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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

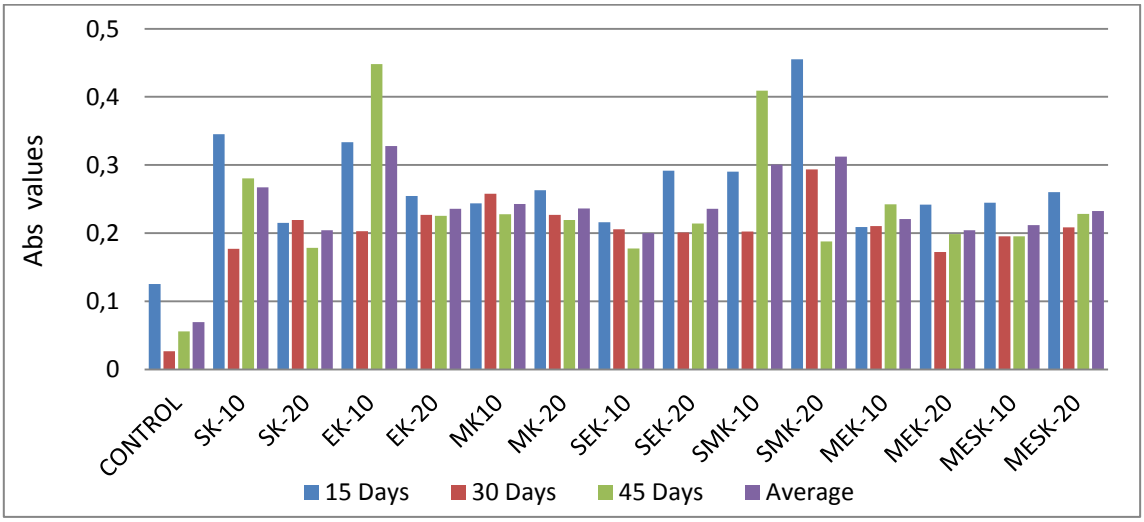
With Drying	mg GAE/ 1g horse chestnut shell
Water (SK)	55.459
Ethanol (EK)	21.35248042
Methanol (MK)	40.39686684
Water-Ethanol (SEK)	77.4308094
Water-Methanol (SMK)	82.05483029
Ethanol-Methanol (MEK)	38.12010444
Water-Ethanol-Methanol (MESK)	74.35770235

(a)

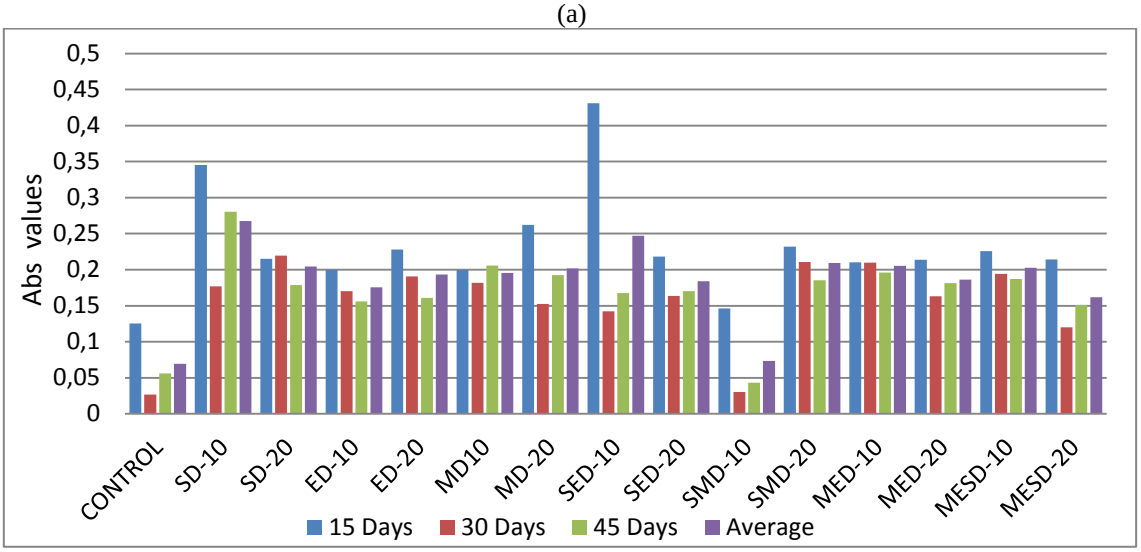
With Simple Distillation	mg GAE/ 1g horse chestnut shell
Water (SD)	55,459
Ethanol (ED)	37.13315927
Methanol (MD)	51.23759791
Water-Ethanol (SED)	75.76501305
Water-Methanol (SMD)	76.91383812
Ethanol-Methanol (MED)	44.71801567
Water-Ethanol-Methanol (MESD)	74.07049608

(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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