

An Investigation of the Effects of the Addition of Pistachio Hull and Testa on the Oxidative Stability of Pistachio Butter

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Information	Abstract
<p>Article Type: Original Article</p>	<p>Introduction: Pistachio butter is susceptible to autoxidation owing to its high content of unsaturated fatty acids.</p> <p>Materials and Methods: In this research, the effects of pistachio hull and testa (0.0, 0.5, and 1.0%) on the oxidative stability of pistachio butter have been investigated. The peroxide value, oxidative stability (using the rancimat method at 110°C), and the refractive index (at 30°C) were evaluated after months 0, 2, and 4 from the storage time at 25°C.</p> <p>Results: The peroxide value of the samples increased with an increase in the storage time. The lowest peroxide value was related to the treatment containing 1.0% of hull and 1.0% of testa. Besides, in the oxidative stability test, the control sample and the treatment containing 1.0% of hull and 1.0% of testa showed the lowest and highest stability time against oxidation, respectively. The refractive index of the samples was determined within the range of 1.464-1.470. According to the results, there was a significant difference just between the control samples of month 0 and month 4, being an indicator of polymerization reactions. There was no significant difference among other treatments at different storage times.</p> <p>Conclusion: Pistachio butter containing the combination of 1% of hull and 1% of testa showed higher oxidative stability after 4 months of storage.</p>
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1. Introduction

Pistachios are from among the most popular and delicious tree nuts used in the salted and roasted forms, or in combination with various desserts (halvah, baklava, cakes, ice creams, etc.). Pistachio butter is one of the nutritious products of the pistachio processing industry, which is produced from ground pistachio nuts with some sugar and a little salt (1). More than 50% of the pistachio butter formula is related to pistachio oil. The high level of the fat in the formula makes the product susceptible to spoilage. Therefore, it is necessary that such spoilage be somehow controlled. Antioxidants are the most important compounds that delay the degradation of oils and fats (2). Ardakani et al. (3) investigated the effects of butylated hydroxytoluene (BHT) antioxidants at the concentrations of 0, 0.01, and 0.02% to extend the shelf life of pistachio butter. The evaluation of the peroxide value after four months of storage at 25°C showed that the effects of antioxidants on pistachio butter were significant. Pistachio nuts contain some natural antioxidants (such as tocopherol) (4). Pistachio hull is a phenolic source, i.e. a natural antioxidant, without the adverse effects of synthetic antioxidants, such as mutagenicity and carcinogenicity (5).

Goli et al. (6) investigated the effects of water and methanol solvents on soybean oil stability at 60°C in an oven. The results showed that the effects of adding 0.06% of pistachio hull was similar to those of 0.02% butylated hydroxytoluene and butylated hydroxy anisole.

Hamedani and Haddad-Khodaparast (7) compared the thermal stability of pistachio oil with that of Kojjuk and virgin olive oil at 170°C for 8 hours. In the end, considering the low peroxide value and the high oxidation index of olive oil

compared to olive, it was concluded that Kojjuk oil had higher oxidative stability. This conclusion can be related to its high antioxidant content, especially tocopherols.

Oxidation results in a change in the refractive index of the oil, so the presence of keto and oxy groups increases the refractive index. In contrast, the refractive index correlates directly with the level of unsaturation in the oil, and by the continuance of oxidation, the amount of unsaturated fatty acids can be reduced in the oil (5). According to Keras-Rogers *et al.* (8), the refractive index increases with an increase in polymerization reactions, the saturation of carbon dioxide double bonds, and the turbidity of the oil.

Chandraskarma and Shahidi (9) studied the effects of roasting on the phenolic and antioxidant compounds of the kernel and testa. The results showed that the antioxidant activity and total phenolic compounds of the kernel and testa increased at high temperatures and low roasting times, but the pro-anthocyanin concentrations decreased.

The third skin of pistachios (the red skin on the kernel) is also a waste of the green kernel production process; the study of these sources shows that no research has yet been conducted on the compounds and their effects on oxidative stability. In this research, the effects of the addition of pistachio hull and testa to the pistachio butter formula have been investigated on the oxidative stability of the product.

2. Materials and Methods

Pistachio butter used in this research was provided from the Pistachio Research Center with the mean fat content of 52%. It was stored in a refrigerator until the process was carried out in a plastic container. To prepare the soft hull, fresh

pistachios from the Ohadi species were used, and their skins were separated manually in the laboratory. The moisture content of the soft hull was determined to be 0.3% after drying. The third skin of pistachios was prepared by soaking the pistachio kernel in hot water at 90°C for 7.5 minutes and then separating the skin from the kernel (10). The two skins were dried in an oven at 70°C for 3 hours and then milled in a laboratory mill for 1 minute to obtain a very soft powder (0.2 micron particles). Next, the mixtures of pistachio butter were prepared in different ratios of zero (control group), 0.5, and 1% of pistachio hull and testa (a total of 9 treatments) and milled for 3 hours in a ball mill (54 Ip model + V500A25, made in Iran). The samples prepared (with the moisture content of less than 1%) were placed in plastic containers in a laboratory at 25°C. The following tests were done at 0, 2, and 4 month intervals.

2.1. Measuring peroxide value

The measurement of the peroxide number was performed based on the method by Wrolstad et al. (11). Oil samples (1.0-2.0g ± 0.01) were weighed inside 250ml Erlenmeyer flasks and dissolved in 30ml of glacial acetic acid, in the chloroform (3:2 v/v) solution being stirred, followed by the addition of 0.5ml of the saturated potassium iodide solution (KI). The flasks were placed in the dark for 1 minute and then mixed with 30ml of deionized water. The mixture was titrated against a standardized solution of 0.01 N sodium thiosulphate (Na₂S₂O₃), while being constantly shaken until the yellow color almost disappeared. Next, 0.5ml of the starch indicator solution (1% w/v) was added to the flasks, and titration was continued until the blue color of the mixture disappeared. Blank titrations were made against each sample titration. The peroxide value (mEq/kg) was calculated according to the following equation:

$$PV = (V_{\text{sample}} - V_{\text{blank}}) \times N \text{ Na}_2\text{S}_2\text{O}_3 \times 1000 / W \text{ Eq1}$$

Where, V= volume of sodium thiosulphate used (ml), N= normality of the thiosulphate solution, and W= weight of sample (g).

2.2. The determination of the oxidative stability using the rancimat method

The induction time (IT) was measured using a rancimat (Model 743, Metrohm Company, Swiss), which was used in this test with an air flow rate of 15 liters/hour and at 110°C (12).

2.3. The refractive index

The refractive index was determined at 30°C using the Abbe-waj refractometer, equipped with a Japanese temperature regulator (12).

2.4. Statistical analysis

The data were analyzed with 3 replications of the treatments based on a factorial experiment in a completely randomized design, using Minitab software version 16. To compare the means, the Tukey test was used at level 5.

3. Results

3.1 The peroxide value

The results showed that the peroxide values of all samples increased with an increase in the storage time (Table 1). In month 0 (freshly produced), the formation speed of the products was low, yet it increased at months 2 and 4. Hence, the peroxide value in the control sample amounted to 2.8 meq/kg, 9.5 meq/kg, and 10 meq/kg in months 0, 2, and 10, respectively.

In month 2, the peroxide value of the treatments with 0.5% of testa and 0.5% of hull did not differ significantly, while in month 4, the difference was significant, and the sample with 0.5% of hull had a lower peroxide value. The comparison of the samples with 1% of hull and 1% of testa showed that although in month 2 the peroxide values of these two samples were significantly different, following 4 months from the storage of pistachio butter, the difference was insignificant.

3.2. The refractive index test

The results showed that the refractive index of pistachio butter samples was within the range of 1.470-1.464 (Table 2). Based on the results, a significant difference was observed in the control sample in month 0 and month 4, which

resulted in an increase in polymerization reactions in month 4. In other treatments, no significant difference was observed between the refractive index of the samples at different storage times.

Table 1. The effects of the storage time and adding pistachio hull and testa on the peroxide value (meq/kg) of pistachio butter

Storage time (month)			
4	2	0	Treatment
10.0± 0.0 ^a	9.5± 0.1 ^{bc}	2.8± 0.0 ^m	Control
9.4± 0.1 ^{cd}	9.2± 0.1 ^d	2.8± 0.0 ^m	0.5% hull
8.3± 0.0 ^{de}	7.8± 0.1 ^{hi}	2.8± 0.0 ^m	1.0% hull
9.8± 0.0 ^{ab}	9.4± 0.1 ^{cd}	2.8± 0.0 ^m	0.5% testa
8.7± 0.1 ^d	8.3± 0.1 ^f	2.8± 0.0 ^m	1.0% testa
8.0± 0.1 ^{gh}	7.5± 0.1 ^{jk}	2.8± 0.0 ^m	0.5% hull, 0.5% testa
8.4± 0.1 ^{ef}	7.7± 0.1 ^{ij}	2.8± 0.0 ^m	0.5% hull, 1.0% testa
7.5± 0.0 ^{ij}	7.3± 0.0 ^{kl}	2.8± 0.0 ^m	1.0% hull, 0.5% testa
7.4± 0.0 ^k	7.0± 0.1 ^l	2.8± 0.0 ^m	1.0% hull, 1.0% testa

The averages of common letters in each column and row do not have a significant difference with each other (P<0.05)

Table 2. The effects of the storage time and the addition of pistachio hull and testa on the refractive index of pistachio butter

Storage time (month)			
4	2	0	Treatment
1.468± 0.0 ^{abc}	1.467± 0.0 ^{a-e}	1.464± 0.0 ^{e*}	Control
1.468± 0.0 ^{abc}	1.469± 0.0 ^{ab}	1.469± 0.0 ^{ab}	0.5% hull
1.468± 0.0 ^{abc}	1.468± 0.0 ^{abc}	1.468± 0.0 ^{abc}	1.0% hull
1.468± 0.0 ^{abc}	1.469± 0.0 ^{ab}	1.468± 0.0 ^{abc}	0.5% testa
1.468± 0.0 ^{abc}	1.471± 0.0 ^a	1.469± 0.0 ^{ab}	1.0% testa
1.467± 0.0 ^{abcde}	1.467± 0.0 ^{a-e}	1.468± 0.0 ^{abc}	0.5% hull, 0.5% testa
1.468± 0.0 ^{abc}	1.469± 0.0 ^{ab}	1.469± 0.0 ^{ab}	0.5% hull, 1.0% testa
1.467± 0.0 ^{abcde}	1.467± 0.0 ^{a-e}	1.469± 0.0 ^{ab}	1.0% hull, 0.5% testa
1.467± 0.0 ^{abcde}	1.467± 0.0 ^{a-e}	1.469± 0.0 ^{ab}	1.0% hull, 1.0% testa

*The averages of common letters in each column and row do not have a significant difference with each other (P<0.05)

3.3. The oxidation stability test

The rancimat test was done for the heat resistance evaluation of the 2 samples of pistachio butter, which had lower peroxide values than other samples, and the control sample. In this connection, the control treatments, including 1% of hull and 1% of

testa as well as 1% of hull and 0.5% of testa were evaluated in storage months 0, 2, 4 in pistachio butter. The results showed that an increase in the percentage of the pistachio skin increased the time of resistance to oxidation (Table 3).

Table 3. The effects of the storage time as well as the addition of pistachio hull and testa on the induction period (hr) of pistachio butter

Storage time (month)			
4	2	0	Treatment
16.52± 0.0 ⁱ	17.82± 0.0 ^h	21.53± 0.0 ^{g*}	Control
24.95 ± 0.0 ^f	26.11 ± 0.0 ^e	29.07 ± 0.0 ^d	1.0% hull, 0.5% testa
34.63 ± 0.0 ^c	35.73 ± 0.0 ^b	38.24 ± 0.0 ^a	1.0% hull, 1.0% testa

*The averages of common letters in each column and row do not have a significant difference with each other (P<0.05)

4. Discussion

Antioxidants can increase the shelf life of food products by delaying the process of lipid oxidation, which is the major reason for the deterioration of oily products during the storage and processing phases. Restrictions imposed on the use of synthetic antioxidants, such as butylated hydroxy anisole (BHA) and butylated hydroxytoluene (BHT) are because of their carcinogenic properties (Goli et al., 2005). Therefore, it is required that other safe and natural sources of food antioxidants be identified. The use of natural plant antioxidants has increased in recent years. The addition of polyphenols to food products is due to their functioning as free radical scavengers and antioxidant properties.

4.1. The peroxide value

The increase in the level of peroxides is due to the formation of oxidation products, i.e.

hydroxides (13). As the results demonstrate, the efficacy of the hull and testa skin in controlling oxidation depends on the percentage of the skin used in pistachio butter formula. Although there was no significant difference among the samples in month 0, over time, pistachio butter samples containing a higher percentage of soft skin and pistachios' third skin showed higher oxidative stability.

The lowest peroxide value was related to the sample containing 1% of hull and 1% of testa, being indicative of the oxidative stability of the sample.

Based on the study results, the combination of hull and testa has been more effective in preventing an increase in the peroxide value. It seems that the low peroxide value in this treatment has been due to the higher percentage of phenolic compounds in the combination of pistachio hull and testa (14). Tavakoli et al. (2) reported that phenolic

compounds, including tocopherols are from among the most important antioxidant compounds. It was also reported that there was a direct correlation between oxidative stability and the sensory properties of edible oils and phenolic compounds. Emami et al. (15) attributed the lower value of the peroxide in the butter samples containing walnut and hazelnut kernel than in the control sample to the presence of antioxidant compounds, such as tocopherols, phytosterols, phenolic compounds, and squalene in walnuts and hazelnuts.

Goli et al. reported that the pistachio hull extract was effective in delaying the oil deterioration process at 60°C, with the activity increasing at the concentrations within the range of 0.02–0.06%. At the concentration of 0.06%, the pistachio hull extract was similar in its activity to the activity of BHA and BHT added at the concentration of 0.02%. Therefore, it is clear that pistachio hull, which is often considered as an agricultural waste, contains antioxidants that may be extracted usefully and added to foods.

4.2. The refractive index test

Although the addition of pistachios' soft skin and testa reduced the pistachio butter refractive index, the type of treatment did not have any effect on the refractive index. This conclusion is consistent with the results of the study by Fayyazi Dastgerdi et al. (5).

4.3. The oxidation stability test

The results showed that with an increase in the pistachio skin, due to an increase in the amount of phenolic compounds and other

antioxidant compounds in pistachio nuts, the stability time of pistachio butter increased. Hence, the control sample had the lowest and the pistachio butter containing 1% of hull and 1% of testa had the highest oxidative stability time. These results are consistent with the research by Kemalirousta et al. (16). Despite the fact that 87.85% of fatty acids in pistachios are unsaturated (17), the presence of antioxidants in the pistachio skin had a significant effect on the resistance of the samples to oxidation. The results of the study by Sedaghat et al. (18) on the shelf life of pistachios using the rancimat method showed that the effects of the storage time on the induction time was significant.

5. Conclusions

Pistachio hull and testa contain a significant amount of phenolic and antioxidant compounds, with these compounds having high antioxidant properties. By adding pistachio hull and testa to the pistachio butter formula, the effects of the delayed oxidative degradation of pistachio butter decreased. The treatment containing 1% of hull and 1% of testa showed the highest antioxidant properties.

Conflict of interest

The authors declare no conflict of interest.

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