

Evaluation of Physicochemical and Antioxidant Properties of *Pinus gerardiana* Nuts and Oil

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Abstract: For optimal usage of new oil resources with high nutritional values, in this study, one chilgoza pine variety was provided, and assays of physicochemical characteristics of its nut such as moisture, ash, thousand nut weight, oil, protein, hull contents, density, pH and also physicochemical characteristics of its extracted oil by n-hexan solvent such as iodine values, saponification and unsaponification, peroxide, specific weight, acid values, moisture of oil, color (Lovibond) and fatty acid profile and extracted oil characteristics by cold press such as total polyphenols, tocopherols, carotenoids, chlorophylls, sterols, color (Hunterlab), DPPH and antioxidant were investigated. Then its effect as a natural antioxidant on rapeseed oil shelf life (without any antioxidant) was studied. The results indicated that chilgoza pine oil is belonged to linoleic-oleic group. Oil of chilgoza pine has α and γ tocopherols and there is no β and δ tocopherols on it. The oil of chilgoza pine, were mixed at two levels of 2% and 5% with rapeseed oil and these mixed were determined by peroxide, TBA and Rancimat indices during 0, 5, 10, 20 and 35 days and compared with control. Statistical results showed no antioxidant effect in shelf life of rapeseed oil at more days. This means that the chilgoza pine oil antioxidant compounds were not enough to prevent oxidative rancidity of rapeseed oil or maybe the antioxidant compounds of nuts were not migrated to oil.

Keywords: Chilgoza pine oil; Physicochemical characteristics; Antioxidant assays; Rapeseed oil.

1. Introduction

Edible nut of the chilgoza pine plant is called chilgoza pine nut (*Pinus gerardiana* W. ex D.), which belong to the Pinaceae family. The first places were observed were near Pakistan, India, Afghanistan and China [1]. For first time it was discovered by Captain Gerard in the year of 1932 and commercially known as chilgoza/neoza pine [2]. The species have different calls in some areas such as Jhalgoza in Afghanistan, Chiri or Chilgoza in Kashmir, Rhi or Neoza in Kinnaur, Chujin in Chitral and Mirri in Pangi (Himachal Pradesh). They grow between 1,600 and 3,300 m above sea level and are abundant in Himachal Pradesh (a total area of about 2,060 ha) and most of the area in Kinnaur (2,040 ha) and a small portion in Chamba district (20 ha). These species can grow under extremely rough site condition and because of the withstand the extremes of cold climate and also aridity, they were described as the “Champion of Rocky Mountains” [3].

Reduction in the regeneration process in the natural habitats of chigoza pine tree is due to the rare and irregular years of the nuts. Chilgoza pine is a rich source of turpentine resins. Its nuts and oils have therapeutic properties; its nuts are useful to improve ulcers and stomach ulcers as they are as anodyne and stimulant and their oils are useful to improve ulcers and stomach ulcers. [3]. Also, the oil of this nut has a hypo-liposomal effect and lower blood cholesterol, and even has the potential to limit appetite and treatment for obesity [4].

Chilgoza pine is known as one of the important internationals commercially goods. It used as roasted nut or as an ingredient in desserts, sauces and salads [1]. Its kernels are rich in fats, proteins and carbohydrates with no cholesterol [5] and also rich in unsaturated fatty acids (especially polyunsaturated fatty acids), which have health benefits such as controlling the blood serum lipid profile (notably the decrease in undesirable low-density cholesterol, very low density lipoproteins and low density lipoproteins) [6].

Chilgoza pine nuts have been considered as an important source of plant proteins. The protein is a well-balanced composition of essential amino acids and is rich in arginine, which is related to many types of bioactivities, including antioxidant, vasodilatation and antiplatelet effects. It also contains a high amount of lysine, therefore these are useful to improve the essential amino acid content in grain-based diets [1].

In general, pine nuts contain approximately 50% fat, 30% proteins, 10% carbohydrate, 4% ash and 6% moisture. They are also rich in unsaturated fatty acids (UFAs). A previous study on a species of pine nuts, reported 51% linoleic acid, and 37% oleic acid, which were known to contain the highest levels of UFAs [7]. Pine nuts contain

high amounts of fats and unsaturated fatty acids, therefore they are prone to hydrophilic and oxidative rancidity, which leads to spoilage during storage [6].

The above contents indicate the importance of chilgoza pine nut and its oil in nutrition and also as a medicine in the treatment of named diseases. In Iran, no similar study has been done on this nut and its physicochemical characteristics and it has been paid very little attention on the global level. Therefore, in this research, the physicochemical characteristics of this oil and nut, as well as the study of the number of effective compounds, their classification to the oleic or linoleic acid group and the mode of antioxidant activity of the oil were investigated.

2. Materials and methods

Chilgoza pine nuts used in this study were supplied from a local market of Mashhad. All reagents used were of analytical grade and were obtained from Merck, Company (Darmstadt, Germany).

2.1 Preparing samples

At first, all the nuts were peeled off and their nuts were separated. Then, physicochemical tests were performed on its nuts. To determine the physicochemical properties of the oil, two types of methods were used as follow.

2.2 Analysis of chilgoza pine nut samples

The nuts were ground by using a mortar and pestle. Then moisture content, ash value, protein content, thousand nut weight, oil, protein, hull contents, density and pH also were determined according to AOCS methods [8].

2.3 Analysis of chilgoza pine nut oil

Physicochemical characteristics of chilgoza nut oil extracted by n-hexane solvent under vacuum at 55-60 °C, such as iodine (Hanus method), unsaponification [9], acidity, saponification, peroxide, TBA and Rancimat values refractive index [8] color (Lovibond) [10] were determined. Also, for investigation of oil antioxidant assays, chilgoza pine nut oil was extracted by cold press equipment (Bekrdaneh BDE65, Iran); Then physicochemical characteristics of chilgoza nut oil, such as total polyphenols [11], carotenoids and chlorophylls [10], color (Hunter lab) [10] contents, DPPH [12] and antioxidant activities were determined [13] and sterols and tocopherols were measured according to INSO 16324 [14] and INSO 7211 [15], respectively.

2.4 Analysis of fatty acid composition

In order to estimating fatty acid composition, which was done by gas chromatography (Unicom 4600, England), extracted oil by n-hexane solvent was used. Split injection, split ratio 1:10, injection temperature at 250 °C, flame ionization detector at 300 °C and Helium with 99.99% purity and 1 ml/min flow rate as carrier gas were device specifications. Fatty acid composition was determined through a fused silica capillary column (BPX70; SGE, Melbourne, Australia) 30 m × 0.22 mm with the film thickness of 0.25 μm. Initial column temperature was 110 °C for 5 minutes and was increased to 210 °C at the rate of 5 °C/min, and held at this temperature for 40 minutes [16].

2.5 Analysis of sterols contents

Sterol compositions were determined by gas chromatography (Young Lin Acme 6000, South Korea). Split injection, split ratio 1:50, injection temperature at 280 °C, flame ionization detector at 300 °C and hydrogen with 1 ml/min flow rate as carrier gas were device specifications. Sterol compositions were determined through a Supelco/Equity-5 column (30 m × 0.25 mm ID, 0.25 mm film thickness). Initial column temperature was 260 °C for 30 minutes and was increased to 290 °C at the rate of 10 °C/min, and held at this temperature for 20 minutes (INSO 16324) [14].

2.6 Analysis of tocopherols content

Tocopherols were analyzed by high performance liquid chromatography (HPLC, Younglin, South Korea) with SP930D pump and equipped with the ultraviolet detector (UV730D), manual injection system with the loop 20 μl and Autochrome 3000 software. Tocopherols were separated on a C₁₈ column (25 cm × 4.6 mm ID, 5 mm particle size). Mobile phase of methanol and acetonitrile (for determination of tocopherol compounds) and flow rate of 1 ml/min at 30 °C and 20 μL injection volume were used [15].

2.7 Statistical analysis

Each of the experiments was performed in three replications. SPSS software and one-way ANOVA were used to determine the significance or absence of differences between the test values and the antioxidant effect of chilgoza pine nut oil. For the difference between treatments and total factors, LSD output test was used.

3. Results and discussion

3.1 Composition of chilgoza pine nuts

The average of physicochemical characteristics of dried chilgoza pine nuts are presented in Table 1. The average hull content was 44.93%. The average moisture content was 2.84%, which shows that the nuts could be stored for a long time than previous research on dried chilgoza pine of Kinnaur region with higher moisture content (6.8%) [5] which could be due to decomposition of fatty acids due to microbial activities and decrease in the storage time. On the other hand, the difference could be resulted of the items such as variety, location, climate zone and so on.

The nuts had approximately 2.86% ash content, which is near to the count of dried chilgoza pine nuts of Kinnaur region (2.7%) and they are due to the soil composition of Kinnaur in absorb minerals by plant. The nut average protein content was 20.82%, which is higher than previous study has been reported to be 12% [5].

The average nut oil content was 48.78 % which is virtually the same with previous study has been reported to be 49.4% [5]. The average pH of nuts was 6.67. According to the researches in this field, no similar studies have been carried out. This test indicated the state of oxidative degradation of unsaturated fatty acids associated with the seedling. According to studies that are not the subject of this research, these nuts are sensitive to fusarium growth and germination, thus, this test can partially measure the states of corruption. Normally, this number for wheat flour is about 6.1 - 6.2 and, as can be seen, the pH of the chilgoza pine nut is close to this number; hence, if the nuts are corrosive, the pH number it will diminish.

Another measured item was such as thousand nut weight (259.71 g) and density (1.10 kg/m³). There were not any similar researches in this case to compare.

The present sample has heavy metals such as iron (0.01 mg/kg), copper (0.04 mg/kg), lead (0.01 mg/kg) and no arsenic metal.

Table 1. Physicochemical characteristics of chilgoza pine nuts and compare it to some edible seeds

Characteristics	Present study	Chilgoza nut [5]	Other studies
Hull (%)	44.93 ± 6.08	-	21-35 (Sunflower seed) [17]
Moisture (%)	2.84 ± 0.03	6.8	12 (Sunflower seed) [17]
Ash (%)	2.86 ± 0.13	4	5 (Soy bean) [17]
Protein (%)	20.82 ± 0.23	12	6-12 (Corn seed) [17]
Oil (%)	48.78 ± 0.23	49.4	45.09 (Peanut; Golden) [18]
Thousand nut weight (g)	259.71 ± 11.58	-	5.10 (Rapeseed) [19]
pH	6.67 ± 0.01	-	-
Density (kg/cm ³)	1.10 ± 0.03	-	1.12-1.21 (Soy bean) [20]
Heavy metals (ppm)			
Cu	0.04 ± 0.00	-	-
Pb	0.01 ± 0.00	-	0.1 (Sesame seed) [21]
As	ND	-	0.1 (Sesame seed) [21]
Fe	0.01 ± 0.00	-	-

* ND= didn't detect. Data are based on mean values of three independent replicate ± SD.

3.2 Composition of chilgoza pine nut oil extracted by n-hexan

Iodine, saponification and unsaponifiable, refractive indexes, acid, peroxide, specific gravity values, moisture content, color and oxidative stability of oil samples extracted from chilgoza pine oil extracted by n-hexane are shown in Table 2. Iodine value determines unsaturation degree in the oil samples. This value for food product is between 75 and 150 g [15, 20]. The iodine value was 121.9 g, which are near to the reported count in previous study [22]. The iodine value, in this case, indicates that the number of unsaturated fatty acids in the chilgoza pine oil is high and this is evident from the amount of acid and free fatty acids values. Saponification value, which is measurement of the average molecular weight of the constituent fatty acids in the lipid, was 97.79 mg KOH/g for chilgoza pine nut oil. This count shows a high different to previous study (295 g) which is due to the length of fatty acid chain, that contained of 51.64% PUFA and thus decrease of saponification value. The unsaponifiable value is almost used for Fraud detection. It was 1.61% for present sample and lower than previous study (5.5 weight percent) [22]. The refractive index is important to determine the type and purity of the oil. The average of sample's refractive index in 20 °C was 1.4739, which was near to the count measured in previous study about 1.4692 [22]. The acid value was 0.49 (mg KOH/g oil), which was so lower than previous study (4 mg KOH/g oil)

[22]. As reported before, the maximum value of acidity accepted for oil used as food product is 0.6% and oil sample shows a good acidity range.

In general, peroxide is the primary product of oxidation of fatty substances, and as a whole, the higher degree of non-saturation of oils, the oil or the fatty substance is more readily oxidized, and peroxide represents the degree of oxidation progression. The average sample's peroxide value was 6.59 (meq O₂/kg oil).

Specific gravity is not the same for all oils and depends on the composition of fatty acids, low-amount compounds in the oil and also the temperature. The average of specific gravity of the sample is 0.9167 g/cm³. This number is similar to the values of oil in previous study with an average value of 0.9187 g/cm³ [22].

The amount of moisture in the chilgoza pine nut oil is an average of 0.0003%. Due to the lower amount of moisture of the nuts and its content of the oil, this oil is expected to have a good resistance to degradation and storage conditions.

The intensity of the color measure by the Lovibond device this oil is an average of yellow: -23.33 and red: 2.25. The color was lower compared to sunflower oil (45 yellow and 2 red) and the oil of wild horseradish of the Pakistani region (31 yellow and 1.28 red) [23].

The average of stability time for the present sample was 15.18 hours. This number is higher than the resistance ranges of safflower oil (9-12 hours) [17] and the oil of wild horseradish of the Pakistani region (8.40 hours) [23]. It's also close to the minimum amount of soybean oil (15 hours) [24]. The average of heavy metals of this oil such as Cu, Pb, Fe were, respectively, 0.05, 0.01, 0.54 ppm. There were not detected any as element in this sample. This oil has no cadmium in comparison to corn oil (0.2 mg/kg) [25] and has a lower lead content and compared with sunflower oil has lower Iron (up to 4.4 mg/kg) and copper (up to 3.1 mg/kg) [26].

Table 2. Physicochemical composition of chilgoza pine nut oil extracted by n-hexane

Characteristics	Amount
Iodin value (GC) (g)	124.24± 0.43
Iodin value (Hanus) (g)	121.9± 0.16
Saponification value (mg KOH/g)	97.79± 0.23
Unsaponifiable value (%)	1.61± 0.09
Refractive index (analog) (20 °C)	1.4739± 0.0001
Refractive index (digital) (20 °C)	1.4661± 0.0004
Acid value (mg KOH/g oil)	0.49± 0.05
Peroxide value (mEq O ₂ /kg oil)	6.59± 0.34
Specific gravity (g/cm ³)	0.9167± 0.43
Rancimat (h)	15.18± 0.32
Moisture content (%)	0.0003± 0.0003
Color	
red	2.25± 0.057
yellow	-23.33± 0.57
Heavy metals (mg/kg)	
Cu	0.05± 0.00
Pb	0.01± 0.00
Fe	0.54± 0.01
As	ND

*ND= didn't detect. Data are based on mean values of three independent replicate ± SD.

According to the table 3, this oil is classified as oleic-linoleic group oils. This group is the most abundant and largest category of edible oils, and the oils of this group usually have high unsaturated fatty acids, and their saturated fatty acids are generally less than 20%. Therefore, this oil is a rich source of fatty acids of ω6 and ω9 because of its high amounts of linoleic acid (50.75%) and oleic acid (40.45%), which are the most important fatty acids. Compared with previous study, the present oil sample has a similar amount of linoleic acid (52.7%), but less oleic acid (36%) [22]. The most abundant saturated fatty acid is palmitic acid with an average of 5.62%. This oil is not a rich source of linolenic fatty acids, and its average is 0.89%.

For measuring the factors such as the amounts of tocopherols, sterols, polyphenols, total carotenoids and chlorophylls of chilgoza pine nut oil, color (Hunterlab), and also the test for determining the antioxidant properties of the oil (Table 4), the chilgoza pine nuts were extracted by cold pressing method. In the cold pressing extraction method, approximately of half of the total weight of the sample, equivalent to 785 g, about 320 g oil was extracted, which was better than solvent extraction. This is due to the application of a temperature lower than hexane boiling point (55-60 °C) in solvent extraction to prevent the effect of temperature on increasing peroxide values.

The average total sterol of the present sample is about 3,995.41 ppm. The chilgoza pine nut oil is rich in beta-sitosterol (69.29 ppm) which is the most abundant plant sterol. Compared to that, stigma sterol is very small

(1.85%). Campesterol with an average of 20.4% is the most abundant plant sterol after beta-sitosterol in this oil. The amount of Δ^5 -avenasterol, which is the index sterol in sesame oil with high antioxidant properties, is very low in this oil and is about 0.47%. Other sterol compounds are less than 1% and this oil is free of cholesterol.

Table 3. Fatty acid composition of chilgoza pine nut oil extracted by n-hexane

Fatty acid composition (%)	Amount
C16:0	5.62± 1.33
C18:0	1.74± 0.36
C18:1	40.45± 0.42
C18:2	50.75± 0.85
C18:3	0.89± 0.29
C20:0	ND
C22:0	ND
∑SFA	3.68± 0.23
∑MUFA	40.45± 0.42
∑PUFA	51.64± 0.57

* ND= didn't detect. Data are based on mean values of three independent replicates ± SD.

Tocopherols are the most important natural antioxidants and are found in many oils. Tocopherols have both antioxidant and vitaminic properties, and these two properties are unique. In the present study, the most abundant tocopherol of chilgoza pine nut oil is γ -tocopherol with an average of 101.51 ppm and followed by the α -tocopherol with an average of 7.72 ppm, is in the second place. This oil does not contain δ and β -tocopherols. The average polyphenols of chilgoza pine nut oil were 168.44 ppm. The amount of these compounds depends on the method of extraction, variety, and so on.

Table 4. Physicochemical composition of chilgoza pine nut oil extracted by cold press

Characteristics	Amount
Sterol composition (%)	
Cholesterol	ND
Brasikasterol	0.01± 0.00
Campesterol	20.4± 0.05
Stigma sterol	1.85± 0.04
Betasitosterol	69.29± 0.1
Sitostanol	8.05± 0.09
Δ^5 -avenasterol	0.47± 0.02
Δ^5 and 24 stigma esta de enol	0.27± 0.02
Total sterol	3995.41± 35.1
Tocopherol (ppm)	
α	7.72± 0.16
γ	101.51± 1.07
Total carotenoid (ppm)	1.39± 0.02
Total chlorophyll (ppm)	0.087± 0.005
Total polyphenol (ppm)	167.26± 1.1
DPPH (%)	57.5± 0.4

* ND= didn't detect. Data are based on mean values of three independent replicates ± SD.

The DPPH test is widely used to test the ability of compounds to act as free radical or hydrogen inhibitors, and it estimates the antioxidant activity of food [27]. The main task of this method is to measure the free radicals created by direct oxidation of the oils. Oxidized oils with a higher content of unsaturated fatty acids (PUFA) than those with lower levels of this type of fatty acid have a larger effect on the absorption of DPPH or the formation of free radicals and therefore are more sensitive to oxidation. Tocopherols play a major role in the determination of antioxidant properties in refined vegetable oils, and in contrast, other antioxidants, such as polyphenols and carotenoids, are removed or degraded during the refining process [28]. As a result, it is expected that in chilgoza pine nut oil obtained by cold pressing method, the most important factor in determining its antioxidant properties is the amount of polyphenols and their type. Antioxidant activity (EC_{50}) (50% reduction in initial DPPH radical absorbance) of chilgoza pine nut oil is 57.5% (100 ppm of the sample).

The amount of total carotenoids in the chilgoza pine nut oil is 1.39 ppm and the total chlorophyll content is 0.087 ppm. In a previous study, the amounts of β -carotene in some types of oil extracted by cold pressing method

was measured. Also the counts of β -carotene were measured in the oils extracted by the solvent. It was observed that β -carotene just only is found in the cold pressed oils; hence, chilgoza pine nut oil is likely to contain β -carotene, because it was prepared by cold pressing method.

The intensity of the color of chilgoza pine nut oil by cold pressing method was according as follow:

a*: -3.02; L*: 56.28; b*: 49.28

Some physicochemical composition of chilgoza pine nut oil was compared with other oils (Table 5-6).

Table 5. Physicochemical composition of chilgoza pine nut oil extracted by n-hexane compare to other oils

Characteristics	Present study	Other studies
Iodin value (Hanus) (g)	121.9	145 (American safflower oil) [17] 112.1 (Canola oil) [29] 114.3 (Cashew) [30]
Saponification value (mg KOH/g)	97.79	224.40 (Peanut oil; Bari 2000) [18] 186-194 (Edible safflower oil) [31]
Unsaponifiable value (%)	1.61	4 (Peanut oil (Mungphalla)) [18] 2.8 (Corn oil) [17] 1.5 (Extra virgin olive oil) [32]
Refractive index (analog) (20 °C)	1.4739	1.4571 (<i>Moringa oleifera</i> seeds oil) [33] 1.4745 (American safflower oil) [17]
Acid value (mg KOH/g oil)	0.49	12.1 (Cashew (BRZ)) [30] 0.28 (Olive oil (Koreniki)) [33] 0.15 (Malaysian virgin coconut oil) [34]
Peroxide value (mEq O ₂ /kg oil)	6.59	6.9 (Sesame oil) [35] <10 (Raw soy bean oil) [17] 1.46 (Virgin olive oil (Barena)) [33]
Specific gravity (g/cm ³)	0.9167	0.9032 (<i>Moringa oleifera</i> seeds oil) [23] 0.9215 (American safflower oil) [17]
Rancimat (h)	15.18	8.40 (<i>Moringa oleifera</i> seeds oil) [23] 9-12 (safflower oil) [17]
Moisture content (%)	0.0003	2 (sunflower seed oil) [17] 0.06 (American safflower oil) [17] ≤ 0.2 (Extra virgin olive oil) [32]
Color		
Red	2.25	1.28 (<i>Moringa oleifera</i> seeds oil) [23] 0.73 (safflower oil (Isfahan)) [31]
Yellow	-23.33	31 (<i>Moringa oleifera</i> seeds oil) [23] 9.8 (safflower oil (Isfahan)) [31]
Heavy metals (mg/kg)		
Cu	0.05	1.3 (Sunflower seed oil) [26]
Pb	0.01	-
Fe	0.54	4.4 (Sunflower seed oil) [26]
As	ND	-

*ND= didn't detect. Data are based on mean values of three independent replicates ± SD.

3.3 Investigation antioxidant effect of chilgoza pine nut oil on refined rapeseed oil without any antioxidant agent

After extraction chilgoza pine nut oil by cold pressing method, it was added in two levels (2% and 5%) to refined rapeseed oil without antioxidant. The major composition of the fatty acids of rapeseed oil was, respectively, oleic acid 62.85%, linoleic acid 20.25%, linolenic acid 7.71%, palmitic acid 5%, stearic acid 1.68%, arachidic acid 0.56%, erucic acid 0.12% and meristic acid 0.02%. The samples were stored at ambient temperature and kept away from light. Oxidative stability, peroxide, thiobarbituric acid and Rancimat content of oil samples were determined among 0, 5, 10, 20 and 35 days, and were compared with control samples (free of chilgoza pine nut oil (0%) and contained of 150 ppm TBHQ antioxidant) (Table 7). This study was done to investigate the effect of both ambient temperatures and accelerated temperature to evaluate the effects of temperature on oil oxidation rate in addition to types and concentrations of antioxidant agents.

Table 6. Physicochemical composition of chilgoza pine nut oil extracted by cold pressing method compare to other oils

Characteristics	Present oil sample	Other studies
Sterol composition (%)		
Cholesterol	0	7-13 (Palm oil) [17] 0.2 (Moroccan sesame seed oil) [35]
Camptsterol	20.4	90-151 (Palm oil) [17] 17.8 (Moroccan sesame seed oil) [35]
Stigma sterol	1.85	44-66 (Palm oil) [17] 6.4 (Moroccan sesame seed oil) [35]
Sitosterol	0	218-370 (Palm oil) [17] 0 (Moroccan sesame seed oil) [35]
Tocopherol (ppm)		
α	7.72	2.2% (Sesame seed oil) [35] 15.19% (Pumpkin seeds) [36]
γ	101.51	90.5% (Sesame seed oil) [35] 61.32% (Pumpkin seeds) [36]
Total carotenoid (ppm)	1.39	81 (Rapeseed oil [37] (β - Carotene)) 27.3 (Soy bean oil [37] (β - Carotene))
Total chlorophyll (ppm)	0.087	26.1 (Flax seed oil (Waihi Bush)) [40]
Total polyphenol (ppm)	168.44	0.015 (Pomegranate seed oil) [39] 123.82 (Green olive oil) [40]
DPPH (%)	57.5	51.8 (Oregano oil) [41] 55.3 (Cinnamon oil) [41]
Color		
a*	- 3.02	3.28 (Flax seed oil (MEL)) [38]
L*	56.28	39.63 (Flax seed oil (MEL)) [38]
b*	49.28	08.91 (Flax seed oil (MEL)) [38]

*ND= didn't detect. Data are based on mean values of three independent replicates \pm SD.

Table 7. Peroxide, TBA and Rancimat results of soybean oil samples containing of three levels of Chilgoza pine nut oil level

	Chilgoza pine nut oil level	Days				
		0	5	10	20	35
Peroxide value (mEq O ₂ /kg oil)						
Chilgoza pine nut oil	0%	2.96 \pm 0.02 ^b	4.2 \pm 0.1 ^c	5.77 \pm 0.02 ^a	9.07 \pm 0.04 ^a	15.9 \pm 0.04 ^b
	2%	3.09 \pm 0.006 ^b	5.62 \pm 0.003 ^a	7.56 \pm 0.02 ^a	10.07 \pm 0.02 ^a	17.05 \pm 0.02 ^a
	5%	3.66 \pm 0.46 ^a	4.82 \pm 0.03 ^b	7.28 \pm 0.02 ^a	10.16 \pm 0.02 ^a	17.58 \pm 0.02 ^a
	150 ppm TBHQ	2.96 \pm 0.02 ^b	4.2 \pm 0.1 ^c	5.77 \pm 0.02 ^b	9.07 \pm 0.04 ^b	15.9 \pm 0.04 ^b
TBA value (mg MDA/kg)						
Chilgoza pine nut oil	0%	0.06 \pm 0.02 ^b	0.07 \pm 0.1 ^b	0.08 \pm 0.02 ^b	0.09 \pm 0.02 ^b	0.13 \pm 0.04 ^b
	2%	0.06 \pm 0.006 ^b	0.08 \pm 0.003 ^a	0.09 \pm 0.02 ^a	0.1 \pm 0.02 ^a	0.16 \pm 0.02 ^a
	5%	0.06 \pm 0.46 ^a	0.08 \pm 0.03 ^a	0.09 \pm 0.02 ^a	0.11 \pm 0.02 ^a	0.15 \pm 0.02 ^a
	150 ppm TBHQ	0.03 \pm 0.02 ^c	0.05 \pm 0.02 ^c	0.06 \pm 0.02 ^c	0.08 \pm 0.04 ^d	0.1 \pm 0.04 ^c
Rancimat result (h)						
Chilgoza pine nut oil	0%	16.07 \pm 0.02 ^b	14.25 \pm 0.1 ^b	14.23 \pm 0.02 ^b	12.81 \pm 0.04 ^c	11.7 \pm 0.04 ^b
	2%	16.06 \pm 0.006 ^b	13.08 \pm 0.003 ^c	12.8 \pm 0.02 ^c	12.29 \pm 0.02 ^c	11.23 \pm 0.02 ^b
	5%	14.14 \pm 0.46 ^c	12.86 \pm 0.03 ^c	12.75 \pm 0.02 ^c	13.6 \pm 0.02 ^b	10.4 \pm 0.02 ^c
	150 ppm TBHQ	27.06 \pm 0.02 ^a	22.36 \pm 0.02 ^a	22.24 \pm 0.02 ^a	19.07 \pm 0.04 ^a	17.8 \pm 0.04 ^a

*Considering that the level of significance (or significant probability) is less than 0.0001, it can be said that there is a significant difference between various treatments at each level (0.05 or 0.01).

The peroxide value indicates the amount of initial oxidation products. Based on the results of comparing the amount of peroxide number of refined rapeseed oil without any antioxidant which have been added 150 ppm TBHQ and 0% (free of chilgoza pine nut oil and antioxidants) as control samples, on days 0, 5, 10, 20 and 35 It can be resulted that chilgoza pine nut oil did not effect on the reduction of rapeseed oil peroxide and initial oxidation products.

Samples containing 5% chilgoza pine nut oil had higher TBA values than 2% and 0% samples, as well as TBHQ. Therefore, with increasing amount of chilgoza pine nut oil, the production of secondary oxidation products increased. It means that, the chilgoza pine nut oil did not effect on the reduction of secondary oxidation products and the TBA value. One of the main reasons for this resulting is the amounts of PUFA in this oil.

The results showed that over time, oil resistance decreased gradually. The highest rate of resistance was observed in oils containing 2% and 5% chilgoza pine nut oil, while the results were significantly different with TBHQ. Generally, the chilgoza pine nut oil did not increase rapeseed oil resistance. Although this test was carried out at ambient temperature, after 20 days, the peroxide value was increased by adding the chilgoza pine nut oil to 10, which is higher than the maximum permitted for rapeseed oil peroxide. It seems that no antioxidant compounds present in chilgoza pine nut, are not migrated to the oil or is not to a level to completely prevent the oxidation of rapeseed oil and increase its resistance.

4. Conclusion

The chilgoza pine nut oil is one of the linoleic oleic acid oil groups; therefore, it is a good source of $\omega 6$ and $\omega 9$ fatty acid groups. The amount of carotenoids and chlorophylls in chilgoza pine nut oil were much lower than other oils (rapeseed, olive, soybeans, etc.). In terms of the amount of tocopherol, this oil contains α and γ tocopherol, which is less than other vegetable oils and in this regard, it has fewer antioxidant properties than other reported cultivars. The results showed that in over time, resistance decreased gradually. So that the highest rate of resistance reduction was observed in oils containing 2% and 5% chilgoza pine nut oils, while the results were significantly different with TBHQ value and generally the chilgoza pine nut oil did not increase rapeseed oil resistance. It seems that no antioxidant compounds present in chilgoza pine nut, are not migrated to the oil or is not to a level to completely prevent the oxidation of rapeseed oil and increase its resistance.

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