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Editorial Board

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Effects of Gallic Acid and Quercetin on the Production of Fruit Puree

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Abstract

Babies of 0-6 months should be fed with mothers milk, but after that period, their nutrient requirements are getting higher and additional nutrients like purees must be used. In that, it is important to give them such foods to support their immunology. Due to polyphenol oxidase activity, apple and banana puree have browning reactions, and thus in production of such puree, rapid preparation and consumption in homes or addition of some protective agents in industry have been used as alternatives. Additives are synthetic materials and some researchers announced that they are capable of cancerous precursors. Reversibly, most of the polyphenols like gallic acid and quercetin are natural molecules having antioxidant properties in plants. In this study, gallic acid and quercetin usability for the production of apple, jerusalem artichoke and banana puree was investigated. In the preliminary experiments with those fruit juices, the concentrations require for stopping browning reactions were determined as 0.001M, 0.01M and 0.1M; so that fruit purees were prepared by glass shredder in gallic acid or quercetin added media. Browning and spoiling were analyzed in all samples and compared with two different control groups; home-made and commercial purees, namely. Quercetin was found more effective on browning than gallic acid in both juices and also in purees. The microbial growth was reduced by gallic acid in a sequence of apple (90.49%), banana (80.24%), and then jerusalem artichoke (68.00%); while quercetin reduction sequence was banana (96.48%), apple (73.56%) and then jerusalem artichoke (46.22%). Therefore, it has been determined that, it may be advisable to add, instead of cancerous materials, gallic acid and quercetin for prevention of browning and to strengthen the immune system of the baby during the period of lacking prebiotics.

Keywords: Browning reactions, Fruit puree, Gallic acid, Quercetin, Spoiling

INTRODUCTION

The World Health Organization recommends that infants be fed only breast milk for the first six months, starting complementary foods at the end of the sixth month (World Health Organization, 2013). Nutritive value of the complementary food depends solely on its composition; the materials used and the proportions of fruit or vegetable content (Čizkova et. al., 2009).

Commercial infant formula contains chemical products with properties that slow down the formation of microorganisms, color protectors, thickeners and sweeteners. These compounds result in a number of diseases, such as mental decline in infants, Alzheimer's and cancer, as a result of certain consumption. It is clear that stopping the use of artificial products will positively affect the baby's health. The majority of the antioxidant capacity of a fruit or vegetable resulted from compounds such as flavonoids, isoflavones, flavones, anthocyanin's, catechins and isocatechins rather than from vitamins C, E or β-carotene (Wang et al., 1996; Kahkonen et al., 1999).

Quercetin, a flavonoid species, is found in plants, especially in vegetables and fruits. It is found in various quantities in many foods, mainly crucifers, grapes, apples, tomatoes and wild mussels (Manach et al., 2004; Kelly, 2011). The other well-known phenolic compound is gallic acid with a chemical formula of 3, 4, 5-trihydroxybenzoic acid. It is also found in a wide variety of vegetables, fruits, tea, coffee and wine (Naira et al., 2016). The structure of gallic acid has phenolic groups that are a source of readily available hydrogen atoms so that radicals produced can be delocalized over the phenolic structure (Nikolic, 2006). The antioxidant activity of gallic acid and its derivatives has been reported in several studies (Fogliani et al., 2005; Kaur et al., 2005). Gallic acid has been shown to possess antimicrobial activity against human pathogens (*Staphylococcus aureus*, *Corynobacterium accolans*), a plant pathogen (*Erwinia carotovora*) and a human pathogenic yeast (*Candida albicans*) (Fogliani et al., 2005). The cytotoxic effects of Triphala, an Indiana herbal drug, on breast cancer cells were attributed to gallic acid (Kaur et al., 2005).

It is known that substances with antioxidant properties have different effects such as binding of free metals, clearing of free radicals, enzyme inhibition and induction of expression of protective enzymes (Nam et al., 2016). It has been proved to have potential preventive and therapeutic effects in many diseases, where the oxidative stress has been implicated, including cardiovascular diseases, cancer, neurodegenerative disorders and in aging (Nikolic, 2006; Kaur et al., 2005).

In this research, the usage of gallic acid and quercetin in inhibition of browning reactions in a fruits was investigated. By this way, they may be used instead of chemicals in the production of especially baby foods. As a result, the baby purees produced with these phenolics will have anticancerous, antimicrobial and antioxidant properties, and thus they will have beneficial effect for baby growth instead of decline observed with chemicals. In addition, since gallic acid and quercetin have antimicrobial properties in themselves, the self-life of the purees will also be increased due to the presence of these compounds in purees.

MATERIAL and METHOD

Quercetin and gallic acid used in the study were purchased from Sigma_Aldrich, and red apples, jerusalem artichoke and banana were supplied from a regional greengrocery. In the preliminary experiments, in order to determine the concentrations that can capable of stopping the browning reaction of the fruits, red apple and jerusalem artichoke juices prepared by Arcelik Fruit Juice were mixed with 5 ml of quercetin or gallic acid solutions, of three different concentrations (0.001M, 0.01M, 0.1M). Control groups were established for each fruit type, which does not contain any solution. Color differences were compared by taking photographs at the end of same time durations.

In the second part of the study, the effect on the duration of microbial deterioration of addition of quercetin and gallic acid into the fruit purees was investigated. In the sterilized ESCO Laminate Flow Cabinet, fruit purees were prepared using glass grater and then added to sterile containers containing 5 ml of gallic acid or quercetin solutions. In this part of the study, both pure fruit puree and commercial fruit puree (banana and carrot-red apple mixture) were used as a control group. The porridge was closed with parafilm and kept at room temperature in the MIPRO water bath until visible microbial deterioration occurred. Color formation and microbial deterioration in the purees were compared with taking their photographs. In order to quantify the microbial growth, at every 24 hours, 500 µl of the sample was inoculated into the test tubes containing 20 ml of sterilized MRS medium in Laminar flow cabinet. The number of microorganisms formed after growth period of 6 hours at 37°C in the ILDAM incubator was expressed as the absorbance values read at 420 nm on an Agilent Technologies Cary 60 UV-Vis spectrophotometer. Analyzes of microorganisms

were also carried out periodically until visible deterioration was observed and compared with control groups.

RESULTS and DISCUSSION

In the first part of the study, control purees, commercial purees (banana and carrot-apple mixture) and experimental purees that had been obtained by mixing with quercetin or gallic acid solutions at different concentrations were stored for 5 days at room temperature in a water bath. In that period they were being photographed to observe the browning reaction results and microorganism formation processes were also followed (Figures 1-4). As seen in Figures 1 and 2, addition of 0.1M quercetin and 0.001M gallic acid solutions into red apple purees were found more effective on delaying browning reaction than commercial and control groups.

In banana puree (Figure 3), the same concentrations of both quercetin and gallic acid solutions were found effective on delay of coloring reaction. When commercial banana puree was compared with the experimental purees containing 0.001M gallic acid or 0.1M quercetin solutions (Figure 4), gallic acid or quercetin were found better. By comparing between two experimental purees, it was concluded that 0.1M quercetin addition was preferable to 0.001M gallic acid.

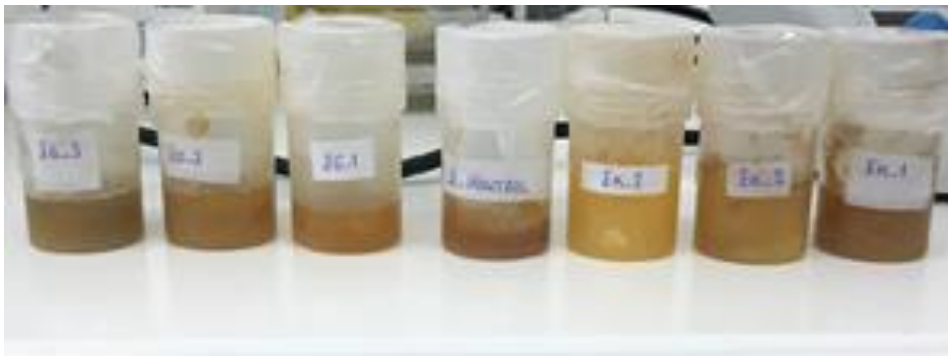

Figure 1. The appearance of the experimental red apple mashes and control group at the end of 5 days.

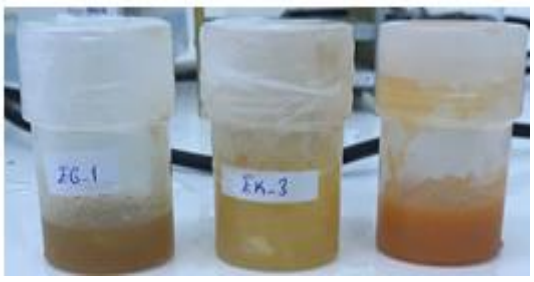

Figure 2. Appearances of Commercial apple juice (apple and carrot mixture) - Experimental apple juice containing (EG-1) 0.001M gallic acid solution - experimental apple juice containing (EK-3) 0.1M quercetin solution.

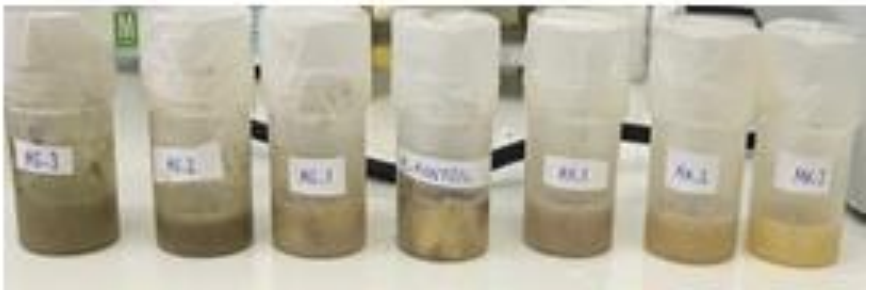

Figure 3. The appearance of the experimental banana purees and control group at the end of 5 days.

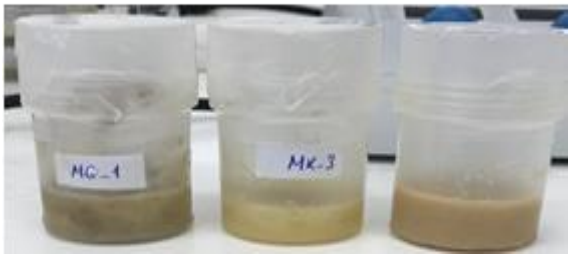

Figure 4. Appearances of Commercial banana puree – experimental puree containing (MG-1)0.001M gallic acid solution – Experimental puree containing (MK-3)0.1M quercetin solution.

The results of microbial growth analysis of all the purees supplied and produced were summarized in Table 1. At the end of 24 hours, 0.001M gallic acid containing experimental red apple puree was found as much durable than commercial red apple puree. In terms of quercetin usage in red apple purees, 0.1M was determined as effective for delaying the microorganism formation. This may resulted from the fact that quercetin is capable of sweeping of reactive oxygen species in the medium (Sakanashi, et al., 2008). The same concentrations for gallic acid and quercetin resulted microbial growth delaying in banana puree, too (Table 1). Although, commercial banana puree has some chemicals to increase the shelf-life, experimental banana purees containing 0.001M gallic acid or 0.1M quercetin solutions had less microbial concentration than commercial one. At the end of the first day, gallic acid resulted microbial growth decrease nearly 90% in red apple, 97% in banana, and 68% in jerusalem artichoke purees, while the usage of quercetin resulted 73%, 96%, and 46% decrease, respectively.

At the end of the second day, it was determined 0.001M gallic acid containing experimental red apple puree was as durable as the commercial puree. The decrease in microbial growth was found less (nearly 46% for gallic acid and 41% for quercetin), but none of the experimental red apple purees were intact. In the banana purees, microbial growth rate was increased in the second day, thus absorbance values were getting close to the commercial banana puree, although they were again so less than the control group. This showed that, in order to prepare much durable banana puree, the more concentrated solutions should be preferred. For jerusalem artichoke purees, the gallic acid and quercetin addition was not found effective, especially after 24 hours.

At the end 3-day period, all of the commercial purees were intact and the visible microbial colonies were observed, except red apple-carrot mixture. In this study, the experimental jerusalem artichoke puree containing 0.001M gallic acid solution was found the most durable against microbial growth (Table 1). Experimental banana purees containing either gallic acid or quercetion solutions were much resistant than to red apple purees.

Microbial growth analyses were continued up to 5-days, but since none of the control group and commercial puree was durable up to that time, making comparison was impossible.

Table 1. Microbial growth in all purees incubated at 37°C during 5-day period

Fruit purees	Microbial growth (absorbance values)					
	First analysis	%	Second analysis	%	Third analysis	%
R.A. control	0.2841		0.5455		-	-
C.R.A-C	0.1879		1.4096		1.8417	-
R.A-G.A-1	0.0270	90.49%	0.2939	46.12%	-	-
R.A-G.A-2	0.2654	41.24%	0.3306	39.40%	-	-
R.A-G.A-3	0.2638	40.39%	0.4298	21.20%	-	-
R.A-Q-1	0.0868	69.44%	0.3202	41.33%	-	-
R.A-Q-2	0.0751	73.56%	0.4089	25.04%	-	-
R.A-Q-3	0.1583	44.28%	0.5518	1.15%	-	-
Control banana puree	0.2247		2.1067		-	-
Commercial banana puree	0.1790		1.24		-	-
Banana-Gallic acid-1 puree	0.0444	80.24%	1.46	30.69%	-	-
Banana-Gallic acid-2 puree	0.1709	23.8.%	1.41	33.07%	1.8618	-
Banana-Gallic acid-3 puree	0.1804	19.71%	1.79	15.03%	1.9630	-
Banana-Quercetin-1 puree	0.3615	60.88%	1.25	40.57%	1.7322	-
Banana-Quercetin-2 puree	0.2702	50.94%	1.26	40.19%	1.7803	-
Banana-Quercetin-3 puree	0.0079	96.48%	1.29	38.76%	1.7952	-
Jerusalem artichoke control puree	0.5519		1.0062		1.9730	
Jerusalem artichoke-Gallic acid-1 puree	0.5003	9.34%	0.93	7.57%	1.4880	24.58%
Jerusalem artichoke-Gallic acid-2 puree	0.6875	24.56%	1.59	58.02%	-	-
Jerusalem artichoke-Gallic acid-3 puree	0.1766	68.00%	1.91	89.82%	2.1029	-
Jerusalem artichoke-Quercetin-1 puree	0.2968	46.22%	1.56	55.03%	-	-
Jerusalem artichoke-Quercetin-2 puree	0.8211	48.77%	1.94	92.8%	-	-
Jerusalem artichoke-Quercetin-3 puree	0.7796	41.25%	1.47	46.09%	1.9787	-

Note: ***** 1; 0.001M, 2; 0.01M, 3; 0.1M.

CONCLUSIONS

It is known that, when some fruits and vegetables such as apples, quince and potatoes are cut or damaged, their colors change. As a result of polyphenol oxidase enzymes oxidation reaction with phenolic compounds, enzymatic browning occurs. This effect is considered as

loss of quality in food, and they are tried to be prevented by various methods during food processing (Anonymous, 2006). Nowadays, commercial thickeners (thickening gels) and/or E-coded additives for cutting of the fruit surface interaction to oxygen and for inhibition of the formation of microorganisms are used, and these substances are known to be detrimental to human health (Boga and Binokay, 2010). Antioxidants can also inhibit the enzymatic reactions required by keeping oxygen, and they can also reduce the activity of enzymes such as polyphenol oxidase in these reactions (Gur and Altug, 2001). In this study, the effects of gallic acid and quercetin, which are well-known antioxidants, on the browning and microbial disruption of fruit purees were investigated.

In the study, in general, experimental fruit purees containing gallic acid and quercetin were found to be more effective in retarding darkness of the color than commercial fruit purees containing additives that could harm to human health. In the experiments, the experimental fruit purees containing quercetin with a concentration of 0.1M produced the most effective result in retarding color decay. During the second phase of the study, the microorganism formation processes of all of the purees whose mouths were covered with parafilm for 5 days at room temperature were followed. At the end of this period, it was concluded that experimental fruit purees containing gallic acid and quercetin in different concentrations were very resistant to other commercial fruits containing preservatives.

Experimental fruit purees containing gallic acid at a concentration of 0.001M, which slows down the growth rate of microorganisms, had been the most effective. It was also concluded that, delaying browning reaction and decreasing microbial growth formation, the concentrations must be taken into account carefully and dependent of the fruit used. This study showed that, instead of consuming commercially available fruit purees containing preservatives that are harmful to human health, natural molecules crush as gallic acid or quercetin having anticancer, antimicrobial and antioxidant properties can be used. Since they can easily be obtained from plants and fruits, the production cost will also be lowered.

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Production of Prebiotic Milk and Investigation of Some Properties

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Abstract

Prebiotics are short chain carbohydrates that cannot be digested by human enzymes. They increase the activity of the colonic bacteria, thus provides beneficial physiological effects to the human body. The well-known prebiotics are inulin and fucrooligosaccharides. Those prebiotics are plant polysaccharides and have low-calorie. Prebiotics present several fruits, vegetables and cereals (jerusalem artichoke, onion, etc.) are used in functional food production. Another group of prebiotics, flavonoids, are antioxidants, and are also present in plant materials. Those compounds are responsible for the taste and color of the material. In this research, it was aimed that production of prebiotic milk by the addition of either *mentha suaveolens ehrh* extracts containing polyphenols or jerusalem artichoke juice containing fructooligosaccharides to its nutrient composition. Single parameter optimization of ultrasonic extraction of *mentha suaveolens ehrh.* was achieved with the parameters of temperature (45-50-60^{0}C), time (5-10-20-30-45-60-75 min) and 1g/10mL solid/liquid ratio by water as a solvent. At the optimum conditions (45^{0}C, 45 minute), 19.59mg gallic acid equivalents of polyphenols were extracted per gram of plant. When the extracts added into 5ml of milk drop by drop, it was determined that 3 drops of extracts changed the color of the milk and 9 drops caused a change of the taste. Also, by following the same procedure with jerusalem artichoke juice it was found that 15 drops caused a significant change in both taste and color of the milk. In the light of these preliminary observations, sugarless and sugar-added milk samples were prepared by mixing different kinds of milks (light, half-fat and whole, pasteurized) with either flavonoid or fructooligosaccharides or both, in two different concentrations (5 or 9 drops of flavonoid; 9 or 15 drops of fructooligosaccharide). Those samples were experienced by volunteers for taste and color acceptability. Addionally, the viscosities of milk samples were also measured and compared.

Keywords: Antioxidant, fructooligosaccharide, *Mentha suaveolens ehrh.*, milk, polyphenol, prebiotic

INTRODUCTION

To increase their quality of life, people prefer to take measures to prevent health problems before they become ill. In today's diet, functional foods that maintain the good state of the immune system, improve it, and reduce the risk of disease are preferred(Roberfroid, 2000).

Another prebiotic food ingredient that provides medical or health benefits, including prevention and treatment of the disease, is the flavonoids in plants. Functional food is a food component that contributes to the prevention and reduction of risk factors for various diseases or to the improvement of some physiological functions in the body (López-Varela, 2002). Inulin and fructooligosaccharides are well known prebiotics. The ground apple are a valuable inulin source with 14-19% inulin content (Lingyun Wei, et al., 2007). Prebiotics are short

chain carbohydrates that can not be digested by digestive enzymes in humans, but selectively support the development of probiotics. These low calorie substances, which have beneficial physiological effects on the body, are found in many vegetables and fruits (banana, banana), cereals (oats), legumes (soybeans)(Roberfroid, 2002). Prebiotics have many benefits, such as reducing the risk of colon cancer, increasing calcium and magnesium absorption (Wollowski, et al., 2001; Pool-Zobel, et al., 2002). When added, it improves sensory properties such as taste and texture, and increases the stability of foams and emulsions in a wide variety of food applications such as dairy products (Slavin, Joanne, 2013).

Helianthus tuberosus, which is used as the best prebiotic source, also contains high amounts of vitamins A and B. It is a feathered, lumpy, perennial plant belonging to the family of *Helianthus*. The resistance of these materials to frost and drought can be explained by the fructan metabolism; because these plants synthesize compounds called fructans instead of starch. The ground apples do not contain the compounds of bitter taste, so it can be used appropriately in the food industry. It does not contain non-interfering components and can therefore be easily extracted(Yildiz, 2006). It strengthens the immune system, fighting harmful bacteria, preventing constipation. It also reduces the risk of diseases such as diabetes, cancer and bone loss. Since they have low calorie due to low glucose content, they can also be consumed by diabetic people(Brownawell, et al., 2012).

Flavonoids are belong to polyphenols and they are pigments as well as the most commonly used like chlorophyll and carotenoids(Constantine, 2007). These compounds act as strong antioxidants and metal chelators. They are also known to have antiallergic, antithrombotic, antiviral and anticarcinogenic effects (Tapas, 2008). Antioxidants act as radical scavengers, hydrogen and electron donors, peroxide scavengers, enzyme inhibitors (Shu-Huei, 2011). An antioxidant is a stable molecule that disassociates unpaired electrons by binding them to free radicals, thus reducing the cell's harmful capacity. These antioxidants retard or inhibit cellular damage by virtue of their free radical scavenging properties (Halliwell, 1995). Low molecular weight antioxidants interact safely with free radicals and terminate the reaction before the cells are damaged. Food antioxidants which cleave free radicals in the body are vitamin E, vitamin C (ascorbic acid) and B-carotene (Levine, et al., 1991). The body can not produce these micronutrients, so these component need to be taken separately.

In order to obtain the health benefits of prebiotics, they should be added into daily-life drinks. Thus, the production of prebiotic-enriched milk was investigated in this study. The prebiotics were extracted from different plant materials chosen by using water and then added into the milk samples. The consumer acceptance and some of the physical properties of prebiotic-containing milks were also evaluated.

MATERIAL and METHODS

In the study, the polyphenols intended to be added into a milk were extracted from *Mentha suaveolens* (mint) plant obtained from a regional herbalist (Gulluce et.al.., 2007). In order to obtain the maximum amount of polyphenol from the plant material, three different temperatures (45, 50, 60 ^{0}C) and seven different extraction times (5, 10, 20, 30, 45, 60 ve 75 minutes) were used in the extraction. In that, the solvent type and solid-to-liquid ratio were kept constants as water and 1g/10 ml, respectively. At the end of extraction by ultrasonic extraction device (Elmasonic S 30 H), the mixture was filtered through FilterLab Filter papers and the materials and extracts were separated. The total phenolic component in the extracts obtained was analyzed by the Folin_Ciocalteu method. The method was carried out with samples prepared by adding 400 µL extract, 100 µL water, 1.5 mL of 20% Na_2CO_3 solution and 5 mL of distilled water to 0.5 mL of Folin Reactant (Yigitarslan, 2017). These samples

were subjected to a colorimetric reaction for 2 hours at 25℃ in the dark, then the absorbance values were measured at 765 nm by a spectrophotometer (Cary 60 UV-VIS) using pure water as a blank. The concentration values expressed as mg GAE/g *Mentha suaveolens* were calculated by inserting absorbances into the calibration curve equation (Abs = 0.01532 * Concentration (µg/ml), R^2 = 0.9989) (Balcı & Yigitarslan, 2017). The conditions providing the highest gallic acid equivalent polyphenol concentration were selected as optimum extraction conditions. Plant extracts of optimum extraction conditions were prepared freshly before adding them into a milk samples.

Another material that is added to a milk is the jerusalem artichoke juice, which is produced by pressing with Arçelik Solid Fruit Juicer. As noted in the literature, in order to have the maximum amount of fructooligosaccharides capable of the highest prebiotic properties, jerusalem artichoke samples were used after being washed and then dried and stored as tightly wrapped at +4°C for 20 days. (Rubel et.al., 2014). Since juices have fast browning reaction, care was taken to transfer them quickly into the 26 mM citric acid added solution (Yigitarslan, 2007).

Flavonoid (F), fructooligosaccharide (P) and flavonoid-fructooligosaccharide (FP) were added to different types of 10 ml milk (dietary (L), semi-oily (Y), whole (T)). Concentrations indicated by the codes 1, 2, 3 and 4 in the study are representing 10 drops and 18 drops of flavonoid, 18 and 30 drops of fructooligosaccharide, respectively. 48 different milk samples were formed in the study with sugar-free and 0.250 g sugar added samples (denoted as *). A control sample that doesnot contain any flavonoid and fructooligosaccharide was also included in the experiments. 25 consumers evaluated the milk samples in the sight of odor, color and taste over 5 full scores. The densities of the control groups and the obtained samples were measured by using a pycnometer and the viscosities were measured by Ostwald viscosimeter. The densities and viscosities were calculated by Equation 2 and 3, respectively. ρ_1 (g/ml) and ρ_0 (g/ml) represent the densities of the sample and water, t_0 (s) and t_1^* (s) represents the disposal time of water and samples, respectively.

$$\rho_{sample} = \frac{m_{sample}}{v_{sample}} \qquad \textit{(Equation 1)}$$

$$c = \frac{\rho_1 * t_1^*}{\rho_0 * t_0} \qquad \textit{(Equation 2)}$$

RESULTS and DISCUSSION

The summary of the sensory analysis of the milk samples were given in Tables 1, 2 and 3, and the results of physical analysis of the milk samples were given in Table 4. The most acceptable sample in terms of color in all of the dietary milk samples was the sample containing the maximum prebiotic (LP4). In terms of odor, the sample containing minimum flavonoid-and-maximum prebiotic content (LFP14) was found the best, whereas in terms of taste, the results showed that a sugary sample having less prebiotics (LFP13*) was accepted much more. Those who have 0 points on the samples submitted for the evaluation of 25 people on 5 full points didn't have got any points from them. The most unacceptable samples in terms of color were LP3 containing only minimal prebiotic and LF2* containing only maximum flavonoid and sugar. The sugary LP3* sample, which is intense in terms of

prebiotic content, was found to be the least favored in terms of odor, while the LF1 sample with the minimum flavonoid content was found to be the least favored in terms of taste.

Table 1. The sensory analysis of dieatary milks

Sample	Color	Odor	Taste
LF1	8	8	1
LF1*	9	9	6
LF2	5	0	0
LF2*	3	5	10
LP3	3	9	7
LP3*	6	2	5
LP4	21	3	2
LP4*	9	6	11
LFP13	4	6	2
LFP13*	10	11	19
LFP14	0	14	11
LFP14*	4	13	13
LFP23	12	0	6
LFP23*	12	6	13
LFP24	10	6	9
LFP24*	16	3	14

As seen in the table below, YFP23, which is the sample containing maximum amount of flavonoid and minimum amount of prebiotic, was accepted highly in terms of color and odor in semi-oily milk samples. YFP13, containing minimal flavonoids-and-prebiotics, and its sugar-containing corresponding (YFP13*) were evaluated as the least favored samples in terms of color and taste within them. At the same time, the YFP14* sample with minimum flavonoid, maximum prebiotic and sugar content was not liked in the same manner as YFP13 sample in taste evaluations. The YF1* sample with flavonoid content was also found to be the least likable sample in terms of odor.

Table 2. The sensory analysis of semi-oily milks

Sample	Color	Odor	Taste
YF1	22	10	7
YF1*	15	4	10
YF2	7	16	11
YF2*	8	3	16
YP3	9	15	11
YP3*	11	15	10
YP4	6	9	7
YP4*	2	13	15
YFP13	10	13	6
YFP13*	1	17	7
YFP14	12	6	10
YFP14*	6	8	6
YFP23	23	18	9
YFP23*	12	8	23
YFP24	20	14	12
YFP24*	17	10	21

When the whole milk samples were evaluated in terms of color, TFP24 containing maximum flavonoid and maximum prebiotic was the most appreciated. When these samples were evaluated for odor, TFP14 containing the minimum amount of flavonoid and the maximum prebiotic was preferred. When evaluated in terms of taste, TFP24*, which was a

sugary sample containing maximum amount in both components, was preferred by the volunteers as in the case of odor evaluation. The least preferred sample in terms of color and odor was the TFP13* sample containing the minimum flavonoid, maximum prebiotic and sugar; while in terms of taste the least favored sample was found as TFP14* sample containing minimum flavonoid maximum prebiotic and sugar.

Table 3. The sensory analysis of whole milks

Sample	Color	Odor	Taste
TF1	15	9	13
TF1*	12	9	17
TF2	4	13	9
TF2*	11	9	17
TP3	9	5	3
TP3*	9	17	17
TP4	2	12	7
TP4*	12	7	7
TFP13	6	0	0
TFP13*	1	4	7
TF14	6	20	9
TFP14*	12	8	2
TFP23	9	6	6
TFP23*	7	6	9
TFP24	18	0	4
TFP24*	9	15	25

Table 4. The results of physical analysis of the milk samples

Sample	Density (g/ml)	Viscosity (cp)
LF1	0.946	2.004
LF1*	0.979	2.13
LF2	0.965	2.072
LF2*	1	2.177
LP3	1.014	2.266
LP3*	1.042	2.298
LP4	0.936	2.227
LP4*	0.965	2.35
LFP13	0.912	2.064
LFP13*	1.025	2.588
LFP14	1.023	2.315
LFP14*	1.063	2.56
LFP23	0.952	2.099
LFP23*	0.966	2.288
LFP24	1.036	2.405
LFP24*	1.054	2.447
Control (dietary milk)	1.032	3.055
YF1	0.968	2.191
YF1*	0.972	2.2
YF2	0.998	2.288
YF2*	1.021	2.37
YP3	0.976	2.181
YP3*	1.034	2.40
YP4	1.012	2.49
YP4*	1.027	2.53
YFP13	0.954	2.16
YFP13*	1.065	2.35
YFP14	1.009	2.372
YFP14*	1.020	2.42
YFP23	0.985	2.086

YFP23*	1.025	2.17
YFP24	0.960	2.20
YFP24*	1	2.32
Control (semi-oily milk)	1.035	3.24
TF1	1.037	2.73
TF1*	1.073	2.865
TF2	1.018	2.599
TF2*	1.047	2.613
TP3	1.004	2.593
TP3*	1.047	2.643
TP4	1.039	2.533
TP4*	1.065	2.59
TFP13	1.027	2.593
TFP13*	1.033	2.638
TFP14	1.025	2.379
TFP14*	1.098	2.517
TFP23	1.053	2.261
TFP23*	1.147	2.73
TFP24	1.033	2.34
TFP24*	1.081	2.415
Control (whole milk)	1.062	2.39

As seen in the table above, it has been determined that the densities of milks changed significantly depending on flavonoid, prebiotic and sugar contents and the amount of these ingredients. The viscosity of the experimental groups was found to be lower than that of the control groups and it was determined that milk samples containing sugar had higher viscosities than sugar-free milk samples. Since prebiotics are also sugars, the viscosities of the milk samples were found as increasing with an increase in prebiotic amount. A similar effect was also observed when the amount of flavonoid was increased. It has been determined that the addition of prebiotic and flavonoids individually or together lead density to increase.

CONCLUSIONS

In this study aiming to add the prebiotic and antioxidant properties to an ordinary milk, the antioxidant feature was supplied from *Mentha suaveolens ehrh.*, while the color was obtained from the jerusalem artichoke extracts. In general, the color coming from jerusalem artichoke was appreciated. Some people declared the taste and odor of the mint extracts were found heavy, while the others declared them as preferable in terms of aroma. The acceptability increased with reducing the extract concentrate in the sample somewhat and when consumed with sugars. Samples with the same content that was not liked on the diet milk samples could found as favorable in semi-oily and whole milk samples. It has been found that the acceptability of the sugary samples containing both flavonoid and prebiotic together was found quite good according to 25 people presented. Finally, it was found in this study that prebiotic and antioxidant enriched milk can be consumed, and if it is needed the taste can also be increased with the usage of some sugar. Thus, by this way, beneficial physiological effects will be provided for the human body.

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Development of Antioxidant Chitosan Films Incorporated with Quinoa Extract

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Abstract

In this study, the antioxidant chitosan (CH) films were prepared by incorporating quinoa extract (QE) (5, and 10%, wt) into CH film solutions. The effect of QE incorporation into CH film was evaluated by physicomechanical, and active properties (antioxidant, and antimicrobial activity). Active compounds were extracted from QE by distilled water at 60°C during 6 hours. The total phenolic content and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of QE were found as 34.35±0.27 mg GAE/100 g (dry weight) and 25.61±1.36 %, respectively. QE included CH films showed higher water vapor permeability values (WVP) and moisture content ($p<0.05$). The transmittance and L^* values of film samples decreased with the increasing QE content whereas opacity and a^* values increased ($p<0.05$). The elastic modulus and tensile strength of CH films did not change significantly by the incorporation of QE, however, the elongation at break values of films increased with the increasing amount of QE. CH films incorporated with QE extract exhibited 15.60±0.68 and 17.92±1.2% of DPPH radical scavenging activity for 5% QE and 10% QE incorporation, respectively. All film samples showed antimicrobial activity against *Escherichia coli*, and *Listeria monocytogenes* but QE incorporation did not promote the antimicrobial activity of CH films. CH films with active properties could have a potential to be used as active food packaging films along with conventional packaging films that could reduce the amount of film used.

Keywords: antimicrobial, antioxidant, chitosan, quinoa extract

INTRODUCTION

Consumption of plastic materials derived from petroleum sources and their poor degradation generate a massive accumulation of plastic waste that has been disposed of in the environment. The use of natural polymers has emerged as an alternative to face up to this problem. Thus, increasing attention has been paid to the development of edible and biodegradable food packaging films as alternatives to the non-biodegradable petrochemical-based plastics. The matrices of edible and biodegradable films are frequently chosen from food grade biopolymers, such as proteins, lipids, and polysaccharides (Mei et al. 2012; Kanmani & Rhim, 2014; Pagno et al. 2015).

Chitosan (CH) is an environmentally friendly material and can be used for edible film production due to its non-toxicity, biocompatibility, biodegradability and film-forming ability (Duan, & Zhang, 2013). In addition, the cationic property of CH offers an opportunity to establish electrostatic interactions with other compounds (Dutta et al. 2004). Besides adding plasticizing agents, disadvantages of the pure CH films can be improved by the incorporation of additional fillers (Rubentheren et al. 2015; Jahed et al. 2017) antioxidant compounds

(Moradi et al. 2012; Friesen et al. 2015), and antimicrobial agents (Hosseini et al. 2009; Ojagh et al. 2010; Rubilar et al. 2013).

Phenolic compounds are also an essential part of the human diet with several valuable biological activities, including antioxidant, antimicrobial, anti-diabetic, anti-inflammatory, anticancer and metabolic regulation properties (Działo et al. 2016). It has been demonstrated that the incorporation of phenolic compounds into CH can improve the physical, mechanical and biological properties of composite films (Ferreira et al. 2014).

Quinoa extract (QE), includes high protein content with a better-balanced amino acid composition, dietary fiber, unsaturated fats, vitamins, and minerals (Alvarez-Jubete et al. 2010). Quinoa seeds also represent a potentially rich source of phenolic compounds, particularly flavonoids (Hirose et al. 2010).

Some researchers suggested that phenolic compounds could be used as a cross-linking agent to enhance the mechanical strength (Rivero et al. 2010) or a plasticizer to eliminate brittleness of CH films (Sun et al. 2014). Therefore, QE with its antioxidant ability could be ideal choice to be added to CH films for the improvement of film properties. The aim of this study was to combine the antimicrobial properties of CH and the antioxidant properties of QE and to characterize the physical properties of films as well as their active properties.

MATERIAL AND METHODS

Materials

Chitosan (CH) (acetylation degree of 75-85%), 2,2-diphenyl-1-picrylhydrazyl (DDPH), Folin-Ciocalteu reagent, magnesium nitrate 6-hydrate, and acetic acid were supplied from Sigma-Aldrich (St. Louis, Missouri, USA). Quinoa seeds (*Chenopodium quinoa* Willd.) were supplied from a local market (Isparta, TURKEY).

Phenolic extraction from quinoa seeds

Distilled water, preheated at 60°C, was added to the quinoa seeds at a ratio of 1:10 (w/w) and the mixture was stirred for 6 h (Carciochi et al. 2015). The obtained solution was filtered under vacuum and freeze-dried (BW-100F, Bluewave Industry Co., Ltd., China) to obtain phenolic compounds (Quinoa seed extract, QE).

Film preparation

Chitosan films were prepared by a casting method. CH film solutions of 1.5% (w/w) were prepared by dissolving the CH in 1 % (w/w) aqueous acetic acid solution with stirring at room temperature for 18 h. Afterward, glycerol was added at 0.3 (w/w), a plasticizer. QE at different concentration (5, and 10%, w/w) was added to CH film solutions followed by homogenization with a homogenizer (DAIHAN HG-15A, Korea) for 5 min. The preliminary studies inferred that CH films with QE lower than 5% showed poor active properties while higher concentration than 10% contributed to the loss of structure. The solutions were then filtered under vacuum and degassed. CH film solutions (50 g) was transferred into a Teflon coated Petri plates (Ø=15 cm). The film solutions were dried at ambient temperature and then dried film samples were conditioned at 25°C for one week. A micrometer (QuantuMike IP65, Mitutoyo, Japan) was used to measure the film thickness at six random positions. Film samples including 5, and 10% QE were coded as CH-5QE, and CH-10QE, respectively.

Characterization of film samples

Water vapor permeability, solubility in water, and water uptake values of film samples

The water vapor permeability (WVP) of films were determined by ASTM E96-95 standard method (ASTM, 1995). Film samples were exposed to 100% RH, and the permeability measurements were carried out gravimetrically at 25°C.

The solubility of film samples in water was determined according to the method described by Pereda et al. (2014). Briefly, film samples (2 × 2 cm) were placed in a beaker

containing 30 mL of distilled water to measure the percentage of dry matter solubilized after 1 h of immersion in distilled water. The undissolved dry matter was determined by removing the film pieces from the beakers and then drying at 105°C for 24 hours.

The water uptake values of film samples (2 × 2 cm) were measured as the ratio of the initial weight of pre-dried samples (at 105°C) to the change in weight after immersion (30 mL distilled water/1 h) (Rubentheren et al. 2016).

Tensile properties of film samples

Tensile properties of film samples were determined by ASTM D882 standard method (ASTM, 2001). Elastic modulus (EM), tensile strength (TS) and elongation (ε) at break point values were determined by a universal testing machine (Lloyd Instruments LR5, London, UK) by stretching the film samples at 50 mm/min. At least eight replicates were carried out for each sample.

Optical properties of film samples

Film opacity values were measured by taking the absorption spectrum of the film samples (1x4 cm) in a range of 400-800 nm with a UV-Visible spectrophotometer (Shimadzu, UV-1601, Tokyo, Japan). Film opacity was then expressed as absorbance unit per thickness (AU nm/mm) (Friesen et al. 2015).

Transmittance values of the films were measured as percent transmittance at 450 nm determined by a UV-visible spectrophotometer (Shimadzu, UV-1601, Tokyo, Japan).

The color of the films was taken with a white standard calibration plate (Y=92.7, x=0.3160, y=0.3321) as a background by a Minolta Chroma Meter (CR-400, Konica Minolta, Inc., Japan) Results were expressed as CIE *L**, *a** and *b** (lightness 'L', red–green 'a' and yellow–blue 'b') coordinates.

Total phenolic content and antioxidant activity of film samples

All film samples were dissolved in acetic acid solution (1% w/w) before the analysis. The amount of phenolic content in dissolved film samples was determined according to Singleton et al. (1999). All solutions were mixed with Folin-Ciocalteu reagent (0.2 N) and sodium carbonate (7.5% w/v) and measured by reading the absorbance of samples at 765 nm using a UV–vis spectrophotometer (Shimadzu, UV-1601, Tokyo, Japan).

The potential antioxidant activity of dissolved film samples was measured based on a radical scavenging ability with the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sanchez-Moreno, 2002). All solutions were treated with DPPH solution (0.1 mM) for 40 min at dark and then the absorbance was taken at 517 nm. The total activity of each sample was expressed as the percentage of reduced DPPH.

The antioxidant activity and total phenolic content of QE were also determined by the same method.

Antimicrobial activity of film samples

The antimicrobial effect of film samples was tested against *Escherichia coli* (ATCC 26922), and *Listeria monocytogenes* (ATCC 19115) with the zone of inhibition assay on solid media. Test microorganisms were grown in brain heart infusion (BHI) broth at 37°C for 18 h. The cells were diluted to a concentration of 10^5-10^6 CFU/mL. Film samples (Ø=15 mm) were placed aseptically on the Petri dishes inoculated with bacterial strains. The plates were incubated at 37°C for 24 h and were then examined for antimicrobial activity.

Statistical analysis

An analysis of variance (ANOVA) and Tukey's multiple comparison tests were used to compare the different treatments at the 95 % confidence level. The statistical analysis was performed by the Minitab 17 software (Minitab Inc., Brandon, UK). Three observations were performed for each sample, and each experiment was replicated three times.

RESULTS AND DISCUSSION

Water vapor permeability, solubility in water, and water uptake values of film samples

The thickness, WVP, water solubility, and water uptake values of film samples are presented in Table 1.

Table 1. WVP, solubility in water, and water uptake values of film samples

Film sample	Thickness (µm)	WVP (g mm/kPa h m^2)	Solubility (%)	Water uptake (%)
CH	46±4[b]	31.53±3.78[b]	21.93±1.92[a]	61.23±7.43[a]
CH-5QE	47±1[b]	66.70±6.40[a]	22.37±0.21[a]	65.36±4.77[a]
CH-10QE	62±5[a]	69.21±5.06[a]	25.27±0.09[a]	79.89±7.31[a]

[a-b] Different letters in the same column indicate significant differences ($p<0.05$)

QE incorporated CH films exhibited higher thicknesses than CH film. The thickness of CH-QE films gradually increased with the increase in QE content. The CH-10QE film showed the highest thickness ($p<0.05$) as an indication that the thickness of CH-phenolic composite film was greatly affected by the amount of phenolic compound added. Similarly, the higher thickness was reported by other studies for CH films including gallic acid (Sun et al. 2014).

As shown in Table 1, QE incorporated CH films showed higher WVP values than CH film ($p<0.05$). The presence of QE scattered in the film could disrupt the inner network of CH film and increase the free volume and segmental motions, leading to the increase of water vapor permeability (Liu et al. 2017).

The water solubility, expressed as the percent of the water-soluble matter in the film, is frequently used to indicate the resistance of film towards the water. The addition of QE into CH film resulted in the elevation of water solubility. The water solubility of CH films increased with the increase of QE content ($p>0.05$). The increase in the water solubility of CH films should be attributed to the hydrophilic property of QE molecules (Kurek et al. 2012). As shown in Table 1, QE added CH films presented higher water uptake values ($p>0.05$) similar to WVP and solubility values. QE caused an increase in both water solubility and water uptake values but there are no significant differences between the film samples. This behavior could be explained by the hydrophilic nature of QE which might offer more free hydrophilic positions to water molecules for hydrogen bonding (Kurek et al. 2012). These results are in agreement with Rubilar et al. (2013) who studied CH films including carvacrol-grape seed extract.

Tensile properties of film samples

The EM, TS, and ε (%) values of film samples are shown in Table 2.

Table 2. Tensile properties of film samples

Film sample	EM (MPa)	TS (MPa)	ε (%)
CH	247.28±87.79	21.29±0.67	24.65±0.82
CH-5QE	493.24±47.33	27.35±0.94	23.59±1.52
CH-10QE	610.13±33.67	30.75±1.50	16.15±2.15

Tensile strength is required to maintain the structural integrity and barrier property of films. In addition, appropriate flexibility is also desired for easy handling of films. With the

increase of QE content, the TS values of CH films increased which could be attributed to the strong molecular interactions between QE molecules and CH chains. However, the elongation at break values of CH-QE films decreased with the increase of QE content. This indicated that the motion of CH film matrices was greatly restricted after incorporation of QE molecules (Ferreira et al. 2014). EM values of CH films, a measure of stiffness and the degree of deformation, increased with the increase in QE concentration. Similar increases in EM and TS values were reported for CH films including cinnamaldehyde (López-Mata et al. 2018).

Optical properties of film samples

Color parameters and optical properties of films including CIE *L**, *a**, *b**, transmittance (T, %) and opacity are summarized in Table 3. With the addition of QE, the *L** and % T values of CH film decreased while the opacity tends to increase, indicating CH film became darker when QE was incorporated into the film. Besides, *a** and *b** values of CH-QE films were higher than that of CH film, suggesting the tendency of composite films toward redness.

Table 3. Optical properties of film samples

Film sample	Transmittance (%)	Opacity (AU nm/mm)	*L**	*a**	*b**
CH	86.30±0.14a	401.04±22.47a	96,50±0.14a	-0.30±0.03a	3.50±0.06b
CH-5QE	83.10±2.12ab	504.96±46.71a	95,99±0.04b	-0.33±0.04a	3.85±0.27b
CH-10QE	80.35±0.21b	594.12±80.00a	95,29±0.26c	-0.45±0.04b	4.74±0.36a

[a-c] Different letters in the same column indicate significant differences ($p<0.05$)

Similarly, other researchers have found a decrease in transparency, and lightness in accordance with higher opacity values upon the addition of tea extract (Wang et al. 2013), carvacrol-grape seed extract (Rubilar et al. 2013), and protocatechuic acid (Liu et al. 2017) into CH films. This could be attributed to the impenetrable matrix created by phenolic interactions which promoted the light scattering through the film.

Total phenolic content and antioxidant activity of film samples

Active packaging such as antioxidant packaging is a very promising technique for extending the shelf life of foods without affecting the integrity of them. The total phenolic content and DPPH radical scavenging activity of film samples are shown in Figure 1. QE has a potential to be used as a natural antioxidant agent with its high phenolic content (Carciochi et al. 2015).

The total amount of phenolic componds and antioxidant activity of QE (aqueous solution) were 34.3±0.3 mg GAE/ml, and 35.6±0.7% respectively. However, film samples including QE showed lower total phenolic content and antioxidant activity. The degree of antioxidant capacity and total phenolic content of CH film was generally proportional to the amount of the QE added ($p<0.05$). Their inclusion in the films confers them antioxidant activity which can be attributed to its hydrogen donating ability due to the formation of the stable end-product by giving hydrogen from the phenolic hydroxyl groups (Carciochi et al. 2015).

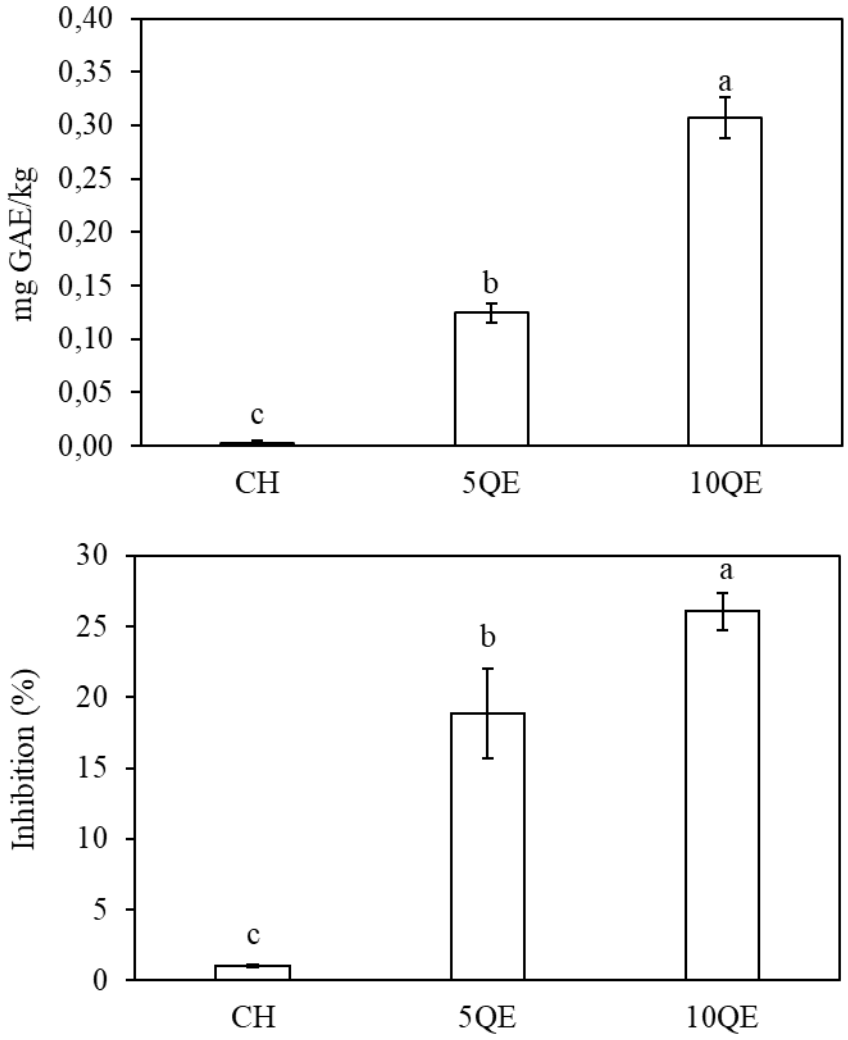

Figure 1. Total phenolic content and DPPH radical scavenging activity of film samples

Antimicrobial activity of film samples

The antimicrobial activity of film samples was tested with zone inhibition by controlling the growth under films and taking the area of clear zones (Figure 2). Although QE includes a high amount of phenolic compounds, results indicated high potential of QE for antimicrobial activity. All film samples showed inhibition against selected bacteria (Figure 2) but distinct clear inhibition zones did not appear when the agar diffusion method was used for determination of CH and CH-QE films activity. This is because of the limitation of CH diffusion in the of film form to diffuse through the agar medium (Coma et al. 2002). Although inhibition zones were not present, the growth of bacteria under CH films was inhibited. It was stated that the effectiveness of antimicrobial activity of film samples is strongly related to retention and diffusivity mechanism of active compounds in the matrix (Fernandez-Pan et al. 2012).

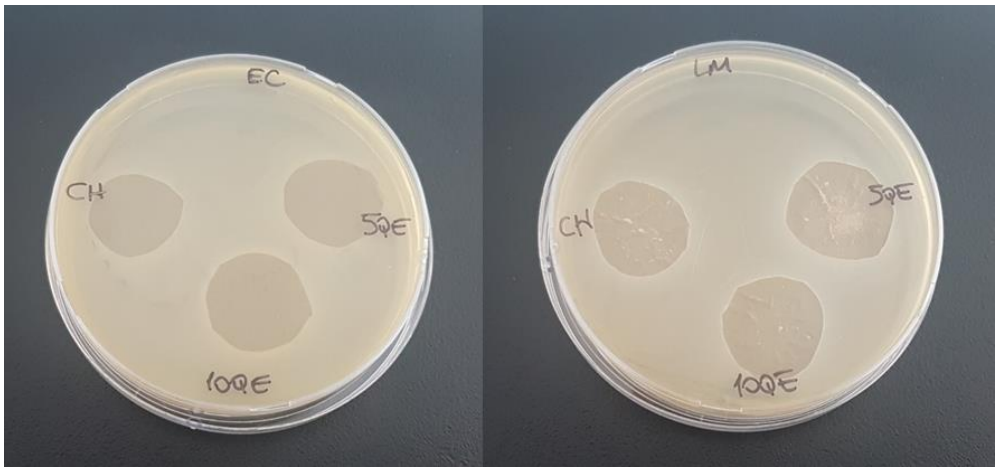

Figure 2. Antimicrobial effect of film samples (EC=*E. coli*, and LM=*L. monocytogenes*)

CONCLUSIONS

The addition of QE significantly affected the physicomechanical, and active properties of CH films. QE promoted an increase in water vapor permeability, solubility, and water uptake values due to its highly hydrophilic nature. QE incorporated CH films showed higher opacity, and lower lightness. QE improved the tensile properties of film samples while causing a decrease in elasticity of film samples. QE included CH films also showed antioxidant activity, but QE did not contribute to the antimicrobial activity of CH films. CH films with active properties could have a potential to be used as active layers along with conventional packaging films that could reduce the amount of film used.

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Modeling of Gallic Acid Diffusion: Case Study on *Cinnamonum zeylaniccum*

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Abstract

Polyphenols, found in vegetables, fruits and grains, are receiving increasing interest in recent years due to their delaying effects especially on the formation of certain types of cancer. However, in the current literature, there is no information on diffusion kinetics, diffusion coefficients of these materials, and the parameters affect on them. In this study, gallic acid-equivalent polyphenols production from *Cinnamonum zeylaniccum* by classical extraction method in ethanol as solvent was investigated the parameters of extraction temperature (25-55°C) and duration (10-90 min), the stirring speed of the medium (minimum-maximum) and the solid/liquid ratio (0.3-1.5g/40ml). Then, multiple parameter optimization was performed with Design Expert Program. For multiple optimizations, solid/liquid, temperature and time parameters resulted from the single optimization (55°C, 40 minutes, maximum mixing speed and 0.3g *Cinnamonum zeylaniccum*/40mL ethanol) were used in Box-Behnken Design construction. It has been determined by a computer program that the maximum gallic acid diffusion (3.267mg/100g) conditions were at 59°C, 37.6 minutes and 44.4 ml of solvent usage in a quadratic model. The most important single and interactive parameters on the extraction was determined as temperature and solid/liquid ratio, respectively. In the study, in order to define diffusion as a mathematical expression, diffusion kinetics data were obtained by performing experiments at different temperatures, without- or optimum-stirring speed conditions. These data were used in evaluating Peleg, Logarithmic, Page and Mass Transfer models. Molecular, effective diffusion coefficients and activation energy of gallic acid-equivalent of total polyphenols were calculated. It was observed that the increase in temperature and stirring speed increased the diffusion coefficients by decreasing activation energy of diffusion.

Keywords: Gallic acid, Modeling, Polyphenols, Diffusion, *Cinnamonum zeylaniccum*

INTRODUCTION

Today, there are many researches on cancer treatment which is one of the most common disease in the world. Cancer treatment-focused drug delivery systems gain importance in these studies, and the use of plant extracts instead of synthetic chemicals is a major factor in reducing side effects. Cinnamon (Lightning et al., 2016), produced mostly in China, Indonesia and Sri Lanka in the world, is a source of gallic acid (Figure 1), a powerful antioxidant. The ability to bind gallic acid free radicals, a phenolic flavonoid, has attractive properties such as interacting with cancer cells without interfering with healthy cells (Mukarami et al., 2008), (Pavun et al., 2014). Gallic acid found in spices and fruits such as apples, grapes and strawberries has properties such as strong antioxidant, antimutagenic,

anticancer and antiinflammatory (Jeong et al., 2009), (Yena et al., 2011), (Balcerzak et al ., 2008). Gallic acid has been shown to inhibit DNA oxidative reactions of free radicals and chelates formed with heavy ions (Canivenc-Lavier et al., 2009), (Moon et al., 2006), (Ulger, 2016), (Verma et. al., 2013).

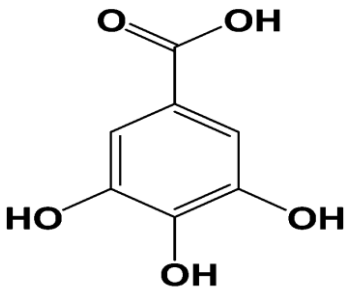

Figure 1. Chemical structure of gallic acid

Since the flavonoids formed by more than four thousand components are obtained by extraction from the plants, the flavonoid content and composition change with the extraction conditions. Both the variability of the extraction method (classical, microwave, ultrasonic, supercritical, etc.) and the solvent diversity cause a world of probability which is considered impossible to realize without optimization methods. For this reason, the response surface method (Turkyılmaz et al., 2014), (Goktas et al., 2015), (Dastianeh et al., 2013), (Levin et al., 2008), (Hesap and Yigitarslan, 2016), (Balci and Yigitarslan, 2017) were developed.

MATERIAL AND METHODS

In the study, the optimization of the extraction process of polyphenols from Cinnamonum zeylaniccum plant in the presence of ethanol as a solvent was carried out in two steps. The plant was supplied from a regional herbalist, and the chemicals such as ethanol, Folin-Ciocalteu and sodium carbonate were bought from Sigma_Alldrich in an analytical purity. In this study, firstly, a single optimization was performed on the parameters and values mentioned in Table 1. Then, in the direction of the results obtained from the single optimization, the Response Surface Method including the three centered-three parameter Box-Benkhen experimental design was used to determine the most effective three parameters optimization has been performed.

Table 1. The parameters of a single optimization

Temperature (°C)	**Solid/Liquid Ratio (g/mL)**	**Time (min)**	**Mixing rate (rpm)**
25	0.3/40	10	50
30	0.5/40	20	100
35	0.7/40	30	150
40	0.9/40	40	200
45	1.0/40	50	250
50	1.2/40	60	
55	1.5/40	70	
60		80	
		90	

In the single optimization, extraction has been performed for each value of the parameter to be worked on, while keeping the other parameters except the parameter to be worked on in Table 1 as constant value. Then the results of gallic acid analysis on the extracts obtained at these conditions were compared. When the parameter being studied was reached its maximum concentration of GAE, then the other parameter was studied by the same way. Thus optimum values for each parameter for the single optimization, which are valuable if they were taken into consideration alone, were determined.

Multiple optimizations were made using a computer program called Design-Expert. In this section, the parameters specified in Table 2 were encoded as minimum (-1), center (0) and maximum (+1) in the results obtained from the single optimization and they were defined into the program. A second-order polynomial function given in Equation 1 is proposed for expressing the extraction surface. In Equation 1, y represent the predicted response (extraction efficiency), x_i term represents the effect of the corresponding parameter affecting the yield, x_ix_j, x_jx_k, x_ix_k terms express the interactive effects of those parameters, β is the coefficient of the term, and finally ε represents the random error.

$$y = ß_0 + \sum_{j=1}^{k} ß_j X_j + \sum_{j=1}^{k} ß_{jj} X^2{}_j + \sum_{i=1}^{j-1} \sum_{j=2}^{k} ß_{ij} X_i X_j + \varepsilon \qquad \text{Equation 1.}$$

After the desired response the proposed function were entered into the program and, the change interval of the parameters wass defined as given in Equation 2, the Box-Behnken experiment design conditions consisting of 15 sets were obtained. Gallic acid analyzes were performed on the extracts, the yields obtained from experiments realized at these conditions were entered in a program and three-dimensional surface graphs expressing the extraction surface were plotted after carrying out statistical analyses. Equation expressing the surface and the coefficients were determined, and finally optimum values of each parameter was determined with numerical optimization.

$$xi = \frac{xi - xo}{\Delta x} \qquad \text{Equation 2.}$$

Table 2. Box-Benkhen Experimental Design parameters and ranges

Parameters	-1	0	+1
Solid/liquid ratio (g/mL)	0.3/35	0.3/40	0.3/45
Temperature (°C)	50	55	60
Time (min)	35	40	45

For the determination and modeling of gallic acid diffusion coefficients, extractions were carried out for 40 minutes at three different temperatures (35-45-55°C) and two different media (mixed and unmixed) and gallic acid measurements were applied every 5 minutes. Four different models namely Peleg, Mass Transfer, Page and Logarithmic Model have been tested with those results in order to determine the best model that provides the mathematical expression of the extraction.

Peleg's Model: Since the extraction curves (concentration of phenolics vs. time) have similar shape with the sorption curves, all of the extraction processes could be described with a non-exponential equation of Peleg (Peleg, 1988):

$$c_t = c_0 + \frac{1}{K_1 + K_2 t} \qquad \text{Equation 3.}$$

where c_t is the concentration of phenolics at time t (mg GAE/g), c_0 is the initial concentration of phenolics at time t=0 (i.e. c_0=0 in all experiments), t is the extraction time, K_1 is Peleg's rate constant (min.g/mg GAE), and K_2 is Peleg's capacity constant (g/mg GAE). In that equation, K_1 relates to the extraction rate (B_o) at the very beginning of the extraction ($t=t_0$):

$$B_0(mg\ GAE\ g^{-1}) = \frac{1}{K_1}$$ Equation 4.

and K_2 relates equilibrium concentration (c_{eq}) at $t\rightarrow\infty$:

$$c_{eq} = \frac{1}{K_2} \quad (mg\ GAE/mg)$$ Equation 5.

Page's Model: Another model used for the mathematical modeling of the extraction proposed by Page as follows (Jokic *et al.*, 2010):

$$c_t = \exp(-kt^n)$$ Equation 6.

where k and n are the constants of Page's Model, and all the other parameters have the same definitions.

Logarithmic Model: In mathematical modeling of extraction processes, Logarithmic model can also be used as follows:

$$c_t = a\ Logt + b$$ Equation 7.

where a and b are the logarithmic model constants.

Mass Transfer Model: Extraction occurs through two steps; Firstly, the solvent penetrates into the solid to dissolve the extractable material, and then the extractable material diffuses from inside the solid to the bulk liquid. The rate determining step of the overall process is the diffusion (Cheung *et al.*, 2012). The rate of this step under unsteady-state conditions is defined by Fick's second law as:

$$\frac{\partial c}{\partial t} = D\ \frac{\partial^2 c}{\partial x^2}$$ Equation 8.

where, c is the concentration of the solute (mg/g), t is time (min), D is the diffusion coefficient (m^2/min), and x is the distance of diffusion. This equation is valid when very dilute solution is used in the extraction and the diffusivity is assumed to be constant (Cacae and Mazza, 2003). If the shapes of the solid particles are assumed to as perfect spheres having the same properties and also if the perfect mixing of the solid-liquid medium occurs, the time of mass transfer at infinity, the general solution of this equation becomes:

$$Ln\left(\frac{c_\infty}{c_\infty - c}\right) = 0.498 + \frac{9.87Dt}{R^2}$$ Equation 9.

where, c is the concentration of the extracted material in the solution at time t (mg/g), c_∞ is the concentration of the extracted material at time t=∞, and R is the characteristic distance (m); i.e. for spheres it is equal to the radius. This equation can be rewritten as:

$$Ln\left(\frac{c_{eq}}{c_{eq} - c}\right) = a + K_{obs}t$$ Equation 10.

Since c_∞ is considered as equilibrium concentration, a is a constant (0.498), and

$$K_{obs} = \frac{9.87D}{R^2}$$ Equation 11.

In this research, Equation 10 was used to fit the experimental data and to obtain a, K_{obs} and diffusion coefficient values.

The model constants were calculated by applying these models to the experimental data and then the estimated gallic acid amount was calculated by using the model constants with these model constants. Furthermore, for each model, the correlation coefficient values were calculated using Equation 12 and these values were compared.

$$r^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - y_{model})^2}{\sum_{i=1}^{n}(y_i - y_{mean})^2}$$ Equation 12.

In the study, Arrhenius law was used to determine energy barriers that must be overcome (activation energy) for the gallic acid extraction from this plant (Equation 13):

$$k = k_{obs}\exp(\frac{-E_a}{RT})$$ Equation 13.

where, k is the extraction rate constant (L/g.min), k_0 is the temperature-independent factor (L/g.min), E_a is the activation energy of the extraction (j/mol), R is the universal gas constant (8.314 j/mol.K) and T is the absolute temperature of the extraction medium (K). Thus, after linearization the plot of ln k versus 1/T, activation energy and k_0 can be determined from the equation:

$$Ln\ k = Ln\ k_0 + (\frac{-E_a}{RT})$$ Equation 14.

At the end of each extraction, the samples were filtered using 110 mm Whatman filter paper. In order to determine the amount of gallic acid in the extract, 50 µL of the extract was mixed with 0.5 mL of Folin-Ciocalteu, 450 µL of purified water, 1.5 mL of Na_2CO_3 (20% by weight), respectively, and again 5 mL of purified water was added and incubated in an incubator for 2 hours at 25°C. Once the color formation was complete, the mixture was analyzed at 765 nm wavelength on the UV_VIS spectrophotometer (Carry 60). These absorbance values were inserted into the calibration curve (Absorbance = 0.001532 * Concentration; R^2 = 0.9174) and the concentrations of gallic acid in the extracts were calculated.

RESULTS AND DISCUSSION

In this study, the most suitable model for the extraction of gallic acid at all temperatures studied was found as the mass transfer model (Figures 2-7). As shown in Figures, the mass transfer coefficient increased as the temperature increased, resulting in a more yield obtained in the mixed condition compared to the unmixed medium. In the mass transfer model, the optimum conditions for this study were found as extractions at 55°C in a mixed environment. Also, it was observed that, the experimental and calculated concentration values were getting closer as the temperature increased. Thus, it was concluded that mass

transfer model can be used effectively at high temperatures. The other models applied did not fit to the experimental data as much as mass transfer model, and thus they were not given.

The diffusion coefficients for different temperatures and different media were calculated by mass transfer model were summarized in Table 3. According to results, the diffusion coefficient increased with the increase in temperature, and, es expected, the effective diffusion coefficients were higher than that of molecular ones, since the agitation produced an extra velocity thus the molecules could move faster, thus increased mass transfer was observed.

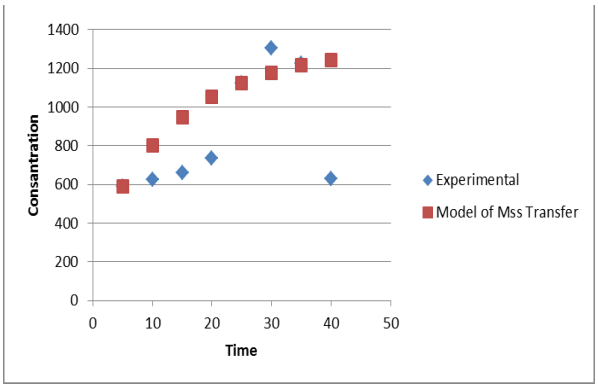

Figure 2. Moleculer Diffusion at 35°C

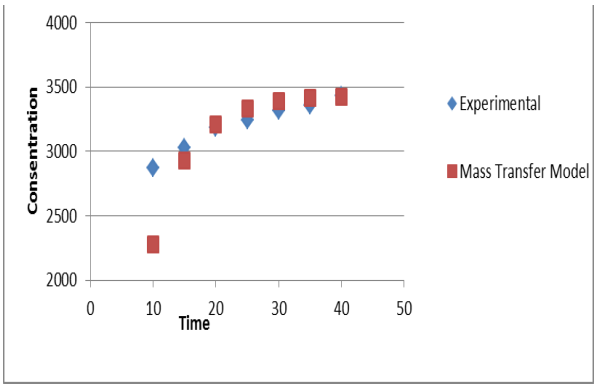

Figure 3. Convective Diffusion at 35°C

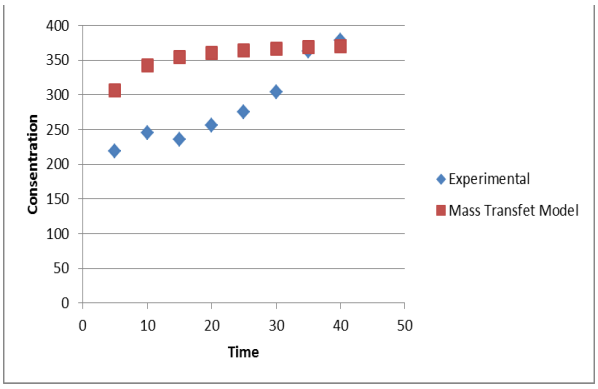

Figure 4. Moleculer Diffusion at 45°C

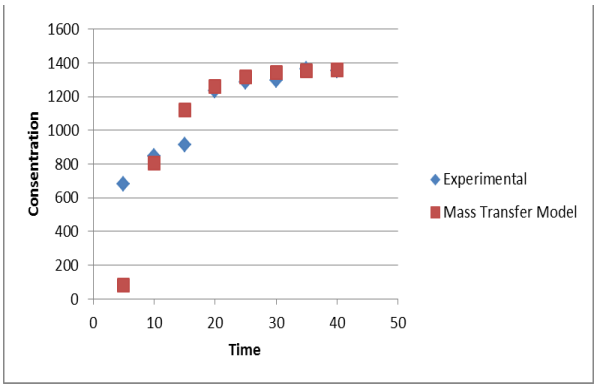

Figure 5. Convective Diffusion at 45°C

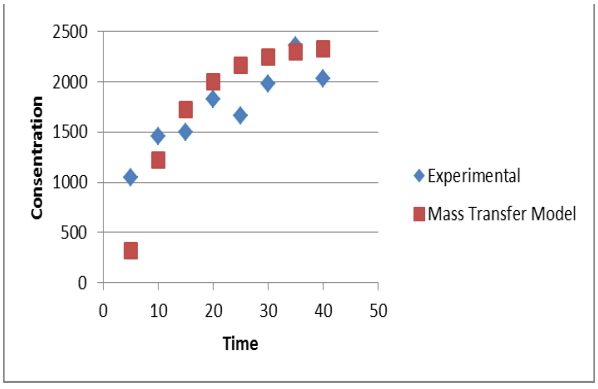

Figure 6. Moleculer Diffusion at 55 °C

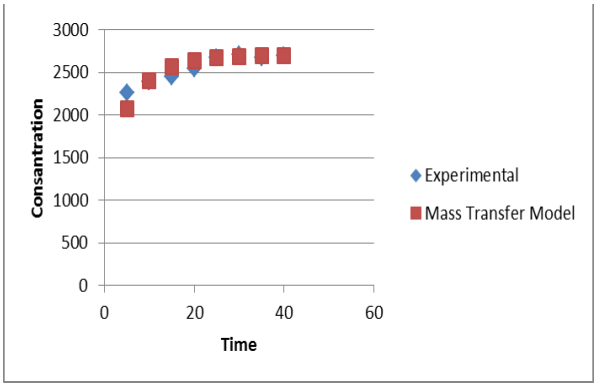

Figure 7. Convective Diffusion at 55°C

Table 3. Diffusion Coefficients

T (°C)	$D_{moleculer}$ (m^2/s)	$D_{convective}$ (m^2/s)	$D_{effective}$ (m^2/s)
35	0.1118	0.1563	0.2681
45	0.1644	0.1053	0.2698
55	0.1861	0.1037	0.2898

In this study, the data given in Table 4 were applied to the proposed design in the Design-Expert program and then statistical tests were applied to each of the proposed functions. The model with the highest regression coefficient and the lowest incompatibility was chosen as the best predicted function for the response surface. For the proposed second-order model, these values were 0.9174 and 0.7686, respectively. The predicted R^2 value was found to be acceptable, since the actual and the calculated data were in agreement confirming this. As a result, according to the statistical analysis, the most suitable model was selected as the quadratic model.

The variance analysis (Table 5) for the quadratic model was applied by ANOVA table of the Design - Expert program; where A was the volume of solvent, and B was the temperature, and C was the extraction time. Taking into consideration of the reality that as the magnitude of F value increases and the p-value decreases the affect of that parameter increases, the most effective single parameter was determined as temperature (as approved also with mass transfer model) and the most effective binary parameters were determined as time and temperature.

Table 4. Design Expert Data

		Factor 1	**Factor 2**	**Factor 3**	**Response 1**
Std	**Run**	**A:Volume of solvent**	**B:Temperature**	**C:Time**	**R1 (Yield)**
		mL	C	dk	mg/100g
15	1	0	0	0	2038.64
8	2	1	0	1	2789.85
14	3	0	0	0	2063.71
1	4	-1	-1	0	1369.04
5	5	-1	0	-1	1455.96
13	6	0	0	0	2067.19
3	7	-1	1	0	2784.32
10	8	0	1	-1	3267.19
4	9	1	1	0	2994.56
12	10	0	1	1	3205.57
7	11	-1	0	1	2569.97
6	12	1	0	-1	3171.80
2	13	1	-1	0	1271.28
9	14	0	-1	-1	1590.95
11	15	0	-1	1	1069.45

Table 5. ANOVA Table

ANOVA for Response Surface Quadratic model						
Analysis of variance table [Partial sum of squares - Type III]						
	Sum of		Mean	F	p-value	
Source	Squares	df	Square	Value	Prob > F	
Model	7,647E+006	9	8,497E+005	6,17	0,0296	significant
A-Hızc Hacmi	5,244E+005	1	5,244E+005	3,81	0,1086	
B-sicaklik	6,039E+006	1	6,039E+006	43,83	0,0012	
C-Sre	2773,02	1	2773,02	0,020	0,8927	
AB	23716,10	1	23716,10	0,17	0,6954	
AC	5,595E+005	1	5,595E+005	4,06	0,1000	
BC	52871,63	1	52871,63	0,38	0,5628	
A^2	63311,17	1	63311,17	0,46	0,5280	
B^2	25227,82	1	25227,82	0,18	0,6865	
C^2	3,535E+005	1	3,535E+005	2,57	0,1701	
Residual	6,890E+005	5	1,378E+005			
Lack of Fit	6,885E+005	3	2,295E+005	946,14	0,0011	Not significant
Pure Error	485,12	2	242,56			
Core Total	8,336E+006	14				

3D Surface graphs were plotted to better analyze the interaction between the parameters. It was seen in Figure 8 that the amount of concentration increased when the temperature and time were increased simultaneously. In the case of decreased tempareures and extraction times, the yield was minimum. The yield would not be affected by increasing the time course of extraction when the temperature was kept at the minimum level, while in the reverse case the yield was increased. If the temperature and the time were at maximum values, the extraction yield reached its maximum. In Figure 9, binary effect of solvent volume and time was investigated on 3D surface. When the graphic is interpreted, it had been observed that, the concentration reached its maximum value at the maximum values of the time and solvent volume.

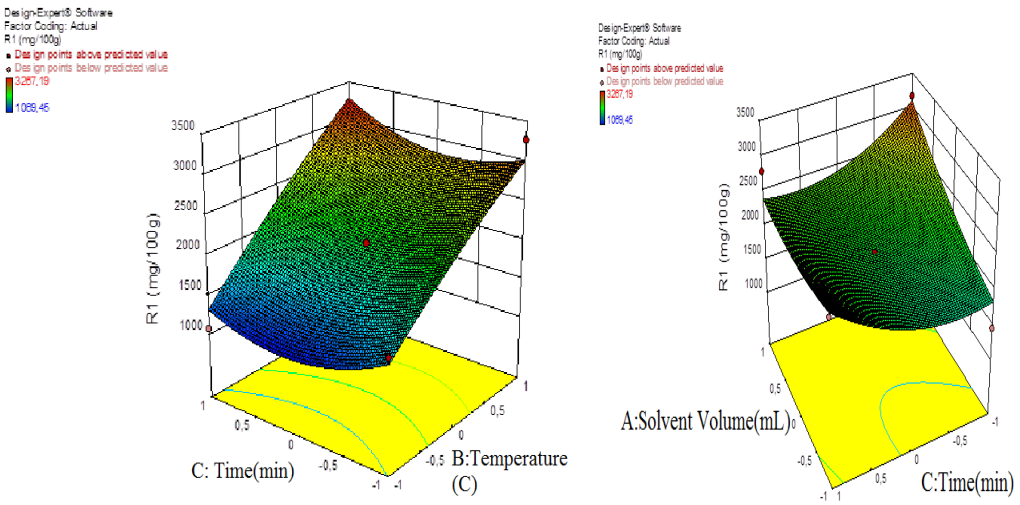

Figure 8. Binary effects of time-temperature (a) and solvent volume-time (b) parameters on the yield of extraction

In Figure 8, the binary effects of solvent volume and temperature parameters were investigated. In that, the yield of extraction reached the maximum in the case of the temperature and solvent volume were at maximum.

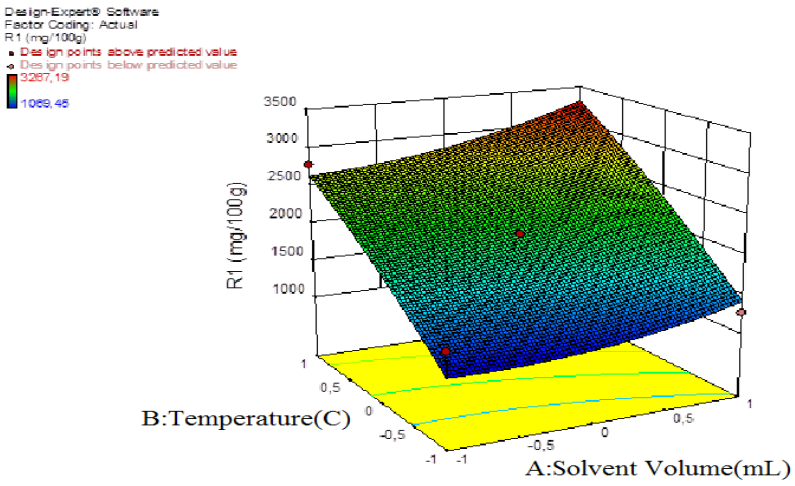

Figure 9. Binary effects of temperature–solvent volume parameters on the yield of extraction

CONCLUSIONS

As a result of the study, the response surface method model equation applicable for the industrial productions was found as in Equation 15:

$$GallicAcid=2056.51+256.02(A)+868.86(B)+18.62(C)+77(A)(B)-373.99(A)(C)+114.97(B)(C)+130.95(A^2)-82.66(B^2)+309.44(C^2)$$

Equation 15.

100 different numerical solutions of the mathematical model were proposed by the Design-Expert program. The highest amount of gallic acid (3219.67 mg/100g) production conditions were determined as 45mL of ethanol used extraction at maximum temperature (60°C) and at maximum mixing speed during 45 minutes. This result was also confirmed with the experimental run realized at those conditions.

Additionally, Mass transfer model was found as the best model representing the experimental data at all conditions. The diffusion coefficients were in the range of 0.1-0.3, and the activation energies of extraction were calculated as 292,776 J/mole İn molecular extractions, 81,9760 J/mole in convective transport.

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EURASIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Determination of Fatty Acid Composition of Iranian Extra Virgin Olive Oils with Respect to Cultivar and Geographic Origin

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Abstract

In this research, 12 Extra Virgin Olive Oil (EVOO) were collected from Iran's northern and southern provinces including Zard, Roghani, Dezful, and Mari cultivars were studied. Free fatty acid content (%), Peroxide value (meq O2/kg sample), and fatty acid composition of Extra Virgin Olive Oil (EVOO) were analyzed. Results showed that Iran's northern EVOOs have more unsaturated fatty acid (Oleic, Linoleic, Linolenic acids) than southern in general. On the other hand, saturated fatty acids content (Palmitic, and Stearic acids) were higher in southern EVOOs. But free fatty acid content (%) and Peroxide value (meq O2/kg sample) showed that oxidation stability of southern EVOOs was higher than northern.

Key words: Iran's Olive oil, Fatty acid composition, Free fatty acid content (%), Peroxide value.

INTRODUCTION

Iran is one of the olive oil producer country in the world. Although, it is not famous in Olive oil producing, but there are many attempts for development of olive trees in this country. Fatty acid composition is an important feature of virgin olive oil. The fatty acid composition affects the taste of virgin olive oil and it is largely responsible for the taste and healthful effects of the people's diet. It is well known that the fatty acid composition of olive oil is quantitatively affected by two main factors: the olive variety used in the production of the oil and the ripening stage at which the olives are harvested. There are only a few types of fatty acids in olive oil, but the proportions of each strongly influence the characteristics and nutritive value of the oil. Palmitic, oleic, and linoleic acids are the main fatty acids commonly found in virgin olive oil, other fatty acids are present in small amounts. Oleic acid is the most important fatty acid in composition of olive oil (55-88%). Oleic acid is a common monounsaturated fat in human diet. Monounsaturated fat consumption has been associated with decreased low-density lipoprotein (LDL) and possibly increased high-density lipoprotein (HDL) resulting in reduced cholesterol level. Apart from oleic acid, the particular minor components present in olive oil, such as polyphenols, hydrocarbons, tocopherols, fatty alcohols and triterpenic compounds and, some of which are known to be anti-inflammatory, make it the quintessential functional food (Boskou, 2006).

Olive fruit are harvested during October to December in Iran. The quality of the virgin olive oil is important factor in determination of its price and is a function of weather during the growing season. Environmental factors, affect olive oil fatty acid composition (Bucci et al., 2002) Due to a high demand for olive oil around the world, not only in the Mediterranean countries, Iranian farmers have been motivated to grow olive trees, therefore olive trees are widely distributed in the northern and southern regions of Iran.

Olive oil fatty acid composition changes may be associated with the zone of production, the latitude, the climate, the variety, and the stage of maturity of the fruit (Ballabio et al.,2006; Lopez-Feria et al., 2008; Di bella et al., 2007; Galtier et al., 2007; Rui Alves et al., 2005; Haddada et al., 2007). Studies showed that Greek, Italian, and Spanish olive oils have low content linoleic and palmitic acids and high content of oleic acid. Tunisian olive oils are high in linoleic and palmitic acids and low in oleic acid. It is not much information on its chemical composition of Iranian Virgin Olive Oil in the literature to date. The aim of this study was to determine fatty acids composition of some Iranian olive oils extracted from different cultivars and geographic origins. Present study tried to investigate fatty acids profile of Iranian Zard, Roghani, Dezful, and Mari olive oils in 2016 season.

MATERIAL AND METHODS

In this research 12 olive oil samples were extracted from the olives including Zard, Roghani, Dezful, and Mari cultivars harvested from different locations of Iran at early stages of maturation in 2016 season. Zard, Roghani, and Mari cultivars were belonged to northern provinces and Dezful cultivar was belonged southern provinces of Iran.

Determination of Free Fatty Acid Content

Total free fatty acids (FFA) of the samples were measured by titrating 1 g sample dissolved in 95% ethanol against phenolphthalein indicator according to American Oil Chemists' Society (AOCS) method Ca 5a-40, and results are given as percent of oleic acid (Ogutcu and Yilmaz 2009).

Determination of Peroxide Value

Peroxide value was determined according to the AOCS Cd 8-53 method . For performing of this analysis 5.00±0.05 g of sample was weighed into a 250-ml erlenmeyer flask with glass stopper and 30 ml of the 3:2 acetic acid-chloroform solution was added. The sample solution was shaked to dissolve. 0.5 ml of saturated KI solution was added. Solution was allowed to stand with occasional shaking for exactly 1 min, and then immediately 30 ml of distilled water was added. Solution must be titrated with 0.1N sodium thiosulfate, by adding it gradually and with constant agitation. Titration should be coutinued until the disappearing of yellow iodine color. 2 ml of starch solution was added and titration was continued with constant agitation, especially near the the end point, to liberate all of the iodine from the solvent layer. At the end part of assay, thiosulfate solution must be added dropwise until the blue color disapearing. PV was calculated with the following formula:

$$\text{POV (mEq/kg)} = (\text{S} - \text{B}) * \text{N} * 1000/ \text{ mass of sample}$$

B:volume of titrant, ml of blank
S:volume of titrant, ml of sample
N:normality of sodium thiosulfate solution

Fatty acid composition analysis

For the determination of fatty acid composition of the oils, fatty acid methyl esters were prepared from olive oil, using a cold transmethylation (Ogutcu and Yilmaz 2009). The fatty acids were converted to fatty acid methyl esters before analysis by shaking a solution of 0.2 g oil and 3

mL of hexane with 0.4 mL of 2 N methanolic potassium hydroxide. A Shimadzu (Kyoto, Japan) gas chromatograph, equipped with a flame ionization detector and a split/splitless injector, was employed. Separations were made on a Teknokroma TR-CN100 (Barcelona, Spain) fused-silica capillary column (60 m×0.25 mm i.d. 0.20 µm film thickness). The carrier gas was nitrogen, with a flow rate of 1 mL/min. The temperatures of the injector and the detector were held at 220 and 250 ◦C, respectively. The initial oven temperature of 90 ◦C was maintained for 7 min, raised to 240 ◦C at a rate of 5 ◦C/min, where it was maintained for 15 min. The injection volume was 1 µL. Peaks were identified by comparison of their retention times with those of authentic reference compounds (Sigma–Aldrich, St. Louis, MO, USA).

Statistical Analysis

Statistical analysis was performed by SPSS 17 (SPSS Inc.Chicago, IL) statistical software and using One-way Anova method. Differences among all groups were determined by Duncan test. All analyses were performed at least duplicate.

RESULTS AND DISCUSSIONS

Free fatty acid content:

Results of free fatty acid content (%) analysis showed that all olive samples were categorized in Extra Virgin Olive Oil class according to International Olive Council regulations (<0.8 %). Dezful Extra Virgin Olive Oil had the lowest content of free fatty acid content among all samples. It may be related to the ecologic condition of Khozestan province of Iran, that has low raining. Roghani cultivar had the highest amount of free fatty acid content, it is because of high raining in this region (Table 1).

Table 1. *Free fatty acid content (%) of Extra Virgin Olive samples*

Olive oil	Zard	Roghani	Dezful	Mari
Free fatty acid content (%)	0.3 ± 0.05^{b}	0.4 ± 0.03^{a}	0.2 ± 0.01^{c}	0.3 ± 0.02^{b}

Different superscript letters in the same column indicate significant difference between mean values ($P < 0.01$).

Peroxide Value:

Results of Peroxide Value assay showed that all olive oil samples were classified as Extra Virgin Olive Oil according to the International Olive Council regulations (PV<20). Roghani cultivar had the highest amounts of Peroxide value, and the lowest amount was belonged to Dezful cultivar. Ecologic conditions of olive cultivars led to increase or decrease of theirs' Peroxide values. The regions with high raining had high peroxide value because of oxidation rate increment (Table 2).

Table 2. *Peroxide value of Extra Virgin Olive Oil samples (meq O2/kg sample).*
Different superscript letters in the same column indicate significant difference between mean values ($P < 0.01$).

Olive oil	Zard	Roghani	Dezful	Mari
Peroxide value (meq O2/kg sample)	16.58 ± 0.021^{b}	18.20 ± 0.122^{a}	12.24 ± 0.035^{c}	16.50 ± 0.085^{b}

Fatty acids profile:

Results of fatty acid composition assay showed that all amounts were in the range of the International Olive Council limitations. Mari cultivar had the highest content of Oleic acid. The highest amount of Palmitic acid was belonged to Dezful cultivar. Zard cultivar had the most value of Palmitoleic acid. The most value of Stearic acid was belonged to the Zard cultivar. Linoleic and Linolenic acids are other important unsaturated fatty acids in extra virgin olive oil. Roghani cultivar had the highest amount of Linoleic acid. Zard and Roghani had the most value of Linolenic acid. The highest amount of Arachidic acid were belonged to the Zard and Roghani cultivars (Table 3). As other features, fatty acids compositions were affected by ecologic condition. The high temperature regions, had the most value of saturated fatty acids. On the other hand, regions with high raining had mono and poly unsaturated fatty acids. Unsaturated fatty acids have important role in decreasing of heart coronary diseases.

Table 3. *Fatty acids compositions of Extra Virgin Olive Oil*

Olive oil	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Linolenic	Arachidic
Zard	12.90±0.42^{c}	2.62±0.12^{a}	5.15±0.61^{a}	69.42±0.85^{b}	7.82±0.41^{b}	1.00±0.01^{a}	0.60±0.01^{a}
Roghani	11.42±0.12^{d}	0.86±0.03^{c}	4.32±0.23^{b}	69.96±0.52^{b}	11.62±0.21^{a}	1.00±0.02^{a}	0.60±0.02^{a}
Dezful	16.50±0.75^{a}	1.55±0.11^{b}	2.36±0.15^{d}	67.31±0.52^{c}	11.00±0.11^{a}	0.82±0.01^{b}	0.46±0.01^{b}
Mari	13.62±0.32^{b}	0.96±0.02^{c}	3.42±0.21^{c}	73.62±0.21^{a}	6.32±0.22^{c}	0.91±0.02^{b}	0.53±0.03^{a}

Different superscript letters in the same column indicate significant difference between mean values ($P < 0.01$).

CONCLUSION

Results of this research showed that Iran's northern EVOOs have more unsaturated fatty acid (Oleic, Linoleic, Linolenic acids) than southern in general. These fatty acids have important role in human diet. On the other hand, saturated fatty acids content (Palmitic, and Stearic acids) were higher in southern EVOOs. But free fatty acid content (%) and Peroxide value (meq O2/kg sample) showed that oxidation stability of southern EVOOs was higher than northern. Because of low data base on Iranian Olive Oil in literature, present study can help to better programming of agriculture plans in Iran.

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EURASIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Application of Gallic Acid Produced from Horse Chestnut (*Aesculus hippocastanum*) Shell in Table Olive Maturation

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Abstract

Some important problems of a table olive processing are high salt content and microorganism occurrence, especially in fermentation. High salt content of black table olives may result an infrastructure for health problems, such as high blood pressure and related diseases and osteoporosis, and also restricts its export area. Additionally, microorganism formations lead to hygiene problems and to degradation that decrease the quality of olives. In this study, the feasibility of the polyphenol-containing vegetable extract usage in the processing of black table olives was investigated for elimination of those problems. For this purpose, as raw material known to be triterpenoid, saponin, escin, coumarin, quercetin, kaempherol and carotenoid containing horse chestnut (well-known in pharmaceutical and cosmetic industries) shells were chosen as a raw material of the extraction. Since different polyphenols components can be extracted by different solvents; water, ethanol, methanol and their binary (1:1 by volume) and triple (5:3:2 by volume of water:ethanol:methanol) combinations were performed in Soxhlet extraction in three-cycle. 16g horse chestnut shell powder/ 400ml solvent ratio were kept constant, and the amounts of polyphenols (as gallic acid equivalents) in the extracts were determined by Folin-Ciocalteu method. Experimental results showed that 55-74mg GAE/1g plant can be obtained by using pure solvents; 74-150mg GAE/1g plant in binary combinations and 12.36mg GAE/1g horse chestnut shell was obtained in triple combination. After separating the alcohol components in the extracts by distillation or completely evaporation, they were added on the brine solutions of black olives containing 0-10% brine. The table olive samples were observed and investigated for their maturation time and the amount of total microorganism formed during that stage. By comparing the results of samples with the control group consisting of 10% brine, it has been concluded that horse chestnut shell can be used in the processing of olives in the food industry.

Keywords: *Aesculus hippocastanum*, Extraction, Gallic Acid, Horse chestnut, Polyphenol, Table olive processing

INTRODUCTION

Polyphenols are compounds containing more than one group of phenols in their molecules. Together with forming the most important natural antioxidant group, they are the secondary metabolites produced by the plants in response to the environment. In addition, the most well- known properties of polyphenols having many effects on human health are anti-oxidant, anti-microbial, anti-viral, anti-mutagenic, anti-hypertensive, anti-cancer, anti-histamine, and hepatoprotective properties (Wang, et. al., 1991; Wang, et. al., 1992, Yamaguchi, 1999; Liu et. al., 2012). Gallic acid (3,4,5-trihydroxybenzoic acid, $C_7H_6O_5$), a hydroxybenzoic acid species found in the non-flavonoid group of polyphenols, is the most important chemical used in the food, pharmaceutical, and cosmetic industries (Curcio et. al.,

2009). Antioxidant, anti-inflammatory and antifungal properties of this compound have been proven by studies (Alberto et. al., 2001; Yilmaz & Toledo, 2004; Chafer et. al., 2007).

Polyphenols are produced by extraction processes, each of the solvents used during this process dissolves different phenolic compound from the plant. The best solvents for gallic acid are ethanol, methanol, water and ethyl acetate, respectively (Daneshfar, 2008). When studies were done in the literature are examined, it has been found that when the appropriate solvent and/or solvent mixtures are used, high yield extraction processes involving different polyphenol components can be made (Kallithraka, et. al.,2007; Alothman, et. al., 2008; Dhawan & Grupta, 2016).

In horse chestnut (*Aesculus hippocastanum*) flowers; flavonoids, rutin and quercetin, saponin, escin, choline, and purine, in its tree bark; esculin, escin, and quercetin, and in its leaves; coumarin glycosides, quercetrin, isoquercitrin, quercetin and carotenoids (lutein) are known to be found (Cronquist, 1981; Stayanov, 1982; McLellan, 2000). As a result of clinical studies considering this material, its several properties including anti- oxidative, anti-spasmatic, anti- microbial and anti- cardiovascular have been (Alternative medicine review, 2009; Dudek- Makuch & Matlawska, 2011).

The antimicrobial effect of polyphenols is used to retard the degradation of food and to prevent undesired microorganism growth (Baysal & Yildiz, 2003; Ozturkcan & Acar, 2017). Since polyphenols have anti- microbial properties they can create a significant advantage in preventing undesirable microorganisms occurring during and in olive production. The main objective of the table olive production, is the diffusion of the oleuropein (the molecule responsible from its sour- taste) present in olives into brine and thus maturating the olives and making them consumable (Ozdemir et. al., 2011). In this context, table olives are processed by harvesting olive fruits, washing and sorting them then when needed them to fermentation, pasteurization, and sterilization after addition of lactic acid and/or other additives (Turkish Food Codex, Table olive communiqué, 2014/33). Brine solution containing more than 8% of salt prevents the development of lactic acid bacteria (*L. plantarum, L. brevis, L. mesenteroides, L. lactis sp.)* that occur during fermentation, leads to the development of undesirable microorganisms such as Candida (%10), Pichia (%15), Saccharomyces (%20) and Debaryomyces (%20). The formation of such microorganisms causes slow fermentation, softening and stinking of the olive and poses a danger to consumer health (Aktan & Kalkan, 1999; Uylaser & Sahin, 2004; Randazzo et. al., 2004).

In the 100 grams of food products sold, 1.5 grams of salt-containing product is called as “highly salted” and 0.6 grams of salt indicates that the product is “low-salted”. Also, when more than 5 g of salt per day is being consumed, health problems such as hypertension, cardiovascular diseases, kidney disease, osteoporosis, stomach cancer and obesity are reported to occur in later times by the World Health Organization (WHO) (T.R. Ministry of Health, Public Health Institution, 2016). In addition, the fact that salt content is high compared to other countries' production techniques is the biggest limitation in Turkey's olive export (Ozkaya et. al., 2010; Aydin et. al., 2014).

When the problems arising due to salt content in table olive production are examined, the aim of the study was to investigate the effect of addition of gallic acid containing polyphenol mixed solutions obtained from horse chestnut instead of salty water on maturation of table olives. Their effects on microorganisms formation observed in olive processing were also evaluated.

MATERIAL and METHODS

It was stated in the literature that horse chestnuts harvested in the weeks of 17-19 after the beginning of the flowering period had the highest polyphenol content (Kedzierski et. al.,

2015), horse chestnuts (barbed hulls) harvested on those specified dates were used as raw materials in this study. The collected horse chestnut shells were cut into pieces of about 2-3 cm and then they were dried in an oven (NUVE, FN-400) at 55℃ for 90 minutes. Finally, they ground to a powder by a grinder (SINBO, SCM-2934).

The polyphenols in the plant raw material were extracted using the three-cycle Soxhlet extraction method. In the extraction process; a total of 16 g horse chestnut husks as quartet filter paper packages were placed in a soxhlet apparatus. Then a volume of 400ml of total solvent composed of individual, double, and triple combinations of water, ethanol, and methanol were added into them to produce extracts having different polyphenol contents and concentrations. 1:1 ratio was used in double combinations of solvents, whereas in the triple combination, 50% of the total solvent was composed of water, 30% was ethanol and 20% was methanol. Extraction was carried out by concentrating the solvents evaporated from the heated vessel into the soxhlet extractor by condensing back into the cooler. After the solid packs were separated, the alcohol in the extracts (if any) was completely dried using a Rotary Evaporator (BUCHI, R-100). By this way solid phenolic were obtained and dissolved in a 50 ml of purified water to form the liquid samples which is then added to the olive.

As a second alternative, the alcohol in the extracts was removed using a simple distillation method. In this method, unlike the literature, 200 ml extract and 200 ml distilled water were added to the balloon and distillation process was initiated. After the distilling the alcohol, the process was stopped and the volume of the mixture in the balloon was measured. Then required distilled water was added to get 400 ml of liquid as a total, and the distillation process was continued up to the time when the material being extracted into the collection vessel began to enter. After simple distillation, the aqueous extract remaining in the balloon was added to the olive samples without any further treatment.

The content of polyphenols in the extracts was calculated as gallic acid equivalent (GAE) using the Folin-Ciocalteu method (Yigitarslan, 2017). According to this method, samples were prepared by addition of 400 µl extract, 100 µl water, 1.5 ml of 20% Na_2CO_3 solution and 5 ml of distilled water, respectively, onto 0.5 ml of Folin Reactant. The mixture was kept in an incubator (ILDAM) at 25°C for 2 hours to realize the colorimetric reaction. At the end of the time, the samples were analyzed on a CARY 60 UV-VIS spectrophotometer at 765 nm. These values were inserted into the calibration curve equation and the results expressed as mg GAE/g horse chestnut shells. (Abs = 0.01532 * Concentration (µg/ml)); R^2 = 0.9989).

The black table olives collected from Aydin, İncirliova region were first placed in containers containing 30 ml of purified water and pre-ripened by changing the waters every 5 days during 25 days. The vessels containing olives during this period; wrapped in parafilm so as not to contact with the air, kept in room temperature and dark place. At the end of this period, different polyphenol-containing solutions (20ml brine-10ml polyphenol mixture and 10ml purified water-20ml polyphenol mixture) were added into the vessels. Mixtures of aqueous polyphenols obtained by distillation were labeled as "D"; solid polyphenol mixtures obtained by drying within a rotary evaporator were coded as "K". Finally, the numbers in the codes represent the volume of the added polyphenol solutions in the olive samples.

200 µl of the liquid, taken from the olive flasks every 15 days, was added to an autoclaved (121 ° C, 15 min) standard MRS liquid medium and analyzed for microorganism development at 420 nm at the end of 20 hours. In these experiments, 30ml of 10% brine was used as a control group. In addition, the maturation characteristics (taste and texture) of the olives were evaluated by comparing with the control group at the end of 45 days.

RESULTS and DISCUSSION

The completion times of the three-cyclic Soxhlet extraction of the solvents used in the study were given in Table 1. Accordingly, the longest and the shortest completion times were obtained with water and with ethanol in pure solvents, while, water-ethanol-methanol triple combination and water-methanol double combination were their corresponding's in the solvent mixtures, respectively. This finding indicates that the solubility of the solvents influences the completion time of the boiling temperatures. It has been determined that the high boiling point of water causes prolonged extraction times in the solvents which contains it.

Table 1. Completion times of three-cyclic soxhlet extraction of the solvents

Solvent type	Completion time (min)
Water	360
Ethanol	180
Methanol	195
Water- Ethanol	240
Water- Methanol	184
Ethanol- Methanol	163
Water- Ethanol- Methanol	285

It was determined that the extracts of double and triple combination solvents used for obtaining high polyphenol content extracts had more GAE compared to single solvents (Table 2). These findings resulted from the reality stated in the literature considered above. The triple combination of water-ethanol-methanol extracted less flavonoid than water-methanol combination did and it extracted more flavonoid than ethanol-methanol combination.

Table 2. The GAE values of polyphenol content extracted by solvent & solvent mixtures

Solvent type	Gallic acid equivalent of flavonoid contents (mg GAE/ 1g horse chestnut shell)
Water	55.459
Ethanol	69.561
Methanol	74.443
Water- Ethanol	149.720
Water- Methanol	120.454
Ethanol- Methanol	74.759
Water- Ethanol- Methanol	135.992

The polyphenol contents (as GAE) calculated according to the Folin-Ciocalteu method in the mixtures obtained after drying and simple distillation were given in Table 3. SK coded sample was determined as having the highest GAE value in pure solvents, whereas it was found that SMK coded sample showed the same property in solvent combinations. The lowest GAE values were observed in EK and MEK coded solvent samples, respectively. In both methods, it was observed that the GAE sequence that the solvents were extracted was the same. This result confirms that the extracted polyphenol content depends on the solvent type. In addition, higher GAE values were obtained in the method with simple distillation than the other methods. This finding might resulted from denaturation some of the polyphenols due to the high temperature used in drying.

Table 3. GAE values of polyphenol mixtures after alcohol removal process
(a) With drying (b) With simple distillation

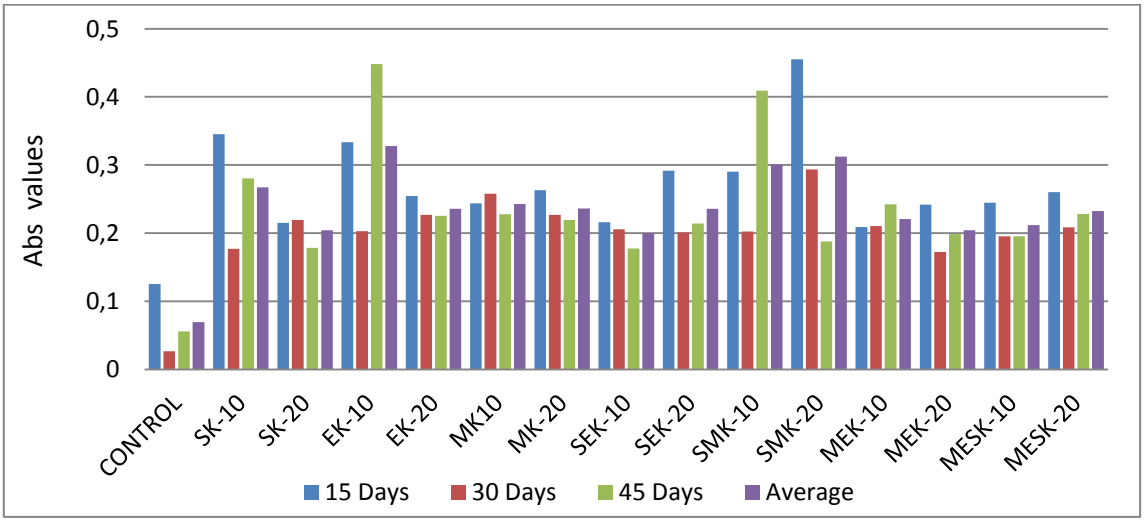
With Drying	mg GAE/ 1g horse chestnut shell
Water (SK)	55.459
Ethanol (EK)	21.35248042
Methanol (MK)	40.39686684
Water-Ethanol (SEK)	77.4308094
Water-Methanol (SMK)	82.05483029
Ethanol-Methanol (MEK)	38.12010444
Water-Ethanol-Methanol (MESK)	74.35770235

(a)

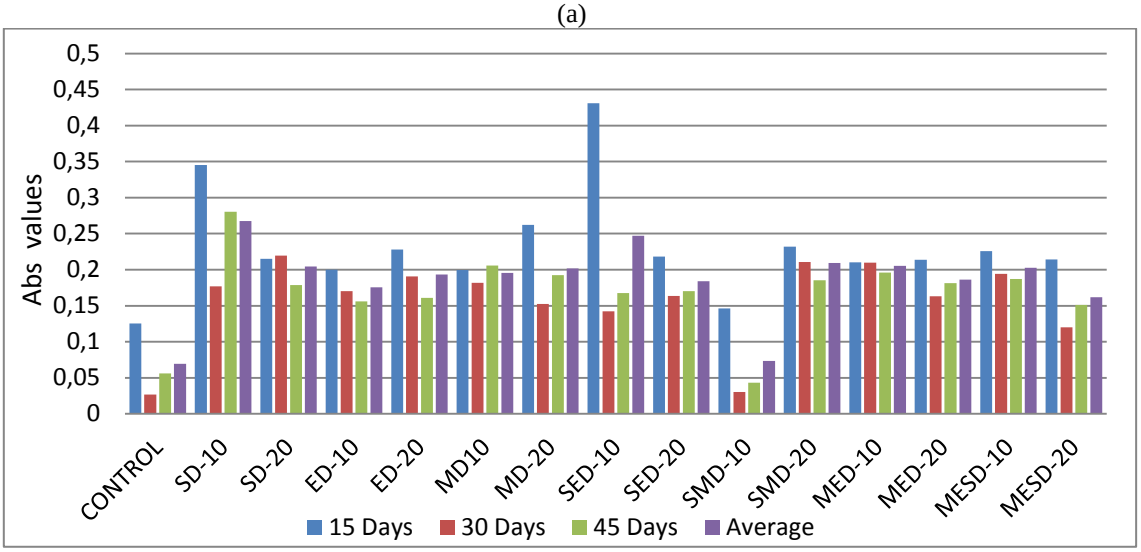
With Simple Distillation	mg GAE/ 1g horse chestnut shell
Water (SD)	55,459
Ethanol (ED)	37.13315927
Methanol (MD)	51.23759791
Water-Ethanol (SED)	75.76501305
Water-Methanol (SMD)	76.91383812
Ethanol-Methanol (MED)	44.71801567
Water-Ethanol-Methanol (MESD)	74.07049608

(b)

The microorganism formations (as absorbance values) examined up to 45 days by taking samples in olive brine containing polyphenol mixtures every 15 days were given in Figure 1.



(a)



(b)

Figure 1. Microorganism growth in polyphenol-containing table olive brines during 15,30 and45 day period (a) polyphenol mixtured obtained after drying (b) polyphenol mixtured obtained after simple distillation

When the mean absorbance values in Figure 1 (a) were taken as reference, the closest microorganism formation to the control group with 0.069 value were determined in the case of using polyphenol-containing mixtures (SEK-20, SK-20 and MEK-20) without salt. Figure 1 (b) showed that the same conclusion could be achieved with the brine-polyphenol mixture (SMD-10) and salt-free polyphenol (MESD-20 and SED-20) respectively. Comparing the polyphenol-containing brine obtained by drying on a rotary evaporator with the control group, the high absorbance values (Figure 1 (a)) were thought to be due to the fact that the polyphenols could undergo denaturation depending on the temperature during the process and thus resulted a reduced antimicrobial property. For this reason, a simple distillation method was proposed as a second alternative in the study.

When maturation periods (taste and texture) were examined, it was determined that the olives in the control group were sweet, edible and medium hardness. In polyphenol-containing blends, the bitterest olives were found to be MK-20 and the sweetest ones were found in ED-10 added mixtures, while the hardest olives were MESK-10 and softest olives were MESK-20 and MESK-20.

CONCLUSIONS

As a result of the three-cycled Soxhlet extraction, the longest extractions lasted for 360 min and the shortest duration was observed by using ethanol-methanol combination. This suggests that extraction completion times were related to the boiling point of the components inside. Triple combination may not be preferred since the polyphenol contents of the extracts with the highest gallic acid equivalent were obtained when a water-ethanol binary solvent mixture was used. However, since each solvent dissolves a different component, although other solvent combination produces lower GAE may be preferred when it is desired to obtain other polyphenol components besides gallic acid. When the amount of microorganism observed in 45 days was examined, it was observed that the lowest (SMD-10) microorganism growth occurred in the mixture having the highest amount of GAE (SMD). For this reason, simple distillation process can be preferred from the industrial point of view because it is easy to use and it has low cost. In addition, SMD-10, which has 33% less salt content, showed the closest microorganism formation to the control group. For this reason, it is predicted that the use of SMD-10 may be a good alternative to the brine for the prevention of the formation of harmful microorganisms which may occur in the future depending on the high salt content.

After 45 days of fermentation, it was determined that ED-10 having the polyphenol-containing mixture had the closest rank to the control group and capable of producing table olives having edible properties. However, taste and texture acceptability of olives fermented with polyphenol mixed solutions containing 33% salt was found higher than salt-free solution. Considering harmful effects of salt content on human health, formation of undesirable microorganisms during fermentation and non- desirability of national table olives in export due to their salt content, the usage of salt-free polyphenol mixed solutions with longer fermentation might contribute some advantages to the olive industry. Also, the olive processing with 33% salt-containing polyphenol solutions will be so "low-salt" and even lower than the low-salt labeled current product. Of course, the detailed information about the microorganism diversity produced during fermentation and the capability of extracting flavonoid component types of each solvent combination must be analyzed.

As a conclusion, this study showed that the horse chestnut shells that are not used for any purpose have been a good alternative to brine used in olive industry if correct solvent combinations and fermentation periods were chosen in table olive processing.

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