

Effects of Pistachio By-Product Polyphenols on Oxidative Stability And α -Linolenic Acid Retention in Extruded Linseed During Long Term Storage

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ABSTRACT

The current study was conducted to determine the effects of pistachio by-product (PBP) polyphenols, on oxidative stability and α -linolenic acid retention in extruded linseed during long term storage. In a completely randomized design, linseed was mixed with PBP and alfalfa hay in 80:10:10 ratios and extruded with (ELINT) or without (ELIN) tert-butylhydroquinone as a synthetic antioxidant. All quality parameters, were similar between treatments ($P < 0.05$). The peroxide value (PV) of treatments after the extrusion process was higher in ELIN treatment ($P > 0.05$). After extrusion, oleic, linoleic, and linolenic fatty acids were higher in ELINT treatment, and the ELIN group had higher palmitic and stearic acid content ($P > 0.05$). Fatty acid composition of treatments changed during storage period ($P > 0.05$), and by increasing the storage period, the α -linolenic acid content of treatment decreased ($P > 0.05$). Moreover, as the storage period increased, the PV of treatments also increased ($P > 0.05$). The α -linolenic acid retention of treatments was different between treatments during the storage ($P > 0.05$). As storage increased, the level of α -linolenic acid retention decreased, and this decrease in the ELIN treatment was higher than the ELINT treatment ($P > 0.05$). In conclusion, phenolic contents of PBP in a 10% extruded linseed, could not be useful as a natural antioxidant for the stability of extruded linseed during the extrusion process and storage period.

Introduction

Pistachio by-products are produced during the dehulling of the pistachio nuts and contain 64.5% soft external hull, 25% twigs, 10% leaves, and 0.5% kernel and bony shells [1,2]. Like pistachio nut and pistachio skin [3], pistachio hulls [4,5] and leaves [6] are good sources of natural phenolic and antioxidants. Phenolic contents of PBP include 7.5%-13.7% phenol and 3.5%-10% (% of dry matter) tannins [7-9] by the addition of phenolic compounds to corn grain, before the extrusion process showed corn oil stability for oxidation during extrusion, significantly increased. Moreover, [10] resulted that vegetative simple phenolic compounds can use as a natural antioxidants during the extrusion process of oat grains. Nowadays, there is an excellent interest in finding new and safe antioxidants from natural sources [11]. There are serious concerns about synthetic antioxidants, given their carcinogenic potential and link to liver damage in humans [12]. However, to date, there is

not enough information about the antioxidant activity of pistachio by-product poly-phenols as use during the extrusion process of oilseeds.

In another side, in the last decade, due to increasing consumer information about a healthier animal products, there is more interested in consuming a dairy products with less deleterious saturated FA and trans-FA [13]. The simplest way to modify milk fat composition is to supplement dairy cow diets with unsaturated lipids [14], such as linseed. Among oilseeds, linseed (*Linum usitatissimum*) has the highest proportion of linolenic acid, averaging 18% of total seed weight and constituting 53% of total FA [15]. Altering the physical structure of oilseeds through heat treatment, like to extrusion processing technology, may help to protect the dietary FA of oilseeds from ruminal biohydrogenation [16,17]. However, there are some inherent problems in the

extrusion process of an oilseed-based feed ingredient. In fact, due to the extrusion process of linseed, the rapid release of intracellular oil may lead to considerable oil loss [18,19]. Besides, the potential production of oxidized compounds and reduced shelf life due to the high ALA content of linseed is also of concern [20]. So, using an appropriate binder (absorbent) material for the production of extruded linseed can be an excellent preventative resolution to reduce oil losses [21,22] and oil oxidation during extrusion. Hence, high natural antioxidants of pistachio hull can make this suitable potential oil binder a good substitute for synthetic antioxidants [23] for the stability of linseed oil during the extrusion process and storage period. Also, alfalfa hay can be an absorbent with functional fat absorption capacity for producing extruded linseed [24]. Therefore, the objectives of this study were to produce an extruded linseed mixed with PBP and alfalfa hay product, and the evaluation of these products for quality parameters, oxidative stability, and FA profile changes during long term storage.

Materials and Methods

Products

Linseed was purchased from a local market (Mashhad, Iran). Pistachio by-product and alfalfa hay were used as absorbent materials for producing extruded linseed. Also, tert-butylhydroquinone (TBHQ) was added as a synthetic phenolic antioxidant to linseed in one treatment mixed with propylene glycol in a 1:3 ratio (TBHQ: propylene glycol). Therefore, the experimental treatments were extruded linseed without TBHQ (ELIN); and extruded linseed with 1000 mg TBHQ/kg linseed (ELINT). Before extrusion processing, at first PBP and alfalfa hay were ground to pass through a 2-mm screen. For treatment with antioxidant, linseed was mixed with TBHQ, and in the following step, a mix of linseed: PBP: alfalfa hay (by mass) (80:10:10 ratio) was prepared. All treatments were triplicated.

Extrusion Process

Extrusion was performed in a double screw extruder (DS56-III, Jinan Saixin Machinery Co., Shandong, China), consisting of three independent zones of controlled temperature in the barrel. The temperature profiles in the first and second zones were kept constant at 70 and 80 °C, respectively, and the die head temperature was about 110 °C. The extruded materials were cut with a die face cutter as they left the extrusion die. After stable conditions were established, about 700 g of extruded product was collected and dried in an air oven at 40 °C for 24 h. Extruded material was stored at 4 °C in plastic bags for further analysis.

Analysis of Quality Parameters

Extrusion effectiveness (EE) is the ability of the extruder to grind whole seeds, measured by comparing the number of whole grains in a sample before extrusion and the number of entire grains remaining after extrusion. In this study, EE was determined using

the technique of Eggie (2010). Oil retention (OR) was measured using the method of Eggie (2010). Water holding capacity (WHC) was determined using a modified version of the AACC 56-30.01 method (AACC International, 1999) as amended by Eggie (2010). Bulk density (BD) was determined using a modified version of ASAE Standard S269.4 DEC 01 (ASAE, 2007), as amended by Eggie (2010). The angle of repose (AR) was measured using the established method for the Carr Angle of Repose (Carr, 1965) and described by ASTM D6393-99 (ASTM, 2006). According to Carr (1965), low angles of 30° to 40° indicate a material with relatively easy-flowing characteristics, while high angles of 50° to 60° represent severe flow conditions [25].

Phenolic Content of Pistachio By-Product

For tannin assay, PBP was dried in an oven at 40 °C to constant weight to minimize changes in tannin content and activity [26]. Dried samples were ground to pass a 2 mm and then 0.5 mm sieve. Samples (200 mg) were extracted in four replicates in 70%, (v/v) aqueous acetone overnight at 4 °C. The extracts were centrifuged at 3000 g at 4 °C for 15 min, and the supernatant was obtained and used in the subsequent assay. The concentration of total phenolic (TP) compounds was determined using the Folin-Ciocalteu procedure [27] and the regression equation of tannic acid (Merck GmbH, Darmstadt, Germany) standard. Total tannins (TT) were estimated indirectly after being absorbed into insoluble polyvinylpyrrolidone (PVPP). The concentration of TT was calculated by subtracting the TP remaining after the PVPP treatment in the assay mixture (Table 1).

Table 1: Fatty acid composition of linseed and pistachio by-products and phenolic contents of pistachio by-product.

Fatty acid content (g/100 g FA)	Linseed	Pistachio by-products
C12:0	-	0.03
C14:0	0.038	1.47
C16:0	5.02	12.34
C16:1	0.048	0.94
C17:0	0.06	0.17
C17:1	0.04	0.08
C18:0	4.27	2.22
C18:1 cis-9	20.2	48.8
C18:2 cis-9, 12	17.11	26.93
C18:3 cis-9, 12, 15	52.83	0.15
C20:0	0.13	4.72
C20:1	0.12	0.63
C22:0	0.09	0.61
C24:0	0.06	0.61
Phenolic contents (% DM)		
Total phenolics	-	9.95
Simple phenolics	-	3.26
Total tannins	-	6.67

Lipid Peroxidation and Fatty Acid Profile

Extruded materials were stored in plastic bags and kept in ambient temperature for 90 days. The stored samples were analyzed for peroxide value (PV) and FA profile on days 0, 10, 20, 30, 60, and 90 after extrusion. Each time, oil was extracted from samples. Also, the FA composition of PBP we analyzed. For oil extraction, dried samples were ground to powder in a grinder (Model 1093; sieve size, 1mm). The powders were extracted with n-hexane (1:4 wt/vol) by agitation in a dark place at ambient temperature for 48 h. The solvent was evaporated in vacuo at 40 °C to dryness. Peroxide value was assessed by colorimetric determination of iron-thiocyanate according to Shantha and Decker. The fatty acid profile was determined by gas chromatography. Fatty acid methyl esters (FAME) were prepared, according to Wijngaarden. A fused silica capillary column (WCOT Fused Silica Capillary, DANI, Model 1000, Rome, Italy) 120 m in length, 0.32 mm internal diameter, and 0.2 µm film thicknesses on an HP 6890 GC equipped with flame ionization detector was used to quantify FAMES. The initial column temperature was set at 180 °C for 20 min, which was increased to 225 °C by increments of 5 °C/min, then to 250 °C by 10 °C/min and held for 12 min. Hydrogen was used as carrier gas with a flow of 1.7 ml/min for the first 10 min. Then, the flow was decreased to 1.3 ml/min, and maintained until the end of the analysis. The detector temperature was set at 300 °C. Identification of FA was performed by comparison with the retention times of FAMES standards (Sigma-Aldrich, Catalog #18919). Separations of all FAME were obtained with a single chromatographic run.

α-Linolenic Acid Retention

The retention of ALA in extruded linseed product was calculated according to expression is given below (Imran et al., 2013):

$$\alpha\text{-linolenic acid retention, \%} = \left(\frac{\text{the content of ALA after extrusion}}{\text{the content of ALA before extrusion}} \right) \times 100$$

Statistical Analysis

Data on quality parameters, PV, and FA changes were analyzed in a completely randomized design, with two treatments and four replicates using the general linear model procedure of SAS (2003).

The statistical model was:

$$y_{ijk} = \mu + T_i + e_{ij}$$

Where y_{ijk} is the observation, μ is the overall mean; T_i is the effect of treatments, and; e_{ij} is the residual error.

Data of PV and FA changes of treatments during the storage period were analyzed as a repeated measures approach using ANOVA with mixed linear models of SAS (2003). The Duncan's t-test compared the means at a 0.05 probability level.

Results and Discussion

There were no differences between treatments for quality parameters (Table 2), and all factors were the same in all treatments ($P < 0.05$).

Peroxide value evaluation of treatments after the extrusion process (Table 3) demonstrated that ELINT treatment had lower PV than the ELIN group ($P < 0.05$). A PV of less than five cannot be rancid for cattle and probably handled fat should not exceed a PV of 10 [28,29] showed that pistachio hulls, which at present are often considered as agricultural waste, contain an antioxidant that may usefully be extracted and added to foods. However, phenolic contents of PBP in a 10% extruded linseed mixture (by mass) could not be useful as a natural antioxidant for the stability of linseed during an extrusion process. [30] confirmed that the high-temperature extrusion process does not deactivate phenolic antioxidant compounds. So, disability of this content of phenolic prepared from PBP as a natural antioxidant can be for two possible reasons;

Table 2: Quality parameters of extruded linseed with or without antioxidants.

Extruded linseed*				
	ELIN	ELINT	SEM†	P-Value
EE‡ (%)	99.44	99.56	0.031	<0.0001
OL (g)	0.071	0.069	0.003	0.0031
WHC§	6.98	6.59	0.133	<0.0001
BD (g.cm-3)	0.72	0.74	0.006	<0.0001
AR (°)	46.68	43.11	-	<0.0001

*ELIN, extruded linseed without antioxidant; ELINT, extruded linseed with TBHQ antioxidant.

†SEM, standard error of the mean;

‡E, Extrusion effectiveness; OL, Oil lost; WHC, Water holding capacity; BD, Bulk density and AR, Angle of repose.

§Measured in g H₂O per gram dry matter.

Table 3: Peroxide value of extruded linseed with or without antioxidant after the extrusion process.

Treatments*				
	ELIN	ELINT	SEM†	P-Value
Peroxide value (meq O ₂ /kg oil)	12.72 ^a	4.63 ^b	1.128	<0.0001

*ELIN, extruded linseed without antioxidant; ELINT, extruded linseed with TBHQ antioxidant.

†SEM, standard error of the mean;

a, b: Within a row, means value with typical letter are not different ($P < 0.05$).

a) the quantity of phenolic which prepared was low to act as an antioxidant in the extrusion process

b) the phenolic compounds of PBP did not release during extrusion process, so could not affect as antioxidant. Because most study which confirmed the antioxidant activity of plant phenolic, extracted the phenolic contents from unusual plants and used them as an aqueous solution containing phenolic compounds, but in our research, we used PBP as a feedstuff include phenolic.

Camire and Dougherty (1998) demonstrated that the addition of phenolic compounds increased lipid stability in extruded corn. Moreover, [31] indicated that simple phenolic compounds could act as antioxidants for extruded oat cereals. The fatty acid composition of extruded linseed (Table 4) differed in treatments with or without TBHQ (P<0.05). The content of C16:0 and C18:0 for ELIN treatment were higher than for ELINT treatment (P<0.05), but ELINT treatment had higher content of C18:1-c9, C18:2-c9, c12 and C18:3-c9, c12, c18 than the ELIN group (P<0.05). (Table 5) It suggests that oxidation of the unsaturated FAs during the extrusion process in ELIN was higher than ELINT. Eggie (2010) used a synthetic antioxidant for extrusion process of linseed mixture with different absorbents (alfalfa, soy hulls, and corn gluten). It demonstrated that there was no significant change in the proportion of individual FA content in any products of extruded linseed, between before and after extrusion analysis. Peroxide value measurement of different treatments during the storage period indicated (Table 6) that treatment without TBHQ had higher PV than ELINT treatment (P<0.05). [32-35] Also, the time of storage had a significant effect on PV (P<0.05), and there was a significant interaction between treatment and time of storage for PV (P<0.05). As storage increased, the level of PV increased, and this increase was higher for ELIN treatment than for ELINT treatment. Also, the FA composition of extruded linseed between treatments (Table 7) during the storage period was different (P<0.05). Also, there was a significant effect for time of storage on the FA proportion (P<0.05). Furthermore, the interaction between treatment and time of

storage on the FA proportion was significant (P<0.05). As storage increased, the percentage of unsaturated FA decreased (P<0.05), and this reducing was higher in ELIN treatment compared to ELINT (P<0.05).

Table 4: Fatty acid composition of extruded linseed with or without antioxidant after the extrusion process.

Treatments*			
FA(g /100g FA)	ELIN	ELINT	SEM†
C16:0	6.59a	5.37b	0.025
C18:0	3.67a	3.53b	0.027
C18:1 cis-9	20.47b	20.83a	0.021
C18:2 cis-9, 12	16.39b	16.53a	0.032
C18:3 cis-9, 12, 15	51.68b	52.14a	0.036

*ELIN, extruded linseed without antioxidant; ELINT, extruded linseed with TBHQ antioxidant.

†SEM, standard error of the mean;

a, b: Within a row, means value with typical letter are not different (P<0.05).

Table 5: α-linolenic acid retention of extruded linseed with or without antioxidant after the extrusion process.

Treatments*			
	ELIN	ELINT	SEM†
α-linolenic acid retention (%)	97.82	98.69	0.152

*ELIN, extruded linseed without antioxidant; ELINT, extruded linseed with TBHQ antioxidant.

†SEM, standard error of the mean;

Table 6: Peroxide value of extruded linseed with or without antioxidant during the storage period.

Treatments*													SEM†		P-value	
ELIN: Storage Day							ELINT: Storage Day						Treat	Time	Treat×Time	
	0	10	20	30	60	90	0	10	20	30	60	90				
‡Peroxid value	12.72	16.67	19.36	29.68	32.02	41.55	4.63	5.02	5.21	5.61	6.53	8.01	1.64	0.013	<0.0001	

*ELIN, extruded linseed without antioxidant; ELINT, extruded linseed with TBHQ antioxidant.

†SEM, standard error of the mean.

‡meq O2/kg oil.

Table 7: Fatty acid composition of extruded linseed with or without antioxidant during the storage period.

Treatments*													SEM†		P-value	
ELINT: Storage Day							ELINT: Storage Day						Treat	Time	Treat×Time	
FA(g /100g FA)		10	20	30	60	90	0	10	20	30	60	90				
C16:0	5.28	6.53	5.26	4.92	6.21	6.59	5.37	6.27	6.93	5.26	6.5	5.23	0.022	0.011	<0.0001	
C18:0	3.69	3.83	3.71	3.89	3.96	3.67	3.53	3.52	3.34	3.59	3.19	3.68	0.037	0.026	0.001	
C18:1 cis-9	20.71	20.82	21.03	20.81	20.51	20.47	20.83	20.81	20.56	21.18	20.21	21.45	0.021	0.052	<0.0001	
C18:2 cis-9, 12	16.91	16.62	17	16.97	16.94	16.34	16.53	16.26	16.21	16.45	16.09	16.85	0.027	0.004	<0.0001	
C18:3 cis-9, 12, 15	52.36	51.75	51.48	50.79	50.41	50.26	52.57	52.25	52.17	52.28	52.56	51.71	0.079	0.012	<0.0001	

*ELIN, extruded linseed without antioxidant; ELINT, extruded linseed with TBHQ antioxidant.

†SEM, standard error of the mean.

The α -linolenic acid is associated with vegetable oil instability [36]. Eggie (2010) reported that the proportions of FAs of extruded linseed with different absorbents (alfalfa, soy hulls, and corn gluten) were affected by the storage period, and it is logical that the concentration of FAs is affected. It is essential to realize that antioxidants are not capable of preventing oxidation, just delaying it [37-40]. (Table 8) demonstrated that milled linseed was stored up to 4 months at ambient temperatures without noticeable

changes in quality. Furthermore, [41-44] reported ground linseed was stable for 280 d when stored at room temperature and in a 12-h light/dark cycle. It seems that extrusion temperature is a more important factor for the stability of unsaturated fatty acids during the extrusion process [45-47]. indicated that extrusion of the semolina-linseed mixture in 45 °C for use in human food was not affected on the stability of triacylglycerol and ALA in the final product.

Table 8: α -linolenic acid retention of extruded linseed with or without antioxidants during the storage period.

	Treatments*											
	ELIN: Storage Day						ELINT: Storage Day					
	0	10	20	30	60	90	0	10	20	30	60	90
‡ALA retention	98.95	97.92	97.44	96.13	95.41	95.13	99.51	98.91	98.74	98.94	99.48	97.85

*ELIN, extruded linseed without antioxidant; ELINT, extruded linseed with TBHQ antioxidant.

†SEM, standard error of the mean.

‡ α -linolenic acid retention (%).

Conclusion

Phenolic compounds of pistachio by-product in 10% extruded linseed were not useful as a natural antioxidant for the stability of extruded linseed during the extrusion process and storage period. The peroxide value of ELIN treatment was higher than the ELINT group after the extrusion process and during the storage period. During the storage period, as storage increased, the level of PV increased. However, α -Linolenic acid retention in extruded linseed was not different between treatments after the extrusion process. Still, it decreased as increasing of storage period, and this decrease in the ELIN treatment was higher than the ELINT group.

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