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Effect of Freezing and Smoking on The Proximate and Mineral Composition of Flat Sardinella (*Sardinella Eba*)

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Abstract

A study was conducted to determine and compare the effects of freezing and smoking on the proximate and mineral composition of *Sardinella eba*. The results of the proximate composition were observed to be higher in the smoked fish samples compared to the frozen samples, with all parameters significantly higher ($P < 0.05$) in the smoked samples than in the frozen samples apart from moisture content. Eight minerals were quantified in *S. eba*, including five macro minerals (Ca, K, P, Na, and Mg) and three trace minerals (Fe, Zn, and Mn). The mineral content values of the smoked fish samples in all the parameters studied were statistically significantly higher than in the frozen fish samples, particularly for the trace minerals such as Zn and Mn, than in the smoked samples. In conclusion, the information obtained in this study could be useful to fish consumers, processors, and nutritionists in the efficient post-harvest management of fish resources.

Keywords: Proximate composition; mineral elements, freezing, smoking, *Sardinella eba*.

Research article

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INTRODUCTION

In the modern world, fish provides 16% of total animal protein consumed globally (FAO, 2016). Its nutritional properties have been highlighted in several studies showing that fish contains important micro-nutrients (riboflavin, iron and calcium) and fatty acids, such as omega-3, all of which are important for human health, particularly during childhood (FAO, 2016). In Nigeria, the demand for fishery products have grown more and more even with the acute shortage of fresh fish there is wide acceptance of frozen fish (Olopade, 2015). Frozen fish dominates other fish forms in total “wet equivalent” of fish consumption in Nigeria (Liverpool-Tasie et al., 2018).

Freezing preserves fish for extended periods because it prevents the growth of microorganisms that cause both food spoilage and foodborne illness. In Nigeria, the complexity of the marketing and distribution of frozen fish, couple with erratic power supply for cold storage warehouses to maintain constant freezing temperature on the fish and with higher ambient temperature make fish quality deteriorates very rapidly (Olopade, 2015). As a result of poor handling of frozen fish outside the low temperature storage space fish are being warmed and even thawed and need refreezing (Olopade 2015). Pourshamasian et al. (2012) reported that during frozen storage some of the deterioration still occurs in the stored food, during which the freezing rate and temperature fluctuation are affecting the extent of quality loss. The quality of raw material before processing is an important factor in determining the product quality.

Lack of cold storage facilities usually forces people to rely on the traditional processing methods of smoking to retain the quality and extend the shelf life of the imported frozen fish. However, the conditions of raw material are a very important factor for the quality and shelf life of the final product (Arason et al. 2014). If the fish is not fresh or is of low quality before processing, the final product quality is compromised. Fish smoking/processing will not improve the quality of the final product if the raw material was not good before processing (Oehlenschläger, 2014). Biochemical composition of the whole body indicates the fish quality. The assessment of the fish's proximate composition is important to know its nutritive value, and its better processing and preservation (Mridha et al. 2005). The principal components of fish muscle include water, protein and fat while the minor components include carbohydrates, minerals and vitamins, and extractives, such as, sugars, free amino acids and nitrogenous bases (FAO, 2014).

The measurement of some proximate profiles such as protein contents, carbohydrates, lipids, moisture contents and ash percentage is often necessary to ensure that they meet the requirements of food regulations and commercial specifications (Watermann, 2000). Minerals components such as potassium magnesium, calcium, iodine, phosphorus are important for human nutrition (Erkan and Ozden, 2007).

Sardines are widely distributed throughout the tropical and subtropical seas including the Mediterranean and Black seas (Froese and Pauly, 2017). The genus *Sardinella* species is an essential source of food for the human population and thus contribute to food security. The demand and consumption of sardines are on the rise due to availability and low cost (Kirema, 2012). *Sardinella eba* is the more commonly consumed frozen fish in Nigeria. The aim of this study was to analyze and document the proximate composition and mineral elements content of frozen (stored at an abused temperature) and later smoked *Sardinella eba*.

MATERIAL AND METHODS

Samples of *Sardinella eba* were purchased from a frozen fish market in Port Harcourt, Nigeria. The fish samples were immediately transported in ice cooled boxes to the Department of Fisheries at the University of Port Harcourt for laboratory analysis. The fish samples were washed and prepared to remove all traces of blood staining and spilled enzymes before being immediately refrigerated to keep them fresh and free of pest and bacterial infestation. The *Sardinella eba* samples were divided into two parts; one was used to determine the proximate compositions of the frozen fish, and the other was dried using a traditional locally constructed smoking kiln, which was made using a drum with the base completely opened to allow free flow of smoke to enhance the smoking of the fish

Proximate composition of fish

Proximate analysis of the muscles was carried out according to standard methods of the Association of Official Analytical Chemists (AOAC, 2000) Moisture determination Moisture was determined gravimetrically by ° desiccation at 105 C and 77 mmHg for 5 h. % Moisture = $\frac{\text{initial weight (g)} - \text{final weight (g)}}{\text{initial weight (g)}} \times 100$ % Ash content Ash content of the sample was determined by incinerating in a muffle furnace at 600°C for 3 h. % Ash = $\frac{\text{weight of ash after heating}}{\text{weight of fresh sample}} \times 100$ % Protein content The total nitrogen was determined by the Kjeldahl method (Vlieg, 1984). Total protein content was obtained by multiplying protein content by 6.25.

Crude fibre content Crude fibre was determined as loss in weight on ash after acid and base digestion of the sample. Lipid Extraction Lipid was extracted from the muscles of the fish, in triplicates, according to methods as described by Bligh and Dyer (1959) with slight modifications, as described by Widjaja et al. (2009). Five grams (5 g) of the muscle sample (1 g of liver) was homogenized with 80 ml methanol, 40 ml chloroform and 28 ml of distilled water for 2 minutes. Chloroform (40 ml) and distilled water (40 ml) was added and homogenization continued for about 2 minutes. After homogenization, it was filtered in a glass funnel, using a Whatmann No. 1 filter paper. The residue was put back in a fresh beaker and was re-homogenized with 40 ml chloroform: methanol (1:1 v/v) for about 30 seconds, then filtered. Filtrates were then combined and transferred to a separating funnel to allow for phase separation. The bottom chloroform layer was then collected after being passed through a 2.5 cm thick layer of anhydrous sodium sulphate (Na_2SO_4). The remaining aqueous layer was washed with 20 ml chloroform. The collected organic chloroform layer, containing the extracted lipids was then evaporated under vacuum at 40°C to remove the solvent and the obtained lipid kept in the refrigerator. The weight of the extracted lipid was recorded. Lipid Content (%) Lipid content (%) = amount of lipid extracted (g) x 100 % weight of original sample (g).

Mineral Determinations

For minerals analysis (Na, Mg, Fe, Mn, K, Ca, P, Zn) the ash samples were digested with 2.5 ml HNO_3 and 60% perchloric acids according to AOAC method (2005). The digested samples were used for selected minerals analysis, using atomic absorption spectrophotometer (Model A A-6200, Shimadzu, Corp., Kyoto, Japan). Two grams from the ash sample were placed in a digestion tube and pre-digested using 10ml of HNO_3 and 1ml of HClO_3 acids were added and temperature maintained at 135°C until the liquor was colourless. The digested liquors were then filtered through a whatman 1 filter paper and diluted to 25ml with distilled water. Suitable standard solutions were prepared and their absorbance measured to prepare a standard curve. The standard curve was used to calculate the concentration of mineral.

Data Analysis

The analyses were performed in triplicate and all data were expressed as mean \pm SD and compared by student's t- test.

RESULTS AND DISCUSSION

Proximate composition of frozen and smoked samples of *sardinella eba*

The proximate compositions of smoked and frozen samples of *Sardinella eba* are presented in Table 1. The results of this study revealed variations in the values of proximate composition with all parameters were significant higher ($P < 0.05$) in the smoked samples than in frozen samples apart from moisture content. Eyo (2001) reported low protein, crude fibre, ash, and high moisture, carbohydrate, and lipid content in frozen fish. It has been observed that the gradual denaturation of protein leads to a decrease in water holding capacity, thus when frozen fish is thawed, drip is produced and nutritional substances are drained away with the drip (Ciarlo et al. 1985).

The apparent increases in some proximate component contents after smoking were mainly due to water loss by evaporation during the process. In agreement with these results, similar changes in the content of essential nutrients in thermally treated fish were reported by Bastías et al. (2017). This finding is in agreement with the observations of Puwastien et al. (1999).

The percentage moisture content value ranged from 35.11±0.02 recorded for the smoked samples to 79.33±0.02 for frozen samples. Pereira et al. (2005) found 71.02% moisture in defrosted fresh sardines. Clucas and Ward (1996) reported that flesh from healthy fish contained 70–80 % water. The decrease in moisture content occurring during smoking is the consequence of water loss during this process. According to Sigurgisladottir et al. (2000), the weight loss is due to dehydration during smoking.

Akinwumi (2014), which found that freezing and smoking were more efficient preservation methods in terms of the retention of the protein value and reduction of moisture content. The percentage value of crude protein recorded for the frozen sample was 16.83±0.05 significantly lower than the smoked sample value of 48.84±0.05. Clucas and Ward (1996) reported that flesh from healthy fish contained (15–24%) protein. This result was similar to values reported in the muscles of other fish species, such as sardines and mackerel (Pourshamsian, 2012). It was observed that the crude fat content was significantly higher in the smoked samples (5.25±0.02) than in the frozen samples (0.33±0.01). Clucas and Ward (1996) reported that the flesh of healthy fish contained 1–22% fat. After the smoking process, the increase in lipid content observed could be the result of moisture evaporation (Eyo, 2001). Based on this result, the fish species can be classified as fatty fish. Fish are often classified according to fat content. Lean fish have <0.5% fat, semi-fat fish contain 0.5–2% and fatty fish have more than 2% (Clucas and Ward, 1996). The ash content in any fish sample is an indication of its mineral content (Huda et al., 2010). The ash contents were found to be in the range of 1.13±0.02 to 4.24±0.02 for frozen and smoked samples, respectively. Szenttamásy et al. (1993) found ash contents ranging from 0.08 to 1% for fresh fish meat. An increase in the ash content in smoked fish can be due to the significant reduction in the moisture content occurring during the smoking process. Fish smoking results in concentration of nutrients such as proteins and ash (Doe and Olley, 1983).

Further results revealed that the percentage of dry matter ranged from 20.47±0.02 for frozen samples to 64.90±0.02 recorded for smoked samples. The values of the nitrogen free extract of smoked samples (6.58±0.00) were significantly higher than the frozen samples (2.18±0.02).

Table 1. Percentage mean proximate values of frozen and smoked samples of *sardinella eba*

Parameters	Frozen	Smoked
% Crude Protein	16.83±0.05 ^a	48.84±0.05 ^b
% Crude Fat	0.33±0.01 ^a	5.25±0.02 ^b
% Crude Fiber	0.00±0.00	0.00±0.00
% Ash	1.13±0.02 ^a	4.24±0.02 ^b
% Moisture	79.33±0.02 ^a	35.11±0.02 ^b
% Dry Matter	20.47±0.02 ^a	64.90±0.02 ^b
% Nitrogen free extract	3.18±0.02 ^a	6.58±0.00 ^b

^{ab}Means with different superscript along same row are significantly different ($p < 0.05$)

Mineral compositions of frozen and smoked *sardinella eba*

Table 2 shows the mineral contents of the frozen and smoked *Sardinella eba*. Eight minerals were quantified in *S. eba*, including five macro minerals (Ca, K, P, Na, and Mg) and three trace minerals (Fe, Zn, and Mn), suggesting that these fish could be used as good sources of minerals. The smoking process contributed to multidirectional changes in the content of mineral elements in the fish. The mineral content values of the smoked fish samples in all the parameters studied were statistically significantly higher than those in the frozen fish samples, apart from the Fe element, which was very high in the frozen sample. Magnesium and Zinc were particularly abundant in the fish analysed. Fish meat is a rich source of minerals and the most abundant microelements are Zinc (Zn), Iron (Fe) and Copper (Cu) (Saadettin et al, 1999). Sofoulaki et al. (2018) reported this health benefit.

Fresh fish contains a significant amount of minerals in general, but processed fish, such as dried fish, have higher values (Kinsella 1986). Marimuthu et al. (2012) indicated that mineral contents in snakehead fish increased depending on the cooking methods. The results of mineral content in this study are in line with the findings of Adewoye et al. (2003) who reported that variations exist in the mineral composition of fish. The concentrations of Zn, Mn and Fe in the frozen and smoked samples are lower than the toxic levels described by (FAO/WHO 2001). The macro minerals (Ca, K, P, Na, and Mg) and three trace minerals (Fe, Zn and Mn) reported in this study were within the limits of FAO (2010) values for fish muscles.

Table 2. Mineral compositions of frozen and smoked *sardinella eba*

Minerals	Frozen	Smoked
%Ca	0.12±0.00 ^a	0.30±0.00 ^b
%P	0.08±0.00 ^a	0.25±0.00 ^b
%Mg	0.07±0.00 ^a	0.17±0.00 ^b
%K	0.09±0.00 ^a	0.21±0.00 ^b
%Na	0.07±0.00 ^a	0.15±0.00 ^b
Fe(mg/kg)	104.16±0.01 ^a	0.12±0.00 ^b
Zn(mg/kg)	10.58±0.02 ^a	18.56±0.02 ^b
Mn(mg/kg)	88.07±0.02 ^a	118.74±0.02 ^b

^{ab}Means with different superscript along same row are significantly different ($p < 0.05$)

CONCLUSION

The effects of freezing and smoking on the proximate composition and mineral contents of *Sardinella eba* were examined. Results reveal that freezing conserves the chemical composition of the fish species and the smoking process reduces the content of water, which contributed to the relative increase in the concentration of nutrients, including crude ash and crude protein, content of mineral elements and reduced the fat content. The overall results obtained from the present study indicated that both freezing and smoking processes are important preservation methods that could enhance the nutritive values of fish and possibly reduce post-harvest losses. It is concluded that *Sardinella eba* subjected to frozen abuse and smoking can still provide healthy fish in terms of minerals and nutrients.

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The Potential Uses of Olive Leaf Extracts in Various Areas

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Abstract

Olive leaves have been used in traditional remedies for centuries. In this study, the antimicrobial activity of the leaf extracts from Ayvalık Yaglik and Manzanilla varieties against food and clinical test microorganisms, fish pathogens and human milk originated lactic acid bacteria (LAB) and also sun protection factor (SPF) were investigated. The results of the disc diffusion assay indicated that the extracts showed antimicrobial activity against the tested microorganisms with inhibition zones from 8.52 mm to 19.36 mm. Most of the olive extracts did not show antibacterial activity on the LAB tested. Therefore, the extracts that has no inhibitory activity can be used together with LAB in various industries. The SPF values of the extract and the extract+cream mixture were between 0.05 and 16.46. The results indicated that the olive leaf extracts may be used as natural sunscreen additive in the cosmetics industry. In addition, the extracts may be used as natural antimicrobial substance in feed, food and pharmaceutical products as an alternative to chemical preservatives.

Keywords: Antimicrobial, Extract, Natural Preservatives, Ultra Violet, Cream

Research article

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INTRODUCTION

Olive is one of the most suitable crop for Mediterranean countries. Olive and its by-products are one of the main foods of the Mediterranean diet (Boudhrioua et al., 2008; Pereira et al., 2007). Synthetic preservatives and antioxidants provide high antimicrobial and antioxidant properties, but most of these chemical preservatives are toxic and have many side effects (Raymond et al., 2009; Chen et al., 2018). Currently, there is an increasing demand for high-quality products with low synthetic chemicals and long shelf life. Therefore, there is an urgent need to develop foods, cosmetics and medicines that used natural substances to provide self-preservation and/or protection from microbial growth. The antibacterial properties of plants have been extensively studied (Ben-Othman et al., 2020). Olive leaf can be obtained during pruning and has both antibacterial and antioxidant properties (Lee & Lee, 2010). As a result, the conversion value products have the potential to become the sustainable and environmentally friendly product that replaces current disposal (Lafka et al., 2013).

Diseases caused by food and clinical microorganisms are an important problem for public health. For example, *Escherichia coli* can be found in undercooked ground beef, raw milk, raw products, grains, as well as soft cheese made from water, unsterilized milk, and fruit juice (Poudyal et al., 2010; Karygianni et al., 2014; Liu et al., 2017). *Salmonella* usually infects humans through water and food. As a result of several studies, the antibacterial activity of olive leaf extract (OLE) against *Salmonella typhimurium* has been reported (Pereira et al., 2007; Erdogan & Turhan, 2012).

Staphylococcus aureus can cause a variety of illnesses. It can cause a variety of skin infections, including abscesses, boil, carbuncle, cellulitis, folliculitis, impetigo, acne, and burned skin syndrome (Ayana & Turhan, 2009). *Bacillus cereus* produces toxins and is common in food poisons (Ahmed et al., 2014). OLE suppressed bacterial growth (Markin et al., 2003). *Candida albicans* is a common pathogenic species that primarily affects people with immunodeficiency (Poudyal et al., 2010; Brahmi et al., 2012). The antibacterial activity of olive leaf extract has been reported to have a significant effect on the suppression of *C. glabrata* (Sudjana et al., 2009).

The healthy development of fish in aquaculture in developed and developing countries is economically and ecologically important (Suez 2019). Fish pathogens that cause disease outbreaks are considered a major threat to aquaculture (Menanteau-Ledouble et al., 2018). *Aeromonas hydrophila* and *Yersinia ruckeri* as fish pathogens causes hemorrhagic septicemia syndrome and leads to increased mortality in aquaculture (Onuk et al., 2015; Brown & Dawson, 2015).

Olive leaf has many effects such as antimicrobial (Korukluoğlu et al., 2010), antioxidant (Benavente-Garcia et al. 2000), hypocholesterolemic (Jemai et al., 2009), cardioprotective (Nekooeian et al., 2014). These beneficial effects of olive leaf are associated with the phenolic compounds in its structure (Vogel et al., 2015).

Today, the demand for probiotic-containing products that support the immune system is increasing. Probiotics are live microorganisms that have beneficial effects on host health by regulating the gastrointestinal tract (Fuller 1989). Lactic acid bacteria (LAB), which are frequently used in biotechnological studies, are of great importance as they are a group of microorganisms used extensively in the production of fermented products, industrial food fermentation, dairy in, industry and food technology. LAB is of great importance due to its use in the health and sensory properties of food products and animal feeds. Recently, scientific interest in LAB has increased due to its importance in food production and various studies have been conducted on the subject (Schaafsma 2008).

Although the sun is a source of life for all living things, it also affects the daily life of living things on earth (Matsui et al., 2009). Exposure to UV radiation can cause harmful effects such as dryness, wrinkles, pigment abnormalities and skin cancer (Nichols & Katiyar 2010). It is recommended to use sunscreen products to protect against the harmful side effects of UV rays. Sunscreen agents have a sun protection factor (SPF) value, which is defined as the ratio of the minimum erythema dose to the sunscreen agent (Riva et al., 2006). Plant extracts obtained by different methods can prevent the acceleration of some transcription factors caused by UV rays in skin cells, and plant extracts may contain bioactive substances that can help prevent harmful rays from the sun and protect the skin (de Oliveira et al., 2016). The purpose of this study is to investigate the antimicrobial effects of olive leaf extracts obtained from Ayvalık Yaglık (AY) and Manzanilla (M) varieties on food/clinical microorganisms and fish pathogens to determine its usage potential as a natural additive in the food and feed industries. In addition, it was aimed to determine the potential use of the olive extracts together with LAB as a natural additive in the food and health industries. The SPF value of the various olive leaves extracts was also purposed to determine for potential use as a cheaper and safer alternative to sunscreens containing harmful chemicals in the cosmetic industry.

MATERIAL and METHOD

Antimicrobial activity

Extract preparation

The olive leaves samples of AY and M varieties were obtained from Izmir Olive Research Institute in September 2019. The plant material was dried in an airy environment without sunlight at room temperature. The dried olive leaves are grounded. In extraction, 10 g powder from olive leaves were extracted with 30 ml of ethanol (96%), methanol, and acetone using a sonicator device (Hielscher) on ice in 3 repetitions in 10 minutes. After extraction, the solvents were evaporated. Then, the obtained solutions were sterilized by 0.45 µm Millipore filters. The extracts were kept at 4°C under dry conditions until they were used.

Determination of Antimicrobial Activity

Disc Diffusion Assay

The antimicrobial activity of olive leaf extracts was determined with disc diffusion assay. *E. coli* O157:H7, *B. subtilis* RSKK 244, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923, *B. cereus* RSKK 863, *E. faecalis* ATCC 29212, *S. sonnei* Mu:57, *Yersinia enterocolitica* ATCC 1175, *E. coli* ATCC 35218, *S. enteritidis* RSKK 171, *A. hydrophila* were cultured in Nutrient/broth (NB) at 37°C. *L. monocytogenes* ATCC 7644, *S. agalactiae* Pas. Ins. 55118, *L. garvieae*, *Y. ruckeri* were growth in Tryptic Soy Broth (TSB)/Agar at 37°C. *V. anguillarum* A4, *V. anguillarum* M1, *V. alginolyticus* in Tryptic Soy Broth/NaCl medium at 25°C, *C. albicans* ATCC 10231 and *C. glabrata* RSKK 04019 in Yeast Extract Peptone Dextrose (YPD)/Agar at 30°C, *L. gasseri* MA-1, *L. gasseri* MA-2, *L. gasseri* MA-6, *L. fermentum* MA-7, *L. fermentum* MA-8, *Lactobacillus delbrueckii* MA-9 in De Man, Rogosa and Sharpe (MRS) at 37°C were cultured. The active cultures of the test microorganisms were washed twice with physiological saline. Then, their concentrations were adjusted to 0.5 McFarland and inoculated into the appropriate solid medium. Sterile discs with a diameter of 6 mm were placed on the inoculated growth medium. Then, 20 µL of the extracts of AY and M varieties were dropped onto the discs. Petri dishes were incubated at appropriate temperatures for 24 h. At the end of the incubation period, the zones around the discs were measured and recorded. In the study, Ampicillin (AM, 10 µg/disc), Kanamycin (K, 30 µg/disc) and Fluconazole (FCA, 25 µg/disc) were used as the control. All experiments were performed in triplicate.

Determination of Minimum Inhibition (MIC) and Minimum Bactericidal or Fungicidal (MBC or MFC) Concentrations with the micro-dilution method

MIC and MBC or MFC values of the extracts were determined by micro-dilution assay against test microorganisms. Test microorganisms at 0.5 McFarland concentration were added to each tube containing the extract and medium and mixed gently. Then, the tubes containing the mixture were incubated at appropriate temperatures for 24 h. After the incubation, the concentration at which there was no growth after incubation was determined as the MIC value. Then, the samples were taken from the tubes, and spot-dropped was made on the specific agar medium and incubated at the appropriate temperatures. At the end of the incubation period, the concentrations of the extracts that prevent the growth of microorganisms on the solid medium were recorded as MBC or MFC values.

In-vitro sun protection factor (SPF)

SPF values of leaf extracts from AY and M varieties was determined in vitro. The extracts were prepared in triplicate in ethanol (96%) at a concentration of 2 µg/µL. The homogeneous mixture was measured in the spectrophotometer (Beckman Coulter) at 5 nm intervals between 290 nm and 320 nm wavelength. The values were calculated using the Mansur equation (Mansur et al. 1986) as below.

$$\text{SPF} = \text{CF} \times \sum_{290}^{320} \text{EE}(\lambda) \times \text{I}(\lambda) \times \text{Abs}(\lambda) \quad (1)$$

CF = Correction Factor (= 10)

abs (λ) = Wavelength absorbance of extracts (λ).

I (λ) = Intensity of sunlight at the wavelength (λ)

EE(λ) = Erythemetogenic effect radiation wavelength (λ)

Sun protection factor of extract and cream mixture

The developed modified method using Imam et al. (2015) and Bambal et al. (2011) protocols was used to determinate SPF value of extract and cream mixtures. For each extract, 1 g of cream was weighed separately and 0.5 g of each extract was added to this cream. The mixture was made up to 10 g with distilled water. 0.1 g of this prepared mixture was taken into another tube and 10 ml was completed with ethanol (40%). It was then sonicated for 5 minutes. After this mixture was filtered through No:1 Whatman filter paper, 0.5 ml was taken into another tube and made up to 5 ml with ethanol. 0.5 ml of the mixture was taken and the volume was completed to 2.5 ml. The mixtures adjusted as 2.5 ml, 5 ml and 10 ml were measured in 3 repetitions in a spectrophotometer at 5 nm intervals in the wavelength range of 290 nm - 320 nm. The values were calculated using the Mansur equation (Mansur et al. 1986).

RESULTS and DISCUSSION

Microbial resistance to antibiotics is increasing day by day. There are many new studies on alternative solutions to reduce the negative effects of antibiotics on human health. The studies show that olive leaves have very good biological activity (Goreishi & Shahrestani, 2009; Khalil et al., 2014). In the current study, the antimicrobial activity of leaf extracts from AY (AYOL) and M (MOL) against food and clinical microorganisms and fish pathogens and LAB were assessed using disc diffusion assay. Micro-dilution assay for determination MIC, MBC, and MFC values of the extracts also was used. The results are presented in Table 1-5. The results indicated that both AYOL and MOL inhibited the growth of all tested microorganisms with inhibition zones ranged between 8.52 - 19.36 mm (Table 1-2). MIC and MBC or MFC concentrations of AYOL and MOL extracts varied in same ranges as 10 - 40 µg/µL and 10 - 80 µg/µL (Table 3-5). All AYOL extracts inhibited the growth of all the tested yeasts. AYOL methanol extract showed the highest inhibition zone diameter for both *S. sonnei* MU:57 (19.07 mm) and *Y. enterocolitica* ATCC 11175 (17.90 mm) among food and clinical pathogens. FCA (25 µg) showed no inhibitory activity against *C. albicans* ATCC 10231. AYOL extracts showed high inhibition zone diameter (12.99 mm) on *C. glabrata* RSKK 04019. AYOL methanol extract against *V. angillarum* M1 (fish pathogen) showed high inhibition zone activity (14.42 mm) (Table 1). AYOL ethanol, acetone, and methanol extracts did not inhibit the growth of five LABs. The methanol extract showed only inhibition activity (13.90±0.7) against *L. gasserii* MA-2. It is predicted that if the olive extract concentrates are in the appropriate concentrate range, they will not prevent the growth of LAB originated from human milk (Table 1).

Table 1. Inhibition zone diameters of Ayvalık Yaglik variety olive leaf extracts and antibiotics (mm ± SD).

Strains	E	M	A	AM	K	FCA
Food and Clinical test Microorganisms						
<i>E. coli</i> O157:H7	12.72±1.5	12.46±0.9	9.83±0.2	17.76±0	19.33±0.4	-
<i>B. subtilis</i> RSKK 244	13.02±0	14.87±0.8	12.68±0.2	30.27±0.9	19.36±0.1	-
<i>B. cereus</i> RSKK 863	11.30±1.2	11.37±0.3	10.99±0.6	16.81±0.2	12.97±0.3	-
<i>S. aureus</i> ATCC 25923	11.60±0.4	13.12±0.8	11.81±0.5	21.04±0.8	19.91±0.5	-
<i>P. aeruginosa</i> ATCC 27853	12.10±0.3	11.21±0.6	10.90±0.2	23.53±0.6	20.37±0.2	-
<i>E. coli</i> ATCC 35218	11.19±0.1	11.69±0.1	11.23±0.71	18.75±0.7	14.11±0.1	-
<i>Y. enterocolitica</i> ATCC 11175	16.71±0.5	17.90±1.7	15.48±0.5	-	18.10±5.7	-
<i>L. monocytogenes</i> ATCC 7644	16.36±0.6	17.05±1	15.92±0.2	29.57±0.1	25.40±1.3	-
<i>S. sonnei</i> MU:57	14.24±0.7	19.07±0.5	15.50±0.6	13.48±1.4	11.08±0.8	-
<i>E. faecalis</i> ATCC 29212	17.32±1.3	17.41±0.6	14.89±1.2	24.02±0.3	13.48±1.4	-
<i>S. enteritidis</i> RSKK 171	12.63±0.5	15.67±0.6	11.50±0.9	14.02±0.3	15.48±1.4	-
<i>C. albicans</i> ATCC 10231	12.10±0.4	12.75±0.7	11.69±0.21	-	-	-
<i>C. glabrata</i> RSKK 04019	12.18±1	12.99±0.2	9.75±0.6	-	-	20.35±0.1
Fish Pathogens						
<i>S. agalactia</i> Pas.Ins. 55118	12.67±0.6	13.39±1.8	12.51±1.1	37.46±0.12	9.89±0.21	-
<i>V. angillarum</i> A4	13.60±1.1	12.16±0.3	11.06±0.9	9.40±0.11	13.76±0.03	-
<i>V. angillarum</i> M1	13.03±0.5	14.42±0.6	12.71±1.5	9.02±0.04	14.81±0.02	-
<i>V. alginolyticus</i>	10.10±0.2	10.33±1.4	8.81±0.2	13.57±0.09	15.05±0.11	-
<i>Y. ruckeri</i>	11.36±0.5	12.44±0.8	9.42±0.7	32.30±0.15	17.54±0.07	-
<i>A. hydrophila</i> ATCC 19570	11.05±0.6	12.33±0.3	10.45±0.9	11.20±0.06	16.16±0.05	-
<i>L. garvieae</i>	9.98±0.9	11.13±0.2	10.33±0.7	33.10±0.12	23.05±0.14	-
LAB						

<i>L. delbrueckii</i> MA-9	-	-	-	23.73±0.75	-	-
<i>L. gasseri</i> MA-1	-	-	-	23.18±2.61	-	-
<i>L. gasseri</i> MA-2	-	13.90±0.7	-	24.31±0.63	-	-
<i>L. fermentum</i> MA-8	-	-	-	28.68±1.29	-	-
<i>L.fermentum</i> MA-7	-	-	-	28.42±1.00	-	-

E: Ethanol, M: Methanol, A: Acetone, AM: Ampicillin (10 µg), K: Kanamycin (30 µg), FCA: Fluconazole (25 µg), -: No Activity, LAB: Lactic Acid Bacteria

Among food and clinical pathogens, MOL methanol extract showed high zone of inhibition activity on *S. enteritidis* RSKK 171 (19.36 mm). All MOL extracts inhibited the growth of *Candida* species. MOL ethanol extract showed high inhibition zone activity for fish pathogens on both *V. angillarum* A4 (13.37 mm) and *V. angillarum* M1 (13.61 mm) (Table 2). The MOL extracts except for ethanol and acetone extracts against *L. gasseri* MA-1 and methanol extract against *L. gasseri* MA-2 did not inhibit the growth of LAB tested. The fact that the extracts do not inhibit the development of the tested LAB indicates that the extract and this probiotic candidate LAB can be used together in the pharmaceutical, food and feed industries.

Oleuropein found in olive fruit and leaves has been reported to inhibit the growth rate of microorganisms (Sudjana et al., 2009; Lee & Lee, 2010). It has been determined that oleuropein and its degradation products have inhibitory effects on *E. coli*, *E. faecalis*, *B. cereus*, *S. aureus*, *S. enteritidis*, *V. alginolyticus*, *C. glabrata* and *C. albicans* (Furneri, 2002). In a study, the antimicrobial properties of phenolic compounds in the aqueous extracts of powdered olive leaves were investigated. It is reported that the inhibitory effect of various concentrations of the extract on microorganisms is found as *B. cereus* ~ *C. albicans* > *E. coli* > *S. aureus* > *P. aeruginosa* > *B. subtilis* (Pereira et al., 2007). In another study with the fruit methanol extract of Alcaparra olives was tested on *B. cereus*, *P. aeruginosa*, *B. subtilis*, *S. aureus*, *E. coli* and *C. albicans* responsible for human gastrointestinal and respiratory tract infection. The olive extract at a concentration of 50 mg/mL is resistant to *C. albicans* and sensitive to other tested microorganisms (Sousa et al., 2006).

In the study investigating the antimicrobial activity of commercially available olive leaf extract against test microorganisms, they found that the extract showed the highest antimicrobial activity against *S. aureus* (Sudjana et al. 2009). In a study investigating the antimicrobial effect of olive leaf extract obtained with 80% ethanol against *L. monocytogenes*, *E. coli* O157:H7 and *S. enteritidis*, it was found that olive leaf extract at a concentration of 62.5 mg/mL inhibited the growth of test microorganisms (Liu et al., 2017).

Table 2. Inhibition zone diameter of Manzanilla variety olive leaf extracts and antibiotics (mm \pm SD).

Strains	E	M	A	AM	K	FCA
Food and Clinical Test Microorganisms						
<i>E. coli</i> O157:H7	12.25 \pm 0.3	12.53 \pm 0.9	11.23 \pm 0.3	17.76 \pm 0	19.33 \pm 0.4	-
<i>B. subtilis</i> RSKK 244	14.83 \pm 0.3	16.06 \pm 0.3	14.34 \pm 0.2	30.27 \pm 0.9	19.36 \pm 0.1	-
<i>B. cereus</i> RSKK 863	11.02 \pm 0.3	11.67 \pm 0.6	10.98 \pm 0.4	16.81 \pm 0.2	12.97 \pm 0.3	-
<i>S. aureus</i> ATCC 25923	11.86 \pm 0.2	12.37 \pm 1.2	11.21 \pm 0.1	21.04 \pm 0.8	19.91 \pm 0.5	-
<i>P. aeruginosa</i> ATCC 27853	11.71 \pm 0.1	12.31 \pm 0.2	11.47 \pm 0.2	23.53 \pm 0.6	20.37 \pm 0.2	-
<i>E. coli</i> ATCC 35218	12.24 \pm 0.1	12.45 \pm 0.2	12.07 \pm 0.4	18.75 \pm 0.7	14.11 \pm 0.1	-
<i>Y. enterocolitica</i> ATCC 11175	15.48 \pm 0.8	17.92 \pm 0.9	15.16 \pm 0.8	-	18.10 \pm 5.7	-
<i>L. monocytogenes</i> ATCC 7644	17.63 \pm 0.9	18.05 \pm 0.2	13.57 \pm 0.7	29.57 \pm 0.1	25.40 \pm 1.3	-
<i>S. sonnei</i> MU:57	17.46 \pm 1.3	17.57 \pm 0.6	14.90 \pm 0.8	13.48 \pm 1.4	11.08 \pm 0.8	-
<i>E. faecalis</i> ATCC 29212	17.18 \pm 0.4	18.58 \pm 0.8	14.53 \pm 1.1	24.02 \pm 0.3	13.48 \pm 1.4	-
<i>S. enteritidis</i> RSKK 171	13.22 \pm 0.2	19.36 \pm 1.5	11.56 \pm 0.3	14.02 \pm 0.3	15.48 \pm 1.4	-
<i>C. albicans</i> ATCC 10231	13.37 \pm 0.7	12.67 \pm 0.1	10.48 \pm 0.5	-	-	-
<i>C. glabrata</i> RSKK 04019	11.45 \pm 0.6	11.92 \pm 0.5	10.53 \pm 0.3	-	-	20.35 \pm 0.1
Fish Pathogens						
<i>S. agalactia</i> Pas.Ins. 55118	12.93 \pm 0.3	12.33 \pm 0.4	10.75 \pm 0.2	37.46 \pm 0.12	9.89 \pm 0.21	-
<i>V. angillarum</i> A4	13.37 \pm 0.5	10.84 \pm 0.5	10.55 \pm 0.6	9.40 \pm 0.11	13.76 \pm 0.03	-
<i>V. angillarum</i> M1	13.61 \pm 0.6	10.28 \pm 0.4	9.02 \pm 0.4	9.02 \pm 0.04	14.81 \pm 0.02	-
<i>V. alginolyticus</i>	11.12 \pm 0.5	10.30 \pm 0.6	8.52 \pm 0.4	13.57 \pm 0.09	15.05 \pm 0.11	-
<i>Y. ruckeri</i>	12.20 \pm 1.2	10.72 \pm 1.1	9.25 \pm 0.6	32.30 \pm 0.15	17.54 \pm 0.07	-
<i>A. hydrophila</i> ATCC 19570	12.10 \pm 1.6	11.66 \pm 1.3	11.80 \pm 0.5	11.20 \pm 0.06	16.16 \pm 0.05	-
<i>L. garvieae</i>	12.32 \pm 0.2	11.39 \pm 0.8	8.53 \pm 0.2	33.10 \pm 0.12	23.05 \pm 0.14	-
LAB						
<i>L. delbrueckii</i> MA-9	-	-	-	23.73 \pm 0.75	-	-

<i>L. gasseri</i> MA-1	11.51±0.2	-	12.58±0.1	23.18±2.61	-	-
<i>L. gasseri</i> MA-2	-	16.48±0.4	-	24.31±0.63	-	-
<i>L. fermentum</i> MA-8	-	-	-	28.68±1.29	-	-
<i>L. fermentum</i> MA-7	-	-	-	28.42±1.00	-	-

E: Ethanol, M: Methanol, A: Acetone, AM: Ampicillin (10 µg), K: Kanamycin (30 µg), FCA: Fluconazole (25 µg), -: No Activity, LAB: Lactic Acid Bacteria

As with many natural products, the composition of the extracts may change due to differences such as geographical location, plant nutrition and variety, and this may have an effect on the antimicrobial activity. It is also suggested that the gathering areas of plants affect antimicrobial activity due to different soil formations (Pereira et al., 2006; Sousa et al., 2006).

Table 3. MIC, MBC or MFC values of AY and M ethanol olive leaf extracts (µg/µL).

Strains	AYOL ethanol extract			MOL ethanol extract		
	MIC	MBC	MFC	MIC	MBC	MFC
Food and Clinical test microorganisms						
<i>E. coli</i> O157:H7	40	40		40	40	
<i>B. subtilis</i> RSKK 244	40	80		40	80	
<i>B. cereus</i> RSKK 863	40	80		40	40	
<i>S. aureus</i> ATCC 25923	20	40		10	10	
<i>P. aeruginosa</i> ATCC 27853	20	40		20	20	
<i>E. coli</i> ATCC 35218	40	80		40	40	
<i>Y. enterocolitica</i> ATCC 11175	20	20		40	40	
<i>L. monocytogenes</i> ATCC 7644	40	40		40	80	
<i>S. sonnei</i> MU:57	40	80		40	80	
<i>E. faecalis</i> ATCC 29212	20	40		40	80	
<i>S. enteritidis</i> RSKK 171	20	40		40	80	
<i>C. albicans</i> ATCC 10231	40		80	40		40
<i>C. glabrata</i> RSKK 04019	40		40	40		20
Fish Pathogens						
<i>S. agalactia</i> Pas.Ins. 55118	20	40		40	80	
<i>V. angillarum</i> A4	20	40		40	80	
<i>V. angillarum</i> M1	20	40		40	80	
<i>V. alginolyticus</i>	10	20		40	80	
<i>Y. ruckeri</i>	40	80		40	40	
<i>A. hydrophila</i> ATCC 19570	40	80		40	40	
<i>L. garvieae</i>	40	40		40	80	
LAB						

<i>L. delbrueckii</i> MA-9	40	80	40	80
<i>L. gasseri</i> MA-1	40	20	40	20
<i>L. gasseri</i> MA-2	40	80	40	80
<i>L. fermentum</i> MA-8	20	20	40	40
<i>L. fermentum</i> MA-7	40	20	20	20

MIC: Minimal Inhibition Concentration, MBC: Minimal Bactericidal Concentration, MFC: Minimal Fungicidal Concentration, AYOL: Ayvalık Yaglik olive leaf, MOL: Manzanilla olive leaf, LAB: Lactic Acid Bacteria

Table 4. MIC, MBC and MFC values of AY and M varieties methanol olive leaf extracts ($\mu\text{g}/\mu\text{L}$).

Strains Food and Clinical test microorganisms	AYOL methanol extract			MOL methanol extract		
	MIC	MBC	MFC	MIC	MBC	MFC
<i>E. coli</i> O157:H7	40	80		40	80	
<i>B. subtilis</i> RSKK 244	40	40		40	40	
<i>B. cereus</i> RSKK 863	40	40		20	40	
<i>S. aureus</i> ATCC 25923	20	20		20	20	
<i>P. aeruginosa</i> ATCC 27853	20	40		10	10	
<i>E. coli</i> ATCC 35218	40	40		20	20	
<i>Y. enterocolitica</i> ATCC 11175	40	40		20	20	
<i>L. monocytogenes</i> ATCC 7644	40	40		20	20	
<i>S. sonnei</i> MU:57	40	80		20	20	
<i>E. faecalis</i> ATCC 29212	40	80		40	40	
<i>S. enteritidis</i> RSKK 171	20	40		40	40	
<i>C. albicans</i> ATCC 10231	20	20		20		20
<i>C. glabrata</i> RSKK 04019	20	20		10		10
Fish Pathogens						
<i>S. agalactia</i> Pas.Ins. 55118	40	80		40	40	
<i>V. angillarum</i> A4	40	80		40	40	
<i>V. angillarum</i> M1	40	40		40	40	
<i>V. alginolyticus</i>	10	10		40	80	
<i>Y. ruckeri</i>	10	20		40	80	
<i>A. hydrophila</i> ATCC 19570	20	20		40	40	
<i>L. garvieae</i>	40	80		40	40	
LAB						
<i>L. delbrueckii</i> MA-9	40	80		40	80	
<i>L. gasseri</i> MA-1	10	10		20	20	
<i>L. gasseri</i> MA-2	40	80		80	80	
<i>L. fermentum</i> MA-8	20	20		10	10	
<i>L. fermentum</i> MA-7	20	20		10	10	

MIC: Minimal Inhibition Concentration, MBC: Minimal Bactericidal Concentration, MFC: Minimal Fungicidal Concentration, AYOL: Ayvalık Yaglik olive leaf, MOL: Manzanilla olive leaf

Table 5: MIC, MBC and MFC values of AY and M varieties acetone olive leaf extracts ($\mu\text{g}/\mu\text{L}$)

Strains Food and Clinical test microorganisms	AYOL acetone extract			MOL acetone extract		
	MIC	MBC	MFC	MIC	MBC	MFC
<i>E. coli</i> O157:H7	40	80		40	80	
<i>B. subtilis</i> RSKK 244	40	80		20	20	
<i>B. cereus</i> RSKK 863	40	80		40	80	
<i>S. aureus</i> ATCC 25923	20	40		40	40	
<i>P. aeruginosa</i> ATCC 27853	20	40		40	40	
<i>E. coli</i> ATCC 35218	40	80		40	80	
<i>Y. enterocolitica</i> ATCC 11175	40	80		40	80	
<i>L. monocytogenes</i> ATCC 7644	20	20		40	80	
<i>S. sonnei</i> MU:57	40	80		40	40	
<i>E. faecalis</i> ATCC 29212	40	80		40	80	
<i>S. enteritidis</i> RSKK 171	40	40		40	40	
<i>C. albicans</i> ATCC 10231	40		40	40		80
<i>C. glabrata</i> RSKK 04019	40		40	20		20
Fish Pathogens						
<i>S. agalactia</i> Pas.Ins. 55118	40	80		40	80	
<i>V. angillarum</i> A4	40	80		40	80	
<i>V. angillarum</i> M1	40	80		40	80	
<i>V. alginolyticus</i>	20	20		40	80	
<i>Y. ruckeri</i>	40	80		20	20	
<i>A. hydrophila</i> ATCC 19570	40	80		40	80	
<i>L. garvieae</i>	40	80		40	80	
LAB						
<i>L. delbrueckii</i> MA-9	40	80		40	80	
<i>L. gasseri</i> MA-1	40	20		20	20	
<i>L. gasseri</i> MA-2	40	80		40	80	
<i>L. fermentum</i> MA-8	40	20		40	20	
<i>L. fermentum</i> MA-7	40	20		40	20	

MIC: Minimal Inhibition Concentration, MBC: Minimal Bactericidal Concentration, MFC: Minimal Fungicidal Concentration, AYOL: Ayvalık Yaglik olive leaf, MOL: Manzanilla olive leaf

In a report studying the activity of extracts from olive leaves, *B. subtilis* was the least sensitive, followed by *E. coli*, *P. aeruginosa*, *S. pneumoniae* and *S. aureus* (Markin et al., 2003). The similar results can be seen in the current study, *B. cereus* was more sensitive to OLE than *B. subtilis*, but was not the most sensitive microorganism.

The research investigating the effect of olive leaf on fish pathogens has been very limited. In a study, the commercially purchased olive extract was applied to hot-smoked rainbow trout (*Oncorhynchus mykiss*) fillets. According to the results of the microbiological evaluation, the shelf life of the control group was observed as 21 days, while the group that was treated with olive leaf extract exceeded the microbiological limit value on the 42nd day.

Thus, it was determined that olive leaf extract was significantly effective on shelf life (Mutlu & Bilgin, 2016). Korukluoğlu et al. (2010) was extracted olive leaf sample with acetone solvent. In the study, it was determined that the MIC value of the extracts on the test microorganisms as 26-170 µg/ml.

In a study conducted by Asan-Ozusaglam & Gunyaktı (2020), it was determined that *L. gasseri* MA-1, MA-2 and MA-6 strains were sensitive to amikacin, gentamicin, kanamycin, penicillin G antibiotics. *L. gasseri* MA-1, MA-2 and MA-6 strains have gamma hemolytic activity, have bile tolerance and the ability to survive at low pH. The LAB strains have an inhibition zone diameter of 14.29 mm -1.87 mm on the pathogenic microorganisms tested. In their study, they reported that the *L. gasseri* MA-2 strain exhibited promising probiotic properties.

In a study, a sensory evaluation of olive leaf ethanol extracts was performed after 7 days by 15 participants on probiotic *Lactobacillus acidophilus* yogurt containing olive leaf extracts of various concentrations. Significant differences between samples ($p > 0.05$) showed that increased olive leaf extract resulted in favourable taste, color, aroma, and thickness (Marhamatizadeh et al., 2013). Another study investigating the effects of spearmint on bacterial growth showed that increased levels of spearmint promoted the growth of *Lactobacillus acidophilus* and bifidobacteria in probiotic milk and yogurt (Marhamatizadeh et al., 2011). The basic characteristics of consumption of probiotic products are their medicinal properties.

The SPF value of AYOL and MOL extracts were also determined. The obtained results were calculated according to the Mansur equation and the results are given in Table 6. The SPF values of AYOL and MOL extracts were varied from 24.02 to 25.69. When the obtained SPF values were compared with the values given in Imam et al., 2015, the percentage of UV protection of AYOL and MOL extracts was found to be approximately 96%.

UV spectrophotometry is used as a simple, fast, low-cost reagent that can be used for in vitro measurements of SPF values in many cosmetic formulations. In recent years, natural compounds and bioactive products have attracted great interest as UV protectors due to their safe use, environmental problems, and few side effects as well as their antioxidant properties.

The SPF of the commercial cream and AYOL or MOL extracts mixture formulation and the commercial cream were tested (Table 6). Generally, it was observed that the mixture of AYOL and MOL extracts and cream mixture showed a higher SPF value than the commercial cream. AYOL methanol extract and cream mixture had the highest SPF value of 11.26 at 10 mL concentration, and acetone extract had the lowest value was 0.5 at 2.5 ml concentration. The highest value of the commercial cream was 1.29 at 10 ml concentration and the lowest value was 0.16 at 2.5 ml concentration. According to Table 6 (Imam et al., 2015), the highest UV protection percentage was evaluated as approximately 90% for the AYOL methanol extract and the commercial cream mixture.

Table 6. SPF values of AY and M variety olive leaf extracts and commercial cream mixture.

Extracts			AY			M			Cream		
	AY Extract	M Extract	Extract + Cream			Extract + Cream			Cream		
			2.5 ml	5 ml	10 ml	2.5 ml	5 ml	10 ml	2.5 ml	5 ml	10 ml
E	26.51	25.31	0.26	0.90	7.74	0.32	1.32	11.73	0.16	0.47	1.29
M	24.02	25.40	0.06	1.09	11.26	0.43	0.45	9.89	0.16	0.47	1.29
A	25.09	25.69	0.05	1.06	10.30	0.23	0.64	5.88	0.16	0.47	1.29

E: ethanol, M: methanol, A: acetone; AY: Ayvalık Yaglik; M: Manzanilla

Sunscreen creams can effectively absorb or reflect sunlight, particularly in the UV range (Wilkinson et al., 1982). Some of the ingredients in sunscreens are synthetic substances that can have toxic effects. Natural substances are harmless and believed to be safer to use. Natural ingredients such as olives, aloe vera, tomatoes, pomegranates, green tea, cucumbers and grapes, and botanical ingredients have the potential to block UV rays and thus have potential as sunscreens (Goswami et al., 2013; Henny & Dachriyanus, 2015). In a study, they observed that olive oil was added to the cream formulation and it was determined that the cream enhanced SPF protection. The best sunscreen formulation for refined tomato extracts, sunscreens have an SPF value of 21.09 and provide excellent protection against UV rays (Sjahjadi & Lucida, 2021).

CONCLUSION

The antimicrobial activity and sunscreen of olive leaf extracts of Ayvalık Yaglik and Manzanilla varieties were investigated. As a result of the research, it was observed that the olive leaf extract has antimicrobial activity against the tested food-borne and clinical and fish pathogen microorganisms. In addition, the results obtained showed that the olive leaf extract was capable of absorbing UV light, thus demonstrating its ability to protect against UV light. It is a better, cheaper, and safer alternative to the harmful chemical sunscreens currently used in the industry. The extracts that do not inhibit the growth of lactic acid bacteria from human milk can be used in fermented products. The fact that the olive plant is an evergreen and its easy production enhances the economic importance of this plant. The research results can be used for the development of functional foods and the preservation of foods. This study creates new alternatives to new health-based food and cosmetics markets to protect human health.

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Effects of Different Solvent Extractions on the Total Phenolic Content and Antioxidant Activity of Lemon and Orange Peels

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Abstract

In recent years, the antioxidant activity of citrus peel and their roles in the prevention of various diseases have attracted more and more human attention. Citrus peels are suggested to be a good source of antioxidants. This study focused on the effects of different solvents (ethanol, methanol, and acetone) on the total phenolic contents and antioxidant activities of lemon and orange peels. The results revealed that both orange peels extract and lemon peels extract exhibited variable antioxidant activity and total phenolic content. The ethanolic extracts of lemon and orange peels showed the highest total phenolic content value (238.1 and 387.7 mg GAE/g, respectively), while acetone extracts had the lowest values. The highest antioxidant value was determined in the ethanolic extract of orange peels as 607.67 $\mu\text{mol trolox/g}$, whereas acetone extract of lemon peels showed the lowest (55.42 $\mu\text{mol trolox/g}$) value. According to results of study, it can be concluded that solvents have a big role on the total phenolic compounds and antioxidant activity of orange and lemon peels.

Keywords: ethanol, methanol, acetone, citrus peel, phenolic compounds, antioxidant activity

Research article

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INTRODUCTION

Waste is defined as “parts of those materials which we use to meet our needs that is not currently used or disposed of after use” (Anonim, 2011; Meneguzzo et al., 2019). The amount and type of waste is increasing day by day and evaluation of different agricultural wastes is gaining importance with supporting by many studies (Ucak, 2019; Yerlikaya et al., 2017; Ucak et al., 2019; Yerlikaya et al., 2015; Ucak et al., 2018; Ucak, 2020). Therefore, solid wastes have become one of the most important environmental problems nowadays. The utilization of waste is one of the important issues, as it consists only some parts of plant grown with intensive labor and high costs while the rest is thrown away (Alkaya et al., 2010; Singh et al., 2014). Although it does not have any important economic value, some of the agricultural industrial wastes can be used as animal feed or fertilizer, while fruit and vegetable wastes such as kernel, fruit and vegetable peel, root, plant peel and leaves are mostly discarded, which creates a serious waste problem in the food and agriculture sector (Ashoush and Gadallah, 2011; Otles et al., 2015; Filimonau et al., 2019).

On the other hand, about 25% to 30% fruits yield are non-edible products such as peels and seeds (Ajila, 2010). Generally these waste by-products include high amounts of antifungal, antibacterial and antiviral compounds that can be successfully applied as a source of phytochemicals and antioxidant agents (Ayala-Zavala, 2004; Bocco et al., 1998; Singh et al., 2002; Guclu et al. 2021; Yerou et al 2017). Orange peels have been found to contain high levels of fiber, calcium, vitamin C, vitamin B6, folate and other essential nutrients in some studies (Hoffman, 1971; Economos, 1992; Okwu, 2008). These peels are rich in phytochemicals like limonoids, synephrine, hesperidin flavonoid, polyphenols and pectin.

About 170 phytonutrients can be found in just one orange and they also contain over 60 flavonoids that prevent many sicknesses and diseases (Karima, 2016; Ibri et al., 2017). Peels contain limonene which has properties that can prevent cancer. They also contain essential oils that serve as immunity boosters due to their anti-inflammatory properties (Guignard, 2000; Ibri et al., 2017). Lemon peels are also rich in fiber, vitamin C which provides 9% of daily value, minerals such as calcium, potassium and magnesium. Further to this, lemon peel has crude fiber of 15.18%, crude fat of 4.98%, protein of 9.42%, and ash content of 6.26%. Lemon juice contains 5% acid at a pH of 2 to 3 giving its sour taste (Jana, 2021). In addition, citrus fruits are the mostly growing fruit all over the world comprising valuable beneficial phytochemicals (Hou et al., 2019; Satari and Karimi, 2018; Rafiq et al., 2017). In this study, the effects of different solvents (ethanol, methanol and acetone) on the total phenolic compounds and antioxidant activity of lemon and orange peels were investigated.

MATERIALS AND METHODS

Lemon and orange peels extraction

Citrus sinensis (orange) and *Citrus limon* (lemon) fruits were purchased from a local market in Niğde, Turkey. Fruits were squeezed to extract the juice and peeled carefully using a sharp knife. Then the peels were washed by distilled water and dried at 45°C for 48 hours. Afterwards, lemon and orange peels were grounded into powder. 10 gram each of the lemon and orange peels powder was extracted in 100 mL of 70% acetone, 70% methanol, and 70% ethanol, respectively. The powder was mixed by an ultrasonic water bath for 30 min and then filtered. Ethanol, methanol and acetone were evaporated with a rotary evaporator at 50°C, 55°C and 45°C under vacuum respectively.

Analysis

Total phenolic content

The total phenolic content (TPC) was determined by using spectroscopic Folin-Ciocalteu colorimetric at 765 nm and showed as gallic acid equivalent (GAE) by using the method described by Spanos and Wrolstad (1990). The samples were put into Folin-Ciocalteu reagent and Na₂CO₃ solution and mixture was mixed by vortex and then kept in a dark site at room temperature for two hours. Total phenolic content value was determined by extrapolating calibration line which was remarked as gallic acid (mg) equivalents/mL sample (GAE/g sample). The standard curve was prepared by 160, 140, 120, 100, and 80 mg/mL solutions of gallic acid in ethanol, methanol and acetone: water with the ratio of 70v:30v.

Antioxidant activity

A 7 mM ABTS solution including 2.45 mM potassium persulfate was made and the radical solution (ABTS + •) was prepared in water by storing 12-16 hours at 24°C to exclude the influence of light. To determine the antioxidant activity of the orange and lemon peel extract as a 22 trolox response, a series of extract concentrations and trolox were prepared. 10 µl of sample was added on 1 mL ABTS + and absorbance was checked for 6 minutes that how much it decreased. The slope obtained from the graphs where the percent inhibition was drawn against the concentrations (Re et al., 1999). The standard curve was prepared by using 40, 60, 80, 100,120, 160, 170 and 180 mg/ml solutions of 1 mM trolox in ethanol, methanol, and acetone: water ratio (70v:30v).

Statistical analysis

All samples were repeated three times and analysis was carried out by using the SPSS software (Statistical Analysis System, Cary, NC, USA). Variance analysis (ANOVA) was used to evaluate the data and P<0.05 significance level of Duncan’s test was based on comparison between the mean differences of parameters.

RESULTS AND DISCUSSION

Total phenolic content of lemon and orange peel extracts

Generally phenolic compounds are known as secondary metabolites in most vegetables and fruits and other things mostly represented as a source of polyphenols like phenolic acids, flavanol, flavanones, and flavones (Singh et al., 2020; M’hiri et al., 2015). Scientists are more concerned about these compounds because of their antioxidant capacity and the aggregation between their utilization and prevention of illness and diseases.

Table 1. Changes in total phenolic content (mg GAE/g) of orange and lemon peels extracted in different solvents

	Ethanol	Methanol	Acetone
Orange peel	387.7±9.14 ^{Aa}	70.60± 0.29 ^{Ba}	33.42± 2.96 ^{Ca}
Lemon peel	238.1± 0.00 ^{Ab}	65.24± 0.29 ^{Bb}	20.62± 0.28 ^{Cb}

Different capital letters indicate a significant difference among solvents, and different lower-cases letters indicate a significant difference between groups (P<0.05).

Table 1 shows the changes in total phenolic content (TPC), after drying and analyzing the TPC of orange and lemon peel extracted with methanol, ethanol, and acetone at 70% concentrations. It was found to be 387.7±9.14 mg GAE/g, 70.60±0.29 mg GAE/g and 33.42±2.96 mg GAE/g, for orange peel in ethanol, methanol and acetone, respectively. The total phenolic content of lemon peel was 238.1±0.00 mg GAE/g, 65.24±0.29 mg GAE/g and 20.62±0.28 mg GAE/g, respectively (P<0.05). While the highest TPC value was found in the 70% ethanol group, the lowest value was observed in the 70% acetone group. Among the groups, the highest TPC value was found in the orange peel group while the lowest value was observed in the lemon peel group. Anagnostopoulou et al. (2006) notified that the TPC of orange peel extract ranged between 3.0 and 105 mg GAE/g dry extract.

While Hegazy et al. (2012) found the TPC of orange peel extract using ethanol to be 169.56 mg GAE/g, methanol 165.38 and acetone 145.79 mg GAE/g dry weight. The total phenolic content in the ethanol extract of orange peel was lower than that reported by Casquete et al. (2015) (222.76 mg GAE/100 g). However, our result was higher than that of Irkin et al. (2015) (11.08± 9.55 mg GAE/100 g). Yerlikaya et al. (2017) reported the total phenolic content of bitter orange as 8.31 g GAE/100 g. In another study, Yerlikaya et al. (2015) reported the lower total phenolic content in bitter orange and grapefruit albedo and flavedo fragments. To conclude, the TPC of citrus species depends on several factors such as the origin of species, extraction temperature and time, extraction solvent and extraction method.

Antioxidant activity of lemon and orange peel extracts

Effects of various solvents on the antioxidant activity of lemon and orange peels were presented in Table 2.

Table 2. Changes in antioxidant activity ($\mu\text{mol trolox/g}$) of orange and lemon peels extracted in different solvents

	Ethanol	Methanol	Acetone
Orange	607.67±18.92 ^{Aa}	237.44 ±4.31 ^{Ba}	224.39±4.37 ^{Ba}
Lemon	203.22±66.95 ^{Ab}	132.90±11.48 ^{ABb}	55.42± 4.37 ^{Bb}

Different capital letters indicate a significant difference among solvents, and different lower-cases letters indicate a significant difference between groups ($P < 0.05$).

In this study, it was found that orange peels have strong antioxidant activity than lemon peel. Moreover, the ethanolic extract of orange peel showed the highest value as 607.67 $\mu\text{mol trolox/g}$. This result agrees with that of Gorinstein et al. (2001) who reported that extracts comes from peel of orange have perfect and potent antioxidant activity. Acetone extract of orange peel showed the lowest antioxidant value (224.39 $\mu\text{mol trolox/g}$). The antioxidant value of lemon peel was reported highest (203.22 $\mu\text{mol trolox/g}$) in the ethanolic extract, whereas acetone extract showed the lowest value (55.42 $\mu\text{mol trolox/g}$). Jayaprakasha et al. (2006) confirmed that the rank order of the navel orange extract by acetone, methanol:water and methanol antioxidant activity result was showed acetone is better than methanol:water and methanol. Park et al. (2014) confirmed that acetone is the best extraction solvent of antioxidant compounds from orange fruit and orange peels. However, our result showed that ethanol is the best extraction solvent of antioxidant compounds from orange peel and lemon peels. It seemed that the antioxidant capacity of the orange peel extract correspond with the quantity of phenolic compounds present in each fraction, so the extracts of the orange peel and lemon peel might be a good source of antioxidants.

CONCLUSION

In the present study the effects of different solvent extraction (ethanol, methanol, and acetone) on the total phenolic content and antioxidant activity of of lemon and orange peels were determined. Orange peels showed higher total phenolic content and antioxidant activity than the lemon peels.

Additionally, the highest antioxidant activity and total phenolic content were determined in the ethanol, methanol and acetone solvents, respectively both in the orange and lemon peels. It can be concluded that the solvent plays a vital role in the extraction of the vegetable and fruit (juice, peel, seed, and pulp) constituents.

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Therapeutic and Functional Properties of Beta-Glucan and Its Effects on Health

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Abstract

Among the dietary fibers, β -glucan is naturally occurring non-starch polysaccharide formed by β -glycoside bonds of D-glucose monomers. Beta-glucan can be present in abundance in cereals, fruits, fungi, algae, yeast and bacteria. Beta-glucan has recently attracted attention with its positive effects on human health. It has effects on many systems in the body containing the immune system and cardiovascular system. Due to the positive effects of beta-glucan, it has been the subject of many studies and its effects in different fields have been demonstrated by these studies. The aim of this review is to evaluate not only the general properties of beta-glucan, but also its effects on the immune system, blood sugar, cholesterol and insulin, anticarcinogenic effect, obesity, antihyperactive effect, prebiotic and antioxidant properties, antimicrobial and antiviral effects. On the other hand, another aim is to demonstrate the purposes of the use of beta-glucan in the food industry. The application of beta-glucan is agreeable for a wide range of food materials, because of the combination of both, health-beneficial properties and technological features.

Keywords: Beta-Glucan, Dietary fiber, Disease, Functional Foods, Health

Review article

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INTRODUCTION

All over the world, the attention in functional foods increases because people understand the significance of health and balanced nutrition to prevent diseases and also the life expectancy increase. Functional foods, which are nutrients or nutritional components, have important roles in organisms. They support the body's primary food elements, regulate physiological and metabolic functions, to prevent the risk of illnesses and protect from diseases and provide a healthier life.

Recently, at the beginning of functional foods, the production of products rich in dietary fiber has started to gain importance because of its benefits to human health. For several decades, it has been observed that β -glucan not only contributes to the functional properties of food but also has important positive effects on human health, and many studies have been carried out to evaluate these effects.

According to studies about the health effects of β -glucan, consumption of β -glucan controls the glycemic response and reduce the total serum cholesterol levels in the blood so it has hypocholesterolemic effect and hypoglycemic activities (Mishra, 2020; Dhewantara 2016; Bulam 2019). Also, it can decrease the risk of coronary heart diseases (Nishantha et al., 2018), moreover it can prevent the risk of obesity and oxidative stress thanks to its prebiotic properties (Kanagasabapathy et al., 2013; Karaçil and Akbulut, 2013). In addition, it improves and modulates the immune system (Silva et al., 2017) and lower the risks of cancer like colorectal cancer (Ciecierska et al., 2019). Moreover, β -glucan shows not only anti-allergic effects (Vlassopoulou et al., 2021), but also it is an effective immune adjuvant for a vaccine (Liu et al., 2021).

Beta-glucan is considered a functional component with positive effects on health and disease prevention. In addition to its health effects, it is also used in developing innovative nutraceutical foodstuffs due to its rheological properties such as gel formation, emulsification and thickening in foods (Mishra, 2020). Furthermore, beta-glucan has abilities to change functional properties such as sensory features, rheology, texture and viscosity of food products (Kaur et al., 2020).

The mechanisms of action and receptors of beta-glucan have been demonstrated by many recent in vivo and in vitro animal experiments and studies. Beta-glucan is preferred because of its cheapness, easy accessibility, reliable consumption based on historical records, its therapeutic and functional effects (Chan et al., 2009). Therefore, it is necessary to demonstrate its benefits and properties with more studies and experiments to utilize in health and food industries.

DIETARY FIBERS

Dietary fiber is mainly plant-derived edible carbohydrate that is not digestible in the small intestines of people because mammals are not able to secrete enzymes that hydrolyze dietary fiber into its monomers (Turner and Lupton, 2011). It can ferment completely or partially in the colons of human. Dietary fiber consists of a special blend of bioactive units such as resistant starches, phytochemicals, minerals, vitamins and antioxidants (Lattimer and Haub, 2010).

Dietary fiber can be put into two main groups as soluble fiber including pectin, guar gum, psyllium, beta-glucan and insoluble fiber such as cellulose, hemicellulose and lignin. Soluble fiber can dissolve in water, however, insoluble fiber cannot dissolve in water. Foods can contain both soluble and insoluble fiber. Therefore, the food that is a good source of soluble fiber may also include some insoluble fiber (Karaçil and Akbulut, 2013). For instance, both fruits and vegetables include pectin (soluble fiber) and cellulose (insoluble fiber). However, fruits contain more pectin and vegetables contain cellulose (Samur and Mercanlıgil, 2008).

The importance of dietary fiber consumption has increased after studies have shown that dietary fiber has positive influences on particular diseases like colon cancer, obesity, and cardiovascular diseases. Moreover, it is stated that dietary fibers have impacts on obesity, blood pressure, hemorrhoids, diarrhea, some intestinal disorders, hypertension, vascular and immune diseases (Dülger and Gahan, 2011). Both soluble and insoluble fibers have various therapeutic impacts on health (Ege and Köseoğlu, 2021). Soluble fibers, form a viscous gel in nature, increase transit time, delay gastric emptying and reduce nutrient absorption. They are smoothly fermented by the gut bacteria in the large intestine and therefore have some prebiotic activities (Mudgil, 2017; Chakrabarty and Chakrabarty, 2019). On the other hand, insoluble fibers, are non-viscous in nature, have fast gastric emptying, reduce the intestinal transit time, and commonly escalate the fecal bulk that can help to lessen constipation (Chakrabarty and Chakrabarty, 2019).

The recommended dietary fiber requirement is 25-30 g per day for adults or 10-13 g per 1000 kcalorie of the daily diet (Samur and Mercanlıgil, 2008). Table-1 shows the recommended daily intake levels for the dietary fibers according to age and gender.

Table 1. Recommended daily intake levels for dietary fibers (Turner and Lupton, 2011)

Age, y	Male (g/day)	Female (g/day)
1-3	19	19
4-8	25	25
9-13	31	26
14-18	38	26
19-50*	38	25
>51	30	21

*Intakes for females rise to 28 g/day during pregnancy and to 29 g/day for lactation.

β-GLUCAN

β-glucan, one of the most significant dietary fibers, are natural and non-starch polysaccharides composed of D-glucose monomers linked by β-glycoside bonds (İşsever et al., 2018). They are hydrosoluble and viscous at low concentrations (Lattimer and Haub, 2010). They can be commonly found in fruits, algae, yeast, some bacteria, cereals (wheat, oat and barley), seaweeds and fungi (Ege and Köseoğlu, 2021; Du et al., 2019; Şimşekli and Doğan, 2015).

β-glucan can be divided into two categories as cereal or non-cereal derived depending on their origin. Cereal sources of β-glucans are oat, rice and barley, besides non-cereal sources are mushrooms, bacteria, algae and seaweed (Murphy et al., 2021).

Differences in molecular weight, degree of branching, compatibility, and intermolecular combination are the factors that can affect the biological activity of β-glucan. Differences in the way glucose molecules bind to each other give each β-glucan unique structural differences (Keser and Bilal, 2008). Beta-glucan's structures and features can also differ according to the isolation source. For instance, the structure of the beta-glucan isolated from the bacteria of *Euglena gracilis* has a linear β 1,3-glucan. In turn, the structure of the fungal type of beta-glucan (*Schizophyllum commune*) has a short β 1,6 branched β 1,3-glucan. Also, the structure of β-glucan isolated from cereal such as Barley has a linear unit-linked via a mixture of β-(1→3) and β-(1→4) bonds (Seo et al., 2019). Briefly, Figure-1 demonstrates that there are different types of beta glucan depending on their structures, respectively.





β-glucan type	Structure	Description
Bacterial		Linear β 1,3-glucan (i.e. <i>Euglena gracilis</i>)
Fungal		Short β 1,6 branched β 1,3-glucan (i.e. <i>Schizophyllum commune</i>)
Yeast		Long β 1,6 branched β 1,3-glucan (i.e. Black yeast)
Cereal		Linear β 1,3 / 1,4-glucan (i.e. Barley)

Figure 1. Structures and description of different types of beta-glucan (Seo et al., 2019)

Beta-glucans can have different types of linkage, the charge of polymers, molecular weight, viscosity, the degree of branching, solution conformation as well as chain length. Therefore, these various types of beta-glucans can have different physical roles and biological effects (Khorshidian et al., 2018).

The viscosity of beta-glucan is positively related to the health-benefiting effects as well as preventing diseases such as diabetes mellitus, cardiovascular diseases, colon dysfunction and cancer (Mishra, 2020). The viscosity changes with the concentration and molecular weight of beta-glucan. At low concentration under the 0,2% , beta-glucan becomes a Newtonian solution and viscosity is not affected by increasing shear rate. On the other hand, if the concentration is above the 0,2% with high molecular weight, the solution becomes more viscous and pseudoplastic form, i.e. Pseudoplastic form is associated with both high molecular weight and high concentration (Anttila et al., 2004). In general, beta-glucan with high molecular weight is more effective in biologically in comparison to beta-glucan with low molecular weight (Mishra, 2020). Although, beta-glucan with high molecular weight shows more biological activity, according to Lei et al. (2015), stated that β -glucan derived from yeast with low molecular weight is better candidate as an immunostimulant and antioxidant as comparison with the high molecular weight of beta-glucan. Another factor is the degree of branching of beta-glucan that affects the biological activities of beta-glucan. If the degree of branching frequencies of beta-glucan are between 0,2-0,33, they show positively more biological activity. For instance, it is known that beta-glucan with the branching ratio between 0,2-0,33 are more powerful immunomodulators (Han et al., 2020). Additionally, branched beta-glucan is more biologically active in comparison with unbranched beta-glucan (Mishra, 2020). Beta-glucan can exist in three different conformation which are single helix, triple helix and random coil, respectively. According to studies, it is declared that beta-glucan with triple helix shows more biological activity (Han et al., 2020). Another study shows that single helix beta-glucan is less able to control growth of tumours as compared to beta glucan with triple helix (Falch et al., 2000). Other feature of beta-glucan is chain length. Beta-glucan with short chain has more mobility and is more able to form junctions with near chains and then easily rearranging itself. With this property, beta-glucan with short chains extensively used to prepare pseudoplastic solution in food industries (Mishra, 2020).

The content of the beta-glucan changes according to environmental factors, cultivators and sources (Mishra, 2020). Table-2 shows the various sources of beta-glucans below. According to this table, the highest content of β -glucan is barley as 2-20 g per 100g dry weight.

Table 2. β -glucan Content (g per 100g dry weight) of some types of cereals (El Khoury et al., 2012)

Type of Cereal	β -glucan Content (g per 100g dry weight)
Barley	2-20
Oat	3-8
Sorghum	1.1-6.2
Rye	1.3-2.7
Maize	0.8-1.7
Triticale	0.3-1.2
Wheat	0.5-1.0
Durum Wheat	0.5-0.6
Rice	0.13

Beta-glucan, has an important place in human nutrition, provides favourable effects on hypertension, total and low-density lipoprotein content, blood sugar, insulin level and gastrointestinal health (Yuca et al., 2019). β -glucan has several effects, containing immunomodulatory effects, anticarcinogenic activities, lipid-lowering effects, as well as the ability to decrease blood sugar levels and control weight (Kim et al., 2006).

Therefore, beta-glucans have a wide application area, particularly in medicine and pharmaceutical, food industries, cosmetics and chemical sectors, as well as in veterinary medicine and feed production because of their properties (Ciecierska et al., 2019).

According to The US Food and Drug Administration (FDA), it is suggested that it is required to consume 3 g or more oat's b-glucan intake per day to get health benefits.

THE EFFECTS OF β -GLUCAN ON HEALTH

β -glucan shows several beneficial effects on human health because of its physicochemical properties. It can be active in all living species from earthworms to people and is able to induce humoral and cellular immunity, regulate diabetes metabolically, urge wound healing, decrease psycho-physical stress, reduce chronic fatigue syndrome and hinder the development of cancer (Sima et al., 2018).

Consumption of β -glucan and dietary fiber, which has been shown and is being proven to have an impact on many different health problems, has an important role in promoting health and preventing diseases (İşsever et al., 2018). Figure-2 below, a summary of the health benefits of beta-glucan is shown. According to this figure, beta-glucan has various properties on health. They are prebiotic properties, hypocholesterolemic properties, immunomodulatory and anti-tumour properties, antioxidant properties and hypoglycemic properties, respectively.

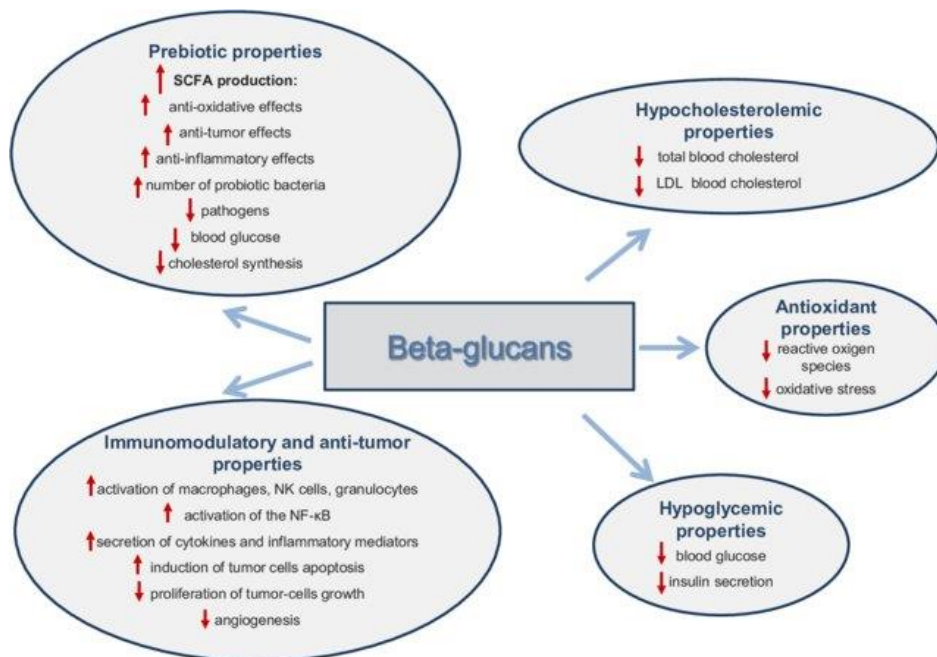


Figure 2. Summary of the health effects of Beta-glucan (Ciecierska et al., 2019)

IMMUNE ENHANCEMENT

β -glucan can be effective in strengthening the response of the fight against microorganisms such as viruses, bacteria, parasites and fungal pathogens in living things (İşsever et al., 2018). It has been shown by many studies that beta-glucan has a stimulating and regulating effect on the immune system in humans, and these effects are stated in much relevant literature. It is known that beta-glucan shows this effect by increasing the functional effects of macrophages, natural killer cells (NK cells) and neutrophils that affect the immune system (Keser and Bilal, 2008).

Additionally, beta-glucan increases the secretion of both cytokines and inflammatory mediators in the immune system (Chan et al., 2009). In addition, beta-glucan can bind to macrophages and white blood cells on the binding system and activate them. Furthermore, the studies demonstrate that beta-glucan increases phagocytosis, lysosomal enzyme activity, IL-1 (interleukin) production function and accelerates macrophage studies (Şöhretoğlu and Uz, 2015).

It has been shown by studies that zymosan, which is obtained from the yeast cell wall and known to contain high amounts of beta-glucan, activates the body's immune system (Ciecierska et al., 2019). According to another study, it was observed that the frequency of catching colds decreased with the use of β -glucan in healthy people (Şöhretoğlu and Uz, 2015). In their study, Talbott and Talbott (2019) showed that the use of β -glucan in marathon runners reduced the symptoms of upper respiratory tract infection and also improve the mood states of runners. Moreover, in in vitro studies on animals and humans, it has been stated that beta-glucan stimulates humoral and cellular immunity, thereby increasing the effect and activity of immune cells (El Khoury et al., 2021).

ANTITUMOR EFFECTS

Another important feature of β -glucan is its anti-cancer effect. Beta-glucan is an important compound used in cancer prevention and cancer treatment such as lung, breast, ovarian and prostate cancer. For example, a beta-glucan known as Lentinan has long been used in the treatment of stomach and colorectal cancer in Japan (İşsever et al., 2018). In studies conducted on cancer patients, it has been shown that with β -glucan therapy tumor size is reduced, infiltration and metastasis are stopped, thus with β -glucan therapy, life expectancy increases (Şöhretoğlu and Uz, 2015). According to Louie et al. (2010), when 200 μ g/ml beta-glucan was applied together with 10000 IU/ml IFN- α 2b, the development of pancreatic cancer cell T24 in vitro was reduced by 75% and more successful results were obtained compared to IFN- α 2b application alone.

Nowadays, one of the new trends in cancer treatment is monoclonal antibody therapy targeting tumor tissue. In studies where monoclonal antibodies and β -glucans were used in combination, these combinations were found to be more advantageous in terms of tumor regression in the short term and survival in the long term compared to the use of monoclonal antibodies alone (Driscoll et al., 2009).

Recent studies in China and Japan has been concentrated on the anti-cancer properties of beta-glucan, also the studies demonstrated that the activities of beta-glucan to be similar to radiation and chemotherapy without the side effects (Lam and Cheung, 2013).

ANTIOXIDANT ACTIVITY

Oxidative stress, which is caused due to imbalance between the production of free radicals and antioxidant defenses, is one of the main reasons for aging and some serious diseases such as cardiovascular disease, diabetes, inflammatory disease, cerebral disease and also cancer (Kofuji et al., 2012; Betteridge, 2000). Beta-glucan has many biological effects, including antioxidant effects, anticancer and scavenging of free radicals in the body. Beta-glucan can prevent the chain reaction caused by reactive oxygen species (ROS) by capturing reactive oxygen by donating hydrogen, thus reducing the damage to the body by ROS (Maheshwari et al., 2019).

(2012) show that β -glucan has a scavenging ability against the hydroxyl radicals that cause various diseases and aging. In an experiment conducted on rats to demonstrate the antioxidant property of beta-glucan, it was determined that orally administered beta-glucan prevented oxidative stress in important internal organs such as the liver and kidneys in rats. As a result of this experiment, it was understood that beta-glucan showed antioxidant properties and prevented the negative effects of free radicals formed in the body (Bayrak et al., 2008).

ANTIMICROBIAL AND ANTIVIRAL EFFECTS

One of the biological effects of beta-glucan is its antimicrobial and antiviral properties. Studies have shown that beta-glucan protects against infections caused by both bacteria and protozoa. In studies on mice, it has been reported that treated with beta-glucan, mice are extra resistant to infections caused by bacteria like *Staphylococcus aureus* and parasites like *Leishmania braziliensis* (Geller and Yan, 2020). In addition, it has been reported that beta-glucan has antibiotic properties especially against infections caused by bacteria resistant to antibiotics. Beta-glucan also has a protective effect against microorganisms such as *Candida albicans*, *Streptococcus suis*, *Plasmodium berghei*, *Staphylococcus aureus*, and *Escherichia coli* (Vetvicka and Fernandez-Botran, 2018).

Geller and Yan (2020) have shown that beta-glucan can protect against infections caused by SARS-CoV-2 and prevent serious clinical symptoms, thanks to its anti-viral properties. Although there is no definitive treatment method for COVID-19 yet and there are authorized/approved vaccine candidates, there are significant obstacles in finding an ideal treatment method without side effects. It is thought that oral beta-glucan can be used as a COVID-19 β -WIFE vaccine adjuvant (Ikewaki et al., 2021).

ANTIHYPOCHOLESTEROLEMIC EFFECT

There are several cholesterol-lowering mechanisms of β -glucans, which ought to be included in the diet in the prevention of cardiovascular disease (CVD) and the treatment of the disease. It is known that β -glucans can form a gel on the mucosal surface of the gut, like a pulp. This gel structure hinders the absorption of bile salts and stimulates bile salt synthesis in the liver. Increasing bile salts, on the other hand, activate the use of circulating cholesterol and lower its levels in the blood (Ege and Köseoğlu, 2021). Since β -glucan has soluble and insoluble forms, which interact with lipids and bile acids in the intestine, and as a result of this interaction, they lower the cholesterol level in the blood (Sima et al., 2018). Othman et al., (2019) indicated that beta-glucan derived from oat has the ability to reduce low-density lipoprotein (LDL) cholesterol and total blood cholesterol levels. According to the rule of the US Department of Health and Human Services (1997), consumption of at least 3 g/day of beta-glucan obtained from oats is recommended for a significant reduction in cholesterol levels and to reduce the risks of coronary heart diseases.

In a study of 2 men with diabetes, patients were given a low glycemic index breakfast for 4 weeks, containing 3 g beta-glucan derived from oat. After comparison with the control group, a 12% decrease was observed in plasma cholesterol levels of this group (Kabir et al., 2002). Another study showed that 0.5 g of concentrated beta-glucan significantly increased the production of short-chain fatty acids. Short-chain fatty acids are effective in preventing hepatic cholesterol synthesis. In this way, it contributes to lowering the level of LDL cholesterol (Queenan et al., 2007).

ANTIHYPERGLYCEMIC EFFECTS

Another important effect of beta-glucan is its antihyperglycemic effect. The molecular weight of β -glucan adjusts the glycemia status. In one study, a beverage containing oat β -glucan (5 g) with a molecular weight of 70000 Daltons (Da) provided lower postprandial glucose and insulin levels than a beverage containing barley β -glucan (5 g) with a molecular weight of 40000.

In research with beta-glucan obtained from *Saccharomyces cerevisiae*, it has been shown that this product can be used as a nutraceutical as well as to lower blood sugar and reduce pain in diabetic patients (Morshed et al., 2013). Kim et al. (2009), showed that regularly consumed barley grains containing beta-glucan reduced insulin response. In addition, it has been stated that obese women at high risk of developing insulin resistance should consume 10 g of beta-glucan per serving. It has been reported that it would be beneficial to add whole-grain foods containing high amounts of beta-glucan to the diets of obese women with hyperglycemia. Additionally, as in the case of glycemia, the amount of β -glucan is crucial in insulin responses. It has been observed that there is a continuous decrease in insulin secretion depending on the amount of β -glucan in oats in overweight individuals, and daily consumption of 3.8 g of β -glucan has significant effects (Beck et al., 2009).

OBESITY

Obesity is defined as the abnormal accumulation of body fat and is associated with increasing serious illness, disability and finally death. Obesity is a condition that should be treated because it is an important risk factor for chronic diseases and its negative effects on quality of life, and the basis of treatment is adequate and balanced nutrition and lifestyle changes (Şahin, 2018). Obese people have excessive adipose tissue and dyslipidemia. Therefore these conditions can cause many chronic diseases, such as metabolic syndrome, hypercholesterolemia, hypertension as well as diabetes, and these diseases are serious risk factors for CVD (Sima et al., 2018).

Beta-glucan from barley and oat has been known to decrease appetite and also lose weight in humans. Oat, which is a functional food, gives satiation along with nutrition thanks to its dietary fiber content of β -Glucan (Maheshwari et al., 2019). In Japan 100 subjects consumed a mixture of rice and barley with high β -glucan (test group, 4.4 g β -glucan per day) and barley without β -glucan (placebo group, 0.0 g β -glucan per day). As a result of the study, it was determined that the group consuming β -glucan barley significantly reduced body weights, waist circumference and body mass index (Aoe et al., 2017). Another study conducted on the mice shows that diet with oat beta-glucan decrease not only fatty liver but also adipocyte size in mice. As a result of this study, beta-glucan inhibits high fat diet caused obesity (Xin-Zhong et al., 2015).

PREBIOTIC ACTIVITY

Beta-glucan shows prebiotic activity with various ways. Thanks to beta-glucan, there is an increase of production of short chain fatty acids (SCFA). Moreover, it can help to increase the growth of health-beneficial probiotic microorganism populations and restrict the development of pathogenic microorganisms (Schmidt, 2020). With these biological effects, beta-glucan acts as prebiotic material. According to Chaikliang et al. (2015), beta-glucan derived from *Auricularia auricula Judae* and *Schizophyllum commune* Fr are possible prebiotics. Another study implies that beta-glucan derived from barley is possible prebiotic material into the manufacturing of beer as well as baked food (Ren et al., 2018).

β-GLUCAN IN THE FOOD INDUSTRY

Nowadays, functional foods have been rapidly growing day to day to respond to the needs of consumers and growing populations all across the world. Apart from the benefits of the field of health and nutrition, beta-glucan has many different functional properties. These properties include thickening, emulsification, stabilization and gelling in the foods (Ahmad et al., 2012). With the aim of developing functional foods, the use of grains in various food formulations and the development of grain-based foods have been the subject of much research in recent years (Özcan et al., 2013). Beta-glucan is used in the food industry as an important ingredient in the content of many food products such as breakfast cereals, sports nutrition products, bakery products, and fat replacers (Bulam et al., 2019).

In a study conducted with yogurt to demonstrate the functional effects of beta-glucan on foods, it was determined that the sensory properties of yogurt with beta-glucan added were improved. For example, Sahan et al., (2008) investigated the possibility of using β-glucan as a hydrocolloid fat replacer in the production of nonfat yogurt. In the study, an increase in the viscosity of yogurt occurred with the addition of β-glucan during 15 days of storage. It has been determined that β-glucan is suitable for use, especially at 0.25-0.50%, with its dietary fiber properties and positive effects on the physical and sensory properties of non-fat yogurts (Sahan et al., 2008). As a result of this study, the storage quality of yogurt increased by decreasing the separation of whey and increasing its water-binding capacity (Kearney et al., 2011).

The potential use of β-glucan as a hydrocolloid is determined by considering its rheological properties. For this reason, it is stated that β-glucan can be used as a thickening agent in ice cream formulations, meal and salad dressings. In addition, it is seen that β-glucan is used in grain-based pasta and various bakery products (Lazaridou et al., 2003). Moreover, thanks to its properties, beta-glucan can be used as an alternative thickener in traditional beverages instead of alginates, pectin, gum arabic, carboxymethyl-cellulose (Giese, 1992). Beta-glucan is applied in different types of food products such as meat products, dairy products and cereal products. In meat industry, beta-glucan can be added to produce low-fat meat, meatballs, probiotic sausages and reduced-fat sausages. In cereal industry, it can be added to bread, noodles, low-fat cakes, muffins, pastas and gluten-free bread. Also, there are applications of beta-glucan in dairy industry. It can be ingredient in cheese, yogurt, milk and probiotic drink. On the other hand, there are other types of applications such as functional beverages, soups and chocolates that beta-glucan is added in (Kaur et al., 2020).

CONCLUSION

Overall, the therapeutic and functional properties of beta-glucan have been investigated through existing studies and literature. Thanks to these properties, it has many effects on human and animal health. In general, it has properties such as reducing total cholesterol and LDL cholesterol levels, acting as anti-cancer, stimulating the immune system, stimulating the glycemic response, preventing obesity, preventing cardiovascular diseases, anti-inflammatory and antioxidant properties. In addition, due to its functional and rheological properties, it is used to improve the sensory properties of products produced in the food industry.

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Turkish Traditional Fermented Plant-Products as Functional Food

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Abstract

Fermentation is one of the oldest techniques to produce microbiologically secure and long-lasting food. The significance of fermented plant food dates heaps of years again. Fermentation grew to become famous with the sunrise of civilization due to the fact it preserves food and gives it a variety of tastes, forms, and different sensory sensations. Moreover, over time, human beings have realized the dietary and therapeutic value of fermented meals and drinks, making fermented ingredients even greater popular. In this study, Turkish traditional plant-based foods' medicinal properties: table olive, turşu, vinegar, fermented carrot juice, boza (fermented millet drink), and tarhana are examined. For this purpose, an overview of functional food and fermented meals books, journals, articles, and websites was once conducted. According to the research, the aforementioned fermented plants are functional foods advisable for health.

Keywords: fermented plant-products, functional food, Turkish fermented food

Review article

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INTRODUCTION

Functional foods are becoming increasingly popular among health-conscious customers who want to improve their overall health and well-being. Food consumption is no longer restricted to satiating hunger or supplying essential nutrients. People are motivated by various health concerns, the adverse effects of unhealthy food, and a desire to live a healthier lifestyle, which has resulted in a dramatic shift in current dietary habits. To some extent, all foods are functional since they provide taste, scent, and nutritional value. Even so, foods are increasingly being studied in-depth for additional physiologic effects that may help to minimize the risk of chronic disease or otherwise improve health (Hasler, 2002).

The name "functional food" was first seen in books and articles in 1984, when the Japanese started to investigate the links between nourishment, sensory satisfaction, fortifying, and the modulation of physiological systems (Siró et al., 2008; Lindner et al., 2013). According to Gibson and Williams (2000), a product can be considered 'functional' if it has been sufficiently proved to beneficially one or more particular functions in the body, beyond appropriate nutrition, in a way that enhances well-being. Functional foods are divided into two extensive categories: plant-origin and animal-origin. Fermented plant products are foods in the plant-origin category.

Fermentation is a gradual breakdown process that occurs in the absence of oxygen and results in converting complex organic compounds into simpler chemicals. Organic acids, carbon dioxide, ethanol, formic and acetic acid, polysaccharides, peptides, hydrogen peroxide, and bacteriocins are among the metabolic products produced by fermentation (Ankolekar and Shetty, 2011). According to Ercoşkun and Ertaş (2003, p. 38), many chemical, biochemical and microbiological changes occur in the fermentation process and as a result improvement of taste, smell, aroma, texture and color characteristic come true. In addition, according to Blandino et al. (2003), fermentation is a natural technique to minimize the volume of material transported, remove undesired components, improve the nutritional value and view, and create a safer product.

Studies on health effects are carried out separately, *in vivo* or *in vitro*, and each product is researched on different diseases. Such studies are necessary because research on health effects is laborious and time-consuming. It is essential to research such products in Turkish Cuisine due to the tendency of people to natural products instead of medicines and the benefits of functional fermented foods. This review attempts to describe the health advantages of the most prevalent Turkish traditional fermented plant products as well as some of the fermented products' microbiological and biochemical features. Firstly, the definition and history of functional food, fermented food and fermented plant products are described. And after, some of fermented plant products used in Turkey and their health benefits are explained.

History of fermented functional food

People who couldn't find food in all circumstances had searched ways to preserve their food (Higman, 2012). Fermentation is one of the most ancient and cost-effective ways of food production and preservation (Prajapati and Nair, 2003; Rhee et al., 2011). Food became spoiled when bacteria developed undesirable odors or tastes in it or toxins that caused illness or death, and humankind learnt to shun it. But, humankind learnt to enjoy and seek foods with appealing scents, flavours, and textures if microorganisms produced them. According to Steinkraus (2004), this was the origin of fermented foods, which now include boza, sauerkraut, wines and beers, lactic acid products like *turşu* and hundreds of other fermented foods. Nowadays, plant-based foods are treated in this manner to achieve the desired sensory attributes of fermented foods and better digestibility and nutritional value. Fermented plant products are nutrient-dense and have a good impact on human health. Vitamins, particularly vitamin C, dietary fiber, mineral salts, and antioxidants, are abundant (Malinowska-Pan'czyk, 2012).

Fermentation aids in the preservation and enhancement of f&b nutritional content. Fermented beverages have thus played essential roles in the development of our technology and culture, helping to the advancement and intensification of agricultural, horticultural, and food-processing skills, owing to their claimed medicinal, nutritious, and sensory advantages (McGovern et al., 2004). Hutkins (2008) indicates that fermented foods were crucial for faraway militaries and fleets because of their greater preservation longevity.

There are various arguments about the first use of the fermentation method. According to Prajapati and Nair (2003), the beginning of fermented products is gone in ancient since the flavor of fermented stuff may have been discovered by chance. Again, according to the authors, the art of fermentation originated in the Indian subcontinent, in the settlements that predate the great Indus Valley civilization back in 10.000 BC. As for Blandino et al. (2003), the earliest records appear in the Fertile Crescent (Mesopotamia) and date back to 6000 BC.

Again, the earliest evidence of these techniques, according to Lindner et al. (2013), trace back to 6000 BC. In addition, regarding the usage of fermentation, especially on drinks since Neolithic times; 7000-6600 BC in China (McGovern et al., 2004), BC 6000 in Anatolia (Vouillamoz et al., 2006) and BC 3150 in ancient Egypt (Cavalieri et al, 2003) there are archaeological researches related to use of fermentation. Table 1 lists the major turning points in the chronology of fermented foods.

Table 1: Turning Points in the Chronology of Fermented Foods

Milestone	Development - Location
ca. 10,000 B.C. to Middle Ages	Evolution of fermentation from salvaging the surplus, probably by pre-Aryans
ca. 7000 B.C.	Cheese and breadmaking practiced
ca. 6000 B.C.	Wine making in the Near East
ca. 5000 B.C.	Nutritional and health value of fermented milk and beverages described
ca. 3500 B.C.	Bread making in Egypt
ca. 1500 B.C.	Preparation of meat sausages by ancient Babylonians
2000B.C.-1200A.D.	Different types of fermented milks from different regions
ca. 300 B.C.	Preservation of vegetables by fermentation by the Chinese
500-1000 A.D.	Development of cereal-legume based fermented foods
1881	Published literature on koji and sake brewing
1907	Publication of book Prolongation of Life by Eli Metchnikoff describing therapeutic benefits of fermented milks
1900-1930	Application of microbiology to fermentation, use of defined cultures
1970- Present	Development of products containing probiotic cultures or friendly intestinal bacteria

Source: Milestones in the History of Fermented Foods, (Abdelrahman et al., 2010; Prajapati and Nair, 2003)

Turkish fermented plant-products as functional food

Nutrition is the most critical factor that affects human’s physical and mental health, productivity and happiness in every division of life. In the past, nutrition with fermented food had great importance in continuing human’s existence. Fermented foods have a long history of being thought to be good for human health in ways that ordinary foods aren't (Machve, 2009). Fermented plant products Table olive, turşu, vinegar, fermented carrot juice, boza (fermented millet drink) and tarhana are the most common Turkish fermented plant products. In the following pages their health benefits are examined individually.

Table olive

Table olive is mainly consumed, especially as breakfast foodstuff in Turkey. The olive is eaten up only after fermentation due to the fact fermentation gets it more digestible and decreases the stinging and cytotoxicity of its phenols (Lindner et al., 2013). Hutkins (2008) states that over 90% of all olives produced in the world are used to make oil, with approximately 7% to 10% being consumed as table olives. Table olives can be prepared in a variety of ways. In olive-producing countries, three commercial table olives are processed: kalamata olives, green olives, and black olives. The black table olives have the highest production volume in Turkey, followed by green and kalamata type olives (Ünal and Nergiz, 2003). Fermentation is required to generate a high-quality end product (Heperkan 2013; Iorizzo et al. 2016; Şanlıer et al., 2019). Olives are fermented with the help of lactic acid bacteria (LAB) and yeast. Fermentation improves the table olive's taste characteristics, creates beneficial volatile chemicals, encourages the growth of LAB, protects against harmful microorganisms, and reduces polyphenols (Şanlıer et al., 2019).

Table olives are high in a variety of critical micronutrients, essential fats, and physiologically active phytochemicals comprising polyphenols, many of which have been linked to various health advantages (Tokoşoğlu et al., 2010). They have a high concentration of monounsaturated fats, particularly oleic acid. Monounsaturated fats, according to Mateljan (2007), are vital in the cell membrane. They have a protective impact on the cells and minimize the chance of cellular inflammation and damage because they are less readily destroyed than polyunsaturated fats. According to the author, table olive is additionally a rich source of vitamin E, the bodies principal liposoluble antioxidant. In addition to vitamin E and monounsaturated fatty acids, Table olive includes several phytonutrients (polyphenolic compounds, oleuropein, and hydroxytyrosol) with significant antioxidant activity and can thus safeguard cellular health. Olives are characteristic of high oil content (12%-30%) (Malinowska-Pan'czyk, 2012). According to García-González et al. (2008), much has been said about the preventive impact of olive oil on cardiovascular illness and cancer. Furthermore, because high levels of free radicals have been linked to health disorders, including osteoarthritis, asthma, and rheumatoid arthritis, the anti-inflammatory activity components present in olives can help lower the harshness of these illnesses. Table olive is also a rich source of various other nutrients that have health-promoting properties. Digestive health promoting fibre, energy producing iron, and free radical scavenging copper are among these nutrients (Mateljan, 2007). Another study (Celik et al., 2019) found that in pregnancy, the mother's daily consumption of Turkish fermented olive can decrease the occurrence of infantile atopic dermatitis.

Turşu (fermented vegetable)

Humanity has practiced the preserving of vegetables by fermentation for 4500 years, according to Malinowska-Pan'czy (2012). It made it possible to eat vegetables out of season and on long journeys Fang (2013) states that in accordance with what archaeologists and anthropologists credit, the old Mesopotamians developed pickled items about 2400 BC. Fang said that cucumbers carried from India 4000 years ago helped start a pickling tradition in the Tigris Valley. Turşu, in its broadest meaning, refers to any vegetable or fruit maintained with salt or acid (Hutkins, 2008). Turşu is one of Anatolia's earliest merchandise of fermentation used by humans; which's name originated from the Persian phrase “torsh”; which meaning “sour” (Kabak and Dobson, 2011). With occasional variations by region, the mixture contains vinegar, salt, water, garlic and spices that drown fresh vegetables such as carrots, beets, eggplant, cauliflower, turnips and cucumber. Turşu is available in two varieties: simple and mixed (Çetin, 2013). In Turkey, cucumber, cabbage, green tomato, carrot, pepper, and garlic are frequently used to make mixed turşu (Erten and Tangüler, 2014). Also, in the preparation of turşu, ginger, parsley, mint leaf, dill leaf, and bay leaf are frequently utilized as flavoring ingredients. (Kabak and Dobson, 2011). Turşu may have many health benefits as its main ingredient is vegetables. According to Liu (2003), eating veggies is a feasible method for consumers to enhance their health and lower the risk of chronic illnesses. Riboli and Norat's (2003) research shows that eating fruit and vegetables lower risks of cancer of the oesophagus, lung, stomach, breast, bladder, and rectum. Turşu is also a good resource of micronutrients. Many research has demonstrated that nutrients like iron, copper, zinc, selenium, beta-carotene, vitamins (A, C, E), and folic acid can affect several immune system constituents, according to Huffnagle and Noverr (2008). Furthermore, they have a function in illness prevention and health promotion. Garlic is used as a flavouring agent in all kinds of turşu. According to Hasler (2002), garlic was proven in clinical research to have a moderate blood pressure-lowering impact, and a growing body of epidemiologic evidence implies a reverse link between garlic consumption and some types of cancer, particularly stomach cancer. Most notably, fermentation can give a product probiotic qualities. The LAB probiotics in turşu can help avoid cirrhosis and diarrhea. (Swain et al., 2014).

Probiotic consumption has been shown to aid in the treatment of diarrhoea, colon cancer, lactose intolerance, cholesterol, immunological function and infections, mineral absorption, blood pressure, irritable bowel syndrome, and colitis in studies (Çetin, 2013).

Vinegar

Vinegar is an old flavouring that can be used as a pickling ingredient or even a medicine due to its germicidal properties (Steinkraus, 2004). The usual course of changes in fruit liquid at ordinary temperatures is alcohol ferment by yeasts, accompanied by oxidizing the alcohol to acetic acid by bacteria. If enough acetic acid is produced, the product is vinegar. The product is described as a sauce made from sweet or amylaceous substances through an alcoholic fermentation accompanied via an acetous one (Machve, 2009). Cider vinegar and vinegar of grapes are the most preferred kinds of vinegar in Turkey. These kinds of vinegar contain several micronutrients, vitamins, enzymes and pectin necessary for a balanced diet (Muller, 2009). Vinegar helps get rid of pathogens in food. Ilkin and Karapinar's (2005) study showed that dressing salad with vinegar would reduce pathogens numbers to a low or undetectable level. Vinegar is also good for high blood pressure. Research (Kondo et al., 2001) showed that vinegar remarkably lowered blood pressure in rats than controls not given vinegar. Vinegar also helps to prevent cancer. Research on human cells by Mimura et al. (2004) showed vinegar have anti-cancer properties due to its polyphenol and acetic acid properties. Shishehbor et al. (2008) showed that vinegar (apple cider) enhanced the blood lipids in diabetic and normal rats by lowering serum triglycerides (TG), increasing serum HDL-c, and decreasing serum LDL-c. With the evidence mentioned above, it would not be wrong to say that vinegar is one of the healthiest products used in kitchens.

Shalgam juice

Shalgam Juice is a hazy, sour soft drink with a purplish red colour that is popular in Turkey's southern provinces (Erten et al, 2008). The juice is made by fermenting black carrots, turnips, bulghur flour, sourdough, salt, and freshwater with lactic acid. The anthocyanins found in the black carrot give shalgam juice its purplish-red colour (Kabak and Dobson, 2011). Because of its rich mineral, vitamin, amino acid, and phenolic content, shalgam juice is a nutritious beverage (Altay et al., 2013). Carrot, the main ingredient of shalgam juice, is high in vitamins (A-D-B-E-C-K) and minerals (potassium, calcium, iron, phosphorus and sodium). Also, carrot contains a high amount of carotenoids, mainly beta-carotene (Kun et al., 2008). Betacarotene, ascorbic acid (Vit C), and "non-nutritive chemicals" such as indoles, flavones, and isothiocyanates are all abundant in turnips the other main ingredient of Shalgam. According to Field (2000), these substances help to avoid food-related diseases and conditions like scurvy and blindness. As Krinsky and Johnson (2005) indicate, the carotenoids and other antioxidants found in carrots are essential for inhibiting and/or interrupting oxidative degradation as well as counteracting free radical activity. According to Field (2000), research has associated high-vitamin A diets with a lower risk of cancer, and studies also suggest that compounds like indoles can counteract the effects of carcinogens. Ascorbic acid is a well-known antioxidant that is claimed to protect against the growth of some malignancies and to slow the progression of the virus HIV. In addition, İncedayi et al., (2008) indicate that turnip bulb is high in cancer-fighting glucosinolates that help the natural immune detoxifying mechanisms work better. Glucosinolates work as "indirect" antioxidants, stimulating the body's inherent antioxidant defenses. Moreover, Baysal et al., (2007) state that shalgam juice helps lose weight, reduces stress and prevents the common cold.

Boza (fermented millet drink)

Boza is a sweet, somewhat bitter to slightly sour, light to dark beige, non-alcoholic beverage popular in Turkey and the Balkans (Blandino et al., 2003). The beverage is made by fermenting barley, oats, maize, millet, wheat, or rice (Botes et al., 2007). According to Akpinar-Bayizit et al., (2010), boza has been recognized for centuries in Central Asia, and it was transferred to Anatolia and Europe through migration. For authors, despite the fact that the Turks were the first ones to create boza, the subject was heavily suppressed by researchers, and boza was released to the market as a national product by other countries. In Turkey, it is typically served with cinnamon and is drunk primarily throughout the winter months. As boza is high in carbohydrates and vitamins, it was used to feed the Ottoman army (Işın, 2011). Boza has been a popular drink consumed as a daily foodstuff by people of any age because of its delicious taste, aroma, and excellent nutritional benefits (Blandino et al., 2003). Boza has a protein content of 0.5–1.6%, a carbohydrate content of 12.3%, and a 75–85% moisture content (Kabak and Dobson, 2011). According to Birer (1987), it is also called liquid bread due to its many nutrient elements. Petrova and Petrov (2011) state that boza has been shown to provide many health benefits such as balancing blood pressure, enhancing milk manufacturing in lactating females and facilitating digestion. It is also vital nutrition for physically active persons because it contains vitamins A, C, E, and four different forms of B. Boza is particularly ideal for vegans and vegetarians because it is fully plant-based and a vital source of vitamins, making it an excellent alternative for dairy-based beverages. In addition, its cereal content, such as barley, oat, millet and others, helps reduce TG; thus, it lowers the chances of cardiovascular disease and paralysis (Mindell, 2009).

Tarhana

Tarhana is breakfast or meal soup typically served with bread and veggies. It is made by combining wheat flour, yoghurt, yeast and various veggies (tomatoes, onions, green peppers, and so on), salt, and spice (mint, paprika), then fermenting it for one to seven days (Daglioğlu, 2000). The mix is sun-dried and crushed to a granular size of 1 mm after fermentation. (İbanoğlu and Ainsworth, 2004). Although yoghurt gets in the mixture, tarhana is a fermented cereal (plant) product. Some other similar foods that match tarhana include "kishk" and "kushuk" in the Middle East, "trahana" in Greece, and "atole" in Scotland (Bilgiçli et al., 2006).

According to Daglioğlu (2000), it is known from the history books that tarhana was first produced by Turks who arrived in Mid-Asia, and it was transported to Anatolia, the Middle East, and the Balkans by Turkish incomers. In Turkey, tarhana is prepared at home byways that have been learned through mother or grandmother since the olden days. Tarhana tastes sour and acidic with a distinct sourdough flavor and is a high source of proteins, vitamins, and minerals, making it ideal for nourishing youngsters and the elderly (Ekinci and Kadakal, 2005).

Tarhana's composition fluctuates between the following ranges: 6.4 – 13.9 % moisture, 12.0 – 29.9 % protein, 41.8 – 77.5 % carbohydrate, 1.6 – 18.2 % fat, 0.1 – 3.1 % fibre, 0.56 – 10.4 % salt, and 1.4 – 14.2 % ash. Tarhana is high in minerals, including sodium, calcium, potassium, iron, zinc, magnesium, and copper. (Kabak and Dobson, 2011).

In addition, research made by Kilci and Gocmen (2014) showed that oat flour had another positive effect on mineral composition, improved phenolic acid composition and increased antioxidant activity of tarhana.

CONCLUSION

Fermentation is the world's oldest technique of food preservation after drying (Prajapati & Nair, 2003; Rhee et al., 2011). Fermented food products have historically become an essential part of humankind's diet and have long been thought to provide health advantages. The purpose of the current study was to determine the health effect of plant-based fermented food in Turkey. For this reason, this study investigated research on plant-based fermented foods. A growing body of data supports the idea that functional foods having physiologically bioactive constituents may promote health. Fermented foods may lower the risk of hypertension, diabetes, obesity, high cholesterol, diarrhoea, thrombosis, and other diseases (Şanlıer et al., 2019). This kind of food is given desired attributes by microorganism and enzyme activities. Functional food may be defined as a comprehensive combination of necessities plus extra dietary elements that can play a vital role in lowering health risks and increasing health. Based on the health benefits of fermented foods, they can be considered functional food. Considering such health benefits of fermented plant-based food, consuming this food should be encouraged instead of convenience food. Plant-based fermented foods have a long shelf life and are available year-round. It is essential that the health benefits of such products are used in the marketing of the products. The study contributes to our understanding of the health advantages of Turkish fermented food. A limitation of this study is that the study was conducted with a literature review. The issue of functional food is an intriguing one that could be usefully explored in further research. The synergistic effects of these foods, which contain both phyto phenolic components and probiotics, should be investigated. More information on functional foods would help us to establish a greater degree of accuracy on this matter. Thus, further research in this field would greatly help the health benefit of plant-based fermented food.

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The Impact of Climate Change on Food Production in Europe

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Abstract

Food availability and food safety are main concerns with globally increased population. There are many factors involve which can affect the food production and food security. Approximately worldwide food production is obtained from agriculture sector. Climate change is a major factor which can influence the food production at maximum level in agriculture sector. In this paper we discuss about effects of climatic variations on food crop production in European countries. It was observed from previous studies that temperature, rainfall and precipitation are main factors which can affect the food crop productivity. Mostly Northern and southern regions of Europe are effected by climatic variations. High temperature, low rain fall, long frost periods all can affect crop productivity in different regions according to geographic locations. In recent years high temperature was noticed as a major factor to disturb the agriculture ecosystem by increasing drought and heat stress. High temperature is also favorable for the reproduction of many insects' pests which can damage the crop and stored food. It is also dangerous for livestock by increasing mortality rate of livestock and due to scarcity of fodder because of high temperature. So there should be a government responsibility to give awareness among farmers to overcome these problems by making new strategies by changing of crop varieties and adjusting crops in alternate cultivating areas.

Keywords: Food production, Climate change, European countries, agricultural ecosystem

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INTRODUCTION

There are numerous factors that influence food security such as international trade, technological and socio-economic progress, and use of farm land. Climate variability is one of the several reasons that can cause changes in the natural ecosystem and happening of food protection vulnerabilities. These vulnerabilities can rise at any stage of the food chain, from prime production level to feeding, and climate variation might have direct and subsidiary effects on these happenings. Increasing or decreasing changes in climatic values affect living things negatively and cause a decrease in productivity, especially in agricultural production (İstanbuluoğlu et al., 2013). Increasing world population, changing climate conditions and economic activities are growing with each passing day makes it more important than water (Bağdatlı and Bellitürk, 2016).

Basically climate variability affects productivity of agricultural crop in six direct or indirect ways which are given following:

- (1) Direct effect of increasing concentration of CO₂ on crop yield and on efficiencies of available sources (Kimball et al., 2002; Ainsworth and Long, 2005). CO₂ and greenhouse gases accumulated in the atmosphere descend to the earth with precipitation. This event is called acid rain. Acid rains change the pH of the water and affect the life of the living creatures in the water. It causes the natural structure of plants to deteriorate (Bağdatlı and Can, 2019). In particular, measures to minimize the impact of greenhouse gases should be taken all over the world and will trigger this increasing the necessary studies and measures to minimize the emissions of carbon emissions will play an important role in reducing the effects of global warming (Bağdatlı and Ballı, 2019). The increment of greenhouse gasses emissions in atmosphere along with the global warming and the changes of temperature and precipitation regimes, have lots of negative effects on agricultural crop production (Bağdatlı et al., 2015).
- (2) Direct effect of rainfall, temperature, radiation, precipitation and humidity on crop growth and development (Olesen and Bindi, 2002). Excessive increase and decrease of temperatures negatively affect the life of living things. It will be difficult to find clean water in the future as the increase of temperatures will increase the evaporation level (Bağdatlı and Can, 2020).
- (3) Direct damage of crop due to extreme weather conditions like heat waves, storm and flood.
- (4) Indirect effect by variations in suitability of diverse crops, basically a north side spreading out of warm-season crops (Carter et al., 1996; Fronzek and Carter, 2007),
- (5) Indirect effect by nutritional modifications in crop food, growth of weeds and pests and occurrence of diseases
- (6) Indirect damage by degradation of the basic source like “soil erosion” and environmental pollution like leakage of nitrate.

There are numerous other ways through which climate associated features can impact food security comprising, marine acidification and warming, and variations in the transference manners of complex pollutants. Increment in temperature and modified patterns of rainfall have an effect on the perseverance and configurations of existence of microorganisms and the configurations of their resultant foodborne infections (FAO, 2008c).

Europe is a major and furthestmost productive provider of food and roughage. It was noticed in 2008 that Europe provided nineteen percent of worldwide meat productivity and twenty percent of worldwide cereal products. Approximately eighty percent of the European meat and sixty three percent of the cereal products are produced in the European countries. The production of European agriculture is mostly high, specifically in Western Europe, and regular cereal yield in the European countries is higher than the world regular yield (Olesen et al., 2011).

Climate variations in different regions of Europe effects agriculture outputs in different ways. In northern regions, climate variation might predominantly have positive influence by increasing yield and types of growing crop species, while there might be negative influence of agriculture on, the quality of surface water.

With the increase in temperature due to climate change, evaporation increases. This causes the evaporation of water resources. (Albut et al., 2018). In southern regions, the drawbacks will be dominated with lesser crop yields, unevenness in yield amount and a decline of appropriate land for customary food crops (Alcamo et al., 2007). In this paper we give brief description of impacts of climate change on food crop productivity in different regions of Europe.

EFFECTS on CROP PRODUCTIVITY in EUROPE

The impacts of climate variation and augmented atmospheric carbon dioxide are probably lead to generally small upsurge in food crop yield in European countries. Though, effects of climate change can be minimize by using advanced technologies like development of novel varieties of crop and advanced agricultural practices (Ewert et al., 2005). In recent times, cereal products have presented significant less yields, showing that climate variation have greater effect on yield than advanced techniques (Kristensen et al., 2010).

Higher yields of food crops were associated with Climate variation in northern regions of Europe (Alexandrov et al., 2002; Richter and Semenov, 2005), whereas the lesser yield of all crops in the Mediterranean regions, including south regions of European Russia (Alcamo et al., 2005; Maracchi et al., 2005). Commonly reduction in crop yield and upsurge in water requirement are estimated for spring season crops in southern region of Europe (Giannakopoulos et al., 2009), while the influence on autumn season crops can vary according to geographic location (Santos et al., 2002).

In northern side European countries duration of the growing period, hoar frost of late spring and initial autumn and availability of sun heat are usual climatic controls (Olesen and Bindi, 2002). In this type of seasonal conditions short growing period is the main reason of less yield of food crops. For instance in the growing period in Germany is one to three months lengthier than in Scandinavian countries (Mela, 1996), but then again it also differs significantly with the elevation as in Austria varies up to three months (Trnka et al., 2009).

In Nordic countries small growing period is the leading cause for the lesser yields of wheat. Cereal productivity is less in Mediterranean regions due to heat stress, availability of water, and less time period of crop maturation. Therefore permanent food crops including olive, grapevine and fruit trees are more vital in this constituency (Alcamo et al., 2007).

The productivity of some spring season crops including maize, soybeans and sunflower can be decreased in southern Europe (Audsley et al., 2006). Whereas, the productivity of autumn season crops including spring and winter wheat depends on the geographic location. Probably the lesser productivity can be found in the furthestmost southern regions and higher productivity in the northern regions including northern areas of Spain and Portugal (Santos et al., 2002; Minguet et al., 2007).

Some food crops that generally grow in southern regions of Europe will turn out to be more appropriate later in northern regions or in higher elevated regions of south.

The prognoses for an assortment of production circumstances demonstrate a 30–50 percent upsurge in appropriate area for maize productivity in Europe at the end of the 21st century, involving Scotland, Ireland, Finland and southern part of Sweden (Hildén and Lethtonen, 2005; Olesen et al., 2007).

The productivity of food crops is less in northern regions of Europe due to cool temperatures (Holmer, 2008), while productivity of food crops in southern regions of Europe is less due to low rainfall and high temperatures (Reidsma and Ewert, 2008). There are many references which shows that growing temperature is a main factor in reduction of food grain productivity globally (Lobell and Field, 2007).

Numerous fruit trees can be easily effected by spring hoar frost during flowering period. A warm climate will develop both the period of the previous spring hoar frost and the periods of flowering, and the threat of flower buds destruction due to late hoar frost are probably endure unaffected (Rochette et al., 2004). Moreover fruit trees also can be damaged by prompt autumn hoar frost, however, there might be a major issue of increased development of pests & diseases (Salinari et al., 2006).

EXTREME WEATHER

The polar ice caps are melting, sea level is rising and soil losses are experienced in coastal areas. Sea level due to melting of glaciers increasing the temperature rose from 10 to 20 centimeters (Bağdatlı and Bellitürk, 2016). Influence of the global climate will have an effect on the change of seasons, especially in the observation of significant changes in temperature and precipitation (Bağdatlı and Arslan, 2020).

The anticipated extreme changes in weather including droughts and high temperature can severely affect the food crop productivity (Meehl and Tebaldi, 2004; Schaër et al., 2004, Jones et al. 2003) and decrease the usual production (Trnka et al., 2004). Specifically, in Mediterranean regions of Europe, frequently weather can be changed during growing period of crops like rainfall during sowing and drought or heat pressure during flowering time are probably decrease the productivity of summer season crops like sunflower (Moriondo et al., 2010).

Warm climate is also more favorable for the production of insect pests, since several insects can complete their reproductive cycles in this warm season (Bale et al., 2002).

LIVESTOCK PRODUCTION

Climatic variations like heat pressure and droughts also can be effected on livestock. The expected upsurges in temperature level in Britain could increase the death rate of broiler chickens and pigs (Turnpenny et al., 2001).

Higher drought stress beside the Atlantic coast can decrease the production of fodder crops to that extent where fodder yield will not be enough for livestock until the provision of water irrigation (Holden and Brereton 2002, 2003; Holden et al., 2003).

High temperatures might also upsurge the danger of diseases in livestock through supporting the insects dispersal, which are main carriers of many viral infections from year to year, and also give strength to those insect carriers which become bounded in cooler temperatures (Wittmann and Baylis 2000; Mellor and Wittmann 2002; Colebrook and Wall 2004; Gould et al., 2006).

CONCLUSION

It is concluded from this study that there are many climate variable factors which are disturbing the food crop yield throughout the Europe. In recent years continuously increase in temperature and diverse patterns of precipitation as extensive upsurges in northern regions of Europe and somewhat lesser reductions in southern regions of Europe was observed. These modifications in climate are probable to disturb agronomic ecosystems at all stages including crop yields, crop protection, environmental effects and livestock. Therefore, adaptation policies should be acquaint to decrease damaging effects and to achieve promising positive effects of climate variation. It is necessary for agriculture advisors to increase awareness about climate variations among farmers, that how to manage and overcome the effects of these variabilities.

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Effect of Chitosan Coating Enriched with Peppermint Essential Oil Emulsion on the Microbiological Quality of Fish Meatballs

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Abstract

In this study, the effects of chitosan coating incorporated with peppermint essential oil (PMO) emulsion on the microbiological quality of fish meatballs were determined. For this purpose, chitosan coating solutions were prepared with (PMO) and without (CF) 1% concentration of peppermint essential oil emulsion. Fish meatballs were coated with these chitosan solutions and stored at 4±1°C for 18 days. One group left as control (C) without chitosan coating. Total psychrophilic bacteria (TPB), total mesophilic bacteria (TMB), total Enterobacteriaceae and total lactic acid bacteria (LAB) were evaluated. According to the results of study, the TPB count of the C and CF groups were reported as 7.59 and 7.15 log CFU/g at the end of storage, while it was found as 6.14 log CFU/g in the PMO group. Total mesophilic bacteria count of fish meatballs coated with only chitosan was determined as 5.93 log CFU/g at the end of the storage and the lowest (5.18 log CFU/g) TMB count was observed in the fish meatballs treated with chitosan coating enriched with peppermint oil emulsion. The highest LAB counts were determined in C and CF groups throughout the storage period. At the end of the storage, PMO group showed the lowest LAB count as 4.54 log CFU/g. The results of the study revealed that the usage of peppermint essential oil emulsion in the chitosan coating is an effective way to inhibit microbial growth in the fish meatballs during the cold storage.

Keywords: chitosan coating, fish meatball, microbial quality, mackerel, peppermint oil, LAB

Research article

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INTRODUCTION

In recent years, the increase in the number of working women and people living alone in search of durable and easy-to-serve food causes ready-made food technology to gain increasing importance (Kılınççeker et al., 2009). The production and consumption of products such as fish burgers, fish balls and fish croquettes are spreading rapidly in the ready-to-eat food sector, and these products are among the ready-to-eat foods that are loved and consumed. Along with the technological developments in the world, important technological developments have been recorded in the fish processing sector in our country as well. Studies show that storage at temperatures below -12°C inhibits microbial growth and slows down enzymatic activity (Rodriguez-Turienzo et al., 2011). However, cooling or freezing alone is not sufficient to completely inhibit lipid oxidation, protein denaturation and microbial activity. For this reason, it has become common today to use more than one processing and packaging technology together.

Packaging technology is widely used to extend shelf life, maintain hygiene and maintain quality, especially in foods sensitive to microbial and oxidative spoilage (Ahmad et al., 2012).

Due to the increasing consumer awareness and sensitivity to the environment recently, there is pressure to avoid the use of synthetic substances and there is an increasing trend towards the use of substances obtained from natural sources. Many studies show that edible coatings made of protein, polysaccharide and lipid-containing materials help extend the shelf life of foods and maintain their edible quality qualifications (Kılınççeker et al., 2009). The most widely used biopolymers are protein and polysaccharides. Chitosan is a natural and cationic polyaminosaccharide obtained from different degrees of alkaline deacetylation of chitin. It is one of the most abundant polysaccharides in nature, and its use as a coating material is very convenient and common.

The use of edible coatings with plant extracts, essential oils and antioxidants can provide some benefits such as improving the organoleptic and nutritional properties of the product they are applied to (Bourtoom, 2008; Falguera et al., 2011). Recently, essential oils have been used as natural antioxidant and antimicrobial agents in edible coatings to increase the shelf life of perishable foods such as fish. While many studies have been conducted on the effects of edible films and coatings on the maintenance of quality of fish and fish products (Renur et al., 2016; Ebadi et al., 2019; Ucak et al., 2021; Ucak, 2020; Hosseini et al., 2016; Alsaggaf et al., 2017; Ucak, 2019; Ucak et al., 2019; Shahbazi et al., 2018), there is not enough information about the chitosan coating supplemented with peppermint essential oil on the quality of mackerel meat balls. Therefore, in this study, it is aimed to create emulsions with peppermint essential oil and to apply the chitosan coating prepared with this emulsion to fish meatballs. It is aimed to preserve the microbiological quality of fish meatballs coated with chitosan during cold storage.

MATERIALS AND METHODS

Materials

Mackerel fillets were supplied fresh from the fishermen in the Niğde region. They were placed in styrofoam boxes together with ice and delivered to the laboratory as soon as possible. The fish were cleaned quickly; the internal organs and bones were removed and grounded into minced meat. Peppermint oil was supplied commercially from a local market in Niğde.

Method

Fish meatballs preparation

Minced fish was placed in a bowl and mixed in a homogeneous way by adding weighed substances in certain sizes. Each meatball was prepared as 30 gr and consist of 83.75% minced fish meat and 16.25% other additives (10% breadcrumbs, 2% salt, 0.5% cumin, 0.5% sweet red pepper, 0.5% allspice, 0.5% ground black pepper, % 1 sunflower oil, 1.25% garlic powder) (Keser, 2019).

Preparation of chitosan coatings

Chitosan coating solution was prepared according to the method of Ojagh et al. (2010). One of the chitosan coating solution was prepared without adding the essential oil emulsion.

Fish meatballs were immersed in the prepared chitosan solutions for 30 seconds and left for 2 minutes, then the fish meatballs were immersed in the solution for a second time for 30 seconds and allowed to dry for 2 minutes (Ojagh et al., 2010). Then the samples were taken into styrofoam plates and covered with stretch film. The control group was not coated with any chitosan solution. All samples were stored at $4^{\circ}\text{C}\pm 1$ for 18 days and quality analyzes were carried out periodically every 3 days.

Microbiological analyzes

For the determination of total mesophilic and total psychrophilic bacteria counts Plate Count Agar (PCA) was used by the spread plate method (ICMSF, 1982). The plates were incubated at 8°C for 7 days for total psychrophilic bacteria counts and at 37°C for 24-48 h for total mesophilic bacteria counts, respectively. Total *Enterobacteriaceae* were enumerated according to the method of FDA (1998) by the use of Violet Red Bile Agar (VRBA). Pour plating method was performed incubating at 37°C for 36-48 h. For the enumeration of lactic acid bacteria, spread plate method was used on Man Rogosa and Shape (MRS Agar) agar. Petri dishes were then incubated in anaerobic jars at 30°C for 48 hours.

Statistical analysis

Statistical analyzes were performed with SPSS software (Statistical Analysis System, Cary, NC, USA) and Duncan multiple comparison test (One-way Anova at $P<0.05$ significance level) were applied to compare the data obtained.

RESULTS AND DISCUSSION

One of the main causes of spoilage of fish and fish products is bacterial growth. The effect of chitosan coating on the microbial quality of fish meatballs is given in Fig. 1. Total psychrophilic bacteria (TPB) count was determined as 2,94 log CFU/g at the beginning of storage and increased in all groups during the storage period. It was observed that the number of TPB in fish meatballs coated with chitosan supplemented with 1% peppermint oil emulsion was significantly ($P<0.05$) lower than the C and CF groups during storage.

While the TPB count of the C and CF groups were 7.59 and 7.15 log CFU/g on the 18th day of storage, it was 6.14 log CFU/g in the PMO group. The lowest TPB count values during storage were observed in fish meatballs samples coated with chitosan supplemented with 1% peppermint oil emulsion. In a study in which rosemary extract was added at different concentrations (0.4% and 0.8%) to mackerel burgers, it was reported that the total number of bacteria during storage was lower than the control group (Ucak et al., 2011). Keser and Izi (2020) found the initial psychrophilic bacteria count as 4.22 log CFU/g in trout meat. In the study where the number of psychrophilic bacteria in fish burgers prepared with different antioxidants (thyme, sage, laurel, green tea) was found to be 4.90 log CFU/g at the beginning, it was reported that the number of TPB in the groups to which antioxidant was added during the frozen storage was lower than the control group (Özoğul and Uçar, 2013).

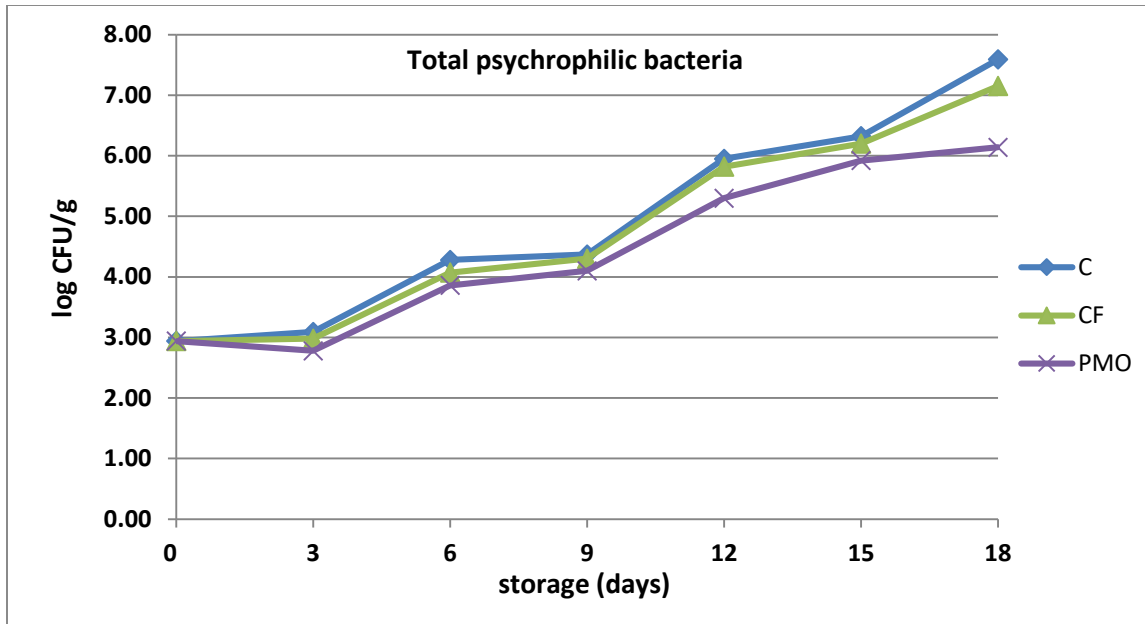


Figure 1. Changes in total psychrophilic bacteria count of fish meatballs coated with chitosan enriched with peppermint essential oil emulsion during storage at 4°C. C: Control without gelatin film, CF: fish meatballs coated with chitosan, PMO: fish meatballs coated with chitosan enriched with 1% peppermint essential oil emulsion.

The changes of total mesophilic bacteria (TMB) count of mackerel meatballs coated with chitosan are presented in Fig. 2. At the beginning of the storage TMB count of mackerel was found to be 2.32 log CFU/g. This value was increased in all groups throughout the storage period and showed the highest value as 6.24 log CFU/g in control group at the end of the storage.

Total mesophilic bacteria count of fish meatballs coated with only chitosan was determined as 5.93 log CFU/g at the end of the storage period, whereas the lowest (5.18 log CFU/g) TMB count was observed in the fish meatballs treated with chitosan coating enriched with peppermint oil emulsion.

In the study conducted by Keser and İzci (2020), the total bacterial count of the trout meatballs prepared with laurel and rosemary essential oils was found to be considerably higher (5.24 log CFU/g) than the current study. They also reported that essential oils of laurel and rosemary slowed the growth of bacteria. Ucak (2020) reported the initial viable count of trout burgers as 2.92 log CFU/g.

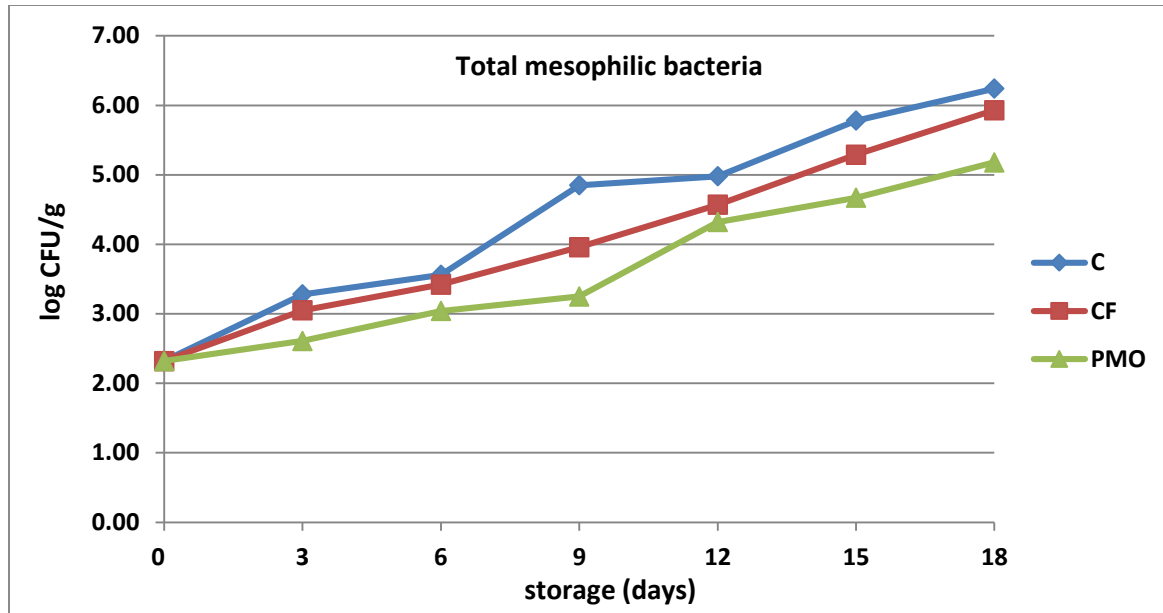


Figure 2. Changes in total mesophilic bacteria count of fish meatballs coated with chitosan enriched with peppermint essential oil emulsion storage at 4°C. C: Control without gelatin film, CF: fish meatballs coated with chitosan, PMO: fish meatballs coated with chitosan enriched with 1% peppermint essential oil emulsion.

Lactic acid bacteria (LAB), which are optional anaerobes, were reported as the part of the natural microbiota of fish fillets. The impacts of chitosan coating on the lactic acid bacteria (LAB) count of fish meatballs were given in the Fig. 3.

The initial LAB count of mackerel was determined as 2.11 log CFU/g and increased in all groups until at the end of the storage. The highest values were observed in control and CF groups, respectively during the storage period. Lactic acid bacteria count of control group was 5.64 log CFU/g at the end of the storage, while this value was 5.49 log CFU/g in the CF group. At the end of the storage, PMO group showed the lowest LAB count as 4.54 log CFU/g. According to Fernandez et al. (2012) LAB, which are gram-positive bacteria, are susceptible to essential oils.

Cai et al. (2014) reported that the LAB counts increased in the sea bass fillets during the storage period, but was low since LAB group grow slowly at refrigeration temperatures. In another study, the initial LAB value of rainbow trout fillets were found as 3.08 log CFU/g and increased throughout the storage period (Agdar GhareAghaji et al., 2021).

Control group showed higher LAB values than the fillets treated with edible coating containing orange peel essential oil. In the present study, control and chitosan coating treated groups also showed higher values than the group coated with chitosan containing peppermint essential oil emulsion.

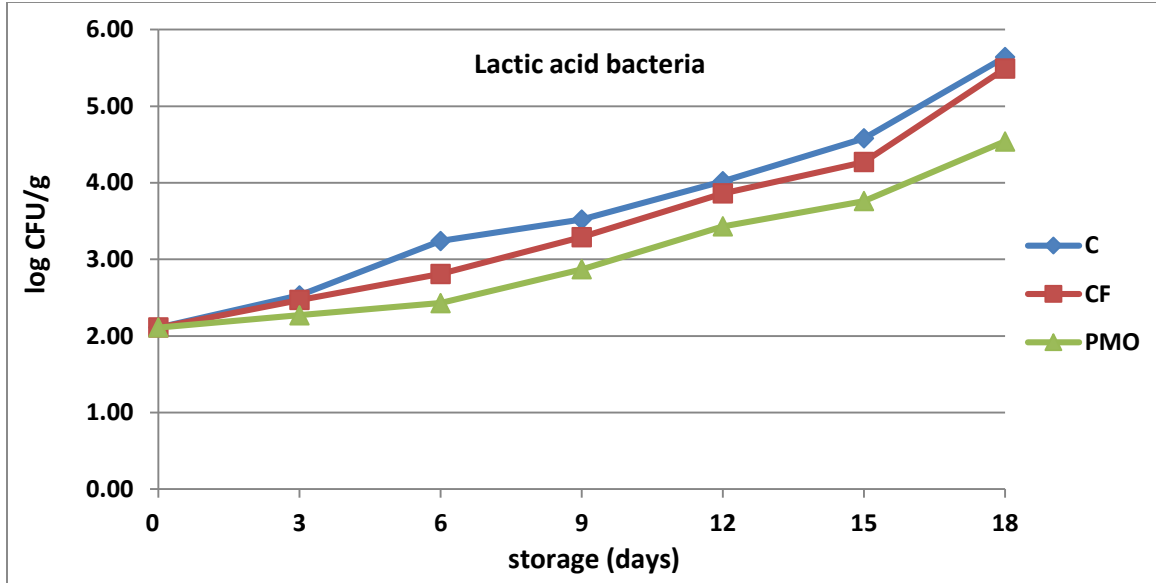


Figure 3. Changes in total lactic acid bacteria count of fish meatballs coated with chitosan enriched with peppermint essential oil emulsion during storage at 4°C. C: Control without gelatin film, CF: fish meatballs coated with chitosan, PMO: fish meatballs coated with chitosan enriched with 1% peppermint essential oil emulsion.

Enterobacteriaceae group is considered as an indicator microorganism which is a part of aquaculture microflora and marine products. Total Enterobacteriaceae changes of fish meatballs coated with chitosan solution enriched with peppermint oil were presented in Fig. 4. The initial total Enterobacteriaceae value was found as 2.35 log CFU/g in mackerel.

Total Enterobacteriaceae is considered the indicator of hygiene in the fish and fish products. The highest values were determined in control group during the storage period, while CF group showed lower values than the control.

Fish meatballs coated with chitosan enriched with peppermint oil emulsion had lowest total total Enterobacteriaceae values during the storage period. At the end of the storage, total Enterobacteriaceae values were 5.67, 5.46 and 4.34 log CFU/g in C, CF and PMO groups, respectively.

Rezaeifar et al. (2020) reported that the chitosan edible coating enriched with lemon verbena extract and essential oil inhibited the total Enterobacteriaceae growth in trout fillets. Similarly, Chamanara et al. (2012) found that total Enterobacteriaceae in the control sample are higher than other treated samples with chitosan incorporated with jujube extract.

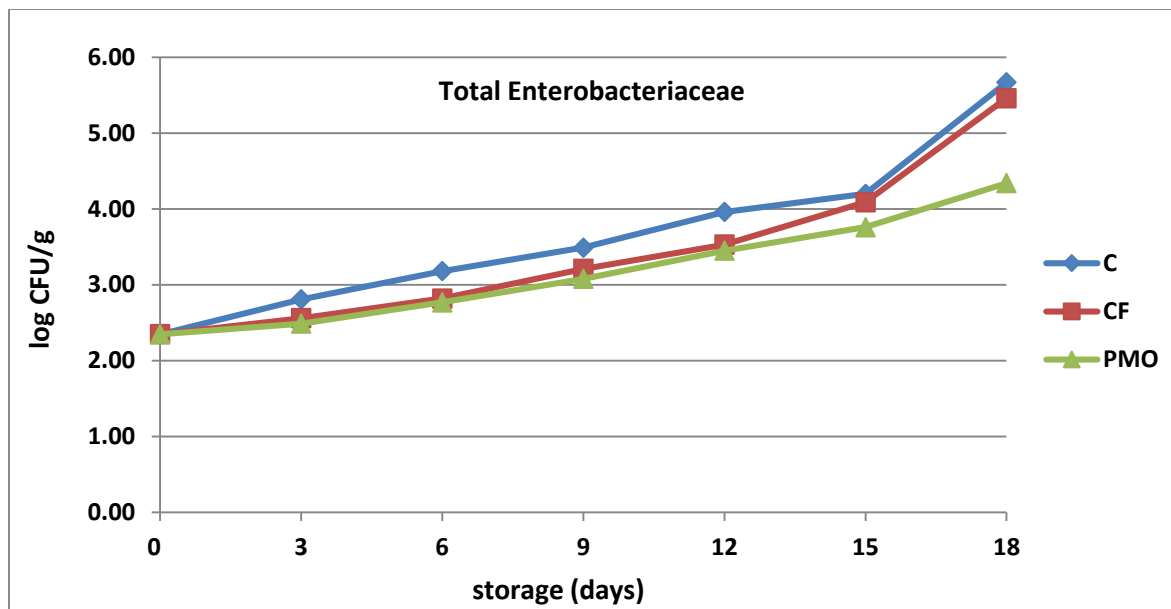


Figure 4. Changes in total Enterobacteriaceae count of fish meatballs coated with chitosan enriched with peppermint essential oil emulsion during storage at 4°C. C: Control without gelatin film, CF: fish meatballs coated with chitosan, PMO: fish meatballs coated with chitosan enriched with 1% peppermint essential oil emulsion.

CONCLUSION

After evaluation of the impact of the incorporation of peppermint essential oil emulsion with chitosan coating for the maintenance of microbiological quality of fish meatballs it can be concluded that the addition of peppermint essential oil emulsion in the chitosan coating inhibited the bacteria growth in the mackerel meatballs.

During the 18 days storage period, fish meatballs coated with chitosan enriched with peppermint essential oil emulsion showed the lowest total psychrophilic bacteria, total mesophilic bacteria, total lactic acid bacteria and total Enterobacteriaceae counts. Besides, these values did not exceeded the limit values in PMO group throughout the storage period.

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