



Effect of Walnut (*Juglans regia* L.) on the Physicochemical, Sensory, Phenolic and Antioxidant Properties of Set Type Yogurts during Storage Time

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ABSTRACT

This study aims to reveal the quality properties of yogurt enriched with walnut during cold storage (4°C) for 28 days. Five sets of yogurt types were produced by using walnut at varying rates (A: 0% (control), B: 1%, C: 2.5%, D: 3.5%, E: 5%). Physicochemical, sensory, phenolic contents and antioxidant properties of yogurt samples was investigated during the 1st, 7th, 14th, and 28th days of storage. The study was conducted to optimize the walnut addition level to obtain better quality yogurt as a functional food. It was determined that the walnut enrichment affects the gross chemical component of yogurt samples. Yogurt sample with %5 walnuts (E) had the highest total phenolic and antioxidant activity content (respectively 1027.50 mg GAE/kg, 19.95 mM TE) among the samples. The yogurt sample with the highest sensory attribute score was the one containing 1% walnuts. Therefore, it was concluded that yogurt added with walnut could serve as functional yogurt beneficial for human health.

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Introduction

Yogurt, the most consumed commodity of dairy products, has an essential role in people's nutrition thanks to its high nutritional value. Yogurt has high protein, lactose, water-soluble vitamins, and calcium (Aryana and Olson, 2017). Food fortification, which means adding one or more ingredients to foods, is carried out regardless of whether the ingredient is available. Food fortification aims to add additional properties to newly designed food products (Świeca et al., 2014). Due to its bioactive components, functional foods are effective in the physical and mental recovery of individuals and reducing the risk of diseases. This is because bioactive compounds contain beneficial components (Biesalski et al., 2009). Since it is a frequently consumed dairy product with antioxidant activity, the researchers added some beneficial components to yogurt. Yogurt can usually be consumed by fruits and vegetables (Oliveira et al., 2015; Kiros et al., 2016) or herbal extracts, seeds, or nuts (Damyanova et al., 2009; Todorova et al., 2009; Halah and Mehanna, 2011). Since

fruits and vegetables contain rich phenolics, herbal extracts, seeds, and nuts are also consumed with yogurt because of their positive impacts on human health. All these additions increase the quality of yogurt. Nuts contain high levels of vitamin C and vitamin E. Thanks to vitamin C; it has a powerful antioxidant feature. Besides, vitamin E prevents tissue damage. It also takes part in the neutralization of free radicals. Hazelnut containing vitamin E is especially important in cancer and cardiovascular diseases (Giugliano, 2000). Moreover, nuts also contain folic acid. Folic acid is the compound required for cell division and the red blood cell generation. Therefore, pregnant women need high amounts of folic acid during pregnancy (Wardlaw, 1999). For instance, walnut (*Juglans regia* L.) has both polyunsaturated fatty acid and high nutritional value (Savage, 2001). Due to its frequent consumption and high nutritional value, walnuts grown in many regions of the world, are a useful food source that people have been consuming since ancient times

(Cheraghali et al., 2018). Walnuts contain high amounts of protein and fat. It is included in the priority plants list of FAO (Gandev, 2007), and contains vitamins and minerals and high protein and fat content. Furthermore, walnuts are rich sources of sterols, flavonoids, phenolic acids, pectic substances, and related polyphenols (Çağlarımak, 2003; Martinez et al., 2010). Walnut, which contains high amounts of omega-6 and omega-3 PUFAs, has a critical role in the coronary heart disease due to the omega-3 PUFA (Davis et al., 2007). Walnut kernels have the highest antioxidant activity among 25 commonly consumed food types. This stems from walnuts' high phenolic content (Gunduc and El, 2003). Walnut phenolics have high levels of antioxidant properties and cleanse free radicals. The total phenolic in 50 grams of walnuts (about a handful) is higher than a glass of apple juice (240 mL), 43 grams of milk chocolate, or a glass of red wine (150 mL) (Colaric et al., 2005).

This study aims to determine the optimal rate of walnut enrichment to obtain a better-quality yogurt as a functional food, and identify the physicochemical, sensory, phenolic and antioxidant properties of yogurt enriched with walnut pieces.

Materials and Methods

Walnut that was bought from a store in Zonguldak was shredded into 3-5 mm. The sterilized full-fat cow's milk purchased from a local store in Zonguldak was used to obtain yogurt. Skimmed milk powder (NFMP) purchased from Pınar Süt Ürünleri in İzmir was utilized. The frozen starter culture (YC-350, a blend of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) was purchased from Chr. Hansen Co. (A/S, Horsholm, Denmark).

Yogurt Production

Cow milk with 3% fat, 3.6% protein, 12.1% total solid content, and 8 SH acidity is used for preparing the yogurt (Tamime and Robinson, 1999). The skimmed milk powder was added to increase the solid content to 15% (15 grams per 100 grams). Afterward, walnuts were added to the milk at concentrations of 0%, 1%, 2.5%, 3.5%, and 5%, and the homogenization process (Homogenizer T 65, Ika, Staufen, Germany) was done. The milk was pasteurized at 85°C for half an hour. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* was added to the mixture at a rate of 2% (w/v). Then the milk was incubated at 42±1°C until the pH was 4.6-4.7 (approximately 3.5 hours). After the incubation period, yoghurt samples were stored at 4±1°C for 28 days. The process was repeated twice and samples were analyzed on the 1st, 7th, 14th, and 28th days of storage.

Analytical Methods

The total solids content (AOAC, 1990) and titratable acidity (Case et al., 1985) were determined. as lactic acid equivalent. A digital device was used to determine the pH value in samples (Schott Instruments, Lab 860, Germany). The whey syneresis was determined by weighing 25 grams of yogurt on filter paper placed in a funnel and measuring the volume of separated whey after 2 hours at 4°C (Tamime et al., 1996).

Determination of Total Phenolic Content

Gallic acid was used as a standard to examine the phenolic content in yogurt by the Folin-Ciocalteu method (Singleton and Rossi, 1965). The absorbance value was measured by spectrophotometer at 760 nm (Shimadzu Scientific Instruments, Inc., Tokyo, Japan). The results obtained were reported as mg gallic acid equivalent (GAE)/kg.

Determination of Total Antioxidant Activity

2,2'-azinobis (3-ethylbenzthiazoline)-6-sulfonic acid (ABTS-TEAC) Assay

2,2'-azinobis (3-ethylbenzthiazolin-6-sulfonic acid) diammonium salt (ABTS⁺) radical cation was prepared by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate. ABTS⁺ radical cation was diluted with PBS, pH: 7.4, to an absorbance of 0.70 (±0.02) at 734 nm equilibrated at 30°C. ABTS⁺ inhibition against Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) was spectrophotometrically measured. at 734 nm (Shimadzu Scientific Instruments, Inc., Tokyo, Japan). TEAC values of samples were calculated from the Trolox standard curve and expressed as Trolox equivalents (in µmol/mL of the sample) (Re et al., 1999).

Sensory Evaluation

Sensory evaluation of the samples was performed by 10 trained panelists (5 male and 5 female) using sensory evaluation scorecards. The panelists' age range was 25-34. Samples were evaluated in five different categories. After each week (1, 7, 14, 28 days) yogurts were evaluated in regard to appearance, consistency, taste-aroma, odor, and general acceptability through a 10-point scale (Lawless and Heymann, 1999).

Statistical Analysis

The whole experimental process and subsequent analyzes were repeated twice. Data were analyzed with the help of SPSS for Windows 16.0. A repeated one-way analysis of variance (ANOVA) measurement was used for the analysis. The differences between the repeated data were analyzed with Duncan analysis (SPSS, 2017).

Results and Discussion

Changes in Chemical Composition and Syneresis Value

Table 1 shows the average composition of yogurts fortified with walnut compared to control group samples. It was observed that the gross chemical composition of the yogurt samples was affected significantly by the walnut enrichment (P<0.05). It was determined that the solid content of the samples increased with increasing walnut level. The pH and serum separation values decreased during storage, but titratable acidity values increased. Moreover, a consistently low pH and increasing acidity were observed throughout the storage process. These results are similar to the previous findings (Ye et al., 2013; Ozturkoglu-Budak et al., 2016; Su et al., 2017; Baba et al., 2018; Ujjirogheneet et al., 2019).

The pH values of the control group samples were lower than the alones with fortified yogurts during the whole storage period.

Table 1. The chemical and physicochemical composition of the samples

Sample	Storage periods (day)	Titratable acidity (LA%)	pH	Whey separation (mL/25 g)	Dry matter (%)
A	1	1.159±0.05 ^{Aa}	4.30±0.10 ^{Aa}	5.43±0.21 ^{Aa}	14.70±0.11 ^{Aa}
	7	1.406±0.06 ^{Ab}	4.14±0.12 ^{Bb}	4.80±0.12 ^{Bb}	14.90±0.10 ^{Bb}
	14	1.496±0.07 ^{Bb}	3.99±0.03 ^{Bb}	4.50±0.00 ^{Ac}	14.92±0.19 ^{Bb}
	28	1.536±0.06 ^{Bb}	3.95±0.06 ^{Bb}	4.00±0.00 ^{Ad}	14.97±0.14 ^{Bb}
B	1	1.149±0.01 ^{Ca}	4.33±0.01 ^{Ba}	5.00±0.00 ^{Bb}	15.81±0.21 ^{Ca}
	7	1.343±0.00 ^{Bb}	4.22±0.00 ^{Ab}	4.50±0.00 ^{Cc}	15.39±0.03 ^{Ca}
	14	1.766±0.02 ^{Ac}	4.18±0.00 ^{Ab}	4.50±0.00 ^{Ac}	15.33±0.07 ^{Ca}
	28	1.688±0.00 ^{Ab}	4.10±0.00 ^{Ba}	3.75±0.00 ^{Aa}	16.02±0.07 ^{Db}
C	1	1.097±0.09 ^{Aa}	4.41±0.14 ^{Ca}	4.13±0.21 ^{Ca}	16.28±0.04 ^{Da}
	7	1.299±0.12 ^{Cc}	4.21±0.14 ^{Cc}	4.25±0.14 ^{Cc}	17.42±1.04 ^{Ab}
	14	1.505±0.16 ^{Cc}	4.14±0.13 ^{Bb}	3.75±0.14 ^{Ba}	16.10±0.59 ^{Da}
	28	1.538±0.09 ^{Bb}	4.14±0.04 ^{Bb}	3.25±0.14 ^{Bb}	16.88±0.08 ^{Ac}
D	1	1.148±0.01 ^{Cc}	4.35±0.00 ^{Ba}	4.00±0.00 ^{Aa}	17.40±0.06 ^{Ea}
	7	1.193±0.00 ^{Da}	4.27±0.00 ^{Ba}	4.00±0.00 ^{Aa}	17.03±0.11 ^{Db}
	14	1.499±0.03 ^{Bb}	4.15±0.00 ^{Ba}	3.85±0.00 ^{Ab}	17.03±0.03 ^{Ab}
	28	1.628±0.00 ^{Cc}	4.10±0.00 ^{Ab}	3.85±0.00 ^{Cb}	18.85±0.06 ^{Cc}
E	1	1.041±0.07 ^{Ba}	4.47±0.06 ^{Ca}	3.95±0.20 ^{Ba}	18.42±0.47 ^{Ba}
	7	1.159±0.06 ^{Cb}	4.26±0.05 ^{Cc}	4.00±0.00 ^{Db}	18.95±0.41 ^{Ea}
	14	1.528±0.04 ^{Cb}	4.15±0.01 ^{Bb}	3.75±0.43 ^{Bb}	18.91±0.16 ^{Ea}
	28	1.623±0.08 ^{Ba}	4.12±0.01 ^{Ac}	3.75±0.14 ^{Ab}	18.12±0.37 ^{Ca}

A: 0% (Plain control sample), B: Yogurt with 1% walnut, C: Yogurt with 2.5% walnut, D: Yogurt with 3.5% walnut, E: Yogurt with 5% walnut. ^a Different lowercase superscripts depict the statistical difference within a row between time (P<0.05). ^{ABC} Different uppercase superscripts depict the statistical difference between means for yogurt samples (P<0.05).

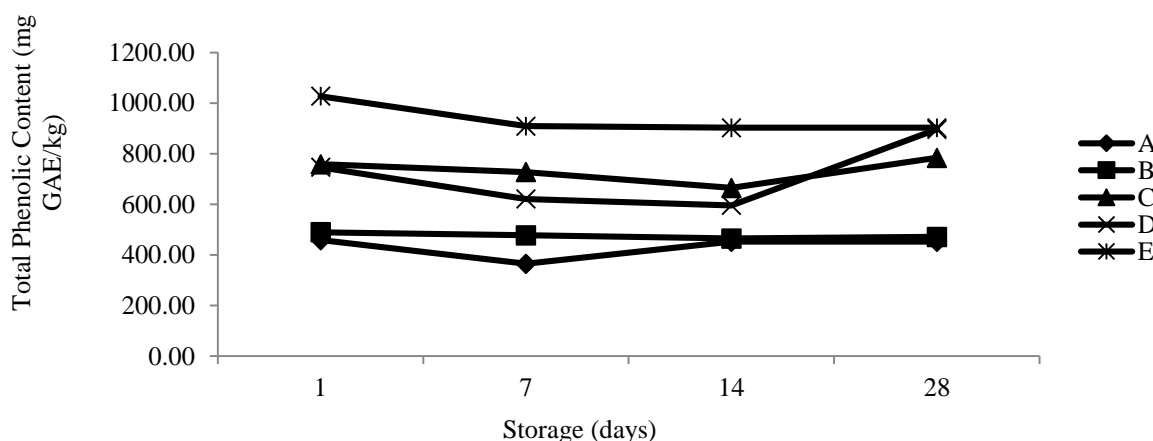


Figure 1. Total phenolic content of the experimental yogurts during storage at 4°C for 28 days: A: 0% (Plain control sample), B: Yogurt with 1%, C: with 2.5%, D: with 3.5%, E: with 5% walnut.

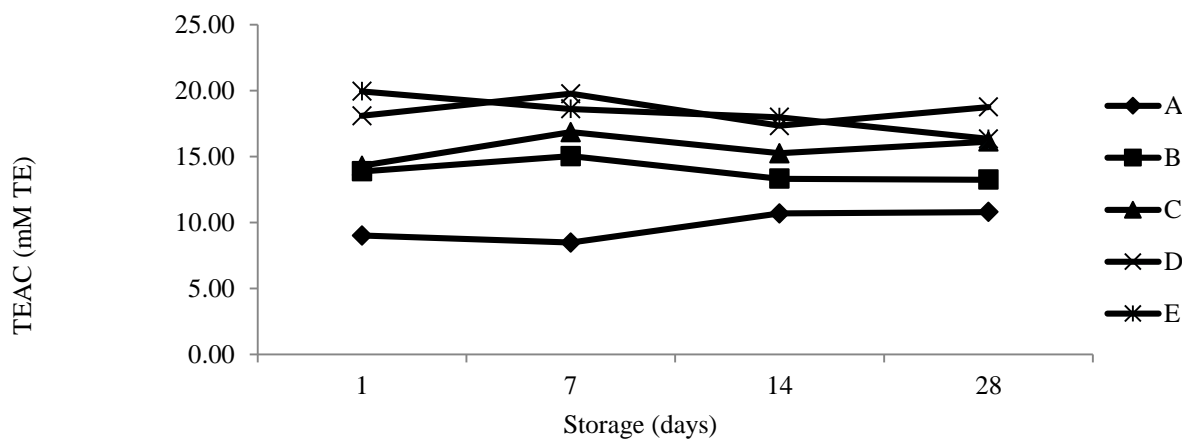


Figure 2. Total antioxidant activity of the experimental yogurts during storage at 4°C for 28 days: A: 0% (Plain control sample), B: Yogurt with 1%, C: with 2.5%, D: with 3.5%, E: with 5% walnut.

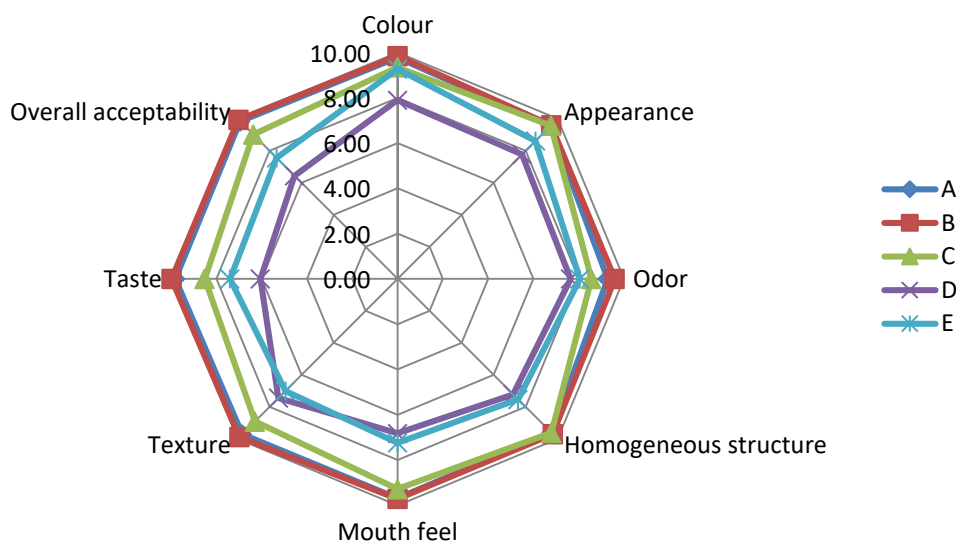


Figure 3. The pattern of variation of sensory properties of yogurts at day 1 of refrigerated storage: A: 0% (Plain control sample), B: Yogurt with 1%, C: with 2.5%, D: with 3.5%, E: with 5% walnut.

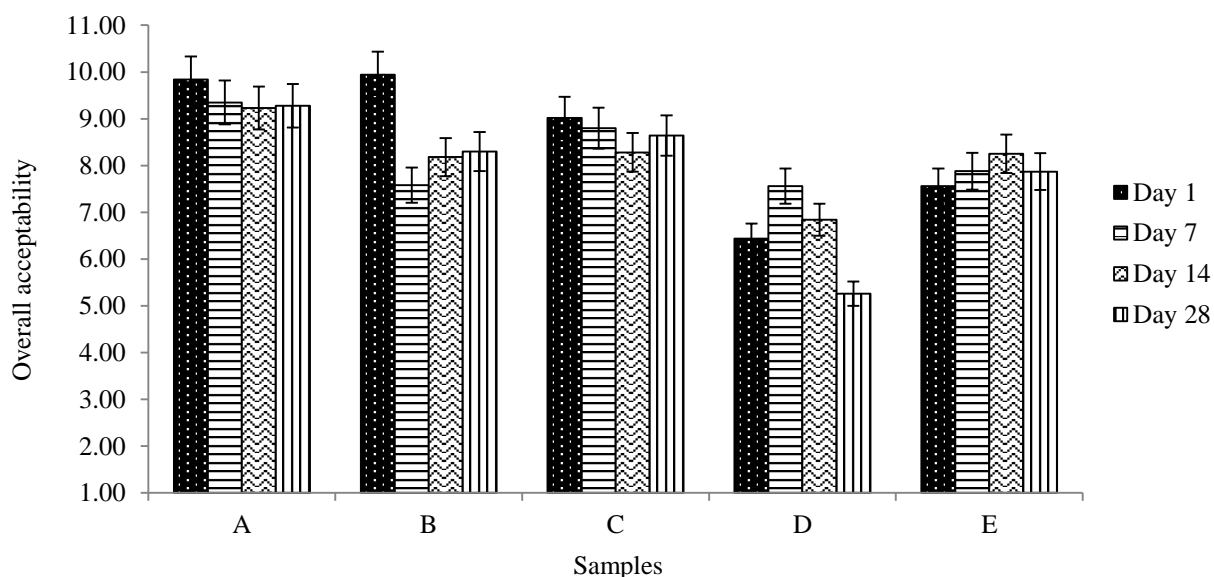


Figure 4. Changes in overall acceptability of yogurt samples during storage: A: 0% (Plain control sample), B: Yogurt with 1%, C: with 2.5%, D: with 3.5%, E: with 5% walnut.

The control group samples had high level of titratable acidities on d 1 and 7 than of fortified ones, whereas acidity was lower on d 14 and 21 for the control yogurt ($P < 0.05$). The increased buffering capacity thanks to the additional proteins in the system caused the initial higher pH, and the lower pH after 14 and 21 day of storage was because of the increased growth of lactic acid bacteria. The higher pH and lower acidity found in the study may stem from the dietary fiber and proteins coming from walnut. All yogurts fortified with walnut had significantly higher values of total solid than control group sample ($P < 0.05$). The syneresis decreased due to the increased dry matter in the yogurt samples. Therefore, the syneresis may have decreased with increased in walnut concentration depending on its ratio.

Evaluation of Total Antioxidant Activity and Phenolic Contents

Total Phenolic Content (TPC)

Figure 1 indicates that the TPC of yogurt samples is significantly ($P < 0.05$) affected by the walnut fortification. Control group samples showed a TPC of 458.75 mg GAE/kg (A) that increased significantly with walnut addition to 1027.50 mg GAE/kg (E) on the first day of storage.

Yogurt sample with %5 walnut (E) had the highest total phenolic content (1027.50 mg GAE/kg) among the samples ($P < 0.05$). Walnut is considered to be a rich source of phenolics. Studies on walnut revealed that it contains several groups of polyphenolics identified mainly as tannins, most of which were demonstrated to contribute the overall antioxidant activity of the walnut extract with different *in vitro* and *in vivo* antioxidant estimation models (Zhang et al., 2009; Amin et al., 2017; Nguyen and Vu, 2021).

Antioxidant Activity

The results in Figure 2 hint that walnuts significantly ($P < 0.05$) affect the antioxidant activity of yogurt samples. It was observed that the walnut addition to yogurt led to a significant increase in antioxidant activity in the samples prepared with walnut compared to the control sample ($P < 0.05$). The highest antioxidant activity content was recorded in the sample with 5% walnut (19.95 mM TE). The walnut addition increased the bioactive ingredients of yogurt, and that may be the main reason behind the higher antioxidant activity (Ye et al., 2013).

Walnut is a rich source of polyphenolic compounds. Thus, an increase in antioxidant values of fortified yogurt samples might be due to the rich polyphenolic content of walnut. Increased antioxidant activity in yogurt is previously determined with the addition of different components (Ye et al., 2013; Su et al., 2017; Baba et al., 2018; Ujiroghene et al., 2019; Anuyahong et al., 2020).

Sensory Analysis

The results of the sensory analysis of the yogurt samples are illustrated in Figure 3 and 4. The sample with 1% walnut had the highest scores for all sensory attributes, while addition of 3.5% walnut resulted with the lowest scores for the sensory attributes. It was observed that there was no negative taste-odor formation in all yogurt samples (A, B, C, D, E) during the storage regarding foreign odor and taste criteria in sensory analysis. The control (A) and 1% walnut added yogurt (B) samples had high scores in the homogeneous structure and mouthfeel criteria. The sensory analysis scores for all yogurts decreased with storage time.

The fact that the control (A) and 1% walnut added yogurt (B) samples had values close to most scales included in the sensory analysis revealed that there is an optimum amount of walnut addition. These findings are also supported by Ye et al., (2013); Su et al., (2017) and Baba et al., (2018).

Conclusions

This study, successfully used walnut (*Juglans regia* L.) to prepare yogurt samples. The results indicated that walnut addition significantly increased antioxidant activity and phenolic content of the samples. Walnut addition resulted higher pH and lower titratable acidity of yogurt during storage. Yogurt samples containing walnut had lower syneresis compared to control during the storage. Yogurt with 1% walnut received the highest overall acceptability score (9.94) on the first day. Walnut could be successfully used as a functional additive in yogurts to enhance health benefits. Therefore, it can be concluded that walnut can be used in producing a new profitable yogurt with good sensory attributes and antioxidant properties.

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Conflict of Interest Statement

No conflicts of interest exist in this study.

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