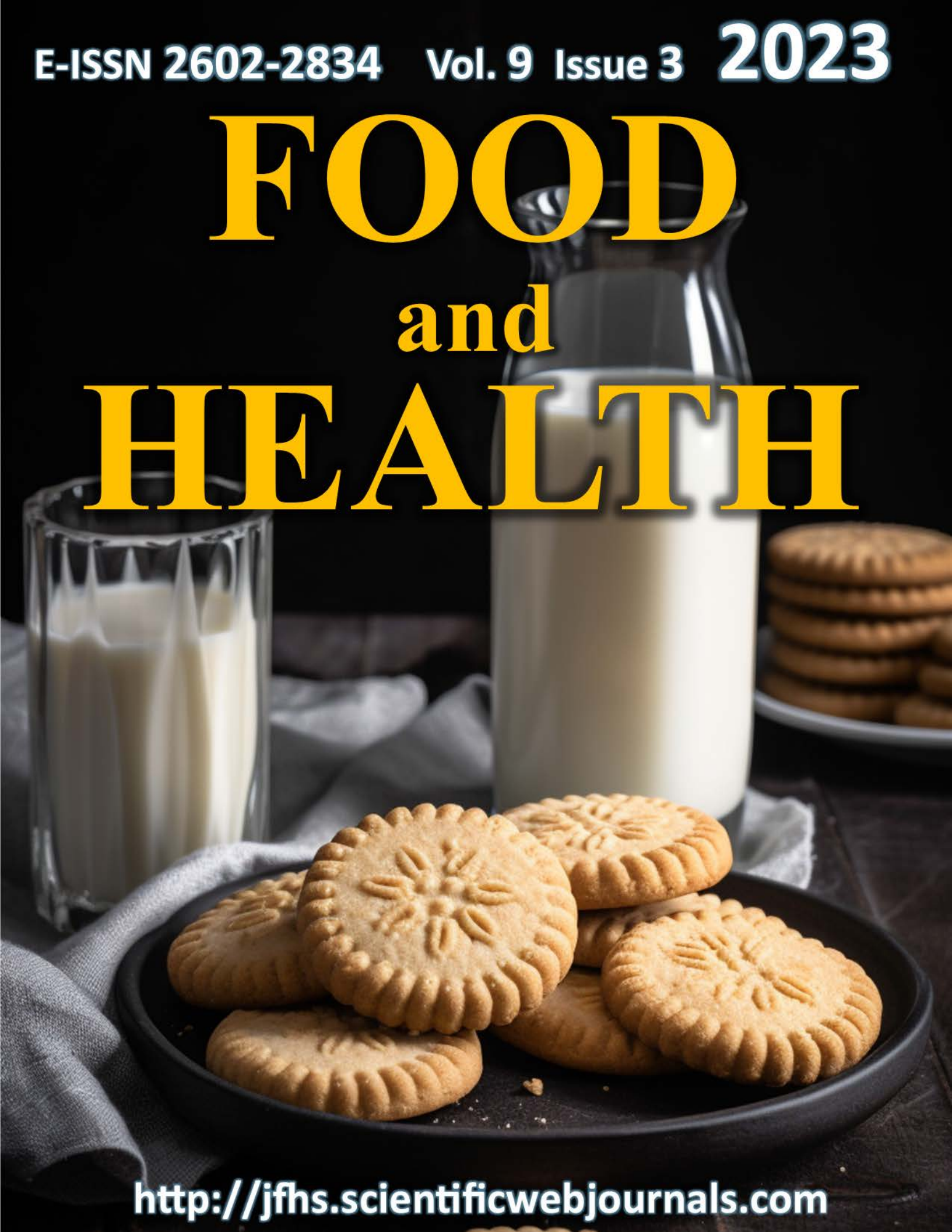
E-ISSN 2602-2834 Vol. 9 Issue 3 2023

FOOD and HEALTH



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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Chief Editor:

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Image by Miray Özden

Editorial Board:

Publisher Özkan Özden

Adress: Abdi Bey Sok. KentPlus Kadıköy Sitesi No:24B D. 435 Kadıköy/İstanbul, Türkiye
E-mail: swj@scientificwebjournals.com
for submission instructions, subscription and all other information visit
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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Aims and Scope

FOOD and HEALTH

Abbreviation: **FOOD HEALTH**

e-ISSN: 2602-2834

Journal published in one volume of four issues per year by

http://jfhs.scientificwebjournals.com **web page**

"Food and Health" journal will publish peer-reviewed (double-blind) articles covering all aspects of **food science and their health effect** in the form of original research articles (full papers and short communications), and review articles. Their team of experts provides editorial excellence, fast publication processes, and high visibility for your paper.

Food / Seafood / Food Technology / Food Chemistry / Food Microbiology/Food Quality/Food Safety/Food Contaminant / Food Allergen / Food Packaging / Modified Food / Functional Food / Dietary Supplements / Nutrition and their health effect is the general topics of the journal.

Manuscripts submitted to the "**Food and Health**" journal will go through a double-blind peer-review process. Each submission will be reviewed by at least two external, independent peer reviewers who are experts in their fields in order to ensure an unbiased evaluation process. The editorial board will invite an external and independent editor to manage the evaluation processes of manuscripts submitted by editors or by the editorial board members of the journal. Our journal will be published quarterly in English or Turkish language.

The journal's target audience includes specialists and professionals working and interested in all disciplines of food and Nutrition Sciences.

The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), and Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 9(3), 184-192 (2023) • https://doi.org/10.3153/FH23017 *Research Article*

A comparative analysis of ten milk samples with differential scanning calorimetry and Fourier transform infrared spectroscopy

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Cite this article as:
Dinç, B., Üstünsoy, R. Ertaş, T., Şen, E. (2023). A comparative analysis of ten milk samples with differential scanning calorimetry and Fourier transform infrared spectroscopy. *Food and Health,* 9(3), 184-192. https://doi.org/10.3153/FH23017

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Submitted: 14.01.2023
Revision requested: 01.03.2023
Last revision received: 07.03.2023
Accepted: 13.03.2023
Published online: 14.05.2023

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Available online at
http://jfhs.scientificwebjournals.com

ABSTRACT

Milk proteins occupy a prominent place in the nutrition of adults and children. Generally, some commercial dairy contains proteins, lactose, other sugar derivatives, and additives. The proportions of the components that make up the milk are different in commercial milk. For this reason, analyzing milk correctly is essential for determining these contents. In this research, analyses of the milk were made by taking differential scanning calorimetry measurements (DSC), and Fourier transform infrared spectrophotometer (FTIR) measurements. Specific heat values and specific values of temperature peaks were examined for ten kinds of milk. DSC curves revealed triacylglycerol dissolution, lactose crystallization, and protein denaturation peaks. Wide variations were observed with the same fat content from 10 milk powders. Most characteristic peaks were not observed when the samples were re-measured after a year at -20°C. The powder samples were compared in terms of protein, fat, lactose content, whey protein casein, and caseinate contents according to differences in FTIR spectra. The FTIR results confirm the DSC curves for most of the analyzed milk types.

Keywords: Differential Scanning Calorimetry, FTIR, Milk quality, Storage effect, Freeze-Drying

Introduction

More than six billion people worldwide consume milk and milk products. Cheese consumption for breakfast and yogurt consumption for other meals are common in Türkiye. Today nearly all commercial milk is being produced by using milk powders. More economical transportation, extended storage conditions, and ease of use make milk powder indispensable for manufacturing businesses. Milk powder is created simply by drying methods such as evaporating water from condensed milk. Milk components' chemical and physical characteristics undergo modifications during the drying process. These are the Maillard reaction, protein denaturation, protein-protein aggregation, protein-fat aggregation, and changes in protein-carbohydrate bonds. Additionally, physical results such as lactose crystallization, stickiness, precipitation, and retention of undesirable aroma (Kaur et al., 2021) are also in these modifications. The occurrence of these adverse modifications, which can damage the milk quality, may depend on many factors, such as the processes applied to milk and their storage conditions. These modifications may be due to the changes in lactose, fat, and protein structure, depending on the storage conditions of the milk (Tsourouflis et al., 1976).

Lactose, a disaccharide, ensures the quality of milk and its long-term healthy preservation. Lactose is generally observed as amorphous glass and is stable under the glass transition temperature (T_g) (Herrington, 1934). The T_g value of lactose was determined as 101°C (Roos & Karel, 1990). The amorphous glass structure of lactose is hygroscopic, i.e., it attracts and accumulates water and could lead to plasticization and a decrease in T_g value (Slade et al., 1991). Amorphous lactose above the T_g increases molecular mobility and lowers the viscosity of milk, which can result in stickiness, molding, and crystallization (Roos & Karel, 1990).

The protein and fat content in milk is also significant for the quality of the milk. These components are very active in determining milk's physical and chemical properties, such as water absorption, glass transition temperature, and crystallization (Shrestha et al., 2007). Jouppila et al. (Jouppila & Roos, 1994) demonstrated that proteins in milk powder may impede the crystallization of lactose, placing a limit on it. In addition, the denaturation and aggregation of proteins create endothermic and exothermic peaks, respectively, that can be determined by differential scanning calorimetry (DSC) measurements (Phosanam et al., 2020). Milk fat, on the other hand, is characterized mainly by triglycerides and produces endothermic peaks that can vary between -40 and +40°C, which can be seen in DSC curves (Kim et al., 2005). In one- or two-component systems, transition links to molecular modification can be analyzed more quickly than in multi-element compounds. Still, the transitions of substances with many components, such as milk powder, can be more complex and challenging to understand. This situation is because the exchange transitions between the substances in the complex compounds may overlap (Rahman et al., 2012). For the analysis of these thermal transitions, the temperature range set by the DSC must be well defined; it can be concluded that this temperature should be below 150°C to gain information about the water content of milk (Jouppila & Roos, 1994). However, to determine the additives in milk, this temperature should be measured at temperatures above 200°C. Potassium nitrate and sodium nitrate are additives in milk, and the DSC peaks of these substances are at temperatures above 200°C, even though chemical additives are becoming increasingly restricted (Smid & Gorris, 2020). Determining the lower limit can also aid in optimizing the sensitivity of the DSC measurement. Setting the lower limit near the sample's glass transition temperature (Tg), for example, can improve measurement sensitivity by maximizing the heat capacity change that occurs in glass transition (Trachenko & Brazhkin, 2011). In addition, the peak size also gives us the amount of content information. For this reason, the differential scanning calorimetry (DSC) system is a method recommended to be used frequently in the quality assessment of foods (Raemy, 2003). In addition, DSC is a highly effective method for detecting physical changes in milk dehydration and storage stages (Vuataz, 2002).

Pellegrino (Pellegrino, 1994) stated that milk oil reduces heat transfer during heat treatment and has a protective effect on the components. The shelf life is prolonged with the participation of various preservatives in the milk, and the essential condition for using these presents is to be unharmful to health. Each country has set a severe limit by making legal regulations regarding the type and quantity of these protectors. In Türkiye, protective use is not alprohibiteheat-treatedated (Güven, 1998). For this reason, it is very important to trace these additives with different chemical analysis methods.

Another important point concerning the storage conditions of milk is that different storage temperatures and times change the protein structures of milk powders (Anema et al., 2006; Howard et al., 2015; Tunick et al., 2016). This research also evaluated this situation by making DSC measurements of milk powders stored at -20 °C for 12 months under the same conditions.

Methodologically, FT-IR spectroscopy is one of the routine methods that can be used in milk analysis and the determination of additives that impair the purity of milk. Infrared spectroscopy is recommended as a unique detection method for determining additives in liquid milk (Ali et al., 2020).

This report aims to understand the effect of storage conditions on milk powders and their composition. Ten different kinds of milk sold in the markets were freeze-dried. Then their components, such as fat, protein, and lactose, were analyzed with DSC, and the same procedure was applied after 12 months. Different amounts of fat and protein were also analyzed and characterized spectrally.

Materials and Methods

Materials

The cow milk of ten well-known brands of UHT milk sold in the market was chosen as samples in this study. The fat, protein, and carbohydrate values given on the milk labels are shown in Table 1.

Table 1. Fat, milk, and protein ratios of milk (grams/100mL)

	Fat	Protein	Carbohydrate
LM1	3.0	2.9	4.7
LM2	3.3	2.8	4
LM3	2.5	3	4.6
LM4	3.3	3	4.7
LM5	3.0	3.1	4.5
LM6	3.4	3.1	4.7
LM7	3.1	2.9	4.7
LM8	3.0	3.0	4.6
LM9	3.0	2.3	8.0
LM10	3.0	1.5	6.9

After the milk was purchased, it was stored at +4°C for three days before freeze-drying; 10 samples were placed in falcon tubes with a capacity of 15 mL, each of 10 mL, under a laminar flow cabinet to avoid contamination.

Freeze-Drying

Freeze-drying was performed at -40°C and 0.05 mBar pressure using a 24 h Lobcanco freeze drier. After the samples were stored at -20°C for 24 hours, the measurements were carried out.

DSC Analysis

DSC measurement was performed with the DSC 60 Plus Shimadzu. Before the measurements, the instrument was calibrated with indium (Melting point 156.6 °C). All samples for DSC analysis were weighed as 10 mg. As a DSC procedure, the reference cell was left empty, and the sample container was placed in the appropriate places in aluminum containers with a closed mouth. The measurement was made between 30°C and 300°C. Studies in the literature take these measurements between -100°C and 250°C (Pugliese et al., 2019). If potassium nitrate is added to milk, a peak specific to this substance may occur above 200°C. Accordingly, measurements were made up to 300°C. The DSC temperature increasing rate is adjusted to 5°C per minute. Measurements were made in an inert nitrogen environment.

FTIR Analysis

FTIR measurements were performed with the ATR FTIR system, Shimadzu IRAffinity-1S, by taking percent transmittance measurements in the wavenumber range of 400-4000 cm^{-1}. Measurements were taken from powder samples.

Results and Discussion

DSC Results

DSC is an instrument that allows measuring the specific heat capacity depending on the temperature and evaluating the phase transitions and enthalpy changes. It can be used in the pharmaceutical industry to examine protein-ligand interactions, determine protein folding and mutations, and define temperature-dependent phospholipid changes (Chiu & Prenner, 2011). For this reason, it is used to examine the protein and lipid structure changes of milk powders and other milk-derived products. Measurements with DSC were used in the 1950s to determine the polymorphism of milk fat due to rapid and slow cooling (Ten Grotenhuis et al., 1999).

Thermal measurement values of fats reveal results depending on fatty acids and triacylglycerol components. Differential scanning calorimetry measurement results in Figures 1 and 2 revealed different fat, lactose, and protein content ratios. The peaks at the beginning of the curves in the range of 11.51 ±0.2

K and 69.97 ±0.35 °C in DSC curves match the cascade melting of triacylglycerol groups dispersed in the lipid droplet (Zouari et al., 2021).

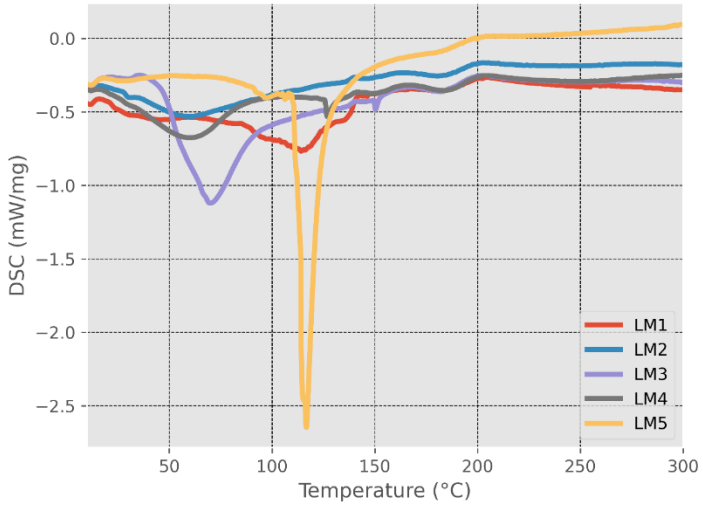

Figure 1. DSC curves of LM1-LM5

While the peaks of fatty acids were prominent in the milk LM3, LM4, LM7, LM8, LM9, and LM10, it was observed that the peaks of fatty acids were not significant in other milk, especially in the follow-on milk LM10 (Figure 1, Figure 2). Interestingly, when the values given on the label are examined, the milk with the least fat ratio is given as LM3. The highest fat-containing milk is LM2, LM4, LM6, and LM7. The values are given on the milk labels, and the values appearing on the DSC curves are different from each other.

The peak of LM10 at -23 °C ±0.33 indicates the polymorphic transition of milk fat from alpha to beta (Ten Grotenhuis et al., 1999). This is because some milk fat still exists in liquid form despite freeze-drying. Additionally, compared to other milk powders, it was in a sticky form, an observation further indicating the presence of polymorphic transition in LM10. These peaks, around -20ºC, are seen in all samples except LM9. The peaks at 11.2°C ±0.21 given by LM7 and LM9 milk in the DSC curves are the first onset temperature peaks and the starting temperature of alpha crystallization. The peaks between 110 and 116 °C are the peaks of lactose, and the highest lactose fraction among these 3 milk powder samples is found in LM9 milk. The peaks of LM2, LM4, and LM8 milk between 204 °C and 205 °C are α-lactose melting peaks. According to Buckton et al., these peaks are due to released water in the collapsed structure of lactose (Buckton et al., 1998). Here the dissolution of crystalline lactose is uneven. The peaks that LM2, LM3, LM4, and LM7 milk give between 140-150 °C are due to the presence of α-lactose monohydrate (Figure 1, Figure 2). The peaks seen here are endothermic (Thomas et al., 2004). On the other hand, determining glass transition temperature effectively determines additives' structural and physiochemical effects in food samples (Cordella et al., 2002).

DSC also determines milk adulteration (Poonia et al., 2017). The glass transition temperature was observed to increase depending on the amount of water added to the milk. The different glass transition temperatures seen between 90 and 120°C are due to the different proportions of water in the milk. The pure milk's glass transition (Tg) value in the previous measurements was 89.2°C. The Tg value was revealed at temperatures varying between 114 and 118°C depending on the amount of water added (Poonia et al., 2017). All the milk except LM3 and LM4 revealed Tg values in this range.

Today, most of the milk is produced from milk powder. Besides, milk powder is used in many products in the food sector, such as cheese, and is stored for months for later usage. During this storage, the physicochemical properties of milk powder change, and its quality decreases (Haque et al., 2010). For this reason, we have stored milk powders for a year at -20°C in closed tubes. When we analyzed the contents of the stored milk powders, we observed that the fat, protein, and lactose peaks of the milk powders disappeared. Only triacylglycerol groups and aquatic peaks were apparent at low amplitude in LM3 and LM9, respectively (Figure 3, Figure 4).

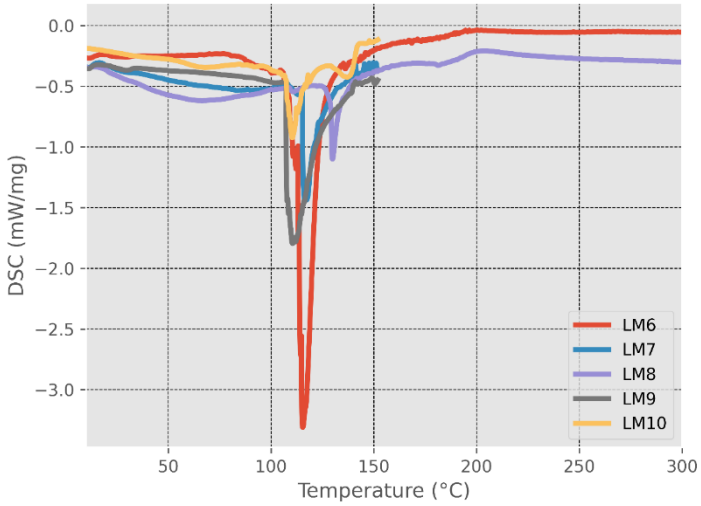

Figure 2. DSC curves of LM6-LM10

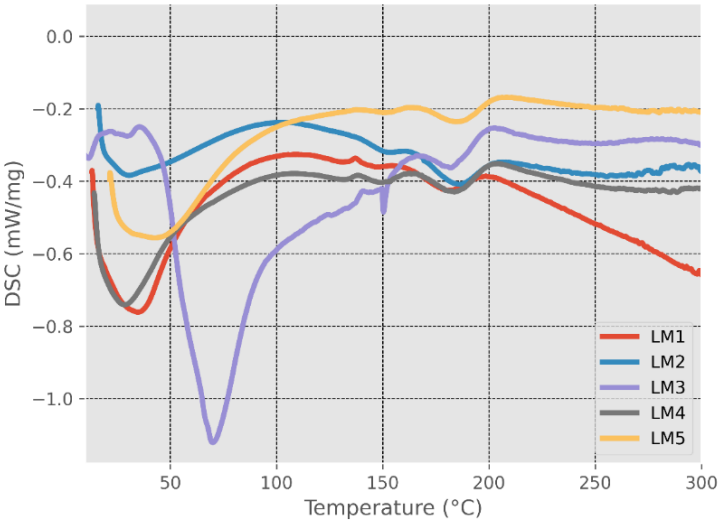

Figure 3. DSC curves of LM1-LM5 after 12 months

During storage, the tendency of milk powder to absorb moisture from the environment creates a bridge between the particles. This causes protein denaturation (Liu & Chaudhary, 2011). From this information, we can say that protein denaturation has occurred, and the physicochemical properties of milk powders have changed. On the other hand, Lactose crystallizes during storage because of the rearrangements of protein-lactose bonds. If amorphous lactose that binds to the active sites of proteins is reduced, it causes destabilization of the proteins (Buera et al., 2005). ΔH values were found by calculating the area of the peak of lactose in LM1 and LM5-10 coded milk. Lactose peaks were not observed in LM2, LM3, and LM4. Looking at the enthalpy values, the milk with the highest lactose content is LM5 and LM9, which have values of 503 and 433 J/g, respectively. Peaks observed in LM2, LM3, and LM4, 59, and 69 have been attributed to the casein glassy transition. It is most significant in the LM4 with a ΔH value of 310 (Table 2) (Jouppila & Roos, 1994; Morgan et al., 2005; Vuataz, 2002).

FTIR spectrums

Examination of the FTIR spectra is also used to determine the differences between milk ingredients. In milk production, protein ratios and nitrogen content are controlled. Adding melamine to milk increases the nitrogen content (Balabin & Smirnov, 2011). In determining melamine contamination, amide I and amide II regions revealed fingerprints in proteins at intervals of 1700-1400 cm^{-1} (Balabin & Smirnov, 2011). In the study of Jawaid et al. on the melamine additive to milk, characteristic peaks were observed between 851-798 cm^{-1} for pure melamine (Jawaid et al., 2013). These peaks are not included in our milk samples. 1800-700 cm^{-1} corresponds to the absorption peaks of carbonyl bonds, which are seen in milky lipids. In addition, the absorption bands between 3000 and 3050 cm^{-1} correspond to olefinic double bonds (-HC=CH-) of unsaturated fatty acids (Araki et al., 2015). Follow-on milk fats showed major peaks in the 753-756 cm^{-1}. Strong absorption of oils and fats are bands between 3000-2800 cm^{-1} caused by stretching vibrations in C-H groups.

One of the amide groups' peaks, visible at 1500 cm^{-1}, disappears in the follow-up milk LM9. The characteristic peaks of C-O vibrations in carbohydrates between 800-1200 cm^{-1} seem to form more and higher bands in follow-up milk LM9 and LM10 compared to the bands of other types of milk (Figure 5, Figure 6).

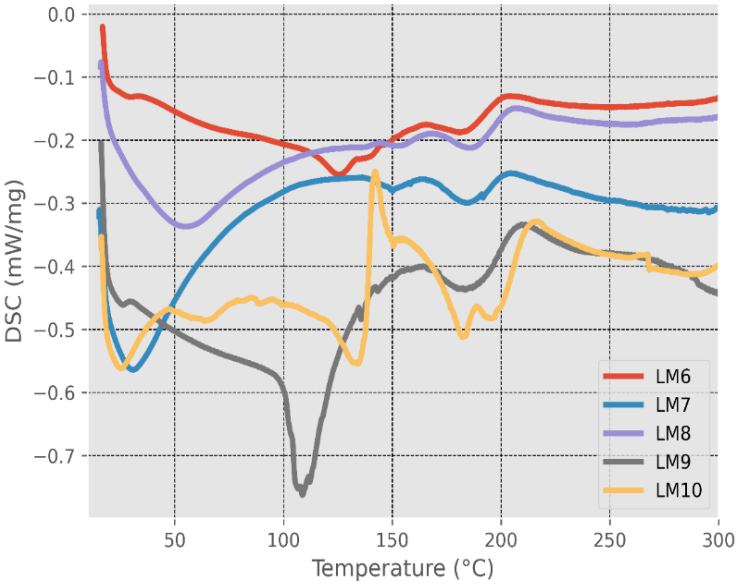

Figure 4. DSC curves of LM6, LM7, LM8, LM9, and LM10 after 12 months

Like DSC curves, FTIR peaks of fatty acids (Rachah et al., 2021) at 1747-1750 cm^{-1} of LM3, LM4, LM7, LM8, and LM9 are evident. The fatty acid peak was not marked in LM10 in DSC curves. Similarly, no peak was observed in LM10 in this band range.

The absorption at 2950 cm^{-1} matches that of C-H (CH_3). The bands at 2915 and 2848 cm^{-1} correspond with -C-H (CH_2) (Dutta et al., 2013). The bands in 1744 and 3004 cm^{-1} belong

to the C=O stretching vibrations of aliphatic esters and C–H stretching vibrations of the cis-CH out-of-plane vibration, respectively (Koca et al., 2010). The wavenumbers at 1320 and 1145 cm^{-1} shows the C–C(=O)–O and O–C–C of the C–O bonds of the esters and the bending vibrations of some methylene groups. The absorption peaks observed at 1467, 1382, 1150, 1117, 1097, and 1057 cm^{-1} are expressing C–H–CH_2, C–H–CH_3, =C–H– (cis), –C–H-bending, –C–H–, and –C–O–CH_2– functional groups, respectively (Figure 5, Figure 6). The bands 753-756 cm^{-1} can be considered an important region for the distinction between bovine and buffalo milk fat fractions and infant formula fat. The differences in the milk fat percentages of follow-on milk, observed in the spectral regions of milk fat, can be attributed to differences in the composition of these fat fractions concerning chain length and degree of unsaturation. It was observed that the total number of bands and their positions were similar in oils obtained from buffalo and bovine milk examined before (Antony et al., 2018).

Table 2. Data are reported as the mean of three replicates ± standard deviation. Tp/ C is the peak temperature of transition. ΔH (Jg-1):change in enthalpy

	>-50 (T_p/°C)	> 10 (T_p/°C)	>20 (T_p/°C)	40-70 (T_p/°C)	105-150 (T_p/°C)	> 150 (T_p/°C)	> 200 (T_p/°C)
LM1	-48.8 ±1.3	11.5 ±0.2	27.25 ±1.5	45.4 ±1.6	114 ΔH= 93 ±24	181.8 ±1.1	254.6 ±1.5
LM2	-30.6 ±1.8	11.6 ±1.5	28.7 ±1.03	59.6 ±1.4 ΔH= 291 ±7.1	140.5 ±1.8	179.8 ±2.6	
LM3		11.5 ±0.5	29.6 ±1.7	69.9 ±0.35 ΔH= 594 ± 2	150.3 ±1.4	181.7 ±1.2	
LM4	-30.4 ±1.6	11.8 ±1.8		59 ±0.7 ΔH= 310 ±3.1	147.8 ±2.8	183.8 ±2	204 ±2.7
LM5	-21.8 ±2.4	11.8 ±1.2			116.7 ±1.3 ΔH= 503 ±13		
LM6	-19.4 ±2.5	17.3 ±1.3			115.4 ± 1.4 ΔH= 43 ±13		
LM7		11.2 ±2.1 16.5 ±1.4			106.1 ±1.2 113.2 ±0.4 ΔH= 113 ±11		
LM8	-31.6 ±1.2 -7.1 ±4.3	11.2 ±0.21			110.8 ±1.8 129.9 ±0.7 ΔH= 42 ±15.4	181.2 ±1.2	204.3 ±1.3
LM9		11.0 ±0.9	29.4 ±1.08		110.4 ±0.6 ΔH= 433 ±16		
LM10	-23 ±0.3			67.7 ±2	110.3 ±2.3 ΔH= 93 ±15 137 ±2.5		

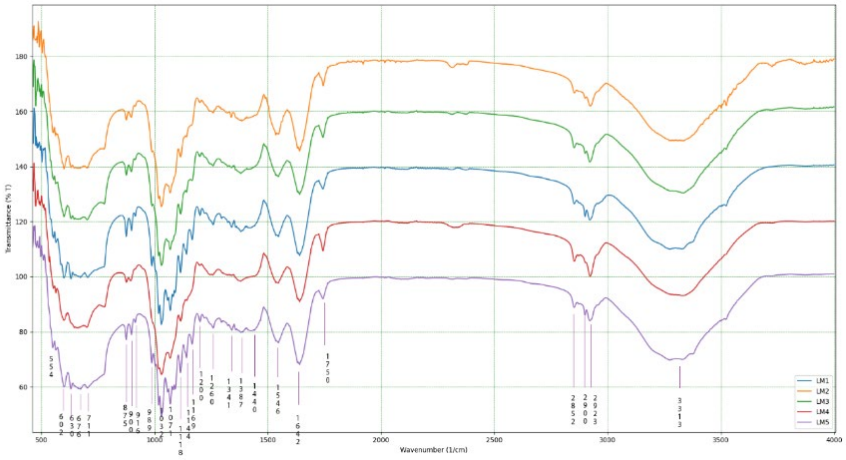

Figure 5. FTIR spectrums of LM1-LM5

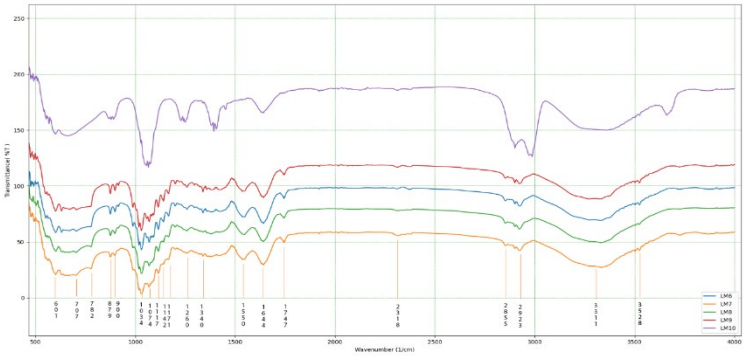

Figure 6. FTIR spectrums of LM6-LM10

Conclusion

Different components added to milk not only reduce milk quality but may also pose a danger to consumers. For example, milk mixed with whey can cause serious problems for people allergic to this substance. For this reason, research on milk content has an important place in the literature. This study identified different water ratios, lactose ratios, protein content, and carbon groups via DSC analysis and FTIR spectra in ten commercial milk brands. The percentages of fat, protein, and carbohydrates in most of the milk sold in the markets should be given on the labels correctly. For this reason, when calculating milk's fat, protein, and carbohydrate content, DSC and FTIR studies should be added to the analysis methods of the brand thanks to their fast, responsive, and accurate analysis. Milk quality also decreases as storage time intervals exceed about 12 months. In markets, packaged foods containing milk are stored at -20°C. For example, ice creams and pastry products using dairy products are stored and sold in the freezer at -20°C in Türkiye. These products are sold with an expiration date of at least one year. However, during this period, the physicochemical structure of milk powder deteriorates, and its nutritional value decreases. These products should not be the priority when purchasing.

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: Authors declare that this study includes no experiments with human or animal subjects.

Funding disclosure: -

Acknowledgments: -

Disclosure: -

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 9(3), 193-200 (2023) • https://doi.org/10.3153/FH23018 Research Article

Determination of consumers' knowledge levels and consumption status on probiotic and prebiotic products

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Cite this article as:
İnce Palamutoğlu, M., Bilgi, E., Horzum, M., Kılıç, Z., Karaca Çelilk, K.E. (2023). Determination of consumers' knowledge levels and consumption status on probiotic and prebiotic products. *Food and Health,* 9(3), 193-200. https://doi.org/10.3153/FH23018

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Submitted: 18.01.2023
Revision requested: 02.03.2023
Last revision received: 14.03.2023
Accepted: 15.03.2023
Published online: 16.05.2023

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Available online at
http://jfhs.scientificwebjournals.com

ABSTRACT

Between March 2022 and May 2022, this study was conducted to determine adult consumers' knowledge and consumption habits of probiotic and prebiotic products. Adults aged 18 to 65 were included in the cross-sectional study using an online questionnaire. Data were collected on participants' knowledge of the terms probiotics and prebiotics, patterns of consumption, frequency of consumption and variables affecting consumption, and diseases treated as a result of their usage. The study involved 447 volunteers, of whom 31.3% were men (n=140) and 68.7% were women (n=307). According to research data, 87.0% of consumers know about probiotics, and 62.2% know about prebiotics. There were statistically significant differences in knowledge of probiotics and prebiotics by sex ($p<0.001$ and $p<0.001$, respectively). When analyzed according to education level, probiotic knowledge improved as education level rose ($p<0.001$). Prebiotic knowledge and education level were statistically significant ($p<0.05$). Although probiotic and prebiotic knowledge is statistically significant according to education level, more research should be done on products, and individuals should be made aware of this issue.

Keywords: Probiotic, Prebiotic, Level of knowledge, Consumption status

Introduction

Awareness of the effect of nutrition on health is increasing daily (Yücel Şengün et al., 2020). It is argued that functional foods should be consumed in addition to nutrition during the day to provide additional benefits to help prevent or deal with diseases. (Pulido et al., 2021). Today, with the growing awareness of healthy nutrition, in addition to meeting the nutritional elements required by the person, the components in its structure are thought to improve current health by lowering the risk of diseases (such as cardiovascular disease, infections, cancer, diarrhea, constipation, and osteoporosis) and have a positive effect on consumer health. Functional foods are foods that improve one's quality of life. In recent years, functional foods have received much attention (Arduzlar Kağan et al., 2019; Demir and Aktaş, 2018; Pradito et al., 2020; Yabancı and Şimşek, 2007; Yücel Şengün et al., 2020). Foods containing probiotics and prebiotics are at the forefront of functional foods (Arduzlar Kağan et al., 2019; Yücel Şengün et al., 2020).

Probiotics are beneficial live microorganisms that provide various health functions to the host by improving the intestinal microbial balance (Pradito et al., 2020). Probiotic bacteria should have a minimum of 10^6 colony-forming units per gram (cfu/g) when they reach the intestines to provide these advantages. Various stress conditions during manufacture, storage, and transit through the gastrointestinal system endanger probiotic bacteria's survival. They must resist gastric juice and bile salts to keep the intestinal environment intact and exhibit their therapeutic functions on the host body (Baş, 2019). Many health benefits, such as overcoming the problem of intestinal infections, controlling pathogenic bacteria in the digestive system, stimulating the immune system, and lowering cholesterol and low-density lipoprotein level,s have been provided by probiotics (Pradito et al., 2020). However, the effect of probiotics varies according to the type of probiotic, the strain used, the dose, and the mode of administration. As a result, the predicted effects do not apply to all probiotic microorganisms. Probiotics are safe and well tolerated when taken orally. Furthermore, probiotic foods should be ingested regularly (Horasan et al., 2021). Fermented dairy products, kefir, boza, tarhana, pickles, soy products, hardly, turnip, table olives, wine, and beer are fermented items that contain probiotic microorganisms (Arpa Zemzem-oğlu et al., 2019a; Demirel, 2018; Özgül et al., 2020).

Prebiotics, on the other hand, are non-microorganic organic components that positively affect the host's health by stimulating the development and activity of one or a limited number of microorganisms in the colon and that cannot be digested in the upper gastrointestinal tract but can be fermented by microorganisms (*Bifidobacterium* and *Lactobacillus* species) in the colon (Yabancı and Şimşek, 2007; Yücel Şengün et al., 2020). It has been determined that prebiotics contribute to the probiotic effect and positively affect the host's health by providing the proliferation of one or more beneficial microorganisms (Taşdemir, 2017). Prebiotics are nutrients not hydrolyzed or absorbed in the stomach or small intestine. It should be selective for beneficial bacteria in the colon microflora and capable of stimulating their proliferation. It should alter the flora to a healthier composition and have positive local and systemic effects on the host (Baş, 2019). In this context, evidence supports the possible positive benefits of some nutrients, such as inulin and fructooligosaccharides (FOS) synthesized from oligofructose, sucrose, and oligosaccharides (xylooligosaccharides) containing galactose and xylose, began to emerge in the 1980s and early 1990s. The consumption of FOS and galactooligosaccharides in human milk has been shown to promote the proliferation of intestinal bifidobacteria (Precup et al., 2022). Microorganisms in the colon, prebiotics, which are taken with food and cannot be digested in the stomach and small intestine, are fermented by the colon microflora. The metabolites are released to form an energy source for the microflora. Prebiotics creates a healthy environment for the host in the intestinal flora, making it possible to use them to treat patients and prevent diseases (Yılmaz, 2004). The health benefits of prebiotics in the gastrointestinal tract have been linked to inhibiting the growth potential of pathogenic microorganisms that produce short-chain fatty acids like acetate, propionate, and butyrate, stimulating the immune system, lowering intestinal pH, and promoting mineral absorption. (Pehlivan, 2020; Precup et al., 2022). Recent studies have shown that prebiotic consumption potentially benefits human and animal health, gastrointestinal tract, obesity, type 2 diabetes mellitus, inflammatory bowel disease, cardiovascular diseases, bones, and neurological disorders. Also, prebiotics and probiotics can be combined into "synbiotics" to achieve synergistic effects. Probiotic strains will stimulate growth by fermenting the prebiotic (Precup et al., 2022).

There needs to be more clarity in society about probiotics and prebiotics. These concepts either need to be discovered or understood. This study aimed to determine consumers' knowledge levels and consumption status about probiotic and prebiotic products when the tendency toward ready-to-eat foods increases. In addition, it evaluated how gender, age,

and health conditions affect the consumption of probiotic and prebiotic products.

Materials and Methods

The study was approved by Afyonkarahisar Health Sciences University Non-Interventional Clinical Research Ethics Committee at the meeting numbered 2022/2 on 04.02.2022. This study was conducted in March-May 2022 with adults aged 18-65, using convenience sampling. According to TUIK (Turkish Statistical Institute) data, in February 2022, 67.9% (57.497.905) of Türkiye's population was between the ages of 18-65 (TUIK, 2022). With a 95% confidence level and a 5% acceptable error, the research was designed to include at least 384 participants (SSC, 2022). An online questionnaire was sent to 447 participants who agreed to participate in the study, and their consent was obtained. Living in the Republic of Türkiye, being willing to participate in the study, and being between 18 and 65 are the inclusion requirements.

The data were collected using a questionnaire form prepared by the researchers using the relevant literature (Horasan et al., 2021; Özgül et al., 2020; Yücel Şengül, 2020; Arpa Zemzemoğlu et al., 2019b) and using the online method. The questionnaire form consists of three parts and 28 questions. In the first part, the demographic information of the participants (age, gender, height, body weight, marital status, education status, income status); in the second part, the knowledge levels, preferences, attitudes, and behaviors of the participants about probiotics and prebiotics, and in the last part, the frequency of consumption of probiotic and prebiotic products. The consumption frequency table in the previous section was questioned as "I never consume" "daily", "2-3 times a week", "once a week", "fortnightly", "monthly" and "yearly".

The gathered data were analyzed in a computer setting using SPSS 26 descriptive statistics tools. The number and percentage frequency tables were used to provide introductory information on the study's participants, and the chi-square test was used to assess the relationship between the variables. The statistical significance threshold was set at $p<0.05$.

Results and Discussion

A total of 447 people participated in the research. The mean age of the participants was 24.3 ±7.66 years, and the mean body mass index calculated body weight/(height)^2 was 23.2 ±4.36 kg/m^2, 68.7% (n=307) of the research group were female and 31.3% (n=140) were male. According to their educational status, 83.0% (n=371) were university graduates, while only 0.4% (n=2) of the participants were primary school graduates. While 90.2% of the participants had no chronic disease, 9.8% reported a chronic disease (Table 1).

Table 1. Socio-demographic Characteristics of Participants

Gender	**n (447)**	**% (100)**
Male	140	31.3
Female	307	68.7
Educational Level	**n (447)**	**% (100)**
Primary School	2	0.4
Middle School	8	1.8
High School	56	12.5
University	371	83.0
Graduate	10	2.2
Diagnosed Disease Status	**n (447)**	**% (100)**
Yes	44	9.8
No	403	90.2
Diagnosed Diseases	**n (44)**	**% (100)**
Asthma - COPD	10	22.8
Celiac	1	2.3
Diabetes	6	13.6
Hypertension	6	13.6
Thyroid	6	13.6
Heart	9	20.5
Migraine	3	6.8
Rheumatic diseases	3	6.8
Level of income	**n (447)**	**% (100)**
Income less than expenses	156	34.9
Same as income	217	48.5
Income more than the expense	74	16.6

Table 2 shows the distribution table of the participants according to their knowledge, usage, and preferences for probiotic-prebiotic. Of 447 people participating in the research, 87% (n=389) stated that they knew the term probiotic and 45.8% (n=178) of the 389 people who knew the term probiotic said that they used probiotics. It was observed that 83.7% (n=149) of the individuals using probiotics preferred probiotics with food/as food, and 16.3% (n=29) preferred it as supplements (capsules, powder, pills, etc.). While only 6.5% of the participants in the study chose supplemental probiotics, only 2.8% (n=5) of them were determined to take probiotic supplements regularly every day. While the most important reason for preferring probiotics is the thought that it is beneficial for digestion (64.7%, n=115), the reasons for not choosing it are that they do not need it (41.2%, n=87).

Again, as indicated in Table 2, while those who knowledge the term prebiotic were 62.2% (n=278), 37.8% did not knowledge the term prebiotic. Prebiotics were found to be effective by 41.0% of the 278 participants who knowledge the term, partially effective by 44.6%, and ineffective by 14.4%.

Statistically significant differences were found both in probiotic and prebiotic knowledge and in the usage of probiotics according to gender ($p<0.001$, $p<0.001$, $p<0.05$, respectively).

Table 4, probiotic knowledge ($p<0.001$), prebiotic knowledge ($p<0.05$), and probiotic usage ($p<0.05$) were found to be statistically significant according to education level (Table 4).

When the research participants' frequency of using probiotics and prebiotics-containing goods was assessed, it was discovered that most did not consume other foods besides banana, tarhana, or garlic. Consumption of probiotic yogurt, kefir, tarhana, vinegar, garlic, banana, leek, rye, and oat are 18.1% (n=81), 18.3% (n=82), 48.1% (n=215), 25.1% (n=112), 38.7% (n=173), 39.6% (n=177), % 25.5 (n=109), 12.8% (n=57), 19.5% (n=87) respectively, consumption once a week was found to be higher, while consumption of Boza, Jerusalem artichoke and artichoke (14.8% (n=66), 17.7% (n=79), 15.0% (n=67) respectively) was determined to be higher once a year.

When 447 people who volunteered for the study were asked if they had heard of the probiotics and prebiotics, 87% (n=389) said they knew the probiotics, while 62.2% (n=278) said they knew the prebiotics. In a similar study of Izmir Province, Yücel Şengün et al. (2020) when asked if the consumers participating in the study were aware of the concept of probiotics and prebiotics, 49% said yes and 51% said they did not know. In a survey conducted for adults, 64.5% of the participants stated that they knew the concept of probiotic food (Arduzlar Kağan et al., 2019). Another study conducted for adults determined that 73.3% knew the term probiotic (Zeren, 2015). In another study conducted for working people, 96% of the participants stated that they knew the term probiotic (Özgül et al., 2020). Arpa Zemzemoğlu et al. (2019b), in their study for university students, 55.6% of the participants stated that they knew what probiotics were.

While 45.8% (n=178) of 389 study participants who were aware of the term "probiotic" reported using them in our study, the percentage for adults was reported as 73.6% (Arduzlar Kağan et al., 2019). In one study, the rate was determined to be 73.5% (n=446), and in another, the rate was determined to be 82.4% (n=593) (Horasan et al., 2021; Arpa Zemzemoğlu et al., 2019b). Özgül et al. (2020) stated that the probiotic consumption rate was 96% (n=24). In another study conducted with university students, a high probiotic consumption rate (99.6%, n=258) was found (Demirel, 2018). This high rate may be because the study was conducted on students who received nutrition education. The most significant factor in the participants' preference for probiotics was their thought that it was beneficial to their digestion (64.7%, n=115). In contrast, the main reason for not choosing was their thought that they did not require it (41.2%, n=87). These results were similar to studies by Horasan et al. (2021), Yücel Şengün et al. (2020), and Özgül et al. (2021), where the benefits of probiotics for digestion were cited by 73.8% (n=329), 63.9% (n=108), and 83.3% (n=20) of participants, respectively. With rates of 62.1% (n=100), and 55.6% (n=15), Horasan et al. (2021) and Yücel Şengül et al. (2020) stated that the reason for not preferring in their study was because they did not know what probiotic products were. (Yücel Şengün et al., 2020; Horasan et al., 2021; Özgül et al., 2020).

Table 2. Distribution of the participants according to the probiotic-prebiotic knowledge, usage, and preference status

Probiotic knowledge	**n (447)**	**% (100)**
Yes	389	87.0
No	58	13.0
Probiotic usage	**n (389)**	**% (100)**
Yes	178	45.8
No	211	54.2
Type of preference in probiotic usage	**n (178)**	**% (100)**
With/As food	149	83.7
With supplements (capsules, powder, pills, etc.)	29	16.3
Frequency of usage of Probiotic Supplements (capsule, powder, pill, etc.)	**n (178)**	**% (100)**
None	149	83.7
Daily	5	2.8
Once a week	3	1.7
2-3 times a week	6	3.4
Fortnightly	5	2.8
Monthly	3	1.7
Yearly	7	3.9
Reason for the usage of probiotics	**n (178)**	**% (100)**
Beneficial for the digestive system	115	64.7
I think it protects against cancer	4	2.2
I find it delicious	4	2.2
It strengthens the immune system	55	30.9
Why don't usage probiotics	**n (211)**	**% (100)**
I don’t know what it is	74	35.1
I do not find it natural	18	8.5
I do not need	87	41.2
I find it expensive	24	11.4
I do not distrust content	8	3.8
Prebiotic knowledge	**n (447)**	**% (100)**
Yes	278	62.2
No	169	37.8
Effectiveness of prebiotics	**n (278)**	**% (100)**
Yes	114	41.0
No	40	14.4
Partially	124	44.6

Table 3. Distribution of Probiotic-Prebiotic knowledge and usage of participants by gender

	Yes		No		Total	p-value /χ^2
Probiotic knowledge	**n**	**%**	**n**	**%**		
Female	281	91.5	26	8.5	307	p=0.001
Male	108	77.1	32	22.9	140	χ^2 =17.6
Probiotic usage						
Female	135	44.0	172	56.0	307	p=0.008
Male	43	30.7	97	69.3	140	χ^2 =7.05
Prebiotic knowledge	**n**	**%**	**n**	**%**		
Female	214	69.7	93	30.3	307	p=0.001
Male	64	45.7	76	54.3	140	χ^2 =23.5

Table 4: Probiotic-Prebiotic knowledge and usage distribution table according to the education level of the participants

	Yes		No		Total	p-value /χ^2
Probiotic knowledge	**n**	**%**	**n**	**%**		
Primary School	0	0.0	2	3.4	2	
Middle School	2	0.5	6	10.4	8	
High School	38	9.8	18	31.0	56	p=0.001
University	340	87.4	31	53.5	371	χ^2 =66.0
Graduate	9	2.3	1	1.7	10	
Probiotic usage						
Primary School	0	0.0	2	0.7	2	
Middle School	0	0.0	8	3.0	8	
High School	22	12.4	34	12.6	56	p=0.034
University	149	83.7	222	82.5	371	χ^2 =10.4
Graduate	7	3.9	3	1.2	10	
Prebiotic knowledge	**n**	**%**	**n**	**%**		
Primary School	2	0.7	0	0.0	2	
Middle School	1	0.4	7	4.1	8	
High School	29	10.4	27	16.0	56	p=0.010
University	239	86.0	132	78.1	371	χ^2 =13.2
Graduate	7	2.5	3	1.8	10	

Table 5. The frequency of usage of Probiotic-Prebiotic products by the participants

Foods	Never		Daily		2-3 times a week		Once a week		Once a month		Once a year	
	n	%	n	%	n	%	n	%	n	%	n	%
Probiotic yogurt	222	50.8	17	3.8	45	10.1	81	18.1	46	10.3	31	6.9
Kefir	219	49.0	4	0.9	30	6.7	82	18.3	71	15.9	41	9.2
Boza	341	76.3	6	1.3	7	1.6	15	3.4	12	2.7	66	14.8
Tarhana	92	20.6	15	3.4	39	8.7	215	48.1	73	16.3	13	2.9
Vinegar	155	34.7	17	3.8	76	17.0	112	25.1	60	13.4	27	6.0
Garlic	94	21.0	26	5.8	102	22.8	173	38.7	46	10.3	6	1.3
Jerusalem artichoke	276	61.7	10	2.2	12	2.7	40	8.9	30	6.7	79	17.7
Artichoke	255	57.0	13	2.9	7	1.6	56	12.5	49	11.0	67	15.0
Banana	60	13.4	34	7.6	130	29.1	177	39.6	43	9.6	3	0.7
Leek	170	38.0	4	0.9	6	1.3	114	25.5	109	24.4	44	9.8
Rye	250	55.9	26	5.8	29	6.5	57	12.8	50	11.2	35	7.8
Oat	186	41.6	25	5.6	66	14.8	87	19.5	61	13.6	22	4.9

It was observed that 83.7% (n=149) of the individuals using probiotics preferred probiotics with food/as food, and 16.3% (n=29) preferred it as supplements (capsules, powder, pills, etc.). Although only 6.5% of our study participants preferred probiotic supplementation, Özgül et al. (2020) stated that 20% (n=5) of participants indicated that they consumed dietary supplements (capsules, powders, pills, etc.).

In the study, it was determined that 91.5% of women (n=281) and 77.1% (n=108) of men knew the terms probiotic and prebiotic ($p<0.001$). In a similar study, when probiotic and prebiotic knowledge was evaluated according to gender, it was determined that 65.3% of women and 31.6% of men knew the concepts of probiotic and prebiotic ($p<0.05$) (Yücel Şengün et al., 2020). Arduzlar Kağan et al. (2019) it was determined that women knew the concept of probiotics at a higher rate (75.8%) in their study on adults. In the study of Zeren (2015) for adults, it was determined that women knew the concept of probiotics at a higher rate (80.0%). Similarly, in a study on individuals who do sports, 52.4% of women declared that they knew the term probiotic (Koç, 2020). The fact that women have a high level of knowledge about probiotic and prebiotic products in studies may be associated with paying more attention to nutrition and health than men. For this reason, women's knowledge level and higher consumption of these products make it meaningful.

In the study, a statistically significant difference was found in the usage of probiotics by gender ($p<0.001$); Arduzlar Kağan et al. (2019) also found no statistically significant difference ($p>0.05$). Horasan et al. (2021) found that probiotic food consumption was higher in women (77.4%), and this difference was found to be statistically significant ($p<0.001$).

Knowledge of the term probiotic according to education level was found to be statistically significant ($p<0.001$), and it was determined that the group with the highest knowledge about probiotics was the group at the university level (87.4%, n=340). No statistically significant difference ($p>0.05$) between knowledge of the term prebiotic according to education level. Yücel Şengün et al. (2020), when assessing the level of knowledge of these concepts by educational level, found that the group with the highest level of knowledge (66.7%) consisted of those at the master's level.

When the frequency of usage of probiotics and prebiotics-containing products of the individuals in the study was evaluated, it was determined that most of them did not consume other foods except bananas, tarhana, and garlic. Yücel Şengün et al. (2020) found that 51% (n=100) kefir and 77.9% (n=152) onion and garlic consumption accounted for the majority.

Conclusion

The demand for probiotic and prebiotic products is increasing rapidly worldwide and in Türkiye. However, it is important to understand consumers' attitudes toward probiotic and prebiotic products and to increase consumer acceptance. With a rise in research and publications demonstrating the benefits of probiotics and prebiotics on health, probiotic and prebiotic awareness and usage rates have been observed. Although

there is a high degree of understanding of probiotics and prebiotics, the utilization percentage has been discovered to be as low as 45.8%. Despite having adequate knowledge, there remains skepticism about using such items. The study found that the people who drank probiotics and prebiotic products consumed tarhana the most frequently, with 48.1% ingesting it once a week. The predicted effect of using probiotic products depends on their frequent usage. More research on probiotics and prebiotic products should be conducted, and individuals should be made aware of the issue.

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: Ethics approval was obtained with the meeting of Afyonkarahisar Health Sciences University Non-Interventional Clinical Research Ethics Committee dated 04.02.2022 and numbered 2022/2.

Funding disclosure: -

Acknowledgments: -

Disclosure: -

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 9(3), 201-211 (2023) • https://doi.org/10.3153/FH23019

Research Article

Hazelnut-enriched diet effectively increases lipoproteins' resistance to oxidation in hypercholesterolemic subjects

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Cite this article as:
Akcan, B., Örem, A., Kural, B., Altınkaynak, Y., Yücesan, F.B., Örem, C. (2023). Hazelnut-enriched diet effectively increases lipoproteins' resistance to oxidation in hypercholesterolemic subjects. *Food and Health,* 9(3), 201-211. https://doi.org/10.3153/FH23019

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Submitted: 23.11.2022
Revision requested: 06.03.2023
Last revision received: 08.03.2023
Accepted: 15.03.2023
Published online: 07.06.2023

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Available online at
http://jfhs.scientificwebjournals.com

ABSTRACT

Oxidative modifications of lipoproteins are crucial in the early stages of cardiovascular diseases (CVD) like hypercholesterolemia. The major aim is to determine the effects of hazelnut consumption on the resistance of low- and high-density lipoproteins to oxidation in hypercholesterolemic subjects. Fifteen hypercholesterolemic subjects (13 men, 2 women) who did not require drug treatment according to the National Cholesterol Education Program Adult Treatment Panel III criteria and had serum levels of TC>200 mg/dL were included in the study. This study was designed as a dual control sandwich model intervention with a single group, isoenergetic three periods. The periods were; Control Diet I (30 days), Hazelnut-Enriched Diet (30 days), and Control Diet II (30 days). The susceptibility of LDL and HDL to copper-mediated oxidation was determined spectrophotometrically by following conjugated diene formation. The resistance of lipoproteins, mainly LDL, was increased by hazelnut consumption. It can be concluded that a hazelnut-enriched diet had protective effects against lipoprotein oxidation in hypercholesterolemic subjects. These changes may play important roles in reducing the development of the atherosclerotic process.

Keywords: Hypercholesterolemia, Hazelnut, Lipids, Oxidation, Lipoproteins

Introduction

Cardiovascular diseases (CVD) are among the most important health problems nowadays. The main underlying cause of cardiovascular disease is atherosclerosis (Nag et al., 2013). Atherosclerosis is a chronic inflammatory disease. Genetic susceptibility, endothelial dysfunctions, hyperlipidemia changes in lipoprotein levels, and oxidation that occurs in the structure of low-density lipoproteins (LDL) are very important in the pathogenesis of atherosclerosis (Borén et al., 2020; Steinberg et al., 1989).

Hypercholesterolemia is a pathological condition caused by increased serum TC and LDL cholesterol levels. It has been shown that there is a strong relationship between hypercholesterolemia and the development of atherosclerosis. Hypercholesterolemia leads to the formation of reactive oxygen species as a result of oxidative stress and may cause the oxidation of lipids and lipoproteins (Negre-Salvayre et al., 2006; Singh et al., 2017). In particular, the oxidation of LDL has important pathophysiological roles in initiating and sustaining the complex cascade of events in atherosclerosis. LDL oxidation is considered an important biomarker in studies examining the effects of bioactive foods on protection against cardiovascular diseases (Winklhofer-Roob et al., 2017).

In terms of heart and vascular diseases, increasing the proportion of fruits, vegetables, nuts such as hazelnuts, and whole grains in the daily diet are the main ingredients of healthy nutrition (Renzo et al., 2019; Segovia-Siapco et al., 2018). Daily dietary intake of nuts and hazelnut oil (not to exceed 20% of the energy from the lipids), which is rich in saturated fatty acids, rich in monounsaturated fatty acids (MUFA), especially oleic acid, high in polyunsaturated fatty acids (PUFA), and high in antioxidant vitamin, vitamin E, would be beneficial in protecting against development atherosclerotic CVD related to hypercholesterolemia (Akhlaghi et al., 2020; Segovia-Siapco et al., 2018).

Cohort studies in nutritional epidemiology reported a 30-50% reduction in CVDs risk associated with consuming nuts (Fraser, 2000). According to the research, people who consumed nuts 1 to 4 times a week and those who consumed less than 1 per week showed a 25% reduction in the risk of death from CVDs. Also, when the frequency of consumption is 5 or more per week, this risk decreases by 50% (Fraser, 2000; Mukuddem-Petersen et al., 2005).

Although many studies investigate the effects of hazelnut consumption on health, studies on hypercholesterolemic individuals are very limited (Brown et al., 2022). In addition, a study examining the impact of hazelnut on HDL oxidation has yet to be done.

In this study, we aimed to investigate the effects of 30 days of hazelnut consumption on LDL and HDL in the hypercholesterolemic subjects who did not require drug treatment.

Materials and Methods

Chemicals

Chemicals; Ethylenediamine Tetra Acetic Acid (EDTA, Disodium salt, CARLO ERBA Barcelona, Spain); Folin-Ciocalteu's Phenol Reagent (SIGMA Missouri, ABD); Sodium Hydrogen Phosphate Dihydrate, Sodium-Hydrogen Phosphate Monohydrate, Sodium Hydroxide, Sodium Bicarbonate, Sodium Potassium Tartrate (MERCK, Darmstadt, Germany); Sodium-Chloride, Sodium Dioxide, Sodium Hydrogen Carbonate (SIGMA-ALDRICH, Missouri, ABD).

Subjects

Fifteen hypercholesterolemic subjects (13 men, 2 women) who did not require drug treatment according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) criteria (Grundy et al., 2004) and had serum levels of TC >200 mg/dL with a mean age 43.6 ±9.5 were included in the present study. Exclusion criteria included nut allergy, smoking status, alcohol or drug abuse, acute and chronic inflammatory diseases, chronic kidney disease, obesity, and endocrine disorders related to lipid and lipoprotein metabolism. All participants were warned not to change their diet, physical activity, or other lifestyle.

Study Design

This study was designed as a dual control sandwich model intervention with a single group, isoenergetic three periods. Each period was 30 days. Calories and nutrients taken in the study periods are given in Table 1. Control Diet I (CDI), hazelnut-enriched diet (HED), and Control Diet II (CDII) were applied to all subjects for the first, second, and last 30 days, respectively. CDI was equivalent to CDII: NCEP ATP III step 2 diet (<7% energy from saturated fatty acids and 200 mg/day dietary cholesterol). During the HED period, hazelnut contributed to 18%–20% of the total daily energy intake, which did not change the daily total energy intake significantly. Two equally weighted packages of daily hazelnuts (49–86 g/day Corylus avellana L. varieties “tombul” from Giresun in Black Sea Region of Türkiye, approximately 82.72% palmitic, 8.89% palmitoleic, 4.85% stearic, 2.73% oleic and

linoleic acids.) was provided to all participants to consume as one portion between breakfast and lunch, and other portion between lunch and dinner. Only water was allowed during hazelnut consumption. Participants were instructed not to consume nuts or nut products other than the hazelnut they were given during the HED period. All diet periods were completed smoothly, and all subjects tolerated daily hazelnut consumption well. A dietitian recorded participants' Food intakes for three consecutive days at the end of each period (two weekdays and one weekend). BeBis computer program (BeBis; Nutrition Information System, Istanbul, Türkiye) were used to estimate calorie. After resting for 15 min, 12h (overnight), fasting venous blood of the participants was taken into serum separator tubes and EDTA-tubes, at baseline and the end of each period. Samples were centrifuged (Centrifuge 5810, Beckman Coulter, Allegra 64R, Germany) at 1500xg for 15 min to obtain serum and plasma, respectively, and stored at -80ºC (Thermo Electron Corp. Farma -86ºC ULT Freezer, Waltham, MA USA) until analysis. Anthropometric measurements were taken at baseline and the end of each period. Body weights (kg), body fat percentages, and body mass index (BMI) were obtained using impedance scales (Tanita Body Composition Analyzer, TBF-300, Illinois, USA).

The study protocol was approved by the local research ethics committee of Karadeniz Technical University Farabi Hospital (file number:2007/10-09). All participants gave written informed consent. The study was conducted according to the recommendations of the Declaration of Helsinki.

Biochemical Determinations

The levels of TC, TG, HDL-C, and LDL-C were determined by using the original reagents in the ROCHE / HITACHI Modular System autoanalyzer, and levels of apolipoprotein AI (ApoAI) and apolipoprotein B (Apo B) by immunonephelometric method (Nephelometer, DADE BEHRING, BN II, Germany) based on monoclonal immunoprecipitation method, after doing the daily quality control applications.

Isolation of Lipoproteins

Isolations of lipoproteins from plasma were performed using the multiple discontinuous density gradient methods used by Sclavons *et al.* (Sclavons et al., 1985).

Beckman Optima LE80K Ultracentrifuge was used with Beckman 90 Ti fixed-angle rotor and polycarbonate ultracentrifuge tubes (Beckman, Lot No: 9.30-99, 10.4 mL). The densities of the prepared NaBr solutions were checked by measuring with a densitometer (Anton Paar DMA 35 N, Austria). VLDL, LDL, and HDL-enriched plasma were isolated by centrifugation at 50000 rpm at 10 ° C for 3 h. Detailed isolated protocols were explained by Vanizor Kural *et al.* (Vanizor Kural et al., 2003). The obtained HDL-enriched plasma was subjected to a second centrifugation process at 37000 rpm at 10°C for 17 h to isolate HDL. Then, isolated HDL and LDL were dialyzed with cellulose membrane (Sigma, D 9777, USA) within PBS (pH=7.4) at 4ºC for 24 h to remove EDTA and other impurities that prevent the oxidation process. Total protein concentrations of the lipoproteins were determined by the method of Lowry *et al.* (Lowry, 1951).

Determination of the Susceptibility of LDL and HDL to Oxidation

Lipoprotein oxidations were performed by the chain reactions method based on the principle that Cu^{2+} binds to molecules to cause chemical, physicochemical, and biological changes, developed by Proudfoot *et al.*(Proudfoot et al., 1997). This type of oxidation causes the loss of tryptophan residues on proteins (Gießauf et al., 1995). The first event in LDL oxidation is hydroperoxide formation in LDL PUFAs. These lipid hydroperoxides are conjugated dienes and give a maximum absorbance of 234 nm. Following this wavelength, the conversion of double-bonded PUFAs to conjugated double-bonded hydroperoxides is determined (Gießauf et al., 1995). Diene conjugation in Cu^{2+}-catalyzed oxidation process [(50 µg lipoprotein/mL)/ (1.67 µM $CuSO_4 \cdot H_2O$) in 10 mM PBS (pH 7.4)] in LDL was monitored at 234 nm for 270 min and at 234 nm for 500 min in HDL, spectrophotometrically (UV-1601, UV Visible Spectrophotometer, Shimadzu) (Hasselwander et al., 1999; Puhl et al., 1994). Time of the resistance to lipoprotein oxidation [t-lag (min), time before onset of lipid peroxidation], the rate of diene conjugation [RDC (nmol/min/mg protein), calculated by using the slope of the propagation phase and the molar absorbance coefficient (ε234=29500) for conjugated dienes], the maximum diene conjugation [MDC (nmol/mg protein), calculated by using the time to reach maximum absorbance and again ε234=29,500] and the time to reach maximum absorbance (t-max, min) were evaluated.

Determination of LDL Oxidation in the Presence of HDL

The effects of isolated HDL on LDL oxidation were also examined. For this purpose, the LDL pool was obtained from mixture samples of all subjects. Cu^{2+}-catalyzed oxidations [(100 µg HDL+ 50 µg pool LDL/mL)/ (1.67 µM $CuSO_4.H_2O$) in 10 mM PBS (pH 7.4)] was monitored at 37°C and 234 nm for 500 min spectrophotometrically (UV-1601, UV Visible Spectrophotometer, Shimadzu) (Hasselwander et al., 1999; Puhl et al., 1994). Time of the resistance to lipoprotein oxidation [t-lag (min), time before onset of lipid peroxidation],

the rate of diene conjugation [RDC (nmol/min/mg protein), calculated by using the slope of the propagation phase and the molar absorbance coefficient (ε234=29500) for conjugated dienes], the maximum diene conjugation [MDC (nmol/mg protein), calculated by using the time to reach maximum absorbance and again ε234=29,500] and the time to reach maximum absorbance (t-max, min) were evaluated.

Statistical Analysis

Data were given as mean and SD for normally distributed variables and median (interquartile range) for non-normally distributed variables. The distribution of variables was assessed by the Shapiro-Wilk test. Parameters that followed the normal distribution were analyzed with repeated measures analysis of variance test. Bonferroni adjustment was used for pair-wise post hoc comparison. The Friedman test evaluated Non-normally distributed variables; the Wilcoxon test was performed for pair-wise comparison for variables with $p<0.05$. Pearson's or Spearman correlation analysis assessed the relationships between intra-group parameters and percentage changes. $p<0.05$ was accepted as statistically significant. F values were given for the parameters by repeated-measures ANOVA. Statistical procedures were performed on SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL) software.

Results and Discussion

Caloric and nutrient compositions of Hazelnut- Enriched Diet and Control Diets are given in Table 1.

The values of body weight, body mass index (BMI), and the levels of lipids and lipoproteins are given in Table 2. Weights, BMI, TC, TG, and LDL-C levels were lower, but Apo AI was higher at the end of the HED period than the CDI period ($p<0.05$). Lipid parameters increased in the CDII period compared to the HED period but were not statistically significant, excluding LDL-C. Percentage changes in the levels of lipid parameters between CDI and HED periods and between HED and CDII periods are shown in Figure 1. While changes % in the levels of HDL-C (3.84 ±7 vs. -1.3 ±10) and Apo AI (4.7 ±15 vs. 6.9 ±13) were not statistically significant ($p>0.05$), changes % in the levels of TC (-8.03 ±6 vs. 8.6 ±12), TG (-12.73 ±15 vs. 8.6 ±33), LDL-C (-8.2 ±7 vs. 9.3 ±13) and Apo B (-1.5 ±13 vs. 11 ±18) were significant ($p<0.05$).

Table 1. Caloric and nutrient compositions of HED and Control Diets (n=15).

Variables	CD I	HED	CD II	F*	*p**
Calories, kcal/day	2395 ±413	2345 ±400[a]	2332 ±401[a]	15.8	0.001
Carbohydrates, energy %	54 ±2.3	44 ±4.6[a]	54 ±1.6[b]	103	<0.001
Protein, energy%	15.1 ±2.2	14.0 ±2.1[a]	14.1 ±1.7[a]	24.8	<0.001
Total fiber, g/day	19.3 ±3.8	25.7 ±2.9	18.6 ±2.9	240	<0.001
Fat, energy%	31 ±1.6	41.9 ±3.6[a]	32.2 ±1.7[a,b]	153	<0.001
MUFA, energy%	13 ±0.5	23.1 ±2.9[a]	14.2 ±1.3[b]	338	<0.001
PUFA, energy%	10.6 ±0.8	13.4 ±1.3[a]	11.9 ±1.1[b]	147	<0.001
SFA, energy%	7.4 ±0.6	6.7 ±0.7[a]	7.1 ±0.6[b]	390	<0.001
MUFA/SFA	1.7 ±0.2	3.4 ±0.3[a]	2.0 ±0.2[b]	282	<0.001
MUFA/PUFA	1.2 ±0.13	1.7 ±0.2[a]	1.19 ±0.16[b]	198	<0.001
SFA/UNSFA	0.3 ±0.04	0.2 ±0.03[a]	0.27 ±0.04[b]	373	<0.001
Cholesterol, mg/day	<200	<200	<200		

Values are expressed as (X±SD)

*; P and F values according to repeated-measures ANOVA.

$P<0.05$ was accepted as statistically significant.

[a]; significant with respect to CDI,

[b]; significant with respect to CDII

CDI: Control Diet I; CD II: Control Diet II; HED: Hazelnut-enriched diet; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; SFA: saturated fatty acid.

Table 2. Values of anthropometric and lipid parameters at the end of each diet period (n=15).

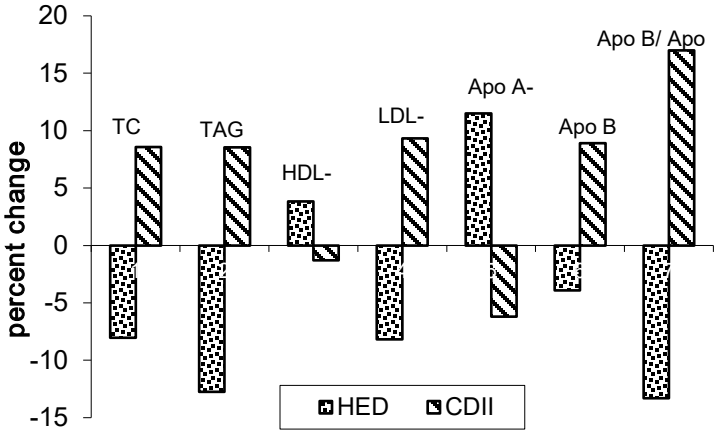
Parameters n= 15	BASELINE (Day 0)	CD I (Day 30)	HED (Day 60)	CD II (Day 90)	F	χ^2	*p*
Age (year)			43.6 ±9.5				
Weight (kg)	82.1 ±14.1	79.5 ±13.7[a]	77.8 ±13.4 [a,b]	77.5 ±13.4[a]	15.3	-	0.001
BMI (kg/m^2)	27.9 ±3.4	27.0 ±3.1[a]	26.4 ±2.9[a,b]	26.3 ±2.9[a]	15.5	-	0.001
TC (mg/dL)	239.1 ±18.4	216.2 ±22.9[a]	198.9 ±24.9[a, b]	214.3 ±22.1[a]	15.2	-	0.002
TG (mg/dL)	170 (115-256)	146 (104-190)	117 [a,b] (95-158)	160 (76-185)	-	4.8	0.091*
HDL-C (mg/dL)	40.6 ±6.9	42.4 ±7.0	44 .1 ±8.5[a,b]	43.2 ±7.5	2.63	-	0.127
LDL-C (mg/dL)	164.9 ±25.1	150.4 ±28.6[a]	137.4 ±24.9[a,b,c]	148.3 ±21.6[a]	13.3	-	0.003
Apo AI (mg/dL)	135.2 ±14.3	128.3 ±16.9	133.2 ±19.3[b]	140.8 ±15.5[b]	6.17	-	0.026
Apo B (mg/dL)	132.3 ±18.5	113.3 ±14.4[a]	111.5 ±20.3[a]	121.9 ±19.8[a]	4.98	-	0.042

P and F values according to repeated-measures ANOVA, posthoc Bonferonni. Data were expressed as mean ± SD.
*; P values according to the Friedman test. Data were expressed as median (interquartile range for 25–75%)
P<0.05 is accepted as statistically significant.
a; significantly different from baseline, b; significantly different from Control diet I, c; significantly different from Control diet II
Apo AI: apolipoprotein AI; Apo B: apolipoprotein B; BMI: body mass index; CDI: Control Diet I; CD II: Control Diet II; HDL-C: high-density lipoprotein-cholesterol; HED: Hazelnut-enriched diet; LDL-C: low-density lipoprotein n-cholesterol; TG: triacylglycerol; TC: total cholesterol.

Table 3. Values of lipoprotein oxidation parameters at the end of each diet period (n=15).

Parameters	CD I	HED	CD II	F *	*p**
LDL oxidation					
t-lag (min)	60.5 ±9.6	70.8 ±12.5[a,b]	58.4 ±12.2	14.1	0.002
RDC (nmol/min/protein)	6.99 ±1.82	6.6 ±2.1	6.9 ±2.6	0.61	0.445
MDC (nmol/mg protein)	420.8 ±132.5	437.8 ±142.4	409.3 ±103.9	0.28	0.601
t-max (min)	135.3 ±12.9	158.6 ±27.8[a]	127.4 ±18.7[b]	18.6	0.010
HDL oxidation					
t-lag (min)	29.1 ±17.5	28.1 ±7.4	24.7 ±7.9	0.77	0.394
RDC (nmol/min/protein)	1.5 ±0.7	1.3 ±0.6	1.2 ±0.6[a]	12.6	0.003
MDC (nmol/mg protein)	128.1 ±19.7	117.9 ±15.4	117.3 ±18.3[a]	5.39	0.036
t-max (min)	154.6 ±45.8	151.6 ±39.1	153.8 ±44.1	0.20	0.661
HDL+ pool LDL oxidation					
t-lag (min)	133.4 ±37.4	163.2 ±48.1[a]	142.5 ±43.8	4.97	0.043
RDC (nmol/min/protein)	3.7 ±1.7	3.6 ±1.6	3.1 ±1.9	1.80	0.200
MDC (nmol/mg protein)	645.1 ±80.1	641.7 ±69.2	614.5 ±114.3	2.01	0.178
t-max (min)	344.2 ±81.3	394.2 ±93.7[a]	379.5 ±105.4	4.22	0.061

a; significantly different from Control diet I, b; statistically different from Control diet II (p<0.05). Values are expressed as (X±SD).
*; P and F values according to one-way ANOVA with repeated measures.
CDI: Control Diet I; CDII: Control Diet II; HED: Hazelnut-enriched diet; MCD: Maximum diene conjugation, RDC: The rate of diene conjugation.



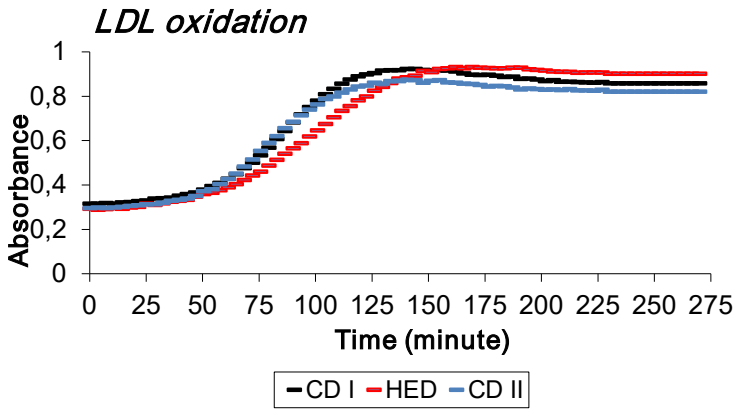
: Percentage changes between CDI and HED periods
: Percentage changes between HED and CDII periods
CDI: Control Diet I, CDII: Control Diet II; HED, Hazelnut-enriched diet

Figure 1. Percentage changes in lipid and lipoprotein levels between diet periods

As seen in Table 3, t-lag and t-max for LDL and HDL+pool LDL oxidation stages increased at the end of the HED period compared to CDI ($P<0.05$), but RCD and MDC did not show any significance. On the other hand, HED did not affect HDL oxidation parameters significantly ($p>0.05$). LDL, HDL, and HDL+ pool LDL oxidation kinetics graphs are given in Figure 2.

When the correlations between parameters were analyzed, it was observed that the values of t-lag during the LDL oxidation process were negatively correlated with LDL-C levels at the end of CDI, and the values of t-lag during HDL+ pool LDL oxidation process with TC levels at the end of HED (Figure 3).

A



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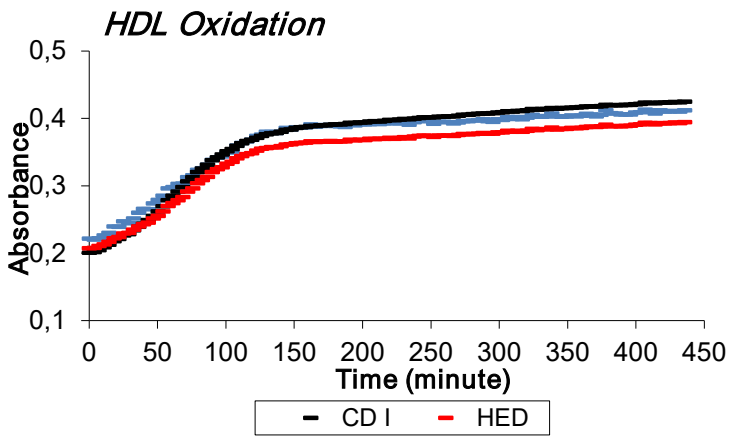

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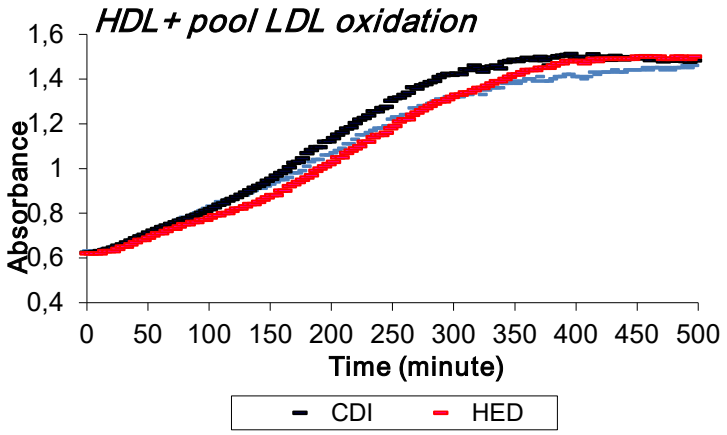


CDI: Control Diet I, CDII: Control Diet II; HED, Hazelnut-enriched diet

Figure 2. Kinetics of lipoprotein oxidations by copper.

Since hazelnut is an oily food, it can be thought that regular consumption may cause an increase in body weight. However, when our study was completed, a significant decrease was observed in body weight and body mass index. Many studies support this result we have obtained. Epidemiological studies show an inverse relationship between the frequency of consumption of nuts and body mass index. In very large cohort studies involving tens of thousands of people, such as the "Adventist Health Study" and the "Nurses' Health Study", a negative correlation was reported between nut consumption and body mass index (Sabate, 2003). Another large cohort study involving tens of thousands of people indicated an inverse relationship between the frequency of the

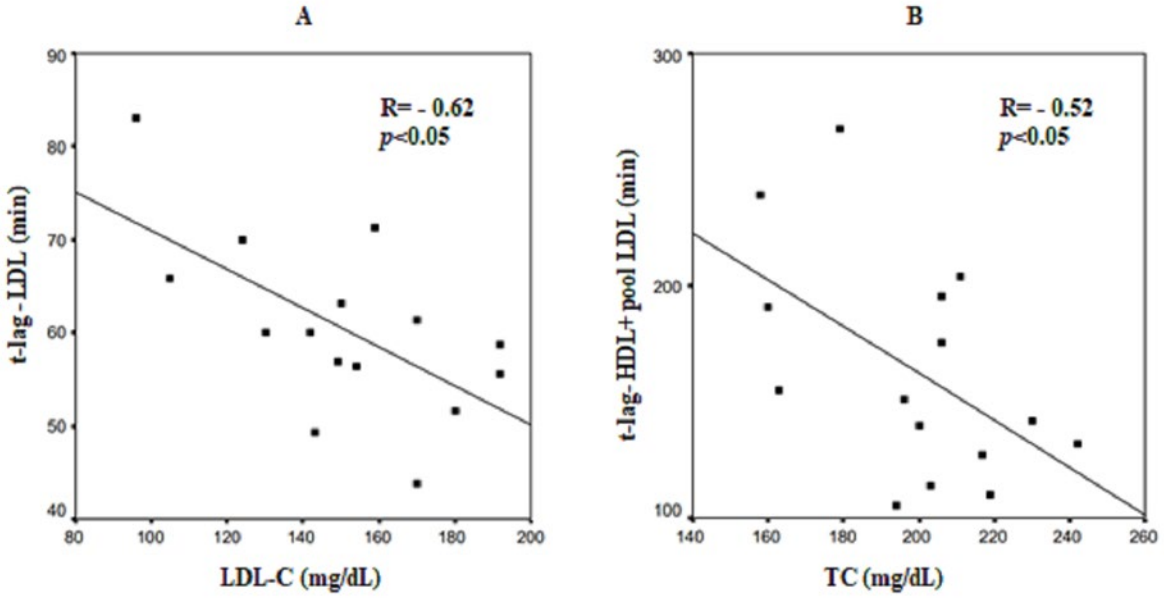
consumption of hard-shelled fruit and body mass index (Segovia-Siapco et al., 2018). Another study involving 6,080 people (Multi-Ethnic Study of Atherosclerosis, MESA) found that nuts consumption caused a reduction in body mass index (Jiang et al., 2006). Studies show that the isocaloric replacement of hazelnuts with other foods in the diet will not increase body weight. In one study, it was reported that there was a significant decrease in body weight in the group that consumed nuts. Frequent consumption of hard-shelled fruits increases the feeling of satiety, increases the metabolic rate at rest due to high protein and unsaturated fatty acid content, and decreases in usable energy due to insufficient digestion of fecal fat are thought to be the causes of weight loss (Jiang et al., 2006; Sabate, 2003). Although the relationship between hazelnut consumption and reduction in body weight has not been fully explained yet, it is attributed to the effect of nuts in delaying gastric emptying, inhibiting anabolic mechanisms, and stimulating catabolic pathways (Akhlaghi et al., 2020).

Nevertheless, studies show no significant change in body mass index with hazelnut consumption. In the study conducted by Mercanlıgil et al., hypercholesterolemic individuals were given a control diet for one month, 40 g of hazelnuts were added to their diets in the second month, and no significant change was observed in their body weights (MercanlIgil et al., 2007). In the study conducted by Durak et al., hazelnuts were added to the regular diets of healthy individuals at 1 g per kilogram per day for 30 days, and it was observed that their body weights did not change (Durak et al., 1999). Koçyiğit et al. investigated the effects of pistachio nut consumption on plasma lipid profile and oxidative status in healthy normolipidemic individuals. Individuals consumed pistachio nuts for 3 weeks, constituting 20% of the daily calorie intake, and it was determined that their body mass index did not change (Kocyigit et al., 2006)

In the present study, serum TC, TG, and LDL-C levels decreased by consuming hazelnuts, and Apo AI levels increased significantly. Some studies, with the consumption of hazelnuts (MercanlIgil et al., 2007), walnuts (Ros et al., 2004), and pistachio nuts (Edwards et al., 1999) in hypercholesterolemic patients, support our findings. Lowering the effects of atherogenic lipid parameters is important to prevent CVDs. It was reported that every 1% reduction in TC and LDL-C concentrations would result in a 1.5% decrease in the incidence of coronary heart disease (Balaban Yucesan et al., 2010). Dietary fatty acids like alpha-linolenic acids and vitamins like γ-tocopherol affect cholesterol levels negatively (Edwards et al., 1999). In addition, diets with a low saturated fat ratio and a high MUFA ratio effectively regulate plasma lipid levels in the protective direction (Fraser, 2000; Renzo et al., 2019).

Hazelnut consumption affected lipoprotein oxidation, such as that t-lag which reflects the resistance of LDL to oxidation, and t-max, which reflects time to reach maximum diene conjugations reduced significantly $P<0.05$ (Table 3). This shows that LDL gained resistance to oxidation through the consumption of hazelnuts. Vitamin E, a chain-breaking antioxidant against lipid peroxidation, finds in high amounts in hazelnut, and consumption of these nuts may lead to an increase in the antioxidant content of LDL. It may affect fatty acid compositions in LDL. In our previous study, Vitamin E levels of serum and LDL particles were higher in hypercholesterolemic individuals in hazelnut-enriched diet periods (Orem et al., 2013). Another study showed that vitamin E levels in plasma and LDL particles were increased by consuming hazelnuts (Puhl et al., 1994). In the same study, when the fatty acid composition of LDL was examined, it was seen that 18:1 (n9) fatty acid increased. This is an expected result considering that 82-83% of hazelnuts contain MUFA and predominantly 18:1 (n9) fatty acid. Increased MUFA contents of LDL particles and increased vitamin E during the HED period could be more effective in decreasing the susceptibility of LDL to oxidation. A decrease in the number of double bonds in the structure of the fatty acids and increased amounts of vitamin E are the factors that cause the elongation of t-lag (Edwards et al., 1999; Spiller et al., 1998). A study on the effects of the peanuts diet on LDL oxidation in healthy individuals showed that the t-lag is increased (Hargrove et al., 2001). Also, it has been reported that polyphenols in walnuts inhibit LDL oxidation in vitro (Hargrove et al., 2001).

HDL of individuals who consume hazelnuts was also effective in reducing LDL oxidation. Alpha-tocopherol affects apolipoproteins, lipids, and antioxidants of HDL positively, and that's way may protect HDL structure and antiatherogenic properties of HDL (Garner et al., 1998; Nicholls et al., 2019; Zabłocka-Słowińska et al., 2019). The enzymes in HDL, mainly paraoxonase, are responsible for HDL's antioxidant properties and, thereby, inhibition effects against LDL oxidation (Negre-Salvayre et al., 2006). We consider that there are changes in the chemical structure of HDL with the hazelnut diet, and thus it can protect LDL against oxidation.



A; Correlation of t-lag in LDL oxidation stage with LDL-C at the end of CDI
B; Correlation of t-lag in HDL + pool LDL oxidation stage with TC at the end of HED period.

Figure 3. Correlations of t-lag with lipid parameters

To support this perspective, according to the findings, while there was no significant change in the amount of HDL-C by the hazelnut consumption, HDL obtained at the HED period leads to a significant increase in LDL oxidation t-lag phase. It has been reported that the quality of HDL contributes to the anti-atherogenic effects (Norata et al., 2006).

According to a systematic review published in 2022 (Brown et al., 2022), although there are many studies on hazelnut consumption and its effects on health, only three of them have been done in hypercholesterolemic individuals (MercanlIgil et al., 2007; Orem et al., 2013; Tey et al., 2011) two of them in hyperlipidemic individuals (Deon et al., 2018; Öngün Yılmaz et al., 2019).

Although the effects of hazelnut consumption on lipid profile and LDL oxidation were shown in these studies, our study is the first to examine the impact of hazelnut on HDL oxidation.

Conclusion

In conclusion, it was thought that the consumption of hazelnut could be led to changes in the structure of the HDL and LDL, can increase the resistance of lipoproteins to oxidation, and have lipid-lowering and weight-loss effects in hypercholesterolemia. Daily consuming certain amounts of hazelnuts (not exceeding 20% of their energy needs) may protect moderately hypercholesterolemic individuals from side effects of hypercholesterolemia, such as atherosclerosis.

The limitation of the study may be the number of subjects. A more significant number of subjects could have been much better regarding results.

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: The study protocol was approved by the local research ethics committee of Karadeniz Technical University Farabi Hospital (File Number: 2007/10-09). All participants gave written informed consent. The study was conducted according to the recommendations of the Declaration of Helsinki.

Funding disclosure: This research was supported in 2007 by the Karadeniz Technical University Scientific Research Projects Coordination Unit. (Project number: 2007.114.01.4.)

Acknowledgments: The authors thank Filiz Kansız, a dietician at the Farabi Hospital, for collecting nutritional data.

Disclosure: -

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 9(3), 212-229 (2023) • https://doi.org/10.3153/FH23020 *Research Article*

Estimating meat consumption based on economic indicators using linear regression analysis approach: A case study of Türkiye

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Cite this article as:
Öztürk, H.M., Öztürk, H.K. (2023). Estimating meat consumption based on economic indicators using linear regression analysis approach: Case Study of Türkiye. *Food and Health,* 9(3), 212-229. https://doi.org/10.3153/FH23020

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Submitted: 21.11.2022
Revision requested: 01.03.2023
Last revision received: 27.04.2023
Accepted: 07.05.2023
Published online: 25.06.2023

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Available online at
http://jfhs.scientificwebjournals.com

ABSTRACT

The main idea of this study is to investigate Türkiye's meat consumption, projection and supplies by using the structure of the Turkish meat industry and Turkish economic indicators. This present study develops several models for the analysis of meat consumption and makes future estimations based on the **R**egression **A**nalysis **M**eat **C**onsumption **M**odel (RAMCM). Four forms of Regression Analysis models are used to estimate meat consumption. These models are named **Mu**ltiple **Li**near **R**egression **A**nalysis (MULIRA), **Li**near **R**egression **A**nalysis (LIRA), **Po**lynomial **Li**near **R**egression **A**nalysis (POLIRA), and **Lo**garithmic **Li**near **R**egression **A**nalysis. The models developed in the linear and non-linear forms are applied to estimate meat consumption in Türkiye based on social and economic indicators; Population, Gross National Product (GNP) per capita, Imports of goods and services (% of GDP), Exports of goods and services (% of GDP), electricity consumption per capita, unemployment, Gross capital formation (% of GDP) figures. It may be concluded that the Multiple Linear Regression Analysis models can be used as alternative solutions and estimation techniques for any country's future meat consumption values.

Keywords: Multiple Linear Regression; Meat Consumption; Estimation; Türkiye; **Po**lynomial **Li**near **R**egression **A**nalysis; **Lo**garithmic **Li**near **R**egression **A**nalysis; **Li**near **R**egression **A**nalysis

Introduction

Türkiye is considered as a bridge between Europe and Asia countries. Türkiye has undergone a significant transformation in a short time. In 1950, 68.1% of the total population in Türkiye was living in rural, and 31.9% was in urban areas; the proportion of people living in rural areas decreased to 22.7% in 2012 (Yilmaz, 2015). The rural population was reduced to 7.7% in 2017 (TURKSTAT, 2017).

On the other hand, Türkiye's Gross Domestic Product (GDP) was 13.995 billion US dollars in 1960. This value increased to 873.98 billion in 2012 and 950.579 billion dollars in 2013. However, it dropped to 851.55 billion dollars in 2017 (World Bank, 2019). During the same period (1960-2017), the population of Türkiye increased from 28 million to 78 million (TURKSTAT, 2019a). Between 1960 and 2017, Türkiye showed significant population, economic and demographic changes. GDP per capita (with current US$) increased from $509.42 in 1960 to $12530 in 2013 but declined to $10540 in 2017 due to the economic crisis experienced in the last 6 years in Türkiye (World Bank, 2019).

The livestock industry has an important place in the Turkish economy. The developments in this sector are directly related to the degree of development and industrialisation of the country and the level of welfare of society. Today, significant changes are experienced in food production and distribution. In the world and Türkiye, while agricultural enterprises decrease in number, they increase in size and tend to produce more single products. Larger producers replace individual producers. At the same time, the tendency towards safer and certain goods in consumer preferences causes significant changes in food production and distribution. All these and similar changes are called agricultural industrialisation. Technological developments and consumer preferences are the main reasons for the agriculture and food sector changes. New technologies have enabled the creation of different products. Due to Türkiye's social and economic development, industrialisation has changed the structure of traditional agriculture.

To grow, develop and lead a long and healthy life, it is necessary to consume sufficient nutrients in a balance. Animal-based foodstuff has importance among the fundamental nutrients. The energy, proteins, vitamins and minerals required for a good and balanced diet are obtained from animal and vegetable sources (Baysal, 2007; Ásgeirsdóttir et al., 2014), and meat consumption in developing countries remains an important food group for consumers (McAfee et al., 2010)

In recent years, food safety, food security and nutrition have been the main topics of concern for all countries worldwide. The growing world population has become increasingly at risk of reaching adequate, healthy and reliable food sources. The unbalanced distribution of food resources has brought important differences between regions in the world. Access to adequate food sources and reliable and balanced nutrition has become a threat to developed, underdeveloped, and developing countries. In line with these developments, all world countries and international organisations have started to take measures and implement food consumption. Due to population growth, global warming, drought and similar problems, it is important to estimate the food demand in the coming period.

People have the right to buy and consume foods that are reliable, affordable, good quality, and have healthy eating habits. Human health is based on adequate and balanced nutrition. For this reason, food production, processing and trade take place in the policy agenda of all countries. As the concepts of food, nutrition and health are inseparable, it is of utmost importance for the health and agriculture sectors to cooperate with food, nutrition and health. Agricultural policies should include health. For this purpose, food and nutrition policies need to be established. Establishing nutritional and nutritional policies requires a broad perspective and cross-sectoral cooperation. Food and nutrition policies should consider economic, cultural and political trends. While food consumption increases due to population growth, on the other hand, economic and social developments cause to increase in food consumption per capita.

Regardless of the level of development, nutrition is one of the major problems of all countries in every period. The production and quality of meat and meat products are important to ensure the population's healthy and balanced nutrition. The meat in the healthy diet of society, the location of red meat in the total meat is very important, especially for children and young people in the age of development. Proteins are not stored in the body and must be removed from the nutrients. The protein required with foodstuffs is 0.8 g per kg/body weight, although it does not vary according to age and sex. This means about 45-55 g of protein per person. For healthy and balanced nutrition, at least half or 2/3 of the proteins required daily should be from animal-origin food (Mutluer, 2005).

At least 50% of the protein required for the balanced nutrition of the human body and 25% of the amount of calories needed must be obtained from animal foods (Gürlük and Turan, 2008)

Meat consumption per capita per year in Türkiye is very low compared to other countries' consumption. Annual meat consumption per capita per year in Türkiye, with data for 2013, was 35.1 kg. Hong Kong is the world's top country by meat consumption per capita. Meat consumption in Hong Kong was 153.1 kg per capita per year. Meat consumption per capita per year in 2013 was 116.23 kg in Australia, 115.13 kg in the United States of America, 90.25 kg in Canada, 107.24 kg in Argentina, 86.76 kg in France, 85.64 kg in Germany and 76.6 kg in Greece, 53.72 kg in Bulgaria and 32.88 kg in Iran. The world's total meat food supply was estimated at 8,598.7 kilograms per capita per year in 2013. Meat consumption per capita for 1961, an average of 1961-2013 and 2013, was shown in Figure 1 for selected countries. (See Figure 1). As seen in the Figure, meat consumption increased in 2013 compared to 1961 for all the selected countries. For all countries except France, meat consumption in 2013 was higher than the average between 1961 and 2013 (Ritchie and Roser, 2017).

China is the top country for domestic meat supply in the world. As of 2013, the domestic meat supply in China was 87,682 thousand tonnes, accounting for 28.42 % of the world's domestic supply. The top 5 countries (others are the United States of America, Brazil, Russian Federation, and Mexico) account for 52.64 % of it. The world's total domestic meat supply was 308,567 thousand tonnes in 2013. Türkiye ranks 23 with 2607 thousand tonnes of production (Ritchie and Roser, 2017).

Estimation of food consumption or demand is of great importance in terms of supply formation. Several studies have been conducted to estimate food consumption. The main idea of this study is also to estimate meat consumption in Türkiye using the economic indicator.

In their studies, Antelo et al. (2017), Aguiar and Hurst (2005), Carroll et al. (2003), and Türkmen-Ceylan (2019) examined the parameters affecting household food expenditure and showed that the unemployment rate decreased household food expenditure, especially during the crisis periods. A similar study was carried out by Azabagaoglu and Oraman (2011) and showed that during the economic and financial crisis 2008, food expenditure was also reduced in Türkiye. On the other hand, in their study, Ivanova et al. (2006) showed that in the years of economic transformation in Bulgaria, food consumption increased due to economic development. Sepúlveda et al., 2008 used logistic regression to determine the factors affecting beef preferences, which are quality labels in Spain.

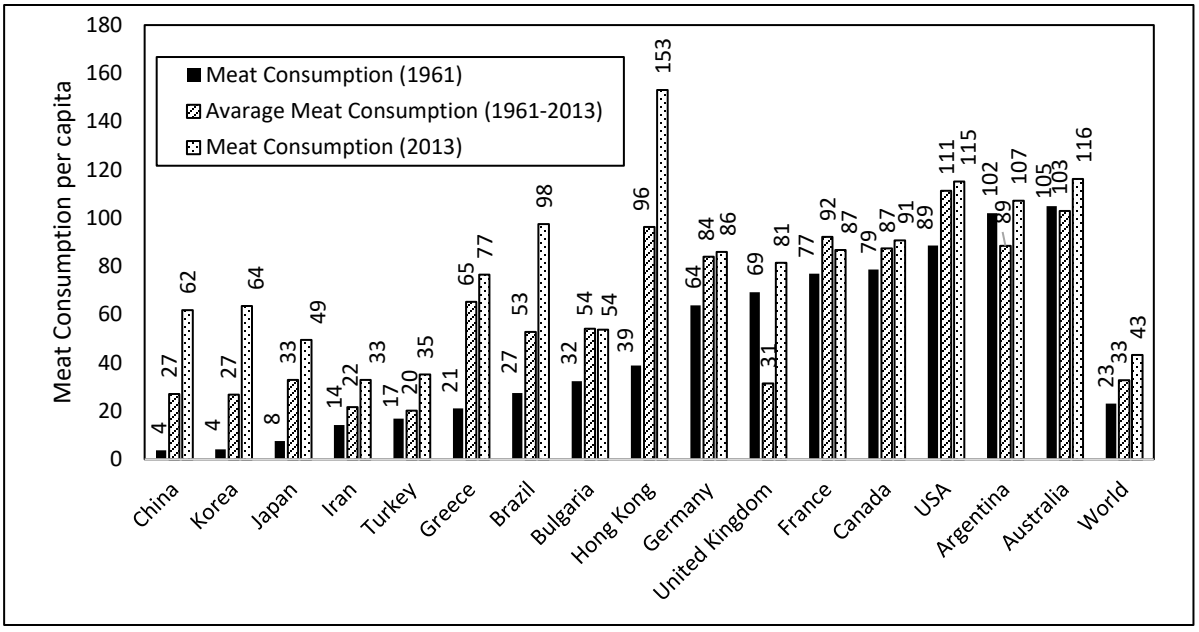

Figure 1. Meat consumption per capita for the selected countries for 1961, an average of 1961-2013 and 2013

On the other hand, Bentolila and Ichino (2008) examined the behaviour of Mediterranean and Northern countries during the crisis period. In the study, it was observed that the decrease in food consumption during the crisis periods was very low in both the Mediterranean and the Northern countries. They concluded that the support of the family in the Mediterranean countries played a role in the government welfare system in Northern countries. However, when economic data and meat consumption are compared for Türkiye, it can be concluded that financial data and meat consumption are related. Meat consumption increases due to increased GDP, and meat consumption decreases due to economic shrinkage and recession (see Figure 2). Yavuz et al. (2013) also reached a similar conclusion, showing that economic meat consumption increases with economic growth. Bilgic and Yen (2013) concluded that there was a decrease in red meat consumption in Türkiye between 2007 and 2009, while there was an increase in the consumption of poultry meat for the same period. They have claimed that the most important factor in this decrease was doubling the red meat prices. Demirtas (2018) showed that the red meat price increase negatively affects meat consumption in Türkiye. Aydogdu and Kucuk (2018) indicated that red meat consumption per capita in Türkiye would be 19.85 kg in 2023, which is 39.5% higher than in 2017 in Türkiye.

Materials and Methods

Model Development

New Meat Consumption Models have been developed in the study to estimate meat demand. Four forms of Regression Analysis Models are used to estimate meat consumption. These models are named Multiple Linear Regression Analysis (MULIRA), Linear Regression Analysis (LIRA), Polynomial Linear Regression Analysis (POLIRA) and Logarithmic Linear Regression Analysis (LOLIRA).

Regression analysis is a mathematical method that examines the relations between a dependent variable and an independent (simple regression) or multiple independent (multiple regression) variables. Regression analysis is a widely used statistical method in social sciences and engineering studies by fitting linear or non-linear equations to observe the relations between one and more independent variables and a dependent variable (Pedhazur, 1997). The linear regression model aims to explain the total change in the dependent variable with independent variables. Regression analysis can provide an inference about how much independent variable(s) predicts the variance of the dependent variable, as well as the extent to which the independent variable(s) predicts the dependent variable (Bagozzi and Yi, 1988; Aiken et al., 2003). When an independent variable is included in the regression model, the analysis is called Simple Linear (Regression) Analysis. In contrast, two or more independent variables are called Multiple Linear Regression Analysis (Dogan and Yilmaz, 2017).

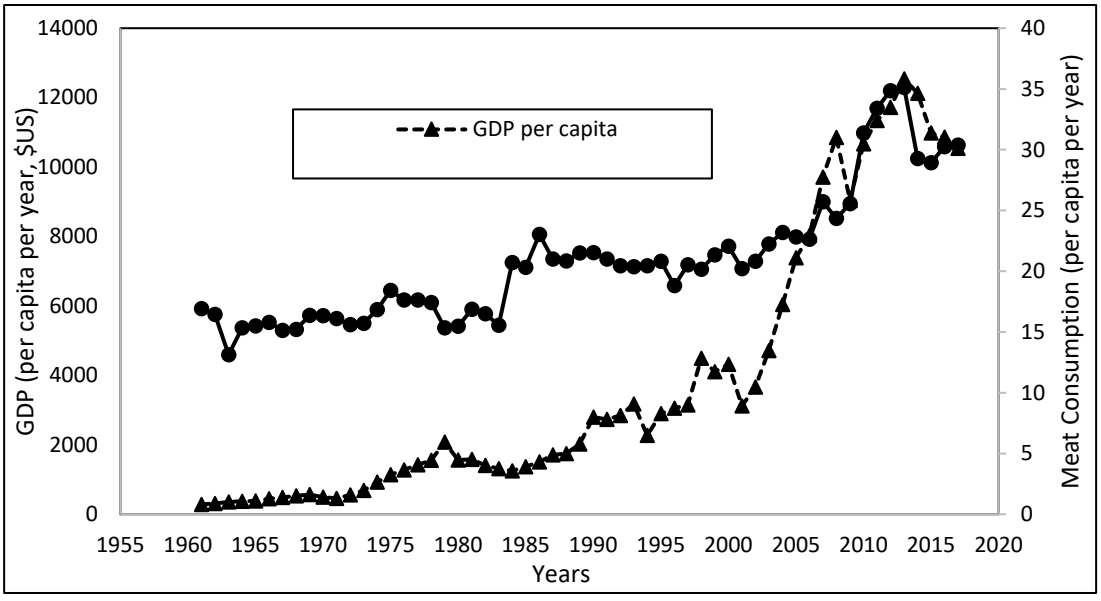

Figure 2. Relation between the GDP per capita and meat consumption per capita

Mathematically, the multiple linear regression equation can be expressed as shown in Equation Multiple Linear Regression Equation

$$Y = \beta_1 + \beta_2 x_2 + \beta_3 x_3 + \cdots + \beta_k x_k \quad (1)$$

In Equation 1, the dependent variables *Y*; x_1, x_2, ..., x_k, are independent variables. k shows the number of independent variables. β_1, β_2, ..., β_k are fixed and unknown parameters for independent variables.

Descriptive statistics were used to describe the basic features of the data set in the study. Correlation analysis was used to examine the relationship between meat consumption and social and economic variables. A linear correlation coefficient was used to determine the degree to which variables were related to covariates. The more the coefficient differed from 1 or -1(close to zero), the weaker the relation.

Linear regression models study the linear relationship between a dependent variable and several independent variables by fitting a linear equation to observed data samples (Coelho-Barros et al., 2008). The fitting is performed by minimising the sum of the squares of the vertical deviations from each data point to the line that best fits the observed data (Agirre-Basurko et al. 2006; Ferraro & Giordani 2012; Kovdienko et al. 2010).

For the MULIRA Model, the following equation, dependent and independent variables, were used to estimate meat consumption.

$$Y = \beta_o + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_5 x_5 + \beta_6 x_6 + \beta_7 x_7 + \beta_8 x_8 \quad (2)$$

Where

Y: Meat Consumption,

β: Regression Coefficients

x_1: *Population,*

x_2: *Gross Domestic Product (GDP) per capita,*

x_3: *Electricity Consumption per capita*

x_4: *Imports of Goods and Services (% of GDP)*

x_5: *Gross Capital Formation (% of GDP)*

x_6: *Exports of Goods and Services (% of GDP)*

x_7: *Unemployment*

The LIRA, POLIRA and LOLIRA Models are the following equation used in the study.

For the **Li**near **R**egression **A**nalysis (LIRA)

$$Y = \beta_o + \beta_1 x_1 \quad (3)$$

For the **Po**lynomial **Li**near **R**egression **A**nalysis (POLIRA)

$$Y = \beta_o + \beta_1 x_1 + \beta_2 x_2^2 \quad (4)$$

Logarithmic **Li**near **R**egression **A**nalysis (LOLIRA)

$$Y = \beta_o e^{\beta_1 x_1} \quad (5)$$

For the Eq. 3, 4 and 5

β is Regression Coefficients and x is Years

The following parameter values were obtained when the Regression Analysis Meat Consumption Model (RAMCM) has applied to the meat consumption problem. The coefficients obtained are given below in the form of Multiple Linear Regression Analysis (MULIRA) models. For meat consumption;

For the ***Mu**ltiple **Li**near **R**egression **A**nalysis (MULIRA)*

$$\begin{aligned} Y = 10.01778 &+ 0.147352 x_2 \\ &+ 0.001671 x_3 - 0.00592_4 x_4 \\ &+ 0.040815 x_5 + 0.072768 x_6 \\ &+ 0.129709 x_7 - 0.14217 x_8 \end{aligned} \quad (6)$$

$$Multiple\ R^2 = 0.9113,\ R^2 = 0.8303,$$
$$Adjusted\ R^2 = 0.8034$$

The value of R^2 for the MULIRA model is 0.98303, but since there is multiple regression, the value of Adjusted R^2 and multiple R^2 should also be considered. These values are calculated as 0.9113 for multiple R^2.and 0.8034 for the adjusted R^2. In other words, independent variables can explain 80.34% of the change in the dependent variable (meat consumption). Multiple regression R^2 is 0.9113; then, the model explains 91.13% of the variance in the dependent variable. These values are quite high and satisfactory for social science and engineering studies.

For the LIRA, POLIRA and LOLIRA Models, the following coefficients have been obtained

*For the **Li**near **R**egression **A**nalysis (LIRA)*

$$Y = -539.281691 + 0.281606 x_1 \quad (7)$$

$$R^2 = 0.7615$$

*For the **Po**linomial **Li**near **R**egression **A**nalysis (POLIRA)*

$$Y = 23111.766156 - 23.501869x_1 + 0.005978x_2^2 \quad (8)$$

$$R^2 = 0.8358$$

Fir ***Lo**garitmic **Li**near **R**egression **A**nalysis (LOLIRA)*

$$Y = 1.1959.10^{-10}e^{1.2999.10^{-2}x_1}$$

$$R^2 = 0.8176$$

Data Source

The data related to the design parameters of Türkiye's; Meat Consumption, Population, Gross Domestic Product (GDP) per capita, Electricity Consumption per capita, Imports of Goods and Services (% of GDP), Gross Capital Formation (% of GDP), Exports of Goods and Services (% of GDP) and Unemployment are obtained from different sources such as Turkish Statistical Institute, (TURKSTAT, 2019b), The Food and Agriculture Organization (FAO) (FAO, 2019), World Bank (World Bank, 2019) Organisation for Economic Cooperation and Development (OECD) (OECD, 2019a). The collected data for the variables are given in Table 1.

Correlation analysis was used to examine the relations between meat consumption and social and economic variables, as given in Table 2. The table shows a high correlation between meat consumption and social and economic indicators. Therefore, these social and economic indicators can be applied to the MULIRA model to estimate Türkiye's future meat consumption and demand.

Multicollinearity, serial correlation, and heteroscedasticity tests are important statistical tests has been applied in the study.

Multicollinearity occurs when two or more independent variables in a regression model are highly correlated. This can create problems in the regression model, such as making it difficult to interpret the individual effects of each independent variable on the dependent variable. Multicollinearity can also lead to unstable and unreliable estimates of the regression coefficients. By performing a multicollinearity test, you can identify if this is a problem in your model and take steps to address it, such as by removing one of the correlated variables or using dimensionality reduction techniques like the principal component analysis.

Serial correlation: Serial correlation, also known as autocorrelation, occurs when there is a correlation between the error terms in a regression model. This can create problems in the model, such as biased regression coefficient estimates and incorrect standard errors. The purpose of performing a serial correlation test is to determine whether this is causing a problem in the model.

Heteroscedasticity: Heteroscedasticity occurs when the variance of the errors in a regression model is not constant across all levels of the independent variables. This can lead to biased and inefficient estimates of the regression coefficients and incorrect standard errors. By performing a heteroscedasticity test, it can be identified if this is a problem in the model and take steps to address it, such as by using weighted least squares regression or transforming the variables in the model. Weighted least squares regression is a regression analysis method that gives more weight to observations with more minor errors, which can help account for heteroscedasticity. Transforming the variables in the model, such as by taking the logarithm or square root of a variable, can also help to reduce heteroscedasticity.

Performing these tests can help ensure that the regression analysis is reliable and valid and that the results are interpretable and actionable. In the study, multicollinearity, serial correlation, and heteroskedasticity tests were performed by developing a Python code; the results are given in Table 3. When Table 3 is analysed, it can be seen that the regression analysis is reliable and the model is statistically significant.

R-squared is a statistic that measures the percentage of variation in the dependent variable explained by the independent variables. R-squared value of 0.875 means that the independent variables in the model explain 87.5% of the total variation in the dependent variable. This suggests that the model has a good fit and a strong predictive power, indicating that the independent variables are good predictors of the dependent variable.

Adj. R-squared, on the other hand, is a modified version of R-squared that considers the number of variables in the regression. An Adj. R-squared value of 0.857 means that the model explains approximately 85.7% of the variance in the dependent variable while adjusting for the number of independent variables in the model. This value is slightly lower than the R-squared value, which is expected when changing the number of predictors in the model. This suggests the model is still a good fit and has strong predictive power. However, it is important also to consider other metrics and diagnostic tests, such as residual plots and significance tests, to ensure that the model is appropriate and reliable.

Prob (F-Statistic) is used to assess the overall significance of

the regression, testing the null hypothesis that all the regression coefficients are equal to zero. A low probability value suggests that the regression is meaningful. The Prob (F-statistic) value of 5.46e-20 indicates that the probability of getting such a large F-statistic by chance alone is extremely small. This means that the overall regression model is statistically significant, indicating that at least one of the independent variables is significantly related to the dependent variable. Therefore, we can reject the null hypothesis that all regression coefficients are zero and conclude that the model provides a good fit to the data.

AIC/BIC are model selection criteria that penalise errors in the model when a new variable is added to the regression equation. The AIC (Akaike Information Criterion) value of 249.4 and the BIC (Bayesian Information Criterion) value of 265.8 are both measures of the quality of the model, specifically, its ability to balance between the goodness-of-fit and the number of parameters in the model. The lower the AIC and BIC values, the better the model. In this case, the AIC value of 249.4 measures how well the model fits the data, while the BIC value of 265.8 measures the model's complexity. Overall, a lower AIC or BIC value suggests that the model has a better trade-off between the fit and complexity of the model. Therefore, comparing different models' AIC and BIC values can help select the best model for the dataset.

The Omnibus test with a value of 0.746 and Prob (Omnibus) value of 0.689 tests the normality assumption of the errors. Since the p-value of Prob (Omnibus) is more significant than 0.05, it suggests that the errors are normally distributed, and hence the normality assumption is met.

The Durbin-Watson value of 0.867 tests the independence assumption of errors. A value between 0 and 2 is preferred, and the importance of 0.867 suggests no significant autocorrelation in the errors.

The Jarque-Bera (JB) test with a value of 0.215 and Test Statistic p-value of 0.0117 also tests the normality assumption of the errors. The p-value is less than 0.05, suggesting that the errors are not normally distributed. This contradicts the result of the Omnibus test, and further investigation is needed to reconcile these findings.

The F-statistic of 3.248 with an associated F-Test p-value of 0.0010 tests the overall significance of the regression model. Since the p-value is less than 0.05, the regression model is statistically significant, indicating that at least one independent variable is significantly related to the dependent variable.

Data were made using the Anova test for statistical relationships between a dependent variable (meat consumption in Türkiye) and various economic and social independent variables. While testing, analysis was made for the actual and predicted data. Looking at the analysis results, the following comments can be made about the overall accuracy of the model:

The R-Squared value (0.9811) shows the rate at which the independent variables explain the dependent variable, and in this case, it shows that the model is correct at the rate of 98.1%.

The adjusted R-Squared value (0.965) considers the number of independent variables in the model and indicates that it is 96.5% accurate.

Anova's results show that at least one of the independent variables in the model has a significant contribution to explaining the change in the dependent variable.

A regression analysis was performed by giving t-statistics and p-values along with these coefficients and error terms. The T statistics results show that the relevant coefficient is not zero, while the p-values indicate whether these coefficients are statistically significant. As can be seen in the table, the P-values for all coefficients are very low at the 0.05 significance level (p-value <0.05), so it can be said that all coefficients are statistically significant. Also, 95% confidence intervals are provided for each coefficient. Therefore, a regression model can be constructed using the data, and the effects of coefficients related to this model can be evaluated.

Table 1. Meat Consumption and the corresponding social and economic indicators for Türkiye

Years	Meat Consumption (kg/capita/year)	Population (Million)	GDP per capita (current US$)	Electricity consumption per capita year (kWh)	Imports of goods and services (% of GDP)	Gross capital formation (% of GDP)	Exports of goods and services (% of GDP)	Unemployment
1961	16.93	28.15	285.01	91.85	6.79	9.97	5.12	3.40
1965	15.49	30.97	385.64	136.79	5.40	11.35	4.56	3.70
1970	16.33	34.88	489.93	209.54	6.36	14.71	4.43	6.40
1971	16.11	35.72	455.11	232.05	8.25	13.77	5.32	6.80
1975	18.43	39.28	1136.38	343.51	11.23	18.71	4.42	7.60
1980	15.48	43.98	1564.25	463.84	11.93	18.16	5.16	8.30
1985	20.32	49.13	1368.40	604.65	18.97	16.51	15.86	7.30
1990	21.54	53.92	2794.35	868.30	17.58	24.55	13.37	8.00
1995	20.81	58.49	2897.87	1152.30	24.35	25.47	19.89	7.60
2000	22.05	63.24	4316.55	1554.33	22.55	23.80	19.45	6.50
2001	20.22	64.19	3119.60	1512.19	22.82	18.14	26.58	8.40
2002	20.81	65.14	3660.07	1580.34	23.00	21.24	24.46	10.30
2003	22.24	66.09	4718.46	1691.23	23.36	22.47	22.24	10.50
2004	23.20	67.01	6040.88	1807.88	25.37	25.21	22.75	10.80
2005	22.83	67.90	7384.26	1918.36	24.42	27.03	21.02	10.60
2006	22.63	68.76	8034.61	2080.63	26.50	29.57	21.65	10.20
2007	25.71	69.60	9709.72	2229.04	26.07	28.71	21.22	10.30
2008	24.36	70.44	10850.87	2299.09	27.08	28.94	22.83	11.00
2009	25.55	71.34	9036.27	2199.27	23.36	23.02	22.57	14.00
2010	31.39	72.33	10672.40	2378.80	25.45	26.97	20.45	11.90
2011	33.42	73.41	11340.82	2535.10	30.40	31.27	22.26	9.80
2012	34.85	74.57	11720.31	2613.96	28.58	28.30	23.67	9.20

Results and Discussion

Fifty-two data (1961–2012) (See Table 1) are used to estimate the weighting parameters of the MULIRA, LIRA, POLIRA, and LOLIRA models, and the remaining data (2013–2017) are used to validate the models.

The values of the independent variables between the years 2013-2040 were estimated using the data between 1961 and 2012. Linear Regression Analysis Models have been used for the estimation. Figure 1a-g shows the actual and estimated data for the Population, Gross Domestic Product (GDP) per capita, Electricity Consumption per capita, Imports of Goods and Services (% of GDP), Gross Capital Formation (% of GDP), Exports of Goods and Services (% of GDP) and Unemployment of Türkiye. As can be seen in Figure 1, for each social and economic indicator, the trend lines are fitted to the observed data with the highest R^2 values. Therefore, they may be used for future estimation. The value of meat consumption between 2013 and 2040 was estimated by using the actual values of the independent variables between 1961 and 2012 and the estimated values of independence between 2013 and 2017. Estimated meat consumption between 2013-2017 was used to validate the model and test data compared with the actual meat consumption data for 2013 and 2017.

The polynomial and linear expressions for each social and economic indicator and their actual values and estimation are illustrated in Fig. 1a–g. Fig. 2 shows actual and estimated meat consumption from 1961-2012 in Türkiye. As can be seen in Figure 2, the MULIRA model estimate is in great harmony with the observed data. LIRA, POLIRA and LOLIRA are also compatible with observed data. On the other hand, Figure 3 shows the meat consumption of Türkiye until 2040.

White Test Results testindeki F-Test p-value=0.00105 ve LM-Test p-value= 0.0117, Breusch-Pagan Test Results testindeki F-Test p-value=0.00027 ve LM-Test p-value=0.0013 ve OLS Regression Results testindeki Test Statistic p-value=0.0117 ve F-Test p-value=0.0010 değerleri açısından nasıl değerlendirebiliriz?

Table 2. Correlation analysis results for meat consumption and social and economic indicators

	Meat Consumption (kg/capita/year)	Population (Million)	GDP per capita (current US$)	Electricity consumption per capita year (kWh)	Imports of goods and services (% of GDP)	Gross capital formation (% of GDP)	Exports of goods and services (% of GDP)	Unemployment
Meat Consumption (kg/capita/year)	1.00							
Population (Million)	0.88	1.00						
GDP per capita (current US$)	0.91	0.89	1.00					
Electricity consumption per capita year (kWh)	0.91	0.96	0.97	1.00				
Imports of goods and services (% of GDP)	0.83	0.96	0.82	0.91	1.00			
Gross capital formation (% of GDP)	0.83	0.92	0.83	0.88	0.90	1.00		
Exports of goods and services (% of GDP)	0.80	0.95	0.78	0.90	0.96	0.84	1.00	
Unemployment	0.71	0.82	0.75	0.77	0.71	0.77	0.67	1.00

Table 3. Multicollinearity, serial correlation, and heteroskedasticity tests result

OLS Regression Results

Dep. Variable:	Meat	R-squared:	0.875
Model:	OLS Adj.	R-squared:	0.857
Method:	Least Squares	F-statistic:	49.13
Date:	Thu, 09 Mar 2023	Prob (F-statistic):	5.46e-20
Time:	22:20:50	Log-Likelihood:	-116.71
No. Observations:	57	AIC:	249.4
Df Residuals:	49	BIC:	265.8
Df Model:	7		
Covariance Type:	non robust		

Omnibus:	0.746	Durbin-Watson:	0.867
Prob(Omnibus):	0.689	Jarque-Bera (JB):	0.215
Test Statistic:	35.634	Test Statistic p-value':	0.0117
F-Statistic:	3.248	F-Test p-value:	0.0010
Breusch-Pagan Test Results			
LM Statistic:	23.592	LM-Test p-value:	0.0013
F-Statistic:	4.943	F-Test p-value:	0.00027
White Test Results			
LM Statistic:	35.6344	LM-Test p-value:	0.0117
F-Statistic:	3.248	F-Test p-value:	0.00105

Table 4. Anova result for variables

Regression Statistics	
Multiple R	0.990504
R Square	0.981099
Adjusted R Square	0.965372
Standard Error	3.687425
Observations	80

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	8	4525.02363	565.628	68.72648	2.28878E-30
Residual	71	584.339339	8.230132		
Total	79	5109.362969			

	Coefficients	***Standard Error***	***t Stat***	***P-value***	***Lower 95%***	***Upper 95%***	***Lower 95.0%***	***Upper 95.0%***
Intercept	0.002739943	0.001202316	2.27888818	0.025640784	0.00034317	0.005136715	0.00034317	0.005136715
Years	-1.18332E-07	2.09611E-08	-5.645324517	3.06406E-07	-1.60117E-07	-7.65469E-08	-1.60117E-07	-7.65469E-08
Population	0.002129779	0.000650369	3.274724551	0.001627646	0.000833292	0.003426266	0.000833292	0.003426266
Actual GDP per capita	-0.012485217	0.003287521	-3.797760975	0.000302198	-0.01903877	-0.005931665	-0.01903877	-0.005931665
Actual Electricity Consumption	-0.540901825	0.2609988	-2.072430312	0.041806705	-1.061193424	-0.020610226	-1.061193424	-0.020610226
Actual Imports of Goods and Services (% of GDP)	0.768984356	0.20158562	3.814678624	0.000285499	0.367130767	1.170837945	0.367130767	1.170837945
Gross capital formation (% of GDP)	1.139232849	0.206576217	5.514830628	5.17122E-07	0.727430686	1.551035011	0.727430686	1.551035011
Exports of goods and services (% of GDP)	0.54719032	0.325607227	1.68052265	0.097189543	-0.101895824	1.196276465	-0.101895824	1.196276465
Unemployment	0.002739943	0.001202316	2.27888818	0.025640784	0.00034317	0.005136715	0.00034317	0.005136715

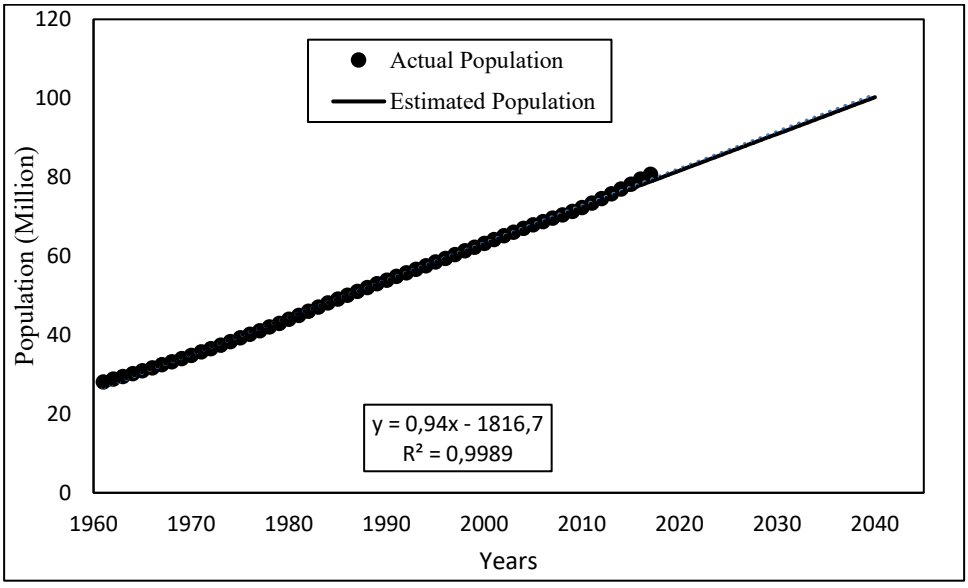

Figure 1a. Trend lines and estimated data of population, figures

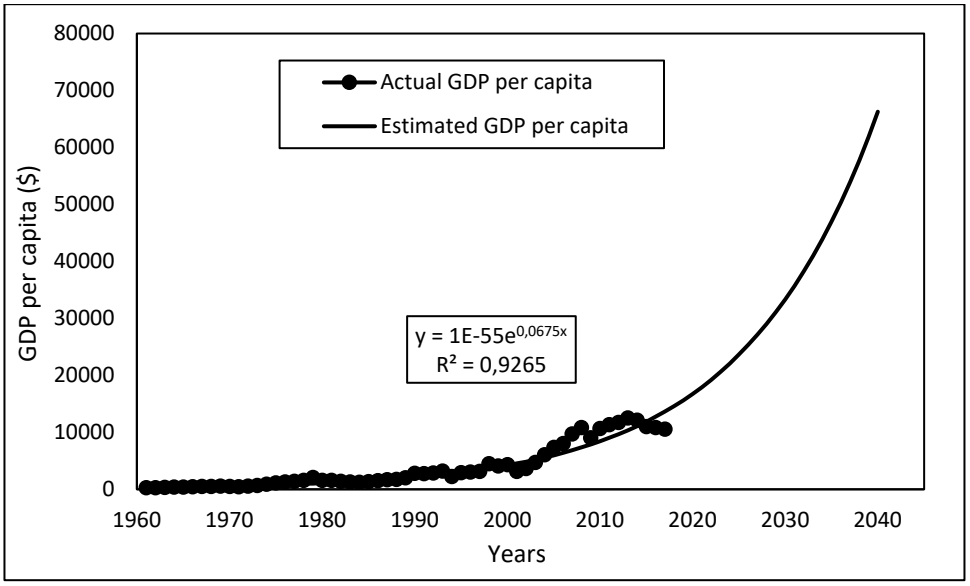

Figure 1b. Trend lines and estimated data of GDP per capita

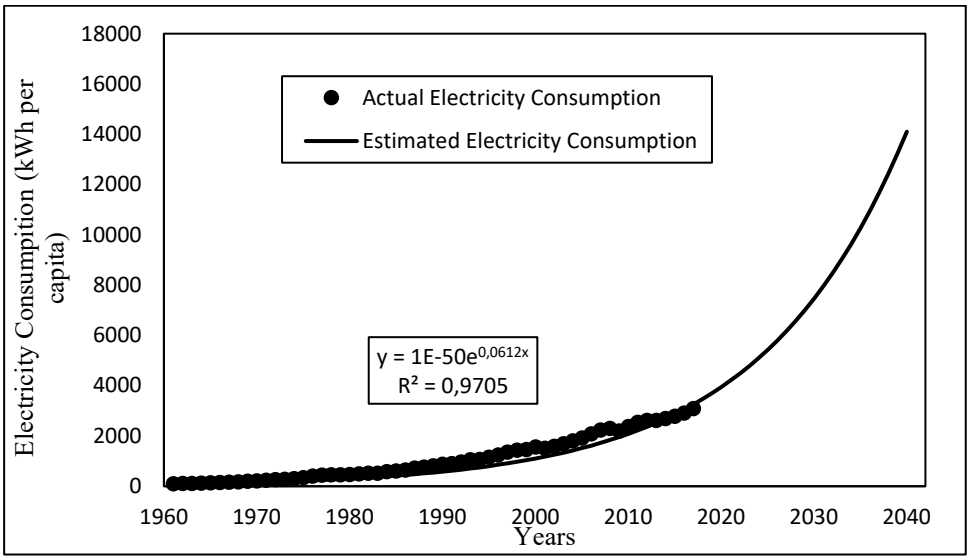

Figure 1c. Trend lines and estimated data of electricity consumption per capita

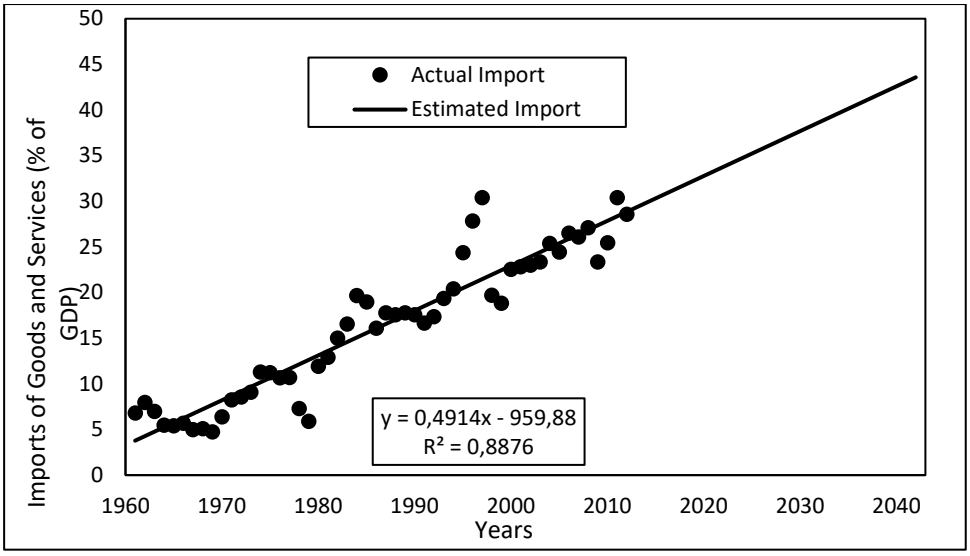

Figure 1d. Trend lines and estimated data of import per capita

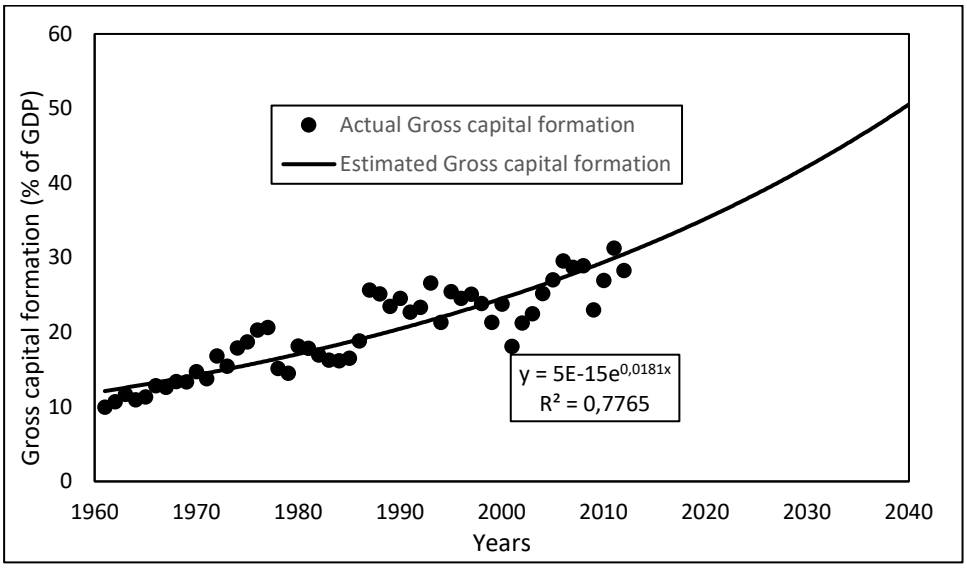

Figure 1e. Trend lines and estimated data of gross capital formation per capita

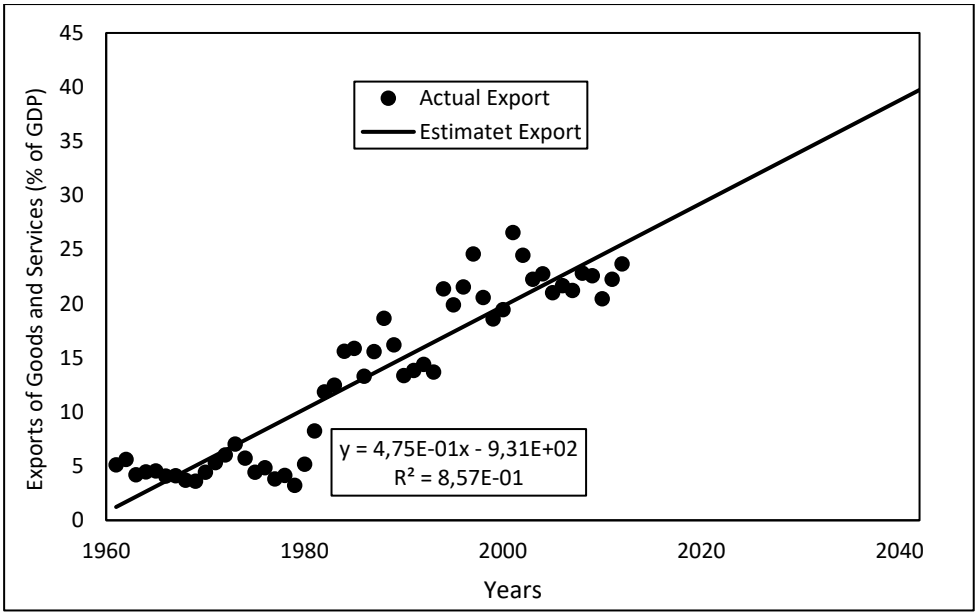

Figure 1f. Trend lines and estimated data of export per capita

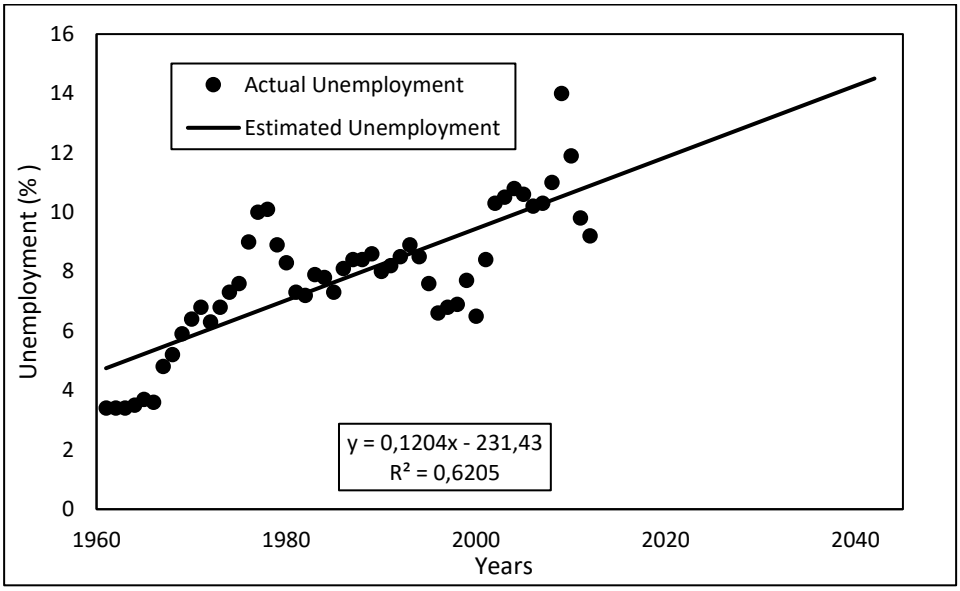

Figure 1g. Unemployment

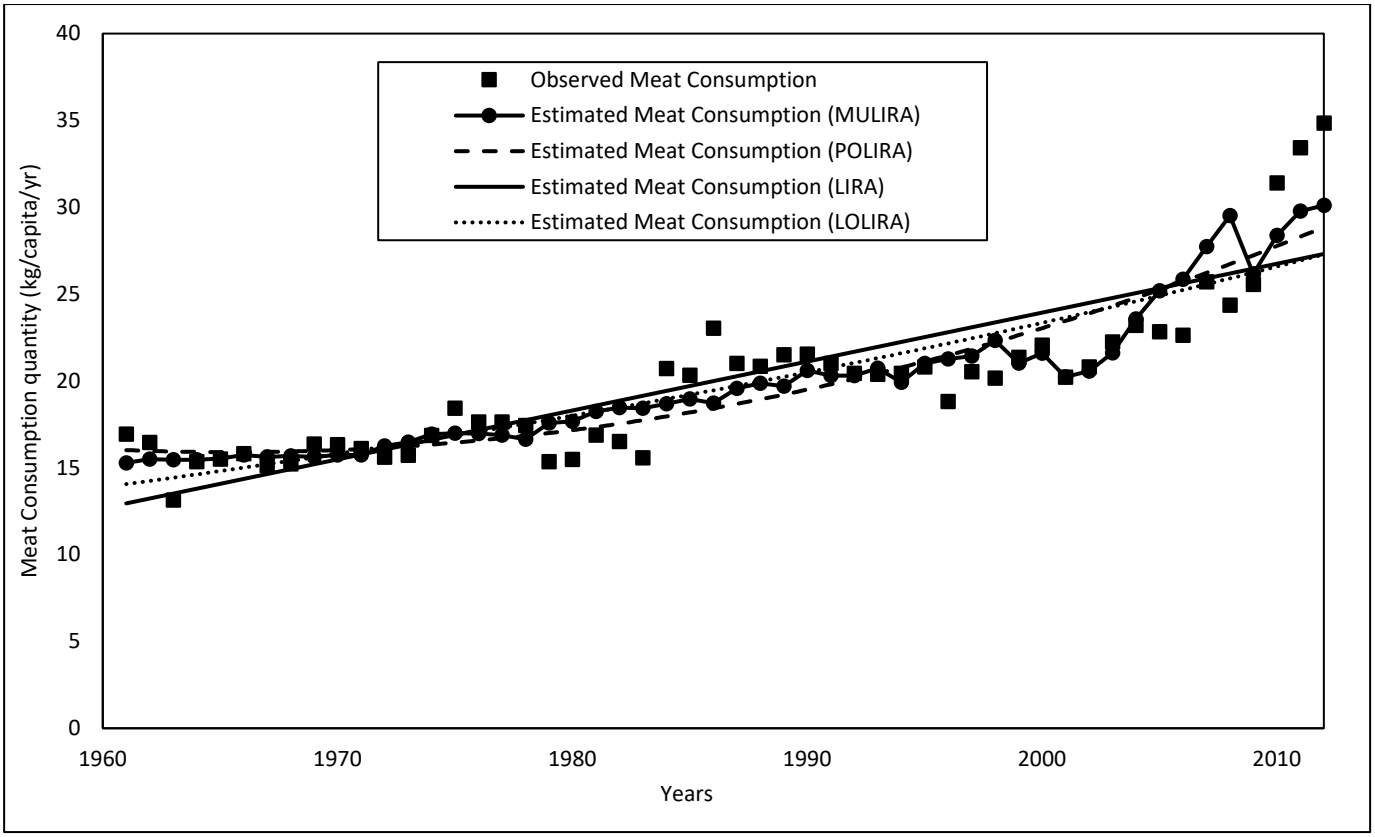

Figure 2. Actual and estimated values of meat consumption for 1961-2012.

Validation is very important for a model before using it for calculation. The model was validated for the five years in the range of 2013-2017. The results obtained from Linear Regression Analysis Models (MULIRA, LIRA, POLIRA, LOLIRA) were validated with observed data (See Table 3). The relative errors between the estimated data of the Minister of Development and observed meat consumption data are reported in Table 4. The MULIRA, LIRA, POLIRA, and LOLIRA model results are also compared with the estimated data of the Minister of Development (2014). As can be seen from Table 3 and Table 4, the MULIRA model provided better results than the Minister of Development (2014). The relative error between the observed and estimated values in the MULIRA model is the maximum for 2013 at -18.97%. For in the remaining years, it is less than 2%. To the Ministry of Development estimates, meat consumption has been predicted to be consistently higher than meat consumption. For example, in 2017, a high estimate of 50% was realised between the actual and estimated values. The estimation errors in 2017 are dramatic when they are compared with each other. The MULIRA model estimation error is 0.84%, while the estimation error of the Ministry of Development is 50.58%. However, the results obtained from other prediction methods (LIRA, POLIRA, LOLIRA) are better than the results of the Ministry of Development but not better than those of the MULIRA (See Tables 5 and 6).

Estimations between 2018 and 2040 using the MULIRA, LIRA, POLIRA and LOLIRA models were compared with the estimation of FAO-OECD (2019a) and FAO-OECD (2019b), and results are shown in Figure 3.

Table 5. Comparison of the linear regression models for meat consumption

	Observed Data FOA (2019)	MULIRA Prediction	Error %	LIRA Prediction	Error %	POLIRA Prediction	Error %	LOLIRA Prediction	Error %
2013	35.12	28.46	-18.97	27.59	-21.44	29.41	-16.26	27.64	-21.30
2014	29.27	28.96	-1.05	27.87	-4.77	29.98	2.44	28.00	-4.33
2015	28.94	29.50	1.92	28.15	-2.71	30.57	5.63	28.37	-1.98
2016	30.25	30.06	-0.64	28.44	-6.00	31.17	3.04	28.74	-5.00
2017	30.39	30.64	0.84	28.72	-5.50	31.78	4.57	29.11	-4.20

, Table 6. Comparison of the Ministry of Development for meat consumption

	Observed Data FOA(2019)	Ministry of Development Prediction (2014)	Error %
2013	35.12	40.52	15.38
2014	29.27	41.78	42.72
2015	28.94	43.08	48.85
2016	30.25	44.40	46.77
2017	30.39	45.76	50.58

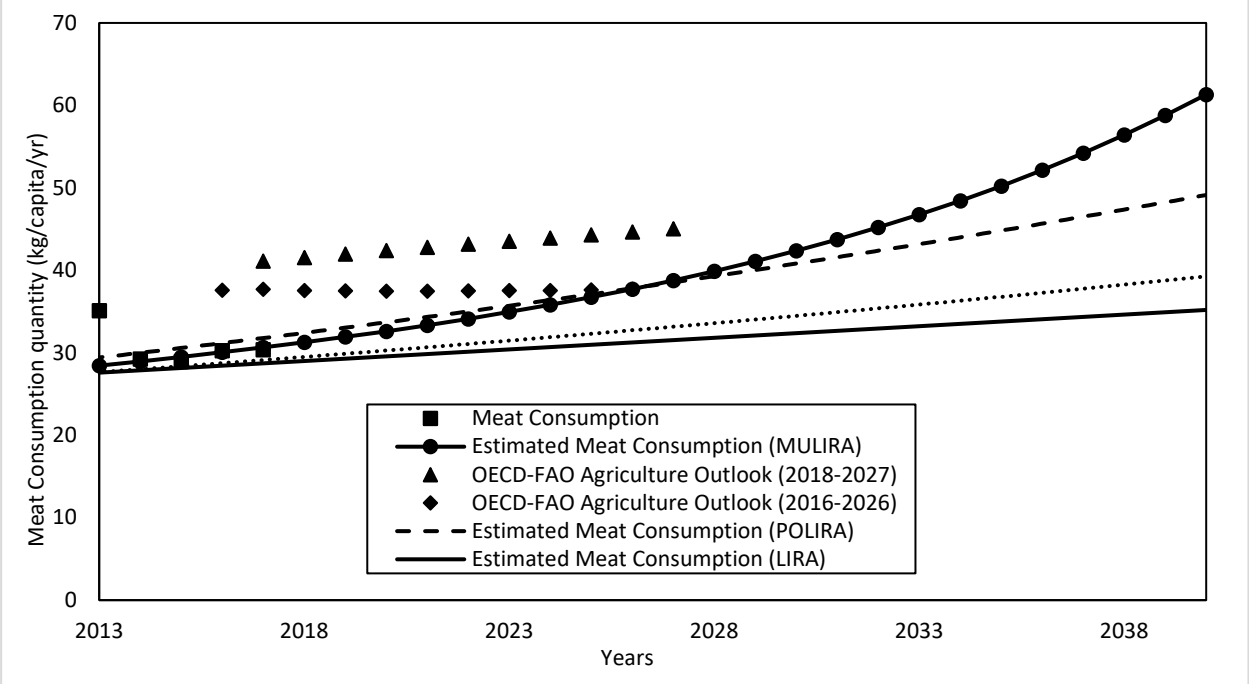

Figure 3. FAO-OECD (2019a) and FAO-OECD (2019b) overestimate than results of the present study.

Conclusion

In this study, the meat consumption of Türkiye is estimated based on Population, Gross Domestic Product (GDP) per capita, Electricity Consumption per capita, Imports of Goods and Services (% of GDP), Gross Capital Formation (% of GDP), Exports of Goods and Services (% of GDP) and Unemployment. The parameters of the Regression Analysis Models developed either in linear or exponential forms were obtained using the observed data. The MULIRA, LIRA, POLIRA and LOLIRA models were validated from 2013 to 2017 with observed data. The four different forms of Regression Analysis Models were performed, while the results obtained were compared projections. The following main conclusions may be drawn from the results of the present study:

(a) All models, Multiple Linear Regression Analysis (MULIRA), Linear Regression Analysis (LIRA), Polynomial Linear Regression Analysis (POLIRA) and Logarithmic Linear Regression Analysis (LOLIRA), can be used as an alternative solution and a prediction of meat consumption of Türkiye.

(b) The linear form of the MULIRA model seems a slightly better-fit solution with the observed data. Therefore, the MULIRA model can be selected for Türkiye's future meat consumption prediction.

(c) Social and economic indicators may be used for meat consumption. Economic development, especially GDP, is the most important factor in meat consumption.

(d) In the test period, the meat consumption estimates made by the Ministry of Development in 2013-2017 are overestimated. Estimates in this study for the same period agree with the actual values.

(e) This study estimates meat consumption in Türkiye using Regression Analysis Meat Consumption Model (RAMCM). The results are compared with the Ministry of Development values. However, an estimation of meat consumption may be investigated with Neural networks, Genetic Algorithms, Fuzzy Logic or other estimation methods. The results of the different techniques could be compared with the result of the present study for the comparison.

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: Authors declare that this study includes no experiments with human or animal subjects.

Funding disclosure: -

Acknowledgments: -

Disclosure: -

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 9(3), 230-241 (2023) • https://doi.org/10.3153/FH23021 Research Article

Antimicrobial and antioxidant activities of Algerian prickly pears and two cultivars dates (Mech-Degla and Frezza)

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Cite this article as:
Temagoult, A., Noui, Y., Zitouni, B. (2023). Antimicrobial and antioxidant activities of Algerian prickly pears and two cultivars dates (Mech-Degla and Frezza). *Food and Health,* 9(3), 230-241. https://doi.org/10.3153/FH23021

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Submitted: 21.02.2023
Revision requested: 11.04.2023
Last revision received: 10.05.2023
Accepted: 15.05.2023
Published online: 25.06.2023

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Available online at
http://jfhs.scientificwebjournals.com

ABSTRACT

This research aims to evaluate the biological activities and physicochemical characterization of the Algerian prickly pear and two varieties of dates (Mech-Degla and Frezza). The phenolic content of these fruits was determined by the Folin-Ciocalteu method. Then, antioxidant activities were studied using two different methods. The antimicrobial activities of the fruit were tested against four bacterial strains and two fungi and then compared to commercial antibiotics. Mech-Degla extract showed a strong antibacterial and antifungal inhibitory effect evaluated against *S. aureus, Streptococcus* spp*, E. coli*, *P. aeruginosa, G. capitatum*, and *Candida* spp. While Frezza date extract and prickly pear showed a weak effect against some bacterial strains, they had no inhibitory effect on fungi. The antimicrobial activity of Mech-Degla is superior to amikacin, ampicillin, and gentamicin. The Frezza variety had the highest antioxidant capacity, followed by Mech-Degla and the low iron reduction capacity is related to prickly pears. The tested fruits had high levels of polyphenols and flavonoids. This study confirms those species' antioxidant activity and antimicrobial properties and the critical effect of natural antibiotics com-pared to synthetic ones.

Keywords: Prickly pear, Dates, Algeria, Polyphenols, DPPH, Antimicrobial and antioxidant effects

Introduction

The nutritional value of the compounds in the fruits is essential for improving general well-being and protecting against several diseases. They may also be beneficial in treating other neurological disorders caused by oxidative stress (Chaouch et al., 2016; El Tanbouly et al., 2017).

Fruits contain beta-carotene, flavonoids, and phenolic compounds (Vaclavik and Christian 2007). The use of these bioactive molecules can improve human health and contribute to the resolution of several problems, such as resistance to synthetic antibiotics (Koubaa et al., 2015).

Cactus, Indian fig, and prickly pears are all names of *Opuntia ficus-indica* (L.) Mill. It occurs naturally in arid and semi-arid areas (El-Mostafa et al., 2014) and is highly environmentally tolerant (Astello-García et al., 2015). It was abundant in Mexico and the United States and was subsequently introduced into Mediterranean regions, notably South Africa and Algeria (El-Mostafa et al., 2014). Prickly pears are available worldwide in a variety of colors, including red, purple, green, and yellow (Aruwa et al., 2018). It was highly recommended for human consumption. Because this fruit is rich in minerals, vitamin C and flavonoids (such as kaempferol, quercetin, and isorhamnetin), phenolic acids, and betalain (Del Socorro Cruz-Cansino et al., 2015; Navarrete-Bolaños et al., 2013).

Palm-Date (*Phoenix dactylifera* L.) is an important crop in warm desert regions. Dates are marketed as high-value fruits worldwide (Abbès et al., 2013). Algeria is considered a date-producing country; annual date production is around 1.5 million euros, with 1136025 tons/ 170082 ha in 2019 (FAOSTAT, 2019).

The nutritional value of dates is attributed to their high levels of dietary fiber, sugars, and minerals such as magnesium, potassium, selenium, and copper (Al-Farsi and Lee, 2008). In addition to vitamins (B and C complexes), and antioxidant richness, polyphenols, flavonoids, and carotenoids (Bouhlali et al., 2016; Sánchez-Moreno and Larrauri, 2016).

This study aims to characterize the physicochemical properties and evaluate the biological activities of the prickly pear and two date varieties (phenolic content, flavonoids, antioxidant activities, and antimicrobial effect). Extracts from these fruits were tested to evaluate their antimicrobial activity against four bacterial and two fungal strains by comparing their effect to commercial antibiotics.

Materials and Methods

Sample Preparation

Fresh yellow-orange (*Opuntia ficus-indica*) fruits were collected in Arris, Algeria. The altitude is 1205 meters above sea level. The fruits of the prickly pear were washed, peeled, and kept at -20 °C until further analysis. In addition, this study was carried out on two varieties of dates (Mech-Degla and Frezza a Deglet Nour variety sorting gap). They were harvested from different palms from Biskra - Algeria. The date samples were stored in plastic bags and kept at -20 °C.

Physicochemical Characterization of Prickly Pear and Dates

To determine the moisture content, the fruits were dried in an oven (Binder, GmbH, Germany) at 103 ± 2° C and atmospheric pressure until a constant mass was obtained. Water content was equal to mass loss (AOAC, 1980).

A pH meter (HANNA, HI 2210, Romania) was used at 20° C to determine the potential difference by immersing the electrodes of the pH meter in the aqueous solution, which was tested according to the AFNOR method (1982).

Ash content was determined by incinerating the sample in a muffle furnace (Nabertherm, Germany) at 500°C. This determination is based on the destruction of any organic matter until whitish ash is obtained (AFNOR, 1982).

Dubois et al. (1956) used the phenol method to determine total sugar contents. Condensation between the formed compounds, by the addition of concentrated sulfuric acid and phenol, gave colored complexes (yellow-orange); (hydroxymethylfurfural in the case of a hexose and furfural in the case of a pentose).

To extract the desired components from the fruit, 1g of the fruit was added to 70 ml of distilled water. The mixture was then heated to 70°C for 30 minutes using a water bath. Subsequently, the mixture was filtered, and the volume was adjusted to 100 mL. Appropriate dilutions were made for each fruit extract. Then, 0.5 ml of sample and 0.5 ml of phenol (0.5%) were added to each test tube. After that, 3 ml of sulfuric acid was added to the test tubes, and the mixture was homogenized thoroughly using a vortex mixer. The test tubes were then placed in a water bath at 100°C for 30 minutes and allowed to cool down afterwards.

The optical density was measured at 490 nm using a visible UV spectrophotometer (Shimadzu, 120-02, Japan) (Fournier,

2001). This was done for each fruit. The standard range was prepared from a stock solution of glucose (mg/mL).

Determination of Total Phenolic Content

Polyphenols Extraction and Measurement

To determine the phenolic compounds, a solvent mixture was used. A 3 g sample was added to 120 ml of methanol: water (80:20 v/v) and allowed steep for 24 h, with agitation. The maceration was carried out at room temperature and in the dark. The extracts were filtered, then concentrated after maceration by the rotavapor (Buchi, Germany) at 45°C and 250 mbar. After that, they were covered with 10 ml of pure methanol and kept at -18°C until its use. This was done for each fruit.

The phenolic compounds were determined according to the method described by Juntachote et al. (2007). All measurements were done in three replicates. Fruit extract in 500 mL was combined with 1 mL of Folin-Ciocalteu reagent and 5 ml of distilled water. After 5 minutes of stirring, the mixture was neutralized with 1 mL of 7.5% Na_2CO_3.

The mixture was incubated in complete darkness at room temperature for an hour. At 760 nm, the absorbance was subsequently determined using a visible UV spectrophotometer (Shimadzu, 120-02, Japan). The results were expressed as Gallic acid equivalents in mg per 100g of fresh fruit (GAE/ 100g).

Determination of Total Flavonoid Content

The total flavonoid content of the extract was determined by spectrophotometry using the method reported by Bahorun et al. (1996). Both prickly pear and date extracts were tested separately. The results were reported as µg quercetin equivalent (QE) / mg extract.

1 ml of each fruit extract was added to 1 ml of trichloride of aluminium (2%). The mixture was stirred for 5 minutes and then incubated for 15 minutes at 25 °C. After incubating, the absorbance was determined at 430 nm using a visible UV spectrophotometer (Shimadzu, 120-02, Japan). The results were expressed as quercetin equivalents in µg per mg of fresh extract (QE/mg).

Radical Scavenging Activity of DPPH

The antioxidant capacity was evaluated using *Farag's* method (Farag et al., 2016) by scavenging the DPPH radical. This method was based on reducing the DPPH radical from purple to yellow DPPH. The lowest absorbance indicates relatively highest activity. It was calculated using Eq. (1)

$$\%\ DPPH\ = [1\ -\ (A\ extract\ /\ A\ control)]\ \times\ 100 \quad (1)$$

DPPH: radical scavenging activity (%)

A extract: absorbances of the sample (nm)

A control: absorbances of the control (nm)

Reducing Power Activity (FRAP)

The reducing power was calculated according to Sánchez-Moreno (2002). At 700 nm, the absorbance was measured. Increased absorbance mixture significantly increases reducing power activity; it was given in terms of Gallic acid equivalents.

Antimicrobial Activity

Four bacterial strains were used to determine the antibacterial activities. *S. aureus and Streptococcus* spp. are Gram-positive strains, *E. coli* and *P. aeruginosa* are Gram-negative strains. *G. capitatum* and *Candida* spp. were tested for antifungal activity. The disc diffusion method described by (Choi et al., 2006) was used for this evaluation.

The central microbiology laboratory, CAC (Anti-Cancer Center) Batna, Algeria, provided us with the bacterial and fungal organisms. Standard drugs, namely Amikacin, Colistin, Ofloxacin, Oxacillin, Tetracycline, Teicoplanin, Fluconazole, and Amphotericin B, were used as positive controls.

A sterile saline solution (0.9 %) was prepared with distilled sterile water and NaCl, also a 10 % of dimethyl sulfoxide (DMSO) solution was prepared.

The microorganisms were cultured in fresh nutrient broth overnight before being incubated at 37°C. Bacterial testing was done on Muller Hinton agar, and fungal testing on Sabouraud dextrose agar plates (EUCAST, 2020). The suspensions of each bacterial strain (0.5 Mc Farland opacity inoculum) were prepared from an 18-hour pure culture.

After inoculating Mueller-Hinton agar and impregnating Whatman paper discs (6 mm) with a small amount of extract (50 µl per disk), these discs were positioned on the surface, and DMSO-impregnated Whatman discs were used as negative controls. Until the visible growth of bacteria, Petri dishes were incubated overnight at 37°C. The same procedure was followed for fungi at 28°C.

The antibiotic susceptibility testing with appropriate antibiotics was performed to serve as positive controls and allow comparison of results. According to EUCAST's recommendations (EUCAST, 2020)

The calliper was used for reading the antibiotic susceptibility tests. An extract was considered active when there was no bacterial growth within the measured area, with a diameter greater than 8 mm.

Statistical Analysis

The results were presented as a mean, standard deviation (n = 3). The XLSTAT v. 14 and the Tukey HSD test were employed to improve variance analysis (ANOVA). When $p < 0.05$, the results were considered statistically significant. Principal component analysis (PCA) and pairwise correlations were applied to gain insight into the main data variation and to interpret the variable's relationships.

Results and Discussion

Physicochemical Characterization of Prickly Pear and Dates (Frezza and Mech-Degla)

The results of the physicochemical analyses of prickly pear and the two date varieties are mentioned in Table 1. They were the average of three repeats.

Table 1. Physicochemical characterization of prickly pear and dates fruits

Parameters	Values		
	Prickly pear	Mech-Degla	Frezza
Moisture (%)	84.00 ±0.72[a]	7.79 ±0.42[b]	11.24 ±0.35[c]
pH	5.5 ±0.01[a]	5.71 ±0.03[a]	5.64 ±0.05[a]
Ash[*]	1.97 ±0.72[a]	2.12 ±0.05[a]	2.13 ±0.01[a]
Total sugars[**]	11.5 ±0.06[a]	57.19 ±1.03[b]	65.49 ±0.98[c]

a,b, and c in the same row indicate a significant value difference at the $P \leq 0.05$ level. [*]: g/ 100g of fresh material [**]: g/ 100g of dry material

Based on the results in Table 1, both date varieties were considered dry fruits, given the low water content (according to the standard, the fruit was dry when the moisture content was <26%) Chibane (2008). On the other hand, the prickly pear was very rich in water and was considered a juicy fruit. This criterion classifies prickly pear as a highly perishable fruit and dates as stable and long-stored fruits at ambient temperatures.

The moisture content of prickly pear pulp was 84%. This value was close to that of Chiteva and Wairagu (2013), who gave levels between 87.4% and 89%. Tonelli and Gallouin (2013) also found 87%. However, the Mech-Degla date was drier than Frezza (7.79 and 11.24 %), respectively. The moisture content found in this study was lower than those reported by Chibane (2008) for the same varieties and those mentioned by Noui (2016).

The three fruits' pH is slightly acidic and varies between 5.5 and 5.7. A slight difference is noted between the pH values of the two date varieties studied. The prickly pear's pH is 5.5. This value was slightly lower than that given by Bouzoubaà et al. (2014), which was between 5.80 and 5.92 for the 'Achefri' and 'Amouslem' varieties. Also, Mazari and Mahdeb (2021) in Souk-Ahras (south Algeria) reported a pH between 5.91 and 5.93.

The Mech-Degla date's pH was identical to that of Chibane (2008) for the same variety, 5.72. But it was superior to the Frezza variety, which was 5.06. It was also superior to that found by Mimouni (2009). The two dates studied have an acceptable pH according to the standard; the latter classifies those with a pH of 5.4 to 5.8 as quality dates (Hachani et al., 2018).

The ash content represents the total amount of mineral salts in the fruit. The ash content was between 1.97 and 2.13 % for the three fruits (Table 1); the Frezza variety was the richest in mineral salts compared with the other two. The ash content of the cultivar Frezza was identical to that studied by Chibane (2008), but the ash content of Mech-Degla was slightly higher. Noui (2018) mentioned 2.14% for the cultivar Mech-Degla. The result of the ash content of these two cultivars was lower than that found by El Arem (2012) for the same Tunisian cultivars in several maturation stages, also those found by Bouhlali et al. (2016) in other Moroccan cultivars.

The content of the prickly pear ash was 1.97 %; this value was higher than that of Mazari and Mahdeb (2021), which was between 0.26 and 0.44 %.

A clear significant difference between the total sugar contents of these three fruits. Sugars were the most important component in dates. According to the literature, the total sugar content of dates varies between 60 and 80 % depending on the variety and stage of maturation (Chibane et al., 2007).

The total sugar content of the two dates studied (Mech-Degla and Frezza) was 57.19 and 65.49 %, respectively (Table 1). Chibane (2008) indicated higher values for the same varieties, 63.8 and 77.3 %, and the Frezza variety was the richest in sugars. Assirey (2015) found higher levels in a study of ten date cultivars in Saudi Arabia. The content of these two varieties studied was like that reported by Tang et al. (2013), which was between 53.98 and 63.16 %.

After comparing the results of the sugar content obtained with those indicated in the standard (Hachani et al., 2018), therefore, it was concluded that the Mech-Degla variety was of poor quality in terms of sugar content (< 60%). At the same time, the Frezza variety was acceptable.

The total sugar content of prickly pear was 11.5 %. This value was lower than the results given by Bouzoubaà et al. (2014), which found values ranging from 13.5 to 15.87 % for different varieties of prickly pear studied in Morocco. Also lower than the results of Mazari and Mahdeb (2021), which were between 13.25 and 14.8 %.

Phenolic Compounds

The results of the phenolic compounds are presented in Table 2.

Table 2. Polyphenol content of prickly pear and dates (Frezza and Mech-Degla)

Parameters	Values		
	Prickly pear	Mech-Degla	Frezza
Total phenolics (mg GAE/100 g FW)	25.18 ±1.03[a]	15.76 ±1.00[b]	11.93±0.84[c]
Total flavonoids (mg QE/100 g FW)	2.63 ±0.03[a]	2.11 ±0.03[b]	1.67 ±0.06[c]

At the P ≤ 0.05 level, different superscript letters in the same row indicate a significant value difference. GAE: Gallic Acid Equivalent; QA: Quercetin Equivalent; FW: Fresh Weight

According to the results presented in Table 2, the difference between the polyphenol content of the three extracts (prickly pear, Mech-Degla, and Frezza) was very clear, which were respectively 25.18 and 15.76 and 11.93 mg Gallic acid equivalent / 100 g of fresh weight.

The polyphenols content of prickly pears was like those obtained by Bouzoubaà et al. (2014), which ranged from 28.94 to 44.72 mg/100 g. However, it was lower than those found by Dehbi et al. (2013); Medina et al. (2007), respectively, between 45.2 mg and 64.36 mg GAE/100 g of fresh weight. However, this polyphenol composition was superior to that of Bargougui et al. (2019). They extracted four cultivars of prickly pear from methanol and ethyl acetate: Ain Amara (Tunisia), Ain Jemaa (Morocco), Longissima (Algeria), and Sanguinea (Italy). Phenolic levels ranged from 2.53 to 0.80 mg/100 g. Several authors found that total polyphenols appear stable during refrigerated storage (Barba et al., 2012).

For date extracts, the results of phenolic levels found by Daas et al. (2014) and Chibane (2008) were even more important than those obtained in this study. Benmeddour et al. (2013) found higher values for these same cultivars and others in a study of ten varieties of Algerian dates (225.57 – 954.57 mg Gallic acid equivalent /100 g of fresh weight).

Total Flavonoid Contents

Flavonoid function was expressed by antioxidant activity and high affinity to polymers and especially heavy metals (Chibane, 2008).

The total flavonoid content of the three fruits ranged from 02.63 to 01.67 mg Quercetin equivalent /100 g (Table 2). This study showed that prickly pears have a higher flavonoid content than the dates studied.

Cruz-Bravo et al. (2019) found high values, ranging from 3.1 to 7.0 mg/100 g, in a comparative study of the flavonoid content of prickly pears during harvesting and storage. Also, El Mostafa et al. (2014) reported higher flavonoid values for prickly pear pulp, cladodes, and seeds.

The Frezza date had the lowest level of flavonoids. These results were lower than Daas et al. (2014) for Mech-Degla 6.01 mg Quercetin equivalent /100 g of fresh weight and Chibane et al. (2007) for the Frezza variety. Benmeddour et al. (2013) reported a significant difference in total flavonoid content between date cultivars in a comparative study (15.22-299.74 mg Quercetin equivalent /100 g of fresh weight). In addition, Chaira et al. (2009) found that the total flavonoid levels of dates varied significantly between some Tunisian cultivars, ranging from 6.41 to 54.46 mg QE/100 g. These variations could be attributed to cultivars, climate, cropping practices, and extraction methods.

Antioxidant Activity

DPPH Radical Scavenging Activity

According to Cruz-Bravo et al. (2019), the important antioxidant activity was due to polyphenols, flavonoids, and carotenoids, which play a protective role in the body.

Table 3 shows the antioxidant activity of the studied fruits. An important inhibitory activity reported for these fruits was their richness in polyphenols and flavonoids. Thus, a significant variation in the ability of the extracts to neutralize the DPPH radical.

As a result, the Frezza date had the highest anti-radical activity (83.33%), followed by prickly pear (80.66 %) and Mech-Degla date (61.11 %).

These results were comparable to Algerian and Italian prickly pear (Bargougui et al., 2019). However, they were higher than those found by Belviranli et al. (2019) for several varieties in Türkiye and Madrigal-Santillán et al. (2013), which showed that the best inhibition activity of prickly pear juice was 65%.

Concerning the inhibitory capacity of DPPH in both date varieties, the results were higher than those obtained by Benmeddour et al. (2013). The results found by Chibanen (2008) were like those obtained in this study for the Mech-Degla variety. However, they were lower than those of the Frezza variety.

Reducing Power Activity (FRAP)

A highly significant difference was estimated from the reductive power of the date (Frezza and Mech-Degla) compared to the prickly pear, which was respectively 19.15 - 18.38 and 05.75 mg/100 g, presented in Table 3.

The results of these date varieties were lower than those found by Benmeddour et al. (2013) in a comparative study of ten Algerian date cultivars. These results were also lower than the findings of Hachani et al. (2018) on five other Algerian date cultivars.

Chougui et al. (2013) and Medina et al. (2007) reported iron reductions of 66.57 mg/100 g and 19 mg/100 g for prickly pear.

Abdel-Hameed et al. (2014) found 81.38 mg/100 g of fresh prickly pear juice with yellow pulp and 123.23 mg/100 g of fruit juice with red pulp.

Table 3. Antioxidant activities of prickly pear and dates (Frezza and Mech-Degla)

	Prickly pear	Mech-Degla	Frezza
FRAP (mg/100 g)	5.75 ±0.34[a]	19.15 ±0.22[b]	18.38 ±0.39[b]
DPPH %	80.66 ±3.28[a]	61.11 ±1.84[b]	83.33 ±2.70[a]

Values are means ± SD. Different letters in each row indicate a significant difference (P< 0.05) among means of varying fruit. Extract

Antibacterial and Antifungal Properties

The extracts' bacteriostatic effects and fungal test were evaluated against six pathogenic strains to determine the presence or absence of inhibition area. The results are presented in Tables 4 and 5.

From the results, it was demonstrated that; Mech-Degla extract exhibited an inhibitory effect on the six strains tested. However, prickly pear extract only inhibited *E. coli* and *P. aeruginosa*. Regarding the Frezza date extract, all strains tested were resistant.

This significant inhibitory activity of these fruit extracts against certain bacteria was the same as that of certain commercial antibiotics, such as Amikacin, Colistin and Teicoplanin. However, it was more important and influential than Cephalexin, Ampicillin and Gentamicin.

It is essential to highlight the high efficiency of Mech-Degla extract., which was equal to that of Amikacin against *P. aeruginosa* and, more important, against *S. aureus* and *E. coli*. Mech-Degla extract showed equal activity regarding the antifungal ketoconazole or bifonazole. (The diameter of the zone of inhibition was more significant than that of the antibiotic used for antibiogram for the same bacterial strain).

The results of this variety of dates were more important than Daas et al. (2014) found.

The results found by Bargougui et al. (2019) confirm the effectiveness of the prickly pear extract against *P. aeruginosa* and *E. coli*.

The results of anti-fungal activity showed that only the Mech-Degla extract has a high efficiency. But these fungi were the most resistant to the effect of Frezza and prickly pear extract.

This antimicrobial activity was due to the ions present in these fruits. It is essential for reducing or preventing the initial adhesion of bacteria. Minerals like Mg^{2+}, Zn^{2+}, Na^{+}, Fe^{2+}, Mn^{2+}, and K^{+} mainly increase antibacterial activity (Meng et al., 2022).

Several authors explain the inhibitory effect against microbial pathogens by the phenolic composition of plant extracts (Balouiri et al., 2016). The inhibitory effect may be enzyme interaction, adsorption to the cellular membrane or metal ions, and substrate deprivation (Baydar et al., 2004).

Taleb et al. (2016) hypothesize that date phenolic compounds can be easily bound to proteins, and the interaction of these with the proteins of bacteria results in hydrogen bond interactions and ionic bonds. This will alter the protein activity of

the microorganism and make it more susceptible to treatment. It behaves like a prooxidant in systems that use redox-active metals such as iron and copper.

Polyphenols mainly compromise bioactive constituents, and these bioactive compounds influence H_2O_2 as an antimicrobial agent. Thus, further confirming the role of polyphenols as antioxidants that scavenge free radicals and reduce H_2O_2 and prooxidants generated in antibacterial activity.

So, depending on the antioxidant content, the bacterial cells proliferate and grow.

This is evident in the antibacterial results, which means that stress responses depend on the concentration of antioxidants.

Several data studies support this result (Abbès et al., 2013; Martín-Sánchez et al., 2014).

Principal Component Analysis

A PCA analysis evaluated the relationships between all the measured biological activities (Figures 1 and 2). The first two axes explain more than 94-98% of the variation. The first axis (F1) had a higher discriminating power of 67.51 % than the second (F 2), which had a discriminating power of 27.47 %. According to the first axis, a separation between the biological activities of the fruit extracts was observed.

The Mech-Degla date was characterized by the height antibacterial level, which correlates with the FRAP capacity on the right of the axis. While in the opposite sense, Frezza date and prickly pear were characterized as less antibacterial. But rich in flavonoids and polyphenols that promote a heightened antioxidant level.

For the second axis, a significant difference showed between prickly pear and the other dates in polyphenol and flavonoid rates.

Table 5. Antifungal activity of prickly pear and date fruits (Frezza and Mech-Degla)

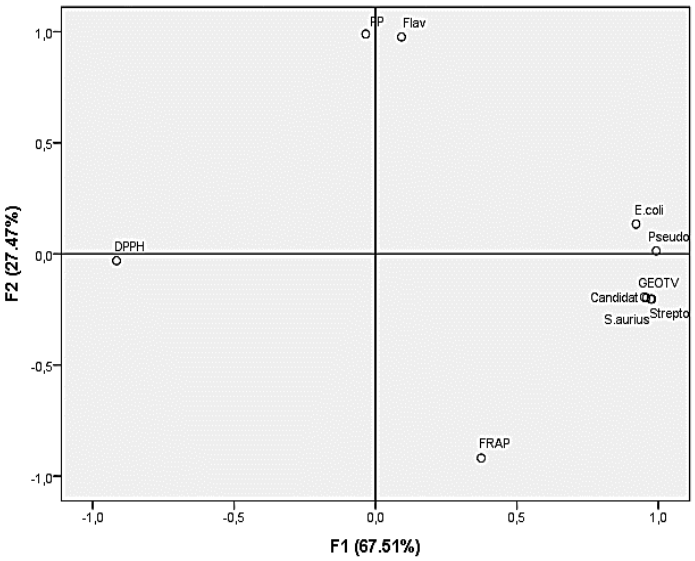
	Diameter of the inhibition zone (mm)		
Fungi	**Prickly P**	**MD**	**F**
***Candida* spp**	0.00[a]	16.50 ±2.48[b]	0.00 [a]
G. capitatum	0.00[a]	14.50 ±1.98[b]	0.00 [a]

Different lower-case letters in the same column indicate significantly different ($P \leq 0.05$)
Candida: Candida spp., G. *capitatum: Geotricum capitatum,* Prickly P: Prickly Pear, MD: Mech-Degla, F: Frezza,

Table 4. Antibacterial activity of prickly pear and date (Frezza and Mech-Degla)

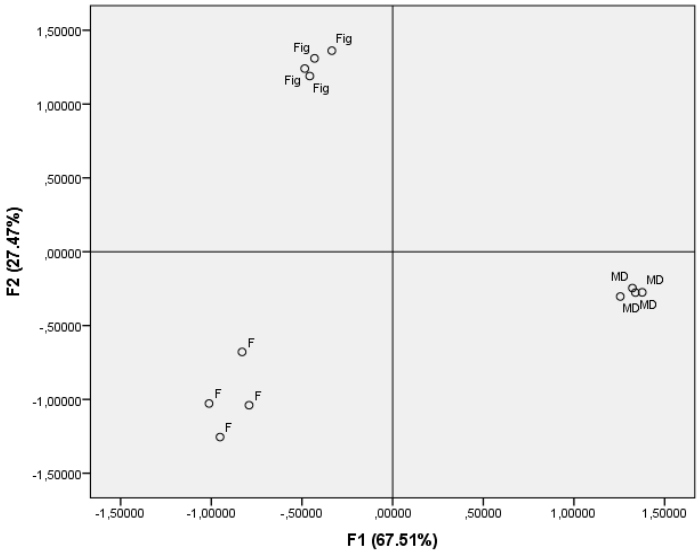
	Diameter of the inhibition zone (mm)								
Bacteria	**Prickly P**	**MD**	**F**	**AK**	**CL**	**TE**	**VA**	**CN**	**OF**
S. Aureus	0.00 [a]	10.50 ±0.7 [b]	0.00 [a]	8.0 ±0.0[c]	-	08.0 ±0.0[c]	14.0 ±0.0[d]	-	11.0 ±0.0[b]
P. aeruginosa	8.0 ±1.41[a]	26.0 ±1.41[b]	2.50 ±0.7[c]	26.0 ±0.0[b]	21.0 ±0.0[d]	-	-	-	30.0 ±0.0[e]
E. Coli	7.50 ±0.7[a]	15.0 ±2.24[b]	3.50 ±0.7[c]	11.0 ±0.0[d]	18.0 ±0.0[e]	-	-	8.0 ±0.0[a]	-
***Streptococcus* spp.**	0.00 [a]	15.0 ±1.41[b]	0.00 [a]	-	-	21.0 ±0.0[c]	0.00 [a]	-	-

Different lower-case letters in the same column indicate significantly different ($P \leq 0.05$)
S. aureus: Staphylococcus aureus, E. coli: *Escherichia coli* and *P. aeruginosa*: *Pseudomonas aeruginosa*, Prickly P: Prickly Pear, MD: Mech-Degla, F: Frezza, AK: Amikacin, CN: Cefalexin, CL: Colistin, TE: Tetracycline, VA: Vancomycin. OF: Ofloxacin



S. aureus: Staphylococcus aureus, Strepto: *Streptococcus* spp. *E. coli*: *Escherichia coli* and *P. aeruginosa*: *Pseudomonas aeruginosa*, GEOTv: *Geotricum capitatum* and *Candida* spp.,
PP: Polyphenols, Flav: Flavonoids

Figure 1. Dispersion plot after space rotating obtained by CPA analysis of three fruits according to the biological characteristics



MD: Mech-Degla, F: Frezza and Fig: Prickly Pear

Figure 2. Dispersion plot after space rotating obtained by CPA analysis of prickly pear and dates (Frezza and Mech-Degla)

Conclusion

This study has shown a significant difference in the biological activities of three fruit extracts (prickly pear, Frezza and Mech-Degla dates) from eastern Algeria, confirming that some biochemical compounds show a significant antimicrobial effect.

The polyphenol content of the prickly pear extract was the highest. But it has the lowest value of FRAP, compared to the date varieties studied (Mech-Degla and Frezza); 19.15±0.2 mg/100g and 18.3 mg/100g, respectively. The high antioxidant activity was noticed in the Frezza extract.

Mech-Degla extract exhibited an antimicrobial effect on the six strains tested. However, the activity of prickly pear extract only inhibited the growth of *E. coli* and *P. aeruginosa*. But the Frezza date extract has no antibiotic effect.

This significant inhibitory activity of these fruit extracts against certain bacteria was the same as that of some commercial antibiotics, such as Amikacin, Colistin, and Teicoplanin. However, it was more important and influential than Cephalexin, Ampicillin, and Gentamicin.

These results demonstrate that these natural products have the potential to be developed into new antimicrobial agents. These characteristics make them an excellent source of functional ingredients in producing many foodstuffs.

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: Authors declare that this study includes no experiments with human or animal subjects.

Funding disclosure: -

Acknowledgments: The authors would like to express their gratitude to all contributors to the realization of this work for their valuable help.

Disclosure: -

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 9(3), 242-253 (2023) • https://doi.org/10.3153/FH23022 Research Article

Farklı basınç büyüklükleri ve çözücülerin böğürtlen (*Rubus plicatus* L.) ekstraktlarının fonksiyonel özelliklerine ve renk stabilitesine etkisi

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Cite this article as:
Başkaya Sezer, D. (2023). Farklı basınç büyüklükleri ve çözücülerin böğürtlen (*Rubus plicatus* L.) ekstraktlarının fonksiyonel özelliklerine ve renk stabilitesine etkisi. *Food and Health,* 9(3), 242-253. https://doi.org/10.3153/FH23022

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Submitted: 14.02.2023
Revision requested: 31.03.2023
Last revision received: 31.03.2023
Accepted: 02.04.2023
Published online: 01.07.2023

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Available online at
http://jfhs.scientificwebjournals.com

ÖZ

Fenolikler, oluşturduğu antioksidan kapasite sayesinde başta kanser olmak üzere, kardiyovasküler hastalıklar, hipertansiyon ve diyabet gibi birçok hastalığa karşı koruyucu etki göstermektedir. Böğürtlen yüksek fenolik madde içeriği sayesinde fonksiyonel gıda olarak anılmaktadır. Bu çalışmada, işlem görmemiş ve hidrostatik basınç uygulanmış (300 MPa veya 600 MPa) böğürtlen püresinden su, etanol, metanol ve bu çözücülerin asetik asit (%1) ya da hidroklorik asit (%1) eklenmiş formları kullanılarak ekstraktlar elde edilmiştir. Ardından 0.gün ve 7.gün fenolik madde içeriği, antioksidan kapasite ve renk değerleri belirlenmiştir. En yüksek fenolik madde içeriği ve antioksidan kapasite hidroklorik asit eklenmiş alkol ekstraksiyonu ile en düşük değerler ise su ekstraksiyonu ile elde edilmiştir. Hidroklorik asitin asetik asitten daha fazla fenolik madde ekstrakte ettiği ve antioksidan kapasiteyi artırdığı belirlenmiştir. Fenolik madde miktarı yüksek örneklerde L^* değerlerinin daha düşük a^* ve b^* değerlerinin ise daha yüksek olduğu bulunmuştur. İşlem görmemiş örnekler ile karşılaştırıldığında basıncın fenolik madde içeriği ve antioksidan kapasiteyi artırdığı belirlenmiştir (p<0.05). 600 MPa'nın etkisi asitlendirilmiş çözücü ortamında daha belirgindir. Fenolik madde içerikleri ile antioksidan ve renk değerleri arasında korelasyon tespit edilmiştir. Sonuç olarak, böğürtlen meyvesinin fonksiyonel özelliklerini arttırmak için hidroklorik asit eklenmiş etanol ortamında ekstraksiyon önerilmiştir.

Anahtar Kelimeler: Böğürtlen, Fenolik, Antioksidan, Renk, Stabilite, Hidrostatik

ABSTRACT

The influence of different pressure magnitudes and solvents on the functional properties and color stability of blackberry (*Rubus plicatus* L.) extracts

Phenolics have a protective effect against many diseases, such as cancer, cardiovascular diseases, hypertension and diabetes, owing to the antioxidant capacity they form. Blackberries are known as a functional food due to their high phenolic content. In this study, the extracts were obtained from untreated and hydrostatic pressure (300 MPa or 600 MPa) treated blackberry puree using water, ethanol, methanol and acetic acid (1%) or hydrochloric acid (1%) added forms of these solvents. Then the phenolic content, antioxidant capacity and color values were determined on the 0th and 7th days. The highest phenolic content and antioxidant capacity were obtained with alcohol extraction with added hydrochloric acid, and the lowest values were obtained with water extraction. It was determined that hydrochloric acid extracted more phenolic substances than acetic acid and increased the antioxidant capacity. It was found that L^* values were lower, a^* and b^* values were higher in samples with high phenolic content. Compared with the untreated samples, it was determined that the pressure increased the phenolic content and antioxidant capacity (p<0.05). The effect of 600 MPa was found to be more pronounced in the presence of acid-added solvents. A correlation was detected between the phenolic contents, antioxidant capacities, and color values. As a result, extraction in hydrochloric acid-added ethanol medium is recommended to increase the functional properties of blackberry fruit.

Keywords: Blackberry, Phenolic, Antioxidant, Color, Stability, Hydrostatic

Giriş

Meyve ve sebzelerden elde edilen biyoaktif bileşenlere olan ilgi son yıllarda oldukça artmıştır. Bunun nedeni bu bileşenlerin vücutta oluşturduğu fonksiyonel özelliklerdir. Fenolik bileşenler, oluşturduğu antioksidan, antikanserojenik, antimutajenik ve antiinflamatuar etkiler sayesinde başta kanser olmak üzere, kardiyovasküler hastalıklar, hipertansiyon ve diyabet gibi birçok hastalığa karşı koruyucu etki göstermektedir (Jiang ve Dusting, 2003; Huang ve ark., 2009; Wahle ve ark., 2010; Rodriguez-Mateos ve ark., 2014; Anantharaju ve ark., 2016). Özellikle oksidatif stresin azaltılması, sağlık üzerine koruyucu etki oluşturan fenoliklerin en önemli fonksiyonel özelliklerindendir (Lorzadeh ve ark., 2022).

Böğürtlen meyvesinin, fenolik madde içeriği yüksek olan üzümsü meyveler (ahududu, yaban mersini, kızılcık, frenk üzümü, çilek) arasında en yüksek içeriğe sahip olduğu tespit edilmiştir (Amakura ve ark., 2000). Oszmiański ve ark. (2015) böğürtlen meyvesinde 15 flavonol, 8 antosiyanin, 6 ellajik asit türevleri, 3 hidroksisinnamik asit ve 2 flavon grubu olmak üzere toplamda 34 fenolik bileşik tespit etmiştir. Bu bileşikler sayesinde böğürtlenin sağlık üzerine koruyucu ve hastalıkların tedavisine yardımcı etkileri rapor edilmiştir (Robinson ve ark., 2020). Bu açılardan bakıldığında fenolik bileşiklerin ve oluşturduğu etkinin artırılmasına yönelik çalışmalar önemlidir.

Fenolik madde miktarı ve antioksidan kapasite değerleri uygulanan ekstraksiyon yöntemine göre değişiklik göstermektedir. Fenolik bileşiklerin çözünürlüğü, alkol ve su gibi polar çözücülerde daha yüksektir (Albert ve ark., 2022). Bunlardan su ile gerçekleştirilen ekstraksiyonlarda işlem süresi uzar, ayrıca organik asitler, şekerler ve çözünür proteinler gibi bileşenlerin de çözülmesi nedeni ile son üründeki safsızlıklar artar (Osorio-Tobón, 2020). Bu nedenle alkol türevleri ekstraksiyon verimini artırmak ve süreyi azaltmak için kullanılmaktadır. Hatta metanol ve etanol gibi çözücülere organik (asetik asit) ve inorganik (hidroklorik asit) asit eklenmesi ile ekstraktın fenolik madde içeriği ve buna bağlı antioksidan kapasitesinin arttığı belirlenmiştir (Kopjar ve ark., 2014; Akhbari ve ark., 2019). Böğürtlende konvansiyonel ekstraksiyon yöntemleri kullanılarak sıcaklık, süre ve çözücü parametreleri optimize edilerek fenolik madde içeriğinin arttırılması mümkün olmuştur (Oancea ve ark., 2013; Soto ve ark., 2016). Fakat bu geleneksel yöntemlerde çözücü maddenin toksisitesi ve seçilen kimyasalın yetersiz ekstraksiyon gücü, yüksek işlem maliyeti, uzayan ekstraksiyon süresi, düşük uygulama etkinliği ve kütle transfer verimi prosesteki en önemli sorunlardandır (Osorio-Tobón, 2020).

Isıl olmayan işlemler, çevre dostu çözücülerin kullanılması ve biyoaktif bileşenlere zararı en aza indirerek ekstraksiyon verimini artırması aynı zamanda da ısıtma maliyetinin getirdiği yükü ortadan kaldırması ile bu sorunların bazılarına çözüm olabilmektedir. Fakat farklı çözücüler kullanılarak fenolik madde, antioksidan kapasite ve renk değerlerinin depolamaya bağlı değişimine ısıl olmayan bir ön işlemin etkisini inceleyen çalışmalar sınırlıdır.

Yüksek basınç uygulaması solvent geçirgenliğini artırarak ekstraksiyon sırasındaki difüzyonu kolaylaştırmaktadır. Böylece işlem süresi azalırken ekstraksiyon verimi artmaktadır (Corrales ve ark., 2008). Literatürde yüksek basınç desteği ile üzüm kabuğu (Casazza ve ark., 2012), domates salçası (Xi, 2006), yeşil çay (Xi ve ark., 2009), armut (Gómez-Maqueo ve ark., 2020), portakal kabuğu (Guo ve ark., 2012) ürünlerinde biyoaktif madde (flavonoid, antosiyanin, karotenoid, likopen, pektin, polifenoller vb.) miktarının artırıldığı tespit edilmiştir.

Bu çalışmanın amacı, gıdanın fonksiyonel özelliklerini hidrostatik basınç büyüklüğüne ve çözücü tipine bağlı olarak açıklamaktır. Bu sayede basınç uygulaması sonrası materyalin farklı çözücü koşullarındaki davranışı da belirlenmiştir. Bunun için işlem görmemiş ve hidrostatik basınç uygulanmış böğürtlen meyvesinden su, etanol, metanol ve bu çözücülerin asetik asit ya da hidroklorik asit kullanılarak hazırlanan karışımları kullanılarak elde edilen ekstraktların 0.gün ve 7.gün fenolik madde içeriği, antioksidan kapasite ve renk değerleri (L^*, a^*, b^*, ΔE^*) karşılaştırılmıştır.

Materyal ve Metot

Örnek Hazırlama ve Ön İşlemler

Analizler öncesi yerel marketten satın alınan ve -18°C'de depolanan böğürtlenlerden, parçalayıcı (MQ 745, Braun, Almanya) yardımıyla 20 saniyede yüksek devir kullanılarak püre elde edildi. Bu örnek kontrol olarak kodlandı. Yüksek basınç uygulanan grup için ise örneklere 25°C'de iki ayrı basınç (300 MPa ve 600 MPa) 15 dakika boyunca uygulandı. Bu işlem için hidrostatik basınç ekipmanı (Avure Technologies, Ohio, ABD) kullanıldı.

Ekstraksiyon çözücüsünün hazırlanması için distile su, etanol ve metanol ile bu sıvıların hidroklorik asit (%1 v/v) ve asetik asit (%1 v/v) (Merck, Darmstadt, Almanya) ile karıştırılmış ve (1) su, (2) su+asetik asit, (3) su+hidroklorik asit, (4) etanol, (5) etanol+asetik asit, (6) etanol+hidroklorik asit, (7) metanol, (8) metanol+asetik asit, (9) metanol+hidroklorik asit

olmak üzere toplamda 9 farklı çözücü hazırlanmıştır. Asit miktarı seçiminde Jiang ve ark. (2004)'nın önerdiği gibi renk pigmentlerine en az zarar verecek oran seçilmiştir.

Ekstraksiyon

Ön işlemler sonucunda elde edilen böğürtlen püresinden 3 g (gram) alınarak 30 mL çözücü ile (1:10 w/v) 50 mL'lik santrifüj tüplerinde vorteks kullanılarak karıştırıldı. Vortekslenen karışım 25°C'de karanlık ortamda 60 dakika ekstraksiyona bırakıldı. Ekstraksiyon süresi bittikten sonra tüpler 2490 *x g* gücünde (4500 rpm) 20 dakika santrifüj (Thermomac, TMC815, Almanya) edildi. Sıvı faz başka bir santrifüj tüpüne alınarak 60 dakika içinde toplam fenolik madde, antioksidan kapasite ve renk ölçümleri yapıldı (0.gün). Depolama stabilitesi için örnekler (15 mL) santrifüj tüplerinde +4°C sıcaklıkta ve karanlık ortamda 7 gün bekletildikten sonra aynı ölçümler tekrarlandı (7.gün).

Ölçüm ve analizler

Toplam Fenolik Madde Miktarı

Toplam fenolik madde miktarının belirlenmesinde Folin Ciocalteu yöntemi kullanıldı (Slinkard ve Singleton, 1977). Analizde 0.1 mL ekstrakt kullanıldı ve sonuçlar 760 nm dalga boyunda ölçülerek 1 g örnek (taze böğürtlen) için mg gallik asit eşdeğeri (GAE) olarak sunuldu. Ölçümlerde kör örnekler 9 farklı çözücü ile hazırlanarak çözücü etkileri hesaplama dışında bırakıldı.

Antioksidan Kapasite

Antioksidan kapasite Brand-Williams ve ark. (1995)'nın sunduğu yöntem ile gerçekleştirilmiştir. Analizde 0.1 mL ekstrakt kullanıldı ve DPPH radikal süpürme değerleri 515 nm dalga boyunda ölçülerek 100 g örnek (püre) için mmol trolox eşdeğeri (TE) olarak sunuldu.

Renk

Böğürtlen püresinin renk değerleri Buslig (1992) tarafından açıklanan yönteme göre Hunter Lab Color Flex kolorimetresinde (Hunter Associates Laboratory, Reston, VA, ABD) açıklık (parlaklık) (*L*), kırmızılık/yeşillik (*a*), sarılık/mavilik (*b*) ve renk farklılığı (*ΔE**) değeri ile tespit edildi. Cihaz, standart karolar (siyah ve beyaz) ile kalibre edildi. *ΔE** hesaplamasında örneklerin beyaz karonun (L_o = 93.73, a_o = -1.04 ve b_o = -0.44) renk değerleri referans olarak alındı.

İstatistiksel analiz

Deneysel verilerin tanımlayıcı istatistikleri, SPSS paket programı (versiyon 23, 2018, IBM Corp., New York, ABD) kullanılarak yapılmıştır. Elde edilen sonuçlar için ortalama alınarak hesaplanan değer "ortalama ± standart sapma" ve istatistiksel harflendirme ile sunulmuştur. Çözücüler arasındaki karşılaştırma ve harflendirme için ANOVA ile Tukey's HSD kullanılmıştır. Ekstraksiyon ve depolama sonrası sonuçların karşılaştırılmasında eşleştirilmiş örnekler t testi (Paired-samples t-test), kontrol ve yüksek basınç uygulanan örnek gruplarının karşılaştırılmasında bağımsız örnekler t testi (Independent-samples t test) ve değerler arasındaki ilişkinin belirlenmesi için iki değişkenli (bivariate) korelasyon testi kullanılmıştır. Analizler %95 güven aralığında (p=0.05) gerçekleştirilmiş ve gruplar arası farklılıklar p<0.05 için ifade edilmiştir. Tüm ölçümler üç paralelli olarak yürütülmüştür. Çalışmanın bağımsız değişkenleri; çözücü tipi (etanol, metanol, su ile bunların asetik asit veya hidroklorik asit ile asitlendirilmiş formları), ön işlem (kontrol, 300 MPa veya 600 MPa) iken bağımlı değişkenleri; toplam fenolik madde, antioksidan kapasite ve renk (*L*, a*, b*, ΔE**) değerleri olarak kodlanmıştır.

Bulgular ve Tartışma

Bu çalışmanın bulgularında, işlem görmemiş (kontrol) ve yüksek basınç uygulanmış (300 MPa veya 600 MPa) örneklerden 9 farklı çözücü ile elde edilen ekstraktların 0.gün ve 7.gün fenolik madde içeriği, antioksidan kapasite ve renk değerleri sunulmuştur.

Toplam Fenolik Madde Miktarı

Çözücülere göre örneklerin fenolik madde içeriklerinin farklı olduğu Tablo 1'de görülmektedir. Buna göre en yüksek fenolik madde içeriğinin hidroklorik asit eklenmiş etanol ve metanol ile ekstrakte edilen örneklerde olduğu belirlenmiştir. En düşük içeriğin ise su ile hazırlanan ekstraktlarda olduğu tespit edilmiştir. Su ile elde edilen fenolik madde miktarı, asit eklemesi ile artırılsa da etanol ve metanol kullanılan denemelere göre daha düşüktür. Bunun nedeni, sulu çözücülerin güçlü polariteye sahip bazı biyoaktif bileşiklerin özütlenmesi için uygun iken etanolün daha geniş polarite aralığına sahip bazı biyoaktif bileşiklerin ekstraksiyonuna da olanak vermesi olabilir. Suyun etanole göre daha düşük ekstraksiyon gücüne sahip olduğu Mello ve ark. (2010)'nın propolis üzerine yaptığı çalışmada da belirtilmiştir. Tablo 1'e göre etanol ve metanol kullanılan işlemlerde asit eklenmesi ile fenolik madde içeriğinin eklenmemiş olan örneklere göre daha yüksek olduğu belirlenmiştir (p<0.05). Etanol+hidroklorik asit ve metanol+hidroklorik asit en yüksek fenolik içeriğin elde edilmesini sağlamış bunu etanol+asetik asit ve metanol+asetik asit takip etmiştir. Su kullanılan ekstraksiyonlarda ise asit eklemesinin ekstrakttaki fenolik madde içeriğini yükseltse de

önemli farklılık oluşturmadığı ortalama değerlere göre belirlenmiştir (p>0.05). Benzer sonuçlara Kopjar ve ark. (2014) çalışmasında da vişne için ulaşılmıştır.

Sonuçlar kullanılan asit türlerine göre karşılaştırıldığında ise hidroklorik asit eklenen çözücülerin bu artışı daha fazla desteklediği ortalama değerler dikkate alındığında görülmüştür. Ekstraksiyon çözücüsüne asit eklenmesi, fenolik maddelerin stabilitesini ve çözünürlüğünü artırarak ekstraksiyonu kolaylaştırır ve bu bileşenlerin yapısının bozulmadan elde edilmesini sağlar. Çünkü hücre duvarına bağlı olan fenolik bileşenler, hücre duvarlarının parçalanması ile ekstrakte edilebilir ve asit eklenmesi bu parçalanmayı kolaylaştırır (Chirinos ve ark., 2007). Hidroklorik asit güçlü bir inorganik asit iken asetik asit nispeten zayıf bir organik asittir. Bu nedenle hidroklorik asit eklenen örneklerin sonuçlarının asetik asit içeren örneklerden daha yüksek bulunması beklenen bir sonuçtur. Diğer taraftan güçlü asitlerin bazı fenolik bileşiklerin hidrolizine ve dolayısıyla ekstrakt içindeki konsantrasyonunun azalmasına neden olabilir (Revilla ve ark., 1998; Wrolstad ve ark., 2001). Fakat bu çalışmada seçilen asit oranının Jiang ve ark. (2004) çalışmasında da belirtildiği gibi ekstraksiyon verimini artırırken fenolik bileşenlere zararı en aza indirdiği bilinmektedir. Akhbari ve ark. (2019) tarafından yapılan çalışmada da patlıcan kabuğundan yapılan ekstraksiyonda en yüksek fenolik madde içeriği etanol+hidroklorik asit ile elde edilmiştir. Aynı çalışmada etanolün metanolden asitler arasında ise hidroklorik asidin asetik asitten daha üstün fenolik ekstraksiyonu gücüne sahip olduğu raporlanmıştır. Kopjar ve ark. (2014)'nın vişne üzerine yaptığı incelemelerde de fenolik madde ve buna bağlı antioksidan kapasite üzerine hidroklorik asit ve etanolün üstünlüğü ortaya konulmuştur. Örneklere uygulanan basınçların etkisi incelendiğinde, basınç uygulaması ile fenolik madde içeriğinin arttığı belirlenmiştir (p<0.05).

600 MPa basınç ile fenolik madde içeriği 300 MPa uygulanan örneklere göre daha yüksek bulunsa da su+asetik asit, etanol+asetik asit, etanol+hidroklorik asit, metanol, metanol+hidroklorik asit ile ekstrakte edilen örnekler haricinde sonuçların 300 MPa ile istatistiksel olarak aynı olduğu tespit edilmiştir. Bu ekstraktlarda farklılığın ortaya çıkmasını sağlayan faktör asit eklemesi olabilir. Basınç ile meydana gelen fenolik konsantrasyonundaki artış, basıncın meydana getirdiği mekanik stresin hücre duvarını parçalaması ile hücre içeriğinin dış ortama salınmasının bir sonucudur (Gómez-Maqueo ve ark., 2020). Diğer bir mekanizma ise mekanik stres nedeni ile hücre duvarı bileşenlerinin çapraz bağlanması veya depolimerizasyonu gevşemeye sebep olarak çözülebilir böylece polifenollerin ortamdaki konsantrasyonu artmış olabilir. Hatta 500-600 MPa arasında yüksek basınç uygulamasının hücre duvarına zarar verdiği için fenolik bileşiklerin ekstraksiyonunu kolaylaştırdığı tespit edilmiştir (Navarro-Baez ve ark., 2022). Pinela ve ark. (2018) da ısıtma işlemi olmaksızın 600 MPa'yı fenolik ekstraksiyon veriminin optimum olduğu basınç olarak bulmuştur. Bunun yanı sıra fenolik madde kaybına yol açan polifenol oksidaz aktivitesi, uygulanan basınç büyüklüğü ile azalmaktadır. Bu sayede fenolik konsantrasyonu artmaktadır. Cao ve ark. (2011) de çilek pulpundaki polifenol oksidaz enzim aktivitesinin 600 MPa ile %50'den fazla azaldığını belirlemiştir. Huang ve ark. (2019) da 600 MPa basınç sayesinde toplam fenolik madde miktarını 300 MPa uygulamasına göre %10 daha yüksek bulmuştur. Bunlara ek olarak 0.gün ile 7.gün fenolik madde içeriği arasında korelasyon olduğu tespit edilmiştir (p=0.00; r=0.91) (Tablo 5).

Antioksidan Kapasite

Antioksidan kapasite ile fenolik madde içerikleri ilişkilidir (Cao ve ark., 2011). Bu çalışmada da bu iki değer arasında pozitif yönlü korelasyon saptanmıştır (p=0.00; r=0.94) (Tablo 5). Fenolik madde içeriği gibi antioksidan kapasiteler de çözücülere göre farklılık göstermiştir (Tablo 1). Yine fenolik içerikler sonuçlarına benzer şekilde, basınç uygulanan örneklerin antioksidan kapasiteleri hem ekstraksiyon sonrası (0.gün) hem de depolama sonrasında (7.gün) farklı bulunmuştur (p<0.05). Tablo 1 ve Tablo 2 verileri karşılaştırıldığında da antioksidan kapasitelerin azaldığı ama 0.gün ile 7.gün sonuçları arasındaki korelasyonu kaybetmediği tespit edilmiştir (p=0.00; r=0.86) (Tablo 5). Basınç büyüklüklerinin antioksidan kapasiteye etkisi önemsiz bulunmuştur (p>0.05).

Renk

Renk, fenolik bileşenler ve antioksidan kapasite sonuçlarını destekleyecek kantitatif bir ölçümüdür. Bunlardan L^* değeri renkteki açıklık ve koyuluğu gösteren bir parametre olduğu için ekstrakte edilen örneklerde yüksek L^* değerinin bulunması rengi meydana getiren bileşenlerin daha düşük konsantrasyonda olduğu yönünde bir fikir verebilir. Çünkü böğürtlendeki antosiyaninler meyvenin renginden sorumlu olan ve renklendirici olarak da kullanılan pigmentlerdir. Bu pigmentler pembe, mavi, mor ve kırmızı renklerde olduğu için a^* ve b^* değerini artırırken L^* değerini düşürmektedir (Haminiuk ve ark., 2012).

Tablo 1. Ekstraksiyon sonrası toplam fenolik madde ve antioksidan kapasite değerleri

Table 1. Total phenolic substance and antioxidant capacity values after extraction

Çözücü grupları	Örnekler	TFM (mg GAE g^{-1})	$\bar{X}_{TFM}$	AK (mmol TE 100 g^{-1})	$\bar{X}_{AK}$
Su	Kontrol	243.80±2.48^{t*}	308.09^{D*}	314.26±3.53^{t}	376.67^{D}
	300 MPa	340.74±1.24^{p}		408.53±1.78^{p}	
	600 MPa	339.73±5.52^{p}		407.20±4.93^{p}	
Su+asetik asit	Kontrol	294.34±2.77^{s}	358.82^{D}	363.46±0.74^{s}	427.17^{D}
	300 MPa	386.38±2.13^{n}		453.84±1.79^{n}	
	600 MPa	395.75±2.08^{m}		464.21±0.48^{m}	
Su+hidroklorik asit	Kontrol	328.49±2.10^{r}	377.83^{D}	397.31±1.03^{r}	445.20^{D}
	300 MPa	399.11±1.56^{km}		465.91±2.01^{km}	
	600 MPa	405.92±3.05^{k}		472.39±2.32^{k}	
Etanol	Kontrol	505.93±2.63^{h}	564.81^{C}	573.62±3.59^{h}	632.44^{C}
	300 MPa	593.52±1.54^{g}		661.61±3.06^{g}	
	600 MPa	594.97±2.55^{g}		662.11±2.55^{g}	
Etanol+asetik asit	Kontrol	614.68±3.06^{f}	712.87^{B}	681.52±2.73^{f}	780.50^{B}
	300 MPa	753.14±2.68^{e}		821.18±1.17^{e}	
	600 MPa	770.79±2.07^{d}		838.80±0.72^{d}	
Etanol+hidroklorik asit	Kontrol	854.90±1.48^{c}	953.94^{A}	922.39±1.71^{c}	1018.98^{A}
	300 MPa	997.08±2.49^{b}		1065.93±4.18^{b}	
	600 MPa	1009.84±1.94^{a}		1077.84±0.62^{a}	
Metanol	Kontrol	447.23±1.02^{j}	483.40^{C}	515.68±3.18^{j}	552.17^{C}
	300 MPa	497.53±1.16^{i}		566.02±3.41^{i}	
	600 MPa	505.46±2.65^{h}		574.80±2.87^{h}	
Metanol+asetik asit	Kontrol	615.66±1.68^{f}	718.66^{B}	683.77±0.89^{f}	786.30^{B}
	300 MPa	767.44±1.30^{d}		834.23±1.43^{d}	
	600 MPa	772.87±1.42^{d}		840.91±1.13^{d}	
Metanol+hidroklorik asit	Kontrol	856.24±1.45^{c}	954.51^{A}	922.85±2.28^{c}	1022.13^{A}
	300 MPa	997.08±1.43^{b}		1064.65±0.75^{b}	
	600 MPa	1010.20±2.37^{a}		1078.89±1.15^{a}	

TFM: Toplam fenolik madde, AK: Antioksidan kapasite.

$\bar{X}_{TFM}$: Çözücülere göre toplam fenolik madde içeriğinin aritmetik ortalaması; $\bar{X}_{AK}$: Çözücülere göre antioksidan kapasite değerlerinin aritmetik ortalaması.

*Küçük harfler, tüm örnekler arasındaki istatistiksel farklılığı göstermekte, büyük harfler ise farklı çözücüler kullanılarak elde edilen örneklere ait sonuçlar arasındaki farklılığı ifade etmektedir.

Tablo 2. Depolama sonrası toplam fenolik madde ve antioksidan kapasite değerleri

Table 2. Total phenolic substance and antioxidant capacity values after storage

Çözücü grupları	Örnekler	TFM (mg GAE g^{-1})	$\bar{X}_{TFM}$	AK (mmol TE 100 g^{-1})	$\bar{X}_{AK}$
Su	Kontrol	210.58±1.66^{t*}	275.31^{D*}	281.04±4.74^{r}	343.89^{D}
	300 MPa	307.52±3.47^{p}		375.32±4.04^{m}	
	600 MPa	307.84±5.52^{p}		373.02±4.93^{m}	
Su+asetik asit	Kontrol	262.04±1.88^{s}	326.63^{D}	331.17±1.88^{p}	394.98^{D}
	300 MPa	354.38±2.19^{n}		421.84±1.91^{k}	
	600 MPa	363.47±1.97^{m}		433.94±1.15^{j}	
Su+hidroklorik asit	Kontrol	296.63±2.12^{r}	345.96^{D}	365.42±1.05^{n}	413.33^{D}
	300 MPa	368.11±3.08^{km}		434.93±3.46^{j}	
	600 MPa	373.16±2.15^{k}		439.65±1.99^{j}	
Etanol	Kontrol	475.29±2.83^{h}	533.77^{C}	542.98±3.44^{h}	601.41^{C}
	300 MPa	562.25±2.47^{g}		631.34±4.03^{g}	
	600 MPa	563.78±3.56^{g}		630.91±3.56^{g}	
Etanol+asetik asit	Kontrol	582.39±2.57^{f}	681.09^{B}	649.23±2.29^{f}	748.72^{B}
	300 MPa	722.42±0.82^{e}		790.44±1.10^{e}	
	600 MPa	738.49±2.76^{d}		806.52±1.31^{d}	
Etanol+hidroklorik asit	Kontrol	823.42±2.07^{c}	922.36^{A}	890.91±2.13^{c}	987.76^{A}
	300 MPa	964.81±2.02^{b}		1033.66±4.16^{b}	
	600 MPa	978.87±0.57^{a}		1050.88±1.33^{a}	
Metanol	Kontrol	415.93±1.93^{j}	451.67^{C}	484.38±4.19^{i}	520.44^{C}
	300 MPa	465.53±1.33^{i}		534.02±3.53^{h}	
	600 MPa	473.57±2.65^{h}		542.91±2.87^{h}	
Metanol+asetik asit	Kontrol	584.35±2.61^{f}	687.52^{B}	652.47±1.75^{f}	755.16^{B}
	300 MPa	736.21±1.77^{d}		803.03±1.44^{d}	
	600 MPa	741.98±1.21^{d}		810.02±2.49^{d}	
Metanol+hidroklorik asit	Kontrol	825.08±2.62^{c}	922.48^{A}	891.68±3.33^{c}	993.10^{A}
	300 MPa	964.43±0.97^{b}		1032.02±0.68^{b}	
	600 MPa	977.94±1.87^{a}		1046.63±0.99^{a}	

TFM: Toplam fenolik madde, AK: Antioksidan kapasite.

$\bar{X}_{TFM}$: Çözücülere göre toplam fenolik madde içeriğinin aritmetik ortalaması; $\bar{X}_{AK}$: Çözücülere göre antioksidan kapasite değerlerinin aritmetik ortalaması.

*Küçük harfler, tüm örnekler arasındaki istatistiksel farklılığı göstermekte, büyük harfler ise farklı çözücüler kullanılarak elde edilen örneklere ait sonuçlar arasındaki farklılığı ifade etmektedir.

Tablo 3. Ekstraksiyon sonrası renk değerleri

Table 3. Color values after extraction

Çözücü grupları	Örnekler	L^*	$\overline{X}_{L^*}$	a^*	$\overline{X}_{a^*}$	b^*	$\overline{X}_{b^*}$	ΔE^*	$\overline{X}_{\Delta E^*}$
Su	Kontrol	$70.90\pm0.40^{de*}$	70.09^{C*}	22.08 ± 0.33^{ghijkm}	22.83^{D}	19.27 ± 0.37^{jkmnp}	19.71^{D}	37.99 ± 0.33^{kmn}	39.17^{E}
	300 MPa	68.93 ± 0.43^{efghi}		23.42 ± 0.91^{efghi}		20.26 ± 0.47^{hijkm}		40.53 ± 0.49^{ijk}	
	600 MPa	70.45 ± 0.46^{defg}		22.98 ± 0.90^{fghijk}		19.60 ± 0.46^{ijkmn}		38.98 ± 0.35^{jkm}	
Su+asetik asit	Kontrol	74.49 ± 0.85^{bc}	73.62^{B}	19.73 ± 0.97^{kmn}	20.42^{E}	18.09 ± 0.31^{mnp}	18.49^{E}	33.83 ± 0.64^{prs}	34.99^{F}
	300 MPa	72.44 ± 0.80^{cd}		21.06 ± 0.59^{hijkmn}		19.02 ± 0.32^{jkmnp}		36.34 ± 0.3^{mnp}	
	600 MPa	73.95 ± 0.75^{bc}		20.56 ± 0.48^{ijkmn}		18.36 ± 0.65^{kmnp}		34.81 ± 0.38^{npr}	
Su+hidroklorik asit	Kontrol	78.41 ± 0.78^{a}	78.09^{A}	18.72 ± 0.09^{n}	19.45^{E}	16.93 ± 0.78^{p}	17.41^{E}	30.46 ± 0.89^{s}	31.60^{G}
	300 MPa	76.89 ± 0.89^{ab}		20.05 ± 0.55^{jkmn}		17.97 ± 0.88^{mnp}		32.91 ± 0.91^{prs}	
	600 MPa	78.98 ± 0.56^{a}		19.58 ± 0.53^{mn}		17.32 ± 0.41^{np}		31.44 ± 0.69^{rs}	
Etanol	Kontrol	55.94 ± 0.22^{pr}	55.11^{F}	30.08 ± 0.33^{a}	30.81^{A}	24.99 ± 0.78^{abc}	25.11^{A}	55.18 ± 0.61^{abc}	56.37^{A}
	300 MPa	53.96 ± 0.48^{r}		31.42 ± 0.98^{a}		25.03 ± 0.78^{a}		57.77 ± 0.77^{a}	
	600 MPa	55.43 ± 0.37^{pr}		30.92 ± 0.92^{a}		25.32 ± 0.46^{ab}		56.15 ± 0.63^{ab}	
Etanol+asetik asit	Kontrol	59.84 ± 1.19^{n}	58.63^{E}	29.73 ± 0.97^{ab}	30.45^{A}	24.94 ± 1.00^{abcd}	25.36^{A}	52.36 ± 1.04^{cd}	53.54^{B}
	300 MPa	56.71 ± 1.25^{np}		31.06 ± 0.57^{a}		25.88 ± 1.02^{a}		54.93 ± 0.84^{abc}	
	600 MPa	59.34 ± 1.13^{n}		30.56 ± 0.49^{a}		25.27 ± 1.03^{ab}		53.34 ± 0.7^{bc}	
Etanol+hidroklorik asit	Kontrol	66.46 ± 0.77^{ijkm}	65.61^{D}	28.74 ± 0.09^{abc}	29.45^{A}	22.69 ± 0.51^{cdefg}	23.15^{B}	46.53 ± 0.54^{efg}	47.71^{C}
	300 MPa	64.41 ± 0.83^{m}		30.05 ± 0.52^{a}		23.73 ± 0.66^{abcde}		49.06 ± 0.37^{de}	
	600 MPa	65.95 ± 0.86^{ijkm}		29.55 ± 0.58^{ab}		23.02 ± 0.82^{bcdef}		47.53 ± 0.18^{ef}	
Metanol	Kontrol	67.85 ± 1.33^{ghijk}	66.66^{D}	25.39 ± 1.16^{def}	26.12^{B}	22.36 ± 0.96^{efgh}	22.77^{BC}	43.45 ± 1.74^{ghi}	44.63^{D}
	300 MPa	65.81 ± 1.29^{km}		26.72 ± 1.66^{bcd}		23.32 ± 0.87^{bcdef}		45.99 ± 2^{efg}	
	600 MPa	66.33 ± 1.31^{ijkm}		26.26 ± 1.79^{cde}		22.63 ± 0.65^{defg}		44.44 ± 1.86^{fgh}	
Metanol+asetik asit	Kontrol	67.96 ± 0.47^{fghijk}	66.43^{D}	24.39 ± 1.16^{defg}	25.16^{BC}	21.38 ± 0.97^{fghij}	21.76^{C}	42.27 ± 1.22^{hij}	43.45^{D}
	300 MPa	65.93 ± 0.40^{jkm}		25.79 ± 1.64^{cdef}		22.23 ± 0.95^{efgh}		44.82 ± 1.51^{fgh}	
	600 MPa	65.41 ± 0.41^{hijkm}		25.31 ± 1.23^{defg}		21.67 ± 0.65^{efghi}		43.25 ± 1.41^{ghi}	
Metanol+hidroklorik asit	Kontrol	70.84 ± 1.05^{def}	70.01^{C}	23.21 ± 0.87^{efghij}	23.95^{CD}	20.19 ± 0.89^{hijkm}	20.61^{D}	39.22 ± 1.52^{jkm}	40.39^{E}
	300 MPa	68.83 ± 1.54^{efghij}		24.55 ± 1.49^{defg}		21.16 ± 0.83^{fghi}		41.75 ± 1.76^{hij}	
	600 MPa	70.35 ± 1.35^{defgh}		24.09 ± 1.25^{defgh}		20.49 ± 0.46^{ghijk}		40.21 ± 1.6^{ijk}	

$\overline{X}_{L^*}$: Çözücü karşılaştırması için örneklerin ortalama L^* değeri; $\overline{X}_{a^*}$: Çözücü karşılaştırması için örneklerin ortalama a^* değeri; $\overline{X}_{b^*}$: Çözücü karşılaştırması için örneklerin ortalama b^* değeri; $\overline{X}_{\Delta E^*}$: Çözücü karşılaştırması için örneklerin ortalama ΔE^* değeri.

*Küçük harfler, tüm örnekler arasındaki istatistiksel farklılığı göstermekte, büyük harfler ise farklı çözücüler kullanılarak elde edilen örneklere ait sonuçlar arasındaki farklılığı ifade etmektedir.

Tablo 4. Depolama sonrası renk değerleri

Table 4. Color values after storage

Çözücü grupları	Örnekler	L^*	$\bar{X}_{L^*}$	a^*	$\bar{X}_{a^*}$	b^*	$\bar{X}_{b^*}$	ΔE^*	$\bar{X}_{\Delta E^*}$
Su	Kontrol	61.80±0.47^{def*}	60.87^{C*}	17.02±0.45^{ghij}	17.77^{D}	14.99±0.21^{jkmn}	15.51^{E}	39.81±0.36^{kmn}	41.11^{E}
	300 MPa	59.77±0.49^{fgh}		18.48±0.84^{efgh}		16.16±0.63^{ghijkm}		42.56±0.59^{hijk}	
	600 MPa	61.03±0.81^{efg}		17.81±1.03^{fghi}		15.39±0.83^{ijkmn}		40.95±0.73^{jkm}	
Su+asetik asit	Kontrol	65.33±1.04^{bc}	64.43^{B}	14.58±0.98^{jk}	15.33^{E}	13.78±0.73^{mn}	14.25^{F}	35.43±0.59^{prs}	36.67^{F}
	300 MPa	63.19±0.76^{cde}		15.85±0.15^{hijk}		14.85±0.61^{jkmn}		38.12±0.38^{mnp}	
	600 MPa	64.76±0.74^{bcd}		15.56±0.55^{hijk}		14.11±0.74^{kmn}		36.46±0.29^{npr}	
Su+hidroklorik asit	Kontrol	69.36±0.99^{a}	68.45^{A}	13.54±0.24^{k}	14.29^{E}	12.91±0.65^{n}	13.26^{F}	31.39±0.91^{t}	32.60^{G}
	300 MPa	67.29±0.74^{ab}		14.78±0.96^{ijk}		13.72±1.21^{mn}		33.93±1.38^{rst}	
	600 MPa	68.70±0.75^{a}		14.55±0.58^{jk}		13.14±0.68^{n}		32.49±0.99st	
Etanol	Kontrol	46.83±0.44^{mn}	45.87^{F}	24.93±0.16^{a}	25.61^{A}	20.82±0.55^{abc}	21.28^{A}	57.71±0.55^{abc}	58.95^{A}
	300 MPa	44.61±0.27^{n}		26.15±0.44^{a}		21.87±0.63^{a}		60.42±0.14^{a}	
	600 MPa	46.16±0.62^{mn}		25.74±1.07^{a}		21.14±0.16^{ab}		58.72±1.01^{ab}	
Etanol+asetik asit	Kontrol	50.62±1.49^{k}	49.79^{E}	24.58±0.77^{ab}	25.34^{A}	20.83±1.18^{abc}	21.18^{A}	54.51±1.06^{c}	55.67^{B}
	300 MPa	48.58±0.93^{km}		25.92±0.27^{a}		21.76±0.97^{a}		57.13±0.89^{abc}	
	600 MPa	50.16±1.46^{k}		25.52±0.44^{a}		20.94±0.85^{abc}		55.36±1.02^{bc}	
Etanol+hidroklorik asit	Kontrol	57.32±1.05^{hij}	56.39^{D}	23.43±0.58^{abc}	24.18^{A}	18.58±0.55^{cdefg}	18.93^{B}	47.83±0.89^{def}	49.09^{C}
	300 MPa	55.21±0.65^{j}		24.87±0.83^{a}		19.44±0.67^{abcd}		50.58±0.45^{d}	
	600 MPa	56.64±1.37^{hij}		24.23±1.04^{ab}		18.76±1.15^{bcdef}		48.85±1.05^{de}	
Metanol	Kontrol	58.88±1.30^{fghi}	57.88^{D}	20.28±1.25^{cdef}	21.00^{B}	17.97±0.78^{defgh}	18.55^{BC}	44.85±1.55^{fghi}	46.20^{D}
	300 MPa	56.51±1.59^{ij}		21.65±1.80^{bcd}		19.08±1.39^{bcde}		47.79±2.47^{def}	
	600 MPa	58.26±1.39^{ghij}		21.07±1.47^{cde}		18.59±0.56^{cdefg}		45.95±1.75^{efgh}	
Metanol+asetik asit	Kontrol	58.69±0.22^{fghi}	58.08^{D}	19.35±1.21^{defg}	20.01^{BC}	17.07±0.79^{efghij}	17.57^{CD}	44.19±0.58^{ghij}	45.18^{D}
	300 MPa	57.09±0.47^{hij}		20.49±1.33^{cdef}		18.03±0.78^{defgh}		46.36±0.81^{efg}	
	600 MPa	58.45±0.40^{ghi}		20.18±1.67^{def}		17.62±0.62^{defghi}		44.99±1.11^{fghi}	
Metanol+hidroklorik asit	Kontrol	61.70±1.11^{def}	60.90^{C}	18.11±0.68^{efgh}	18.91^{CD}	16.01±0.78^{hijkm}	16.43^{DE}	40.82±1.32^{jkm}	41.98^{E}
	300 MPa	59.75±1.44^{fgh}		19.59±1.51^{defg}		16.91±0.81^{efghij}		43.39±1.72^{ghij}	
	600 MPa	61.26±1.44^{efg}		19.02±1.38^{defg}		16.38±0.41^{fghijk}		41.73±1.59^{ijk}	

$\bar{X}_{L^*}$: Çözücü karşılaştırması için örneklerin ortalama L^* değeri; $\bar{X}_{a^*}$: Çözücü karşılaştırması için örneklerin ortalama a^* değeri; $\bar{X}_{b^*}$: Çözücü karşılaştırması için örneklerin ortalama b^* değeri; $\bar{X}_{\Delta E^*}$: Çözücü karşılaştırması için örneklerin ortalama ΔE^* değeri.

*Küçük harfler, tüm örnekler arasındaki istatistiksel farklılığı göstermekte, büyük harfler ise farklı çözücüler kullanılarak elde edilen örneklere ait sonuçlar arasındaki farklılığı ifade etmektedir.

Tablo 5. Korelasyon matrisi

Table 5. Correlation matrix

	Korelasyon katsayıları (r)										
	^{1}L	^{1}a	^{1}b	$^{1}\Delta E^*$	^{7}L	^{7}a	^{7}b	$^{7}\Delta E^*$	^{1}TFM	^{1}AK	^{7}TFM	^{7}AK
^{1}L	1											
^{1}a	-.918**	1										
^{1}b	-.949**	.953**	1									
$^{1}\Delta E^*$	-.986**	.969**	.977**	1								
^{7}L	.834**	-.819**	-.845**	-.815**	1							
^{7}a	-.822**	.881**	.852**	.870**	-.923**	1						
^{7}b	-.846**	.843**	.894**	.873**	-.943**	.948**	1					
$^{7}\Delta E^*$	-.872**	.858**	.871**	868**	-.872**	.951**	.946**	1				
^{1}TFM	-.288**	.441**	.359**	.386**	-.301**	.501**	.402**	.403**	1			
^{1}AK	-.310**	.428**	.372**	.429**	-.323**	.522**	.418**	.411**	.938**	1		
^{7}TFM	-.321**	.522**	.447**	.436**	-.344**	.529**	.457**	.409**	.912**	.841**	1	
^{7}AK	-.354**	.537**	.460**	.441**	-.351**	.537**	.461**	.417**	.881**	.864**	.943**	1

**: $p<0.05$ önem düzeyindedir.
TFM: Toplam fenolik madde miktarı, AK: Antioksidan kapasite.
[1]Ekstraksiyon sonrası değerler, [7]Yedi günlük depolama sonrası değerler.

Bulgular çözücüler açısından değerlendirildiğinde; Tablo 3'te belirtildiği gibi, böğürtlen ekstraktları arasında en yüksek L^* değerleri hidroklorik asit eklenmiş su ortamında ekstrakte edilen örneklerde tespit edilmiştir. Bunu asetik asit eklenmiş su ve sadece su ile elde edilen örnekler takip etmiştir. Bunun nedeni diğer çözücüler ile elde edilen renk maddelerine göre su ile eksrakte edilenlerin daha az olması ve güçlü bir asit olan hidroklorik asidin eklenmesi ile rengin daha yüksek parlaklığa ulaşmasıdır. Bu asit ve renk ilişkisi Karaaslan ve ark. (2011)'nın üzüm suyu çalışması ile de desteklenmiştir. Metanol grubunda ise L^* değerleri büyükten küçüğe; metanol+hidroklorik asit, metanol+asetik asit ve metanol ekstraktlarında tespit edilmiştir. Örnekler içinde en düşük L^* değeri ise etanol ile ekstrakte edilen örneklere aittir.

Tablo 3'e göre ekstraktların L^* değerlerindeki değişiklikler a^* değerlerindeki farklılıklar ile örtüşmektedir. Ayrıca fenolik madde içeriğinin a^*, b^*, ΔE^* değerleri ile pozitif, L^* değeri ile negatif korelasyonu olduğu belirlenmiştir (Tablo 5). Buna göre renk bileşenlerinin konsantrasyonunun azalmasına bağlı olarak L^* değerlerinde artış meydana geldiği a^* değerlerindeki düşüş ile desteklenmiştir. Alkol grubunda (metanol veya etanol), asidin a^* ve b^* değerleri üzerine etkisi görülmemiştir ($p>0.05$). Ölçülen diğer renk değerlerine bakıldığında en yüksek a^*, b^* ve ΔE^* değerlerinin etanol grubunda olduğu belirlenmiştir. Bunu metanol ile ekstrakte edilen örnekler takip etmiştir. Kopjar ve ark. (2014) da alkol (metanol) ile ekstrakte edilen vişne posalarının renk yoğunluklarını sudan daha yüksek bulmuştur. Tablo 4'te etanolün a^*, b^* ve ΔE^* değerlerindeki üstünlüğü 7.gün sonuçlarında da gözlemlenmiştir.

Ekstraksiyonda asit kullanımları karşılaştırıldığında ise hidroklorik asit eklenen ortamlardan elde edilen örneklerin L^* değerleri daha yüksek bulunmuştur. Bunun nedeni Jiang ve ark. (2004)'nın da açıkladığı gibi hidroklorik asidin polifenol oksidaz enzimini inhibe etmesine bağlı koyulaşmanın engellenmesi olabilir. Fakat renk farklılığına bakıldığında a^* ve b^* değerlerinin düşük olmasına bağlı en düşük ΔE^* değerleri, hidroklorik asit eklenen çözücülerden elde edilen örneklerde tespit edilmiştir. Depolama sonrasında da en yüksek L^* değeri ile en düşük a^* ve b^* değerlerinin hidroklorik asit ile muamele edilen örneklerde olduğu saptanmıştır (Tablo 4). Cao ve ark. (2011) da çilek pulpunun renk değerlerinde (L^* ve b^*) kontrol ve 600 MPa basınç uygulanan örnekler arasında bir farklılık saptayamamıştır. Bu durum, yüksek polifenol oksidaz kapasitesi ile açıklanmıştır.

Elde edilen tüm bulgular değerlendirildiğinde 7 günlük depolama sonrası en fazla toplam fenolik madde kaybı (%11) su ile ekstrakte edilen örneklerde görülürken en az (%3) kayıp hidroklorik asit eklenmiş alkol ile ekstrakte edilen örneklerde belirlenmiştir. Ekstraktlara uygulanan işlemler açısından en yüksek kayıp oranı %13 ile kontrol örneklerinde gözlenirken bunu %10 ile 300 MPa ve %9 ile 600 MPa büyüklüğünde basınç uygulanan örnekler takip etmiştir. Hidroklorik asit eklenen alkol ile ekstrakte edilen örneklerde de sıralama aynı olup toplam fenolik madde miktarındaki kayıp, kontrol örneklerinde %3.6, 300 MPa basınç uygulanan örneklerde %3.2, 600 MPa işlem uygulanan örneklerde de %3 bulunmuştur. Bu sonuçlar antioksidan kapasite sonuçları ile örtüşmektedir. Antioksidan kapasitede en fazla (%9) düşüş su ile ekstrakte edilen örneklerde gözlemlenirken en az (%3) kayıp hidroklorik asit

eklenmiş alkol ile ekstrakte edilen örneklerde saptanmıştır. Ekstraktlara uygulanan işlemler açısından antioksidan kapasitede azalma en fazla kontrol örneklerinde ölçülmüş bunu sırayla 300 MPa ve 600 MPa basınç uygulanan örnekler takip etmiştir. Benzer sonuçlara Kopjar ve ark. (2014) çalışmasında da vişne için ulaşılmış, depolama sonrasında en az (%5) fenolik madde kaybı alkol ve hidroklorik asit karışımı ile elde edilmiştir. Depolama süresince fenolik madde ve antioksidan kapasitedeki bu değişim pH, sıcaklık farklılıkları ve oksijen varlığından kaynaklanabilir (Chirinos ve ark., 2007). Fakat bu çalışmada depolama süresince örneklerin pH ve sıcaklık değerleri kontrol edildiği için değişimin en büyük nedeni uygulanan işlemler ve depolama sırasında örneklerin oksijen ile teması olabilir. Oksijenin başlattığı oksidasyon, bir zincir reaksiyon olduğu için fenolik maddelerin az olduğu ortamda daha hızlı gerçekleşerek daha fazla düşüşe neden olabilir. Bu sayede su ile ekstrakte edilen ve kontrol örneklerinde alkol ortamında ekstrakte edilen ve basınç uygulanmış örneklere göre daha hızlı oksidasyona bağlı fenolik madde miktarında ve buna bağlı antioksidan kapasitede düşüş gözlemlenmiş olabilir.

Sonuç

Bu çalışmada hidrostatik basıncın, polar çözücüler ve bunların asitlendirilmiş formlarının böğürtlen meyvesinin fenolik madde miktarı, antioksidan kapasite ve renk değerlerinde meydana getirdiği değişim, ekstraksiyon ve depolama sonrasında belirlenmiştir. Böylece uygulanan işlemlerin ürünün fonksiyonel özelliklerinde ve zamana bağlı renk stabilitesinde meydana getirdiği farklılaşma karşılaştırmalı olarak ortaya konulmuştur. Çözücüler açısından en iyi çözücünün alkol grubu olduğu belirlenmiştir. Tüm örneklerde hidroklorik asidin asetik asitten daha fazla fenolik madde ekstrakte ettiği ve antioksidan kapasiteyi artırdığı tespit edilmiştir. Basıncın fenolik madde içeriği ve antioksidan kapasiteyi anlamlı düzeyde yükselttiği belirlenmiştir. Fakat asit eklenen ortamlarda daha yüksek basınçların etkisinin daha belirgin olduğu gözlemlenmiştir. Buna göre böğürtlenin fonksiyonel özelliklerini en çok artıran ekstraksiyon koşullarının 600 MPa basınç ve hidroklorik asit eklenmiş etanol ortamı olduğu söylenebilir. Fenolik madde miktarı yüksek örneklerde ise L^* değerlerinin daha düşük a^* ve b^* değerlerinin ise daha yüksek olduğu bulunmuştur. Ayrıca fenolik madde içerikleri ile antioksidan kapasiteler arasında yüksek korelasyona rastlanmıştır. Bu ilişki depolama aşamasında da korunmuştur. Gelecek çalışmalarda ısıl ve ısıl olmayan ön işlemler beraber kullanılarak süre, sıcaklık, çözücü tipi ve çözücü miktarı gibi ekstraksiyon parametrelerinin optimize edilmesi faydalı olabilir.

Etik Standart ile Uyumluluk

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

Etik izin: Araştırma niteliği bakımından etik izin gerektir-memektedir.

Finansal destek: -

Teşekkür: -

Açıklama: -

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 9(3), 254-261 (2023) • https://doi.org/10.3153/FH23023

Research Article

Generation Z consume animal-free milk? A Türkiye experience

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Cite this article as:
Aydemir, M.E., Okan, Y.T., Takım, K. (2023). Generation Z consume animal-free milk? A Türkiye experience. *Food and Health,* 9(3), 254-261. https://doi.org/10.3153/FH23023

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Submitted: 29.03.2023
Revision requested: 22.05.2023
Last revision received: 29.05.2023
Accepted: 31.05.2023
Published online: 02.07.2023

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Available online at
http://jfhs.scientificwebjournals.com

ABSTRACT

This study aims to examine the perceptions of young people between the ages of 18-22 in Türkiye, studying at universities and representing Generation Z regarding the consumption of animal-free milk and to determine whether there will be a consumer base for animal-free milk in Türkiye in the coming years. For this purpose, 620 students were informed to participate in the research, but data were collected from 215 individuals. A face-to-face interview was conducted with the individual. 32.55% of the participants stated that they could consume animal-free milk. 27 (30%) of 90 female participants and 42 (33.6%) of 125 male participants stated that they could consume animal-free milk. Participants stated that they would not consume animal-free milk because they were concerned that animal-free milk is unhealthy, unnatural, insufficient in terms of nutritional value, unsafe, negatively affecting animal husbandry, and posing a problem in terms of belief. As a result, it has been seen that the Z-generation individuals living in Türkiye have the potential to consume animal-free milk. Suppose the participants' concerns are clarified, and their familiarity with this product is increased. In that case, it is predicted that there will be a more severe increase in the animal-free milk consumption potential future in Türkiye.

Keywords: Animal-free milk, Artificial milk, Generation Z

Introduction

Proteins are the biggest basic building blocks of the human body after water. Proteins, which have many vital functions in the physiology and metabolism of living things, are found in foods of plant and animal origin (Ebcim et al., 2021). Milk proteins are the primary component in animal milk, which have various biological activities such as easy uptake and digestion, high nutrition, immunity enhancement and antioxidation (Zhengfu et al., 2021). In recent years, with the increasing human population and changing consumer trends, there has been an increase in various research and biotechnological studies on alternative protein sources. Today, products such as microbial proteins, alternative plants, insects, algae, fungi, in vitro or artificial meat, animal-free vegan cheese and biofermentation are emphasised and recommended as alternative protein sources (Ünver Alçay et al., 2018).

It is stated that consumer preference for non-animal foods has recently increased due to the increasing interest in health and animal welfare (Short et al., 2021). Especially in conventional milk production, it is reported that there is a trend towards alternative dairy products due to concerns such as animal welfare (treatment of farm animals), environmental impact (pollution, land use and greenhouse gas emissions) and human health (increased antibiotic resistance and exposure to zoonotic diseases) (Clay et al., 2020; Falkeisen et al., 2022). Companies that produce animal-free milk have been established to meet this consumer trend.

Animal-free milk was first produced by California-based company Perfect Day. Later, Legen Dairy Foods and Real Vegan Cheese companies made animal-free milk with the same method (Mendly-Zambo et al., 2021). The animal-free milk production method of these companies is based on fermentation. Perfect Day reported the company's way of producing animal-free milk as follows: Bovine DNA encoding the production of casein and whey proteins (alpha-lactalbumin and beta-lactoglobulin) is inserted into the plasmid DNA of yeast cells. This yeast is added to a mixture of water and plant-based sugar. Here, yeast ferments sugar to produce milk proteins. Then, filtering removes the yeasts from the environment, and the final product is obtained by combining them with certain proportions of plant-derived oils, minerals, sugar and clean water (Pandya, 2014). This product is claimed to have a longer shelf life than regular milk. It will be a safer food because it does not contain hormones, antibiotics and lactose, and artificial milk will be mass-produced and commercialised soon (Perfect Day, 2019).

Since age is one of the main factors affecting people's food choices, it is important to determine the food preferences of age groups (Grasso et al., 2019). Generation Z, which represents a specific age group, refers to people born in the digital age between the late 1990s and early 2000s. In 2020, approximately 30% of the world's population (2 billion) comprised the Z generation (Zuo et al., 2022). It is predicted that Generation Z will play an important role in the economic, political and social change of countries in the future, as well as affect the preferability of food, purchasing ability and consumption of food (Bogueva & Marinova 2020; Su et al., 2019). Generation Z especially prefer foods that make a good impression, reliable and transparent food(s) sources, branded products that meet expected standards, and foods that are globally trending on social media (Szakály et al., 2018). Therefore, it is important to determine the food preference of the Z generation and to develop products according to the needs of this generation.

This study aims to examine the perceptions of young people between the ages of 18-22 in Türkiye, studying at universities and representing Generation Z regarding the consumption of animal-free milk and to determine whether there will be a consumer base for animal-free milk in Türkiye in the coming years.

Materials and Methods

Working Group

The study group of this research consists of individuals aged 18-22 and representing the Z generation, studying at Harran University in the autumn semester of the 2021-2022 academic year. Participants were briefed about animal-free milk before the questions were asked. First, the definition of animal-free milk is made, and then comprehensive information about the production technology of animal-free milk is given. Within the scope of the study, 620 students were reached, but some did not accept to participate since the study's data collection was based on volunteerism. Due to this limitation, the data of 215 participants, 90 (41.86%) women and 125 (58.13%) men, who voluntarily participated in the study, were analysed.

Research Pattern

This research is a survey study in which different question patterns are used. Our research consists of open-ended and closed-ended survey questions. Quantitative descriptive statistics were used for closed-ended questions, and qualitative

descriptive analysis was used for open-ended questions. Büyüköztürk & Demirel (2018) stated that open-ended survey studies are included in qualitative patterns.

Data Collection Tool

Firstly, permission was obtained from the participants who answered our questionnaire for the study, and the interviews were recorded. They preferred a discussion in this way aimed to be prepared for the interview in line with the study plan and to provide a sincere and natural response to the questions about the study in a natural environment (Karasar, 2008). Since the age criterion we determined for our research is important, it was chosen from young people between 18-22. First of all, questions about demographic information were prepared for the participants within the scope of the study, and two closed-ended questions were asked "yes" and "no" about whether they would consume animal-free milk or not within the scope of the study. Following this, open-ended questions were asked to reveal the reasons according to the answers given by the participants to the closed-ended questions. The researchers prepared the questions.

Data Analysis

After the interviews were completed, the data analysis process was started. First, the data were adapted to the SPSS 24 package program to analyse the answers to the closed-ended questions. Then, a percentage frequency analysis was performed. Audio recordings were converted into text for the study of open-ended questions. Afterwards, these interviews were transferred to the Maxqda 20 Package Program, and the analysis steps were started. Out of 215 participants, 114 participants made detailed explanations about whether they would consume animal-free milk, and the data of these participants were included in the qualitative analysis. The obtained data were first read once to ensure the data's intelligibility. Then, coding was done with the second reading. After coding, themes and sub-themes were created by combining the relevant codes.

Results and Discussion

Data were collected from 215 individuals, 90 (41.86%) female and 125 (58.13%) male participants. All of the participants are 18-22 age group university students. With the analysis of the data obtained from the participants, it is understood that the statements of the participants are shaped around two main themes. The first theme's reasons for preferring animal-free milk, and the second theme's reasons for not consuming animal-free milk.

Animal-Free Milk Consumption Preferences

The question of whether they would consume animal-free milk was asked to 215 individuals participating in the study, along with its justification. While 70 (32.55%) people who participated in the research stated that they could consume animal-free milk, 145 (67.45%) people said that they would not consume animal-free milk. 27 (30%) female participants and 42 (33.6%) male participants stated that they could consume animal-free milk (Figure 1). The findings regarding the reasons for both consumption and non-consumption of these people are presented below.

Reasons for Consuming Animal-Free Milk

This section presents the sub-themes of the emerging theme regarding the reasons for consuming animal-free milk. When Figure 2 is examined, it is seen that the participants mentioned some themes related to consuming animal-free milk. A few statements related to these themes are shown below.

- If it meets my nutritional needs, I consume it (Participant(P)42)
- I consume to prevent global warming caused by cows (P84)
- I would wonder and consume. If it is proven to be healthy, I will consume it constantly (P103)
- I consume to protect animals (P114)
- I consume if the nutritional values are the same to protect the environment (P 154)
- I will consume if the price is appropriate (P177)
- I consume for economic and ecological reasons (P198)

The question we posed to the participants regarding animal-free milk ranked first in the statements the participants gave: "I would consume animal-free milk if it is proven to be healthy", 16.8% (24 individuals). In addition, when we look at the other reasons for consumption respectively, they stated that they would consume it if it meets my daily nutritional needs 9.8% (14 individuals), out of curiosity 8.4% (12 individuals), to protect the environment 7% (10 individuals), if the price is affordable 4.9% (7 individuals), to protect animals 4.2% (6 individuals), if I have to 2.8% (4 individuals).

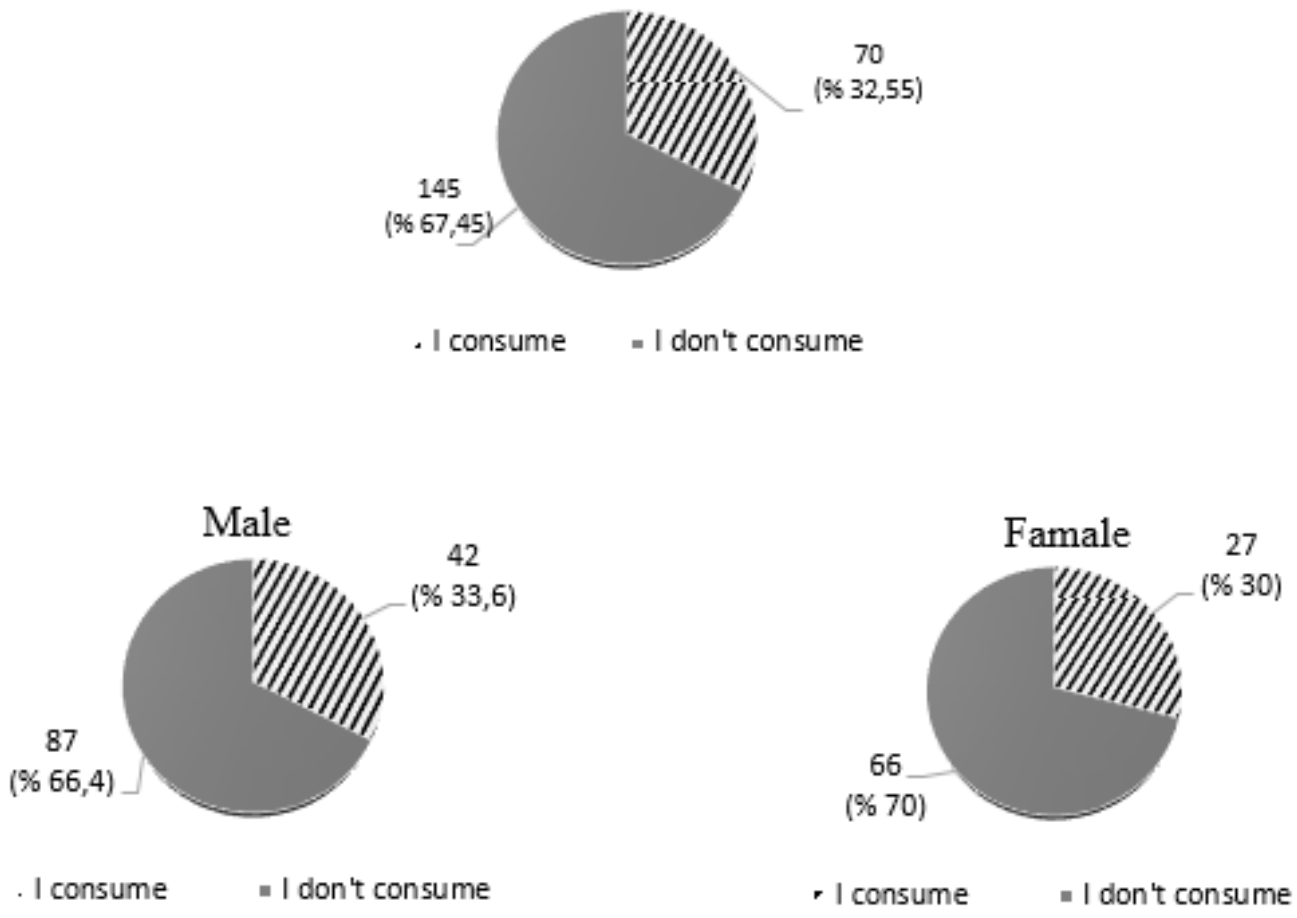

Figure 1. Animal-free milk consumption preference rates of the participants

Reasons for Not Consuming Animal-Free Milk

This section presents the sub-themes of the emerging theme regarding the reasons for not consuming animal-free milk. Figure 3 shows the sub-themes obtained in the research based on the question asked to the participants regarding the reasons for not consuming animal-free milk. Some statements regarding participant attitudes are presented below:

- Because I think it is harmful to health (P16)
- I think its nutritional value will be very low (P53)
- I prefer natural foods (P128)
- I think it is harmful to health. I am against its consumption as it will adversely affect animal husbandry (P181)
- I do not think it is halal (P184)
- I do not trust and consume because it is not natural, and I do not have enough knowledge about its production (P187)
- My concern is that it will harm my body in the future (P223)

In the question we asked the participants regarding the reasons for not consuming animal-free milk, it is seen that the participants stated that they do not prefer to consume animal-free milk, with the highest answer because it is unhealthy 85.5% (59 individuals) and may have side effects related to this theme 14.5% (10 individuals). Considering the statements they gave respectively, they stated that they would not consume animal-free milk for reasons such as it is not natural 76.8% (53 individuals), it does not have sufficient nutritional value 24.6% (17 individuals), in order not to negatively affect animal husbandry 8.7% (6 individuals), it may contain negative additives 7.2% (5 individuals), it is not halal 4.3% (3 individuals), it may harm the ecological environment 2,9% (2 individuals) and not safe 2.9% (2 individuals).

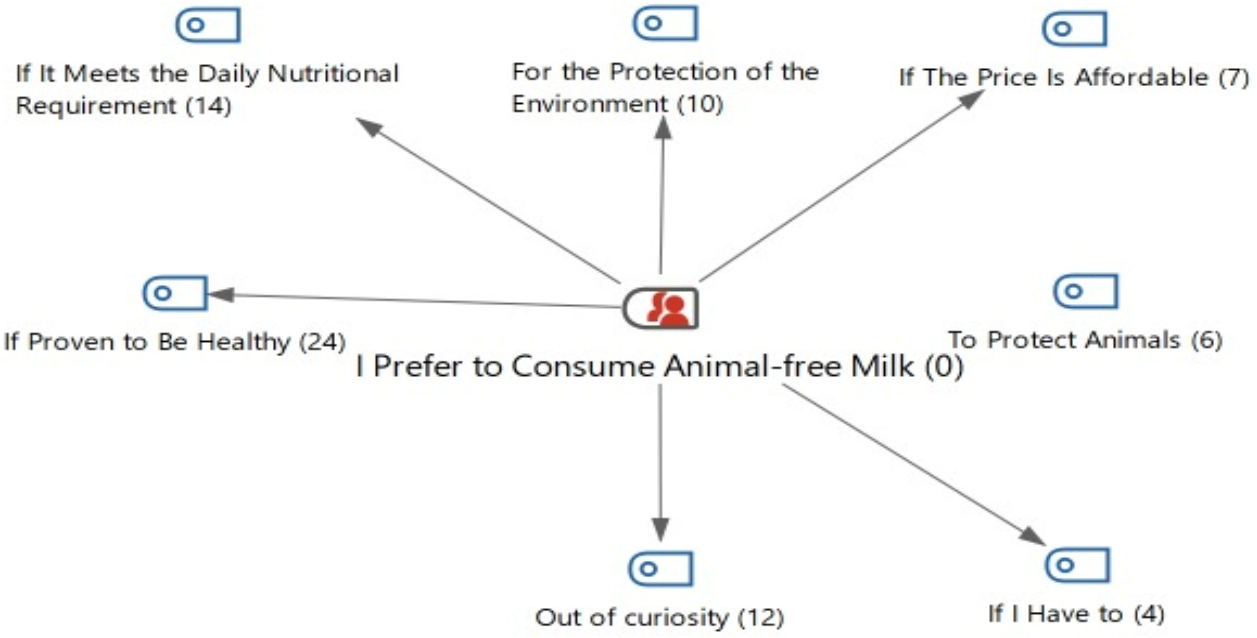

Figure 2. Sub-themes of the emerging theme about the reasons for consuming animal-free milk

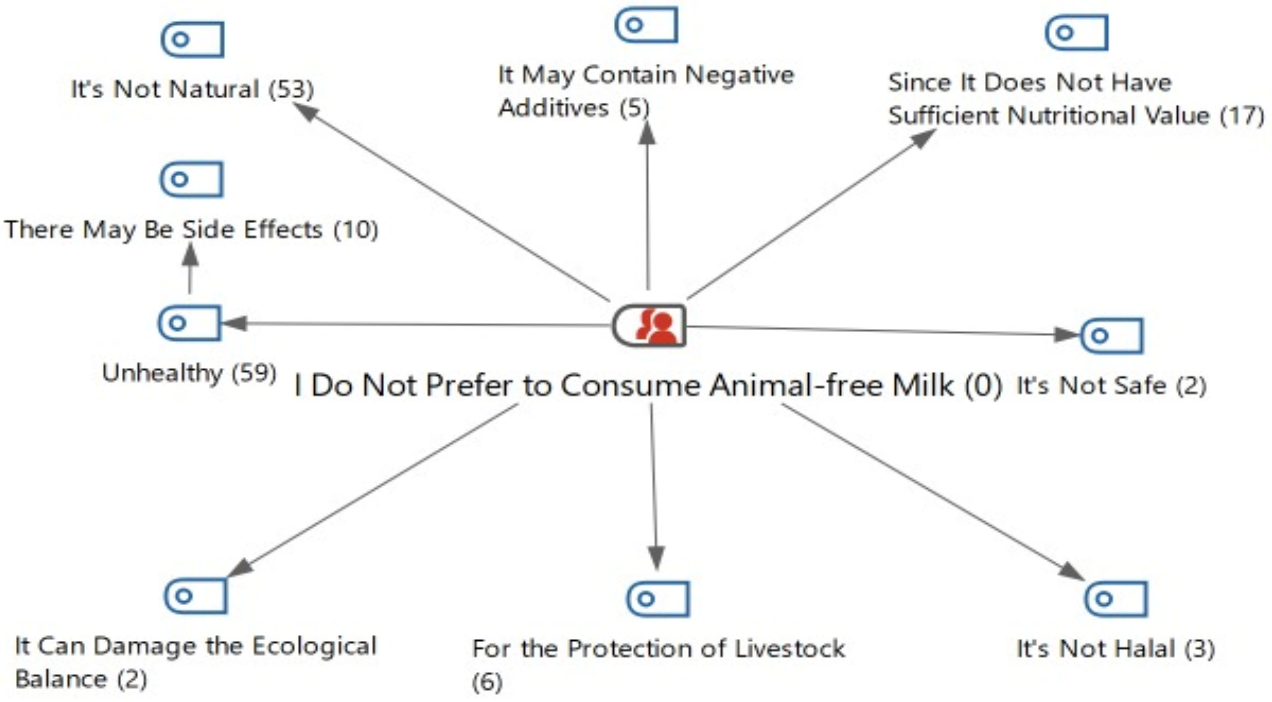

Figure 3. Sub-themes of the emerging theme about the reasons for not consuming animal-free milk

This research contains the first information about consumer acceptance of the al-free milk product, produced by the ferthe mentation method in Türkiye. Data on animal-free consumer acceptance in other countries are also limited. According to the results of this study, 70 (32.55%) of the 215 individuals who participated in the survey stated that they could consume animal-free milk, wh. In comparison, (67.45%) stated that they would not consume animal-free milk. Similar to our work, Zollman Thomas & Bryant (2021) surveyed 5,054 individuals from Brazil, Germany, India, the United Kingdom, and the United States to determine the acceptability of dairy products derived from fermentation. They stated that 78.8% of the participants would try such a product, and 70.5% would buy it. They reported that Brazil has the highest percentage of consumers willing to consume and buy, while the USA has the lowest. In a study conducted by The Grocer in the United Kingdom in 2018 that investigated the primary consumer responses to animal-free milk, 28% of the participants stated that they were willing to buy animal-free milk, while 40% of the participants reported that they completely refused to buy non-animal milk (Grocer, 2018). The variation in consumption preferences according to the results of the studies can be explained by the way the questionnaire questions are expressed, the age or maturity of the participants and the variation in the number of participants. In addition, consumer acceptance of animal-free milk in Türkiye is lower than in countries such as Brazil, Germany, India and the USA is thought to be due to the lack of familiarity with the right to animal-free milk technology. It is stated that familiarity with food technologies strongly indicates the desire to consume foods produced in new ways (Bryant & Barnett 2018). Therefore, as the knowledge of animal-free milk production technology increases in Türkiye, it is predicted that the desire to consume will probably increase in parallel.

Age and gender are major factors affecting food choices (Grasso et al., 2019). Studies show younger participants are more willing to consume animal-free milk than older participants. In the survey conducted by Zollman Thomas & Bryant (2021), it was reported that young people in countries in Brazil, Germany, India, the United Kingdom and the USA were more inclined to consume animal-free milk. A survey by The Grocer 2018 observed that young consumers are more open to incoming animal-free milk than older consumers (Grocer, 2018). While only 19% of people over 55 agree to consume animal-free milk, 44% of people between 18 and 24 reported that they approve of its consumption. In our study, the acceptance of animal-free milk consumption varied depending on gender. 30% of female participants and 33.6% of male participants stated that they could consume animal-free milcouldIt is seen that men are more inclined to consume animal-free milk.

One of the most critical concerns about animal-free milk consumption is the concern that animal-free milk is not natural, safe and healthy (Broad et al., 2022). In a survey conducted by Grocer in 2018, it was reported that 50% of the participants were worried that animal-free milk is not natural, 43% were concerned that it would have side effects in the future, and 37% feared it might contain dangerous chemicals. The current study shows that 85.5% of the participants who do not prefer animal-free milk consumption are concerned about being unhealthy, 76.8% being unnatural, and 14.5% containing additives. Interestingly, it is seen that 16.8% of the participants who said they would consume animal-free milk answered that it is proven to be healthy. Therefore, the findings of the study are compatible with the literature. Unsurprisingly, the participants did not find this new product, animal-free milk, healthy and natural. The fact that it is a new product produced with biotechnological methods worries people. Since this product is not widely consumed for a long time, its side effects are also known. So these reasons raise concerns. In addition, people are more inclined to consume organic and natural products. There is a belief that if a product is organic, it is healthier (Carfora et al., 2019). Therefore, it is unsurprising that a product produced in the laboratory using biotechnological methods is suspicious.

The strongest arguments for choosing animal-free milk include animal welfare, the adverse effects of animals on the environment, climate change concerns and the potential health risks of animal foods (Broad et al., 2022). The results of the present study also support this opinion. Of the participants who said they would consume animal-free milk, 4.2% stated that they would consume animal-free milk to protect animals and 7% to protect the environment. On the contrary, it is seen that 2.9% of the participants who say that they do not consume animal-free milk think that animal-free milk harms the ecological environment. In addition, it is seen that 8.7% of the participants who say that they do not consume animal-free milk answer not to consume in order not to affect animal husbandry adversely. However, it is seen that the rates are low in both groups. The reason for this is thought to be due to the indifference of the participants to environmental protection. It has been determined that people who believe that it will harm animal husbandry have received training on animal husbandry. Therefore, it was concluded that individuals interested in agriculture and animal husbandry are against animal-free milk.

According to the results of the study, it is seen that 24.6% of the participants who say they do not consume animal-free milk think that they do not consume animal-free milk because it does not have sufficient nutritional value. It is seen that 9.8% of the participants who say they consume animal-free milk think they will consume it if it meets their daily dietary needs. Therefore, it is believed that animal-free milk should be carefully controlled and documented in terms of nutritional value, and its content should be shared with the public.

According to the results of the study, it was seen that the price of animal-free milk was among the reasons for the preference of the participants. It is seen that 4.9% of the participants who say they will consume animal-free milk answer that if the price is appropriate, they will finish it. It is thought that the fact that the participants are young and students who are just starting to be economically independent affects their orientation to alternative and cheap products.

It is reported that young individuals in the Z-generation prefer globally trending foods on social media (Su et al., 2019). In the study, it is seen that 8.4% of the participants who said they would consume animal-free milk answered that they would consume animal-free milk because I was curious. Animal-free milk is thought to be a new product and is on social media's agenda, arousing the participants' curiosity.

Biotech applications such as animal-free milk raise numerous ethical, philosophical, and religious questions. Because of the uncertainties in cellular biotechnological applications, religious authorities are still discussing and sceptical of these issues (Loike, 2018). In the current study, 4.3% of the participants who say that they do not consume animal-free milk, even if it is low, state that they will not consume it because they think it is not halal.

Conclusion

As a result, it has been seen that the Z-generation individuals living in Türkiye have the potential to consume animal-free milk. We think the low consumption rate compared to the rates reported in other countries is because the participants are worried about many points and unfamiliar with animal-free milk production technology. The participants' main concern regarding the consumption of animal-free milk is that they do not find it healthy and cannot be a natural product. Therefore, efforts should be made to eliminate consumers' concerns about animal-free milk and inform consumers about this product before commercially putting animal-free milk on the market in Türkiye. Suppose the participants' concerns are clarified, and their familiarity with this product is increased. In that case, we foresee that there will be a more severe increase in the animal-free milk consumption potential of the Z generation, which will represent the Turkish population in the future.

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: Ethics committee certificate numbered 02.01.2023-194043 was obtained from Harran University Social and Human Sciences Ethics Committee for this study.

Funding disclosure: -

Acknowledgments: -

Disclosure: -

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 9(3), 262-281 (2023) • https://doi.org/10.3153/FH23024 Review Article

Bitkisel yağlarda mikotoksin varlığı

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Cite this article as:
Can, N., Duraklı Velioğlu, S. (2023). Bitkisel yağlarda mikotoksin varlığı. *Food and Health,* 9(3), 262-281. https://doi.org/10.3153/FH23024

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Submitted: 15.03.2023
Revision requested: 13.04.2023
Last revision received: 27.04.2023
Accepted: 28.04.2023
Published online: 04.07.2023

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Available online at
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ÖZ

Bitkisel yağlar, yağlı tohum ve meyvelerden elde edilen yağlardır. Gıda, ilaç ve kimya sanayinde yaygın olarak kullanılan bitkisel yağlar, son yıllarda tüketiciler tarafından daha fazla tercih edilir hale gelmiştir. Bunun en önemli sebebi yüksek enerji verme, esansiyel yağ asidi kaynağı olma, yağda çözünen vitaminleri taşıma, antioksidan aktivite gösteren bileşikleri içerme gibi faktörlere bağlı olarak sağlık ile ilişkilendirilmeleridir. Öte yandan bitkisel yağlar çeşitli yollarla bazı kontaminantlar ile bulaşabilmektedir. Bitkisel yağlar, uygun olmayan koşullara maruz kalan ham maddeler yoluyla mikotoksinlerle kontamine olabilir. Bu çalışmada bitkisel yağlarda mikotoksinlerin varlığına dair yapılmış çalışmalar incelenmiş olup, bitkisel yağların aflatoksinler, fumonisin, okratoksin, deoksinivalenol, zearalenon gibi mikotoksinler ile değişik seviyelerde kontamine olabildiği görülmüştür. Buradan hareketle bitkisel yağlarda kontaminasyonun önlenmesi için tedbirlerin alınması, özellikle hammaddelerde oluşabilecek küf gelişimi ve mikotoksin oluşumunun önüne geçilmesi gerektiği sonucuna varılmıştır.

Anahtar Kelimeler: Bitkisel yağ, Soğuk pres yağ, Mikotoksin, Toksijenik küf, Mikrobiyal kalite

ABSTRACT

Mycotoxins in vegetable oils

Vegetable oils are obtained from oil seeds and fruits and are widely used in the food, pharmaceutical, and chemical industries. In recent years, consumers' interest in vegetable oils has increased. The most important reason is that they are associated with health due to factors such as providing high energy, being a source of essential fatty acids, carrying fat-soluble vitamins, and containing compounds with antioxidant activity. On the other hand, they may contain some contaminants, such as mycotoxins. Vegetable oils can be contaminated with mycotoxins through raw materials exposed to unsuitable conditions. In this review, studies on the presence of mycotoxins in vegetable oils were examined. As a result, it has been observed that vegetable oils can be contaminated with mycotoxins such as aflatoxins, fumonisin, ochratoxin, deoxynivalenol, and zearalenone at different levels. It was concluded that contamination should be prevented and measures should be taken against mould growth and mycotoxin formation in raw materials.

Keywords: Vegetable oil, Cold press oil, Mycotoxin, Toxigenic moulds, Microbial quality

Giriş

Yağlar, üç değerlikli bir alkol olan gliserin ($C_3H_5(OH)_3$) ile yağ asitlerinin (R-COOH) esterleşmesinden meydana gelmekte olup, yapılarına göre katı yağlar, yarı katı yağlar ve sıvı yağlar; fonksiyonlarına göre organ yağları ve depo yağları; bulundukları yere göre ise bitkisel ve hayvansal yağlar olarak sınıflandırılmaktadır (Demirci, 2012). Türk Gıda Kodeksi Bitki Adı ile Anılan Yağlar Tebliği'nde bazı yağların tanımları yer almaktadır. Buna göre sadece bitkisel kaynaklardan elde edilen ve temel olarak yağ asitleri gliseridlerinden oluşan yağ, bitkisel yağ adını almaktadır. Çözücü ekstraksiyonu ve/veya mekanik yöntemle elde edilen ve doğrudan tüketime uygun olmayıp, rafinasyon veya teknik amaçlı kullanıma uygun olan yağ, ham yağ olarak tanımlanmaktadır. Rafine yağ, doğal trigliserid yapısında değişikliğe yol açmadan rafine edilen yağ olarak ifade edilmektedir. Mekanik yöntemle ve ısı uygulaması ile elde edilen ve sadece su ile yıkama, çöktürme, filtrasyon ve santrifüj işlemleri ile saflaştırılmasına izin verilen yağ, natürel yağ olarak adlandırılmaktadır. Soğuk preslenmiş natürel yağ ifadesi ise doğrudan tüketime uygun olan, ısıl işlem olmaksızın sadece mekanik yöntemle elde edilen yağı tanımlamaktadır (Anonim, 2012).

Gıda ve Tarım Örgütü ve Dünya Sağlık Örgütü tarafından paylaşılan ve bilimsel araştırma sonuçlarına dayanan bir raporda, yağlardan sağlanan kalorinin diyetteki tüm kalorinin %15-30'unu oluşturması ve bunun büyük bir bölümünün bitkisel sıvı yağlardan karşılanması tavsiye edilmektedir (Taşan ve Geçgel, 2007). Esansiyel yağ asidi kaynağı olmaları, yağda çözünen vitaminleri taşımaları, antioksidan etkileri gibi faktörler, tüketicilerin bitkisel sıvı yağ tüketimine olan ilgisinin artmasına yol açmıştır (İmer ve Taşan, 2018). Bununla birlikte, tüm gıdalarda olduğu gibi bitkisel yağların da bazı kontaminantlara maruz kalması, gıda güvenliği yönünden başta tüketiciler olmak üzere, resmi makamlar ve bilim insanları için bir endişe kaynağıdır. Kontaminantlar, gıdaya kasıtlı olarak eklenmemiş fakat yetiştirme, işleme, üretim, hazırlama, taşıma, paketleme ve depolama işlemleri sırasında ortaya çıkabilecek maddelerdir. Bunlar, örneğin çevresel kimyasallar gibi dış kaynaklı olabileceği gibi, üretim sırasında yağ asitlerinin ya da diğer bileşenlerin bozulmasıyla oluşan ürünler gibi içsel kaynaklı da olabilmektedir (Matthäus ve ark., 2016).

Yağ hammaddelerinin de aralarında yer aldığı tarımsal ürünlerin, küfler ile kontamine olabildikleri bilinmektedir (Tabuc ve ark., 2009). Bu durum bitkisel yağlarda potansiyel mikotoksin tehlikesine işaret etmektedir (Bhat ve Reddy, 2017). Mikotoksinler, tahıllar, meyveler, kuru yemişler, baharatlar, bitki çayları ve yağlı tohumlar gibi ürünlerde gelişen küfler tarafından üretilen sekonder metabolitlerdir (D'Mello ve MacDonald, 1997; Wilson ve ark., 2002; Can ve Duraklı Velioğlu, 2017). Sağlık üzerindeki olumsuz etkileri yapılan çalışmalarla gösterilmiş olan mikotoksinlerin gıdalarla birlikte alınmasının karaciğer ve sindirim sistemi ile ilgili hastalıklara ve kansere yol açabildiği ifade edilmektedir (Fung ve ark., 2004).

Bitkisel yağlarda görülebilen başlıca kimyasal kontaminantlar arasında mikotoksinlerin yer aldığını gösteren çeşitli çalışmalar mevcuttur (Finoli ve ark., 2005; Bao ve Trucksess, 2010; Qian ve ark., 2015; Ma ve ark., 2016). Bitkisel yağlara uygulanan rafinasyon işlemi mikotoksinlerin uzaklaştırılmasında kısmen etkili olmakla birlikte, soğuk pres yağlar gibi bazı bitkisel yağlara ısıl işlem uygulanmamaktadır (Brühl, 1996). Bunun sonucunda bitkisel yağların tüketilmesiyle birlikte mikotoksinlerin vücuda alınması ve zararlı etkilerine maruz kalınması tehlikesi ortaya çıkmaktadır.

Bu çalışmada, bazı yağ hammaddelerinde ve bitkisel yağlarda küflerin ve mikotoksinlerin varlığını tespit etmeye yönelik dünyada ve ülkemizde yapılmış çalışmalar derlenmiştir.

Bitkisel Yağ Hammaddelerinde Toksijenik Küfler ve Mikotoksinler

Gıdaların ve tarımsal ürünlerin doğal mikroflorasını oluşturabildiği gibi, bu ürünlere sonradan da bulaşabilen mikroorganizmalar arasında küfler, önemli bir yer tutmaktadır. Ürünlerde gelişerek bozulmalara, istenmeyen değişikliklere yol açabilmesinin yanı sıra küfler uygun şartlarda toksik metabolitler üretebilmektedir (Elden Taydaş ve Aşkın, 1995; Can ve Duraklı Velioğlu, 2018).

Yağlı tohumlar ve meyveler, hasat öncesini ve sonrasını kapsayan süreçte toksin üreten çok sayıda küf ile kontaminasyona açıktır (Abdolmaleki ve ark., 2021). Yağ bitkilerinde yaygın florayı, aralarında toksijenik türlerin de bulunduğu *Aspergillus*, *Fusarium* ve *Penicillium* oluşturmaktadır (Şahin ve ark., 1999; Demir ve ark., 2002; Demirci, 2008). Bu durum yağ bitkilerinin mikotoksinler ile kontamine olması ve mikotoksinlerin yağa taşınması riskini doğurmaktadır (Markaki, 2010). Nitekim fındık, yer fıstığı, ceviz, badem, ayçiçeği tohumu, keten tohumu, aspir, pamuk tohumu, susam ve kolza tohumları gibi yağ hammaddelerinin mikotoksinlerle bulaşabildiği, özellikle aflatoksinler açısından riskli oldukları bildirilmektedir (Öksüztepe ve Erkan, 2016; Bhat ve Reddy, 2017).

Yer fıstığı muhtemelen küf, mikotoksin ve özellikle de aflatoksin kontaminasyonu yönünden en yaygın çalışılan yağlı tohum olup, yer fıstığının aflatoksinlerin yanı sıra fumonisin,

okratoksin, zearalenon ve siklopiozonik asit ile kontamine olduğunu gösteren çalışmalar mevcuttur (Lansden ve Davidson, 1983; Sangare-Tigori ve ark., 2006). Yer fıstığında mikotoksin kontaminasyonu, topraktan bulaşan küfler yoluyla yetiştirme aşamasında ya da hasat sonrası depolama sırasında gerçekleşebilmekle birlikte hasat öncesi kontaminasyon daha büyük bir tehdittir. Kurutulmuş-kavrulmuş yer fıstıklarının da önemli düzeyde aflatoksin içerdiği bilinmektedir (Bhat ve Reddy, 2017). Aflatoksin üreticisi *Aspergillus flavus*, *A. niger*, *A. parasiticus*, ve *A. nominus* yer fıstığında karşılaşılan en yaygın küfler arasındadır (Li ve ark., 2009).

Ayçiçeği tohumlarının, sitrinin, aflatoksin, alternariol, alternariol monometil eter ve tenuazonik asit gibi mikotoksinleri üretebilen *Fusarium verticillioides*, *Penicillium chrysogenum*, *Alternaria alternata*, *Aspergillus* spp., *Cladosporium* spp., *Drechslera* spp. ve *Curvularia* gibi küfleri barındırabildiği Pozzi ve ark. (2005) tarafından ortaya konulmuştur. Nahar ve ark. (2005), aralarında toksijenik türlerin yer aldığı *Aspergillus ochraceus*, *Cladosporium cladosporioides*, *Emericella nidulans*, *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *A. terreus*, *Fusarium pallidoroseum*, *F. solani*, *Penicillium* spp. ve *Rhizopus stolonifer* gibi çok sayıda küfün ayçiçeği tohumunda bulunduğunu bildirmektedir. Banu ve Muthumary (2005), ayçiçeği tohumunda, yağlı kek ve yağsız kekinde, çözücü ile ekstrakte edilmiş yağında ve rafine yağında küf oluşumunu incelemiştir. En baskın küflerin tohumda *A. flavus*, yağlı ve yağsız kekte *R. stolonifer*, çözücü ile ekstrakte edilmiş yağında *A. flavus* ve *A. japonicus* olduğu belirlenirken, rafine yağında küf kontaminasyonu bulunmamış, bunun sebebinin rafinasyon sürecinde uygulanan yüksek sıcaklık olabileceği belirtilmiştir. Diğer taraftan toksijenik küfler ile bulaşmasının bir sonucu olarak ayçiçeği tohumlarının alternariol ve alternariol monometil eter gibi mikotoksinleri içerdiği gösterilmiştir (Pozzi ve ark., 2005). Aflatoksin, ayçiçeği tohumlarında tespit edilen bir diğer mikotoksin çeşididir. Mohammed ve ark. (2018), toplam aflatoksin (aflatoksin B_1 [AFB_1] + aflatoksin B_2 [AFB_2] + aflatoksin G_1 [AFG_1] + aflatoksin G_2 [AFG_2]) düzeyinin 243 µg/kg ve AFB_1 düzeyinin 218 µg/kg'a ulaştığını belirledikleri ayçiçeği tohumu örneklerinin bir kısmının AFB_1 seviyesinin Avrupa Komisyonu/Avrupa Birliği tarafından yağlı tohumlarda bulunmasına izin verilen maksimum limiti (2 µg/kg) aştığını bildirmiştir.

Mısır, toksijenik küf ve mikotoksin kontaminasyonu yönünden yaygın şekilde incelenen yağ hammaddelerinden biridir. Mısırın, *A. flavus*, *F. verticillioides* (syn. *Fusarium moniliforme*) ve *Fusarium proliferatum* gibi çesitli toksijenik küfler ile bulaşabildiği bilinmektedir (Demirci, 2008). Adejumo ve ark. (2007) tarafından yapılan bir çalışmada mısırdan *F. verticillioides* (%71), *F. sporotrichioides* (%64), *F. graminearum* (%32), *F. pallidoroseum* (%15), *F. compactum* (%12), *F. equiseti* (%9), *F. acuminatum* (%8), *F. subglutinans* (%4) ve *F. oxysporum* (%1) izole edilmiştir. 180 mısır örneğinin incelendiği bu çalışmada 66 örnekte trikotesen, deoksinivalenol (DON), 3, monoasetildeoksinivalenol ve diacetoxyscirpenol saptanmıştır. Çeşitli araştırmacılar tarafından mısırın yüksek düzeyde DON (9.6-745.1 µg/kg), zearalenon (ZEA) (0-230.8 µg/kg), moniliformin (21-699 µg/kg), fumonisin (26-774 µg/kg), aflatoksin (0-126.5 µg/kg) içerebildiği ortaya konmuştur (Abbas ve ark., 2002; Adejumo ve ark., 2007; Broggi ve ark., 2007; Trung ve ark., 2008).

Yağ hammaddelerinden biri olan aspir tohumları, *Alternaria carthami*, *Fusarium* spp., *A. flavus*, ve *Penicillium* spp. gibi toksijenik küfleri barındırabilmekte ve DON, vomitoksin; T-2 toksin ve trikotesen ile kontamine olabilmektedir (Miller ve ark., 2001). Kolza tohumunun *Aspergillus niger*, *A. clavatus*, *A. vesicolor*, *Fusarium oxysporum*, *F. avenaceum*, *Penicillium expansum*, *P. palitans*, *P. roquefortii*, *P. viridicatum*, *Alternaria alternata* ve *Rhizo-mucor pusillus* gibi türleri içerebildiği, ayrıca *Cladosporium*, *Alternaria*, *Penicillium* ve *Fusarium*'un en baskın küfler olduğu, ilaveten DON (164-183 µg/kg) ve aflatoksin ile kontamine olabildiği bildirilmektedir (Kačergius ve ark. 2005; Brazauskiené ve ark., 2006).

Aspergillus niger, *A. flavus, A. ochraceus*, *A. tamarii*, *Penicillium citrinum*, *Fusarium* spp. ve *Alternaria* türleri susamda bulunabilmektedir (Bhat ve Reddy, 2017). *A. flavus* ile inoküle edilen susam tohumunun 20 günlük inkübasyon sonucu 25 µg/kg aflatoksin B_1 içerdiği gösterilmiştir (Mbah ve Akueshi, 2009). Susamın jüt, polipropilen ve hermetik çuvallarda depolanmasının fungal gelişim ve mikotoksin oluşumu üzerine etkisinin incelendiği bir çalışmada 6 aylık depolamanın ardından susam tohumlarının *Aspergillus*, *Penicillium* ve *Fusarium* türleri ile kontamine olduğu ve kontamine olmuş susam tohumları oranının jüt çuvallarda daha yüksek olduğu tespit edilmiştir. Benzer şekilde tohumların aflatoksin, fumonisin, DON ve okratoksin A (OTA) içerdiği belirlenmiş ve jüt çuvallarda depolanan tohumlarda mikotoksin miktarı daha yüksek bulunmuştur (Alemayehu ve ark., 2023). Pirinç tohumu ve kepeği de toksijenik küfler ve aflatoksinleri içerebilmektedir (Jayaraman ve Kalyanasundaram, 2009; Sempere Ferre, 2016). Kepek ve tohumundan yağ elde edilen yulafta küf ve DON, nivalenol, T-2 toksin, HT-2 toksin ve ZEA kontaminasyonu bildirilmektedir (Müller ve ark., 1998; Krysińska-Traczyk ve ark., 2007). Sacchi ve ark. (2009), yulaf örneklerinin *Alternaria alternata* ve *Aspergillus flavus* en baskın türler olmak üzere *Alternaria*, *Aspergillus*, *Fusarium*, *Arthrinium*, *Acremonium*, ve *Curvularia* gibi toksijenik küfleri barındırdığını saptamış ve iki örnekte 105.0-108.0 µg/kg fumonisin B_1 (FB_1) tespit etmiştir.

Colletotrichum, *Fusarium*, *Rhizoctonia*, *Alternaria*, *Aspergillus*, *Penicillium* cinsleri keten tohumunda bulunabilen küflerdir (Kumud ve ark., 1997). Keten tohumunda küf kontaminasyonu genellikle kapsüllerin olgunlaşması sırasında nem ve sıcaklık gibi hava koşullarına bağlı olarak ortaya çıkmaktadır. Bir çalışmada, keten tohumlarında hasat ve depolama sırasında küf florasının *Alternaria* (*A. alternata*, *A. linicola*, *A. dianthi* ve *A. pluriseptata*), *Fusarium* spp. ve *Penicillium* spp. tarafından oluşturulduğu bildirilmiş, tohumlarda DON bulunmuş ve 8 aylık depolama sonrası aflatoksin (2.5 µg/kg) ve OTA (1.2 µg/kg) oluşumu gösterilmiştir (Gruzdevienė ve ark., 2006). Benzer bir çalışmada Sahay ve ark. (2006) tarafından keten tohumlarında *A. flavus*'un en baskın küf olduğu belirlenmiş ve incelenen 105 örnekten 46'sının 120-810 µg/kg aflatoksin B_1 içerdiği saptanmıştır. Keten tohumlarında mikotoksin kontaminasyonuna yönelik bir başka çalışmada *Alternaria* toksinleri alternariol (104 µg/kg), alternariol monometil eter (30 µg/kg) tespit edilmiştir (Královà ve ark., 2006). Soyanın çeşitli toksijenik küfler ile bulaşabildiği ve aflatoksin, trikotesen gibi mikotoksinleri üreten küflerin gelişimini desteklediği ifade edilmektedir (Bhat ve Reddy, 2017).

Fındık, badem, ceviz, Antep fıstığı, kaju, kestane, Avustralya fındığı gibi yemişler genellikle hasat öncesi veya depolama sırasında küf kontaminasyonuna uğrayabilmektedir. Hasat öncesi yemişler açılmış bir kabukta olgunlaştığında hava ve böcek kaynaklı küf sporları ile bulaşmasının yanı sıra, hasat sonrası kabuk ayırma, yıkama ve sınıflandırma gibi aşamalarda küf kontaminasyonu oluşabilmektedir. *Aspergillus* spp, *Penicillium* spp. bu ürünlerde en sık karşılaşılan küflerdir (Demir ve ark., 2002; Fernane ve ark., 2010). Sung ve ark. (2021), inceledikleri yemişlerin %35'inde aflatoksin, fumonisin ve zearalenone tespit etmiştir. Ayrıca aflatoksin, DON, OTA ve beauvericin kontaminasyonu bildirilmektedir (Pour ve ark., 2010; Rubert ve ark., 2012).

Zeytinler de uzun süre toprak ve zemin ile temas, jüt çuvallarda bekletme, yetersiz havalandırma gibi küflerin gelişimine uygun koşullara sıklıkla maruz kalabilmektedir (Ferracane ve ark., 2007; Markaki, 2010). Yapılan çalışmalar zeytinlerde *Cladosporium*, *Alternaria*, *Geotrichum*, *Mucor*, *Rhizopus*, *Trichoderma* küflerinin bulunabildiğini, *Aspergillus* ve *Penicillium* türlerinin ise en sık rastlanan küfler olduğunu ortaya koymaktadır. Ayrıca bunlar arasında *A. flavus*, *A. parasiticus*, *A. ochraceus P. citrinum*, *P. expansum* gibi toksijenik küflerin yer aldığı da bildirilmektedir (Yassa ve ark., 1994; Eltem ve Öner, 1995; Eltem, 1996; Adlouni ve ark., 2006; Heperkan ve ark., 2006; Roussos ve ark., 2006). Buna bağlı olarak zeytinlerin aflatoksin, okratoksin, patulin, sitrinin, penisilik asit gibi mikotoksinler ile kontamine olduğunu gösteren çalışmalar mevcuttur (Oral ve Heperkan, 1999; Korukluoğlu ve ark., 2000; Leontopoulos ve ark., 2003; Finoli ve ark., 2005; Dazkır, 2010). Bu durum, kontamine zeytinlerden elde edilen zeytinyağında mikotoksin bulaşması olabileceğini düşündürmektedir. Meyvedeki küf kontaminasyonuna ilaveten, zeytinyağı üretimi sırasında yeterince temizlenmemiş presleme sistemindeki kek kalıntılarında oluşabilecek küf gelişimi de mikotoksin kontaminasyonu için risk yaratabilmektedir (Karaca ve Yemiş, 2008). Zeytinyağlarına uygulanabilen rafinasyon işleminin aflatoksin gibi bazı mikotoksinleri yağdan kısmen uzaklaştırdığı bilinmekle birlikte zeytindeki aflatoksinlerin %18-47'sinin zeytinyağına geçtiği bildirilmektedir. Ayrıca rafinasyon işleminin soğuk pres yağlara ve natürel sızma zeytinyağı gibi natürel yağlara uygulanmadığını vurgulamak gerekmektedir (Letutour ve ark., 1983; Ferracane ve ark., 2007).

Bitkisel Yağlarda Mikotoksinler

Dünya çapında en çok ihtiyaç duyulan bitkisel maddelerden biri olan bitkisel yağlar, geniş uygulama alanına sahip olduğundan çeşitli ürünlerde kullanılmaktadır. Bitkisel yağlar iklim, toprak, hasat, depolama ve işleme koşulları gibi dış etkenlerin yanı sıra materyalin çeşidine göre de değişebilen düzeyde safsızlık ve bulaşan içeren doğal hammaddelerden elde edilmektedir. Yağların üretiminde kullanılan hammaddeler, çoğu zaman küf gelişimi ve mikotoksin üretimine olanak sağlayan koşullarda haftalarca depolanmaktadır (Bordin ve ark., 2014). Buna bağlı olarak bitkisel yağlarda mikotoksinlerin değişen seviyelerde bulunabildiği çeşitli çalışmalarda gösterilmiş olup, bu çalışmaların bazıları Tablo 1'de özetlenmiştir. Ülkemizde bazı ürünlerde bulunabilecek maksimum mikotoksin limitleri yönetmelik ile düzenlenmiş olup, Türk Gıda Kodeksi Bulaşanlar Yönetmeliği'ne göre doğrudan insan tüketimine sunulan veya gıda bileşeni olarak kullanılan yer fıstığı, diğer yağlı tohumlar ve bunların işlenmiş ürünlerinde bulunmasına izin verilen maksimum aflatoksin B_1 miktarı 5 µg/kg ve toplam aflatoksin miktarı 10 µg/kg'dır. Aynı yönetmelikte rafine mısır yağında zearalenon için maksimum limit 400 µg/kg olarak belirlenmiştir (Anonim, 2011).

Aflatoksin, bitkisel yağlarda varlığı araştırılan başlıca mikotoksindir. Ji ve ark. (2022), yerfıstığı, mısır, susam, zeytin, soya, kolza, ayçiçek, pirinç kepeği, pamuk tohumu, kamelya ve palm yağından oluşan 63 örnekte HPLC-FLD ile aflatoksin analizi gerçekleştirmiş ve örneklerin 25'inde (%39.7) 1.10-88.3 µg/kg aflatoksin tespit etmiştir. Satış noktalarından temin edilen 11 yer fıstığı yağı örneğinin 9'unun (%81.8) 1.74-17.5 µg/kg aflatoksin içerdiğini belirleyen araştırmacılar mısır yağında da yüksek seviyede aflatoksin bulunduğunu, yer fıstığı ve mısırın yanı sıra susam ve zeytinin de aflatoksin

üreten küf istilasına karşı daha duyarlı olduğunu gözlemlediklerini bildirmiştir. Çalışma sonucunda yer fıstığına ait 4, susam, mısır ve zeytinyağına ait 1'er örnekte tespit edilen aflatoksin düzeyinin, Avrupa Birliği tarafından AFB_1 ve toplam aflatoksin için sırasıyla 2 µg/kg ve 4 µg/kg olarak belirlenen maksimum limitleri aştığı ortaya konmuştur. Waqas ve ark. (2021), Pakistan'da yerel ve ithal tohumlardan Soxhlet aparatı kullanarak elde ettikleri ayçiçek, soya, kanola, mısır ve hardal yağında AFB_1 ve toplam aflatoksin düzeyini araştırmıştır. Toplam 393 örneğin HPLC ile incelendiği çalışmada yerel tohumlardan elde edilen yağ örneklerinin aflatoksin düzeyi daha yüksek bulunmuştur. Bu örneklerde ortalama AFB_1 ve toplam aflatoksin miktarı sırasıyla ayçiçek yağında 8.7-15.1 µg/kg, soya yağında 14.29-25.61 µg/kg, kanola yağında 7.41-11.80 µg/kg, mısır yağında 6.34-8.83 µg/kg ve hardal yağında 7.71-13.43 µg/kg olarak belirlenmiştir. Yer fıstığı ve susam yağları aflatoksinler açısından sıklıkla incelenen yağlar arasındadır. Deng ve ark. (2018), 52 yer fıstığı yağı örneğinden 43'ünde 0.5-69.4 µg/kg düzeyinde AFB_1 saptamıştır. Yer fıstığı yağının AFB_1 varlığı yönünden incelendiği bir diğer çalışmada 427 örnekten 80'inin (%18.7) 20.1-234.8 µg/kg AFB_1 içerdiği bulunmuştur (Qi ve ark., 2019). Poormohammadi ve ark. (2021) HPLC-FLD ile analiz ettikleri yer fıstığı yağında 0.56-30.95 µg/kg, susam yağında 0.03-13.11 µg/kg toplam aflatoksin tespit etmiştir. Aynı çalışmada araştırmacılar, 18 örnekte toplam aflatoksin, 16 örnekte AFB_1 miktarının Avrupa Birliği tarafından izin verilen maksimum limitleri aştığını bildirmiştir. Chen ve ark. (2019), HPLC-MS/MS ile analiz ettikleri yer fıstığı yağı örneklerinin 134.03 µg/kg'a varan düzeyde toplam aflatoksin içerdiğini belirlemiştir. Bao ve Trucksess (2010) zeytinyağı, yer fıstığı yağı ve susam yağında RPLC/FLD ile AFB_1, AFB_2, AFG_1, AFG_2 analizi gerçekleştirmiştir. Araştırmacılar, 2 yer fıstığı yağında 6.0 ve 8.5 µg/kg, bir susam yağında yaklaşık 0.1 µg/kg aflatoksin tespit etmiştir. Elzupir ve ark. (2010), 21 yer fıstığı, 14 susam, 19 ayçiçeği ve 27 karışım tohum yağını HPLC ile AFB_1, AFB_2, AFG_1, AFG_2 kontaminasyonu yönünden analiz etmiştir. Pozitif sonuç veren 80 örnekte toplam aflatoksin konsantrasyonu 0.43-339.9 µg/kg arasında değişirken, tüm susam yağı örneklerindeki toplam aflatoksin düzeyi Gıda ve İlaç Dairesi'nin belirlediği kabul edilebilir sınırın (20 µg/kg) üzerinde bulunmuştur. Yang ve ark. (2011), 31 yer fıstığı, 7 soya, 5 mısır, 1 ayçiçeği ve 31 karışım yağında LC-MS/MS ile AFB_1, AFB_2, AFG_1, AFG_2 kontaminasyonunu incelemiştir. Araştırmacılar, soya, mısır ve ayçiçeği yağlarında aflatoksin tespit edilmediğini bildirmiştir. Yer fıstığı örneklerinin AFG_2 içermediği, 0.01-2.72 µg/kg AFB_1, AFB_2, AFG_1 içerdiği bulgulanmıştır. Karışım yağlarda ise AFB_2, AFG_1, AFG_2 bulunmazken, 6 örnekte 0.14-0.24 µg/kg AFB_1 saptanmıştır. Bitkisel yağ örneklerinde AFB_1, AFB_2, AFG_1 ve AFG_2 kontaminasyonunu belirlemek amacıyla gerçekleştirilen bir çalışmada 25 bitkisel yağ örneği HPLC ile analiz edilmiştir. Örneklerde AFG_1 ve AFG_2'ye rastlanmadığını bildiren araştırmacılar, 7 örnekte 0.27-0.89 µg/kg düzeyinde AFB_1 ve AFB_2 bulunduğu sonucunu ortaya koymuştur (Ma ve ark., 2013). Idris ve ark. (2010), ham ve rafine edilmiş toplam 56 yer fıstığı, susam ve pamuk yağında HPLC ile AFB_1, AFB_2, AFG_1, AFG_2 varlığını incelemiştir. Örneklerde AFB_2, AFG_1 ve AFG_2 kontaminasyonu saptanmamış, 1 yer fıstığı ve 7 susam yağında 0.2-0.8 µg/kg AFB_1 tespit edilmiştir. Aflatoksin tespit edilen örneklerin tamamının rafine edilmemiş ham yağlardan oluştuğuna dikkat çeken araştırmacılar, bu yağların aflatoksin kontaminasyonu için daha uygun olabileceğini vurgulamıştır. Hindistan cevizi, palm, ayçiçek, susam, zeytin, soya ve mısır yağlarına ait toplam 59 örnekte aflatoksin kontaminasyonu ELISA ile incelenmiş ve Hindistan cevizi yağı dışında kalan örneklerin hiçbirinde 0.8 µg/kg olan tespit limitinin üstünde aflatoksin bulunmamıştır. Aflatoksin içerdiği belirlenen 12 hindistan cevizi yağı örneğinde HPLC ile yapılan analiz sonucunda 1.76-60.92 µg/kg AFB_1, 0.13-3.43 µg/kg AFB_2, 0.15-8.35 µg/kg AFG_1 ve 2.25-72.70 µg/kg AFG_2 saptanmıştır (Karunarathna ve ark., 2019). Bitkisel yağlarda aflatoksinlerin, zaman zaman yasal düzenlemeler ile belirlenen maksimum limitleri de aşan, farklı düzeylerde bulunabildiği çeşitli çalışmalar ile gösterilmiştir. Bununla birlikte mısır, yer fıstığı, soya, kanola, pirinç, ceviz ve badem yağı gibi bazı yağlarda aflatoksin tespit edilmediğini bildiren çalışmalar da mevcuttur (Yu ve ark., 2019a; Yu ve ark., 2019b; Zhang ve ark., 2020).

Mısır yağlarının başta ZEA olmak üzere, fumonisin ve DON ile kontamine olduğu görülmektedir. Escobar ve ark. (2013), 25 rafine mısır yağı ve 25 mısır yağı margarini örneğinde fumonisin, DON ve ZEA varlığını ters fazlı sıvı kromatografisi ile incelemiş ve UPLC/MS/MS ile doğrulamıştır. Rafine mısır yağı örneklerinden birinde 7 µg/kg FB_1, ikisinde 106-216 µg/kg DON, sekizinde 0-67 µg/kg ZEA saptanmış, fumonisin B_2 (FB_2) ise hiçbir örnekte tespit edilmemiştir. Margarin örneklerinde ise FB_2 ve DON tespit edilmezken, %8'inde 109 µg/kg'a varan FB_1'in ve %24'ünde 86 µg/kg'a varan ZEA'nın bulunduğu bildirilmiştir. Üzüm çekirdeği, keten tohumu, mısır, zeytin, yer fıstığı, kabak çekirdeği, kolza, pirinç, susam, soya, ayçiçeği, buğday rüşeymi, devedikeni, ceviz ve karışım salata yağlarına ait 44 örnekte ZEA varlığının HPLC ile araştırıldığı bir çalışmada, 4 mısır yağı örneğinde 57-117 µg/kg ZEA saptanmıştır (Siegel ve ark., 2010). Çin'de yapılan benzer bir çalışmada yerel satıcılardan temin ettikleri 16 mısır, 6 yer fıstığı, 6 kolza, 6 soya ve 6 ayçiçeğine ait 40 yağ örneğinde 5 türevi ile birlikte ZEA mikotoksinlerinin varlığını GC-MS yöntemi ile araştıran Qian ve ark. (2015), 9 mısır

örneğinde 5.2-184.6 µg/kg ve 1 kolza örneğinde 40.7 µg/kg ZEA tespit etmiştir. Mısır yağına ait 50 örneğin incelendiği bir çalışmada örneklerin aflatoksin içermediğini belirleyen araştırmacılar, 5 örnekte 49.16-69.13 µg/kg ZEA, 8 örnekte 5.69-9.68 µg/kg FB_1 ve 42 örnekte 32.64-101.41 µg/kg FB_2 bulunduğunu ortaya koymuştur. Aynı çalışmada incelenen palm, ayçiçek ve pirinç kepeği yağlarında bu mikotoksinler tespit edilmezken 1 adet soya yağında 59.31 µg/kg ZEA bulunmuştur (Junsai ve ark., 2021). Zhang ve Xu (2019), bitkisel yağlarda 12 mikotoksinin eşzamanlı tespiti için kararlı izotop seyreltme LC-tandem MS (LC-MS/MS) yöntemini geliştirmiş ve bu yöntemi kullanarak inceledikleri mısır yağında 37-390 µg/kg ZEA, 7-10 µg/kg T-2 toksin, 3 µg/kg FB_2 ve 1 µg/kg fumonisin B_3 tespit etmiştir. Aynı yöntemle inceledikleri yer fıstığı yağında ise 17 µg/kg AFB_1 ve 3 µg/kg AFB_2 saptamışlardır. ZEA ve alternariol mikotoksinlerinin eşzamanlı tespitinde kullanılmak üzere bir yöntem geliştiren ve bu yöntemi mısır ve ayçiçek yağlarına uygulayan Moya-Cavas ve ark. (2023), mısır yağının 45 µg/kg ZEA ve ayçiçek yağının 21 µg/kg alternariol içerdiğini bildirmiştir. Ayçiçek yağı (34 adet), ham ayçiçek yağı (3 adet), soya yağı (3 adet) ve mısır yağında (1 adet) aflatoksin, ZEA ve metaboliti α-zearalenol varlığının araştırıldığı bir çalışmada 1 ayçiçek yağı örneğinde 0.7 µg/kg AFB_2 ve 2 µg/kg ZEA belirlenmiştir. Ham ayçiçek yağı örneklerinde 0.5-2.0 µg/kg AFG_2 tespit eden araştırmacılar, soya ve mısır yağı örneklerinde hiçbir mikotoksine rastlanmadığı sonucunu ortaya koymuştur (Hidalgo-Ruiz ve ark., 2019).

Soğuk Pres Yağlarda Mikotoksinler

Son yıllarda tüketilen gıdalar ile insan sağlığı arasında yakın bir ilişki olduğunun ortaya konulması, tüketicileri "doğal", "organik" ve "daha az işlenmiş" gıda arayışına yönlendirmiştir. Bu durum soğuk pres yağların da dahil olduğu natürel yağlara olan talebin artmasına yol açmıştır (Matthäus, 2008). Kendine özgü özelliklere ve lezzete sahip olan soğuk pres yağlar, değerli biyoaktif maddeler içermektedir. Sağlık açısından önemli doymamış yağ asitlerini bileşiminde bulundurmasının yanı sıra tokoferol ve fenolik bileşikler gibi doğal antioksidanlarca rafine yağlara oranla daha zengindir. Doğal minör bileşenlerinin içeriğinden kaynaklanan duyusal özellikleri ve sağlık üzerine olumlu etkileri, soğuk pres yağların tüketiciler tarafından giderek daha fazla değer görmesini sağlamaktadır (Prescha ve ark., 2014). Bunun sonucu olarak, önceleri ilaç ve kozmetik endüstrisinde kullanım alanı bulunan soğuk pres yağlar mutfaklara girmiş ve besin olarak tüketilmesi yaygınlaşmıştır (İmer ve Taşan, 2018).

Soğuk pres yağların üretimi incelendiğinde, yabancı maddelerden arındırılan hammaddenin preslerde sıkma işleminin maksimum 40°C sıcaklıkta gerçekleştirilmesinin ardından basit bir filtreleme işleminin yapıldığı ve üretimde organik çözücüler gibi kimyasal kirleticilerin kullanılmadığı görülmektedir (Geçgel ve ark., 2017). Bu durum, faydalı bileşenlerin korunmasını sağlamakla birlikte soğuk pres yağları, bulaşmış hammaddeden elde edilmesi halinde mikotoksin açısından riskli hale getirmektedir. Literatürde bunu ortaya koyan çeşitli çalışmalar mevcut olup, bu çalışmalardan bazıları Tablo 2'de özetlenmiştir.

Soğuk pres yağlarda mikotoksin varlığını araştıran bazı çalışmalarda yağdaki mikotoksin miktarının yanı sıra yağın elde edildiği hammadde ve hammaddedeki yağın ayrılmasının ardından geriye kalan kekteki mikotoksin düzeyi de ortaya konmuştur. Bu çalışmalarda hammaddedeki mikotoksinlerin bir kısmının yağa geçtiği, mikotoksin çeşidine bağlı olarak değişen düzeyde bir kısmının ise kekte kaldığı gösterilmiştir. Sidhu ve ark. (2009), *Madhuca indica* Gmel. tohumunda, bu tohumdan elde edilen soğuk pres yağda ve kekinde ELISA ile aflatoksin düzeyini araştırmıştır. Tohumlarda 282.36 µg/kg AFB_1 ve 315.51 µg/kg toplam aflatoksin, soğuk pres yağında 201.57 µg/kg AFB_1 ve 220.66 µg/kg toplam aflatoksin, geri kalan kekte ise 74.35 µg/kg AFB_1 ve 87.55 µg/kg toplam aflatoksin tespit eden araştırmacılar yağ ve kekteki aflatoksin oranının 7:3 olduğunu saptamıştır. Xu ve ark. (2018), 10 mısır özü örneğinde, örneklerden solvent ekstraksiyonu ve soğuk pres yoluyla elde ettikleri mısır özü yağında ve geriye kalan kekte DON miktarını incelemiştir. Mısır özünde 310.5-2684.4 µg/kg, solvent ekstraksiyonu ile elde edilen yağlarda 69.7-293.6 µg/kg ve soğuk pres ile elde edilen yağlarda 30.4-254.4 µg/kg DON tespit edilmiştir. Araştırmacılar tarafından, solvent ile ekstrakte edilmiş yağ ve soğuk pres yağındaki DON içeriğinin 6 örnekte önemli bir farklılık göstermediği bildirilmiştir. Solvent ekstraksiyonu ve soğuk pres ile yağ eldesinin ardından geriye kalan kekte sırasıyla 209.8-2311.8 µg/kg ve 281.7-2317.7 µg/kg olan DON düzeyinin soğuk pres kekinde, soğuk pres yağdan 8.4-15.7 kat fazla olduğu ve DON'un keke geçme eğiliminde olduğu belirlenmiştir. Bu görüşü destekleyen ve Smeu ve ark. (2022) tarafından gerçekleştirilen bir çalışmada, ayçiçeği ve kolza tohumu, ceviz, susam ve üzüm çekirdeğinin de aralarında yer aldığı ürünlerden soğuk pres yağ eldesinin ardından geriye kalan pres keki gibi yan ürünlerdeki aflatoksin, DON ve ZEA düzeyi incelenmiştir. Bazı örneklerde aflatoksin ve ZEA tespit edilmezken, DON tüm örneklerde tespit edilmiştir. Örneklerin aflatoksin düzeyi 0.23-1.51 µg/kg, ZEA düzeyi 1.03-79.22 µg/kg olurken DON düzeyi 25.57-980.09 µg/kg olarak belirlenmiştir.

Tablo 1. Bitkisel yağlarda mikotoksinlerin araştırılmasına yönelik çalışmalar

Table 1. Studies on the investigation of mycotoxins in vegetable oils

Yağın türü	Mikotoksin	Örnek sayısı	Pozitif örnek	Miktar (µg/kg)	Yöntem	Kaynak
Mısır* Üzüm çekirdeği Keten tohumu Zeytin, Yer fıstığı Kabak çekirdeği Kolza, Pirinç, Susam, Ceviz Karışım salata, Soya, Ayçiçeği Devedikeni, Buğday ruşeymi	Zearalenon	44	4*	57-117	HPLC	Siegel ve ark. (2010)
Mısır Yer fıstığı Kolza Soya Ayçiçeği	Zearalenon	40	9 - 1 - -	5.2-184.6 - 40.7 - -	GC-QqQ MS	Qian ve ark. (2015)
Mısır	Zearalenon	1	1	460	HPLC	Lauren ve Ringrose (1997)
Mısır Ayçiçeği Karışım	Zearalenon	104	104	22.5-70.78 2.24-2.67 0.63-4.45	HPLC	Sadeghi ve ark. (2016)
Mısır	Zearalenon Deoksinivalenol HT-2 Toksin T-2 Toksin			386-651 45-51 5-10 23-58	HPLC	Schollenberger ve ark. (2008)
Rafine Mısır	Fumonisin B_1 Fumonisin B_2 Deoksinivalenol Zearalenon	25	1 0 2 8	0-77 0 106-216 0-67	HPLC	Escobar ve ark. (2013)
Mısır Soya Buğday ruşeymi	Zearalenon	38 20 11	38 14 10	?-921 0-41.4 0-46.2	HPLC	Kappenstein ve ark. (2005)
Buğday ruşeymi	Zearalenon Deoksinivalenol	25	25	<8-44 <22-163	HPLC	Giménez ve ark. (2013)
Mısır	Fumonisin B_1 Fumonisin B_2 Fumonisin B_3	20	0	<10	HPLC	Patel ve ark. (1997)
Soya Mısır Ayçiçeği	Aflatoksin B_1 Aflatoksin B_2 Aflatoksin G_1 Aflatoksin G_2	7 5 1	0	0	LC–MS/MS	Yang ve ark. (2011)
Karışım yağ	Aflatoksin B_1 Aflatoksin B_2 Aflatoksin G_1 Aflatoksin G_2	31	6 0 0 0	0.14–0.24	LC–MS/MS	Yang ve ark. (2011)
Bitkisel yağ	Aflatoksin B_1 Aflatoksin B_2 Aflatoksin G_1 Aflatoksin G_2	7	0	-	LC–MS/MS	Fan ve ark. (2015)
Bitkisel yağ	Aflatoksin	245	9	0.1-5.8	HPLC	Anonim (2001)

Bitkisel yağ	Aflatoksin B_1 Aflatoksin B_2 Aflatoksin G_1 Aflatoksin G_2	25	7	0.27-0.89	HPLC	Ma ve ark. (2013)
Yer fıstığı	Aflatoksin B_1	57	56	0-40		Opadokun (1992)
Yer fıstığı	Aflatoksin B_1 Aflatoksin B_2 Aflatoksin G_1 Aflatoksin G_2	31	15 6 3 0	0.15-2.72 0.15-0.36 0.01-0.02 0	LC–MS/MS	Yang ve ark. (2011)
Yer fıstığı	Aflatoksin B_1 Aflatoksin B_2 Aflatoksin G_1 Aflatoksin G_2	63	45	0.11-24 0.1-21 0-7.1 0	HPLC	Salau ve ark. (2017)
Ayçiçeği Yer fıstığı* Soya* Mısır Keten tohumu Zeytin	Zearalenon Aflatoksin B_1 Aflatoksin B_2 Aflatoksin G_1	62	6*	<0.04–42.5 <0.05–11.0 <0.04–4.56 <0.04–0.59	HPLC-MS/MS	Zhao ve ark. (2017)
Yer fıstığı-soya	Aflatoksin B_1	39	39	0.2-114.4	ELISA	Sun ve ark. (2011)
Ham yer fıstığı	Aflatoksin B_1 Aflatoksin B_2 Aflatoksin G_1 Aflatoksin G_2	3	3	26.1 18.1 18.5 13.2	TLC	Abalaka (1984)
Rafine yer fıstığı	Aflatoksin B_1 Aflatoksin B_2 Aflatoksin G_1 Aflatoksin G_2	3	1 0 0 1	6.6 - - 4.8	TLC	Abalaka (1984)
Yer fıstığı Pamuk tohumu	Aflatoksin B_1			>98 >65	TLC	Abalaka ve Elegbede (1982)
Ham Pirinç kepeği Rafine Pirinç kepeği	Aflatoksin B_1	20 20	15 6	236-956 İz-28	TLC	Jayaraman ve Kalyanasundaram (2009)
Yer fıstığı Susam Rafine Pamuk	Aflatoksin B_1 Aflatoksin B_2 Aflatoksin G_1 Aflatoksin G_2	56	1 7 0	0.6 0.2-0.8 - -	HPLC	Idris ve ark. (2010)
Yer fıstığı Susam	Aflatoksin Aflatoksin B_1	2 1	2 1	6-8.5 0.1	RPLC/FLD	Bao ve Trucksess (2010)
Susam Yer fıstığı Ayçiçeği Karışım tohum	Toplam aflatoksin	81	80	41.2-339.9 5.7-100.0 0.6-175.7 0.4-45.7	HPLC	Elzupir ve ark. (2010)

Tablo 2. Soğuk pres yağlarda mikotoksinlerin araştırılmasına yönelik çalışmalar

Table 2. Studies on the investigation of mycotoxins in cold pressed oils

Yağın türü	Mikotoksin	Miktar (µg/kg)	Yöntem	Kaynak
Mahua tohumu	Aflatoksin B_1 Aflatoksinler	201.57 220.66	ELISA	Sidhu ve ark. (2009)
Mısır özü	Deoksinivalenol	30.4-254.4	HPLC	Xu ve ark. (2018)
Yer fıstığı	Aflatoksin B_1 Aflatoksin B_2 Aflatoksin G_1 Aflatoksin G_2	5.98-105.71 1.02-36.33 - -	HPLC-FLD	Şahin ve ark. (2021)
Yer fıstığı	Aflatoksin B_1 Aflatoksin B_2 Aflatoksin G_1 Aflatoksin G_2	1.09-11.10 0.08-0.09 - -	HPLC	Ji ve ark. (2020)
Ayçiçeği	Aflatoksin B_1 Aflatoksin B_2 Aflatoksin G_1 Aflatoksin G_2	1.20-2.55 0.37-0.79 0.94-2.01 0.25-0.42	HPLC-FLD	Var ve Uçkun (2021)
Ayçiçeği	Tenuazonik asit Altenuen Alternariol Tentoksin Alternariol metil eter	12.8 - - 7.1 -	LC-ID-MS/MS	Tölgyesi ve ark. (2020)
Ayçiçeği* Kolza tohumu	Tentoksin Dihidrotentoksin	0.64-6.73 4.48*	LC-MS/MS	Liu ve Rychlik (2013)
Susam	Aflatoksin B_1 Aflatoksin B_2 Aflatoksin G_1 Aflatoksin G_2	0.63-1.04 <0.1-0.19 - -	HPLC	Akbari ve ark. (2021)
Susam	Aflatoksin B_1 Aflatoksin B_2 Aflatoksin G_1 Aflatoksin G_2	0.2-1.2 0.1-0.2 0.1-0.6 -	HPLC	Ramezani ve ark. (2022)
Kabak çekirdeği	Aflatoksin B_1 Aflatoksin B_2 Aflatoksin G_1 Aflatoksin G_2	- - - -	UHPLC-MS/MS	Arslan ve ark. (2017)

Tablo 3. Zeytinyağında mikotoksinlerin araştırılmasına yönelik çalışmalar

Table 3. Studies on the investigation of mycotoxins in olive oil

Yağın türü	Mikotoksin	Örnek sayısı	Pozitif örnek	Miktar (µg/kg)	Yöntem	Kaynak
Zeytinyağı	Aflatoksin B_1			5-10		Toussaint ve ark. (1977)
Zeytinyağı	Aflatoksin B_1	50	36	0.0028-0.0463	HPLC	Daradimos ve ark. (2000)
Zeytinyağı	Aflatoksin B_1 Aflatoksin B_2 Aflatoksin G_1 Aflatoksin G_2	35	3	<0.04 - - -	LC-MS/MS	Cavaliere ve ark. (2007)
Zeytinyağı	Aflatoksin B_1 Aflatoksin B_2 Aflatoksin G_1 Aflatoksin G_2	50	2	0.10	RP-HPLC	Yorulmaz ve Bircan (2013)
Zeytinyağı	Zearalenon			0	HPLC	Siegel ve ark. (2010)
Zeytinyağı	Aflatoksin B_1 Okratoksin A	30	1 25	0.03 0.36-2.10	HPLC	Dazkır (2010)
Zeytinyağı	Aflatoksin B_1 Okratoksin A	30	3 24	0.5-2.4 0.1-17	HPLC	Ferracane ve ark. (2007)
Zeytinyağı	Aflatoksin B_1 Okratoksin A	50	12 44	<0.056-0.06 0.06-1.030	HPLC	Papachristou ve Markaki (2004)
Zeytinyağı	Aflatoksin B_1 Okratoksin A	60	0 3	- <40	TLC	Letutour ve ark. (1983)
Zeytinyağı	Aflatoksinler Okratoksin A	28	13	0.006-0.04 0.052-0.244	HPLC	Finoli ve ark. (2005)

Soğuk pres yöntemi ile elde edilen yağlardaki mikotoksin düzeyinin diğer ekstraksiyon yöntemleri ile elde edilen yağlardaki mikotoksin düzeyi ile karşılaştırıldığı çalışmaların çoğunda soğuk pres yöntemi ile elde edilen yağların daha yüksek miktarda mikotoksin içerdiği belirlenmiştir. Şahin ve ark. (2021) tarafından farklı ekstraksiyon yöntemlerinin yer fıstığından yağa aflatoksinlerin geçişine etkisini incelemek amacıyla gerçekleştirilen çalışmada yağlardaki AFB_1 ve AFB_2 miktarı üzerine farklı işleme yöntemlerinin istatiksel olarak anlamlı olduğu bulunmuştur. Sıcak presleme ile ekstraksiyon ve rafinasyon işlemlerini kapsayan endüstriyel yöntemle elde edilen yağlarda AFB_1 ve AFB_2 tespit edilmemiştir. AFB_1 miktarı solvent ekstraksiyonu ile elde edilen yağlarda 2.51-96.97 µg/kg, kavrulmuş fıstıkların soğuk presyonu ile elde edilen yağlarda 4.33-128.68 µg/kg, soğuk pres yağlarda 5.98-105.71 µg/kg olurken AFB_2 miktarı solvent ekstraksiyonu ile elde edilen yağlarda 0.46-17.22 µg/kg, kavrulmuş fıstıkların soğuk presyonu ile elde edilen yağlarda 0.84-33.0 µg/kg, soğuk pres yağlarda 1.02-36.33 µg/kg olarak bulunmuştur. Buna göre yer fıstıklarından solvent ekstraksiyonu, kavrulmuş yer fıstığının soğuk preslenmesi ve soğuk pres yöntemleri ile elde edilen yağlara AFB_1 geçiş oranları sırasıyla %9.0-79.8; %11.3-75.3; %9.3-77.6 ve AFB_2 geçiş oranları sırasıyla %10.1-83.3; %18.4-86.2; %10.5-92.0 olarak tespit edilmiştir. Sonuç olarak sırasıyla endüstriyel yöntem ve solvent ekstraksiyonu yağa aflatoksin geçişini azaltma üzerine en etkili birinci ve ikinci yöntem olurken soğuk pres en az etkili yöntem olarak belirlenmiştir. Ji ve ark. (2020), kabuklu ve kabuksuz yer fıstığından solvent ekstraksiyonu, soğuk pres ve sıcak pres yoluyla elde ettiği ham yer fıstığı yağlarında aflatoksin düzeyini araştırmıştır. AFB_1'in yer fıstığı ve yer fıstığı yağında en sık rastlanan aflatoksin olduğu, AFB_1 ve toplam aflatoksin miktarlarının yağda, elde edildiği yer fıstığına kıyasla daha düşük miktarda bulunduğu, solvent ekstraksiyonu ile elde edilen yağların presleme ile elde edilen yağlardan daha az aflatoksin içerdiği ve sıcak presleme ile elde edilen yağların soğuk presleme ile elde edilen yağlardan daha az aflatoksin içerdiği bildirilmiştir. Yer fıstığındaki aflatoksinlerin %5-20 oranında ekstraksiyon ile elde edilen yağa geçtiği, kabuk soyma işleminin ardından bu oranın azaldığı tespit edilmiştir. Var ve Uçkun (2021), aflatoksin içermeyen ayçiçeği tohumlarına 5 ve 10 µg/kg aflatoksin ekleyerek ekstraksiyon yöntemlerinin yağa aflatoksin geçişi üzerine etkisini araştırmıştır. Araştırmacılar aflatoksinlerin yağa transferi üzerine eklenen aflatoksin miktarı ve uygulanan ekstraksiyon yöntemlerinin etkisinin istatistiksel olarak önemli olduğunu bildirmiştir. 5 µg/kg ve 10 µg/kg aflatoksin eklenmiş ay çekirdeğinden elde edilen yağda tutulan toplam aflatoksin miktarı

soğuk preste sırasıyla 2.76 µg/kg (%55.2) ve 5.77 µg/kg (%57.7), kavrulmuş tohumların preslenmesi ile 1.87 µg/kg (%37.4) ve 4.69 (%46.9) µg/kg, solvent ekstraksiyonunda 0.91 (%18.2) ve 1.96 (%19.6) µg/kg olarak belirlenmiştir. Yağa aflatoksin geçişini azaltan en etkili yöntemin solvent ekstraksiyonu olduğu ortaya konmuştur. Diğer taraftan, soğuk pres ayçiçek yağının *Alternaria* toksinleri ile bulaşabildiği çeşitli çalışmalarla gösterilmiştir. Puntscher ve ark. (2019), soğuk pres ayçiçeği yağı örneklerinin 2.9 µg/kg'a varan düzeyde alternariol, 2.9 µg/kg'a varan düzeyde alternariol monometil eter ve 458 µg/kg'a varan düzeyde tenuazonik asit içerdiğini ortaya koymuştur. Örneklerin %69'unun alternariol, %75'inin alternariol monometil eter ve tenuazonik asit, %81'inin tentoksin, %31'inin altertoksin I ve %19'unun alterperylenol ile kontamine olduğunu bulgulayan araştırmacılar, inceledikleri rafine edilmiş yağların, soğuk preslenmiş yağlara kıyasla önemli ölçüde daha düşük kontaminasyon gösterdiğini bildirmiştir. Benzer bir çalışmada bir soğuk pres ayçiçek yağı örneğinde *Alternaria* toksinlerinden olan tenuazonik asit (12.8 µg/kg) ve tentoksin (7.1 µg/kg) tespit eden araştırmacılar, rafine edilmiş iki ayçiçek yağı örneğinde ise 4.5-5.0 µg/kg düzeyinde tentoksin saptamıştır (Tölgyesi ve ark., 2020). *Alternaria* toksinlerinin tespit edilmesi amacıyla yapılan bir başka çalışmada, Liu ve Rychlik (2013), 1 adet soğuk pres kolza tohumu yağında 0.64 µg/kg tentoksin bulunduğunu, dihidrotentoksin ise bulunmadığını belirlemiştir. Aynı çalışmada 3 adet soğuk pres ayçiçek yağı örneğinde 6.73 µg/kg'a varan düzeyde ve ortalama 4.83 µg/kg tentoksin ile 4.48'e varan düzeyde ve ortalama 2.54 µg/kg dihidrotentoksin tespit edilmiştir.

Susam yağı da mikotoksin kontaminasyonunu tespit etmek üzere incelenen ürünler arasındadır. Akbari ve ark. (2021), soğuk pres yöntemi ile susam yağı üretiminin farklı aşamalarından toplam 17 adet ham susam, 17 adet filtre edilmemiş yağ ve 17 adet filtre edilmiş yağ örneği ile geleneksel yöntemle susam yağı üretiminin farklı aşamalarından toplam 9 adet ham susam, 9 adet filtre edilmemiş yağ ve 9 adet filtre edilmiş yağ örneğinde aflatoksin B_1, B_2, G_1 ve G_2 düzeyini araştırmıştır. Kabuk soyma işleminin toplam aflatoksini %79.79 oranında azalttığı tespit edilen çalışmada, geleneksel ve soğuk pres yöntemlerinin ham susamdan elde edilen yağa sırasıyla %8.2 ve %70.22 oranında toplam aflatoksin aktarımına neden olduğu belirlenmiştir. Sonuç olarak araştırmacılar, susam yağının aflatoksin içeriğini azaltmada geleneksel yöntemin soğuk preslemeye göre daha etkili olduğunu bildirmiştir.

Öte yandan soğuk pres yağlarda mikotoksin tespit edilmeyen veya yasal düzenlemelerle belirlenen limitleri aşmayan oranda mikotoksin tespit edilen çalışmalar da bulunmaktadır. Ramezani ve ark. (2022), yerel satıcılardan geleneksel soğuk pres susam yağı ve marketlerden ticari markalara ait endüstriyel soğuk pres susam yağı temin ederek bunların AFB_1, AFB_2, AFG_1 ve AFG_2 düzeylerini incelemiştir. 10 endüstriyel susam yağı örneğinin 7'sinde (%70'inde) 0.1-0.7 µg/kg ve 20 geleneksel susam yağı örneğinin 13'ünde (%65'inde) 0.1-1.2 µg/kg düzeyinde aflatoksin belirlenmiştir. Endüstriyel ve endüstriyel olmayan susam yağı örnekleri arasında aflatoksin kontaminasyonu yönünden anlamlı bir farklılık bulunmamıştır. AFB_1 ve toplam aflatoksin düzeylerinin tüm örneklerde İran (5 ve 15 µg/kg, sırasıyla) ve Avrupa Birliği (2 ve 4 µg/kg, sırasıyla) tarafından izin verilen yasal limitlerin altında olduğu ortaya konmuştur. Arslan ve ark. (2017), Anadolu'nun 4 farklı bölgesinden temin ettikleri kabak çekirdeklerini kullanarak soğuk pres yağ elde etmiş ve bu yağlarda aflatoksin B_1, B_2, G_1 ve G_2 kontaminasyonunu belirlemiştir. UHPLC-MS/MS yöntemiyle gerçekleştirilen analiz sonucunda kabak çekirdeği yağlarında aflatoksin kalıntısına rastlanmadığı bildirilmiştir.

Zeytinyağında Mikotoksinler

Zeytinler, bileşimleri ve çevre koşulları gibi faktörlere bağlı olarak hasattan itibaren son ürünün depolanmasına kadar geçen her aşamada çeşitli toksijenik küfler ve mikotoksinler ile bulaşabilmektedir. Bunun neticesinde kontamine zeytinlerden elde edilen zeytinyağında mikotoksin varlığı söz konusu olabilmektedir (Var ve ark., 2011). Tablo 3'te zeytinyağında mikotoksin kontaminasyonunu inceleyen çalışmalar özetlenmiştir.

Aflatoksin zeytinyağında incelenen başlıca mikotoksindir. Toussaint (1977) incelediği zeytinyağı örneklerinde 5-10 µg/kg düzeyinde AFB_1 bulmuştur. Poormohammadi ve ark. (2021), 10 zeytinyağı örneğini HPLC-FLD ile aflatoksin kontaminasyonu yönünden incelemiş ve 6 örnekte 0.9-11.45 µg/kg toplam aflatoksin tespit etmiştir. Pakistan'da yerel ve ithal zeytinlerden Soxhlet aparatı kullanılarak elde edilen zeytinyağı örneklerinde AFB_1 ve toplam aflatoksin düzeyinin incelendiği bir çalışmada örnekler HPLC ile analiz edilmiştir. İthal zeytinlerden elde edilen 20 yağ örneğinden 8'inde ortalama 4.38 µg/kg AFB_1 ve 7.47 µg/kg toplam aflatoksin bulunmuştur. Yerel zeytinlerden elde edilen 18 yağ örneğinden 8'inde ortalama 8.51 µg/kg AFB_1 ve 12.78 µg/kg toplam aflatoksin belirlenmiştir (Waqas ve ark. 2021). Bununla birlikte zeytinyağındaki aflatoksin seviyesinin genellikle düşük düzeyde olduğu bildirilmektedir. AFB_1, AFB_2, AFG_1 ve AFG_2 varlığının 15 natürel sızma, 15 natürel ve 5 ham zeytinyağı örneğinde LC-MS/MS ile incelendiği bir çalışma sonucunda 3 natürel zeytinyağı örneğinde metot tayin limiti olan 0.04 µg/kg'ın altında AFB_1 tespit edilmiştir (Cavaliere ve ark., 2007). Daradimos ve ark. (2000) AFB_1 kontaminasyonunu tespit etmek amacıyla inceledikleri 50 zeytinyağı örneğinden

14 tanesinde aflatoksin tespit etmediklerini, geri kalan örneklerde ise 0.0028-0.0463 µg/kg düzeyinde AFB_1 saptadıklarını bildirmiştir. Zeytinyağlarında aflatoksin kontaminasyonunu belirlemek amacıyla gerçekleştirilen bir diğer çalışmada 50 adet zeytinyağı örneğinde AFB_1, AFB_2, AFG_1 ve AFG_2 varlığı RP-HPLC ile incelenmiştir. Örneklerin %96'sında hiçbir aflatoksin bulaşısı olmadığı, yalnızca iki örnekte ortalama 0.10 µg/kg AFB_1 bulunduğu ortaya konmuştur (Yorulmaz ve Bircan, 2013). Nabizadeh ve ark. (2018), rafine edilmemiş 15 zeytinyağı örneğinde AFB_1, AFG_1 ve AFG_2'ye rastlamadığını, örneklerin 2'sinde 0.2-0.4 µg/kg AFB_2 saptadığını, rafine edilmiş örneklerde ise aflatoksin tespit etmediğini bildirmiştir. Zeytinin bileşiminde yer alan çeşitli fenolik bileşiklerin bu toksinin üretimini kısıtlayıcı yönde etki etmesi, aflatoksinin zeytinyağında düşük düzeyde tespit edilmesinin nedenleri arasında gösterilmektedir. Bu fenolik bileşiklerden bazıları küf gelişimini engellememekle birlikte aflatoksin üretimini sınırlandırmaktadır. Örneğin, esas olarak kafeik asit ve daha az miktarda kateşin ve kumarinleri içeren bir zeytin ekstraktının, *Aspergillus flavus* gelişimini durdurmaksızın aflatoksin üretimini %90 oranında engellediği ifade edilmektedir (Paster ve ark., 1988). Benzer şekilde oleuropein, misel kitlesi yönünden değerlendirildiğinde, *A. parasiticus* küfünün gelişimini teşvik ederken aflatoksin üretimini %83-93 oranında azaltmıştır (Gourama ve Bullerman, 1987).

Okratoksin, zeytinyağında incelenen bir diğer mikotoksindir. Letutour ve ark. (1983), 60 ham çiftlik zeytinyağı örneğinde AFB_1 ve OTA varlığını TLC ile incelemiş ve 3 örnekte iz miktarda OTA tespit edildiğini, hiçbir örnekte ise AFB_1 bulunmadığını bildirmiştir. Finoli ve ark. (2005) tarafından yapılan bir çalışmada 28 natürel sızma zeytinyağı örneğinde aflatoksin ve OTA kontaminasyonu incelenmiştir. Örneklerin %54'ünde mikotoksin tespit edilememiştir. Bir örnekte AFB_1 ve OTA'nın birlikte bulunduğu bildirilen çalışmada, örneklerin aflatoksin düzeyleri 0.006-0.04 µg/kg, OTA düzeyleri 0.052-0.244 µg/kg olarak belirlenmiştir. 30 sızma zeytinyağı örneğinde HPLC ile AFB_1 ve OTA varlığını araştırdığı çalışmasında Dazkır (2010), 1 örnekte 0.03 µg/kg AFB_1, 25 örnekte ise 0.36-2.10 µg/kg OTA tespit etmiş ve AFB_1 bulunan örneğin aynı zamanda OTA içerdiğini ortaya koymuştur. Natürel zeytinyağlarında AFB_1 ve OTA kontaminasyonunun araştırıldığı bir çalışmada HPLC ile 30 adet örnek incelenmiştir. %80'inin OTA ile kontamine olduğu belirlenen örneklerin kontaminasyon seviyesi 0.1-17 µg/kg olarak bulunmuştur. Üç örnekte ise 0.5-2.4 µg/kg düzeyinde AFB_1 saptanmış olup, araştırmacılar tarafından örneklerin %10'unda iki mikotoksinin birlikte bulunduğu ortaya konmuştur (Ferracane ve ark., 2007). Natürel zeytinyağı örneklerinde AFB_1 ve OTA varlığını araştıran Papachristou ve Markaki (2004), 50 örneği HPLC ile analiz etmiştir. OTA, incelenen 6 örnekte tespit edilemezken, geri kalan örneklerde 0.06-1.030 µg/kg düzeyinde bulunmuştur. On bir örnekte aflatoksin B_1 izine rastlanmış, 1 örnekte 0.06 µg/kg düzeyinde tespit edilmiştir.

Zeytinyağında mikotoksin kontaminasyonunu inceleyen çalışmalar daha çok aflatoksin ve okratoksin üzerine yoğunlaşmakla birlikte son yıllarda *Alternaria* toksinleri, zearalenon ve fumonisin de incelenen mikotoksinler arasında yer almaktadır. Yirmi natürel zeytinyağı örneğinde tenuazonik asit, tentoksin, alternariol ve alternariol monometil eter olmak üzere 4 farklı *Alternaria* toksininin incelendiği bir çalışmada 12 örneğin incelenen mikotoksinlerden en az birini içerdiği gösterilmiştir. Örneklerin %60'ında bulunan alternariol monometil eter en yaygın tespit edilen mikotoksin olurken onu sırasıyla örneklerin %25 ve %20'sinde bulunan alternariol ve tenuazonik asit izlemiştir. Tentoksine ise hiçbir örnekte rastlanmadığını bildiren araştırmacılar, örneklerin 4.40 µg/kg'a varan düzeyde tenuazonik asit, 7.53 µg/kg'a varan düzeyde alternariol ve 13.7 µg/kg'a varan düzeyde alternariol monometil eter içerdiğini belirlemiştir (Lin ve ark., 2022). Natürel zeytinyağı ve organik zeytinyağında alternariol tespit edilen bir başka çalışmada HPLC-FLD ile gerçekleştirilen analiz sonucunda natürel zeytinyağında 24 µg/kg, organik zeytinyağında 28 µg/kg alternariol saptanmıştır (Moya-Cavas ve ark., 2023). Zeytinyağı (35 adet), natürel sızma zeytinyağı (33 adet), ham zeytinyağı (31 adet), rafine zeytinyağı (11 adet), pirina yağı (15 adet) ve ham pirina yağına (28 adet) ait toplam 153 örneğin UHPLC-QqQ-MS/MS ile analiz edildiği bir çalışmada örneklerin hiçbirinde AFB_1 ve AFB_2 tespit edilmemiştir. Natürel sızma zeytinyağı örneklerinin %18'inde 0.8-1.9 µg/kg AFG_1, rafine zeytinyağı örneklerinin %9'unda 1.1 µg/kg AFG_2 ve ham pirina yağı örneklerinin %82'sinde 1.4-6.8 µg/kg AFG_2 bulunmuştur. Aynı çalışmada örneklerde ZEA ve metaboliti α-zearalenol varlığı da araştırılmış ve α-zearalenol hiçbir örnekte tespit edilmemiştir. Ancak zeytinyağı örneklerinin %51'inde 1.1-21.1 µg/kg, natürel sızma zeytinyağı örneklerinin %3'ünde 1.3 µg/kg, ham zeytinyağı örneklerinin %55'inde 0.6-25.6 µg/kg, rafine zeytinyağı örneklerinin %73'ünde 0.7-20.2 µg/kg, pirina yağı örneklerinin %7'sinde 0.7 µg/kg ve ham pirina yağı örneklerinin %7'sinde 0.6 µg/kg ZEA saptanmıştır. Araştırmacılar, örneklerde belirlenen ZEA miktarı her ne kadar Avrupa Komisyonu tarafından belirlenen 400 µg/kg sınır değerinin altında olsa da, rafinasyon işleminin son üründe bu bileşikleri giderebileceği veya en aza indirebileceği bilinmesine rağmen rafine zeytinyağı örneklerinin çoğunun (%73) ZEA içermesini dikkat çekici bulmuştur (Hidalgo-Ruiz ve ark., 2019). Elli adet zeytinyağı örneğinin incelendiği bir başka çalışmada 12 örnekte 29.17-208.54 µg/kg ZEA, 2 örnekte 17.25-57.79 µg/kg FB_1, 11 örnekte

13.25-71.42 µg/kg FB_2, 20 örnekte 0.23-0.92 µg/kg beauvericin ve 18 örnekte 1.11-2.32 AFB_2 tespit edilmiştir (Junsai ve ark., 2021).

Sonuç

Tarımsal gıda ürünlerine yaygın olarak toksijenik küfler ve mikotoksinler bulaşabilmektedir. Toksijenik küfler ile bulaşmış yağ bitkilerinde uygun koşullar oluştuğunda, küfler tarafından mikotoksinlerin üretimi yetiştirme aşamasında meydana gelebileceği gibi, yağlı tohum ve meyvelerin yüksek sıcaklık ve nem değerlerinde depolanması da mikotoksin oluşumuna yol açabilmektedir. Rafinasyon işlemi, mikotoksinleri yağlardan kısmen uzaklaştırmakla birlikte soğuk pres yağlar ve natürel yağlara bu işlem uygulanmamaktadır. Dolayısı ile bu ürünlerin söz konusu kontaminantlar ile bulaşması, artan tüketimleri göz önüne alındığında insan sağlığı açısından tehlike yaratabilir. Bu durumu engellemek için iyi üretim uygulamaları ile iyi hijyen uygulamalarına bağlı kalınarak bitkisel yağlarda üretimin her aşaması kontrol altında tutulmalıdır. Yağ hammaddelerinin yetiştirilmesi, depolanması ve işlenmesi sırasında küf kontaminasyonunu minimum düzeyde tutacak önlemler alınmalı ve mikotoksin üretimine uygun koşulların oluşumunun önüne geçilmelidir.

Diğer taraftan bitkisel yağlar ve hammaddelerinde toksijenik küf kontaminasyonu, mikotoksin varlığı, bulaşmanın hangi aşamalarda meydana geldiği ve nasıl önlenebileceği, dekontaminasyon yolları gibi alanlarda yapılacak ayrıntılı çalışmaların konuya farklı bir yaklaşım sunabileceği 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.

Finansal destek: -

Teşekkür: -

Açıklama: -

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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). 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., Ertaş, N. (2021). Development of a functional cake formulation with purpl e 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 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

FOOD and HEALTH
E-ISSN 2602-2834

is over.

Accepted manuscripts are copy-edited for grammar, punctuation, and format. Once the publication process of a manuscript is completed, it is published online on the journal's webpage as an ahead-of-print publication before it is included in its scheduled issue. A PDF proof of the accepted manuscript is sent to the corresponding author and their publication approval is requested within 2 days of their receipt of the proof.