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Effect of chitosan as a coating material on the chemical properties of local walnut oil

ABSTRACT

The study aimed to identify some of the chemical properties of the local walnut kernel oil, and to identify the effect of immersing the pulp in chitosan solution with three different concentrations (0.5%, 1.0% and 1.5%) on the quality of the cold extracted oil from these samples when stored for a period of four months. The acid value and free fatty acids % and peroxide value increased during storage, and the least increased was the samples treated with chitosan, especially the concentration 1.5%, but the Iodine value was decreased in all stored samples, but the lowest was the samples treated with chitosan, especially the concentration of 1.5%. The results showed that walnut oil contained many saturated and unsaturated fatty acids, but the highest amount of fatty acid was linoleic, then oleic, then linolenic, there was no change in the amount of saturated fatty acids, while the amount of unsaturated fatty acids decreased for untreated and chitosan-treated samples during storage, except that treatment with chitosan reduced the amount of losing.

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INTRODUCTION

Chitosan is a dietary fiber in the form of chains of linear polysaccharides derived from the chitin compound extracted from the exoskeleton of clams, oysters and crustaceans (Kaur and Vasundhara, 2014), however it undergoes some simple chemical treatments that give it certain advantages (Newkirk, 2013). Many applications of the food industry, especially in the field of food preservation, as biodegradable packaging materials and in the manufacture of health-promoting products. It was noted that its use in these products expands greatly, especially in products used to reduce weight or as a reducer of their fat content, and because of its ease of use and circulation, it can be used For packaging of fruits and vegetables (Giaconia *et al.*, 2020). Chitosan wrappers are ideal because of their gas permeable properties that are attributed to the hydrogen bonds between its chains (Lim *et al.*, 2012), and are flexible and conformable (Rabea *et al.*, 2003).

Walnut oil is a product of high nutritional value and contains a high percentage of bioactive compounds, including ω -3 and ω -6 unsaturated essential fatty acids. There is also a problem to protect the quality of the oil from oxidative changes when extracting the oil from the walnut kernel, in particular preventing the oxidation of polyunsaturated fatty acids. (Buranasompoba *et al.*, 2007).

The purpose of the study is to identify some of the chemical properties of the local walnut kernel oil, and to identify the effect of immersing the pulp in chitosan solution with three different

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concentrations (0.5%, 1.0% and 1.5%) on the quality of the cold extracted oil from these samples when stored for four months.

MATERIALS AND METHODS

Preparation of samples for packing

Chitosan Powder was obtained from Biorigins UK. Fresh *Juglans regia* Local walnut fruits were obtained after harvesting from walnut trees located in Al-Horman area in Sulaymaniyah Governorate / Iraq, the green husk and hard shell were removed to obtain the pulp only.

Chitosan solutions were prepared in three concentrations: 0.5%, 1%, and 1.5% in a solution of 1% acetic acid. Walnut pulp were immersed in the three solutions of chitosan 0.5%, 1% and 1.5% for 30-40 seconds separately, and then drying in an air oven at a temperature of 45 °C for 3 hours (Maghsoudlou *et al.* 2012). For comparison, the treated and untreated walnut kernel samples were stored in plastic containers after hermetically closed for 4 months at a temperature of 25 °C, and the tests were conducted for fresh samples and for treated and untreated samples after every two months of storage for a period of four months.

Extraction of walnut oil

walnut oil was extracted from the pulp, whether treated or not treated with chitosan, for fresh samples and for untreated samples, and treatment every two months from storage for four months on cold with a mechanical juicer, then centrifugation of the resulting oil for 30 minutes at a speed of 3500 rpm to produce a clear oil Free of impurities, ready for laboratory testing.

Chemical tests

The percentage of moisture in the oil was estimated based on what was mentioned in IUPAC, (2007) and the percentage of free fatty acids was estimated according to the method mentioned by AOCS (2009) and numbered Ca-Sa-40, and the acid value of walnut oil was estimated according to what was mentioned In AOCS (2009) numbered CD-3D-63, the peroxide value of walnut oil was estimated according to the method mentioned by AOCS (2009) numbered Cd-8-53, and the iodine value of walnut oil was determined using the method mentioned by AOCS (2009) numbered Cd. -1-25, fatty acids were estimated using a GLC (Gas Liquide Chromatography) device and according to what was stated in AOAC (2005) numbered 969.33 by a GLC gas liquid chromatography device produced by Hewletl Packard Company (438) University of Baghdad / College of Education for Girls / Laboratory Ibn Sina, and he used a metal column (Se: 30) 3 meters long and 1/8 mm in diameter, and the stationary phase was (DEGS) Diethylene Glycol Succinate at a concentration of 15% with the presence of the support material ChromosorbW with a diameter of 80-100 Mesh was the oven temperature 100 -300 ° C, with an average temperature rise of 10 ° C, and the temperature of the blue area of the sample was 300 °C and the temperature of the detector was 325 °C. The carrier gas was helium, with a flow rate of 30 milliliters per minute, and the sample volume was 1 microliter of heptane-dissolved oil.

The data were statistically analyzed according to the Complete Randomized Design (CRD) design and using the (SAS) Statistical Analysis System (2001) program, and the transactions were tested by Duncan's test to compare between the means and at the level of significance ($P \leq 0.05$).

RESULTS AND DISCUSSION

%Moisture

Table (1) shows that the moisture content of fresh walnut kernel oil is 0.20%, and that the highest moisture value was in the oil extracted from samples of walnut untreated with chitosan and stored for four months (0.24%), and also the same moisture value in the oil extracted from Walnut core samples treated with 1.5% chitosan and stored for two and four months, and the lowest value of moisture (0.20%) in the oil extracted from samples of walnut core treated with 0.5% chitosan and stored for four months. These results do not agree with what Jokić *et al.* (2014) mentioned that the moisture content in walnut oil is 0.11%. The reason for the difference in the results of the study with the results of other researchers is due to the different conditions and methods of extracting the oil from the walnut kernels. In the process of mechanical pressing and genetic factors of different varieties and breeding conditions, the results of the statistical analysis showed that there was no significant effect of chitosan treatment on the moisture content of stored oil.

Table (1): Percentage of moisture in treated walnut kernel oil with different concentrations of chitosan during storage

%Chitosan	% Moisture at storage period (day)		
	0	60	120
0	0.21 a	0.22 a	0.24 a
0.5	0.21 a	0.21 a	0.20 a
1	0.21 a	0.23 a	0.22 a
1.5	0.21 a	0.24 a	0.24 a

* Dissimilar letters differ significantly at the level (0.05) according to Duncan's test to compare between means.

% free fatty acids

It is noted from the results in Table (2) that % of free fatty acids are estimated as oleic acid for fresh walnut oil 0.23%, and this value increased during the storage period for these samples after two months of storage to 0.31% and to 0.36% after four months of storage to record the highest value, while the increase for this trait was significantly less compared to its values for the untreated samples during the storage period. 1.5% and 1%, which was 0.24%, these results are close to what Leahu *et al.* (2016) stated that the free fatty acids of walnut oil estimated as oleic acid ranged from 0.20% - 0.23%, and the results of the study also agree with what researchers Patras and Dorobantu (2010) found that the % of the free fatty acids of walnut oil ranged between 0.20% - 0.31% (oleic acid), and thus the chitosan used in our study and the percentages used to coat the walnut pulp had maintained the level of free fatty acids within the limits mentioned by the researchers. Therefore, in addition to the role of chitosan as an inhibitor of lipolytic enzymes (lipase) and its ability to impede oxidative rancidity and reduce the triple products of this phenomenon, including the formation of short-chain organic acids (Jin *et al.*, 2017). The results of the statistical analysis showed a significant effect of adding chitosan at the concentrations used in the study on the percentage values of acids. The estimated free fatty acids as oleic acid, where significant differences were observed at the level (0.05) between the mean values of % of free fatty acids for chitosan-treated and untreated samples during storage for a period of four months.

Table (2): % of free fatty acids (oleic acid) for treated walnut kernel oil with different concentrations of chitosan during storage

%Chitosan	% free fatty acids at storage period (day)		
	0	60	120
0	0.23 f	0.31 b	0.36 a
0.5	0.23 f	0.26 d	0.28 c
1	0.23 f	0.24 f	0.27 c
1.5	0.23 f	0.24 f	0.25 e

* Dissimilar letters differ significantly at the level (0.05) according to Duncan's test to compare between means.

Acid value

Table (3) shows the acid values of walnut oil extracted from kernel samples treated and not treated with chitosan. The results showed an increase in the acid value during storage for all samples give varying degrees. The result was before storage is 0.16 mg / KOH gm of oil, this result is close to the limits reported by Sandulachi *et al.* (2019) that the acid value of walnut oil ranges from 0.17 mg / KOH gm oil - 5.05 mg / KOH gm oil) and close to the value mentioned by Gharibzahedi *et al.* (2012) that the acid value of walnut oil ranges from 0.12 mg/KOH gm oil - 0.14 mg/KOH gm oil, while it does not agree with what Popovici *et al.* (2012) found that the acid value of walnut oil is 0.94 mg/KOH gm oil, and it does not agree with what was mentioned by Martinez *et al.* (2013). that the acid value of walnut oil is 0.08 mg / KOH gm of oil, and this difference may be attributed to genetic factors, different varieties, breeding conditions and estimation methods used to estimate this trait. The highest value was recorded after storage for four months and which amounted to 5.98 mg/KOH gm of oil, which indicates the occurrence of the phenomenon of

decomposition rancidity in the comparison samples at high rates during the storage process. The oil extracted from kernel samples treated with chitosan at a concentration of 0.5% after two months of storage (1.66 mg/KOH gm of oil), and it rose to 2.54 mg/KOH gm of oil after four months of storage, while the acid value in the oil extracted from treated walnut kernel samples with chitosan at a concentration of 1% after two months of storage, it was 0.92 mg/gm of oil, and it increased to 1.43 mg of KOH/g of oil after four months of storage, and it increased to 0.57 mg/KOH gm of oil after four months of storage, in light of these results it is clear that chitosan has a role in reducing the rate of rancidity (Nowzari *et al.*, 2013). The results of the statistical analysis showed significant differences ($P \leq 0.05$) between the average acid values For samples treated with chitosan and with concentrations For used and untreated samples during storage for a period of four months.

Table (3): Acid value (mg KOH/gm oil) of treated walnut kernel oil with different concentrations of chitosan during storage

%Chitosan	Acid value at storage period (day)		
	0	60	120
0	0.16 h	3.42 b	5.98 a
0.5	0.16 h	1.66 d	2.54 c
1	0.16 h	0.92 f e	1.43 e d
1.5	0.16 h	0.36 h g	0.57 g f

* Dissimilar letters differ significantly at the level (0.05) according to Duncan's test to compare between means

.Peroxide value

Table (4) shows a significant difference ($P \leq 0.05$) between the peroxide values of walnut oil extracted from kernel samples treated and not treated with chitosan. / kg oil, and an increase in this value was observed during storage in samples treated and not treated with chitosan, while the highest increase in oil extracted from samples of walnut core not treated with chitosan reached 2.23 mm equivalent O₂ / kg oil after four months of storage, while this The rise gradually decreased with the increase in the concentration of chitosan solution used in covering the kernels of the walnut, where the lowest rise in the value of peroxide was recorded in the oil extracted from samples of walnut pulp treated with chitosan at a concentration of 1.5% after two months of storage (0.98 mEq O₂ / kg oil), observed through these results are within the limits mentioned by Arslan (2010) that the peroxide value of walnut oil ranges from 0.81-1.27 mEq O₂ / kg oil, excluding the peroxide value of the oil extracted from samples of treated walnut kernels ,after four months of storage (2.23 mEq O₂ / kg oil), while the peroxide values of the oil extracted from the rest of the walnut samples ranged within these limits. Storage, which indicates that it possesses an antioxidant property and in turn led to a reduction in the oxidative rancidity rate of walnut oil during storage (Ferreira *et al.*, 2018). The results of the statistical analysis showed that there were significant differences at the level of 0.05 between the average values of peroxide for samples treated with chitosan at the concentrations used in the study and for untreated samples during Storage for four months.

Table (4): Peroxide values (mO₂ equivalent / kg oil) of treated walnut kernel oil with different concentrations of chitosan during storage

%Chitosan	Peroxide values at storage period (day)		
	0	60	120
0	0.93 g	1.24 b	2.23 a
0.5	0.93 g	1.08 d	1.25 b
1	0.93 g	1.04 e	1.19 c
1.5	0.93 g	0.98 f	1.07 d

* Dissimilar letters differ significantly at the level (0.05) according to Duncan's test to compare between means.

Iodine value

Table No. (5) shows the iodine value of oil samples extracted from local walnut pulp treated and not treated with chitosan during storage. the iodine values in the oil extracted from fresh walnut core samples not treated with chitosan was (129.76), and this value decreased during storage in the samples treated and non-treated with chitosan, as it reached the lowest value in the oil extracted

from samples of walnut core not treated with chitosan after four months of storage (123.74, which is the highest significant level of change in the iodine value, while the decrease gradually decreased with the increase in the concentration of chitosan, which was treated with walnut pulp, to reach 129.56 in the sample of walnut oil treated with chitosan at a concentration of 1.5% and stored for two months, 129.56, then stored for four months (129.13). , Martínez *et al.* (2013) reported that the iodine value of fresh walnut oil is about 157, while Singh *et al.* (2010) reported that the iodine value of fresh walnut oil is about 96.39, in which the results were close to Saxenaa *et al.* (2009) mentioned that the iodine number of fresh walnut oil is 127.64, these differences are due to the difference in environmental conditions and genetics. In general, it is inferred from the results that the loss in unsaturated fatty acids was less for chitosan-treated samples compared to untreated samples, which indicates that chitosan possesses the effectiveness Antioxidant, which gave the oil a protective action against the catabolism of unsaturated fatty acids.

Table (5): Iodine value of treated walnut kernel oil with different concentrations of chitosan during storage

%Chitosan	Iodine value at storage period (day)		
	0	60	120
0	129.76 a	126.82 f	123.74 h
0.5	129.76 a	127.64 e	125.34 g
1	129.76 a	128.37 d	127.63 e
1.5	129.76 a	129.56 b	129.13 c

* Dissimilar letters differ significantly at the level (0.05) according to Duncan's test to compare between means.

The type and quantity of fatty acids

Table (6 and 7) shows the type and quantity of fatty acids of the local walnut kernel oil. The results showed that the fresh oil contained the following saturated fatty acids: Lauric acid (C-12) with an amount of 0.46% and Myristic acid (C -14) in an amount of 0.33% as medium-chain fatty acids, palmitic acid (C-16) in an amount of 6.29% and stearic acid (C-18) in an amount of 2.44% as long-chain fatty acids, and it contains the following unsaturated fatty acids: Palmitoleic acid Δ 9. (16:1) ω -7 acid in the amount of (0.55%), oleic acid (18:1, ω -9, Δ 9) in the amount of 19.83%, and linoleic acid (18:2, ω -6, Δ 9 , 12) with an amount of 59.70% and linolenic acid (18:3, ω -3, Δ 9, 12, 15) in an amount of 10.52%. From these results, it is clear that unsaturated fatty acids are dominant in walnut and linoleic acid constitutes the largest proportion followed by Oleic acid then linolenic acid, and hence walnut oil can be considered a source of the essential fatty acid linoleic acid. Low levels of saturated fatty acids, so that their quantity did not exceed 10% of the total fatty acids of walnut oil. Tables 6 and 7 show the change in the fatty acid content of walnut oil during storage for a period of four months, whether for oil extracted from untreated samples or treated with chitosan with the concentrations used in The study, especially with regard to unsaturated fatty acids, it was observed from the results that there was no significant change in the oil content of saturated fatty acids, whether for samples treated with chitosan or not treated with it during storage for a period of four months, and this may be attributed to the stability of these acids towards the factors causing their demolition, especially the interactions of Oxidative rancidity, and it is noticed from the results, with regard to unsaturated fatty acids and for samples not treated or treated with chitosan, a significant decrease in their quantity, especially for samples not treated with chitosan, while the least decrease in its quantity was in samples treated with chitosan, especially the concentration of 1.5% during storage for four months. The results also showed that the most affected fatty acid in the storage process is linolenic acid because it is considered one of the many fatty acids, It is unsaturated and then quickly destroyed by oxidative rancidity reactions.

Table (6): Type and quantity (%) of saturated fatty acids of treated walnut kernel oil with different concentrations of chitosan during storage

%Chitosan	of saturated fatty acids at storage period (day)%		
	0	60	120
Lauric acid			
0	0.460 a	0.450 a	0.405 a
0.5		0.411 a	0.454 a
1		0.445 a	0.431 a
1.5		0.414 a	0.434 a
Myristic acid			
0	0.33 a	0.31 a	0.34 a
0.5		0.33 a	0.30 a
1		0.30 a	0.33 a
1.5		0.32 a	0.33 a
Palmitic acid			
0	6.290 a	6.193 a	6.142 a
0.5		6.131 a	6.116 a
1		6.159 a	6.197 a
1.5		6.131 a	6.229 a
Stearic acid			
0	2.440 a	2.419 a	2.409 a
0.5		2.444 a	2.402 a
1		2.439 a	02.441 a
1.5		2.440 a	2.433 a

* Dissimilar letters differ significantly at the level (0.05) according to Duncan's test to compare between means.

Table (7): Type and quantity (%) of unsaturated fatty acids of treated walnut kernel oil with different concentrations of chitosan during storage

%Chitosan	of unsaturated fatty acids at storage period (day)%		
	0	60	120
Palmitoleic acid			
0	0.55 a	0.35 c	0.19 e
0.5		0.40 b	0.30 d
1		0.44 b	0.34 c
1.5		0.50 a	0.41 b
Oleic acid			
0	19.83 a	19.51 e	19.37 f
0.5		19.60 b	19.44 d
1		19.61 b	19.50 c
1.5		19.70 a	19.63 a b
Linoleic acid			
0	59.70 a	59.33 d	59.01 f
0.5		59.50 b	59.19 e
1		59.50 b	59.30 d
1.5		59.55 b	59.41 c
Linolenic acid			
0	10.52 a	10.18 c	10.09 d
0.5		10.41 b	10.20 c
1		10.40 b	10.25 c
1.5		10.49 b	10.37 c

* Dissimilar letters differ significantly at the level (0.05) according to Duncan's test to compare between means.

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تأثير الكيتوسان كمادة تغليف على الصفات الكيميائية لزيت الجوز المحلي

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الخلاصة

استهدفت الدراسة التعرف على بعض الخواص الكيميائية لزيت لب ثمار الجوز المحلي, والتعرف على تأثير غمر اللب بمحلول الكيتوسان بثلاثة تراكيز مختلفة (0.5% و 1.0 % و 1.5 %) على نوعية الزيت بطريقة الإستخلاص البارد من هذه العينات عند الخزن لمدة أربعة اشهر.

إزدادت قيمة الرقم الحامضي والنسبة المئوية للأحماض الدهنية الحرة و قيمة البيروكسيد بإستمرار الخزن إلا إن أقلها إرتفاعاً العينات المعاملة بالكيتوسان وخاصة التركيز 1.5% ، كما إنخفض الرقم اليودي بإستمرار الخزن إلا أن أقل إنخفاض كان في العينات المعاملة بالكيتوسان وخاصة التركيز 1.5 % , وبينت النتائج إحتواء زيت الجوز على العديد من الأحماض الدهنية المشبعة وغير المشبعة ألا إن أعلاها كمية الحامض الدهني اللينوليك ثم الأوليك ثم اللينولينيك , كما لم يحدث تغير في كمية الاحماض الدهنية المشبعة بينما إنخفضت كمية الأحماض الدهنية غير المشبعة للعينات غير المعاملة والمعاملة بالكيتوسان خلال الخزن إلا إن المعاملة بالكيتوسان خفض من مقدار الفقد في كميتها.

الكلمات المفتاحية:
الكيتوسان ، الأغلفة الحيوية ، الجوز ، زيت الجوز.