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Research Article

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The Effect of Pistachio Green Hull Extract on the Phenolic Compounds, Peroxide Value and Carbonyl Value of Salmon Oil Emulsion in Water

Ahmad Shakerardekani[1*], Sara Banihashemi[2], Elham Taghavi[3*] and Afsaneh Morshedi[4*]

[1]*Pistachio Research Center, Horticultural Sciences Research Institute, Agricultural Research Education and Extension Organization (AREEO), Rafsanjan, Iran*

[2]*Pistachio Safety Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*

[3]*Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, Kuala Nerus, Terengganu, Malaysia*

[4]*Facultad de Ciencias Gastronomicas y Turismo, Universidad UTE, Quito, Ecuador*

[*]Correspondence to:
Ahmad Shakerardekani
Pistachio Research Center,
Horticultural Sciences Research Institute,
Agricultural Research Education and Extension Organization (AREEO),
Rafsanjan, Iran.
E-mail: shaker@areeo.ac.ir

Elham Taghavi
Faculty of Fisheries and Food Science,
Universiti Malaysia Terengganu,
Kuala Nerus, Terengganu, Malaysia.
E-mail: elham.taghavi@umt.edu.my

Afsaneh Morshedi
Facultad de Ciencias Gastronómicas y Turismo,
Universidad UTE,
Quito, Ecuador.
E-mail: Afsaneh.morshedi@ute.edu.ec

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Abstract

In this research, the extract of pistachio hull was used to delay the oxidation of fish oil emulsion in water. Total phenolic compounds, peroxide value and carbonyl value of salmon oil was measured for 3 months storage. The total phenolic content of methanolic extract was 11.7 mg/g and the main components of methanolic extract included gallic acid, 4-hydroxybenzoic acid, protocatechuic acid and naringenin. In ethanolic extract, almost the same result was obtained. The lowest and highest peroxide value (0.6 and 51.3 meq/kg) and carbonyl number (0.007 and 0.41 μmol/g oil) were observed on day 0 and 90, respectively. Pistachio green hull extract delayed the oxidation rate and confirmed the antioxidant properties of phenolic compounds in the pistachio hull.

Keywords

Oily fish, Peroxide value, Carbonyl number, Extraction, Phenolic compound

Introduction

Recently, extensive research has been done on the oxidation of oil emulsions in water. Factors affecting the oxidative properties of the emulsion include the chemical structure of the lipids, the interfacial properties and the droplet properties, and the reaction with the aqueous phase [1].

Fish body oil is mainly made up of triglycerides, a combination of glycerol and fatty acids, along with varying amounts of phospholipids and other constituents, and its pH is neutral. Fish oil fatty acids are not saturated, it stinks quickly and consequently, will have no nutritional value. Therefore, it must be stored and used properly and for a short time. Both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are major omega-3 acids. Omega-3 fat is prone to oxidation, which affects the sensory properties of the product, and the oil gets fishy taste and smell, having a negative effect on the expiration date. Fish oil is a good supplement for the treatment of arthritis, diabetes, atherosclerosis, cancer, neurological and mental disorders, and skin diseases [2]. Fish oil reduces the risk of coagulation, lowers blood pressure, and produces prostaglandins, a physiological regulator that prevents the buildup of fats in the artery wall [3]. In order to minimize the risk of heart attacks, it is necessary to tailor the consumption of fish oil to the daily need of our body [4].

Antioxidants are compounds added, especially to foods, to reduce the rate of spoilage in these products. In recent years, the tendency to add natural polyphenols to biological and food systems has increased due to the free radical scav-

enging property of polyphenolic compounds. The extract of pistachio green hull has been considered as one of the sources of natural antioxidants [5].

The main by-product of pistachio is green pistachio hull. Due to the enormous amount of this product, it can be used in food and cosmetic products [6, 7]. Pistachio hull is a rich source of phenolic and flavonoid compounds; hence, it has beneficial antioxidant, and antimicrobial properties [5]. Hosseinzadeh et al. [8] studied the antioxidant properties of aqueous and ethanolic extracts of pistachio leaves and fruits. All extracts showed antioxidant properties but the performance of ethanolic extracts was better than the aqueous extracts [8]. Tsantili et al. [9] investigated the effects of cultivar, drying and storage conditions on total phenolic content, total flavonoid content and total antioxidant capacity of pistachios nuts. The results indicated that cultivar, temperature and packaging atmosphere affect the mentioned factors [9]. Rajaei et al. [10] investigated total phenolic content and antioxidant activities of methanolic extract of pistachio hull. Concentration-dependent antioxidative capacity was verified for all cultivars. The results showed that pistachio green hull can be used as a cheap and easily accessible source of natural bioactive compounds [10].

Since the consumption of industrial compounds as antioxidants in food products causes various problems and considering the importance of pistachios as good sources of polyphenolic compounds, this study was conducted to use pistachio hull extract as a natural antioxidant in salmon oil (one of the most perishable oils) in order to evaluate the quality parameters of this compound for 3 months storage.

Material and Methods

Raw materials

The Akbari pistachio variety was collected from the fruits of pistachio trees in Rafsanjan (Kerman, Iran) and stored at 4 ± 1 °C until the test was conducted.

Extraction

The extraction was carried out according to Barreca et al. [11]. The frozen hulls (at -20 °C) were ground to a powder with a frozen mortar and, the samples (3.0 g each) were extracted at 20 °C under continuous stirring for 12 h, with methanol or ethanol solvent. The samples were then centrifugated at 1900 g for 15 min and the supernatants were filtered with filter paper and evaporated to dryness in a rotavapor. This procedure was repeated three times.

Emulsion preparation

Fish oil was extracted by separating adipose tissue manually and the process was completed by adding ether. The aqueous phase was prepared by dissolving potassium sorbate, sodium benzoate, citric acid and 0.05% extract of green pistachio hull [12] in water and gradually adding 3% Persian gum and stirring it for 5 min. At this stage, after 8 h of hydration, the oil phase containing 5% fish oil was added to the above mixture and homogenization was done at a pressure of 25 MPa for 1 min.

Test methods

Phenolic and flavonoid compounds

Phenolic compounds in methanolic and ethanolic extracts of pistachio green hull (in micrograms per gram of dry matter) were examined using gas chromatography based on Farhoosh et al. [13] method. Total phenolic content was measured using Folin reagent. A calibration curve of gallic acid in methanol was performed in the concentration range 0.04 - 0.40 mg/ml. To draw the standard curve, 1 ml of a standard solution of gallic acid, 6 ml of methanol, 2.5 ml of the Folin reagent, and 5 ml of 7.5% Na_2CO_3 were added. The absorbance was read at 765 nm. For the sample solution, 2.5 g of oil was diluted with 2.5 ml of *n*-hexane and centrifuged three times for 5 min at 5000 rpm with chloroform: water (CH_3OH:H_2O) at a ratio of 80:20 (v/v). Finally, 2.5 ml of Folin reagent, 5 ml of 7.5% Na_2CO_3 was added, reaching a volume of 50 ml. The rest of the process was similar to the standard sample [13].

Total flavonoid was determined according to the procedure devised by Barreca et al. [11] with some modifications. Absorbance at 440 nm was recorded. All determinations were carried out in triplicate. Flavonoid content is expressed as mg of quercetin equivalents (QE) per fresh weight sample (g).

Peroxide value

The oil peroxide value of the samples was measured on days 0, 30, 60, and 90. The oil sample (0.3 g) was added to 9.8 ml chloroform-methanol in a ratio of 7:3 v/v and mixed for 2 - 4 s. Ammonium thiocyanate solution (50 ml, 30% w/v), was added to the solution and mixed again for 2 - 4 s. Then, 50 ml of iron (II) chloride solution was added and mixed on a vortex mixer for 2 - 4 s. After 5 min incubation at room temperature, the absorbance of the sample was read at 500 nm in a spectrophotometer. The results were expressed in milli-equivalents of oxygen per kg of oil [13].

Carbonyl value

The carbonyl value of oil samples was measured on days 0, 30, 60, and 90. To remove any trace of carbonyl compounds in the solvent, 0.5 g of sodium borohydride was added to 1000 g of 2-propanol. Then it underwent the distillation process. Oil sample (1.0 g) of was introduced into the flask and the flask was filled to the mark with the solvent containing triphenylphosphine (0.4 mg/ml) to remove peroxide. Standard 2,4-decadienal was prepared at concentrations of 50 - 500 µmol in the solvent. 1 ml of standard or sample was introduced into a 15 ml test tube, and then mixed with 1 ml of DNPH solution. The mixture underwent heating for 20 min at 40 °C. It was cooled in water, and then 8 ml of 2% KOH solution was added. The test tube was centrifuged at 2000 g for 5 min. The absorbance of the upper layer was measured at 420 nm and the results were expressed in µmol of 4,2-decadhanal per gram of oil [13].

Statistical analysis

Data analysis was performed in a completely randomized design. Significant differences between means were determined by Duncan's multiple range tests. The data analyses were carried out in triplicates using SPSS 11.5.

Results and Discussion

Total phenolic and flavonoid contents

The total phenolic and flavonoid contents in the methanolic extract (11.7 ± 0.48 and 0.68 ± 0.01 mg/g) were higher than the total phenolic and flavonoid contents in ethanolic extract (6.74 ± 0.42 and 0.34 ± 0.01 mg/g). The presence of these compounds in pistachio hull extract is the cause of anti-radical and antioxidant properties of this part of the plant [5]. In a study conducted by Rajaei et al. [14], the amount of phenolic content varied from 15.3 to 31.1 (mg of phenol per gram of dry sample) and Ahmad Aghaei cultivar had the highest amount of phenolic compounds.

According to Arcan and Yemenicioğlu [15], phenolic content of pistachio kernels was 4.61 mg/g, which is far less than the phenols in pistachio hull reported in the current research. The main differences between the extracts are the presence/absence of quercetine, kaempferol and apigenin and their amounts (Table 1). The major constituents of methanolic extract were gallic acid, 4-hydroxybenzoic acid, protocatechuic acid and naringenin, almost the same result that was seen in ethanolic extract. The medicinal and biological properties of many of these compounds have been studied and properties such as anti-cancer, antioxidant, antibacterial, anti-inflammatory, anti-viral, anti-hepatitis and anti-aging have been identified [11].

Peroxide value

The peroxide value increased for 90 days (Figure 1). The lowest peroxide value (0.6 ± 0.03) was observed on the first day and the highest amount was observed on day 90. The rate of peroxide value changes in the control sample and the sample containing pistachio hull extract was the same and a significant difference was observed between the results of the sample containing pistachio hull extract and the control sample from day 30 onwards. Fish oil is sensitive to oxidation due to the high amount of unsaturated fatty acids and according to the results obtained, the increase in peroxide value was significant over time ($p \leq 0.05$). The results of this study were consistent with the results of Rezaei et al. [16] who studied the impact of walnut husk on soybean oil [16]. Hosseinzadeh et al. [8] studied the antioxidant properties of aqueous and ethanolic extracts of pistachio leaves and fruits. Both extracts showed good antioxidant properties and were effective in reducing peroxide value and carbonyl value.

Carbonyl value

According to figure 2, the lowest amount of carbonyl was observed on the first day (0.007 µmol/g oil) and the highest amount was observed from day 90 (0.41 µmol/g oil). No significant difference was observed between the samples on days 30 and 60; however, the amount of carbonyl value was significantly less than the control sample. Carbonyl compounds are the result of the decomposition of peroxides. Over time and

Table 1: Phenolic and flavonoid compounds in methanolic and ethanolic extracts of pistachio green hull (in micrograms per gram of dry matter).

Compounds	Post	R_t (min)	Wavelength (nm)	Methanolic extract	Ethanol extract
Phenolic acid	-	-	-	-	-
Hydroxy benzoic acid	-	-	-	-	-
Gallic acid	1	7.35	268	57.2 ± 3.86	14.5 ± 0.62
Protocatoic acid	2	18.45	293; 258	10.89 ± 1.2	3.82 ± 0.19
4-hydroxybenzoic acid	4	31.41	279	72.09 ± 2.74	3.97 ± 0.22
Vanillic acid	7	39.53	291; 269	4.46 ± 0.28	0.06 ± 0.01
Hydroxy cinnamic acid	-	-	-	-	-
Chlorogenic acid	6	38.2	319; 291	1.13 ± 0.06	0.2 ± 0.01
Flavonoids	-	-	-	-	-
Flavonol	-	-	-	-	-
Eriyo D cetiol-7-glycoside	8	57.56	283	73.29 ± 1.68	40.26 ± 2.4
Eriyo D cetiol	14	70.26	287	2.80 ± 0.05	0.72 ± 0.02
Naringinin	17	79.3	289	2.41 ± 0.23	0.2 ± 0.01
Naringin	11	64.37	284	9.73 ± 0.51	77.7 ± 0.44
Flavonol	-	-	-	-	-
Isorhamentin-3-glucoside	13	69.71	354; 254	26.74 ± 2.3	17.54 ± 2.33
Quercetin	16	78.11	370; 255	0.77 ± 0.01	-
Campfrol	19	84.34	365; 264	2.22 ± 0.13	-
Quercetin-3-rotinoside	12	65.59	354; 254	340.01 ± 8.31	137.25 ± 6.52
Quercetin-3-galactoside	10	64.34	354; 254	2.51 ± 0.06	1.14 ± 0.01
Isorhamentin-3-rotinoside	15	71.02	354; 254	42.74 ± 3.72	1.11 ± 0.08
Flavonol	-	-	-	-	-
Luteolin	18	80.84	370; 259	1.27 ± 0.06	0.47 ± 0.04
Apigenin	20	85.72	338; 268	0.11 ± 0.01	-
Flavonols	-	-	-	-	-
Catechin	3	24.13	279	60.64 ± 1.38	31.64 ± 4.53
Epicatechin	5	33.9	279	0.88 ± 0.05	0.37 ± 0.01

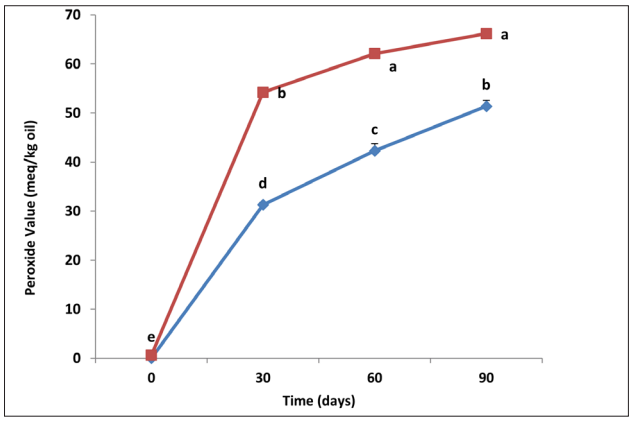


Figure 1: Changes in the value of fish oil peroxide value in the emulsion, the letters above the dots indicate a significant difference. Lozenge points: sample containing pistachio hull extract; Square points: control sample; and non-common letters have a significant difference at the 5% statistical level.

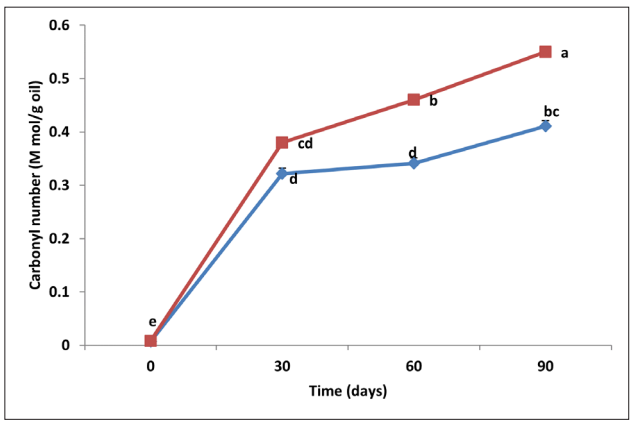


Figure 2: Changes in the carbonyl value of fish oil in the emulsion, the letters above the dots indicate a significant difference. Lozenge points: sample containing pistachio hull extract; Square points: control sample; and non-common letters have a significant difference at the 5% statistical level.

with an increase in the amount of initial oxidation compounds, the amount of carbonyl compounds increases, which is consistent with the results of this study. The results of this study were consistent with the results of Rezai et al. [16] who investigated the effect of walnut green husk on soybean oil for 16 days. Dehghan et al. [17] studied the effect of methanolic Bene hull extract on the physicochemical properties of soybean oil for 12 days and the results of measuring carbonyl value over time were consistent with the results of this research.

Conclusion

Antioxidant compounds are abundant in plants. The consumption of synthetic antioxidants has adverse effects on human health and brings about carcinogenic effects. As a result, there has been a growing tendency to use natural antioxidants.

In this research, in order to reduce the oxidation rate of oil, pistachio hull extract was added to the salmon oil. The results showed that pistachio green hull extract prevented the increase of peroxide value and carbonyl number, and as a result, it was effective in reducing the oxidation of salmon oil. Therefore, due to its high phenolic and flavonoid compounds, it is suggested that pistachio green hull extract is used as a natural antioxidant in foods or biological systems.

Acknowledgements

None.

Conflict of Interest

None.

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