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Evaluation of Extraction Percentage and Physicochemical Properties of Walnut Oil

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ABSTRACT

In this study, walnut oil was first extracted by conventional method using hexane in three different temperatures 25, 35 and 45 ° C, three times for 5, 10 and15 h, and the ratio of solvent to walnut at 2, 3 And 4 (w / v). Through the study of the effect of parameters on the extraction, it was found that the increase in extraction time leads to significant increases in total efficiency of extraction, acid number, saponification value, the percentage of wax and the refractive index; while on the other hand, it leads to a significant decrease in the amount of phenolic compounds. The increase in temperature leads to a significant increase in the total efficiency of extraction, acid index, saponification index, phenolic compounds, wax content and refractive index. Among the ratio of solvent to nut, the ratio of 3 was considered as the optimal level. Determination of the structure of walnut oil fatty acids revealed that the major fatty acids of walnut oil are linoleic acid (54.54 percent), oleic acid (23.89%) and linolenic acid (12.80%), respectively, which in total; they constitute over 91 percent of the total amount of fatty acids. Percentage of oil extraction, acid number, saponification value, phenolic compounds, wax content, specific gravity, refractive index and structure of the fatty acid was calculated to evaluate the physicochemical properties of walnut oil.
KEYWORDS: Walnut oil, solvent extraction, extraction percentage, physicochemical properties

INTRODUCTION

Walnut is known as the oldest tree dating back to 7000 BC of which the fruit is used as food. In fact, walnut tree is among the few useful plants that naturally grow in both Eastern and Western hemispheres of the earth (this is a strong evidence for the existence of trees on the earth before the continents separated from each other) [23]. The scientific name of the genus is Juglans; from the Latin word Jovis-Glans meaning Jupiter Hazelnut [20].

From the nutritional point of view, walnut is a very nutritious nut and it has different composition depending on variety. Most of walnut varieties have 60% oil approximately, the value of fat varies between 70-52% depending on variety and region [28].

The benefits of walnuts on cholesterol in human diet have been proven. It is proved that a balanced consumption of walnuts reduces cholesterol or low-density lipoprotein levels to about 16 percent in men. It has been proven that walnuts are effective in preventing heart disease. Dried fruit nuts are often rich in unsaturated fatty acids with a monounsaturated bind such as oleic acid, while the nuts od walnut are rich in two unsaturated fatty acids with polyunsaturated bonds including linoleic acid and linolenic acid [32].

In general, the type of fatty acids consumed in human diet is more important than the total oil consumed. In addition, the ratio of fatty acids in nutritional and economic value of oil is very important. The higher proportion of unsaturated fatty acids with one double bond cause additional durability against oxidation and increases its shelf life, but polyunsaturated fatty acids with multiple double bonds is more susceptible to oxidation, but they are more important in terms of human health and nutrition [18].

As mentioned earlier, walnuts are a rich source of linoleic acid, linoleic acid [14].

According to FAO statistics, in 2011, the total annual world production of walnuts estimated almost three million and 420 thousand tons and China about a million and 700 thousand tons, Iran 450 thousand tons, America 425 thousand tons and Turkey 195 thousand tons were the most important nut-producing countries in the world. Iran is the most important producer of walnuts in the world [19]. Persian walnut is the only species of this family that its nut is economical in terms of food consumption. Choosing a kind of oil for specific purposes is determined by its fatty acid structure and its properties. Oil extracted from one source is not suitable for all purposes, as different oils have different fatty acid structures. Therefore, to prove their usefulness a raw material, a comprehensive study of their physicochemical properties is required [25]. The purpose of this study is the extraction percentage and the physicochemical properties of walnut oil.

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MATERIALS AND METHODS

Materials

Walnut used in this study were obtained from the city of Kalat, Khorasan province, the variety is known as paper-like shell walnut, and Hexane used in this study were obtained from Iran's Mojalali company, and the other chemicals were obtained from Merck, Germany.

Equipment and Devices

The instruments and devices used in present study are listed in Table 2:

Table 2 Equipment and devices used in present study

Devices	Production Model	Manufacturer
Gas Chromatography	Varian Star 3 400	America
Spectrophotometer, visible and ultraviolet radiation[1]	Shimadzu UV 160A	Japan
Magnetic Mixer	IKA RCT basic	Germany
Electric mill	IKA A11 basic	Germany
Refractometer	ModelDTM-1	Japan

The oil extraction

At first, samples of walnut (obtained from Kalat city in Khorasan province) are broken and the nuts were separated from walnut shell with the least harm possible. Then the prepared samples were stored at -18 degree centigrade until use. In the next step, the nuts were grinded and became ready for extraction by electric mill for 30 seconds in 10 seconds sequences. It is noteworthy that in order to maintain qualitative properties and to prevent damage, grinding is done just before each extraction stage. The resulting powder were mixed with n-hexane in a ratio of 1: 2, 1: 3 and 1: 4 w/v, and oil extracted while stirring at 5, 10, 15 hours and at three temperature levels of 25, 35, and 45 ^{oC}. After this period, the solvent was evaporated in vacuum and at a temperature of 40 ^{oC}. The extracted oil was kept in a dark container in the freezer until performing related tests.

Tests

Fatty acid structure

Fatty acid structure was measured using Cert et al. (2000) method [15].

Measuring the acid number

Acid number was measured using Hosseini (1998) method [3].

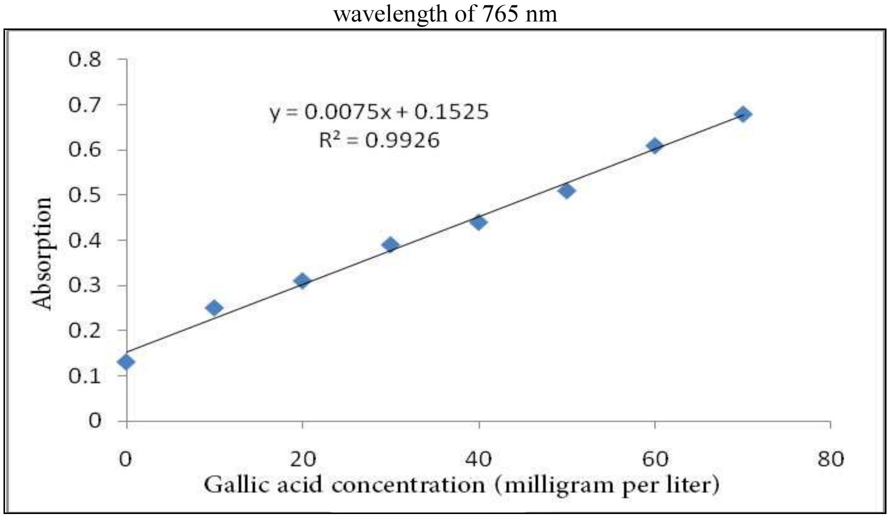
Saponification value

Saponification value was measured using Hosseini (1998) method [3].

Phenolic compounds

Phenolic compounds measured using Shahidi, F. and Naczk. M. (2004) method [31] and absorption curve of gallic acid concentration (mg per ml) was plotted (Fig. 2) and the equation 4 with the coefficient of determination equal to 0.99 was obtained:

Figure 2 Calibration curve of the concentration of phenolic compounds against the absorption read at a wavelength of 765 nm



[1] Atomic absorption spectroscopy

Measurement of waxy compounds
Waxy compounds were measured using Hosseini (1998) method [3].
Measurement of Specific gravity
Specific weight was measured using Hosseini (1998) method [3].
Measurement of the refractive index
Refractive index was measured using Hosseini (1998) method [3].

Statistical Analysis

In this study, to compare physicochemical characteristics of walnut oil, a factorial completely randomized design with three replications using analysis of variance (ANOVA) at five percent level ($p < 0.05$) was assigned. Comparison of the data was performed according to Duncan test using SPSS software and EXCEL 2010 graph drawing software.

RESULTS AND DISCUSSION

Percentage of oil extraction from walnuts

Walnut oil content was determined as of 49 to 63 percent, and this content is much higher than common sources of edible oils such as soybean (18 to 22%), canola (18 to 22%) and olive (12 to 50 percent) [26]. In addition, the amount of walnut oil was higher than oil seeds such as sesame (51.61 percent) [4], Cannabis (30.5 percent) [27], flax (44.25 percent) [2] and corn oil (3.1 to 5.7 percent) [29].

Studying the effect of the solvent on oil extraction content shows -according to Figure 3-, that with increasing solvent content, the extracted oil content also increases significantly ($P < 0.05$). However, the point here is that by increasing the amount of solvent, due to the higher proximity of oil and solvent, oil extraction increased while oil content of the nut is reduced, and this reduces the driving force of extraction. Therefore, it is expected that further increase in the amount of solvent decreases the extraction efficiency [6].

The results also showed that with increasing extraction time, due to increased contact time of solvent and oil, the content of extracted oil, and hence the percentage of oil extraction, increases significantly ($P > 0.05$) (Fig. 4).

On study the effect of temperature on the extraction of oil, increasing the temperature from 25 $^{\circ C}$ to 45 $^{\circ C}$ has a positive and significant impact ($P > 0.05$) on maximum extraction of oil from walnuts (Figure 5). The reason for this is an increase in kinetic energy as well as boosting effect of temperature on increasing the rate of dissolution of oil in the solvent [6].

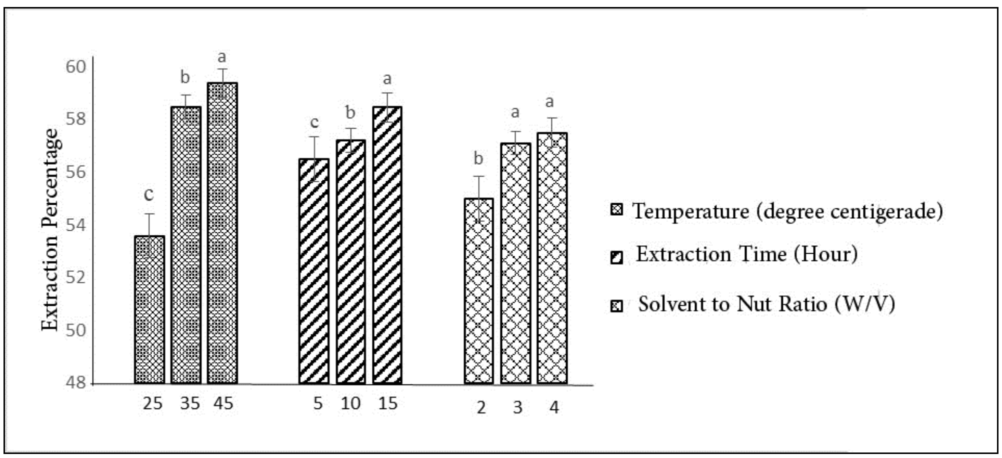


Figure 2 Effects of temperature, time and amount of solvent on extraction percentage

Physicochemical properties of walnut oil

Choosing a kind of oil for specific purposes is determined by its fatty acid structure and its properties. Oil extracted from one source is not suitable for all purposes, as different oils have different fatty acid structures. Therefore, to prove their usefulness a raw material, a comprehensive study of their physicochemical properties is required [16-25]. The extracted walnut oil was yellow in color and was liquid even at refrigerator temperatures.

Fatty acid structure

The composition of fatty acids affects the physical, chemical and nutritional properties of fats [2]. The structure of fatty acids extracted from walnut oil is shown in Table (2), as it is evident from the table, the major fatty acids of walnut oil are linoleic acid (54.54 percent), oleic acid (23.89%) and linolenic acid (12.80%), respectively, which in total, they constitute over 91 percent of the total amount of fatty acids. The amount of linoleic acid of walnut oil was close to soy oil (53.7 percent), and was higher compared to the raw canola oil (21%) And virgin olive oil (6.3 percent) [29]. High levels of linoleic acid may lead to the tendency of oil to oxidation. However, these fatty acids have favorable nutritional applications; they also have beneficial physiological effects in the prevention of cancer and heart disease [30].

Linolenic acid content of walnut oil, was also higher compared to sunflower oil (0.1 to 0.2 percent) and fish oil (20 to 26%) [2], omega fatty acids have a protective effect on breast cancer risk [33], walnut oil contains 8.3 percent saturated fatty acids, which is lower than the amount of soybean oil (15.5 percent), olives (12.3 percent) and higher than canola (7 percent). In addition, compared to the polyunsaturated fatty acids of soybean oil (84.7 percent), olive oil (12.3 percent) higher, and unsaturated fatty acids of walnut oil, compared to canola oil (91.7 percent) was lower [29].

Ratio of polyunsaturated to saturated fatty acids (PUFAs / SFAs) is called Poly-n Index, and is usually a measure of the amount of unsaturation of oils and fats as well as their tendency to lipid self-oxidation. Higher values of this index mean higher oxidation of oil or fat [9]. According to Table 2, this ratio was 8.11for walnut oil, which is higher than soybean, canola and olive oil, which are 3.95, 4.25 and 0.5, respectively [29]. Considering the higher poly-n index of walnut oil, it is expected that it has worse oxidative stability than soybean, canola and olive oils.

Table 2 Composition of fatty acids in walnut oil

Fatty acid	**Walnut oil**
Lauric acid (12: 0)	0.004
Myristic acid (14: 0)	0.021
Palmitic acid (16: 0)	5.186
Stearic acid (18: 0)	2.833
Oleic acid (18: 1)	23.897
Linoleic acid (18: 2)	54.543
Linolenic acid (18: 3)	12.808
Arachidic acid (20: 0)	0.175
Behenic acid (22: 0)	0.050
The total saturated fatty acids	8.3
The total unsaturated fatty acids	91.4

Acid number

Acid index is defined as the milligrams of KOH required to neutralize the free fatty acids in one gram of test sample (unit of measurement: mg / g).This index indicates whether the test sample is healthy or decayed. The oil of which the hydrolysis reaction is taken place, the acid index is high. Acid index indicates the degree of acidity of oil. Acid index usually increases gradually with a gentle slope. Oils undergo qualitative changes over time by many factors such as heat and pollution, and acid index represents one of the indicators of these changes [7].

On study of the effect of solvent on extracted walnut oil acidity index, according to Figure 3, with increasing solvent levels from 2 to 3, the acidity index increased significantly ($P<0.05$), however, with higher levels, we don’t observe significant impact on acid index. Increasing Acid index with increasing amount of hexane is examined and confirmed by other researchers as well [10].

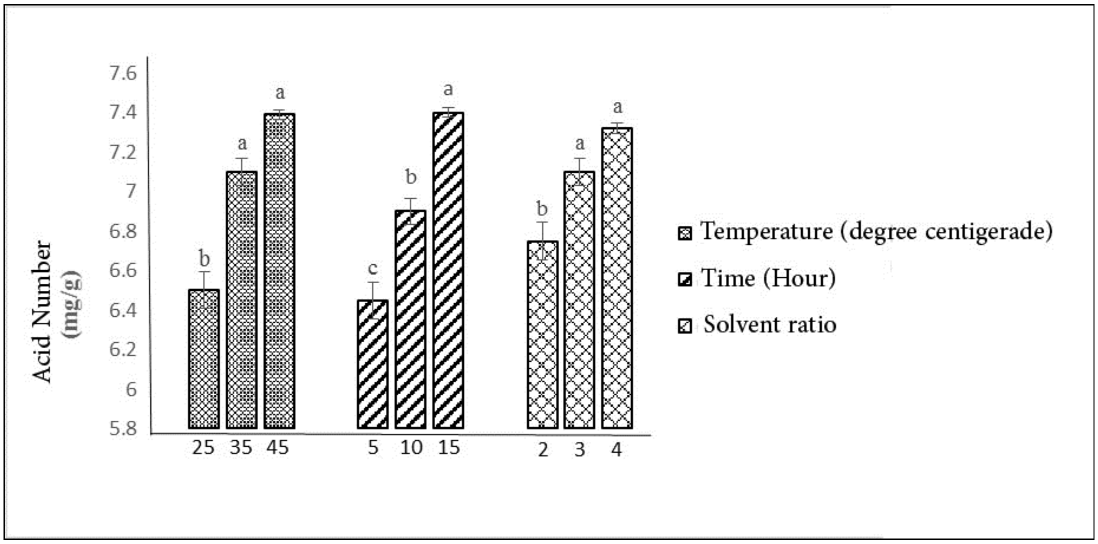


Figure 3 Effects of temperature, time and amount of solvent on acid number

The results showed that by increasing the extraction time, the acid index of the extracted acid increases significantly ($P<0.05$) (Fig. 3). When fats and oils are exposed to air, they become hydrolyzed, and this process produces free fatty acids and cause undesirable flavors in the oils and fats. Also, the higher the percentage of unsaturated fatty acids contains in the oil, their vulnerability to oxidation is greater [35]. As mentioned earlier, walnut oil is rich in unsaturated fatty acids [28]. With increasing time of exposure to solvents, the probability of oil hydrolysis and producing free fatty acids increases [8]. On the study of the effect of temperature on the acid index, increasing temperature from 25 to 35 degree centigrade has positive and significant effect ($P<0.05$) on acid index, but applying higher temperatures (45 degree centigrade) did not show a significant effect on acid index (Figure 3). Another important factor that plays a role in the hydrolysis of fats and oils is the temperature in which they are being kept. The high temperature causes a change in color and taste of the oil and to exhaust a blue and then a black smoke, indicating the production of free fatty acid and hydrolysis of oil [8] .

Saponification value

By definition, saponification index indicates the amount of KOH per grams which is required to neutralize the fatty acids derived from hydrolysis of one gram of fat. It is in fact a measure to express the mean molecular weight of fatty acids that have been used in structure of fat. Put it simply, it show us the required amount of KOH for the saponification (hydrolysis) of a gram of fat. In a given weight, with the higher amount of consumed KOH, the fatty acid molecules are smaller and have lower molecular weight. For example, butterfat, of which the fatty acid molecules are small, has partially greater saponification index than corn oil, which is basically made up of large molecule fatty acids.

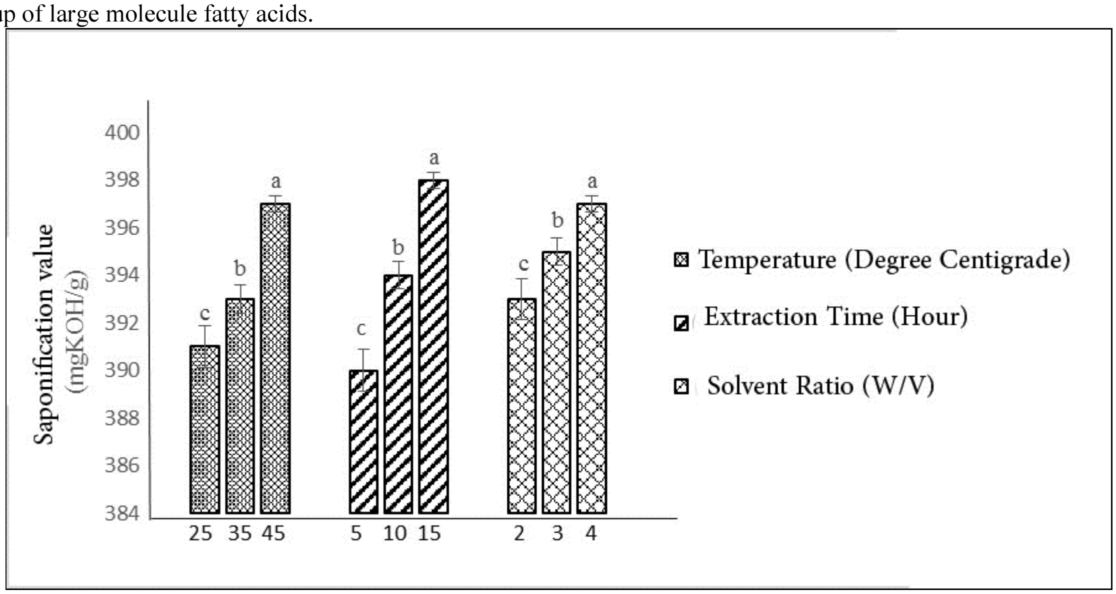


Figure 4 Effects of temperature, time and amount of solvent on saponification value

Saponification index provides useful information on the way fatty acids are composed with glycerol, such as: [7] on study of the effect of solvent on saponification index of extracted walnut oil, according to Figure 4, by increasing the amount of solvent used, the saponification index increased significantly ($P < 0.05$). In a similar study, the effect of hexane solvent on increasing the saponification index has been confirmed by Adewoye and Ogunleye (2012) [10] .

The results also showed that with increasing extraction time, the amount of saponification index in extracted oil increases significantly ($P < 0.05$) (Figure 4). On the effect of temperature on saponification index, the results showed that increasing the temperature from 25 to 45 degree centigrade had significant effect ($P < 0.05$) on saponification index of extracted walnut oil (Fig. 4), as the observations of other researchers also confirm these results. For example Adewoye and Ogunleye (2012) studied the effect of temperature on saponification index of Neem oil and observed that with increasing temperature, soap index of neem oil increases [10] .

Phenolic compounds

Most of known phenolic compounds in walnut are phenolic acids, and tannin, which is a phenolic molecule with a low weight exits in high densities, and these compounds, can be found in the shell around the nut, which have higher antioxidant properties [21]. Phenolic compounds would prevent lipid oxidation and formation of oxidation products such as malondialdehyde, which changes the smell, color, and decreases nutritional value and causes food decay, by absorbing free radicals, releasing hydrogen, single oxygen absorption as well as chelating ions [13].

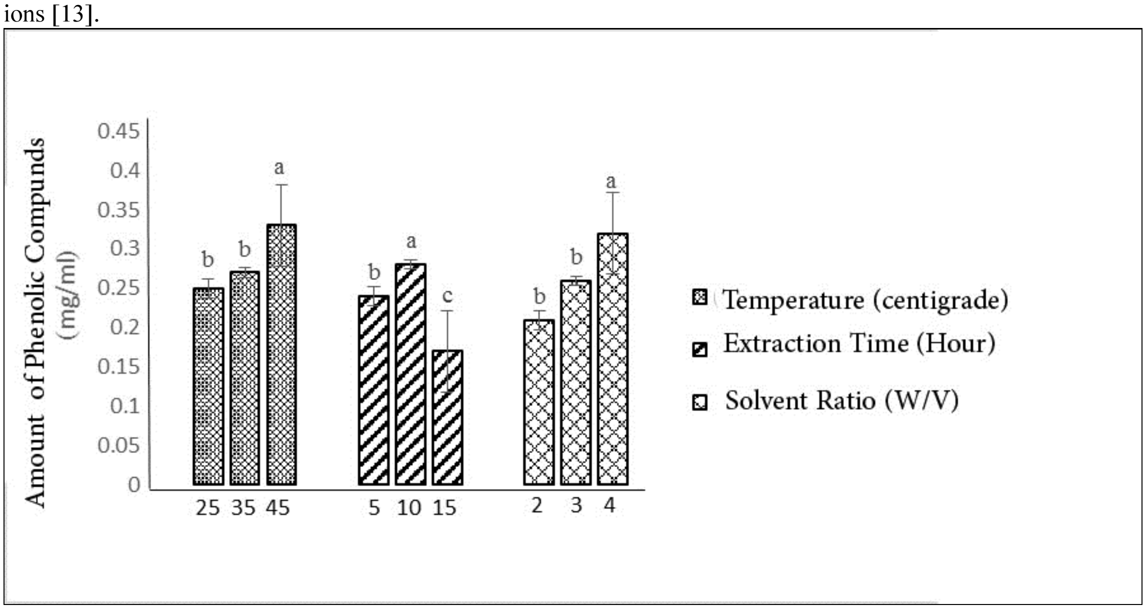


Figure 5 Effects of temperature, time and amount of solvent on phenolic compounds

Typically in traditional method of extraction, higher amount of solvent, as well as longer extraction time would increase active compounds [36] .

Considering the temperature used, the time it takes for extraction can increase or decrease the amount of phenolic compounds extracted. Spigno and Tramelli (2007) studied the effects of temperature and time on the antioxidant activity and the amount of phenolic compounds extracted from the marc of grapes. According to their results, increasing extraction time has first a positive effect and then a negative effect (due to thermal degradation) on amount of phenolic compounds extracted [34]. Considering that in traditional method of extraction, phenolic compounds are exposed to air and temperature, the possibility of damage is too high [8].

On the study of the effect of temperature on the amount of phenolic compounds, the results showed that with increasing temperature from 25 to 35 degree centigrade, no significant change in the amount of phenolic compounds extracted was observed, but by further increase in temperature up to 45 $^{\circ}C$, the amount of phenolic compounds obtained from walnut oil increased significantly ($P < 0.05$) (Fig. 5). Rising temperature causes breaking down of cell membranes and faster exertion of phenolic compounds from plant tissues [22]. However, given that phenolic compounds are sensitive to heat, further increase in temperature leads to thermal destruction of phenolic compounds in the extracted oil [8].

Wax

Waxes are a group of high melting point, insoluble compounds that are found naturally in crude vegetable oils. These compounds, due to making the refined oils hazy, are considered undesirable. On the other hand, these compounds are used in cosmetics, pharmaceutical, food, lubricants, leather industries and polymers [24] . Agzu and Diosun expressed that waxy compounds are in fact hydrated phospholipids that are considered boosters of oxidation due to the large amounts PUFA in their structure, and thus they can have a negative affect the taste, odor, color and appearance of oil [17]. On the study of the effect of temperature on percentage of wax extracted, the results showed that with the increase of extraction temperature, the wax index increases (Figure 6). This increase in amount of wax compounds with increasing extraction temperature is expected because the solubility of wax to temperature changes is very high [7]. The same way that for separation and deposition of was, wintering process is used, the reverse process of increasing the temperature leads to an increase in the percentage of wax extracted [5].

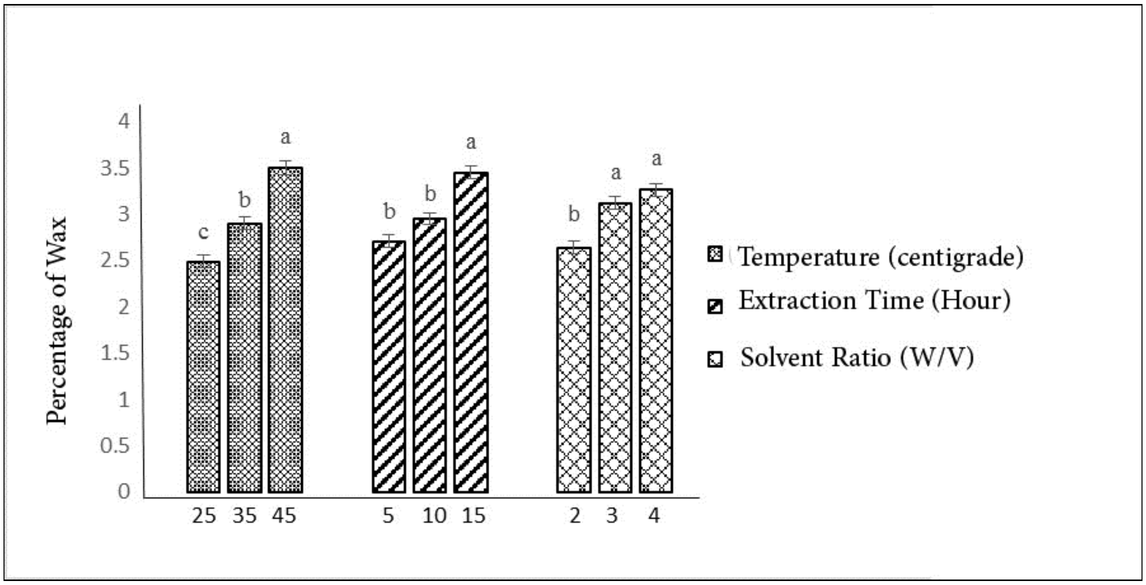


Figure 6 The effects of temperature, time and amount of solvent on percentage of wax

The refractive index

The refractive index of a material is the ratio of the speed of light in vacuum to the speed of light in that material, and since vacuum cannot be used in practice, the air is used instead. Each type of oil has its own refractive index and this is related to the saturation ratio of oil, however, the amount of free fatty acid, oxidation and thermal processes can affect it. This method is applicable to all conventional oils and liquid fats [11].

Refractive index of oils is usually between 1.7 - 1.3 degrees. The refractive index is used for measuring the purity of the oil in oil fraud and in hydrogenation of oils. This parameter increasing with chain length (although this relation is not linear), and unsaturation ratio increases.

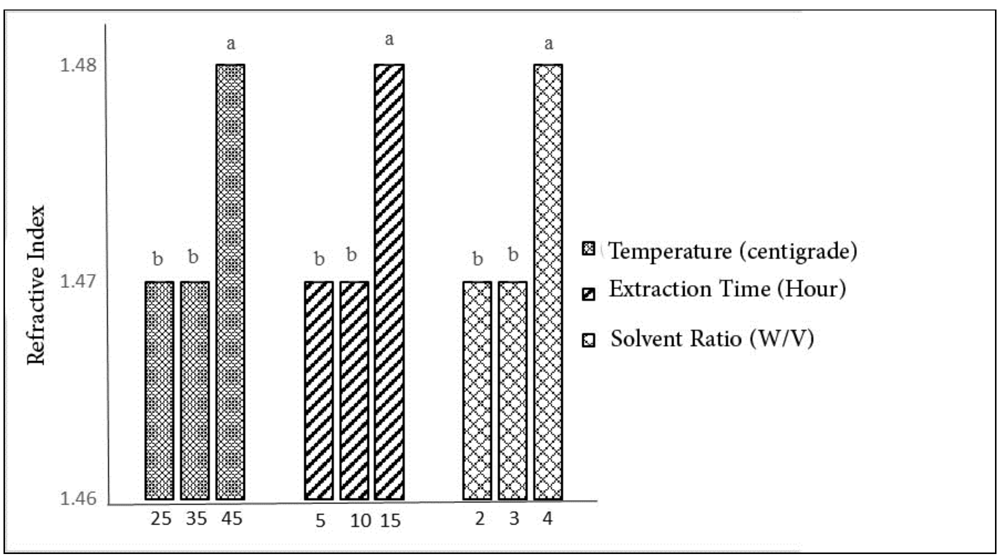


Figure 7 Effect of temperature, time and amount of solvent on refractive index

On the study of the effect of solvent on refractive index in the extracted walnut oil, with increasing solvent from 1.2 to 1.3, the refractive index showed no significant change, but with greater increase in the amount of solvent ($P< 0.05$) we observe a significant effect on refractive index [7]. Akpan et al. (2006) in a study of the refractive index of virgin and refined castor oil expresses that the impurities in the oil cause an increase in the refractive index [11]. As mentioned earlier, by increasing the amount of the solvent, the efficiency of the oil extraction increases, however, it should be noted that at the same time, the amount of impurities getting into the oil will also increase, and these impurities would increase the refractive index. On the study of the effect of temperature on refractive index, the results showed that increasing the temperature from 25 to 35 $^{\circ C}$, leads to a significant increase in refractive index of walnut oil, however, increasing temperature up to 45 $^{\circ C}$, the refractive index increases again significantly ($P< 0.05$) (Fig. 7). One of the reasons for the change in refractive index in oils is the presence of conjugated double bonds in fatty acids, which causes a distinct increase in the refractive [5].

Specific weight

Specific weight is the ratio of the weight of a certain volume of a substance to the weight of the same volume of distilled water at 25 degrees Celsius. The specific weight is a factor that is used to identify oils or fats, and as the ratio of long-chain fatty acids and saturated increases, the specific weight increases [1]. On the study of the effect of solvent and temperature on specific weight of extracted walnut oil, by increasing solvent from 1.2 to 1.4, specific weight shows no significant change (8). The specific weight of oils determines oil purity [12]. Richard (2004) stated the specific weight of crude soybean, canola and olive oils as 0.917, 0.914 and 0.909 gram per cubic centimeter, respectively. The higher specific weight of the walnut oil compared to soybean, canola and olive oils can be contributed to its greater saturation and the presence of long-chain fatty acids, as the results in Table 2 confirm this.

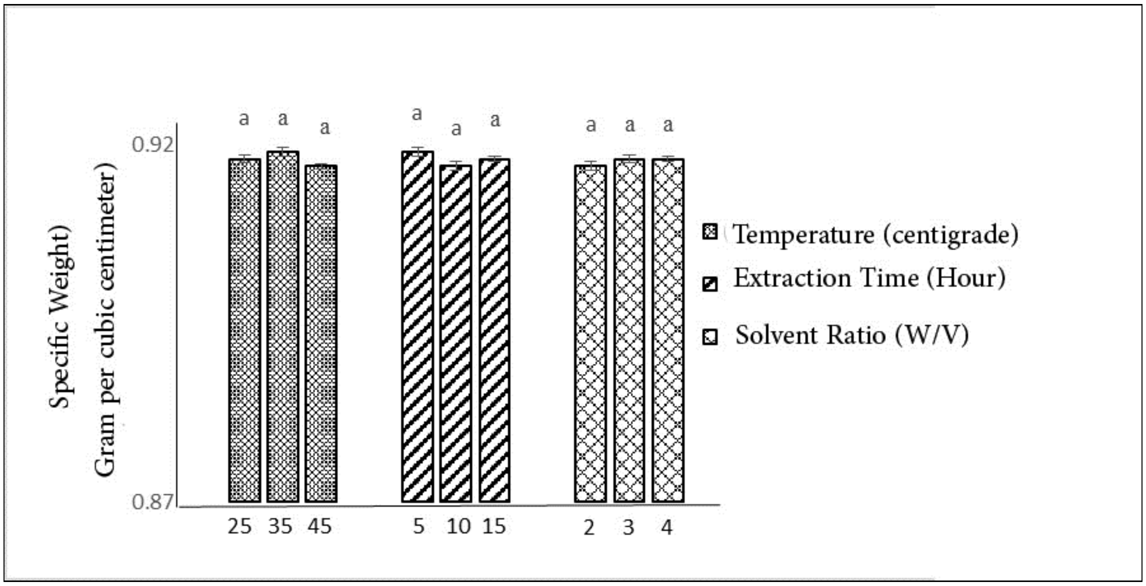


Figure 8 The effects of temperature, time and amount of solvent on specific weight

Table 3. Total average values of the test conducted on walnut oil

Test	Calculated value
Percentage of extracted oil	56.47
Acid number (milligrams per gram)	6.84
Phenolic number (milligrams per kilogram)	403. 43
Saponification value (milligrams per gram)	393. 84
Wax number (percentage)	2.99
The refractive index (22 ^{oC})	1.47
Specific Weight (g per cubic centimeters)	0.91

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