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CHANGES OF QUALITY CHARACTERISTICS OF FUNCTIONAL FRUIT YOGURTS FORTIFIED WITH HUSK EXTRACTS OF VARIOUS NUTS DURING COLD STORAGE

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ABSTRACT

Walnut, almond, and pistachio husk extracts were used to enrich the peach yogurt. The effects of the extracts on the physicochemical, biochemical, microbiological, and sensory properties of yogurt were investigated during 21 days of cold storage. Husk extracts slowed down the increase in acidity of yogurt and, correlatedly, the syneresis values of extract fortified yogurts were lower than the control yogurt. Inevitably, husk extracts caused a difference in ΔE of yogurts. However, this difference did not affect the sensory appearance scores of yogurts negatively. All extracts, especially those of walnut husk, significantly increased the antioxidant and antidiabetic activities of yogurt, but this effect was partially reduced with prolonged storage. Also, the extracts reduced the lipid oxidation of yogurt by almost half. Although some sensory values of almond and walnut samples were relatively lower than control yogurt, the panelists liked pistachio yogurts at least as much as control yogurt. The findings of this study revealed that husk extracts of various nuts can be used as both a natural preservative and a functional ingredient in fruit yoghurt.

Keywords: Yogurt, Husk, Walnut, Almond, Pistachio

INTRODUCTION

Yogurt, which is thought to have been made in Mesopotamia for the first time in 5000 BC, is a popular food consumed with different presentation forms today **(Clark *et al.*, 2009; Trachoo, 2002)**. Yogurt is a fermented dairy product with a high nutritional value obtained from lactic acid fermentation and containing live lactic acid bacteria **(Lourens-Hattingh & Viljoen, 2001)**. Antimicrobial **(Chuayana *et al.*, 2003)**, anti-carciogenic **(Wollowski *et al.*, 2001)** and anti-obesity effects **(Zarrati *et al.*, 2014)** of yogurt are known. Nutritionally, yogurt is rich in highly digestible protein, many important minerals and vitamins such as calcium, phosphorus and B vitamins **(O'Sullivan *et al.*, 2016)**, but poor in phenolics **(Hashemi Gahruie *et al.*, 2015)**. Although plain yogurt is common in a few countries, fruit yogurt is more popular in the rest of the world because the fruit flavor masks the excess acetaldehyde flavor **(Barnes *et al.*, 1991)**. In addition, the color and functionality of the added fruit make the fruit yogurt more attractive. Consumers no longer want to just fill their stomachs, they demand more functional and healthy foods that can buy many benefits at the same time. With this trend, researchers have focused on using many different bioactive sources as ingredients in plain or fruit yoghurts **(Balpetek Külcü *et al.*, 2021; Hamad *et al.*, 2020; Moussa *et al.*, 2019)**.

Culinary nuts, including walnuts, almonds and pistachios, are at the forefront with both their taste and many beneficial components. Thus, their worldwide production has nearly doubled over the past decade to reach 5.33 million metric tons **(Shahbandeh, 2021).** A nut consists of three basic parts: the edible inner seed, the hard shell that protects the seed from external influences, and the outermost pericarp, often called a hull or husk. The husk is separated from the fruit after harvest and is usually disposed of by incineration, as it has no widespread industrial use. However, waste materials produced by an industry with millions of tons of input every year have many phytochemical effects. In this context, it has been reported that walnut, almond and pistachio husks have a wide range of phenolic contents with antioxidant and antimicrobial effects **(Barreira *et al.*, 2010; Fernández-Agulló *et al.*, 2013; Rajaei *et al.*, 2010)**. Moreover, the researchers documented that husks protect the cell from the degradative aspect **(Meshkini, 2016**) and have anti-proliferative effects on bone tumor cells **(Khani & Meshkini, 2021)**.

In previous studies, the biochemical and medicinal properties of walnut, almond, and pistachio husks were examined, but their use as a food ingredient was rarely questioned (**Jahanban-Esfahlan *et al.*, 2019)**. To the best of our knowledge, no study has yet been reported in the literature in which the husks of culinary hazelnuts are used in yogurt production. Therefore, the aim of this study was to determine the effects of walnut, almond, and pistachio husk extracts on the physicochemical, antioxidant, antidiabetic, antioxidative, microbiological, and sensory properties of peach yogurt during the 21 days of cold storage period.

MATERIAL AND METHODS

Husks of nuts, other raw materials, and chemicals.

Husks of walnuts, almonds, and pistachio were obtained from local producers in respectively Kahramanmaş, Muğla and Şanlıurfa province of Turkey. The husks were brought to the laboratory, laid out in a thin layer out of the sun and left to dry. Dried samples were ground to an average particle size of 0.3 mm with a grinder and stored at -18 °C. Fruit yogurts with extracts were produced with commercial yogurt culture (YC-381, Chr Hansen, Turkey) in Azık Company (Turkey). Peaches and sugar were purchased from a local market in Kayseri (Turkey). Enzymes, indicators, solvents, and other chemicals were purchased from Merck (Germany) unless otherwise noted.

Extraction of husks of nuts

Ground pistachios, walnuts and almonds husks were kept at + 4 ° C for 24 hours in order to thaw. Completely thaw ground samples were prepared for extraction by drying in an oven at 45 ° C for 6 hours. In the extraction of herbal materials, the method applied by (Fernández-Agulló et al., 2013) was used with a minor modification. 10 g of sample was weighed and 100 mL of water was added on it. After that, it was kept in a shaking water bath at 60 °C for 12 hours, and then it was filtered with the help of a coarse filter and then Whatman filter paper no.4. After the liquid part was centrifuged at 200 rpm for 10 minutes, the supernatant was separated from the sediment and stored at -18 °C.

Production of fruit-yogurt with husks extract

Stirred type fruit yoghurts were produced based on **(Farahat & El-Batawy, 2013)** with a minor modification. After the fat ratio of raw cow milk was standardized to 1.5%, it was pasteurized at 85 ºC for 30 min. Then, the milk was cooled to 45 ± 1 ºC, 2% yoghurt culture was inoculated and milk was incubated at 42 ± 1 ºC until the pH reached 4.7. The yoghurts were cooled to 4 ºC and were stirred with a mixer. The ratio of extract to be added was determined by preliminary studies (data not shown). It was aimed to maximize the extract ratio in order to increase the bioactive potential of yogurt. However, sensory scores of yoghurts with an extract of more than 1.5% were below acceptable limits. Also, since 10% fruit is used in the production of many commercial peach yoghurts, yoghurt production was designed

accordingly. Experimental groups were created by adding 10% fruit pulp prepared according to Figure 1 and 1.5% nuts husks hydrosol extract to yogurt. The control group was prepared using distilled water instead of extract. Yoghurt samples were stored at +4 ºC for 21 days and analyzed on days 1, 7, 14 and 21, respectively.

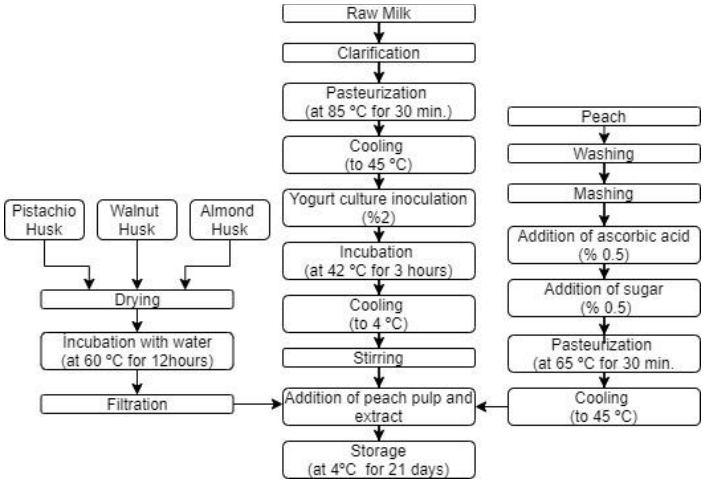

Figure 1 Process flow chart for the production of yogurt fortified with nuts husk extract

Physicochemical analysis

Measurement of pH and lactic acid

Three drops of phenolphthalein were dropped onto 9 grams of homogenized yogurt sample with a pasteur pipette. It was titrated with 0.1 N NaOH solution until it became a light pink color. The lactic acid percentage of the samples was calculated by the following equation:

$$Lactic\ Acid\ (\%) = \frac{Amount\ of\ NaOH\ spent\ (mL) \times 0.009 \times 100}{Amount\ of\ yogurt\ sample} \qquad \text{Eq.1}$$

The pH values of yoghurt samples were measured by dipping the probe of a digital pH meter with a composite electrode (HANNA HI 98127, HANNA Instruments, USA) into the yoghurt samples.

Measurement of viscosity

Viscosities of yoghurt samples were measured at 4 ± 1 ºC with a viscometer (Visco Basic Plus R, Fungilab SA, Spain) based on **(Falade *et al.*, 2015)** with minor modifications. The disc tip number 5 of the device was immersed in the samples until it was completely covered. The value on the digital display in 10 sec. at 20 rpm. was read and the viscosity was recorded as centipoise (cp).

Syneresis analysis

A 25 grams of the yogurt sample was spread evenly on a filter paper. The filter paper (Whatman no 589/2) was placed on a glass funnel, the tubing of which was in a conical flask. After 2 hours of natural filtration at 4 ± 1 ºC to the total sample amount, percentage of syneresis was calculated by proportioning the serum collected in the conical flask.

Determination of total color differences

The surface color values of the yoghurt samples were measured with a colorimeter (HunterLab, Color Flex, USA) based on the CIELAB color space. After the samples were placed in the device, the numerical values representing the lightness (L^*) and basic colors of red, green, blue and yellow (a^*, b^*) were read from the digital display and recorded. The total color differences (ΔE), using the color value of the control samples as a reference, was calculated by the following formula:

$$\Delta E = \sqrt{\left[\left(L_{control} - L_{sample}\right)^2 + \left(a_{control} - a_{sample}\right)^2 + \left(b_{control} - b_{sample}\right)^2\right]}$$

(Eq. 2)

Biochemical analysis

Extraction for biochemical analysis

The extracts to be used to determine the bioactive potential of yoghurt samples were prepared based on **(Karaaslan *et al.*, 2011)** with minor modifications. 20 g of yogurt sample and 30 mL of methanol acidified with 30 µl concentrated HCL were mixed. Then the mixture was left to incubation at 4 ± 1 ºC for 24 hours. At the end of the incubation, the mixture was passed through Whatman filter paper no.1 under vacuum condition. The filtrate was dried in a rotary evaporator at 50 °C and used in bioactive potential analysis.

Total phenolic content and antioxidant-antidiabetic activity

Total phenolic content (TPC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activities of yogurt samples were determined based on **Singleton *et al.*, (1999)** and **Brand-Williams *et al.*, (1995)** with minor modifications as detailed in the our previous study **(Doğan *et al.*, 2020)**.

The reducing power of yogurt samples was determined based on **(Oyaizu, 1986)** with slight modification. 2.5 mL of 200 mM potassium hydrogen phosphate (KH_2PO_4) buffer (pH 6.6) and 2.5 mL of 1 % potassium ferricyanide ($K_3Fe(CN)_6$) solutions were added to 1 mL of yoghurt extracts with a concentration range of 1-10 mg.ml^{-1}. After incubation 50 °C for 20 min., 2.5 mL of 10% trichloroacetic acid (TCA) was added to the mixture in order to terminate the reaction. 2.5 mL of the supernatant collected by centrifugation at 2700 xg for 10 minutes was vortexed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride ($FeCl_3$) solution. After the mixture was filled into the spectrophotometer cuvette, the absorbance at 700 nm. was read and recorded. Using the linear regression curve, the concentration corresponding to an absorbance of 0.5 was calculated and accepted as EC_{50}.

Antidiabetic activity was determined according to previous methods of enzyme inhibition against α-glucosidase and α-amylase **(Doğan *et al.*, 2021)**.

Lipid oxidation analysis

The level of lipid oxidation that yoghurts are exposed to during the storage period was determined by the thiobarbituric acid reactive substances (TBARS) test based on **(Bakry *et al.*, 2019)** with minor modifications. 1% 2-thiobarbituric acid (TBA) was added into 5% trichloroacetic acid (TCA). 1 mL of the prepared solution, 1 gram yogurt and 1 mL of 0.8 % (prepared with ethanol) butylated hydroxytoluene (BHT) were homogenized. The mixture was centrifuged at 1000 rpm for 5 minutes, then the supernant was filtered with Whatman filter paper no.40. After heat treatment at 100 °C for 10 min, the supernatant was cooled to room temperature. The absorbance at 532 nm of the liquid filled into the spectrophotometer cuvette was recorded. The amount of malondialdehyde (MDA) corresponding to the absorbance was calculated using the standard calibration curve, and the results were expressed in mmol MDA.kg yogurt^{-1}.

Microbiological analysis

Microbiological properties of yogurt samples during the storage period were determined on the 1st, 7th, 14th and 21st days. 10 g of yogurt and 90 mL of Ringer's solution was homogenized for 2 min. using a stomacher. 1 mL of the serial dilutions of the prepared stock solution was used in the standard plate count method. *Streptococcus thermophilus* counting was performed on petri dishes containing approximately 12.5 g M17 agar incubated at 42°C for 48 hours under aerobic conditions. In order to establish anaerobic conditions for *Lactobacillus delbrueckii* subsp. *bulgaricus*, petri dishes with approximately 12.5 mL MRS agar were inoculated and covered with 5 mL more agar. After 48 hours of incubation at 37 °C, yellow-colored colonies in the form of bacilli were counted. Potato Dextrose agar was used for yeast-mold counting of yogurts samples. In order to increase the selectivity, 10% tartaric acid was added to the medium before inoculation until the pH reached 3.5. After 5 days of incubation at 25 °C, all colonies on the inoculated petri dishes were counted. Coliform bacteria tests in yogurt samples were performed in petri dishes with Violet Red Bile Agar by incubating at 32 °C for 24 hours **(Wehr, 2004)**. All data obtained from microbiological counts were subjected to logarithmic transformation and expressed as log cfu / g.

Sensory evaluations

The sensory evaluation of yoghurt samples was based on appearance, structure-texture, taste-aroma and overall acceptability. The semi-trained panelists were composed of ten (the genders were equal) member of Yozgat Bozok University, ages 18-36. The specification for selection of panelists based on their experience and background related to yoghurt products. Approximately 50 g yoghurt samples at 4 °C were served to the panelists in lidded white plastic cups. Panelists were asked to mark the sensory scores of the samples from extremely dislike (1 point) to extremely like (5 points). Each sample was coded with a random three-digit number, and panelists were served water and salt-free crackers between assessments. The arithmetic mean of the points of the parameters was used as the final score for each sensory evaluation.

Statistical analysis

The experiments were arranged according to the split plot trial pattern in randomized blocks. The data of the study were analyzed by one-way ANOVA, and the difference between the significant means was determined by the Tukey multiple comparison test with using SPSS 22.0 statistics software (SPSS Inc.,

Chicago, IL). Principle component analysis (PCA) used to determine the correlation between data, was performed with Minitab v18 software (Minitab Inc., PA, USA). All analyses were performed in triplicate and results were expressed as mean±standard deviation.

RESULTS AND DISCUSSION

Physical and physicochemical properties of yogurt with extract

The change in pH and % lactic acid value of yogurts containing husk extracts of different nuts during the storage period is shown in Table 1. Initially, both pH and % lactic acid values of the samples were not statistically different ($p > .05$). However, while pH values tended to decrease in all samples during storage, % lactic acid increased slightly. This can be explained by the fact that lactic acid bacteria hydrolyze the lactose in yogurt to lactic acid over time. On the other hand, a decrease in pH and an increase in % lactic acid value during storage were more severe in control yogurt compared to yogurts containing the extract ($p < .05$). The antimicrobial effects of the extracts may have caused this difference by reducing lactic acid fermentation. Based on the end of the storage period, the highest pH values belonged to almond, walnut, and pistachio samples, respectively, while the % lactic acidity values were the opposite of this order. Similar observations were reported in studies using the banana peel **(Kabir *et al.*, 2021)**, mint **(Bakry *et al.*, 2019)**, and date palm spikelets **(Almusallam *et al.*, 2021)** in yogurt.

Viscosity is an important quality parameter that measures whether yogurt is watery or dense, sticky or fluid. The viscosity values of the extract added yogurts were statistically higher ($p < .05$) than the control yogurts, as shown in Table 1. The husk extracts and, therefore, the yogurts produced using these extracts contained plenty of phenolic substances. Presumably, these phenolic compounds formed more viscous complexes with milk proteins such as casein, causing the viscosity of the yogurts to increase. Viscosity values of all yogurts increased in a similar trend to acidity values during the storage period. Meanwhile, a positive correlation between viscosity and % lactic acidity and a negative correlation between viscosity and pH can clearly observed in Figure 2. Increase in viscosity during the storage period was probably due to related as the acidity increased, the milk proteins gained firmer and more rigid structure. Similar increase in viscosity of yogurt during the cold storage period has been reported **(Atallah *et al*., 2022; Keshavarzi *et al*., 2021; Falade *et al*., 2015)**

For yogurt, syneresis can defined as a textural defect affected by the complex relationship between many different factors. The amount of serum separated from the extract-added fruit yoghurts was less compared to the control yoghurt, as in Table 1. The increase or decrease in the acidity level of fermented milk products is an effective factor on the structure and syneresis value of the product. While the water holding capacity of low acidity proteins is insufficient, an increase is observed in water holding capacity at high acidity. Therefore, in ideal acidity, the water holding capacity of proteins increases and their syneresis values decrease inversely **(Nguyen *et al.*, 2017)**. It was determined that there was a very high correlation (-0.927) between the syneresis value and the titration acidity of the extract-added fruit yoghurt samples during the storage period. Similar results were reported in yoghurts containing juniper molasses **(Çelik *et al.*, 2009).**

Table 1 Physical and physicochemical, biochemical, and microbiological properties of yogurts during the storage period

		Storage period (Days)			
Parameter	Yogurt sample	1.	7.	14.	21
pH	Control	4.61±0.03 ^{aA}	4.03±0.13 ^{cB}	4.01±0.03 ^{cB}	3.92±0.07 ^{cC}
	Walnut	4.60±0.08 ^{aA}	4.23±0.05 ^{aB}	4.21±0.04 ^{aB}	4.18±0.13 ^{aB}
	Pistachio	4.62±0.09 ^{aA}	4.16±0.08 ^{bB}	4.09±0.06 ^{bC}	4.08±0.08 ^{bC}
	Almond	4.63±0.08 ^{aA}	4.20±0.07 ^{abB}	4.12±0.07 ^{bC}	4.19±0.09 ^{aB}
Lactic acid (%)	Control	1.12±0.01 ^{aC}	1.16±0.01 ^{aC}	1.26±0.01 ^{aB}	1.38±0.01 ^{aA}
	Walnut	1.13±0.02 ^{aB}	1.17±0.02 ^{aB}	1.23±0.02 ^{aA}	1.26±0.01 ^{cA}
	Pistachio	1.11±0.01 ^{aC}	1.16±0.01 ^{aBC}	1.21±0.02 ^{aB}	1.29±0.01 ^{bcA}
	Almond	1.11±0.03 ^{aC}	1.18±0.01 ^{aB}	1.23±0.03 ^{aB}	1.33±0.03 ^{abA}
Viscosity (cp)	Control	41365±642 ^{dD}	42211±1023 ^{dC}	43243±923 ^{dB}	44961±1367 ^{dA}
	Walnut	42364±1459 ^{cD}	44962±1276 ^{bC}	45328±1038 ^{cB}	46376±1973 ^{cA}
	Pistachio	43456±1978 ^{bD}	44351±1453 ^{cC}	45932±1083 ^{bB}	47853±1553 ^{bA}
	Almond	44634±1346 ^{aD}	45756±1438 ^{aC}	46103±1543 ^{aB}	48196±1273 ^{aA}
Syneresis (whey %)	Control	37.42±0.16 ^{dA}	35.72±0.42 ^{dB}	31.81±0.34 ^{cC}	30.27±0.38 ^{dD}
	Walnut	39.42±0.35 ^{bA}	37.46±0.34 ^{bB}	33.33±0.47 ^{bC}	30.62±0.34 ^{cD}
	Pistachio	40.39±0.24 ^{aA}	37.75±0.91 ^{aB}	34.38±0.23 ^{aC}	31.32±0.63 ^{bD}
	Almond	38.14±0.28 ^{cA}	37.02±0.63 ^{cB}	33.35±0.43 ^{bC}	31.59±0.71 ^{aD}
ΔE	Control	-	1.31±0.04 ^{dC}	1.51±0.03 ^{dB}	1.63±0.05 ^{dA}
	Walnut	17.23±0.23 ^{aA}	17.1±0.09 ^{aA}	16.43±0.27 ^{aB}	17.14±0.37 ^{aA}
	Pistachio	10.06±0.34 ^{cA}	9.67±0.13 ^{cAB}	9.85±0.09 ^{cAB}	9.56±0.43 ^{cB}
	Almond	12.66±0.29 ^{bC}	13.22±0.19 ^{bAB}	13.41±0.16 ^{bA}	12.88±0.21 ^{bBC}
TPC (mg GAE.kg^{-1})	Control	25.76±0.13 ^{dC}	26.82±0.32 ^{dB}	23.19±0.75 ^{dD}	28.14±0.26 ^{dA}
	Walnut	713.76±4.62 ^{bA}	573.92±7.16 ^{bB}	412.96±8.14 ^{bC}	382.75±7.12 ^{bD}
	Pistachio	831.13±8.23 ^{aA}	621.86±8.81 ^{aB}	549.12±6.13 ^{aC}	492.76±3.26 ^{aD}
	Almond	326.53±4.91 ^{cA}	279.71±3.42 ^{cB}	152.11±3.21 ^{cC}	146.93±2.41 ^{cD}
DPPH EC_{50} (mg.mL$^{-1)}$	Control	1716.13±49.14 ^{aD}	2714.13±46.79 ^{aC}	2982.81±86.92 ^{aB}	3079.16±96.75 ^{aA}
	Walnut	23.18±3.82 ^{dD}	108.86±12.15 ^{dC}	321.12±35.15 ^{dB}	574.83±26.48 ^{dA}
	Pistachio	308.06±21.94 ^{cD}	584.23±53.75 ^{cC}	1324.56±64.34 ^{cB}	1845.73±61.08 ^{cA}
	Almond	724.14±36.15 ^{bD}	1041.76±92.67 ^{bC}	1482.09±63.25 ^{bB}	2367.42±71.12 ^{bA}
Reducing Power EC_{50} (mg.mL^{-1})	Control	3846.95±91.16^{aD}	4381.51±24.97^{aC}	4837.64±37.14^{aB}	5149.11±73.14^{aA}
	Walnut	118.94±1.38^{dD}	384.57±9.47^{dC}	873.91±4.37^{dB}	1217.37±19.34^{dA}
	Pistachio	371.98±6.73^{cD}	673.84±12.78^{cC}	1567.97±37.68^{cB}	2670.29±47.19^{cA}
	Almond	1890.42±22.79^{bD}	2973.62±59.61^{bC}	3267.26±46.59^{bB}	3597.16±36.42^{bA}
α-glucosidase IC_{50} (μg.mL^{-1})	Control	389.14±4.55 ^{aD}	487.71±5.78 ^{aC}	524.92±4.89 ^{aB}	580.56±9.14 ^{aA}
	Walnut	79.65±2.48 ^{dD}	95.47±6.48 ^{dC}	193.49±2.49 ^{dB}	241.39±9.75 ^{dA}
	Pistachio	179.37±7.32 ^{cD}	297.95±2.94 ^{cC}	371.68±9.47 ^{cB}	497.37±10.17 ^{cA}
	Almond	297.36±3.48 ^{bD}	354.67±5.34 ^{bC}	498.34±7.39 ^{bB}	562.93±4.66 ^{bA}
α-amylase IC_{50} (μg.mL^{-1})	Control	624.95±7.09 ^{aD}	1124.67±8.34 ^{aC}	1375.49±3.78 ^{aB}	1384.31±9.70 ^{aA}
	Walnut	168.34±4.39 ^{dD}	216.36±8.27 ^{dC}	403.79±7.39 ^{dB}	546.95±2.99 ^{dA}
	Pistachio	321.94±7.31 ^{cD}	679.88±8.64 ^{cC}	701.98±9.24 ^{cB}	1002.43±18.02 ^{cA}
	Almond	514.31±5.46 ^{bD}	702.69±6.14 ^{bC}	1097.31±9.21 ^{bB}	1281.36±10.07 ^{bA}
S. thermophilus (log cfu.g^{-1})	Control	8.38±0.03 ^{aB}	9.20±0.04 ^{aA}	8.24±0.05 ^{aB}	6.20±0.05 ^{aC}
	Walnut	8.30±0.02 ^{aB}	9.08±0.05 ^{aA}	8.12±0.03 ^{aB}	5.60±0.05 ^{bC}
	Pistachio	8.34±0.02 ^{aB}	9.21±0.04 ^{aA}	8.26±0.01 ^{aB}	6.04±0.04 ^{abB}
	Almond	8.34±0.01 ^{aB}	9.36±0.03 ^{aA}	8.18±0.03 ^{aB}	5.90±0.03 ^{abC}
L. bulgaricus (log cfu.g^{-1})	Control	8.45±0.05 ^{aA}	8.34±0.01 ^{aA}	8.30±0.03 ^{aA}	8.22±0.02 ^{aA}
	Walnut	8.47±0.01 ^{aA}	8.41±0.03 ^{aA}	8.23±0.02 ^{aA}	8.04±0.02 ^{aA}
	Pistachio	8.47±0.03 ^{aA}	8.48±0.04 ^{aA}	8.43±0.04 ^{aA}	8.26±0.04 ^{aA}
	Almond	8.44±0.07 ^{aA}	8.08±0.06 ^{aAB}	8.04±0.02 ^{aAB}	7.95±0.03 ^{aB}

All mean data are presented with standard deviation. Significant differences at $p < 0.05$ are expressed in lowercase letters in the columns of the same parameter group or in capital letters in the rows denoting the storage period.

The color of fruit yoghurts is one of the most important criteria for consumer acceptability. The color of fruit yoghurt should be compatible with the fruit used in production. In order to measure this, *ΔE* was calculated by comparing the color of the control yoghurts on the first day of the storage period with both the control yoghurts after the first day of storage and all extract added yoghurts. The *ΔE* of the extract added yogurts were walnut>almond>pistachio and this sequence did not change during the entire storage period. This may be due to the unique color of the husk of nuts and the extract from them. Minor differences in *ΔE* occurred throughout the storage period. The most important reason for this differentiation is probably the fact that the fruit used in yogurt production was exposed to the Maillard reaction and was relatively browned. In addition, the coloring matter in both the fruit and the extracts may have degraded over time. Significant differences were reported in the L, a and b values, which are functions of *ΔE*, during the storage period in yoghurts produced with mint **(Bakry *et al.*, 2019)** and date extracts **(Almusallam *et al.*, 2021)**.

Bioactive potential of yogurts

TPC was revealed in fruit yogurts containing walnut, almond and pistachio husk extract and control yogurts (Table 1). Considering all samples and storage period, the highest TPC belonged to the pistachio sample on the first day of storage (831.13 mg GAE.kg^{-1}), and the lowest TPC belonged to the control sample on the 14th day of storage (23.19 mg GAE.kg^{-1}). Although the control yoghurt had relatively stable but low TPC during the storage period, the addition of the extract caused a dramatic increase on the TPCs of the yoghurts. However, this high TPC at the beginning of the storage decreased by 46%, 40% and 55% in walnut, pistachio and almond samples, respectively, on the 21st day, the last day of the storage period. The decrease in TPC during storage period could be due to degradation of polyphenols in the presence of lactic acid bacteria in yogurt **(Rodríguez et al., 2009)**.
Antioxidant activities of yogurt samples were measured using DPPH radical scavenging activity and reducing power value. Since the results were expressed as EC_{50} concentration in both methods, the low amount of concentration indicated that the activity was high. DPPH radical scavenging activity and reducing power value were significantly affected ($p < .05$) by both samples and storage period, as shown Table 1. Antioxidant activity for both methods was highest in the order of walnut > pistachio > almond. As expected, the antioxidant activity of the control yoghurt (1716.13 mg.mL^{-1} for DPPH and 3846.95 mg.mL^{-1} for reducing power) was lower than the extract added yoghurts. It should be noted that, based on the averages of EC_{50} concentrations calculated in both methods, walnut, pistachio and almond samples lost their activity by approximately 16.5, 5.5 and 1.5 times, respectively, at the end of the 21-day storage period. There is an intense relationship between the phenolic content of the plant materials and their antioxidant activities **(Aryal *et al.*, 2019)**. Already, the correlation was quite clear on the bi-plot (Figure 2) with an angle of almost 180 degrees between the TPC and DPPH - Reducing Power EC_{50} vectors.
The α-amylase enzyme secreted from the pancreas breaks down complex starches into oligosaccharides in the small intestine lumen. Furtherly, α-glucosidase enzyme breaks down these oligosaccharides into trisaccharides, disaccharides and ultimately glucose and other monosaccharides in the small intestines. Inhibition of these enzymes greatly reduces the digestion of complex carbohydrates. In this way, the amount of glucose going into the circulation decreases and an important defense mechanism is established for type 2 diabetes **(Israili, 2011)**. In this study, inhibition of α-glucosidase and α-amylase were measured during the storage period of yoghurts produced with various nuts husk extracts and results were expressed as IC_{50} (µg extract.mL^{-1}). Inhibition of α-glucosidase and α-amylase was significantly affected by the variety of extracts added to yoghurts. Phenolic compounds in extracts obtained from different parts of plants were reported to have anti-diabetic effects by different mechanisms. Masticadienolic and masticadienonic acids in pistachios **(Lawali *et al.*, 2020)**, quercetin and quercitrin in almonds **(Adefegha *et al.*, 2017)**, and juglone in walnuts **(Atila Uslu & Uslu, 2019)** have enzyme inhibition potential. The most potent α-glucosidase and α-amylase inhibitors were in the same order and based on the last day of storage as follows: walnut ($IC_{50\ \alpha\text{-glucosidase}}$=241.39, $IC_{50\ \alpha\text{-amylase}}$ =546.95) > pistachio ($IC_{50\ \alpha\text{-glucosidase}}$=497.37, $IC_{50\ \alpha\text{-amylase}}$ =1002.43) > almond ($IC_{50\ \alpha\text{-glucosidase}}$=592.93, $IC_{50\ \alpha\text{-amylase}}$ =1281.36). The enzyme inhibitory capacity of all samples for both α-glucosidase and α-amylase decreased approximately 1.5 to 3.2-fold from day 1 to day 21 of storage. However, this decreasing trend during the storage period was relatively higher in samples containing extracts compared to control samples. As a result of the relationship between phenolic compounds, proteins and the fermentation process, insoluble compounds may be formed that adversely affect both phenolics and proteins with anti-diabetic activity **(Akan *et al.*, 2021)**. In this study, it can be said that the use of walnut, pistachio and almond shell hydrosols in fruit yoghurt helped to increase α-amylase and α-glucosidase inhibitory activities.

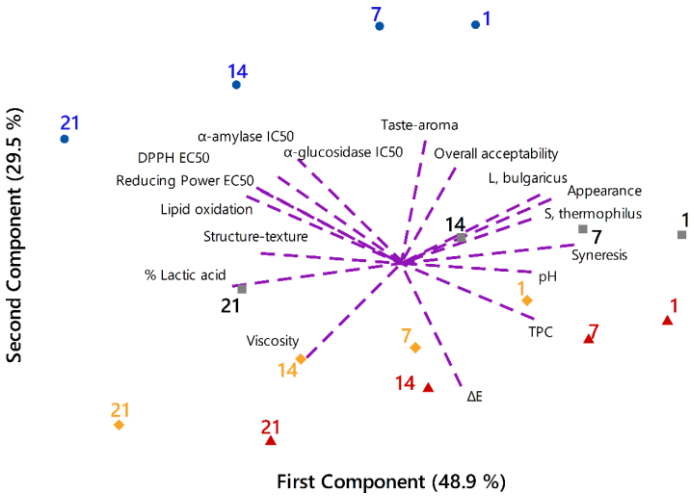

Figure 2 Bi-plot showing the distribution of yogurt samples in quality parameters with principal component analysis. Control samples are expressed with ●, walnut samples with ▲, pistachio samples with ■, almond samples with ◆ . The numbers next to the icons indicate the day of the storage period.

The degree of oxidation of yoghurts

The effect of the addition of the extract on the oxidative stability of yoghurts was measured by the TBARS value. The TBARS value of all samples increased significantly during the storage period ($p < .05$), but the addition of the extract suppressed the oxidation level by approximately half regardless of the storage day, as shown in Figure 3. Based on the 21-day storage period, the TBARS values of yoghurts containing walnut, pistachio and almond husk extract were determined as 79.78, 87.36 and, 98.92 mmol MDA.kg^{-1} yogurt, respectively. However, at the end of the same period, the oxidation level of the control yogurt reached 169.37 mmol MDA / kg yogurt, a level much higher than that of the other samples. Similar to the results, it was reported that seaweed extract (**O'Sullivan *et al.*, 2016**), grape pomace **(Tseng & Zhao, 2013)**, and banana peel extract **(Kabir *et al.*, 2021)** used in yoghurts remarkably reduced oxidation levels.

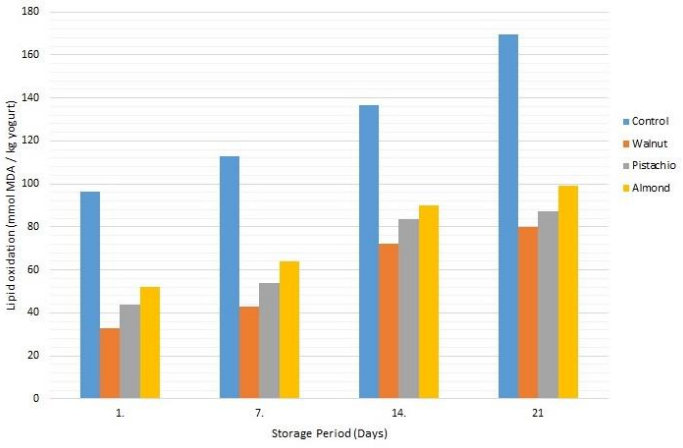

Figure 3 Graph of lipid oxidation of yogurts during the storage period

Microbiological properties of yogurt with extract

Yeast, mold, or coliform bacteria were not found in any yogurt samples during the storage period due to the heat treatment of the raw material before production and the hygienic production environment.
Nuts husks extracts caused a change ($p < .05$) in the activity of lactic acid bacteria (LAB) in yogurt (Table 1). *Lactobacillus delbrueckii* subsp. *bulgaricus*, which averaged 8.46 log cfu.g^{-1} in all samples on the first day of storage, changed in different ways as the storage period prolonged. On the 7th day of storage, *Lactobacillus delbrueckii* subsp. *bulgaricus* in almond samples decreased sharply to 8.08 log cfu/g and diverged negatively from other samples ($p < .05$). Although the *Lactobacillus delbrueckii* subsp. *bulgaricus* numbers of all samples decreased during the storage period, the decrease only in the almond sample was statistically significant ($p < .05$). The highest *Lactobacillus delbrueckii* subsp. *bulgaricus* on the last day of storage belonged to the pistachio sample with 8.26 log cfu.g^{-1}, which was slightly higher than that of the control yogurt. Therefore, it can be said that the pistachio husk extract in yogurt stimulated the growth of *Lactobacillus delbrueckii* subsp. *bulgaricus* to a small extent.

The growth of *Streptococcus thermophilus*, which increased up to 7 days regardless of the yogurt sample, decreased after peaking on the 7th day and reached the minimum level at the end of 21 days. A similar effect of pistachio extract on the growth of *Lactobacillus delbrueckii* subsp. *bulgaricus* was observed on days 7 and 14 of the storage period for *Streptococcus thermophilus*. On these days of analysis, *Streptococcus thermophilus* was slightly higher in the pistachio-added yogurts than in the control yogurts. However, inhibition of *Streptococcus thermophilus* was higher in extract added yoghurts than control yoghurt on the last day of storage.

The effect of phenolic compounds on LAB in yogurt can be very variable when combined with storage conditions. Components in herbal extracts are often cited for their inhibitory effect **(Zaika *et al.*, 1983)**. However, some phenolic compounds and their complex interactions may contribute to the growth of bacteria **(Amirdivani & Ahmad Salihin Hj, 2015)**. Even during the storage period, these components may derive and exacerbate the existing effect **(Sun-Waterhouse *et al.*, 2013)**. Moreover, there are many factors affecting microbial activity in yogurt, such as the relationships between milk and plant components, acidity and temperature. Similarly, many findings have been reported that herbal ingredients added to yogurt have both inhibitory and provoking effects on LAB proliferation **(Demirkol & Tarakci, 2018; Kabir *et al.*, 2021; Mohamed Ahmed *et al.*, 2021).**

Sensory properties of yogurt with extract

Sensory parameters of extract added and control yoghurt are presented in the radar chart in Figure 4. There was no significant change in the appearance, structure-texture, taste-aroma and overall acceptability scores of the yoghurts until the 14th day of the storage period. The most striking differences between the samples in the first 7 days was that the almond sample for appearance, and both almond and walnut samples for taste-aroma scored on average approximately 1 unit lower than other samples. Most panelists found the distinctive bitter taste of these samples unfamiliar. Interestingly, at day 14 of storage, the sensory scores of the pistachio sample were able to compete with the control yogurt and even it had slightly higher appearance and overall acceptability scores than control sample. Although the structure-texture and taste-aroma scores of all samples were good at 21 days of storage, their overall acceptability and appearance scores came very close to the limit value of 4. At the end of storage, all the yogurts to which the extract was added in particular had better appearance scores than the control yogurt. Undoubtedly, apart from the complex interactions in human evaluation, the high syneresis value of the control yogurt also played a role on low appearance score. In addition, there was no significant difference between the overall acceptability scores of the other samples, except for the almond sample. To summarize briefly, the findings showed that pistachio shell extract improved some of the sensory parameters of yogurt. However, most of the panelists stated that they could ignore the partial decreases in sensory values for a healthier product.

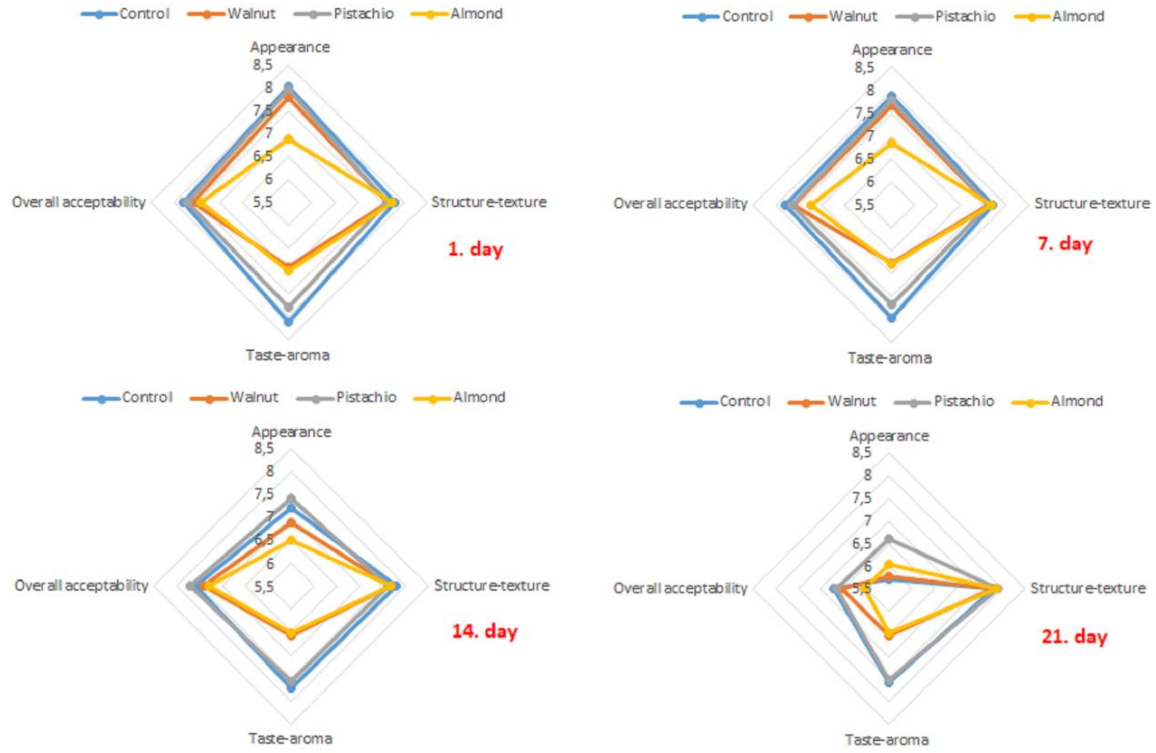

Figure 4 Radar plot of sensory parameters of yogurts during the storage period

CONCLUSION

Walnut, Almond and Pistachio husk extracts improved many characteristics of the original yogurt. Notably, lipid oxidation of yogurts was reduced by about half with the addition of extract. In addition, the sensory scores of the yogurts to which walnut, almond, and pistachio husk extract were added during the storage period were not bad at all compared to the control yogurt, but the sensory performance of the pistachio sample, especially at the end of the 21-day storage period, was outstanding. Moreover, the Pistachio sample had the highest TPC and it was observed that the Walnut sample showed the highest antioxidant activity, by far than the control yogurt, according to both DPPH and reducing power. In this context, the increase in TPC and antioxidant activities of yogurts fortified with extracts was remarkable. Additionally, the findings showed that the extracts used in yogurt, especially the walnut husk hydrolysate, have the potential to inhibit amylase and glucosidase, which are key enzyme roles for type 2 diabetes. However, it should be noted that the prolonged storage period was reduced the bioactive potential of the extract fortified yogurt. For this reason, further studies, including instrumental analysis methods, are essential to reveal the mechanism of this decrease. In addition, different studies such as folic acid analysis, determination selenium, and tocopherol can be designed in the future. In this study, it was concluded that the husk of culinary nuts such as walnuts, pistachios, and almonds, an underutilized by-product, have the potential for the dairy industry.

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