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Process Conditions of Table Olive Fermentation

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Abstract

Table olive is one of the important fermented products in the food industry. There are mainly two types of table olive production methods in which fermentation is used. These are natural fermentation methods and Spanish style methods. Fermentation process in these methods take time and during this time the process progresses under the influence of different factors and so it must be managed well in order to achieve the desired quality, standard and safety production and minimum economic losses. In table olive fermentation, phenolic compounds contents, reducing sugar content, microbial profile, salt concentration, acidity and temperature are the main parameters that should be considered for providing a proper fermentation conditions. Understanding the basic parameters that determine the progress of the fermentation process in table olive production and the effects of these parameters on the process is of key importance for process control, acceleration and development of new production methods in table olive production. In this study, the factors determining the process conditions in table olive production were investigated.

Keywords: Fermentation, Olive, Process, Table olive, Parameter, Condition

Review article
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INTRODUCTION

Fermented food production has been carried out since ancient civilizations (Erten et al., 2015; Mannaa et al., 2021). Fermentation is the process of breaking down organic molecules by enzymatic activity of microorganisms (Sharma, 2020). Microorganisms used for fermentation are bacteria, yeasts and molds. In food technology, fermentation provides increased product shelf life and good organoleptic properties (Smid and Hugenholtz, 2010). Table olive is one of the important fermented products in the food industry (Kara ve Özbaş, 2013). Table olive production process aims to remove the bitterness caused by oleuropein in the fruit, improve the sensory quality and to increase the shelf life of the product (Gomez et al., 2006). There are mainly two types of table olive production methods in which fermentation is used. These are natural fermentation method (untreated) and Spanish style method (treated). In natural fermentation, olives are brought to eating maturity by fermentation (6-9 month) by removing their bitterness directly in brine (containing salt, 5-10 %). Since there is no alkali application in this method, the diffusion of phenolic compounds and fermentable components out of the olive and into the brine is limited, the removal of bitterness is delayed and the fermentation time is prolonged. (Gomez et al., 2006; Lanza, 2012).

However, this is a situation that increases the nutritional value and antioxidant activity of olives (Conte et al., 2020; Rocha et al., 2020). Spanish style production method is applied to green olives and the bitterness of the olive is removed with alkali. Removal of bitterness occurs in the form of hydrolysis of oleuropein glycoside, which causes bitterness in olives, to non-bitter components with alkali effect. With the alkali application, the permeability of the olive skin increases and a suitable medium for fermentation is provided. Then, the alkali is removed from the olive by washing processes. Afterwards, the olives are fermented (2-3 months) in brine (containing salt 5-10 %) so that brought to eating maturity (Minquez-Mosquera et al., 2008).

Although Spanish style treated olive fermentation takes a shorter time than natural fermentation, both of these two processes take time and during this time the fermentation process progresses under the influence of different factors. This process must be managed well in order to achieve the desired quality, standard and safety production and minimum economic losses.

PROCESS CONDITIONS OF TABLE OLIVE FERMENTATION

In table olive fermentation, phenolic contents, reducing sugar content, microbial profile, salt concentration, acidity and temperature are the main parameters that should be considered for providing a proper fermentation conditions (Corsetti et al., 2012; Fendri et al., 2012).

Microorganisms

Table olive fermentation is a complex biochemical process involving different microorganisms (Lanza, 2013). This process is generally a spontaneous process. The microbial composition is affected by several factors such as the composition of the brine, ambient acidity, salt concentration, olive variety, olive phenolic content, temperature, oxygen availability, alkaline concentration during fermentation (Botta and Cocolin, 2012; Santos et al., 2017). Mainly microorganisms that can be found in the fermentation medium are *Enterobacteriaceae*, lactic acid bacteria (LAB) and yeasts (Romeo, 2012). However, yeasts and LAB have the main role in the fermentation process and thanks to the synergy between them, high quality products are obtained (Botta and Cocolin, 2012; Ertan et al., 2015; Kiai et al., 2020). While in treated olive fermentation, LAB have a major role, in untreated natural olive fermentation yeasts or LAB can play a major role depending on factors like salt content and acidity of the brine (Özdemir, 1997; Santos et al., 2017). In a study (Tassou et al., 2002) conducted, it was reported that LAB dominate the medium in brine with 4% salt concentration, while yeasts dominate the medium in brine with 8% salt concentration. LAB are highly sensitive to salt concentration and phenolic compounds, whereas yeasts are more resistant to these compounds. The main LAB species involved in olive fermentation are *Lb. plantarum* and *Lb. pentosus* and yeasts species are *Saccharomyces cerevisiae*, *Wicherhamyces anomalus*, *Pichia klayveri*, *Candida boidinii* (Pereira et al., 2015; Perpetuini et al., 2020).

The main actions that yeasts perform in fermentation are: The formation of flavour and aroma compounds such as ethanol, glycerol, acetaldehyde, esters and higher alcohols, the formation of organic acids (acetic acid, succinic acid, formic acid), the formation of nutrients in the medium such as vitamins, amino acids, purines and reduced carbohydrates necessary for LAB development, the reduction of phenolic compounds and the prevention of the development of spoilage microorganisms (Botto and Cocolin, 2012; Bleve et al., 2014).

Esterase positive species take an important role in fermentation by forming esters from free fatty acids. Also, since many yeasts have lipase enzymes, they many cause an increase in free fatty acids in the medium (Botto and Cocolin, 2012). This contributes positively to the development of taste and aroma of the product. In addition, the use of sugar remaining in the product at the end of fermentation by yeasts prevents microbiological development in the product after packaging (Perpetuini et al., 2020). However, in some cases, it has been reported that some yeast species may cause softening in the product by causing degradation of pectic substances in the olive cell wall and in the middle lamella during fermentation, and gas packets may form on the product surface due to the carbon dioxide production (Fendri et al., 2012; Fadda et al., 2014). The all effects of yeasts on fermentation are not yet clear (Anagnostopoulos et al., 2017). The reasons for yeasts to form the majority in the fermentation medium are the increase in the acidity as a result of microorganisms activity, high salt concentration, lactic acid bacteria being more sensitive to phenolic compounds, higher presence of phenolic compounds in untreated olives obtained without alkali treatment, and higher acidity at the beginning of fermentation in olives preparing without alkaline application (Kara ve Özbaş, 2013). Oleuropein degradation is one of the main purposes for table olive processing. Many yeasts are known to exhibit β-glucosidase activity, which degrades oleuropein (Perpetuini et al., 2020; Zhang et al., 2021). So, some yeasts probably have a role in oleuropein degradation (Barilacqua et al., 2012). As for the source of yeasts in fermentation, Pereira et al. (2015) reported that yeasts were found in similar amounts in olive brine and olive pulp, and the source of these yeasts in fermentation was probably the fruit itself and the vats in which fermentation was carried out.

LAB increase the acidity of the medium by producing lactic acid from fermentable sugars, thus prevent the development of spoilage and pathogenic microorganisms and increases the shelf life of the product. In addition, they contribute to the development of taste and aroma of final product. LAB also perform the debittering process by hydrolyzing oleuropein (Botto and Cocolin, 2012; Bleve et al., 2014; Bonatsou et al.,2017; Romeo et al., 2018). It is known that LAB also form antimicrobial components such as organic acids, bacteriosins, diacetyl and reuterin that inhibit spoilage microorganisms (Corsetti et al., 2012). *Lb. plantarum* and *Lb. pentosus* are the main bacteria involved in olive fermentation. They stand out by their resistance to acidity and salt and their ability to degrade oleuropein (Romeo et al., 2018). It was reported that, in fermentation, yeasts were predominantly found on the olive surface and stomatal opening, while bacteria predominate in the intracellular cavities of the sub stomal cells (Nychas et al., 2002).

The microbial composition of the medium is quite complex during fermentation, depending on the changes in the biochemical conditions of the brine (Botto and Cocolin, 2012). Ambient acidity is low at the start of fermentation. Gram (-) bacteria are dominant in this medium. This period should be as short as possible and the acidity should increase so that the product does not deteriorate. Otherwise, these microorganisms will cause negative taste in the product and the formation of gas pockets on the olive surface (Botto and Cocolin, 2012; Lanza, 2013). Acetic acid can be added to inhibit Gram (-) bacteria (Medina et al., 2010). Bleve et al. (2014) studied the Cellina di Nardo and Leccino variety table olives obtained by natural fermentation. They found that microorganisms Gram (-) belonging to the *Enterobacteriaceae* family were present in the beginning and were not found in the fermentation medium a few days later. Low acidity allows undesirable microorganisms such as *Clostridium spp.* to growth in the medium. In the same study, it was reported that *Clostridium* and *Pseudomonas* microorganisms were not found at the end of fermentation and this was due to high acidity (pH <4.3).

In another study with similar results, it was determined that while *Enterobacteriaeceae* and Pseudomonas decreased in brine, LAB and yeast populations increased during fermentation in black olives produced by natural fermentation method (Nychas et al., 2002). As the fermentation progresses, the acidity rises and the LAB dominates medium, especially in treated olives production. The optimum growth acidity for the LAB is between pH 5.5-5.8 (Lanza, 2013; Botto ve Cocolin, 2012; Perpetuini et al., 2020). Since alkali treatment is applied in treated olives, the acidity at the beginning of fermentation (pH 8.0-9.0) is lower compared to olives obtained by natural fermentation (pH 5.0-6.5) (Lanza, 2013). Slow development of acidity is usually associated with slow growth of the LAB population (Martorana et al., 2017). In the untreated olive fermentation, if the salt content is not high and depending on the presence of phenolic secoiridoid derivatives in the medium, LAB and yeasts can develop together. Or one of them dominate the medium (Hurtado et al., 2012; Fendri et al., 2012; Fadda et al., 2014). It is stated that when the final pH of the product approaches 3.8-4.0 in untreated olives, yeasts become dominant in the fermentation medium (Kara ve Özbaş, 2013). Bleve et al. (2014) reported that the concentrations of yeasts in *Cellina di Mondo* and *Leccino* variety table olives obtained by natural fermentation increased from 3.0 log cfu/mL to 4.0-5.0 log cfu/mL throughout fermentation and they have an important role in fermentation. Aeration of the vat where fermentation takes place and also its size and types influence the fermentation development too. The size of the vat affects the ambient temperature. Fermentation takes place more irregularly in small volume vats (Hurtado et al., 2012).

It is possible to use starter culture to accelerate, control and standardize the fermentation process, to improve taste and aroma properties, and to increase the microbial safety of the product (Lanza, 2013; Bonatsou et al., 2017). By using starter culture, acidity formation takes place in a shorter time and thus the fermentation process is accelerated (Fendri et al., 2012). *Lb. plantarum* and *Lb. pentosus* are used as starter cultures, however it is possible to adding yeasts to the starter culture (Santos et al., 2017; Cosmai et al., 2018). The success of the starter culture in fermentation is directly related to the olive variety and the process method used (Hurtado et al., 2012). Perpetuini et al. (2018) in their study conducted with table olives inoculated with *Lb pentosus* (C8 ve C11) strains and spontaneously fermented, they determined that oleuropein was completely degraded in the samples produced by inoculation, LAB developed faster and a better acidification rate was provided. Pino et al. (2018) study conducted in which *Lb. paracasei* and *Lb. plantarum* strains were used as starters and they reported that starter culture accelerated fermentation, increased acidity from the 7th day, and inhibited spoilage bacteria also in this study, it was seen that yeast growth was correlated with main alcohols and phenols that cause undesirable taste and aroma and the use of starter suggested to prevent the growth of these autochthonous yeasts and spoilage bacteria. In another study, *Lb. plantarum* and *Lb. pentosus* strains were used as starters. In this study, it was determined that the fermentation time was shortened in the samples using starter, the antioxidant capacity was higher than the spontaneous samples, and the spoiling microbiata was lower (Comunian et al., 2017).

Features expected from starter culture are rapid development, high tolerance to salt, acidity and phenolic compounds, capacity to reduce phenolic compounds, showing enzyme activities that can contribute to the sensory properties of the final product, and being able to develop at low temperatures to improve the fermentation activities that continue especially in winter (Corsetti et al., 2012; Değirmencioğlu, 2016; Perpetuini et al., 2020).

Temperature

Ambient temperature is an important factor in the course of fermentation. The optimum temperature for fermentation is around 20 ºC. Therefore, fermentation slows down in the winter months when the temperature is low (Kayguluoğlu, 2018). By controlling the process temperature, fermentation can be accelerated and positive effects on product quality can be achieved. An increase in the amount of nutrients diffused from the fruit to the brine, enhancement of LAB growth and increase in acidity can be achieved with the application of high temperature (>18 ˚C). Also, less bitter product is obtained with the increased degradation of oleuropein due to the better growth of LAB (Campus et al., 2017; Vertedor et al., 2021). Although it accelerates the fermentation and has positive effects on the product, temperature control is not generally applied in the industry due to the energy cost (Aponte et al., 2010; Medina et al., 2010; Vertedor et al., 2021). There are limited number of studies in the literature about effects of temperature on the end product quality of table olives. Tassou et al. (2002) studied in their study, effects of different temperatures (25 ºC, 18 ºC and ambient temperature) and salt concentrations (4%, 6%, 8%) applications on the untreated olive fermentation. They reported that the best conditions for fermentation was providing with the sample of (25 ºC, %6) after five months of brining. The best acidity development was achieved with this sample. Also in this sample no off-flavors were detected indicating unfavorable fermentation by the panelists . In another study conducted, the effects of high temperature (20-24 ºC) applications on the brines of Spanish style treated table olives, during the fermentation was studied. It was reported that at the end of three months, the growth of LAB and yeasts and the development of acidity are higher and better color and optimum firmness are achieved in heated samples compared to the control samples without heat treatment (Vertedor et al., 2021).

Phenolic Compounds

The phenolics are minor compounds in olive fruit however they provide to gain functional properties to the fruit as they are the major antioxidant compounds in fruit. The phenolic compounds found in olives are mainly from classes the phenolic acids (gallic acid, caffeic acid), phenolic alcohols (hydroxytyrosol and tyrosol), flavonoids (luteolin-7-glucoside, luteolin) and secoiridoids (oleuropein, verbascoside) (Sahan et al., 2013; İzli, 2017). The phenolic fractions and content in product depends on different factors such as cultivar, climate, location, process conditions and stage of maturity (Perpetuini et al., 2018). Although it varies, the main phenols in olive fruits are oleuropein, tyrosol, hydroxtyrosol and verbascoside (Sahan et al., 2013). The most important of these compounds is oleuropein. Because, one of the main purposes of the table olive production process is to degrade oleuropein to eliminate the bitter taste caused by it. Oleuropein is degraded by microorganisms firstly to oleuropein-aglycone and glucose by the enzyme β-glucosidase and then to hydroxytyrosol and elenolic acid by the esterase enzyme. Also, oleuropein is degraded directly into hydroxytyrosol and elenolic acid glucoside with the application of alkali (NaOH) in the treated olive process. Then, during fermentation, elenolic acid glucoside is broken down into glucose and elenolic acid with the effect of acid (Ozdemir et al., 2014). During fermentation, the phenolic compounds in olives diffuse into the brine, and thus the amount of phenolic compounds in olives decreases. In a study conducted, in which using Gemlik and Edincik varieties of olive, it was determined that the amount of phenolic compounds of olive flesh in untreated olive fermentation decreased continuously during the fermentation of the fruit. Phenolic content decreased to 1.25% of the olive, about half of the amount in raw olives after 250 days of fermentation.

Also, the amount of phenolic compounds in the brine reached 1% towards the end of fermentation for both varieties (Borcakli et al., 1993). In another study conducted with four different olive cultivars, it was determined that flavonoid loss in olive flesh was 60% and total phenol loss was 79% during fermentation in olives produced by natural fermentation method. The main phenolic compounds detected in the brine after 71 days of fermentation were hydroxytyrosol, tyrosol, catechine and quercetin (Kiai and Hafidi, 2014). In a study using green and purple olives, it was determined again that there was a significant decrease in flavonoid and total phenol content in olive fleshes during fermentation, and this decrease was accompanied by a decrease in antioxidant activity. At the end of fermentation, it was determined that while the amounts of hydroxytyrosol and caffeic acid increased in both olive fleshes, the amounts of protocatechuic acid, ferulic acid, *p*-coumaric acid and quercetine decreased. Also, the main phenolic compound found in both brines was hydroxytyrosol (Kiai et al., 2020). Phenolics have important effects on colour, flavour and nutritional properties of products. They also show protective effects and extend shelf life of product because they have antimicrobial and antioxidative properties. They have antimicrobial effects on many microorganisms including LAB which have roles in the olive fermentation. They also can increase the shelf life of the product, thanks to their antioxidative properties (Borcakli et al., 1993; Özdemir, 1997; Pereira et al., 2006; Charoenprasert and Mitchell, 2012). Phenolic compounds show direct effects on table olive production processes and the final product with these properties.

Acidity

Acidity development in fermentation occurs mainly by the conversion of sugars to organic acids as a result of LAB metabolism, as well as the diffusion of organic acids from olives to the brine and hydrolysis of oleuropein (Pereira et al., 2015; Campus et al., 2017). Acidity is of prime importance for the development of the fermentation process because of it provides preservation of the product and LAB growth by preventing the development of undesirable bacteria (Borcakli et al., 1993; Pino et al., 2018). In treated olive fermentation, acidity is lower than in natural fermentation at the beginning due to the transition of the alkali remaining in the olive to the brine (Medina et al., 2010; Lanza, 2013). However, at the end of fermentation, acidity is lower in olives produced by natural fermentation method. The decrease in sugar content as the olive ripens and the lower permeability of the olive skin when alkali application is not used are some possible reasons for this situation (Gomez et al., 2006; Lanza 2012). It was reported that, the pH at the end of fermentation is 3.4-4.4, and as lactic acid total acidity value is 0.8-1.2 %, in treated olive fermentation and the final pH value is 4.3-4.5 and as lactic acid total acidity value is 0.5-1.0 %, in natural fermentation (Erten et al., 2015). The use of starter culture ensures that this acidity progress is faster and higher. In the study in which two different LAB strains are used as starters, it was determined that the pH value of the brine started at approximately 5 at the beginning of the 30-day fermentation, decreased to approximately 4 on the 7th day of fermentation, and was 3.7 at the end of the fermentation. In addition, it was observed that the acidity increased more slowly in the control samples without starter culture and the pH values determined as 4.8 at the end of fermentation were higher than the values of the inoculated samples (Perpetuini et al., 2018). Organic acids such as acetic acid, citric acid, sorbic acid, benzoic acid or their salts can be added with the purposes of accelerating the fermentation process and as preservative for product (Gomez et al., 2006; Fendri et al., 2012; Hurtado et al., 2012; Lanza, 2013; Erten et al., 2015).

Salt

One of the main parameters affecting the development of the process in table olive fermentation is the salt concentration of brine (Aponte et al., 2010). The brine salt concentration determines the diffusion rates of the soluble components. Because of osmotic pressure difference between brine and olive flesh, water soluble components diffuse from the olive flesh to the brine and vice versa during fermentation. In this context, as the sodium content increases in olives, some of nutrients of microorganisms such as reducing sugars, phenolic compounds, pectic substances, vitamins, alcohols and organic acids moves to the brine (Borcakli et al., 1993; Bautista-Gallego et al., 2013). This transition between brine and olive flesh continues until the amounts of ingredients on both sides reach equilibrium (Kanovouras et al., 2005).

In a study conducted with naturally fermented green olives brines with two different salt concentrations (4% and 7%), it was determined the diffusion of phenolic compounds and reducing sugars from olive flesh to brine was higher in samples containing 4% salt, brines containing 7% salt had higher polyphenol content and antioxidant activity. Also as expected, the salt content of olive fleshes in brine containing 7% salt was reported to be significantly higher (Fadda et al., 2014).

Salt is also used to prevent the growth of undesired microorganisms and improve the sensorial properties and texture of the product (Marsilio et al., 2002; Medina et al., 2010; Campus et al., 2015; Pino et al., 2018). Recently, the use of salt in olive production in industry tends to be reduced. The main reasons for this can be stated as masking of the fruit aroma by the excess salt, the prevention of lab growth by high salt concentrations, environmental causes related to chlorides, recommendation of low sodium intake in the diet for health reasons and shriveling of olives at high salt concentration (Borcakli et al., 1993; Medina et al., 2010; Değirmencioğlu, 2016).

Reducing Sugars

The water soluble compounds, must initially transition from the olive flesh to the brine, for the fermentation of table olives to take place. The most important of these soluble components for fermentation are reducing sugars. Mainly reducing sugars in olive are glucose, fructose and sucrose (Kiai et al., 2020). The transition of reducing sugars from olive flesh to the brine occurs depending on parameters such as olive skin permeability, salt concentration, temperature and olive/brine ratio (Borcakli et al., 1993; Kiai and Hafidi, 2014). Due to the transition of sugars to the brine, the amount of sugar in olive flesh is significantly reduced during fermentation (Özdemir, 1997).

Sugars in brine are the main energy source for microorganisms involved in fermentation. Microorganisms convert these sugars into organic acids (mainly lactic acid) and thus increase the acidity of the medium (Ünal and Nergiz; 2003; Kiai and Hafidi, 2014; Alak, 2016). In a study using green and purple olives, it was determined that the reduction in sugar content during fermentation was 73% in green olives and 60% in purple olives (Kiai et al., 2020).

Another study was done with Gemlik and Edincik varieties of olive. In the process using the Edincik variety, which has a higher reducing sugar content, it was determined that the transition of reducing sugars to the brine was faster, the pH value decreased faster and the final pH value was lower than the process in which Gemlik variety was used (Borcakli et al., 1993). In general, olives used in table olive production are required to have a high sugar content (Kara ve Özbaş, 2013).

CONCLUSION

Table olive, which is an industrial and high added value product, is a product that is consumed all over the world and has an important place in the food industry with its functional properties and nutritive value. However, the production of table olives is a process that progress under the influence of different parameters and usually takes time. Understanding the basic parameters that determine the progress of the process in table olive production and the effects of these parameters on the process is of key importance for process control, acceleration and development of new production methods in table olive production.

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Investigation of Antimicrobial and Photoprotective Activity of *Hylocereus undatus* Methanol Extracts

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Abstract

Hylocereus spp, which is very rich in bioactive substances, is known to be protective against various metabolic disorders. Probiotics are known to be useful microorganisms and have potential use in the cure and prevention of some disorders. In this study, it was aimed to investigate the use potential of cream formulations containing *Hylocereus undatus* extracts in the pharmaceutical and cosmetic industries. Firstly, the antimicrobial activity of *H. undatus* extracts against some pathogenic microorganisms and probiotic candidate strains were determined. Inhibition zone diameters of the extracts against the tested pathogenic microorganisms were obtained in the range of 7.00-12.14 mm. Then, a cream formulation containing *H. undatus* fruit methanol extract and probiotic strain *L. fermentum* MA-7 against the pathogenic microorganisms was tested to determine its antimicrobial activity. Among the cream formulations, cream+ *H. undatus* fruit methanol extract + *L. fermentum* MA-7 (CEL) showed the highest inhibition zone diameter (17.59 mm) on *Escherichia coli* O157:H7. The solar protection factor (SPF) of the extracts and the extract-cream mixture was also determined in vitro. The peel extract exhibited the best SPF value of 25.92. The highest SPF values of peel and fruit extract cream mixtures were determined as 22.76 and 10.58 at 10 ml concentration. The result of the study indicated that *H. undatus* extracts and *L. fermentum* MA-7 cream formulation containing may have the potential to inhibit the growth of pathogenic microorganisms in the cosmetic and pharmaceutical industries as natural antimicrobial additives. In addition, *H. undatus* methanol extracts with high UV blocking capacity can be used as a natural protective additive in sunscreens for the cosmetic industry.

Keywords: White pitahaya, probiotic, cream formulation, antibacterial, antifungal, photoprotective activity

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INTRODUCTION

Hylocereus spp., known as pitahaya or dragon fruit, is a vine cactus species belonging to the cactus family Cactaceae. It is one of the tropical fruits newly introduced to the Mediterranean region of Turkey. It is generally known as dragon fruit in Turkey (Le Bellec et al., 2006; Attar et al., 2022).

Hylocereus spp. contains carbohydrates, steroids, proteins, tannins, alkaloids, flavonoids, and phenolic compounds. Pitahaya fruits are gaining popularity due to their exotic appearance and health benefits (Sushmitha et al., 2018; Mahdi et al., 2018). It has been reported that *Hylocereus* spp., which is very rich in bioactive materials, can be consumed as a preventative against obesity, type 2 diabetes, cancer, etc. (Attar et al., 2022). It has also been reported that both red and white dragon fruit peels are cytotoxic to cancer cell lines Bcap-37, MGC-803 and PC3 (Luo et al., 2014). Enhancement awareness of the benefits of plant products is becoming increasingly widespread in society in order to provide an adequate intake of macronutrients-micronutrients, vitamins, dietary fibers and phytochemicals, which are very important for human health (Attar et al., 2022).

The skin is an immunogenic organ that functions as a biological sensor for us and is the first defense against external allergens (Schmidt 2004). The skin is the largest organ of our body and is constantly exposed to chemical, physical, fungal, and bacterial threats. Probiotics are well known to be beneficial for skin conditions and have been clinically studies (Roudsari et al., 2015). The probiotics may have potential uses in the prevention and treatment of skin diseases in many areas such as skin hypersensitivity, allergic inflammation, UV-induced skin harm, wound protection.

Creams are semi-solid topical products designed to be applied to the mucous membrane or skin. Medicated creams are products containing active ingredients for therapeutic purposes in cases of inflammation, infection, etc. Non-medicated creams do not contain active components, they are used in cosmetics as a moisturizer, emollient, etc. in some skin conditions (Adeleye et al., 2019). There is an increment requisition for personal care products with natural ingredients, which are less hypo-allergenic compared to synthetic cosmetics and do not need to be hesitant about skin irritations. Natural cosmetic products provide the skin with nutrients such as vitamins A, C and E, which provide anti-inflammatory, antioxidant, anti-aging, anti-methanogenic effects and improve skin health (Lohani et al., 2019).

The intention of the study is to investigate the potential usage of the new cream formulations in the cosmetic and pharmaceutical industries. Firstly, the potential of using *H. undatus* extracts as a natural preservative instead of synthetic preservatives was investigated. New cream formulation containing *H. undatus* fruit extract was then developed, and its antimicrobial activity was evaluated. Afterwards, the solar protection factors (SPF) of *H. undatus* extracts were tested to obtain the potential use of *H. undatus* extracts as an additive in natural herbal sunscreens in the cosmetic industry. Finally, the SPF values of mercantile cream added *H. undatus* extracts was determined in vitro.

MATERIAL and METHOD

Plant Material and Preparation of Extracts

The *H. undatus* fruit was purchased from Antalya-Turkey. *H. undatus* fruits were washed and then fruit and peel were separated dried at room temperature (Figure 1). After grounding, the powder from *H. undatus* peel and fruit were separately extracted with methanol (99.7%) using a sonication device in 2 repetitions in 10 minutes every day (2 days) each. Then, the organic solvents were removed by a rotary evaporation. *H. undatus* peel and fruit extracts dissolved with dimethyl sulfoxide (DMSO) were sterilized by sterile filters (0.45 μm).

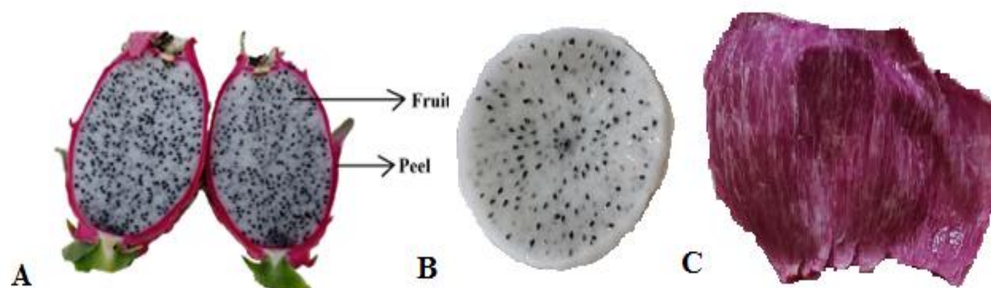


Figure 1. A: *H. undatus*, **B:** *H. undatus* fruit, **C:** *H. undatus* peel

Microorganisms

Food-borne and Clinical Pathogens

Enterococcus faecalis ATCC 29212 and *Listeria monocytogenes* ATCC 7644 were cultured at 37ºC in Tryptic Soy Broth (TSB) for 24 hours. *Salmonella enteritidis* RSKK 171, *Escherichia coli* O157:H7 and *Pseudomonas aeruginosa* ATCC 27853 were grown in Nutrient Broth (NB) for 24 hours.

Yeast

Candida albicans ATCC 10231 and *C. glabrata* RSKK 04019 were cultured at 30ºC in Yeast Peptone Dextrose (YPD) medium for 24 hours.

Probiotic Candidate Strains Originated from Breast Milk

Limosilactobacillus fermentum MA-7*, Lactobacillus gasseri* MA-1*, Lactobacillus delbrueckii* MA-9 were cultured at 37ºC in Man, Rogosa and Sharpe (MRS) media for 24 hours (Asan-Ozusaglam and Gunyakti, 2019; 2020; 2022).

Determination of Biological Activity

Disc Diffusion Method

The biological activity of peel and fruit methanol extracts obtained from *H. undatus* was determined using disc diffusion assay. The prepared bacterial suspension (0.5 Mcfarland) was inoculated on agar medium and sterile discs were placed on the agar**.** *H. undatus* peel and fruit extracts (4 mg/disc for pathogenic test microorganisms and 1, 2 and 4 mg/disc of for probiotic candidate strains) were then dropped onto the discs. Ampicillin (AM-10 µg/disc) antibiotics disc was used as controls for the pathogenic test microorganisms and Fluconazole (FCA-25 µg/disc) for the yeasts. The inoculated petri was incubated for 24 hours at suitable temperatures indicated previously. The experiment was carried out in duplicate.

Determination of Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) or Minimum Fungicidal (MFC) Concentration

The micro-dilution assay was used to determine MIC and MBC or MFC values of the *H. undatus* extracts. The extracts were added to each tube containing growth medium to obtain a final concentration of 80 µg/µl and diluted to 40, 20, 10, 5 and 2.5 µg/µl. Microbial suspension (0.5 McFarland concentration) was added to each tube. The tubes were then incubated under the conditions required for each microorganism. After the incubation, the values in the tube without microbial growth were recorded as the MIC concentration of the extract. MBC and MFC concentrations were determined by inoculating samples from the tubes onto solid medium.

The petri dishes were incubated for 24 hours at the appropriate temperature for the test microorganisms. After incubations, the lowest concentration without growth was defined as MBC or MFC values.

Cream Formulation Containing *H. undatus* Fruit Extract and Probiotic

The antimicrobial activity of the cream formulations was obtained using the modified method used in our previous study (Asan-Ozusaglam and Celik, 2023). In the developed antimicrobial cream formulations, a mercantile cream, *H. undatus* fruit extract and/or a probiotic candidate strain *L. fermentum* MA-7 (Asan-Ozusaglam and Gunyakti, 2018) isolated from human milk were used. The antimicrobial activity of the developed cream groups was determined against the test microorganisms (*L. monocytogenes* ATCC 7644, *S. enteritidis* RSKK 171, *E. coli* O157:H7, *E. faecalis* ATCC 29212, *P. aeruginosa* ATCC 27853, *C. albicans* ATCC 10231 and *C. glabrata* RSKK 04019) using the well diffusion method. The experiment was performed in triplicate. The culture dishes were incubated in the conditions mentioned above for the test microorganisms.

Determination Photoprotective Activity of Extracts

The solar protection factor (SPF) of *H. undatus* peel and fruit extracts was determined by spectrophotometric method in vitro conditions. The extracts (0.002 g/ml) were mixed with 96% ethanol. The mixture was read in the UV-VIS spectrophotometer in the wavelength range of UV-B (290-320 nm). The Mansur equation was used to calculate the SPF value.

Mansur's equation (Mansur et al., 1986)**:**

$$\textbf{SPF} = \mathrm{CF} \times \sum_{290}^{320} \mathrm{EE}(\lambda) \times \mathrm{I}(\lambda) \times \mathrm{Abs}(\lambda)$$

Determination of Photoprotective Activity of Cream Mixtures Containing Extract

The solar protection factor (SPF) of *H. undatus* peel and fruit methanol extracts and mercantile cream mixture was determined by spectrophotometric method under in vitro conditions. The cream and peel and fruit extract were mixed and then added distilled water to make up the final volume (10 g). The mixture was homogenized and diluted to three different concentrations (2.5-5-10 ml). The cream groups that did not contain the extract were used as the control group. The mixtures were measured in triplicate using UV-VIS spectrophotometer in the wavelength range of UV-B. The SPF values of the cream mixtures with containing the extract were calculate using the Mansur equation (Mansur et al., 1986).

Statistical Analysis

The data of antimicrobial activity of *H. undatus* extracts on lactic acid bacteria and the cream formulations on pathogenic test microorganisms were analyzed using GNU SPSS version software. The statistical significance was confirmed by One-Way analysis of variance (ANOVA) with Tukey's post-hoc test. *P* value below 0.05 ($P<0.05$) will be considered statistically significant.

RESULTS and DISCUSSION

Antibacterial and antifungal activities of *H. undatus* peel and fruit extracts were investigated on test microorganisms using disc diffusion and micro-dilution assays. The disc diffusion assay results against food-borne and clinical and yeast microorganisms are presented in Table 1. The inhibition zone diameters ranged from 7.00 mm to 11.85 mm for the peel extract and 7.55 mm to 12.14 mm for the fruit extract.

Among the test microorganisms, the highest inhibition zone diameters of *H. undatus* extracts were detected against yeasts. *H. undatus* peel and fruit extracts can be used to prevent and/or treat both bacterial and fungal infections.

Table 1. Disc diffusion assay results of *H. undatus* extracts.

Food-borne and Clinical Microorganisms	**Inhibition Zone Diameters (mm±SD)**		
	HPME	**HFME**	**AM**
***L. monocytogenes* ATCC 7644**	8.31±1.85	7.55±0.33	29.57 ±0.1
***S. enteritidis* RSKK 171**	9.43±0.11	9.08±0.94	-
***E. coli* O157:H7**	7.81±0.38	7.86±0.61	17.76 ±0
***E. faecalis* ATCC 29212**	7.00±0.72	8.14±1.82	24.02 ±0.3
***P. aeruginosa* ATCC 27853**	9.68±0.10	8.90±0.57	23.53 ±0.6
Yeast			**FCA**
***C. albicans* ATCC 10231**	11.85±0.44	10.64±0.06	-
***C. glabrata* RSKK 04019**	11.23±1.15	12.14±0.91	20.35 ± 0.10

*HPME: *H. undatus* Peel Methanol Extract, HPME: *H. undatus* Fruit Methanol Extract, -: resistant

In the study of Rohin et al. (2012), the inhibition zone diameter of white pitahaya (*H. undatus*) peel and fruit methanol extracts against *E. coli* (7.00 mm-ND) and *Enterococcus faecalis* (11.00 mm-8.00 mm) were determined using the disc diffusion assay. In the current study, the inhibition zone diameters of peel and fruit extracts against *E. coli* O157:H7 were found to be 7.81 mm and 7.86 mm. The zone of inhibition against *E. faecalis* ATCC 29212 was determined as 7.00 mm and 8.14 mm. In a recent study, the antimicrobial activity of peel and pulp methanol extracts from *H. undatus* against *Pseudomonas sp.* was investigated using the well diffusion method. The inhibition zone diameter was not observed in the peel extract against the *Pseudomonas* sp. test microorganism, while the inhibition zone diameter of 25 mm was detected in the pulp extract (Ishnava and Patel, 2019). The observation of various antimicrobial activity against the test microorganisms may be due to differences in the extraction method, extract concentrations, strains of test microorganisms and growing conditions of the fruit such as climate, soil conditions etc.

In our previous study, methanol extracts of *H. undatus* fruit and peel obtained by using different extraction method (with hot water bath) showed inhibition activity between 6.17 mm and 13.15 mm against test microorganisms (*L. monocytogenes* ATCC 7644, *S. enteritidis* RSKK 171, *E. coli* O157:H7, *E. faecalis* ATCC 29212, *P. aeruginosa* ATCC 27853, *C. glabrata* RSKK 04019 and *C. albicans* ATCC 10231). The difference in the inhibition zone between our previous work may be due to the difference in the extraction method used (Asan-Ozusaglam and Celik, 2023).

MIC is the lowest concentration that inhibits microorganism growth in vitro. The minimum bactericidal or fungicidal concentration is the lowest concentration that reduces the number of microorganisms in the medium containing the bacterial inoculum by 99.9 in vitro conditions (Kowalska and Dudek, 2021). Micro-dilution assay results are presented in Table 2. The results showed that MIC values of *H. undatus* peel extracts against test microorganisms were determined as 20-80 µg/ml and MBC values as 40-80 µg/µl.

The peel extract inhibited the *P. aeruginosa* ATCC 27853 and *C. glabrata* RSKK 04019 strains at the lowest concentration of MBC (40 µg/µl). The MIC values of the fruit extract against the test microorganisms were determined to be 40-80 µg/ml and the MBC values to be 40->80 µg/µl.

The lowest MFC value in fruit extract was determined against *C. glabrata* RSKK 04019 (40 µg/µl). Therefore, the *H. undatus* extracts may be used as a bactericidal or fungicidal agent in food and pharmaceutical industries.

Table 2. MIC, MBC or MFC values of *H. undatus* extracts.

Test Microorganisms	**MIC (µg/µl)**		**MBC or MFC (µg/µl)**	
	HPME	**HFME**	**HPME**	**HFME**
***L. monocytogenes* ATCC 7644**	40	80	80	>80
***S. enteritidis* RSKK 171**	80	40	80	80
***E. coli* O157:H7**	80	40	80	80
***E. faecalis* ATCC 29212**	80	80	80	>80
***P. aeruginosa* ATCC 27853**	40	40	40	80
***C. albicans* ATCC 10231**	40	40	80	80
***C. glabrata* RSKK 04019**	20	40	40	40

*HPME: *H. undatus* Peel Methanol Extract, HPME: *H. undatus* Fruit Methanol Extract

In a study conducted by Nurmahani et al. (2012), MIC and MBC values of hexane, chloroform and ethanol *H. undatus* fruit extracts against *S. aureus, Campylobacter jejuni, Bacillus cereus, L. monocytogenes, E. faecalis, Yersinia enterocolitica, E. coli, Salmonella typhimurium* and *K. pneumoniae* were determined in the range of 1.25-10 mg/ml and 2.5->80 mg/ml, respectively.

In the current study, the antimicrobial activity of *H. undatus* extracts against some probiotic candidate strains was also determined. Disc diffusion assay results are presented in Table 3. As the concentration of *H. undatus* extracts decreased, the inhibition zone diameters of the extracts on the probiotic candidate strains also decreased. The statistical analysis results showed that the difference between LAB in peel extract at 4 mg/disc concentration was not significant ($P>0.05$).

There was significant difference between *L. fermentum* MA-7 and other LAB tested ($P<0.05$) at 4 mg/disc concentration of fruit extract. The visual data indicates that the lowest inhibition zone diameter was obtained in *L. fermentum* MA-7 (9.62 mm) at the concentration 4 mg/disc in fruit extract. This means that *L. fermentum* MA-7 will be less affected by the *H. undatus* fruit extract and maintain its viability compared to other LAB tested.

In recent years, the studies on the development of mercantile products containing *H. undatus* fruit extract have been increasing. The appropriate concentrations of the *H. undatus* extracts and the probiotic candidate *L. fermentum* MA-7 strain may have the potential use in new cream formulations against infections.

Table 3. Antimicrobial activity of *H. undatus* extracts against some probiotic strains.

Test Microorganisms	Extracts Inhibition Zone Diameters (mm±SD)					
	1 mg/disc		2 mg/disc		4 mg/disc	
	HPME	HFME	HPME	HFME	HPME	HFME
***L. fermentum* MA-7**	6.40±0.47[a]	6.96±0.14[a]	7.66±0.12[a]	8.19±0.39[a,b]	10.31±0.34[a]	9.62±0.07[a]
***L. gasseri* MA-1**	6.36±0.10[a]	6.80±0.53[a]	8.99±0.48[b]	7.86±0.87[a]	10.33±0.52[a]	11.36±0.69[b]
***L. delbrueckii* MA-9**	6.56±0.02[b]	7.93±0.37[a]	7.59±0.25[a]	9.57±0.23[a,b]	9.61±0.04[a]	12.82±0.25[c]

*HPME: *H. undatus* Peel Methanol Extract, HPME: *H. undatus* Fruit Methanol Extract, the different letters in the same column shows statistically significance ($P<0.05$)

Thengi et al. (2019) isolated two *Leuconostoc* sp. strains from pitahaya fresh fruits. The strain showed antibacterial activity against *Staphylococcus aureus*. They also indicated that fruits are very important because they contain beneficial probiotic strains as well as their nutritional properties.

Micro-dilution assay results are presented in Table 4. The MIC values for all extracts were determined in the range of 20-40 µg/µl and MBC values were determined as 40 µg/µl. The results indicated that the tested probiotic strains were sensitive to *H. undatus* extracts, however, appropriate concentrations of the extracts can be used together with the probiotic strains.

Table 4. MIC and MBC values of *H. undatus* extracts against probiotic strains.

Test Microorganisms	MIC (µg/µl)		MBC (µg/µl)	
	HPME	HFME	HPME	HFME
***L. fermentum* MA-7**	40	20	40	40
***L. gasseri* MA-1**	20	40	40	40
***L. delbrueckii* MA-9**	40	20	40	40

*HPME: *H. undatus* Peel Methanol Extract, HPME: *H. undatus* Fruit Methanol Extract

The well diffusion test results of the cream formulations are presented in Table 5. Since *L. fermentum* is known to skin moisture and improve skin health (Lee et al.,2022), it has been used as a probiotic strain in the cream formulations in the current study. In the control (cream) group, inhibition zone was not observed against the tested microorganisms except for *P. aeruginosa* ATCC 27853 (6.44 mm) and *C. glabrata* RSKK 04019 (2.76 mm). In the CEL group, *H. undatus* fruit extract and probiotic candidate strain have been shown to increase the antimicrobial activity of commercial cream by creating a synergistic effect when compared to the control group.

The highest inhibition zone diameter was obtained to be 17.59 mm against *E. coli* O157:H7 in the CEL cream formulation group. *E. coli* O157:H7 is one of the food-borne pathogenic microorganisms that is important for public health. *E. coli* O157:H7 causes serious intestinal infections in humans (Turgut and Kaya, 2015). Another important feature of *E. coli* O157:H7 is that the minimal infection dose is very low (Özkuyumcu, 2009). The developed antimicrobial cream can be an alternative natural antimicrobial agent for protection from *E. coli* O157:H7 contaminations.

Unexpectedly, in *C. glabrata* RSKK 04019, the highest inhibition zone diameter was detected in the C (2.76 mm) and CL (8.73 mm) groups, while the lower inhibition zone diameter was found in the CEL (5.58 mm) group. This may be due to the fact that the extract may have a slight inhibitory effect on *L. fermentum* MA-7. The difference between CEL group and C group is significant in *C. glabrata* RSKK 04019 ($P<0.05$). The results of the presented study showed that the developed cream formulations may inhibit these pathogens when such pathogens infect to our skin. *Candida spp*. is one of the most common causes of colonization in burns and wounds. *Candida spp.* is associated with persistent infections that cause tissue loss on the surface of the skin and inhibit wound healing (Farjah et al., 2020). The high antimicrobial activity of the cream formulation prepared against *C. albicans* ATCC 10231 (8.52 mm) and *C. glabrata* RSKK 04019 (5.58 mm) test microorganisms indicated that it may has potential for use in the pharmaceutical and cosmetic industries. The high antimicrobial activity of the cream formulation prepared against test microorganisms showed that it has potential for use in the pharmaceutical and cosmetic industries.

Table 5. Well diffusion assay results of cream formulations.

Test Microorganisms	Inhibition Zone Diameter (mm) of Cream Formulation Groups			
	C	CL	CE	CEL
***L. monocytogenes* ATCC 7644**	ND^{a}	ND^{a}	ND^{a}	2.78±0.19^{b}
***S. enteritidis* RSKK 171**	ND^{a}	1.62±0.82^{b}	1.10±0.82^{a}	3.03±0.49^{c}
***E. coli* O157:H7**	ND^{a}	6.40±0.24^{b}	1.44±0.23^{a}	17.59±1.39^{c}
***E. faecalis* ATCC 29212**	ND^{a}	ND^{a}	1.46±0.36^{b}	2.69±0.74^{c}
***P. aeruginosa* ATCC 27853**	6.44±0.85^{a}	3.28±0.30^{b}	4.53±0.54^{a,b}	7.62±1.16^{a,c}
***C. albicans* ATCC 10231**	ND^{a}	6.76±0.60^{b}	ND^{a}	8.52±1.36^{b}
***C. glabrata* RSKK 04019**	2.76±0.53^{a}	8.73±0.62^{b}	4.57±0.14^{c}	5.58±0.96^{c}

*C: Cream (control), CL: Cream+ *L. fermentum* MA-7, CE: Cream+ fruit Extract, CEL: Cream+ fruit Extract+ *L. fermentum* MA-7, ND: Not Determined, the different letters on the same line indicate significance ($p<0.05$)

In a study, it was reported that topical applications of *H. undatus* aqueous extract showed improvement in excision (59%) and incision (52%) wound test in the treated group of diabetic rats (Perez et al., 2005). Sunscreens constitute an important part of the cosmetic industry. In our study, the SPF values of *H. undatus* peel and fruit extracts are given in Figure 2. The peel extract (25.92) has more solar protection potential than fruit extract (24.84).

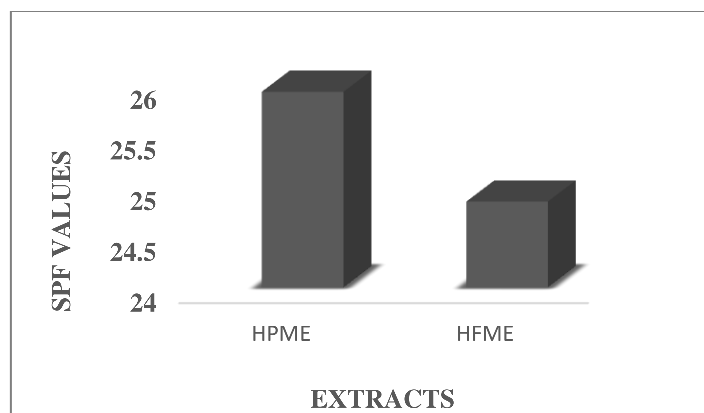
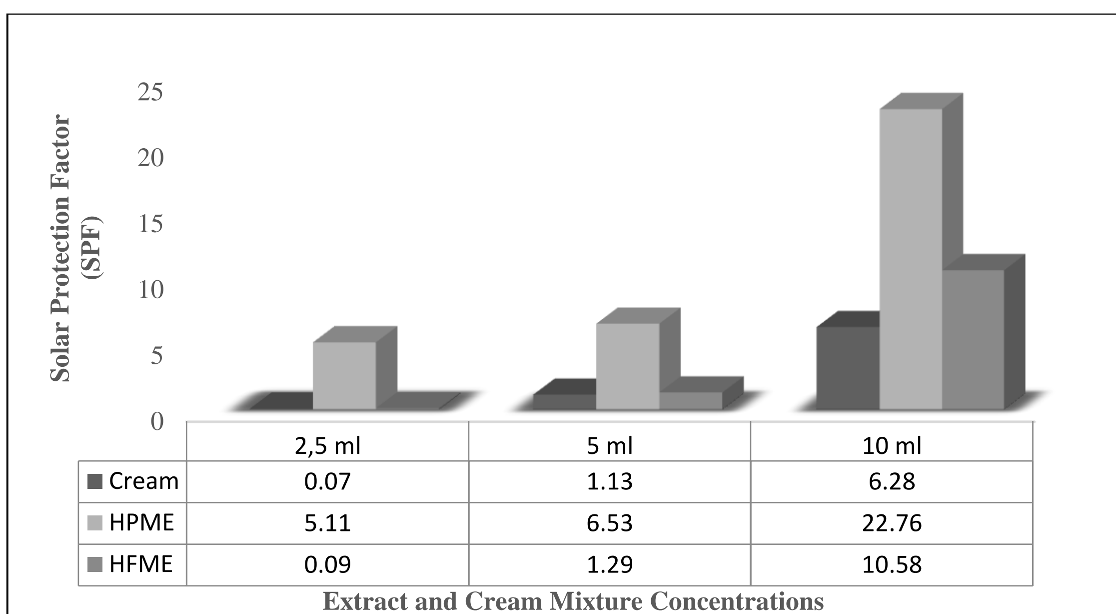


Figure 2. SPF values of *H. undatus* extracts (HPME: *H. undatus* Peel Methanol Extract, HPME: *H. undatus* Fruit Methanol Extract)

Vijayakumar et al. (2020) evaluated the solar protection factor of *Hylocereus polyrhizus* peel ethanol extract at different concentrations. In their studies, SPF values were determined as 15.38 - 35.02.

After determining the SPF values of *H. undatus* extracts, the extracts were mixed with mercantile cream and the SPF values of the extract and cream mixtures were determined at different concentrations. The SPF values of extract-cream mixtures are given in Figure 3. The extract and cream mixtures showed higher SPF values than the control group at all concentrations (2.5 ml, 5 ml and 10 ml). The peel extract exhibited the best SPF value of 22.76 at 10 ml concentration. This indicated that the peel extract had 93% UV blocking rate according to Imam et al. (2015). In our previous study, the SPF of white pitahaya fruit and peel methanol extracts (obtained by using a hot water bath) and cream mixtures was determined as 9.26 and 23.34 at a concentration of 10 ml (Asan-Ozusaglam and Celik, 2023).



	2,5 ml	5 ml	10 ml
Cream	0.07	1.13	6.28
HPME	5.11	6.53	22.76
HFME	0.09	1.29	10.58

Figure 3. SPF values of cream-*H. undatus* extracts mixture (HPME: *H. undatus* Peel Methanol Extract, HPME: *H. undatus* Fruit Methanol Extract)

CONCLUSION

Biological activities of *H. undatus* peel and fruit extracts were investigated to evaluate the potential usage of *H. undatus* fruit in the pharmaceutical and cosmetic industry. The peel and fruit extracts of *H. undatus* have showed antimicrobial activity against tested microorganisms. The prepared cream formulation has a static/cidal effect against test microorganisms. Therefore, the use of *H. undatus* extracts can be an alternative solution to the prevention of various skin problems by reducing the use of synthetic preservatives. These cream formulations, they can be used in our daily life as an antibacterial moisturizing cream. Also, UV filtering capacities of *H. undatus* extracts were determined to be high. It was determined that when the extracts are mixed with the cream, the UV filtering capacity of the cream increases. Mercantile sunscreens raise various biosecurity and environmental pollution concerns, so these problems will be avoided when plant extracts are used. In our study, it was determined that *H. undatus* extracts have the potential to be used as a natural preservative in the cosmetic or pharmaceutical industry, instead of expensive topical cream formulations containing synthetic preservatives.

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Olive Leaf: Antimicrobial and Antioxidant Properties, Food Applications, and Current Studies

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Abstract

Olive leaves are an important natural source of phenolic compounds with many bioactive properties. It is important to use them in different areas instead of throwing them as waste. Its antioxidant and antimicrobial properties have been proven by many studies and have recently been used in medicine, cosmetics, pharmaceuticals and food products. In this review, the importance of olive leaves, their composition, antimicrobial and antioxidant effects and food applications are briefly explained by giving the recent studies on this subject as examples. This review is likely to be valuable to interested academic and industrial researchers in their studies and knowledge of olive leaves, natural antimicrobials and antioxidants, and innovative food formulations with functional qualities.

Keywords: Food waste, Functional foods, Natural food additives, Olive leaves, Phenolic compounds

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INTRODUCTION

Many wastes and by-products contain valuable compounds that arise during the cultivation, harvesting, and industrial processing of agricultural crops. It is extremely important in terms of economy, environment, social and human health to evaluate these products in different ways and transform resources in order to protect natural sources, minimize waste and ensure sustainability. One of the most valuable compounds in agricultural wastes and by-products are phenolic compounds. They are secondary metabolite products in plants and important in the plant's defense mechanism against insects, microorganisms, and UV radiation. Because of their antimicrobial and antioxidant activity properties, they are used in many areas for example medicine, food, and cosmetics.

The olive tree (*Olea europaea* L.), a member of Oleaceae family and a symbol of Mediterranean civilization, peace, and abundance, is widely spread plant in the world. Nearly 98% of the world's olive harvest comes from the 8 million hectares of trees that are found in countries in the Mediterranean region (Ahmad-Qasem et al., 2014). The olive fruits are used in the production of olive and olive oil, and the leftover pieces and residues developed as a result of the processing of the olive and pruning are regarded as by-products of olive (Souilem et al., 2017; Talhaoui et al., 2015). Olive mill wastewater, olive pomace, olive leaves, and olive seeds can be given as examples of these by-products, which are rich in phenolic substances. (Souilem et al., 2017; Talhaoui et al., 2015). Mostly, these are regarded as waste,

and their management and disposal constitute an environmental problem. On the other hand, because they are important sources of bioactive compounds such as phenols, sterols, squalene, tocopherols, etc., they are valuable in human nutrition. The biochemical properties of these compounds have been better recognized with studies on olives, olive leaves, and olive oil, and the use of these products in daily nutrition, as ingredients in the food sector and in the pharmaceutical industry has become widespread.

Olive leaf (OL) is one of these products. It contains high amount of polyphenolic compounds and other beneficial phytochemicals, such as flavonoids, triterpenes, and chalcones, which have positive effects on health and has been used in traditional medicine for a long time for its health advantages, such as boosting the immune and cardiovascular systems, supporting energy levels, decreasing blood pressure, treating diarrhea, respiratory and urinary tract infections, stomach and intestinal diseases, eye infection, and as mouth cleanser (Borjan et al., 2020; Hashmi et al., 2015; Benavente-Garcia et al., 2000). The European Food Safety Authority (EFSA) considers that olive oil polyphenols offer protection against oxidative damage and LDL (cholesterol indicator) and recommends a minimum consumption of 5 mg/day (EFSA, 2011). In the same report, the Panel also considered that there was insufficient evidence to establish a cause and effect relationship between the consumption of olive oil polyphenols and the maintenance of normal blood HDL-cholesterol concentrations. In the scientific opinion report of EFSA on the substantiation of a health claim related to olive leaf extract (OLE), it has accepted OLE as a safe products and the positive effect of OL on health was supported by citing scientific studies, but the scientific evidence is insufficient to establish a cause and effect relationship between the consumption of OLE and an increase in glucose tolerance (EFSA, 2014).

Besides positive effects on human health, recent research has shown that it is possible to use OLE in food formulations and food packaging as an antibacterial and antioxidant additive instead of synthetic agents to increase the shelf life and safety of foods. For these reasons, studies on phenolic component recovery from OLs, the use of OLs, OLE and the main phenolic compound of the extract (oleuropein) in foods, cosmetics, and pharmaceutical industries keep increasing in the literature. The products obtained from olive leaves also have an important commercial value. Generally, you can find several OL preparations in the markets in the form of dried whole leaf as herbal tea, powders made from dried plant material, liquid extracts, tablets, capsules, and food supplements (Arslan et al., 2021; Romani et al., 2019; Özcan and Matthäus, 2017). Especially the consumption of whole dried OLs as tea attracts attention from consumers. It is also possible to find several products sold as supplements having immune stimulator, antioxidant, anti-inflammatory, anti-aging, and blood sugar-regulating effects that use OLs in their formulations. Thus, the objective of this paper is to provide an overview of the antioxidant and antibacterial properties of phenolic acids, as well as their application in the food industry.

OLIVE LEAF AND ITS PHENOLIC COMPOUNDS

OLs are discarded in large amounts as a residue during pruning, harvesting and production of olive-related products. The amount of OLs released by pruning varies between 12 to 30 kg/tree, depending on the pruning type and age of the tree. They represent 10% of the overall weight of harvested olives (Khemakhem et al., 2017; Talhaoui et al., 2015). The European Medical Agency has acknowledged OL as an approved herbal preparation with a wide range of health-promoting qualities among several plants that have been the subject of investigations (EMA Monograph., 2017). In the scientific database titled "Compendium of

botanicals reported to contain naturally substances of possible concern for human health when used in food and food supplements" published by EFSA in 2016, information on plants used in food applications in European Union countries was compiled. The olive tree, and thus the other olive-related parts, are not included in the list in this database (EFSA, 2012a). Furthermore, the olive tree is included in the "Allocation List of Herbals Considered as Food" published by the Tea & Herbal Infusions Europe (THIE) association, and it is stated that the part of the plant used is the leaf (THIE, 2019).

The beneficial bioactive properties and potential health benefits of OLs like antioxidant, hypoglycemic, antimicrobial, antihypertensive, anti-inflammatory, anticarcinogenic, and antiatherosclerotic have been reported by many studies on humans and animals, and are attributed to their phenolic compound content (Qabaha et al., 2018; Difonzo et al., 2017; Liu et al., 2017; Boss et al., 2016; Wainstein et al., 2012; Lee and Lee, 2010; Sanchez et al., 2007). The major phenolic substances found in OLs are secoiridoids (oleuropein, dimethyloleuropein, verbascoside, ligtroside, and oleuroside), phenolic acid and its derivatives (vanilic acid, caffeic acid, vanillin), phenolic alcohols (tyrosol, hydroxytyrosol), flavonols (quercetin, isoramnetin, rutin), flavones (luteolin-7-glucoside, apigenin7-glucoside, luteolin, diosmetin, and diosmetin-7-glucoside), and flavon-3-ols (catechin, gallocatechin) (Jung, 2019; Vizza, 2019; Giacometti, 2018; Lockyer, 2016; Talhaoui et al., 2015).

The most prevalent phenolic compound, oleuropein, is a secoiridoid, heterosidic ester of hydroxytyrosol and elenolic acid, representing 1-14% of olive leaf weight (EFSA, 2012b). It gives a bitter taste to olives and is found in different amounts in various parts of olive tree, such as branches, leaves, roots, buds, fruit, and flowers (Benavente-Garcia et al., 2000). OLs are shown to be the richest source of oleuropein in nature and their levels can range between 10 to 140 g/kg OLs (Barbaro et al., 2014; Japón-Luján et al., 2008). The composition of commercial OLEs varies, with oleuropein normally contributing 20–40 % of the total phenolic compounds (EFSA, 2012b). The other important phenolic substances in OL is hydroxytyrosol. It is degradation product of oleuropein and has high antioxidant activity as well. Oleuropein decomposes into elenolic acid and hydroxytyrosol under the influence of β-glucosidase enzyme, light, temperature, acid, or base. Hydroxytyrosol has an amphiihilic structure, is a lipid- and water-soluble molecule and has the catechol group in its chemical structure (Rietjens et al.., 2007). It can be found in extra virgin olive oil esterified with elenolic acid or in simple phenol form. During the ripening and processing of the olive, the amount of oleuropein decrease, while the amount of hydroxytyrosol increase (Abaza et al., 2017; Özcan and Matthäus, 2017). So, olive oil contains higher amounts of hydroxytyrosol than oleuropein, and the amount of oleuropein is quite low compared to OL (Malik and Bradford, 2008).

The amount of phenolic substances and their composition in OLs are variable depending on the age of the tree, origin, proportion of branches, harvest time, climate, moisture, storage conditions, etc. It is stated in various studies that the quantity of phenolic substances in the leaves is its the highest level between November and March, which also includes the harvest and pruning periods (Di Meo et al., 2022; Abaza et al., 2017; Blasi et al., 2016; Çetinkaya and Kulak, 2016). In addition, the quantity and composition of OLE's phenolic substances depend on the drying method, such as drying in the sun or shade or in the oven, and the extraction methods. The extraction method type, temperature, pH, solvent type, and extraction time are fundamental factors affecting the quality and quantity of polyphenolic compounds recovered from olive leaves (Giacometti et al., 2021; Alcántara et al., 2020;

Ghomari et al., 2019; Rahmanian et al., 2015; Ahmad-Qasem et al., 2014). Compounds vary in their ability to be extracted using various techniques based on their chemical properties. For instance, it has been found that supercritical fluid extraction or pressurized liquid extraction are better suited for obtaining phenolics with less polarity, such as luteolin and apigenin, whereas polar compounds, such as derivatives of oleuropein, are better obtained through maceration (Taamalli et al., 2012).

ANTIOXIDANT EFFECTS OF OLs

It is stated that fruit and vegetable-rich diets have protective effects against several diseases. This has been attributed partly to the antioxidant effects of their phenolic compounds (Benavente-Garcia et al., 2000). Although synthetic antioxidants are used in many products, because of their possible mutagenic and carcinogenic effects, there is a growing interest in the use of antioxidants derived from herbal origins. Natural polyphenols found in foods have antioxidant activity and may prevent or reduce oxidative damage occurring at cellular levels. Utilizing them in many industries, such as cosmetics, pharmaceutical industries and food supplements, is becoming more popular due to their rich phenolic content. OLs are a frequently emphasized and studied product as a natural antioxidant. It has exhibited powerful antioxidant activities by preventing or delaying the oxidation of oxidative substrates. Because of their high oleuropein and hydroxytyrosol content, OLs have the highest antioxidant properties compared to other olive tree sections (Dekanski et al., 2011; Japon-Lujan et al., 2006; Benavente-Garcia et al., 2000). The amount and placement of hydroxyl groups in respect to the carboxyl functional group are often important factors in antioxidant action (Robards et al., 1999). The structure of phenolic compounds, such as the glycosylation site and positions and numbers of the hydroxyl groups on the carboxyl functional group, glycosidic moiety, is an important factor in both their metal chelating and radical scavenging action (Xie et al., 2015; Balasundram et al., 2006). Research has shown that the biological activity of aglycone part is higher than that of the glycoside portion (aglycones bound to glycones) (Oniszczuk et al., 2019).

Polyphenols (like hydroxytyrosol) and their secoiridoid derivatives (like oleuropein) in olive products react with free radicals, donate their own hydrogen to oxidants, neutralize them and scavenge free radicals, thus forming more stable variants (Quiñones et al., 2012; Saija and Uccella, 2000). They scavenge radicals such as DPPH, ABTS·+, OH and O2· –. (El and Karakaya, 2009; De la Puerta et al., 1999). They may also reduce reactive oxygen species generation by increasing the activity of antioxidant-acting enzymes or preventing enzyme activity that induces pro-oxidant activities. Another important point for antioxidant activity is the number and location of aromatic hydroxyl groups and the stability of the formed aroxyl radicals (Xiang and Ning, 2008; Benavente-Garcia et al., 2000). The aroxyl radical can delocalize electrons extensively. This is necessary in order to generate several mesomeric structures and radical stabilization. Antioxidant activity by oleuropein and hydroxytyrosol can also be associated with their hydrogen-donating and chelating metal ions like as Fe^{3+} and Cu^{2+}, which catalyze reactions that generate free radicals and have the power to block a number of inflammatory enzymes, including lipoxygenases, without having an impact on the cyclo-oxygenase pathway (Özcan and Matthäus, 2017; El and Karakaya, 2009; Andrikopoulos et al., 2002; Visioli et al., 2002). Oleuropein and hydroxytyrosol have also been found to be scavengers of hypochlorous acid and superoxide anions, two powerful oxidants formed at the inflammatory location and a key ingredient in bleaches based on chlorine that frequently contact food during production (Visioli et al., 2002).

ANTIMICROBIAL EFFECT OF OLs

OLs' antibacterial properties have long been utilized to treat various illnesses and fevers. Studies showed that OLE has antimicrobial activities against a wide range of bacteria, yeasts, molds, viruses, and other parasites including *Listeria monocytogenes, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Yersinia enterocolitica, Salmonella typhi, Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa, Helicobacter pylori, Vibrio parahaemolyticus, Campylobacter jejuni,* and *Candida albicans* (El-Sohaimy et al., 2021; Sánchez-Gutiérrez et al., 2021; Liu et al., 2017; Gökmen et al., 2016; Techathuvanan et al., 2014; Aliabadi et al., 2012; Keskin et al., 2012; Lee and Lee, 2010; Sudjana et al., 2009; Pereira et al., 2007; Markin et al., 2003). Although the mechanism of antimicrobial action has not yet been fully elucidated, it is reported that phenolic compounds have the ability to denature proteins, damage cell membranes, break down cell peptidoglycans, adversely affect cell membrane permeability, and thus create leakage of cytoplasmic elements such as potassium, glutamate or inorganic phosphate, which delays and inhibits the growth rate of microorganisms (Lee and Lee, 2010; Sanchez et al., 2007). The potential of hydroxyl groups in phenolic substances to attach to important enzymes' active sites and change microbial metabolism is another factor contributing to their antimicrobial effects (Gyawali and Ibrahim, 2014). The length of the saturated side-chain and position of the hydroxyl group in aromatic ring both affect the antimicrobial activity (Cueva et al., 2010). Caffeic acid, for instance, has been shown to have greater antibacterial activity than p-coumaric acid because it contains more hydroxyl groups replaced in the phenolic ring (Stojković et al., 2013).

In studies demonstrating the antioxidant and antimicrobial activities of OL, although phenolic substances show antimicrobial or antioxidant properties individually, it has been shown that they show a stronger activity when used together (Giacometti et al., 2021; Lee and Lee. 2010; Pereira et al., 2007). The level of antimicrobial effect and the sensitivity of microorganisms vary according to the type of microorganism. Pereira et al. (2007) indicated that *B.cereus* was the most susceptible microorganism among the strains tested, while *B.subtilis* was found to be the most resistant. In the same study, *E.coli* (Gram-negative bacteria) was found to be more sensitive compared to *S.aureus* (Gram-positive bacteria). Contrary to this study, it was determined that the oleuropein showed greater antimicrobial activity against *St.aureus* than its effect on *E.coli* and this was due to variations in cell structures by Sanchez et al. (2007). Markin et al. (2003) reported that OLE killed *E.coli*, *St.aureus, K.pneumonia,* and *P.aeruginosa* in 3 hours of exposure at 0.6% (w/v) concentration. However, due to *B.subtilis*'s ability to form spores, it was possible to inhibit when OLE was used up to 20% (w/v) concentration.

Keskin et al. (2012) also demonstrated that OLE significantly decreased the growth of all studied Gram-positive and Gram-negative bacteria, with the exception of *B.cereus* CCM 99, *Enterobacter aerogenes* ATCC 13048, and *Enterobacter cloacae* ATCC 13047. In the study by Sudjana et al. (2009), *C.jejuni*, *Helicobacter pylori,* and *S.aureus* were the most susceptible bacteria to OLE, whereas *E. coli*, *P.aeruginosa, B.subtilis*, *K.pneumoniae*, and *Serratia marcescens* were the least susceptible. Liu et al. (2017) found OLE, when used at a certain concentration, almost completely inhibited growth of major foodborne pathogens such as *E.coli* O157: H7, *L.monocytogenes*, and *S.enteritidis*. It reduced cell mobility in *L.monocytogenes* which corresponded with the lack of flagella, as seen by scanning electron microscopy, and inhibited biofilm formation by *S.enteritidis*. and *L.monocytogenes*.

Gökmen et al. (2014) determined the antibacterial efficacy of OLE on *L.monocytogenes* with MIC values greater than 32 mg/ml. OLE exhibited antifungal effects with a MIC of 24 mg/ml, minimal fungicidal concentration of 48 mg/ml, and 21 mm inhibitory zone diameter against *C.albicans* PTCC-5027 in the study of Nasrollahi and Abolhasannezhad (2015) and stated that it can be beneficial for treatment and prevention of infections like oral thrush caused by *Candida* spp. Zoric et al. (2016) determined the antifungal effects of OLE against *C.dubliniensis* CBS 7987 and *C.albicans* ATCC 10231 strains and found the MIC value for *C.albicans* to be 46,875 mg/mL and for *C. dubliniensis* to be 62.5 mg/mL. By Markin et al. (2003), the antifungal activity of OLE was demonstrated against *C.albicans* as well as against three dermatophytes, *Microsporum canis, Trichophyton mentagrophytes* and *T.rubrum*. It was suggested that it can be used in cases of fungal opportunistic infections. Unlike these studies, Shialy et al. (2015) determined that OLE did not show antifungal activity on *C.tropicalis, C.albicans*, *C.krusei,* and *C.glabrata*.

FOOD APPLICATIONS

Awareness of the importance of diet in health has been raised among consumers, and it has shown that foods should be considered for their active positive impacts on health as well as the quality and amount of nutrients they contain. To promote the health benefits of natural resources, research is being done on the creation and marketing of functional foods, dietary supplements, and nutraceuticals made from natural sources. In this respect, OLs products, abundant sources of phenolic compounds, have potential to be used as natural food additives and supplements in foods. In fact, OLs arise from the pruning of olive trees and are also waste products from table and olive oil production. Nowadays, considering zero-waste approaches, it is very important to reduce waste generation and transform it into other sources and their valorization. The waste or by-products of one sector can become the raw materials for another one through industrial symbiosis. Generally, several OL preparations can be found at the markets in the form of dried whole leaf as herbal tea, powders made from dried plant material, liquid extracts, tablets, and food supplements (Arslan et al., 2021; Romani et al., 2019; Özcan and Matthäus, 2017). Especially use of dried whole OLs to be consumed as tea attracts attention by consumers.

One of the most common uses of OLs in foods as an antioxidant. Oxidation of lipids can happen during food preparation and storage as a result of free radicals, light, heat, enzymes, metals, metalloproteins, and the activity of microorganisms. Oxidative balance is very important in maintaining the quality of products, especially with high oil-content foods. In food products, oxidative degradation causes rancidity, potentially toxic oxidative products, degradation of colors, sensory decay, loss of nutritional quality, and decrease in shell life. Commercial chemical antioxidants are mostly used in food industry to prevent or slow down oxidation because they are effective, cheap, and highly stable. On the other hand, it is known that they are suspected of being promoters of carcinogenesis process and consumers have recently preferred to use natural additives instead of synthetic ones (Farag et al., 2007). Due to their great antioxidant potential, OLs have drawn more attention as the significance of natural antioxidants has increased. Besides their antioxidant effects, enriching food products with OLs might also improve nutritional value and provide health benefits.

Studies have revealed that olive leaves are generally used in foods as natural food additives for enrichment with antioxidant and bioactive compounds, extending shelf life by increasing oxidation stability and producing functional products. OLE is thought to be useful in extending the shelf life of vegetable oils by improving oxidation stability and maintaining

nutritional components. Ammar et al. (2017) carried out research on the phenolic profile of Tunisian cultivars' olive oil, to which olive leaves were added before extraction process. The recovered oil from the Chétoui cultivar that had been treated with 3 Oueslati variety of Tunisian olive oil has been proven to contain more polyphenols (44%), chlorophyll (about 67%), and carotenoids (about 62%) when olive leaves (3%) are added to the oil (Tarchoune et al., 2019). According to Sevim et al. (2013), adding olive leaves to olive oil may help the oil's bioactive profile after 18 months of storage. The leave-treated oils showed a significant increase in chlorophyll, alpha-tocopherols, phenolic contents, and antioxidant activity.

Studies have been done to enhance olive oils with the addition of phenolic substances as a result of the growing interest in olive oils with high phenol content in recent years. Thus, both nutritionally rich olive oil with high phenol content and high oxidation stability are obtained. In particular, studies on the addition of phenolic substances obtained from olive leaves by different extraction methods to olive oil have attracted attention (Arfaoui et al. 2022). The studies show that the enrichment of OLEs improves the virgin olive oil's oxidation by lowering the peroxide value and extinction coefficients during the storage period. Also from a sensory perspective, high overall acceptability and a better flavor and odor were observed in enriched olive oil after six months compared to the control. Arfaoui et al. (2022) incorporated OLE as a natural antioxidant into extra virgin olive oil from "Chetoui" variety to improve nutritional quality and oxidative stability. Results showed that the enrichment maintained organic standards and had no impact on the olive oil quality.

Oxidation and off-flavour can occur easier in refined vegetable oils, like soybean oil, palm oil, and sunflower oil since there are unsaturated fatty acids and low amount of antioxidant substances. For this reason, butylated hydroxytoluene (BHT) is generally added to these oils to improve their oxidative stability. As mentioned above, studies on switching to natural antioxidants from synthetic ones are among the most emphasized. Salta et al. (2007) added OLE to olive oil, sunflower oil, palm oil, and vegetable fats to enrich them with polyphenols and determined that OLE contributes to increasing the capacity to scavenge radicals and oils' oxidative stability, showing more antioxidant activity when compared to BHT. Hassan et al. (2022) assessed the impact of OLE addition at various concentrations on lipid oxidation in refined soybean oil and BHT at a concentration of 200 ppm and showed that OLE had a strong antioxidant capacity similar to BHT at 200 ppm during accelerated storage, making it possible to use it as natural antioxidant in soybean oil.

Dauber et al. (2022) compared the performance of OLEs produced by using various extraction methods in preventing oxidative damage in canola oil. During five-week storage at 60 °C, the ability of canola oil to resist oxidation with or without the addition of extract was evaluated. When OLEs were added to canola oil, the oxidation process was delayed relative to the control oil, extending the oil's shelf life. In another study, the addition of olive hydroalcoholic leaf extracts (OHE) increased the resistance of canola oil and high oleic sunflower oil to heat during frying of French potatoes. When compared to oils without extracts, oils with OHE reduced the generation of polar substances and displayed an anti-polymeric impact (Jimenez et al., 2017). In another study in which refined olive and husk oils were enriched with OLs, rich in oleuropein and hydroxytyrosol, it was determined that OLs showed significant resistance to oxidative deterioration and increased their stability and prevented deterioration during storage (Bouaziz et al., 2008). They examined the antioxidant capacity of OLs and hydrolyzed OLEs in pomace and refined olive oils and found that they could lower the production of conjugated dienes and trienes as well as lipid hydroperoxides when compared to the control, improve the quality of the pomace olive oil, and bring it closer

to that of virgin olive oil. Compared to OLE itself, hydrolysate was more efficient. During storage, the two main components of enzymatic OLE hydrolysate, oleuropein aglycone and hydroxytyrosol, demonstrated the strongest antioxidant capacity and the lowest observed stability, followed by oleuropein. There are also studies showing that an extract rich in hydroxytyrosol has antioxidant effects on food lipids and increases oxidative stability (De Leonardis et al., 2008).

Malheiro et al. (2013) added different amounts of OL (1%, 2.5%, 5% and 10%; w/w) during oil extraction from olives and verified the effect of adding OLs on the quality and composition of olive oils. They determined that the OLs addition significantly improved the resistance of olive oils to oxidation and 10% addition of leaves increased the amount of vitamin E in oils by approximately 30% due to α-tocopherol in the leaves. Similarly, Kritsakis et al. (2017) determined that the adding OLE at the malaxation stage increased oxidation stability and phenol content overall in olive oils and a darker green olive oil with the desired properties was obtained due to the increase in carotenoid and chlorophyll contents. When OLE is used during olive oil extraction, the olive oil obtained has more fruity, bitter and positive properties. As a result, it was stated that OLE can be used as a natural additive to extend the shelf life of olive oils and increase their functional properties.

As for frying oil; during cooking, thermal oxidation rate is faster than autoxidation and the unstable hydroperoxides quickly break down into secondary oxidation products. Foods are heated to about 180 °C and above in frying processes and several chemical reactions take place, including autoxidation, polymerization, isomerization, and hydrolysis. Farag et al. (2007) investigated if sunflower oil obtained directly by pressing OL would increase the stability of reused oil during intermittent frying for 5 h/day for five consecutive days. During intermediate heating, the researchers discovered that sunflower oil blended with olive phenols had a greater smoke point and fewer primary oxidation products. Additionally, there was a correlation between the content of OL phenols and the beneficial effect against thermal oxidation. Accordingly, the high presence of phenolic substances in the OL resulted in low production of substances that react with thiobarbituric acid (TBA). Also, an activity against polymerization was found, as the amount of polar substances and triacylglycerol polymers, was also found to be lower in the treated oil compared to the control. Depending on the frying technique, there are differences in the amount of oil absorbed by the food. Therefore, the efficiency of phenolic transfer to fried food may vary. The addition of OLE did not considerably reduce the total polar material or the conjugated dienoic acids during the frying cycles, but it did significantly increase the oxidation stability compared to the control oil (Zribi et al. 2013).

OL and OLE can be incorporated as active components in milk and yogurt to extend thier shelf life and/or functional properties. Adding a certain amount of OL to milk and yogurt can increase the functionality of fermented milk products without influencing the survival of bacteria such as *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Str. thermophiles* (De Leonardis et al., 2008; Zoidou et al., 2014; Barukčić et al., 2022). OLE at concentrations of 0.2, 0.4 and 0.6% was applied in milk and yoghurt to investigate the effect on growth and viability of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* (Marhamatizadeh et al., 2013). OL milk and yoghurt had much more *L.acidophilus* and *B.bifidum* than control milk and yoghurt, and positive correlation was found between bacterial growth and OL concentration. The addition of OLE during the preparation of lowfat apricot yogurt considerably impacted *Str.thermophilus* count, protein, pH value, dry matter, and ash contents. The water-holding capacity values decreased throughout the storage period and

antioxidant activity was higher at the end of the storage (Peker and Aslan, 2017). Barukčić et al. (2022) enriched yogurts with OLE (1.5, 3, and 5% v/v) and observed shorter fermentation and lower pH, probably due to the starter culture's stimulation to produce acids more quickly, with no negative impact on the survival of yogurt starter bacteria. Despite having slightly unfavorable sensory properties, OLE-enriched yoghurts had improved phenol content, syneresis, and antioxidant activity, whereas viscosity and water-holding capacity decreased. Taking into account all of the results, 1.5% OLE addition seemed ideal for enriched yogurt products. Pourghorban et al. (2022) compared the effects of OL on the microbiological, physicochemical, antioxidant, and sensory properties of pre- and post-fermented yogurt samples enriched with various amounts of OLP and OLE during 21 days of storage. The results of the sensory evaluation indicated that acceptability was dependent on the concentration, OLE and OLP concentrations lower than 1.5 and 5 mg/ml, respectively, were acceptable. It was found that OLP or OLE had no adverse effect on yogurt starter culture bacteria and the total phenol amount, antioxidant capacity, and shelf life of the samples with OLE or OLP added were higher. Further phenolic compound reduction was seen when OLE and OLP were introduced to the samples during the pre-fermentation stage. This may be related to the starter bacteria's activity during fermentation. Similar to how the starting bacteria might use them as a source of carbon or energy. Additionally, in the samples of enhanced yogurt, the lactic acid formation during fermentation may result in the breakdown of phenolic compounds at an acidic pH.

In bakery products such as bread, crackers, cakes and biscuits, OLP or OLE is used in formulations to both enrich the product and increase its oxidation stability. The studies show that, these products with OLE added have higher antioxidant activity and longer shelf life. In addition, it has been stated that a functional product can be produced due to its high phenolic content (Palmeri et al., 2016). To develop breadsticks that are nourishing and healthful with a long shelf life, phenolic rich extracts from OL were incorporated into gluten-free formulations. Compared to the control, fortified breadsticks exhibited increased moisture and a_w, lower hardness, same color, and significant phenolic composition changes, as shown by a decline in the insoluble/soluble polyphenol ratio and a parallel rise in polyphenol bioaccessibility and antioxidant activity. Furthermore, all reinforced breadsticks showed a notable shelf life extension (Conte et al., 2021). Cedola et al. (2020) illustrated OLE valorization as a food ingredient instead of white wine in a popular cereal-based food named "taralli". The enhanced product was usually deemed satisfactory from a sensory perspective because the control sample displayed less friability than the standard product did. According to the data, OLEs enhance the nutritional value of enriched Taralli, and cooking did not change the amount of polyphenols, antioxidant activity, or flavonoids.

OLE and OLP can be added to minced beef meat to prevent lipid and myoglobin oxidation during cold storage (Aouidi et al., 2017). OL shows a capacity to prevent lipid oxidation and myoglobin oxidation. OL also improves the meat's technological quality by reducing storage and defrosting losses. It was assessed how varying oleuropein concentrations (0.3%, 0.6%, and 0.9%) affected the antioxidant activity and storage quality of Tabaq-Maz, a well-known type of traditional fried ribs in India. Oleuropein significantly reduced the TBARS (thiobarbituric acid-reactive substances) and free fatty acid (as % oleic acid) values compared to control, indicating an impact on oxidative stability. The count of total bacteria, psychrophills, yeast and mold was found to be lower in OLE-added samples, thus it was observed that it had a significant effect on microbiological properties (Dua et al., 2015). Hayes et al. (2010) examined the effects of lutein, sesamol, ellagic acid, and OLE on total

viable bacteria, TBARS value, lipid oxidation, oxymyoglobin oxidation, color, pH, and sensorial qualities of raw beef patties. All the nutraceuticals reduced total viable counts, or TBARS in food products. While sesamol addition to beef increased oxymyoglobin oxidation, OLE and lutein decreased oxymyoglobin oxidation in comparison to the control (Hayes et al., 2010).

Ranchman et al. (2021) also determined how OLE affected the physical characteristics of chicken breast sausage (CBS) and how OLE protected CBS from lipid oxidation during frozen storage. From 15 to 60 days in storage, the weight loss of control CBS without OLE upon thawing increased, but that of OLE-CBS did not change. Control CBS's viscosity, elasticity, breaking strength, and water-holding capacity reduced after being frozen, but those of OLE-CBSs remained the same. SEM analysis of CBSs revealed that, in contrast to control CBS, OLE-CBSs that were held frozen kept their unfrozen counterparts' structural similarity. Additionally, the addition of OLE to CBS prevented the oxidation of lipids, lowering of pH, and discoloration brought on by frozen storage.

Romeo et al. (2021) evaluated impact of the OLE addition on the mayonnaise's oxidative resistance during storage. It was greatly improved by enriching it with various quantities of OLEs. Individual phenolic content, oxidative stability, microbiological and sensory criteria, and antioxidant parameters were all checked to ensure its purity. The antioxidant capacity of the studied materials was functionalized and improved by hydroxytyrosol and hydroxyl acetyl derivate, as demonstrated by their connection with DPPH assay. No microbiological contamination was found, and enriched mayonnaises displayed good pH values. On the other side, using too much extract can alter the finished product's flavor and render it unpalatable to consumers.

Moudache et al. (2017) developed a packaging material with OLE that has antioxidant capacity for food and is used for fresh minced pork meat. OLEs were incorporated in an adhesive formula used to create the multilayer polyethylene film that served as the packaging material. This packaging was used to keep fresh pork minced meat in storage for 16 days at 4 °C. The study showed that active films containing 10% and 15% of OLEs effectively improved the antioxidant capacity of a fresh meat and can be used to increase the storage life of fresh minced meat for around two days (Moudache et al., 2017). It was observed that there was a development in the physical characteristics of chitosan-based composite films developed by adding OLEs and olive pomace extracts (OPEs) (Khalifa et al., 2016). The film containing 20 g/L of OLE had the lowest water solubility (17.82%). Besides, the composite films showed a stronger antifungal effect against *Penicillium expansum* and *Rhizopus stolonifier* than chitosan films as the inhibition percentage of these molds was increased by roughly two times by the addition of chitosan-20 g/L OLE and chitosan-20 g/L OPE. Chitosan-20 g/L OLE-based coatings maintained the qualities of coated products like apples and strawberries while delaying mold growth during cold storage.

The other common use of OL in foods is as an antimicrobial agent. The rise of drug-resistant bacteria around the world has begun to threaten human health. Therefore, intensive research continues to develop new effective antimicrobials. Antimicrobial drugs, which have a wide range of clinical uses, are replaced by plant-derived antimicrobial substances such as oleuropein due to their narrow antimicrobial spectrum and the resistance created by microorganisms over time, as well as causing serious side effects (Bisignano et al., 1999). Plant derived antimicrobials are generally considered safer compared to synthetic food preservatives and attract more attention as they can have beneficial impacts on human health.

In addition to prolonging the storage period of foods, natural plant antimicrobials can also benefit from their flavoring properties (Liu et al., 2017). High temperature treatments and chemical preservatives are generally used for food preservation, delaying food deterioration, and inhibiting the development of microorganisms in foods. Since high heat treatments can adversely affect physical, sensory and nutritional qualities of food, and chemical preservatives have negative effects on human health, studies on alternative preservation methods and the use of natural compounds in foods continue to increase. For this purpose, studies on the effects and usage possibilities of bioactive substances with antimicrobial activity, especially in some plants, come to the fore.

In a study conducted by Eraslan (2017), the effect of the use of commercial OLE on the microbiological properties of tomato paste was investigated as an alternative to chemical preservatives. It was determined that the total mesophilic aerobic bacteria, yeast and mold numbers in tomato paste samples decreased significantly with OLE supplementation. The researcher stated that the addition of OLE at different rates to tomato paste improves the microbiological quality and has a positive impact on the storage life.

Ahmed et al. (2014) evaluated the antimicrobial performance of OLE at different concentrations on raw, peeled shrimp samples. While *E.coli*, *P.aeruginosa*, *K.pneumoniae,* and *S.aureus* sensitive to OLE, a higher OLE concentration was needed to inhibit *B. subtilis* possibly due to its spore forming ability. The levels of total viable bacteria and total coliform bacteria in samples immersed in OLE for 3 hours at 4 °C were reduced by 1 log level and it has been stated that it is possible to use OLE as a natural preservative in seafood. The antimicrobial effect of adding OLE to meatballs at different rates on the growth of *St.aureus, S.typhimurium*, and *E.coli* O157 was investigated (Gökmen et al., 2016). In the control group meatball samples, *St.aureus, S.typhimurium*, and *E.coli* O157 numbers at 3 log and 6 log inoculation levels, respectively, increased in the range of 0.17-0.36, 0.05-0.40, and 0.15-0.20 logarithmic units at the end of the 7th day of storage. After the storage time, *St.aureus, S.typhimurium,* and *E.coli* O157 numbers decreased in the range of 0.23-0.94, 0.15-0.60, and 0.23-0.25 logarithmic units at 3 and 6 log inoculation levels, respectively, in the samples to which OLE was added at different rates. It was determined that the antimicrobial effect was significant. In conclusion of the study, the addition of OLE to meatballs at different concentrations enhanced the microbiological properties, had no negative effects on sensory aspects and had a positive effect on the shelf life. In this study, encouraging findings were revealed regarding the use of OLE as an antioxidant and antimicrobial in foods such as meatballs (Gökmen et al., 2016). OLE was applied to rainbow trout fillets that had been hot smoked and the changes in microbiological and sensory properties during storage at 4 ºC were examined. It was found that OLE application increased the shelf life of the product, did not cause any negative changes in sensory properties, and recommended to be used as a natural preservative in seafood (Mutlu and Bilgin, 2016). Kurt and Ceylan (2017) determined the effects of OLE at various concentrations (500, 250 and 125 ppm) on the chemical, physical, and microbiological features of Turkish dry fermented sausage. While OLE decreased the overall number of aerobic mesophilic bacteria and the number of lactic acid bacteria (LAB) during storage, it did not significantly affect the pH or color of sausage. It also decreased the level of free fatty acids and thiobarbituric acid. To assess the shelf-life extension and safety of raw minced beef during retail/display, Djenane et al. (2019) added OLE at 1 and 5% (v/w). Depending on its concentration, OLE has much stronger antibacterial and antioxidant properties. Shiga toxin-producing *E.coli* O157:H7 and *S.enterica* ser. Enteritidis pathogens levels, as well as psychrotrophic counts and TBARS valuse, were all decreased by OLE

addition. In another study, the effects of jujube, blueberry extracts, and OLEs on the shelf life and quality of meatballs during storage were examined, and it was found that the highest protective effect was obtained with OL (Gök and Bor, 2012). In a study using sausages, the effects of OLE, green tea and nettle on the physicochemical, textural, microbiological, and sensory features of Frankfurter (German type) sausages were evaluated. It has been determined that it reduces at least 2 logs and does not have an adverse effect on product quality (Alirezalu et al., 2016).

Lahreche et al. (2020) studied the impact of OLE as a natural antimicrobial and antioxidant on the quality characteristics of white and dark muscles from fillets of frigate mackerel maintained at a fridge temperature (3±1°C) under vacuum packing. The OLE application extended the storage-life of muscles, initiated lipid peroxidation in white muscle at an initial stage of storage and improved the microbiological properties of both muscles by restricting bacterial growth. The effect of OLE on *L.monocytogenes*'s heat resistance was examined both in Tryptic Soy Broth (TSB) and sous vide packed ground beef (Akdemir and Kıymetli, 2021). Total reductions in TSB and sous vide packed ground beef supplemented with OLE were higher than control samples for all heating temperatures (55, 60 and 65°C). The results obtained indicate that the use of OLE in the formulation may be an extra barrier to the control of *L.monocytogenes* growth in heat-treated ground beef.

The milk fortification with OLE reduced total mesophilic bacteria and fat and lactose losses. The OLE can be used to make a novel functional milk with a longer shelf life. OLE has also a strong antibacterial effect particularly against *B.cereus* and can inhibit α-glucosidase enzyme at 0.2 mg oleuropein/ml concentration (Palmeri et al., 2019).

Aliyari and Rezaei (2021) enriched the French sauce samples with OLE and investigated the oxidative stability, the microbiological and sensory properties of samples. It was observed that OLE could enhance the samples' oxidative stability and could be applied as a preservative in place of the commercial preservatives. The French sauce sample containing OLE indicated a lower total mesophilic aerobic bacteria value than the control French sauce sample. According to the results of the microbiological tests, in addition to preventing the growth of LAB at the end of the examined shelf-life time, OLE was able to retard the growth of microorganisms during the storage period.

Recently, studies on the utilization of natural bioactive substances in the development of active and functional packaging used to increase food safety and provided a longer shelf life for foods have attracted attention. In this sense, OLE has been used especially in chitosan-based films formulation. According to the results of the study of Musella et al. (2021), chitosan films with OLE incorporated had significant antimicrobial efficacy against *C.jejuni* and *L.monocytogenes* , mainly prolonging the lag phase of the microorganisms. However, with the exception of *C.jejuni,* the OLE does not appear to enhance the chitosan's inherent antimicrobial properties. The antimicrobial impact of CH + OLE films on *L.monocytogenes* at room temperature was more obvious than it was at 37 °C. The results imply that CH + OLE may have a significant impact in preventing the growth of microorganisms in food at cold temperatures. The chitosan films with OLE exhibited increasing antioxidant activity and were more soluble in food-like substances, and had a lower water vapor permeability. Famiglietti et al. (2022) developed chitosan-based films containing dried OLE and investigated the amount of polyphenols and the antioxidant and antimicrobial activity of these CH-based-edible films entrapped this extract. The antimicrobial effect of the films was tested against *Ent.faecalis*

ATCC® 29212, *S.enteritidis* RIVM 706, and *S.enterica* subsp. *enterica* serovar Typhimurium ATCC® 14028 in vitro.

All the bacteria studied were effectively inhibited by the dried OLE component of the edible films, depending on the concentration used. It was shown that these films might behave as active bioplastics when used for hamburgers rather than traditional baking paper. Bastante et al. (2019) showed that polyethylene terephthalate/polypropylene films made by the integration of phenolic compounds from OLE by supercritical solvent impregnation have antimicrobial effects against *P.aeruginosa* and *S.enteritidis*. Additionally, gelatin films containing 5.63% OLE were successful in lowering L.monocytogenes counts in fish that had been cold-smoked (Albertos et al., 2017).

CONCLUSIONS and FUTURE PROSPECTS

Because they have valuable compounds, OL has many positive effects on both human health and food quality. For this reason, instead of being thrown away as waste, it can be used for foods to improve food storage life and to develop functional foods, as well as in the fields of medicines, cosmetics and pharmaceuticals. Different authors showed antioxidant activity of OLE in delaying oxidation in a variable food matrix and antimicrobial activity against several microorganisms in foods at certain concentrations and conditions. However, OLE concentration is an important factor and should be considered before application because of its pro-oxidant activity and bitter taste. Furthermore, OLE's stability in food products is important for achieving its intended use. On the other hand, in using OLE to produce functional foods, more *in vivo* studies should be conducted to better understand functional effects of the OLE on human health. Although several human studies have examined the bioavailability and metabolism of the compounds in OLE, the information is still limited and unable to provide a complete grasp of this subject. OL extraction methods and conditions are another important issue. A desired approach is to increase extraction effectiveness (taking into account both yield and functionality) while reducing overall cost. More extraction studies are needed to improve OL extraction, especially by green processes suitable for application in foods.

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Impact of Temperature on the Salty Taste Perception of Reduced Salt Ayran

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Abstract

In recent years many efforts have been made to limit the use of salt in processed foods. Ayran is among the dairy foods that contain salt and is a type of fermented milk drink widely consumed in Turkey. Temperature of the food at consumption can influence many of its sensorial properties as well as the salt perception. In this study we investigated the impact of serving temperature of ayran on the saltiness scores at sensory panel. Ayran samples were produced at three salt levels; 0.2, 0.5 and 0.8%; and they were served at 3 different temperatures (4, 15 and 24˚C) for the sensory evaluation. Chemical composition, pH, viscosity, whey separation and sensory properties were determined. Temperature influenced the sensory saltiness scores significantly ($p<0.05$). Saltiness scores were higher at low temperatures.

Keywords: Saltiness perception, Temperature, Ayran, Salt reduction, Sensory

Research article
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INTRODUCTION

Ayran or so-called yogurt drink is a popular fermented milk drink in Turkey. It is principally produced by diluting the yogurt with water and adding salt. According to fermented dairy products bulletin (TGK, 2009), salt concentration should not exceed 1% in Ayran. High salt intake is associated with several health problems such as cardiovascular diseases and hypertension. According to World Health Organization, daily salt intake should be less than 5 g, and for children under 15 years old much lower levels (less than 2 g/day) are recommended (Anonymous, 2012). Many studies have been done in order to reduce the salt content of foods. However, it has always been a challenge for the food industry to reduce the salt. Enhancing the saltiness perception of the food is another approach that can help reducing the salt content without sacrificing the consumer acceptance. Saltiness of the food is detected in the mouth when Na^+ ions react with taste receptor cells located on taste bud cells. An epithelial sodium channel (ENaC) in the taste cell wall allows sodium cations to enter the cell. Previous studies have shown the thermal sensitivity of taste nerves. Some taste fibers respond to thermal stimuli, and temperature dependency of salt responses was explained by the cold activation of ENaC and heat activation of the vanilloid receptor (TRPV1t). Cooling can enhance the detection of saltiness and/or sourness in the anterior part of the tongue (Talavera et al., 2007; Cruz and Green, 2000).

There are several studies showing the influence of temperature on saltiness perception, however most of these studies are employed using salt solutions solely. Few studies involved interactions between salt, acid and bitter compounds.

No previous study has been done before, examining the temperature dependency of saltiness perception of ayran to the author's knowledge. Objectives of this study include determining the impact of serving temperature on sensory scores and particularly saltiness perception, and to reduce the salt concentration of ayran to an acceptable level.

MATERIAL and METHOD

Preparation of Ayran Samples

Ayran samples were made at Harran University Department of Food Engineering laboratory by diluting the yogurt (Pınar A.Ş.) obtained from the market at 1:0.9 ratio. Ayran samples were made uniform by a hand blender after the water addition. Salt was added after dividing each batch into 3 parts at the concentrations of: 0.2%, 0.5% and 0.8%. Trial was replicated twice.

Composition and pH

Total solids determined by gravimetric method according to IDF (1982). % Fat was analyzed by Gerber method (IDF, 1991). pH was measured directly by inserting the probe of the pH meter (Thermo Scientific, USA) into samples. Acidity was measured according to Bradley et al. (1992).

Viscosity

Viscosity was measured using Brookfield Viscometer (Brookfield Model RVDV-II+, Brookfield engineering Laboratories. Inc. Middlesbrough UK.) with spindle no 3 at 100 rpm and 15s (Ozer et al., 1997). Sample temperature was 4˚C during measurement.

Whey Separation

100 ml sample was placed in graduated cylinder and kept at 4˚C until measurement. Once placing the samples, measurements were taken after 1 day, 2, 6, 15, 20 and 33 days from the same cylinders. Volume of the separated whey on top was measured (Ozunlu, 2005).

Sensory Evaluation

Sensory evaluation was done by a group of 10 experienced sensory panelists. Sensory panelists were elected after a screening test for determining their detection limits of basic tastes (salty, sour and sweet) (Meilgarrd et al., 2007). At first step, each panelist candidate was presented with salt (0,2 %), citric acid (0.07) and sugar (1.2%) solutions and they were expected to recognize and name salty, sour and sweet tastes correctly. At the second step, panelist candidates were presented with a set of salty (0,1, 0,2 and 0,4 NaCl), sour (0,035, 0,07 and 0,14% citric acid) and sweet (1, 2 and 4 % sugar) solutions and asked for ranking them according to their intensity. 10 panelists who passed the screening tests were participated in sensory analysis of ayran samples.

Each sensory panel was done with one set of 3 ayran samples at 4, 15 and 24 ˚C at one salt concentration level. Sensory panel was completed after all panelists evaluated all concentration levels (3 sets: 0,2%, 0,5% and 0,8% salt). Ayran samples were evaluated for saltiness, sourness, flavour and consistency using a just-about-right (JAR) 5-point intensity scale with 3 being just about right, < 3 not enough salt and >3 too much salt. Samples were evaluated using ranking test model according to Drake (2008).

Statistical Analysis

Statistical analysis was performed by SPSS version 16 (SPSS Inc., Chicago, IL). Analysis of variance (ANOVA) was done to establish statistical differences between the chemical, physical and sensory properties of the samples depending on ayran temperature, salt concentration and interaction between those two factors.

RESULTS and DISCUSSION

Chemical and Physical Properties

Chemical analysis results and viscosity of ayran samples are given in Table 1. No significant difference was observed between the pH, acidity, total solids and fat contents of ayran samples at different salt levels (P>0,05). Increasing the salt content to 0.5% resulted in higher viscosity. Köksoy and Kılıç (2003), observed a decrease in viscosity of the Ayran when salt content was increased from 0.5 to 1.0%. The decrease in viscosity was attributed to the increase in the repulsive forces created by salt ions on the surface of the micelles reducing the tendency of aggregation. In our study increasing the salt content from 0.5 to 0.8% lowered the viscosity a little but it was not significant (P>0.05). On the other hand, our results showed that at very low salt levels (0.2%) viscosity was significantly reduced possibly as a result of reduced protein-protein interactions due to low ionic strength.

Table 1. Chemical analysis results and viscosity of ayran samples having 0.2%, 0.5 and 0.8% salt

	Salt level (%)		
	0,2	0,5	0,8
pH	4,45 ^{a}	4,47 ^{a}	4,41 ^{a}
Acidity (lactic acid %)	0,57 ^{a}	0,57 ^{a}	0,53 ^{a}
Total solids (%)	8,2 ^{a}	8,3 ^{a}	8,3 ^{a}
Fat (%)	1,4 ^{a}	1,4 ^{a}	1,5 ^{a}
Viscosity (cP)	109,5^{a}	130^{b}	125,5^{b}

^{a,b,c} Means in the same row with different superscript letters are statistically different (P< 0,05).

Whey separation results of the ayran samples for 33 days are given in Fig. 1. Whey separation occurs in ayran due to the sedimentation of destabilized proteins by time. Whey separation is an inevitable phenomenon for ayran, however the time it takes for phase separation to occur is a quality parameter for ayran and should not be too fast.

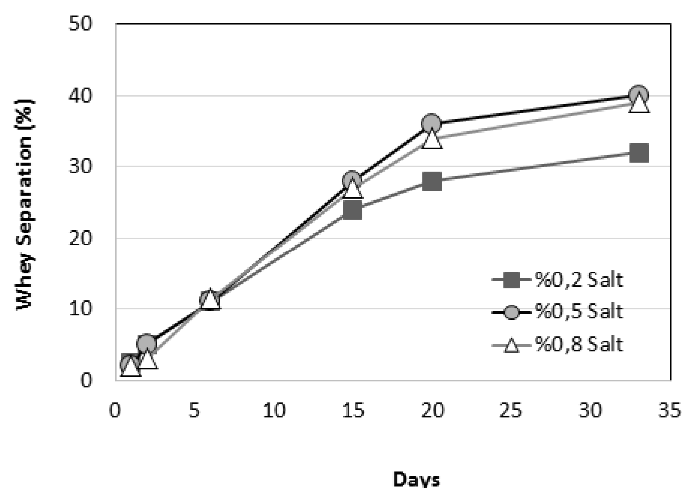

Figure 1. Whey separation results of ayran samples having 0.2% , 0.5 and 0.8% salt

Statistically significant increase was observed in whey separation during 33 days of storage. Whey separation levels didn't differ during the first week between samples having different levels of salt. However higher separation was observed at higher salt levels after day 15. Köksoy and Kılıç (2003), had also observed higher whey separation at high salt levels, where they only measured the whey separation on day 15. They attributed the higher whey separation at high salt levels to the rearrangement of the casein micelles involving increased protein–protein interactions.

Sensory Properties

Both salt level and temperature had significant influence on saltiness scores. According to fermented dairy products bulletin (TGK, 2009), salt concentration should not exceed 1% in ayran. In this study we found that, samples with 0,5% salt were scored (3) ideal, above 0,5% were found to be too much salty and samples with 0,2% salt were scored less than 3, meaning not enough salt (Table 2). Serving temperature influenced the saltiness scores significantly ($P<0.05$).

Saltiness perception of samples at low temperatures was higher. 70% of the panelists scored the 0.2% salty samples as JAR at 4˚C, while the same samples were found to be JAR by only 20% of panelists at 24˚C, and rest of the panelists scored them <3 (Fig. 2). At 0.5% salt level most of the panelists scored the samples 3 and >3. While no panelist found the samples having "not enough salt" at 4 and 15˚C, same samples were scored <3 by 36% of the panelists at 24˚C. Samples having 0.8% salt were found to be too salty by 60% of the panelists at 4 and 15˚C, while at 24˚C same samples were scored >3 by 40% of the panelists and some (10%) found them having not enough salt.

Ayran samples did not show any significant differences in terms of sourness and consistency as seen in Table 2. Different salt levels didn't influence the flavor except for 24°C samples, where samples with 0.2 % salt received low scores. Flavor scores tended to be close to ideal at low temperatures and as the temperature increase, 0.2 % samples were found to be below the ideal; and at 0.8 % salt level, samples had scores >3. No significant flavor difference was observed between different temperatures at 0.5% salt level.

Table 2. Sensory analysis results of ayran samples having 0.2% , 0.5 and 0.8% salt

Parameter	Salt level (%)	4 °C Mean		SD	15 °C Mean		SD	24 °C Mean		SD
Saltiness	0,2	2,60 [aA]	±	0,30	2,50 [abA]	±	0,30	1,90 [bA]	±	0,24
	0,5	3,18 [aB]	±	0,40	3,09 [abB]	±	0,30	2,81 [bB]	±	0,15
	0,8	3,80 [aC]	±	0,19	3,70 [abC]	±	0,17	3,30 [bC]	±	0,27
Sourness	0,2	3,00 [aA]	±	0,89	2,82 [aA]	±	0,60	2,91 [aA]	±	0,94
	0,5	3,00 [aA]	±	0,47	2,91 [aA]	±	0,54	3,27 [aA]	±	1,10
	0,8	3,27 [aA]	±	0,65	3,18 [aA]	±	0,40	3,36 [aA]	±	1,03
Flavor	0,2	3,18 [aA]	±	0,40	3,00 [aAB]	±	0,77	2,27 [bA]	±	0,90
	0,5	3,00 [aA]	±	0,00	2,73 [aA]	±	0,47	2,64 [abA]	±	0,92
	0,8	3,09 [aA]	±	0,70	3,27 [aB]	±	0,65	3,36 [aB]	±	1,12
Consistency	0,2	3,09 [aA]	±	0,30	3,00 [aA]	±	0,45	2,91 [aA]	±	0,70
	0,5	3,00 [aA]	±	0,00	3,09 [aA]	±	0,70	2,91 [aA]	±	0,70
	0,8	3,18 [aA]	±	0,60	3,09 [aA]	±	0,30	2,91 [aA]	±	0,30

[a,b,c]Shows differences between ayran samples at different temperatures. Means with different superscript letter in the same row are significantly different (p<0.05).
[A,B,C]Shows differences between ayran samples at different salt levels. Means with different superscript letter in the same column are significantly different (p<0.05).
SS: Standard deviation

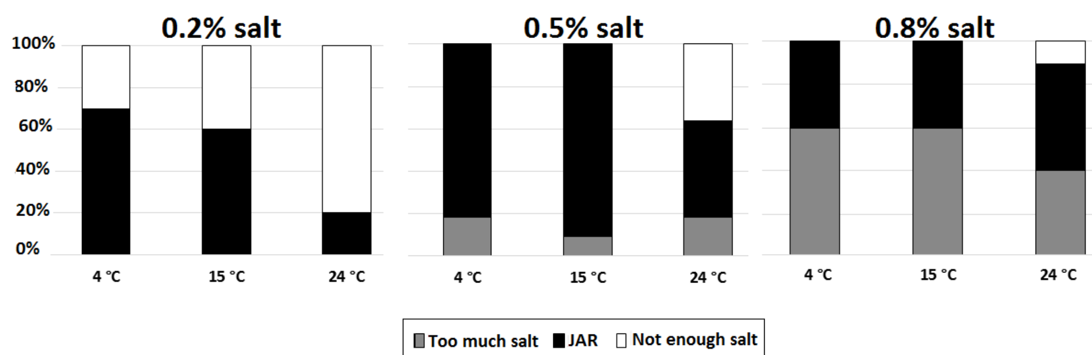

Figure 2. Saltiness intensity responses of ayran samples at 0.2, 0.5 and 0.8% salt levels

Several studies have shown that temperature influences the taste sensation, and many neurons in mammalian taste pathways respond to temperature. Kanno et al. (2016), studied the perception and thresholds of basic tastes under thermal stimulation of the tongue and they found that salty taste threshold increased for both the cool (10-13˚C) and hot (37-39˚C) stimulus. Lipscomb et al. (2016) did not find any influence of temperature on salty taste intensity when salt solutions were consumed alone. However, they have seen a significant effect of the temperature on the salty taste intensity when salt combined with acid. McBurney et al. (1973) reported that perceived salt intensity was higher at 17, 37 and 42°C as compared to intermediate serving temperatures of 22, 27 and 32°C, respectively.

They also showed that the detection threshold for salt was higher at temperatures 4 and 42°C and lower at temperatures 22 to 32°C. Paulus and Reisch (1980) suggested that perception of saltiness decreases as the temperature is increased. Cruz and Green (2000) reported that cooling the anterior edge of the tongue (chorda tympani nerve) can arouse sourness and/or saltiness. Our findings were also in accordance with most of the previous studies suggesting that cooling increase the salty taste intensity and perception of higher saltiness levels.

CONCLUSION

In this study our results showed that, serving temperature has a significant role on saltiness perception of ayran. Saltiness scores were higher at low temperatures. More than half of the panelists found the samples having "just about right" level of salt even at only 0.2% salt. This study demonstrated that reducing the salt content of the ayran to a certain extent could be unnoticeable at low temperatures.

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Determination of Microbiological Quality of Fish Burgers Enriched with Orange Peel Extract

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Abstract

In this study, the effect of orange peel extracts (OPE) on the microbiological quality and shelf life of fish burgers was determined. To complete this objective, orange peels were extracted by using 70% ethanol. Fish burgers were divided into three groups. First group (G1) were treated with 1% concentration of OPE, second group (G2) were treated with 2% concentration of OPE and third group were prepared without OPE known as control group. All fish burgers were stored at 4±1°C for 15 days. Total mesophilic bacteria (TMB) and total psychrophilic bacteria (TPB) count were determined. Conferring to the results of analysis, the TMB count was 6.28 log CFU/g and 5.44 log CFU/g in G1 and G2 groups, respectively by the end of the storage period, whereas in control it reached to 7.42 log CFU/g. Total psychrophilic bacteria count was 6.8 log CFU/g and 5.96 log CFU/g in G1 and G2 groups, respectively by the end of the storage period while in control it was determined as 7.19 log CFU/g. So it was concluded that the TMB and TPB counts in fish burgers treated with 1% and 2% OPE were significantly ($P<0.05$) lower than the control group throughout storage. Results of this study showed that the usage of OPE is an effective approach to prevent microbial growth in the fish burgers during the refrigeration storage.

Key words: Orange peel extract, psychrophilic bacteria, mesophilic bacteria, shelf life, fish burger

Research article
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INTRODUCTION

Fish has high quality nutrients like well-balanced proteins, great amount of essential n-3 polyunsaturated fatty acids (PUFA), vitamins and minerals (P, Se, Mg, and I) (Atitallah et al., 2019). Fish fatty acids are much important to humans for the inhibition of cardiovascular diseases that's why fish is a good substitute of red meat protein which can cause heart diseases (Conner, 2000; Mozaffarian et al., 2005; Vicente and Torres, 2007). Seafood is the most perishable food so it's more susceptible to microbiological and enzymatic spoilage due to oxidative reactions (Metin et al., 2002).

Fish and readymade fishery products like fish burgers, fish fingers and fish meat balls are the much important amongst the globally dealt food item however also they are most fragile food products because of great amount of polyunsaturated fatty acids. To stop the decline in fish product quality because of microbial growth and lipid oxidation, artificial food preservers like Butylated hydroxyl toluene, Butylated hydroxyanisole, and Tert-Butylhydro quinine are used (Frankel, 1993). These artificial antioxidants can be dangerous for human health as they could be carcinogenic (Ito et al., 1986). Therefore, it is essential to find out natural antioxidants, specifically plant derived compounds to increase the shelf life of fish and readymade fishery products through preventing and deferring lipids oxidation (Sanchez-Alonso et al., 2008). Many plants, herbs and spices which have been used in several food formations have antioxidant activities (Liu et al., 1992). The bioactive compounds naturally existing in plants have been proven to have antimicrobial, antioxidant and anti-carcinogenic properties (Ahn et al., 2007; Kanatt et al., 2007).

Citrus fruits are the globally prevalent fruit type by annual yield of more than 100 million tons (Shehata et al., 2021), and their industrial usage produce a great content of fruit wastes include pulp, seed and peel, which has come to be an ecological threat if not used and predisposed well. The peels of these citrus fruits are not eatable, they have enough bioactivities mostly antioxidant, antimicrobial, and anti-carcinogenic (Kumar et al., 2020). Citrus fruit waste have great importance because it consists high content of several flavonoids, polyphenols, carotenoids, sugars, dietary fiber, ascorbic acid, essential oils, and few other minor elements (Sharma et al., 2017).

Total bioactive compounds are considerably higher in peels of oranges, lemons, and grapefruits as compared to their fruits (Ramful et al., 2011). Therefore Recycling these fruit byproducts in a proper manner might give substantial economic advantage to food manufactures because of their valued industrial and nutritious specificities which provide possible health security for consumers, similarly, minimalize the worst ecological effect and enhance worth of this remains. It was also reported that many of these fruit byproducts might be utilized as functional elements during processing and manufacturing of healthy food products because of its high amount of bioactive compounds worked as natural stabilizers, antimicrobials, antioxidants, flavorings, pigments, and emulsifying mediators (Marın et al., 2002; Ayala-Zavala et al., 2011).

Approximately 25 to 30% fruits productivity are non-eatable products like peels and seeds (Ajila et al., 2010). Generally, fruit peels comprise about 50 to 65% of the whole fruit mass and known as the major byproduct of fruit industry. If they are not administered properly, they converted to waste and caused bad odor, insect’s production leads to severe ecological pollution (Mandalari et al., 2006). Orange (*Citrus sinensis*) is a citrus fruit links to the family “Rutaceae”, and orange peel waste by-product contained many nutrients, high content of fiber, vitamin C, vitamin B6, calcium, folate and other essential nutrients and minerals, which works as antimicrobial and antioxidants (Okwu, 2008). In this study it was aimed to determine the antimicrobial effects of orange peel extract in fish burgers.

MATERIALS and METHODS

Orange peel extraction

Oranges *(Citrus sinensis)* were bought from a local market and they peeled prudently by using a sharp knife. Then the peels were washed through distilled water and dried for 48 hours at 45°C. After that, orange peels were converted into powder by grinding. Subsequently 10 gram powder of orange peels was added in 100 mL of 70% ethanol for extraction and put into ultrasonic water bath for 30 minutes. When powder was mixed after 30 minutes it was filtered. Ethanol was vaporized by means of a rotary evaporator at 50°C under vacuum and then orange peel extract was obtained (Ucak and Khalily, 2022).

Preparation of fish burgers

Fish (anchovy) bought from local market, Nigde, Turkiye and quickly brought to laboratory in styrofoam boxes filled with ice. In the laboratory, fish were cleaned with distilled water, then cut the head, clean the fish gut, cut into fillets and minced them by using a meat mixer. After that minced fish meat was distributed into three groups known as Control (C), Group 1 (G1) and Group 2 (G2). Subsequently mixture for fish burgers formation was prepared by using the process proposed by Tokur et al. (2004) by means of slight modification. All spice ingredients were mixed with minced fish meat separately in each group, and two groups were supplemented with 1% and 2% concentration of orange peel extract in Group 1 and Group 2 respectively and mixed completely by using domestic mixer. Orange peel extract was not added to the control group. After mixing fish burgers were shaped from each group by using shaper. Each group has sixty burgers and each burger have a weigh of 50 g, comprising of 87.8% minced fish meat, 4% wheat flour, 6% corn flour, 0.2% onion powder, 0.2% garlic powder, 1.2% salt and 0.6% sugar. Then, burgers were kept at 4°C in refrigeration up to 15 days for periodic investigation of microbial growth (Ucak et al., 2011).

Microbiological analyzes

Fish burgers samples from each group were taken for the evaluation of microbiological analyses including total mesophilic and psychrophilic bacteria counts. 10 gr of fish burger from each group was taken and mixed with 90 mL of Ringer solution for 3 minutes to make it homogenized. More dilutions were prepared up to 10^{-8} concentration and then 0.1 mL from each dilution was spread on plate count agar plates (ICMSF, 1982). Then these 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.

Statistical analysis

Statistical analyzes were done 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 for data comparison.

RESULTS and DISCUSSION

Total mesophilic bacteria count

According to the recommended limited value (7 log CFU/g) for fresh fish (ICMSF, 1986), the results of this study specify that fish burgers which were preserved by using OPE of 1% and 2% concentration were in good quality throughout the storage period as compared to control group. The influence of OPE on the growth of mesophilic bacterial growth on fish burgers is given in Figure 1. Total mesophilic bacterial count was 2.17 log CFU/g at the beginning of storage which increased to 6.28 log CFU/g and 5.44 log CFU/g in G1 and G2 groups, respectively at the end of the storage period, whereas in C it reacheD to 7.42 log CFU/g. It was perceived that the total mesophilic bacteria counts in fish burgers prepared with 1% and 2% concentration of OPE were significantly ($P<0.05$) lower than the C group through the storage period.

It was noticed in another study in which peppermint essential oil was used with chitosan coating to preserve mackerel fish meat balls and it was concluded that Total mesophilic bacteria count in C and CF groups were 6.24 and 5.93 log CFU/g respectively on the 18th day of storage, the lowermost Total mesophilic bacteria count 5.18 log CFU/g was found in fish meatballs samples coated with chitosan added with 1% peppermint oil emulsion (Ucak and Afreen, 2022). There was a study in which scientists reported that rosemary and laurel essential oils decelerated the growth of bacteria however in that study total bacterial count of the trout fish meatballs supplemented with rosemary and essential oils was found to be significantly higher (5.24 log CFU/g) than the present study (Keser and İzci, 2020).

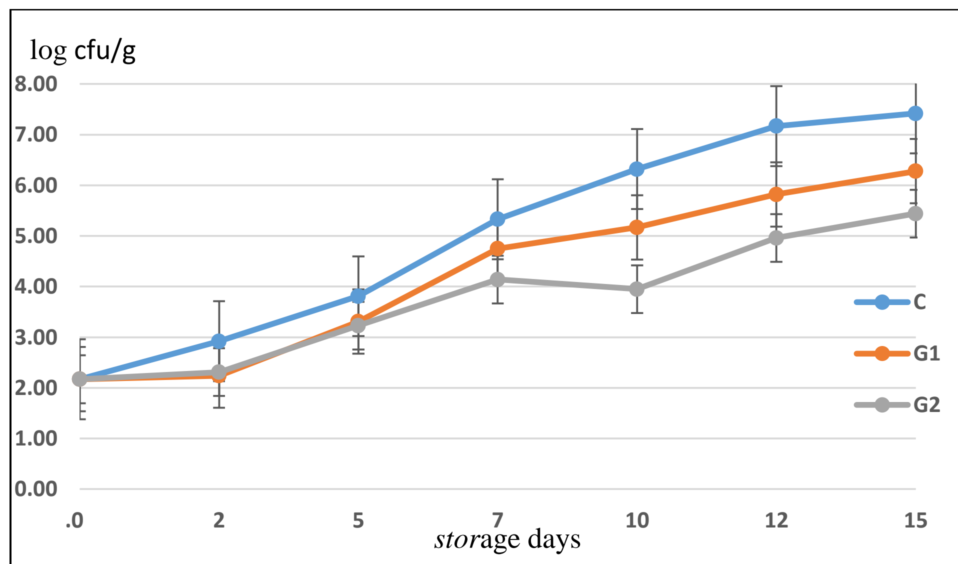

Figure 1. Effect of OPE on the growth of total mesophilic bacterial growth in fish burgers

Total psychrophilic bacteria count

The main reason of fish and fishery products spoilage is bacterial growth. The influence of OPE on the growth of total psychrophilic bacterial in fish burgers is given in Figure 2. Total psychrophilic bacteria count was determined as 2.04 log CFU/g at the beginning of storage which increased to 6.8 log CFU/g and 5.96 log CFU/g in G1 and G2 groups, respectively at the end of the storage period. In control group this value reached to 7.19 log CFU/g. Total psychrophilic bacteria count value in fish burgers treated with 1% and 2% OPE was significantly ($P<0.05$) lower than the control group during the storage period.

It was noticed in another study in which peppermint essential oil was used with chitosan coating to preserve mackerel fish meat balls and it was concluded that Total psychrophilic bacteria count in C and CF groups were 7.59 and 7.15 log CFU/g on the 18th day of storage, while 6.14 log CFU/g in the PMO group.

The lowest Total psychrophilic bacteria count was found in fish meatballs samples coated with chitosan added with 1% peppermint oil emulsion (Ucak and Afreen, 2022). It was observed in another study, in which rosemary extract was added with concentrations of 0.4% and 0.8% in the formation of mackerel fish burgers, and the total bacterial growth during storage was lesser in rosemary concentrated groups as compared to the control group (Ucak et al., 2011). It was found in one study that initial psychrophilic bacterial count was 4.22 log CFU/g in trout meat (Keser and Izci, 2020). Different antioxidants including thyme, laurel, sage and green tea were used to preserve fish burgers and initial count of psychrophilic bacteria was found to be 4.90 log CFU/g, and it was detected at the end of the study that total psychrophilic bacteria in those groups which were supplemented with antioxidant during the frozen storage was lesser as compared to the control group (Ozogul and Ucar, 2013).

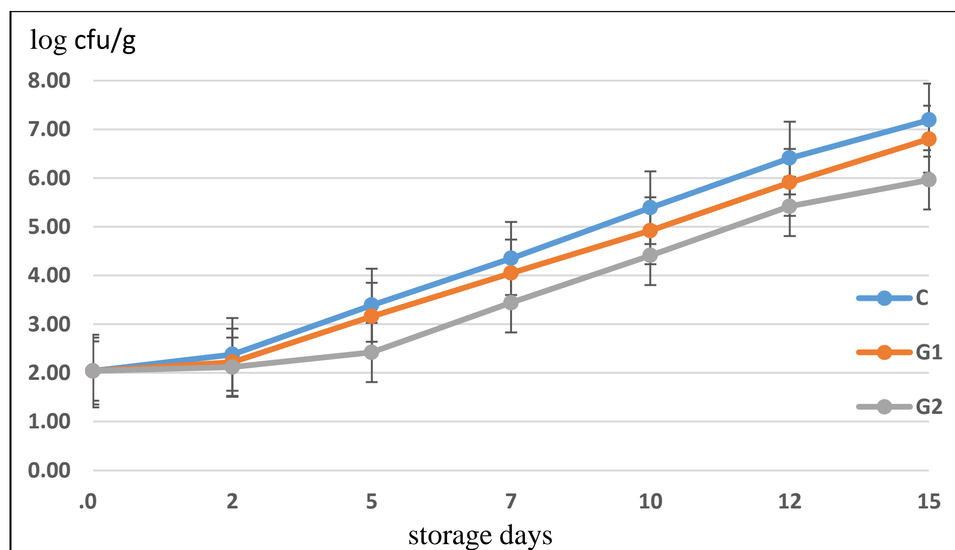

Figure 2. Effect of OPE on the growth of total psychrophilic bacterial growth in fish burgers

Many previous studies shows that microbiological growth was considerably (($P<0.05$) influenced by adding pomegranate peel extract and edible coating. It was noticed that those fish fillets which treated with pomegranate peel extract have longer shelf life throughout refrigerated storage and have less growth of total viable count as compared to control samples (Prashanth et al., 2000; Shahidi and Naczk, 2004; Tomas-Barberan et al., 2001). Rosemary extract was also establish efficient in controlling the microbial growth of in fishery products (Sacchetti et al., 2005).

CONCLUSION

It was concluded from the present study that supplementation of fish burgers with orange peel extract suppressed the bacterial growth in the fish burgers. Throughout the 15 days of storage period, fish burgers incorporated with 1 and 2% orange peel extract presented the lowest total mesophilic bacteria and total psychrophilic bacteria counts as compared to the control group. Moreover, these bacterial count did not exceed the limit values of microbiological quality of fish.

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