmasından kaynaklandığı belirtilmiştir. Bununla birlikte, kırmızı dut meyvesinin mor ve mor-kırmızı dut meyve ekstraktlarından daha yüksek bir askorbik asit ve β -karoten içerdiği rapor edilmiştir.

Dut meyvelerinde ayrıca birçok uçucu bileşik bulunmaktadır. Calín-Sánchez, ve ark., (2013) İspanya'da yetiştirilen beyaz (*Morus alba* L.) ve kara (*Morus nigra* L.) dut meyvelerinde bulunan uçucu bileşiklerin asetik asit, 3-hidroksil-2-bütanon, etil butirat, etil asetat, 3-metilbütanal, 2-metilbütanal, heptanal, metional, hekzanal, trans-2-hekzanal, 2-oktenon, hekzanoik asit, benzaldehit, metil hekzanoat, 2-etilhekzanal, oktanal, limonen, 6-metil-5-hepten-2on, etil hekzanoat, 2,4-nonadienal, fenilasetaldehit, trans-2-oktenal, cis- α -ocimene, terpinonen, 2-nonanon, nonanal, oktanoik asit, cis-2-nonenal, dodekanoik asit, terpinen-4-ol, etil oktanoat, etil dodekanoat, dekanal, dekanolik asit ve etil dekanolat olduğunu bildirmişlerdir.

Yüksek miktardaki antosiyanin içeriğinden dolayı *M. nigra* L. meyvesi siyah renklidir. Kostić ve ark., (2019) tarafından yapılan çalışmada Güneydoğu Sırbistan'daki *Morus alba*, *Morus nigra* ve *Morus rubra* dutların biyoaktif bileşenleri etanol ile ultrases destekli ekstraksiyon yöntemiyle ekstrakte edilmiş ve *Morus nigra*'nın flavonoid ve antosiyanin içeriğinin *Morus alba* ve *Morus rubra*'dan daha yüksek olduğu tespit edilmiştir. Ayrıca yapılan diğer araştırmalarda *Morus nigra*'daki baskın antosiyaninin siyanidin-3-O-glikozit olduğu bildirilmiştir (Pawlowska ve ark., 2008; Qi ve ark., 2014; Qin ve ark., 2010). D'urso ve ark., (2019) İtalya'da yetişen *Morus nigra*'da 4 farklı antosiyanin tespit etmiş olup bunların siyanidin ve pelargonidin bir, iki veya üç heksoz şekeriyle konjuge formları olduğunu bildirmişlerdir. Ayrıca *Morus nigra*'da antosiyanin biyosentezindeki öncül bileşenlerden dihidrokuersetin (taksifolin) heksosit ve dihidrokamferol heksosit flavonollarını bulmuşlardır. Benzer şekilde yapılan bir başka çalışmada ise *Morus nigra*'daki kuersetin-glikozidlerinin kamferolden daha yüksek olduğu tespit edilmiştir (Sánchez-Salcedo ve ark., 2015). Türkiye'de yetişen *Morus nigra* ve *Morus rubra* çeşitlerinin incelendiği bir başka çalışmada da *Morus nigra*'daki antosiyanin miktarının (ana bileşen siyanidin-3-O-glikozit) *Morus rubra*'dan yüksek olduğu bulunmuştur (Özgen ve ark., 2009). Yapılan farklı çalışmalarda ise *Morus nigra*'daki fenolik asitlerin flavonol ve antosiyanin miktarından daha yüksek olduğu ve fenolik asitlerden benzoik (protokateşik) ve sinamik asit (klorojenik) konsantrasyonunun flavonol ve antosiyaninlerden daha fazla bulunduğu tespit edilmiştir (Jin ve ark., 2017; Khalifa ve ark., 2018; Mena ve ark., 2016; Veberic ve ark., 2015).

Yang ve ark., (2010), dondurularak kurutulmuş *M. alba* dut meyvesinin toplam fenolik ve flavonoidler ile antosiyanin içeriğinin sırasıyla 23.0 mg/g gallik asit eşdeğeri, 3.9 mg/g rutin eşdeğeri, 0.87 mg/g siyanidin-3 glukoze eşdeğerleri olduğunu bildirmişlerdir. Dut meyve tozundaki flavonoidleri belirlemek için HPLC'nin kullanıldığı analizlerde başlıca bileşen olarak rutin (0.43 mg/g), ardından morin (0.16 mg/g), kuersetin (0.01 mg/g) ve mirisetin (0.01 mg/g) varlığı saptanmıştır. Ayrıca, dut meyvelerinin dondurularak kurutulmuş tozunda ise 1.20 mg/g askorbik asit, 0.32 mg/g vitamin E ve 243.0 mg/g diyet lif tespit edilmiştir. Ayrıca dutun yağ asidi içeriği ve bileşiminin, farklı ekolojik koşullara göre değiştiğini ifade eden araştırmacılar, dut meyvelerinin toplam yağ asidi içerdiğini, tespit edilen en yüksek miktardaki yağ asidinin linoleik asit C18:2 (%79.4) olduğunu bunu palmitik asit C16:2 (%8.6) ve oleik asidin C18:1 (%7.5) izlediğini, örneklerde %0.6 oranında α -linolenik asit C18:3 tespit edildiğini bildirmişlerdir. Türkiye'de yapılan bir başka çalışmada ise örneklerde yüksek oranda linoleik asit C18:2 (%57.3) tespit edildiği, bunu palmitik asidin C16:0 (%22.4) izlediği bildirilirken örneklerde linolenik asit C18:3'ün varlığının tespit edilmediği belirtilmiştir (Ercisli ve Orhan., 2007). Jiang ve Nie (2015)'nin yapmış olduğu çalışmada ise beyaz (*Morus alba* L.), rus (*M. alba* var. *tatarica* L.) ve kara (*Morus nigra* L.) dut meyvelerinin esansiyel amino asitlerden izolösin, lösin, treonin, lizin, valin, fenilalanin, tirozin, triptofan, histidin, metionin ve sistein ile esansiyel olmayan amino asitlerden arginin, alanin, prolin, glutamik asit, glisin, serin ve aspartik asit içerdiğini bildirmişlerdir. Meyvelerin yağ asitlerinden linoleik asit, miristik asit, stearik asit, palmitik asit ve α -linoleik asit içerdiği de tespit edilmiştir. Ayrıca araştırmacılar malik asit, süksinik asit, sitrik asit, tartarik asit, asetik asit gibi organik asitlerin varlığını da bildirmişlerdir.

Yapılan farklı çalışmalarda *Morus nigra*'da bulunan ana vitaminlerin (mg/100 g) niasin (0.620), riboflavin (0.101), C (21.8) ve E (0.87) vitaminleri olduğu tespit edilirken majör minerallerin (mg/100 g) ise potasyum (922), kalsiyum (132), sodyum (59) ve magnezyum (106) olduğu bildirilmiştir (Ercisli ve Orhan., 2007; Lochynska ve ark., 2015). Benzer şekilde bir başka çalışmada askorbik asit, niasin ve riboflavin sırasıyla majör vitaminler olarak bulunmuştur (Imran ve ark., 2010). *Morus nigra*'da ki yağ asidi içeriği (%) ise cis-C18:2 w6 (61.85), cis-C18:1 w9 (14.75), C16:0 palmitik asit (12.06) ve C18:0 stearik asit (5.86) olarak bildirilmiştir (Ramappa ve ark., 2020). Ayrıca bir başka çalışmada dut meyvelerinin potasyum, kalsiyum, magnezyum, demir, sodyum, çinko, bakır, selenyum ve manganez gibi mineralleri içerdiğini saptamışlardır (Jiang ve Nie., 2015).

Kang ve ark., (2006), Amberlite IRC-50 iyon değişim kromatografisini kullanarak %1 HCl-MeOH dut meyve (*M. alba* L.)

macroura türlerinde, 1-pentanolün ise *M. alba* çeşidinde olduğu bulunmuştur. Benzer şekilde *M. nigra* ve *M. alba*'nın düşük miktarda uçucu bileşenlerden 2,4-nonadienal, metil hegzanat, limonen, oktanol ve etil hegzanat içerdiği bildirilmiştir (Sánchez-Salcedo ve ark., 2015).

Farmakolojik Özellikler

Dut meyvesi, sağlık üzerindeki potansiyel farmakolojik aktiviteleri nedeniyle araştırmacıların ve tüketicilerin dikkatini çeken, antosiyaninler açısından zengin fonksiyonel bir gıdadır (Bhattacharjya ve ark., 2021; Lee ve ark., 2017). Dut yapraklarının farmakolojik aktivitelerinin incelendiği çok sayıda çalışma (Bai ve ark., 2021; Ma ve ark., 2022) olmasına rağmen meyvelerinin farmakolojik özellikleri üzerine yapılmış sınırlı sayıda çalışma bulunmaktadır (Bhattacharjya ve ark., 2021). Dut meyvelerinin *in vitro* ve *in vivo* farmakolojik özelliklerinin araştırıldığı çalışmalar aşağıda özetlenmiş ve Tablo 1 ve 2 ile Şekil 2'de sunulmuştur.

Hipolipidemik Etki

Kardiyovasküler hastalıklar (KVH), her yıl dünya çapında yaklaşık 17 milyon insanın KVD'den (inme ve koroner kalp hastalığı dahil) ölmesiyle en yaygın ölüm nedenleri arasında yer almaktadır (Ma ve Lee., 2016; Townsend ve ark., 2016). KVH'nin gelecekte küresel mortaliteye en büyük katkıyı sağlayacağı tahmin edilmektedir (Lu ve ark., 2016). Hiperlipidemi KVH için başlıca risk faktörlerinden biridir (Nelson., 2013). Hipolipidemik ilaçların çoğunun potansiyel yan etkiler göstermesi ve pahalı olmaları (Yang ve ark., 2010; Zhang ve ark., 2013), alternatif tıbbın kan lipid düzeylerini düşürmedeki etkinliğinin belirlendiği çalışmalarda artış olmasına sebep olmuştur (Rabito ve Kaye., 2013).

Yang ve ark., (2010), %5 veya %10 dut meyve tozu ile takviye edilmiş yüksek yağlı diyetle beslenen sıçanların serum ve karaciğer trigliserit, toplam kolesterol ve serum LDL kolesterol konsantrasyonlarında önemli bir düşüş olduğunu bildirmiştir. %5 veya %10 *M.alba* dut meyve tozu ile takviye edilmiş yüksek yağlı diyetle beslenen sıçanlarda ise serum yüksek yoğunluklu lipoprotein (HDL) kolesterolünde bir artış olduğunu saptamışlardır. Ayrıca araştırmacılar, dut meyvelerinin yüksek diyet lifi ve linoleik asit içeriğine sahip olması nedeniyle, dut meyvelerinin hipolipidemik bir etkiye sahip olabileceğini öne sürmüşlerdir.

Chen ve ark., (2005), Yeni Zelanda beyaz tavşanlarını 10 hafta boyunca yüksek kolesterol diyeti (HCD) (%95.7 stve art Purina yemi, %3 domuz yağı ve %1.3 kolesterol) ve HCD ile

%0.5 veya 1.0 oranında *M.alba* dut meyvelerinin % su ekstraktı ile beslenmişlerdir. HCD yanında %0.5 veya 1.0 oranında dut meyvelerinin % su ekstraktı verilen tavşanların sadece domuz yağı diyeti ile beslenenlere göre daha düşük toplam kolesterol, LDL kolesterol ve trigliserit seviyelerine sahip olduğu tespit edilmiştir. Ayrıca bu çalışmada HCD yanında %0.5 veya %1.0 dut meyvesi su özütü ile beslenen tavşanların aorttaki şiddetli aterosklerozunun %42-63 gibi önemli bir oranda azaldığı ve bu bulguların tavşanların kan damarlarının histopatolojik incelemesi ile desteklendiği bildirilmiştir. Çalışmada dut meyvelerinin su ekstraktının toplam kolesterol ve LDL kolesterol düzeyleri üzerindeki etkisinin doza bağlı olduğu ve dut meyvelerinin %0.5 veya %1.0 su ekstraktı ile beslenen tavşanlarda karaciğer veya böbrek fonksiyonlarındaki değişiklikler üzerinde olumsuz bir etki gözlenmediği bildirilmiştir.

30-60 yaşları arasındaki 58 hiperkolesterolemik yetişkin üzerinde yapılan randomize kontrollü diğer bir çalışmada ise, Sirikanchanarod ve ark., (2016) haftalık 45 g dondurularak kurutulmuş *M.alba* dut meyvesi tüketiminden (325 mg antosiyanin) sonra, deney grubunun kontrol grubuna göre önemli ölçüde daha düşük toplam kolesterol ve LDL değerlerine sahip olduğunu bildirmiştir. Çalışmada ayrıca dut meyvelerinin tüketiminin ateroskleroz riskini azaltabileceği ve LDL'nin oksidasyonunu önlemek için anti-hiperlipidemik ve anti-oksidatif yeteneklere sahip olduğundan hiperkolesterolemik hastalar için alternatif bir tedavi olarak kullanılabilirliği rapor edilmiştir (Chen ve ark., 2005).

Bir başka çalışmada ise *M. nigra* meyvesinin etanolik ekstraktlarının düzenli olarak Sprague-Dawley sıçanlarına verilmesiyle malondialdehit içeriğinin azaltıldığı, karaciğer yağlanmasının zayıflatıldığı ve lipid metabolizması anormalliklerinin düzenlediği bildirilmiştir. Böylece kara dut ekstraktının sıçanlarda arteriyel ateroskleroz gelişimini baskıladığı tespit edilmiştir (Jiang ve ark., 2017).

Anti-Diyabetik Etki

Diyabet, insülin sekresyonunun kusurlarından kaynaklanan hiperglisemi ile karakterize bir hastalık olup, KVH ve çeşitli organların yetersizliği gibi bir dizi sağlık komplikasyonu ile ilişkilidir (Kalofoutis ve ark., 2007). Yapılan *in vivo* çalışmalarda *M.alba* dut meyvesinin anti-diyabetik aktivitesi tespit edilmiş olup, *M.nigra* dut meyvesinin anti-diyabetik etkisinin incelendiği bir çalışmaya rastlanmamıştır.

Tablo 1. Dut meyvelerinin *in vitro* farmakolojik özellikleri**Table 1.** *In vitro* pharmacological properties of mulberry fruits

Farmakolojik Etki	Tür	Çözücü	Etki mekanizması	Analiz Modeli	Kaynaklar
	<i>M. nigra</i>	Etanol	Lipoliz enzimini durdurucu etki	<i>In vitro</i>	Chen ve ark., (2017)
Anti-inflamatuar	<i>M. alba</i>	Etanol	İnflamatuar yanıtlara karşı koruyucu etki sağladığı tespit edilmiştir.	Liposakkarit (LPS) stimüle edilmiş RAW 264.7 makrofaj hücreleri	Yu ve ark., (2021)
	<i>M. alba</i>	Su	İçerisinde ekstraktın bulunduğu Carbon dots (C-dots) ambalajların <i>Listeria monocytogenes</i> aktivitesini durdurduğu tespit edilmiştir.	<i>In vitro</i>	Salimi ve ark., (2021)
Antimikrobiyal	<i>M. nigra</i> ve <i>M. alba</i>	<i>M. nigra</i> ve <i>M. alba</i> tozu	<i>E. coli</i> , <i>S. aureus</i> ve <i>P. aeruginosa</i> karşı antibakteriyal aktiviteler incelenmiş ve <i>M. nigra</i> 'nın MBC testinde <i>E. coli</i> ile karşılaştırıldığında <i>S. aureus</i> 'a karşı daha güçlü inhibitör aktivite gösterdiği bulunmuştur.	<i>In vitro</i>	Chen ve ark., (2017)
	<i>M. alba</i>	Su	İmmüoglobulin G (IgG) antikor seviyeleri önemli ölçüde arttığı tespit edilmiştir.	<i>In vitro</i> ve xenograft fare modeli	Chang ve ark., (2015)
Bağışık artırıcı	<i>M. alba</i>	Etanol	Makrofajların nitrik oksik salgısı ve fago-sitozu artırıcı etkisi tespit edilmiştir.	<i>In vitro</i>	Wang ve ark., (2018)
	<i>M. alba</i>	Metanol	Meyvede bulunan pirol alkaloidlerinin RAW 264.7 hücrelerinde makrofaj hücrelerinin aktivitesini başlattığı bulunmuştur.	<i>In vitro</i> - RAW 264.7 makrofaj hücreleri	Kim ve ark., (2013)

**Şekil 1.** Dut meyvelerinin başlıca biyoaktif bileşimi
Figure 1. Major bioactive composition of mulberry fruits**Şekil 2.** Dut meyvelerinin farmakolojik özellikleri
Figure 2. Pharmacological properties of mulberry fruits

Tablo 2. Dut meyvelerinin *in vivo* farmakolojik özellikleri**Table 2.** *In vivo* pharmacological properties of mulberry fruits

Farmakolojik Etki	Tür	Çözücü	Etki mekanizması	Analiz Modeli	Kaynaklar
	<i>M. nigra</i>	Su	DeneySEL olarak periodontitis oluşturulan sıçanlarda bölgesel alveoler kemik rezorpsiyonunun inhibe edilmesine bağlı olarak periodontal iyileşme gözlemlenmiştir.	Erkek Wistar sıçanlar	Talo Yildirim ve ark., (2019)
Anti-inflamatuar	<i>M. alba</i>	Su	Siyanidin-3-glikozidin PC12 hücrelerinde antioksidan, MCAO sıçanlarında ise serebral iskemik hasar üzerinde nöroprotektif etkileye sahip olduğu görülmüştür. Ayrıca endotel disfonksiyonu önlediği bildirilmiştir.	PC-12 hücreleri ve MCAO sıçan	Kang ve ark., (2006)
	<i>M. nigra</i>	Meyve suyu	Meyve suyunun verildiği parkinson indüklü farelerde Levodopa-induced dyskinesia (LID)'in engellendiği görülmüştür.	Parkinson hastalığı indüklenmiş erkek BALB/c farelere haf-talık 10-15 ml/kg ve-rilmiştir.	Fahimi ve Jahromy (2018)
Antiparkinson	<i>M. alba</i>	Su	Beş hafta boyunca farelere verilen meyve suyunun parkinson hastalığı semptomlarını iyileştirici etkisi göz-lenmiştir.	Parkinson hastalığı indüklenmiş erkek C57BL/6 fareler	Gu ve ark., (2017)

Jiao ve ark., (2017), yedi hafta boyunca *M. alba* dut meyvesinin iki farklı polisakkarit fraksiyonu (MFP50 ve MFP90) ile beslenen diyabetik sıçanların açlık glukozu, açlık serum insülini, homeostaz modeli insülin direnci, trigliserit ve oral glukoz toleransı değerlerini düşürdüğünü saptamışlardır. Kontrol grubu olarak saf su ile beslenen diyabetik sıçanlarla karşılaştırıldığında, MFP50 ve MFP90 ile beslenen diyabetik sıçanlar, sırasıyla %26.5 ve %32.5 oranında daha düşük serum insülin düzeyine sahip olduğunu bildirilmişlerdir. Ayrıca hem MFP50 hem de MFP90'ın serum alanin transaminaz (ALT) düzeylerini düşürdüğünü bulmuşlar ve aynı zamanda bunların potansiyel hepatoprotektif etkileri olduğunu düşünmüşlerdir. MFP50'nin MFP90'dan daha stabil bir hipoglisemik etkisi olmasına rağmen, MFP90'ın MFP50'den daha iyi bir hipolipidemik etkisi tespit edilmiştir. Benzer bulgular Guo ve ark., (2013) tarafından da bildirilmiş ve 2 hafta boyunca *M. alba* dut meyvesi polisakkaritleri ile beslenen diyabetik sıçanların açlık kan şekerinde düşüş olduğu saptanmıştır.

Wang ve ark., (2013) tarafından yapılan bir başka çalışmada, 2 hafta boyunca *M. alba* dut meyvesinin etil asetat ekstraktı ile beslenen diyabetik sıçanların, açlık kan şekeri ve glikozile

edilmiş serum protein seviyelerinde önemli bir düşüş olduğu bildirilmiştir. Ayrıca, dut meyvelerinin etil asetat ekstraktının diyabetik sıçanlarda katalaz (CAT), glutatyon peroksidaz (GSH-Px) ve süperoksit dismutazın (SOD) antioksidan aktivitelerini önemli ölçüde arttırdığını ve 2,2-difenil-1-pikrilhidrazil (DPPH) ile süperoksit anyon radikallerine karşı güçlü α -glikozit inhibitör aktiviteye ve radikal süpürücü aktivitelere sahip olduğu rapor edilmiştir.

Yan, ve ark., (2016) ise 8 hafta boyunca günde 50 ve 125 mg/kg vücut ağırlığı dozlarında *M. alba* dut meyvesinin antosiyanin ekstraktı ile beslenen erkek (db/db) farelerin kolesterol düzeylerinde önemli bir düşüş olduğunu, açlık kan şekeri, leptin, serum insülin ve trigliserit ile adiponektin düzeyinde ise artış olduğunu bildirmişlerdir. Araştırmacılar aynı zamanda dut meyvesinin, dut meyvesinin antosiyanin ekstraktının insülin ve leptinin direncini arttırmak için kullanılabileceğini öne sürmüşlerdir. Bu sonuçlar, diğer araştırmacıların (Wang ve ark., 2013, Guo ve ark., 2013 ve Jiao ve ark., 2017) elde ettiği veriler ile birlikte değerlendirildiğinde, dut meyvelerinin anti-hiperglisemik ve anti-hiperlipidemik etkileri nedeniyle diyabet tedavisinde önemli bir rol oynayabileceğini düşündürmektedir.

Diğer taraftan *M. nigra* meyvesinin anti-diyabetik etkisinin incelendiği çalışmaya rastlanmazken yaprağı ile ilgili sınırlı sayıda çalışmanın olduğu tespit edilmiştir (Abd ve ark., 2011; Kojima ve ark., 2010; Wei ve ark., 2018).

Anti-Obezite Etkisi

Obezite geleneksel olarak belirli bir boy, cinsiyet ve yaştaki bireyler için en düşük ölüm oranına karşılık gelen ideal ağırlığın ≥ 20 üzerinde bir ağırlık olarak tanımlanmakta olup tip 2 diyabet, kalp hastalığı ve kanser gibi hastalıkların ortaya çıkma riskini arttırmaktadır (Lee ve ark., 2017). Yapılan bazı çalışmalar obezitenin dislipidemiye katkıda bulunmada önemli bir rol oynadığını göstermiştir (Ebbert ve Jensen 2013; Jung ve Choi 2014; Klop ve ark., 2013; Turan ve Tuncay 2020).

Lim ve ark., (2013) düşük (133 mg dut yaprağı ekstresi ve 67 mg dut meyve ekstresi/kg/gün) ve yüksek (333 mg dut yaprağı ekstresi ve 167 mg dut meyve özütü/kg/gün) dozlarda *M.alba* dut yaprağı ekstresi ve dut meyvesi ekstresi kombinasyonu ile beslenen yüksek yağlı diyetle indüklenen obez farelerin vücut ağırlığı artışında, açlık plazma glukozunda, insülin ve insülin direncinin homeostaz model değerlendirilmesinde önemli bir azalma olduğunu bildirmiştir. Dut yaprağı özütü ve dut meyve özütü kombinasyonunun yüksek dozu, glikoz kontrolünü büyük ölçüde iyileştirdiği, manganez süperoksit dismutaz, indüklenabilir nitrik oksit sentaz, monosit kemoatraktan protein-1, C-reaktif protein (CRP), tümör nekroz faktör- α protein ve interlökin-1 düzeylerini de düşürdüğü bildirilmiştir. Bu nedenle, dut yaprağı ekstresi ve dut meyvesi ekstresi kombinasyonunun, obezitenin neden olduğu oksidatif stres ve iltihabı modüle ederek anti-obezite ve anti-diyabetik özelliklere sahip olduğu ileri sürülmektedir.

Kim ve ark., (2014) yapmış oldukları çalışmada *M.alba* meyve pyrrol alkaloidlerinin pankreatik lipaz aktivitesini durdurucu etkisinden dolayı obeziteyi önleyici etkisinin olduğunu bildirmiştir.

Peng Peng ve ark., (2011), 12 hafta boyunca *M. alba* dut meyve suyu ekstresi ile beslenen erkek hamsterlarda, yüksek yağlı diyetle bağlı vücut ağırlığı ve iç organlarda yağlanmanın daha düşük olduğunu, bunun yanında serum triasilgliserol, kolesterol seviyelerinde, LDL/HDL oranında bir azalma olduğunu bildirmişlerdir. Aynı zamanda, dut meyve suyu ekstraktının yağ asidi sentezini, 3-hidroksi-3-metilglutaril-koenzim A (HMG-CoA) redüktazını ve hepatik peroksizom proliferatörü ile aktive olan reseptör α ve karnitin palmitoiltransferaz-1'i yükselttiği ifade edilmiştir. Araştırma sonucunda, dut meyve ekstraktının vücut ağırlığını azaltmak için kullanılabileceği ve lipoliz ile lipogenezi düzenlediği öne sürülmüştür.

Literatür taramasında karşılaşılan *M.alba* ile ilgili çalışmaların aksine *M. nigra* meyvesinin anti-obezite etkisine dair bir araştırmaya rastlanmamıştır.

Anti-Tümör ve Anti-Kanser Etki

Dünya Sağlık Örgütüne (DSÖ) bağlı Uluslararası Kanser Araştırma Ajansı (IARC)'nın 2020 yılında hazırladığı raporda her yıl dünya genelinde 10 milyon yetişkinin hayatını kanser sebebiyle kaybettiği belirtilmiştir. Kanser sayılarında gözlenen artışın yanı sıra tedavide uygulanan kimyasallara karşı oluşan ilaç direnci ve yan etkiler sebebiyle alternatif tedavi arayışı artmaktadır (Wild ve ark., 2020).

M. alba, kök, kök kabuğu ve yapraklarından ekstrakte edilen fenolik bileşikler vasıtası ile antikanser etkiler göstermektedir. Yapıda en çok flavonoidler bulunmaktadır, ayrıca morasinler, fenolik asitler, alkaloidler, kumarinler, kalkonlar ve stilbenler de elde edilmiştir (Chan ve ark., 2020). Literatürde; kolon, mide, meme ve karaciğer kanseri ile, lösemi ve myelom hücre dizileri üzerine yapılan araştırmalar mevcuttur (Chen ve ark., 2021). Özellikle, *M. alba* metanol ekstraktları ile yapılan çalışmalarda, ekstrelerin antiproliferatif etkileri olduğu gösterilmiş; kolon ve mide kanserinde yapılan hücre dizisi çalışmalarında, en etkin ekstrenin kökten hazırlananlar olduğu bildirilmiş, bunu sırasıyla yaprak, dal ve meyve izlemiştir (Chon ve ark., 2009).

Gastrointestinal sistem kanserleri dünyadaki en yaygın kanser türlerinden biridir (Pourhoseingholi ve ark., 2015; Sung ve ark., 2021) ve yaygın olarak *Helicobacter pylori* 'nin mide karsinogenezini tetiklediği bilinmektedir (Nishizawa ve Suzuki 2015; Tan ve ark., 2018; Xiang ve ark., 2021).

Huang ve ark., (2011), 5 haftalık balb/c erkek farelerin 7 hafta boyunca antosiyanin açısından zengin *M.alba* dut meyvesi ekstraktı ile beslenmesinden sonra, farelerde atipik glandüler hücreler (AGS) tümör ksenograft büyümesinin inhibe edildiğini bildirmişlerdir ve bu sonuçlar dut meyvelerinden elde edilen antosiyaninlerin mide kanseri oluşumunu önlemek için kullanılabileceğini düşündürmektedir.

M. nigra meyvesinin içerdiği biyoaktif bileşenlerden dolayı antikanser etki gösterdiği çeşitli çalışmalarda bildirilmiştir (de Pádua Lúcio ve ark., 2018; Lobo ve ark., 2010; Mujtaba ve ark., 2021). Benzer şekilde kara dut meyvesinin içeriğindeki siyanidin ve pelarginidin bileşenlerinin tümör gelişimini durdurucu etkisi ve kanser hücrelerinin canlılığını azaltıcı etki gösterdiği rapor edilmiştir (Cho ve ark., 2017). Cho ve ark., (2017) yaptıkları çalışmada meyvedeki siyanidin-3 glikozidin MDA-MB-453 hücrelerinde kanser oluşturulmuş dışi farelerde tümör gelişimini durdurduğunu tespit etmişlerdir.

Kikuchi ve ark., (2010), *Morus alba* kök kabuklarından elde edilen Albanol A'nın insan lösemi hücre dizisinde (HL60) ve insan DNA topoizomeras I ve II'de etkilerini incelediğinde, prokaspaz-3, 8 ve 9'un inhibe edildiğini gösterirken, kaspaz 2, 3, 8 ve 9 seviyesinde artış olduğunu saptamış ve bax/bcl-2 oranında da artış olduğunu göstermiştir. Çalışmada, Albanol A'nın, apoptozisi belirgin şekilde indüklediğini, hücre dizisi üzerinde sitotoksik özellik gösterdiğini ve şu anda lösemi tedavisindeki aktif kullanılan hedeflerden olan topoizomeras I ve II üzerine inhibitör etki gösterdiğini tespit etmişlerdir. Bir başka çalışmada, *M. alba*'dan ekstrakte edilen flavonoid 4'-O-methylkuwanon E'nin, All-trans retinoik asit ile benzer kimyasal yapısı olması üzerine insan monositik hücre kültürü üzerine etkisi incelenmiş ve sonuçta doz bağımlı büyüme inhibisyonu ve p38 kinaz bağımlı monositik diferansiyasyonun engellendiği ve apoptozisin indüklendiği gösterilmiştir (Kollar ve ark., 2015).

Kronik Lenfositik Lösemi ile, en sık görülen hematolojik malignite olan Multiple Myelom ile ilişkili yapılan başka bir hücre dizisi çalışmasında, *M. alba* kök kabuklarından elde edilen ursolik asitin intrasellüler olarak hastalık patogenezinde önemli rol oynayan β -katenin, c-myc ve siklin d1'in hücre içi seviyelerini azalttığı ve böylece hücre proliferasyonunun engellendiği ve apoptozisin indüklendiği gösterilmiştir (Song ve ark., 2021).

Hepatoprotektif Etki

M. alba ve *M. nigra* dut türlerinin meyve ekstraktlarının karaciğeri koruyucu etkilerinin bildirildiği çalışmalar bulunmaktadır (Li ve ark., 2016; Youssef ve ark., 2017).

Dut meyvesi kabuğunun (sıkıldıktan sonraki katı bileşen) antosiyaninlerinin erkek Sprague Dawley sıçanlarında karbon tetraklorür (CC14) ile indüklenen karaciğer fibrozu üzerindeki koruyucu etkisini araştıran bir çalışmada, Li ve ark., (2016) dut meyve antosiyaninleri ile beslenen sıçanların ALT, aspartat amino transferaz, kolajen tip-III hiyalüronidaz asit ve hidroksiprolin düzeylerinde azalma olduğunu bildirmişlerdir.

Chang ve ark., (2013) ise hücre modeli olarak insan hepatom hücresi HepG2'yi kullanarak *M. alba* meyve antosiyanin ekstraktlarının (MAE) hepatosit üzerindeki koruyucu etkilerini ve mekanizmalarını araştırdıkları çalışmada, MAE'nin yağ asidi sentezini baskıladığını ve yağ asidi oksidasyonunu artırarak oleik asit tarafından indüklenen lipid birikiminin iyileşmesine katkıda bulunduğunu bildirmiştir. Çalışma kapsamında, MAE'nin hipolipidemik etkilerinin, AMPK'nin (Aktive Edici Protein Kinaz) fosforilasyonu, lipid biyosentezinin inhibisyonu ve lipolizin uyarılması yoluyla meydana geldiği

ifade edilmiştir. Bu nedenle, dut meyvelerinin alkol kullanımına bağlı olmayan karaciğer yağlanması hastalığını önleyebileceği düşünülmektedir.

M. nigra sulu meyve ekstraktlarının incelendiği bir hücre kültürü (HepG2) çalışmasında içeriğindeki fenolik bileşenlerin karaciğerde toksin birikmesini önlediği ve hepatoprotektif etkiye sahip olduğu bildirilmiştir (Youssef ve ark., 2017).

Antioksidan, Sitotoksitite ve Oksidatif Strese Karşı Koruyucu Etki

Serbest radikaller vücuttaki biyolojik moleküllerin redoks durumunu değiştirerek oksidatif stresin artmasına neden olmaktadır. Ancak vücuttaki eksojen antioksidan seviyesi artırılır ise antioksidanlar, reaktif oksijen türlerinin ve reaktif nitrojen türlerinin neden olduğu hasarı önleyerek serbest radikallerin neden olduğu oksidatif hasarın etkilerini ortadan kaldırmaktadırlar (Memete ve ark., 2022). Buna bağlı olarak metabolik sendrom (kardiyovasküler hastalık, diyabet vb) riskinin azalması ve bağışıklık savunmasının artması sağlanabilmektedir.

M. nigra ve *M. alba* meyvelerinin antioksidan aktivitelerinin karşılaştırıldığı farklı çalışmalarda *M. nigra*'nın *M. alba*'dan daha yüksek antioksidan kapasiteye sahip olduğu bildirilmiştir (Afran 2012; Sánchez-Salcedo ve ark., 2015).

Geçmiş yıllarda yapılan bir çalışmada dondurularak kurutulmuş dut meyvelerinin streptozotosin ile indüklenmiş Sprague Dawley sıçanların eritrositlerindeki zararlı oksidatif maddeleri azaltarak anti-oksidatif savunma mekanizmasını güçlendirdiği bildirmiştir (Hong ve ark., 2004). Türkiye'de yetişen *M. nigra* meyve ve yaprağının antioksidan aktivitelerinin farklı çözücüler kullanılarak karşılaştırıldığı bir başka çalışmada en yüksek antioksidan aktivitenin (%33.1) yaprağın metanol ekstraktında gözlendiği bunu ise meyvenin metanol ekstraktının (%28.7 inhibisyon) takip ettiği bildirilmiştir (Yiğit ve ark., 2008). Benzer şekilde, Sırbistan'da yetişen *M. nigra* meyvesinin etanol, etanol-su ve su ekstraktının antioksidan aktivitesinin araştırıldığı bir diğer çalışmada da etanol-su karışımının da %71.41 DPPH aktiviteye sahip olduğu rapor edilmiştir (Kostiæ ve ark., 2013).

Farklı meyvelerin resveratrol içeriğinden kaynaklanan antioksidan aktivitelerinin incelendiği bir çalışmada *M. rubra*'nın en yüksek resveratrol içeriğine ve Java eriği (*Syzygium cumini*) ile Jak meyvesi (*Artocarpus heterophyllus*)'den daha fazla antioksidan aktiviteye sahip olduğunu bildirmiştir (Shrikanta ve ark., 2015).

Mahmood ve ark., (2017) dut meyvesinin farklı türlerinin antioksidan aktiviteleri üzerine olgunlaşma düzeyi ve kurutma

tiplerinin etkisini inceledikleri çalışmalarında *M. alba* ve *M. nigra*'nın antioksidan aktivitesinin olgunlaşma ilerledikçe arttığını, bununla birlikte havalı kurutucularda kurutulan tamamen olgunlaşmış dut meyvesinin daha yüksek antioksidan aktiviteye sahip olduğunu bildirmişlerdir.

Dut meyve ekstraktının insan karaciğeri HepG2 hücrelerinde etil karbamat (EC) kaynaklı sitotoksiteye karşı koruyucu etkisini araştıran bir çalışmada, Chen ve ark., (2017), *M. alba* dut meyvesi ekstraktı tedavisi (0.5 mg/mL, 1.0 mg/mL ve 2.0 mg/mL) ile hücre canlılığında azalma olmadığını bildirmişlerdir. Bu nedenle araştırmacılar, dut meyvesinin EC kaynaklı sitotoksite ve oksidatif strese karşı koruma sağlamak için kullanılabileceğini öne sürmüşlerdir.

M. alba dut meyvesi tüketiminin farelerde anti-yorgunluk aktivitesi üzerindeki etkisini araştıran Jiang ve ark., (2013), dut meyvelerindeki antosiyanin varlığının, egzersize bağlı oksidatif stresi ve fiziksel yorgunluğu azaltmak için bir antioksidan görevi görebileceğini ileri sürmüşlerdir.

Beyin Hasarına Karşı Koruyucu (Nöroprotektif) Etki

Esansiyel yağ asitleri (omega 3, omega 6), nörol fonksiyonların güçlendirilmesinde gerekli olan hormonlardan eikozanoidlerin (tromboksanlar, lökotrienler ve prostaglandinlerin) üretilmesinde görev almaktadırlar (Feng ve ark., 2015). Dut meyvesi içeriğindeki esansiyel yağ asitlerinin beyin aktivitesini güçlendirici, LDL değerini düşürücü ve kolesterolden kaynaklı nörotoksite oluşumunu engelleyici etkisi olduğu rapor edilmiştir (El-Sayyad 2015). Yang ve ark., (2016) farklı olgunluklardaki *M. alba* meyvesinin nöroprotektif aktivitesini tip-2 diyabet indüklenmiş C57BL/6/J fareler kullanarak araştırmış ve olgunlaşmış meyvelerin nöroprotektif aktivitesinin en yüksek olduğunu bildirilmiştir.

Kang ve ark., (2006), dut (*Morus alba* L.) meyve ekstraktlarından izole edilen C3G'nin *in vitro* hidrojen peroksit maruz kalan canlılarda PC12 hücreleri üzerinde sitoprotektif bir etki ve oksijen glikoz yoksunluğunun neden olduğu serebral iskemik hasar üzerinde bir nöroprotektif etki gösterdiğini bildirmiştir. Bu nedenle, dut meyvelerinin *in vivo* ve *in vitro* iskemik oksidatif stresin nöroprotektif etkileri olduğu ileri sürülmektedir.

Yürütülen farklı bir çalışmada Alzheimer hastalığının araştırılmasında kullanılan APP/PS1 transgenetik farelere 1.5-3 hafta boyunca *M. alba* ekstraktı (100 mg/kg vücut ağırlığı) verilmiş ve sonrasında yapılan histolojik analizler ile meyve ekstraktının farelerdeki korteks ve hipokampus dokularında A β plaklarının ve nöron apoptozunu azalttığı tespit edilmiştir (Liu ve Du 2020).

Shin ve ark., (2021) *Morus alba* ekstraktının oksidatif stresin uygulandığı hipokampal nöronal HT-22 hücrelerindeki ve skopolaminin indüklediği hafıza bozukluğu olan farelerdeki nöroprotektif etkisini analiz etmişlerdir. Elde edilen sonuçlarda meyve ekstraktlarının TrkB/Akt yolunun aktivasyonunu stabilize ederek beyin-türevli nörotrofik faktör (BDNF) ve antioksidan enzimlerin ekspresyonunu düzenleyerek nöronal hücreleri oksidatif stres kaynaklı apoptoza karşı koruduğunu göstermişlerdir (Shin ve ark., 2021).

Sonuçlar ve Gelecek Araştırmaları

Kendine has rengi, tadı ve aroması ile gıda endüstrisinde önemli bir yer tutan dut meyvesi, başta flavonoller, antosiyaninler, flavanonoller ve fenolik asitler olmak üzere zengin bir mineral ve vitamin kaynağıdır. Epidemiyolojik çalışmalar, üzümü meyvelerden özellikle dut meyvesinin kullanıldığı diyetlerin biyoaktif bileşenlerinden dolayı antidiyabetik, antioksidan, nöroprotektif, hipolipidemik, anti-obezite, anti-inflamatuar, hepatoprotektif, antiparkinson, anti-tümör- anti-kanser, antimikrobiyal etkileri olduğunu ve bağışıklığın artmasına olumlu katkı sağladığını göstermektedir.

Son yıllarda, böğürtlengillerden dut türlerinin üretimi dünya çapında artış göstermektedir. Bu durum gıda ve sağlık endüstrisinin dut meyvesinin sağlığa faydalarını daha detaylı araştırması için önemli bir fırsat yaratmaktadır. Dut meyvesinin bileşimindeki antosiyaninler, alkaloidler, flavonoidler ve polifenoller gibi biyoaktif bileşiklerin miktarı ve bileşimi türlere, çeşitlere ve hatta aynı türün farklı renklerine göre değişmektedir.

Yüzyıllardır dut ağacının yaprakları, kabuğu, kökleri ve meyveleri geleneksel olarak şeker hastalığı, yüksek tansiyon, karaciğer, böbrek ve göz hasarlarını tedavi etmek için çeşitli dozlarda çay şeklinde kullanılmıştır. Bununla birlikte, sınırlı sayıda *in vivo* (hayvan ve klinik) çalışma olması nedeniyle, dut türlerinin önerilen günlük alım miktarı (dozajı) ve tedavi süresi ile ilgili yeterli bilimsel kanıt olmadığından tüketici güvenliğini değerlendirmek zordur.

Sonuç

Sonuç olarak, bazı dut türleri ve çeşitlerinin biyoaktif bileşikleri ve bunların farmakolojik etkileri *in vitro* olarak çalışılmış olmasına rağmen, dut meyvesi günlük tüketim miktarı, tedavi için tüketim süresi ile sağlık arasındaki nedensel ilişkiyi kurmak için daha detaylı klinik çalışmalara ihtiyaç vardır.

Etik Standartlar ile Uyumluluk

Çıkar çatışması: Yazarlar bu yazı için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan etmişlerdir.

Etik izin: Araştırma niteliği bakımından etik izne tabii değildir.

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Aralıklı orucun bağırsak mikrobiyotası üzerine etkisi

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ÖZ

Beslenme alışkanlıklarının bağırsak mikrobiyotasını etkilediği bilinmektedir. Son dönemlerde besin alımının çeşitliliğinden ziyade besin alınan zamanın mikrobiyota üzerine etkisine odaklanılmış ve bunun mikrobiyomu etkilediği görülmüştür. Aralıklı orucun mikrobiyotadaki yararlı mikroorganizmaları arttırdığı, patojen mikroorganizmaları azalttığı rapor edilmiş olsa da etki mekanizması tam olarak açıklanamamıştır. Bu derlemenin amacı aralıklı oruç ve mikrobiyota ilişkisini inceleyen güncel çalışmaları yorumlamaktır.

Anahtar Kelimeler: Aralıklı oruç, Bağırsak beyin eksenini, Beslenme, Mikrobiyota

ABSTRACT

The effect of intermittent fasting on gut microbiota

Nutritional habits are known to affect the gut microbiota. Recently, it has been focused on the effect of the time taken in food on the microbiota rather than the variety of food intake, and it has been seen that this affects the microbiome. Although it has been reported that intermittent fasting increases beneficial microorganisms in the microbiota and decreases pathogenic microorganisms, its mechanism of action has not been fully explained. The purpose of this review is to interpret current studies examining the relationship between intermittent fasting and microbiota.

Keywords: Intermittent fasting, Gut brain axis, Nutrition, Microbiota



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Giriş

Aralıklı Oruç

Beslenmenin insan sağlığını yakından ilgilendirdiği eski çağlardan bilinmektedir. Son zamanlarda küresel hastalık yükünün artması ile bu hastalıkların tedavisinde enerji alımının veya besin alınan zamanın kısıtlanmasına giden bir takım beslenme tedavileri uygulanmaktadır (Di Daniele, 2019). Bu uygulamalardan popülerliğini koruyan aralıklı açlık diyetleri; dönüşümlü açlık, zaman kısıtlı beslenme ve özellikle Müslüman toplumların dini gereği tuttıkları İslami oruç olarak sınıflandırılmaktadır (Mirmiran ve ark., 2019).

Aralıklı açlık diyetlerinin çıkış noktası 5:2 oranında beslenmeyi ön gören diyetlerdir. Buna göre bireyler haftanın 5 günü normal beslenirken diğer 2 gününde ise kadınlar 500 kkal/gün; erkekler ise 800 kkal/gün ve altında enerji alırlar (Yumuk ve ark., 2015). Dönüşümlü açlık bireylerin besinleri istedikleri miktar ve zamanda istedikleri gibi tükettikleri beslenme günlerinden sonra dönüşümlü olarak ihtiyaç duyduğu enerjinin sadece %25'ini tükettikleri açlık günlerini içermektedir (St Onge ve ark., 2017). Zaman kısıtlı beslenme genellikle 8 saatlik yeme içmenin serbest olduğu zaman diliminden sonra 16 saatlik süre içinde yeme içmenin tamamen kesildiği, günümüzde de sıklıkla uygulanan beslenme çeşididir (Longo ve Mattson, 2014). İslami oruç ise Müslüman toplumlara göre her yılın farklı dönemlerine denk gelen Ramazan ayında, bir gün içerisindeki imsak vakti ile güneş batımına kadar geçen süreyi Allah'ın emirleri doğrultusunda yemeden, içmeden vb. birtakım hususlardan uzak durarak geçirmektir (Patterson ve Sears, 2017). Ramazan ayında oruç süresi mevsimsel ve coğrafik farklılıklara göre 29-30 gün ve 12-22 saat arasında değişmektedir. Oruç aç kalınan süre ile kıyaslandığında zaman kısıtlı beslenme ile benzerlik göstermektedir.

Bu tarz beslenme programlarında yemek yeme zamanındaki değişiklikler ve beslenme kalitesinin değişmesi ile vücutta birtakım değişiklikler meydana gelmektedir. Ramazan orucunun lipid parametrelerini olumlu yönde etkileyerek koroner kalp hastalığı riskinin azaltılabileceği rapor edilmiştir (Akanji ve ark., 2000). Buna karşılık Ramazan ayında gerek uzun süreli açlığa karşılık yağ dokusundan serbest yağ asidinin fazla salınması (Aybak ve ark., 1996) gerekse de Ramazan ayında toplam yağ ve karbonhidrat alımında artış olması nedeniyle serum trigliserit düzeylerinde artış olduğunu gösteren çalışmalar da mevcuttur (El Ati ve ark., 1995). Benzer olarak aralıklı açlık diyetlerinin kan glikoz seviyelerinin korunması, vücuttaki glikojen depolarının azaltılması, keton oluşumu, leptin seviyelerinin azalması ve adiponektin seviyelerinin yükselmesi gibi metabolik değişiklikleri üzerine çalışmalar da mevcuttur (Harvie ve ark., 2011).

Sağlıklı bireyler üzerinde yapılan çalışmaların değerlendirildiği bir meta analizde Ramazan orucunun erkeklerde kadınlara kıyasla vücut ağırlığı ve bazı biyokimyasal parametrelerde daha yararlı sağlık sonuçları geliştirdiği bildirilmiştir. Buna göre her iki cinsiyette LDL kolesterol ve açlık kan şekeri düzeyleri azalırken; kadınlarda vücut ağırlığı, toplam kolesterol ve trigliserit seviyelerinin arttığı, erkeklerde toplam kolesterol, LDL kolesterol ve vücut ağırlığında önemli bir azalma olurken trigliserit düzeylerinde küçük bir düşüş yaşandığı bildirilmiştir (Kul ve ark., 2014). Mirmiran ve ark. (2019) yaptıkları bir meta analizde Ramazan ayındaki aralıklı orucun lipid ve lipoprotein parametrelerinde iyileşme gösterdiğini rapor etmiştir. Fernando ve ark. (2019) meta analiz çalışmasında Ramazan orucunun hafif şişman ve şişman bireylerde yağ yüzdesi ve vücut ağırlığında önemli bir azalma sağladığını göstermiştir. Bu özelliği ile orucun obezite tedavisi ve oluşumunun önlenmesinde etkili olabileceği düşünülebilir.

Aralıklı oruçta uzun süreli açlık beraberinde otofajiyi doğurur. Otofaji, hücrenin uzun süreli açlıkla karşı karşıya kalması durumunda besin elde etmek için hücre içi yapılarının parçalanması olayıdır. Besin yokluğunda otofajinin gerekli ihtiyaçları karşılayarak hücre içi dengesinin korunmasında önemli bir faktör olduğu bildirilmiştir (Ohsumi, 2001; Shintani ve Klionsky, 2004). Ayrıca otofajinin metabolizmanın düzenlenmesi, hücre farklılaşması, hücre yaşlanması ve ölümü, hücre içi patojenlerin yıkılarak bağışıklık sisteminde önemli bir etkiye sahip olduğu yönünde çalışmalar da mevcuttur (Mizushima ve ark., 2008). Orucun insan sağlığı üzerine etkilerinin popülerliği günden güne artmaktadır. Özellikle Japon bilim adamı Yoshinori Ohsumi'nin uzun yıllar yaptığı çalışmalar sonucu aç kalmanın hücrelerin sindirimden çok, kendini yenilemeye uyum sağlayarak vücut direncini arttırdığını göstermesi (Takeshige ve ark., 1992; Tsukada ve Ohsumi 1993; Mizushima ve ark., 1998; Ichimura ve ark., 2000) ve bu çalışmalar sayesinde 2016 yılında Nobel Ödülü alması konuya ilgiyi arttırmıştır (Anonim, 2016).

Mikrobiyota

Genel anlamıyla mikrobiyota bakteri, mantar, arkealar, virüs ve protozoanlar gibi çeşitli mikroorganizma popülasyonunun belirli bir yeri kolonize etmesi olarak tanımlanmaktadır. Başka bir deyişle vücudumuzun deri, ağız, boşaltım sistemi, akciğer ve gastrointestinal sistemin (GİS) farklı bölgelerinde yaşayan mikroorganizmaların tümüdür ve bu mikroorganizmaların konakçı üzerinde önemli faaliyetleri mevcuttur (Sekirov ve ark., 2010).

İnsan mikrobiyotasının kendi hücre sayısının yaklaşık 10 katı kadar yani 100 trilyondan fazla simbiyotik mikroorganizma tarafından kolonize edildiği düşünülürken (Qin ve ark., 2010) yakın tarihte bu sayının 1,3 katı kadar olduğu tespit edilmiştir (Sender ve ark., 2016). Vücudun her bölgesi farklı sayıda mikroorganizma barındırırken, bu bölgeler arasında en çeşitli bakteri popülasyonu bağırsaklarda bulunmaktadır (Human Microbiome Project 2012; Blum 2017). Yetişkin bir insanın ortalama ince bağırsak yüzölçümü 400 m²'dir. Yaklaşık olarak 6 metre uzunluğunda olan ince bağırsak ve 1,5 metre uzunluğundaki kolon, bütün mikrobiyotanın %70'ini barındırmaktadır. Bağırsak mikrobiyotasının toplam ağırlığı 1-2 kg kadardır ve bu kütlelenin önceleri yaklaşık 500-1000 mikroorganizma türü barındırdığı düşünülürken (Ramakrishna ve Krishnan, 2007) bu sayının 35.000'den fazla olduğu bildirilmiştir (Frank ve ark., 2007). GİS'te toplam 10¹³ - 10¹⁴ mikroorganizma olduğu düşünülmektedir (Gill ve ark., 2006). Ayrıca, toplam bakteri genleri perspektifinden tanımlanırsa, İnsan Mikrobiyom Projesi ve İnsan Bağırsak Yolunun Metagenomu (MetaHIT) çalışmaları, insan mikrobiyomunda 10 milyondan fazla gen olabileceğini düşündürmektedir (Qin ve ark., 2010).

Her ne kadar bağırsak mikroorganizmaları konakçının bağırsıklık sistemi tarafından patojen olarak görülse de bağırsak bakterilerinin çoğu patojenik değildir, aksine enterositlerle birlikte simbiyotik bir ilişki içindedirler. ABD İnsan Mikrobiyom Projesi (HMP) (Human Microbiome Project, 2012) ve Avrupa İnsan Bağırsak Yolu Metagenomikleri (MetaHIT) (Qin ve ark., 2010) çalışmaları sağlıklı bir bağırsak florasının, insanın genel sağlığı ile yakından ilişkili olduğunu kanıtlamıştır.

Yetişkin bir bireyin bağırsak mikrobiyota kompozisyonu dört büyük gruba içermektedir. Bunlar *Firmicutes*, *Bacteroidetes*, *Actinobacteria* ve *Proteobacteria* (Tablo 1) olup; intestinal mikrobiyotasının yaklaşık %95'ini *Firmicutes* ve *Bacteroidetes*'ler oluşturur (Claesson ve ark., 2011; Mariat ve ark., 2009).

Bağırsak mikrobiyotası beslenme, yaşam tarzı, egzersiz, stres, antibiyotik kullanımı gibi birçok faktörden etkilenmektedir. Modern insanla en az temas etmiş, hiç işlenmiş gıda tüketmeyen, hiç antibiyotik kullanmamış, cep telefonu maruziyeti olmayan en ilkel yaşam biçimine sahip Kızılderililer'den Yanomami Kabilesi'nin mikrobiyotasının incelenmesi sonucu, modern insanların yaklaşık %30'luk bir mikrobiyom kaybı yaşadığı ortaya konmuştur (Clemente ve ark., 2015).

Tablo 1. Yetişkin Bir Bireyin Temel Bağırsak Mikrobiyota Kompozisyonu (Tap ve ark., 2009)

Table 1. Basic Gut Microbiota Composition of an Adult Individual (Tap et al., 2009)

Bakteri Âlemi	Bakteri Cinsi
<i>Firmicutes</i> (çoğunluğu Gram +) %79.4	<i>Clostridium</i> , <i>Ruminococcus</i> , <i>Eubacterium</i> , <i>Roseburia</i> , <i>Ruminococcus</i> , <i>Butyrivibrio</i> , <i>Feacalibacterium</i> , <i>Lactobacillus</i>
<i>Bacteroidetes</i> (Gram -) %16.9	<i>Porphyromonas</i> , <i>Prevotella</i>
<i>Actinobacteria</i> (Gram +) %2.5	<i>Bifidobacterium</i>
<i>Proteobacteria</i> (Gram -) %1	<i>Eschericia coli</i> <i>enetobacteriaceae</i>

Bağırsak Beyin Eksenini

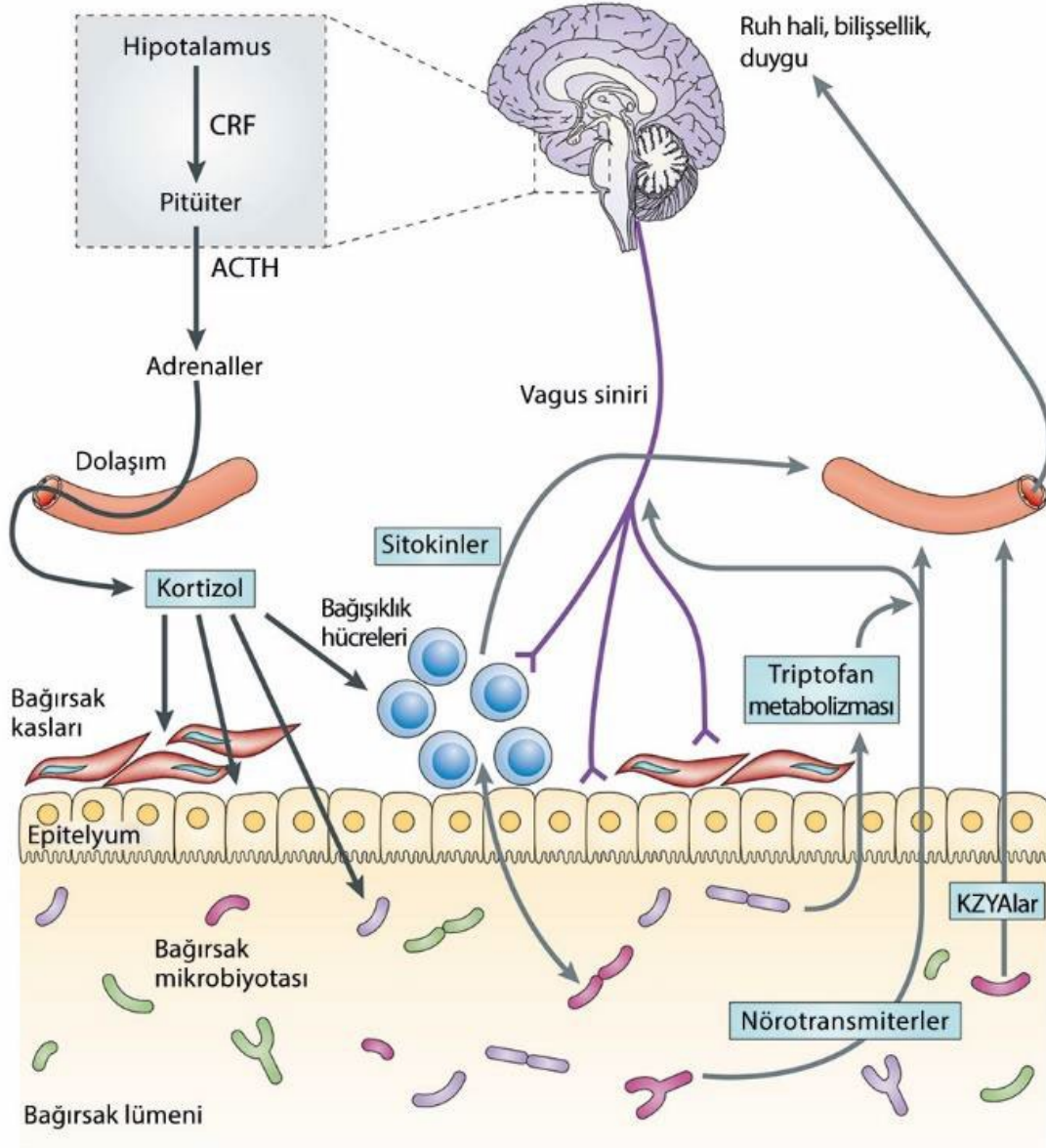
Bağırsak mikrobiyotası ve beyin birbiriyle çift yönlü iletişimde olan iki sistemdir. Çift yönlü bu iletişim bağırsak mikrobiyotasının beyinden etkilenmesini içerdiği kadar; bağırsak mikrobiyotasının da beyni etkilemesi anlamına gelmektedir. Beyin ve bağırsak etkileşimine dair birçok bilgi GİS'te bulunan bakteriler üzerinden elde edilmektedir (Morais ve ark., 2020).

Vücuda alınan ve sindirilemeyen posa ve prebiyotik besinler bağırsak mikrobiyotasında bulunan mikroorganizmalar tarafından fermente edilerek kısa zincirli yağ asitleri (KZYA) oluşturulur. Bu KZYA beyni yöneten nörotransmitter salgılanmasında görev alır. Bu önemli nörotransmitterlerin başında dopamin, serotonin, asetilkolin ve gama amino bütirik asit (GABA) gelir. Büyük çoğunluğu bağırsaklarda üretilen bu nörotransmitterler Nervus Vagus siniriyle beyne iletilir. Bu iletiye tam olarak bağırsak beyin eksenini denilmektedir. Bu nörotransmitterler hipotalamus, hipofiz ve böbrek üstü bezlerinin çalışmasında görev almaktadır. Bu bölgelerin doğru çalışmaması vücutta kortizol salınımına neden olarak kronik inflamatuvar hastalıkların (diyabet, hipertansiyon, inme, metabolik sendrom vb.) oluşmasına neden olmaktadır (Martin ve ark., 2018).

Vücutta dopamin salınımının azalması Parkinson (Zhang ve ark., 2022); serotonin salınımının azalması depresyon, anksiyete, unutkanlık (Huang ve Wu, 2021; Liu ve ark., 2021); asetilkolin salınımının azalması Alzheimer (Doifode ve ark., 2021; Qian ve ark., 2021) ve GABA salınımının azalması fibromiyaljiye (Clos-Garcia ve ark., 2019) neden olmaktadır. Kortizol üretimini azaltan önemli nörotransmitter GABA'dır (McCarroll ve ark., 2019).

Dünya Sağlık Örgütü (DSÖ) tarafından vücutta sağlığı bozacak derecede aşırı yağ birikimi olarak tanımlanan obeziteden dünyada yaklaşık 600 milyon yetişkin ve 100 milyon çocuğun etkilendiği bilinmektedir (GBD 2015 Obesity Collaborators, 2017). Obezite özellikle Tip2 Diyabetes Mellitus (DM),

kardiyovasküler hastalıklar ve dislipidemiye neden olarak beraberinde metabolik sendromu getirmektedir (Abdelaal ve ark., 2017; Ajala ve ark, 2017). İnsan GİS'nde bulunan patojen ve yararlı bakteriler arasındaki dengenin bozulmasıyla oluşan disbiyozis tablosunun da metabolik sendroma neden olduğu düşünülmektedir (Festi ve ark., 2014).



Şekil 1. Bağırsak Beyin Ekseni (Aydın ve Tuli, 2021)

Figure 1. Intestinal Brain Axis (Aydın and Tuli, 2021)

Aralıklı Oruç ve Mikrobiyota İlişkisi

Aralıklı oruç genellikle ne yenildiğinden ziyade ne zaman yenildiğine odaklanır. Genel olarak dışlanan bir besin olmakla birlikte çalışmalar aralıklı oruç diyetlerinde alınan enerjinin normal beslenmeye kıyasla %25-30 oranında azaldığını rapor etmektedir. Bu durum vücut ağırlığı kaybı sağlarken beraberinde bir takım metabolik değişiklikleri de getirmektedir (Most ve ark., 2017). Bunlardan bir tanesi de insanın genel sağlığıyla yakından ilişkili olan mikrobiyotadaki değişimlerdir.

Tablo 2’de bağırsak mikrobiyotası ve aralıklı oruç ile ilgili çalışmalar ve bu çalışmalardan elde edilen sonuçlar özetlenmiştir. Aralıklı oruç ve mikrobiyota ilişkisini inceleyen ilk çalışmalar 2017 yılında yayımlanmıştır. Buna göre aralıklı

oruç beyaz yağ dokusunu azaltırken bej yağ dokusunu arttırmakta; *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Tenericutes* ve *Firmicutes/Bacteroidetes* bolluğunu arttırmaktadır (Li ve ark., 2017). Beli ve ark. (2018) tarafından 7 ay süren bir çalışmada aralıklı orucun *Firmicutes* bakterilerinde artışa neden olmasına karşın diyabetik retinopati belirteçlerinde anlamlı bir iyileşme sağladığı gösterilmiştir. Wei ve ark. (2018) 8 haftalık çalışmasında da benzer sonuçlar gösterilmiş olup, DM belirteçlerinde iyileşme olduğu gösterilmiştir. Ülkemizde 2019 yılında yayınlanan bir çalışmada aralıklı oruçla *Akkermansia Muciniphila* ve *Bacteroides Fragilis* mikroorganizmalarında artış gözlemlenirken açlık glikoz ve total kolesterolde düşüş yaşandığı (Özkul ve ark., 2019), yakın tarihli yapılan bir çalışmada da benzer sonuçlara ek olarak nörotansmitter salgılanmasında görev alan KZYA salınımında artış olduğu rapor edilmiştir (Guo ve ark., 2021).

Tablo 2. Aralıklı Oruç ve Mikrobiyota İlişkisini İnceleyen Çalışmaların Genel Özeti

Table 2. Summary of Studies Examining the Relationship between Intermittent Fasting and Microbiota

Çalışma Tipi	Kaynak	Yıl	Hedef	Müdahale Süresi	Müdahalenin Etkisi
Hayvan	Li ve ark.	2017	Bağırsak mikrobiyotası	30 gün	↓ Beyaz yağ dokusu ↑ Bej yağ dokusu ↓ Obezite ↑ <i>Firmicutes</i> ↑ <i>Bacteroidetes</i> ↑ <i>Actinobacteria</i> ↑ <i>Tenericutes</i> ↑ <i>Firmicutes/Bacteroidetes</i>
Hayvan	Beli ve ark.	2018	Bağırsak mikrobiyotası Diyabetik retinopati (DR)	7 ay	↑ <i>Firmicutes</i> ↓ <i>Bacteroidetes</i> ↓ <i>Verrucomicrobia</i> ↓ DR uç noktaları ↓ Lökosit infiltrasyonu ↓ TNF- α mRNA ↓ Bağırsak mäsini ↑ Goblet hücre sayısı ↑ Villus uzunluğu ↑ Plazma peptidoglikan
Hayvan	Cignarella	2018	Bağırsak mikrobiyotası	4 hafta	↓ Yağ kütlesi ↓ Yağsız kütle ↑ Bağırsak bakteri zenginliği ↑ <i>Lactobacillaceae</i> ↑ <i>Bacteroidaceae</i> ↑ <i>Prevotellaceae</i> ↑ Antioksidatif mikrobiyal metabolik yollar ↓ IL-17 üreten T hücreleri ↑ Düzenleyici T hücreleri
Hayvan	Wei ve ark.	2018	Bağırsak mikrobiyotası	8 hafta	↓ Kan şekeri

			DM		↑ İnsülin duyarlılığı ↑ β hücre fonksiyonunda iyileşme ↓ <i>Prevotellaceae</i> ↓ <i>Alistipes</i> ↓ <i>Ruminococcaceae</i> ↑ <i>Parabacteroides</i> ↑ <i>Blautia</i>
Hayvan	Li ve ark.	2019	Bağırsak mikrobiyotası	30 gün	↑ <i>Bacteroides</i> ↑ <i>Akkermansia</i> ↑ <i>Erysipelotrichaceae</i>
Hayvan	Deng ve ark.	2020	Bağırsak mikrobiyotası Lipid profili	30 gün	↓ Vücut ağırlığı ↓ <i>Firmicutes/Bacteroidetes</i> ↑ <i>Allobaculum</i>
Hayvan	Li ve ark.	2020	Bağırsak mikrobiyotası	1 ay	↑ <i>Akkermansia</i> ↓ <i>Alistipes</i>
Hayvan	Liu ve ark.	2020	Bağırsak mikrobiyotası DM	28 gün	↓ İnsülin direnci ↓ Bilişsel bozukluk ↓ Beyaz yağ dokusu ↓ Adiposit ↓ Plazma LPS ↑ <i>Lactobacillus</i> ↑ <i>Odoribacter</i>
Hayvan	Shi ve ark.	2021	Bağırsak mikrobiyotası Kan basıncı	10 hafta	↑ Mikrobiyotada iyileşme ↓ Kan basıncı
İnsan	Özkul ve ark.	2019	Bağırsak mikrobiyotası	29 gün	↑ <i>Akkermansia muciniphila</i> ↑ <i>Bacteroides fragilis</i> ↓ Açlık glikozu ↓ Total kolesterol
İnsan	Guo ve ark.	2021	Bağırsak mikrobiyotası Kardiyometabolik risk faktörleri	8 hafta	↓ Yağ kütlesi ↓ Oksidatif stres ↓ İnflamatuar sitokinler ↑ Vazodilatör parametreler ↑ KZYA üretimi ↓ Lipopolisakkarit ↑ Mikrobiyotada iyileşme
İnsan	Su ve ark.	2021	Bağırsak mikrobiyotası	30 gün	↓ Vücut ağırlığı ↑ Bağırsak mikrobiyotasının çeşitliliği ↑ <i>Lachnospiraceae</i> ↑ <i>Firmicutes</i> ↑ <i>Clostridiales</i> ↓ <i>Bacteriodes</i> ↓ <i>Prevotellaceae</i> ↑ AST ↓ ALT ↑ AST/ALT

Sınırlı sayıdaki çalışma aralıklı orucun bağırsak mikrobiyotası üzerine olan temel mekanizmasını tam olarak açıklayamamaktadır. Bağırsak mikrobiyotamızın önemli bir bileşeni olan *Akkermansia muciniphila*'nın mukoza tabakasına güçlü bir şekilde yapıştığı (Derrien ve ark., 2004), diğer mikroorganizmalara oranla diyet değişiklikleri ve değişen dışkılama alışkanlıklarından daha az etkilendiği; diğer bakteri gruplarının tükenmesine bağlı olarak daha bol hale geldiği şeklinde yorumlanmıştır (Özkul ve ark., 2019). Benzer olarak aralıklı orucun *Lachnospiraceae* türlerinde bolluk sağladığı ve *Lachnospiraceae*'nin *Clostridiales* ile insan bağırsağındaki butirojenizin ana kaynağını oluşturduğu rapor edilmiştir (Su ve ark., 2021) ancak bu bolluğun tam olarak nasıl sağlandığı açıklanamamıştır. Bu gibi sonuçlar mikrobiyotanın bütün bileşenleri için de geçerli değildir. Beslenme periyotları kadar, tüketilen besinlerin içeriğinin; CHO, yağ, protein ve özellikle posanın mikrobiyotayı etkilediği bilindiğinden tek başına aralıklı orucun etkisi açıklanamamaktadır.

Sonuç

Aralıklı oruç diğer diyet türlerinde olduğu gibi belirli bir prosedüre dayanmaktadır. Buna göre aralıklı orucun temeli günün üçte birlik zamanında yeme içmenin serbest olduğu üçte ikilik bölümünde ise sınırlandırıldığı bir protokole dayanır. Bu tarz beslenme programı kişinin fizyolojik durumuna, yaşına, cinsiyetine, günlük beslenme biçimine ve kültürel özelliklere göre uygulanabilir.

Aralıklı orucun bağırsak mikrobiyotasına etkisi yapılan çalışmalara rağmen henüz tam olarak netleştirilememiştir. Aralıklı orucun *Lactobacillus* ve *Akkermansia* gibi ağırlık kazanımının önüne geçen mikroorganizmaların artışı sağlayarak vücut yağını kontrol edip, insülin duyarlılığını iyileştirdiği ve obezite ile ilgili diğer metabolik değişiklikleri düzenlediği rapor edilmiştir.

Genel olarak aralıklı orucun bağırsak mikrobiyotasındaki yararlı mikroorganizmaların sayısını artırıp, patojen mikroorganizmaların sayısını azaltarak modifiye ettiği yönünde çalışmalar olsa da gerek çalışmaların gerekse çalışmalardaki katılımcı sayısının az olması bu konunun daha detaylı olarak büyük örneklem sayısı ile çalışılması gerektiğini göstermektedir. Ayrıca çalışmalarda mikrobiyota sayı ve çeşitliliğini etkileyen besinlerin (makro ve mikro besin içeriği) kompozisyonu ve biyoyararlanımının net olmaması bu konudaki eksikliklerden sayılmaktadır.

Sonuç olarak aralıklı orucun mikrobiyota üzerindeki etkilerini inceleyen çalışmalar oldukça kısıtlı olmakla birlikte aralıklı orucun uzun dönemdeki etkilerini araştıran mevcut bir çalışma da bulunmamaktadır. Daha büyük örneklem sahip

yapılacak ayrıntılı çalışmalar, aralıklı oruç ve bağırsak mikrobiyotası arasındaki ilişkinin daha iyi anlaşılmasına yardımcı olacaktır.

Etik Standartlar ile Uyumluluk

Çıkar çatışması: Yazarlar bu yazı için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan etmişlerdir.

Etik izin: Araştırma niteliği bakımından etik izne tabii değildir.

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The "FOOD and HEALTH" journal provides a platform for the open public discussion of the journal contents. To secure accountability and to encourage sincere professional inputs without incivilities the system is set up to require registration and logging for the recording of inputs. Some of the website contents will be available without logging but no peer review comments can be posted on the website without the disclosure of the reviewer's identity to the journal editors.

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The editor of the "FOOD AND HEALTH" journals is responsible for deciding which of the articles submitted to the journal should be published. The editor may be guided by the policies of the journal's editorial board and constrained by such legal requirements as shall then be in force regarding libel, copyright infringement, and plagiarism. The editor may confer with other editors or reviewers in making this decision.

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An editor at any time evaluates manuscripts for their intellectual content without regarding race, gender, sexual orientation, religious belief, ethnic origin, citizenship, or political philosophy of the authors.

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The editor and any editorial staff must not disclose any information about a submitted manuscript to anyone other than the corresponding author, reviewers, potential reviewers, other editorial advisers, and the publisher, as appropriate.

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Unpublished materials disclosed in a submitted manuscript must not be used in an editor's own research without the express written consent of the author.

Research Ethics

An approval of research protocols by the Ethics Committee in accordance with international agreements (World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects," amended in October 2013, www.wma.net) is required for experimental, clinical, and drug studies. If required, ethics committee reports or an equivalent official document will be requested from the authors.

For manuscripts concerning experimental research on humans, a statement should be included that shows the written informed consent of patients and volunteers was obtained following a detailed explanation of the procedures that they may undergo. Information on patient consent, the name of the ethics committee, and the ethics committee approval number should also be stated in the Materials and Methods section of the manuscript. It is the author's responsibility to carefully protect the patients' anonymity. For photographs that may reveal the identity of the patients, signed releases of the patient or of their legal representative should be enclosed.

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“FOOD and HEALTH” journal requires experimental research studies on vertebrates or any regulated invertebrates to comply with relevant institutional, national, and/or international guidelines. The journal supports the principles of the Basel Declaration (<https://animalresearchtomorrow.org/en>) and the guidelines published by the International Council for Laboratory Animal Science (ICLAS) (<https://iclas.org/>). Authors are advised to clearly state their compliance with relevant guidelines.

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Submitted manuscripts that pass preflight are scanned for plagiarism using iThenticate software. Authors will be notified if plagiarism/self-plagiarism is detected. Editors can resubmit the article for any peer review or similarity check during production, if necessary. High similarity scores can cause an article to be rejected before or even after it is accepted. Depending on the type of article and the percentage of similarity scores from each article, the overall similarity score is generally expected to be less than 20%.

Double-Blind Referee Evaluation

After the plagiarism check, the editors evaluate the appropriate ones in terms of originality, methodology, the importance of the subject, and suitability to the journal's scope. The editor directs the submitted articles to a fair double-blind peer review (submits the articles that comply with official rules to at least two national/international referees for evaluation) and gives approval by managing the processes for publication after they are modified by the authors in accordance with the referees' rules.

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Reporting Standards

Authors should present an accurate account of the work performed as well as an objective discussion of the significance of the reports of original research. Underlying data should be represented accurately in the paper. The Manuscript should contain sufficient detail and references to permit others to replicate the work. Fraudulent or knowingly inaccurate statements constitute unethical behavior and are unacceptable.

Data Access and Retention

Authors are asked to provide the raw data in connection with a paper for editorial review and should be prepared to provide public access to such data (consistent with the [ALPSP-STM Statement on Data and Databases](#)).

Originality and Plagiarism

The authors should ensure that they have written entirely

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original works, and if the authors have used the work and/or words of others that should be appropriately cited or quoted.

By submitting articles to "FOOD and HEALTH" journal, the author attests to the following:

- Proper reference is provided for all contents extracted from other sources
- Strong action will be taken against cases of plagiarism
- All the papers submitted have to pass through an initial screening and will be checked through the Advanced Plagiarism Detection Software (iThenticate, etc.).

Multiple, Redundant or Concurrent Publication

An author should not publish manuscripts describing essentially the same research in more than one journal or primary publication. Submitting the same manuscript to more than one journal concurrently constitutes unethical publishing behavior and is unacceptable.

Acknowledgment of Sources

Proper acknowledgment of the work of others must always be given. Authors should cite publications that have been influential in determining the nature of the reported work.

Authorship of the Paper

Authorship should be limited to those who have made a significant contribution to the conception, design, execution, or interpretation of the reported study. All those who have made significant contributions should be listed as co-authors. Where there are others who have participated in certain substantive aspects of the research project, they should be acknowledged or listed as contributors.

The corresponding author should ensure that all appropriate co-authors and no inappropriate co-authors are included on the paper and that all co-authors have seen and approved the final version of the paper and have agreed to its submission for publication.

Hazards and Human or Animal Subjects

If the work involves chemicals, procedures or equipment that have any unusual hazards inherent in their use, the author must clearly identify these in the manuscript.

Disclosure and Conflicts of Interest

All authors should disclose in their manuscript any financial or other substantive conflicts of interest that might be construed to influence the results or interpretation of their manuscript. All sources of financial support for the project should be disclosed.

Fundamental Errors in Published Works

When an author discovers a significant error or inaccuracy in his/her own published work, it is the author's obligation to promptly notify the journal editor or publisher and cooperate with the editor to retract or correct the paper.

Responsibility for the Editor and Reviewers

General duties and responsibilities of the editor;

- Actively seek the views of authors, readers, reviewers, and editorial board members about ways of improving their journal's processes
- Encourage and be aware of research into peer review and 'journalology' and reassess journal processes in the light of new findings
- Work to persuade their publishers to provide them with appropriate resources, guidance from experts (e.g. designers, lawyers) and adequate training to perform their role in a professional manner and raise the quality of their journal
- Support initiatives designed to reduce academic misconduct
- Support initiatives to educate researchers about publication ethics
- Assess the effects of their journal policies on author and reviewer behavior and revise policies, as required, to encourage responsible behavior and discourage misconduct



- Ensure that any press releases issued by the journal reflect the message of the reported article and put it into context

Duties of Reviewers;

Contribution to Editorial Decisions: Peer review assists the editor in making editorial decisions and through the editorial communications with the author may also assist the author in improving the paper.

Promptness: Any selected referee who feels unqualified to review the research reported in a manuscript or knows that its prompt review will be impossible should notify the editor and excuse himself from the review process.

Confidentiality: Any manuscripts received for review must be treated as confidential documents. They must not be shown to or discussed with others except as authorized by the editor.

Standards of Objectivity: Reviews should be conducted objectively. Personal criticism of the author is inappropriate. Referees should express their views clearly with supporting arguments.

Acknowledgment of Sources: Reviewers should identify relevant published work that has not been cited by the authors. Any statement that an observation, derivation, or argument had been previously reported should be accompanied by the relevant citation. A reviewer should also call to the editor's attention any substantial similarity or overlap between the manuscript under consideration and any other published paper of which they have personal knowledge.

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All submissions are screened by similarity detection software. The similarity rate in the articles to be sent to the journal should be below 20%.

In the event of alleged or suspected research misconduct, e.g., plagiarism, citation manipulation, and data falsification/ fabrication, the Editorial Board will follow and act in accordance with [COPE](#) guidelines.

Each individual listed as an author should fulfill the authorship criteria recommended by the [ICMJE](#). The ICMJE recommends that authorship be based on the following 4 criteria:

1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work;
2. Drafting the work or revising it critically for important intellectual content;
3. Final approval of the version to be published;
4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

In addition to being accountable for the parts of the work he/she has done, an author should be able to identify which co-authors are responsible for specific parts of the work. In addition, authors should have confidence in the integrity of the contributions of their co-authors.

All those designated as authors should meet all four criteria for authorship, and all who meet the four criteria should be identified as authors. Those who do not meet all four criteria should be acknowledged on the title page of the manuscript.

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during the initial submission process in order to act appropriately on authorship rights and to prevent ghost or honorary authorship. If the editorial board suspects a case of “gift authorship,” the submission will be rejected without further review. As part of the submission of the manuscript, the corresponding author should also send a short statement declaring that he/she accepts to undertake all the responsibility for authorship during the submission and review stages of the manuscript.

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Manuscript Preparation

Manuscripts can only be submitted through the journal’s online manuscript submission and evaluation system, available at

<http://dergipark.gov.tr/journal/1646/submission/start>

Manuscripts submitted to the journal will first go through a technical evaluation process where the editorial office staff will ensure that the manuscript has been prepared and submitted in accordance with the journal’s guidelines. Submissions that do not conform to the journal’s guidelines will be returned to the submitting author with technical correction requests.

Authors are required to submit the following forms during the initial submission.

- Copyright Transfer, Ethic, Authorship Contribution Forms (one pdf file available from <https://dergipark.org.tr/tr/download/journal-file/19582>)
- ICMJE Potential Conflict of Interest Disclosure Form (should be filled in by all contributing authors) Download this form from <http://www.icmje.org/conflicts-of-interest/> fill and save. Send this to the journal with your other files.

Preparation of the Manuscript

Manuscripts prepared in Microsoft Word must be converted into a single file before submission. Please start with the title page and insert your graphics (schemes, figures, *etc.*), and tables in the one main text (word office file).

Title (should be clear, descriptive, and not too long)

Full Name(s) and Surname (s) of author(s)

ORCID ID for all author (s) (<http://orcid.org/>)

Authors complete correspondence Address (es) of affiliations and e-mail (s)

Abstract

Keywords (indexing terms), normally 3-6 items

Introduction

Material and Methods

Results and Discussion

Conclusion

Compliance with Ethical Standards

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- **Acknowledgment:** Acknowledgments allow you to thank people and institutions who assist in conducting the research.
- **Disclosure:** Explanations about your scientific / article work that you consider ethically important.

References

Tables (all tables given in the main text)

Figures (all figures/photos given in the main text)

Manuscript Types

Original Articles: This is the most important type of article since it provides new information based on original research. **The main text should contain “Introduction”, “Materials and Methods”, “Results and Discussion”, “Conclusion”, “Compliance with Ethical Standard” and “References” sections.**

Statistical analysis to support conclusions is usually necessary. Statistical analyses must be conducted in accordance with international statistical reporting standards. Information on statistical analyses should be provided with a separate subheading under the Materials and Methods section and the statistical software that was used during the process must be specified.

Units should be prepared in accordance with the International System of Units (SI).

Review Articles: Reviews prepared by authors who have extensive knowledge of a particular field and whose scientific background has been translated into a high volume of publications with a high citation potential are welcomed. These authors may even be invited by the journal. Reviews should describe, discuss, and evaluate the current level of knowledge of a topic in research and should guide future studies. The main text should start with the Introduction and end with the Conclusion sections. Authors may choose to use any subheadings in between those sections.

Short Communication: This type of manuscript discusses important parts, overlooked aspects, or lacking parts of a previously published article. Articles on subjects within the scope of the journal that might attract the readers’ attention, particularly educative cases, may also be submitted in the form of a “Short Communication”. Readers can also present their comments on the published manuscripts in the form of a “Short Communication”. The main text should contain “Introduction”, “Materials and Methods”, “Results and Discussion”, “Conclusion”, “Compliance with Ethical Standard” and “References” sections.

Table 1. Limitations for each manuscript type

Type of manuscript	Page	Abstract word limit	Reference limit
Original Article	≤25	180	40
Review Article	no limits	180	60
Short Communication	≤5	150	20

Tables

Tables should be included in the main document, and presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text).

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Tables should be created using the “insert table” command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

Figures and Figure Legends

Figures, graphics, and photographs should be submitted in main document WORD files (in JPEG or PNG format) through the submission system. Any information within the images that may indicate an individual or institution should be blinded. The minimum resolution of each submitted figure should be 300 DPI. To prevent delays in the evaluation process, all submitted figures should be clear in resolution and large (minimum dimensions: 100×100 mm). Figure legends should be listed at the end of the main document.

All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company, should be provided in parentheses in the following format: “Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)”

All references, tables, and figures should be referred to within the main text, and they should be numbered consecutively in the order they are referred to within the main text.

Limitations, drawbacks, and shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

References

Reference System is APA 6th Edition (with minor changes)

The APA style calls for three kinds of information to be included in in-text citations. The author's last name and the work's date of publication must always appear, and these

items must match exactly the corresponding entry in the references list. The third kind of information, the page number, appears only in a citation to a direct quotation.

....(Erkan, 2011).

....(Mol and Erkan, 2009).

....(Özden et al., 2021).

....(Mol and Erkan, 2009; Erkan, 2011; Özden et al., 2021).

Citations for a Reference Section:

An article

Olcay, N., Aslan, M., Demir, M.K., Ertas, N. (2021). Development of a functional cake formulation with purple carrot powder dried by different methods. *Food and Health*, 7(4), 242-250. <https://doi.org/10.3153/FH21025>

A book in print

Harrigan, W.F. (1998). Laboratory Methods in Food Microbiology. Academic Press, pp. 308. ISBN: 9780123260437

A book chapter

Craddock, N. (1997). Practical management in the food industry A case study. In Food Allergy Issues for the Food Industry; Lessof, M., Ed.; Leatherhead Food RA: Leatherhead, U.K., pp 25-38. ISBN: 4546465465

A webpages

CDC (2020). Rift Valley Fever | CDC. <https://www.cdc.gov/vhf/rvf/index.html> (accessed 20.08.2020).

Revisions

When submitting a revised version of a paper, the author must submit a detailed “Response to the reviewers” that states point by point how each issue raised by the reviewers has been covered and where it can be found (each reviewer’s comment, followed by the author’s reply and line numbers where the changes have been made) as well as an annotated copy of the main document. Revised manuscripts must be submitted within 15 days from the date of the decision letter. If the revised version of the manuscript is not

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submitted within the allocated time, the revision option may be cancelled. If the submitting author(s) believe that additional time is required, they should request this extension before the initial 15-day period is over.

Accepted manuscripts are copy-edited for grammar,

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