A COMPARATIVE STUDY TO EVALUATE THREE IMPORTANT PLANT OILS FOUND IN EGYPT

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ABSTRACT: *Oils are a very important component in different diets, which makes it important for researchers to constantly search for a variety of sources of these oils that are acceptable to consumers on the one hand and are characterized by high nutritional value and storage properties on the other hand. This study was carried out to determine physical properties of linseed, soybean and walnut oils (Refractive index, color, specific gravity) as well as chemical properties (Acid value, saponification value, ester value, iodine value and peroxide value). The percentage of unsaponifiable matter and stability induction period at 100◦C were also estimated. To determine the nutritional value of these oils, both their fatty acid content (assessed by the gas chromatography apparatus) and sterol content (assessed by GC-MS). Physical properties of linseed, soybean or walnut oils showed that; refractive index were 1.48, 1.47 and 1.84,   respectively, while color (red) intensity were 9.5, 3.5 and 2.6, respectively and specific gravity were 0.93, 0.92 and 0.92, respectively. On the other hand, chemical properties of linseed, soybean or walnut oils showed that; saponification value were 192, 191 and 190.73(mg KOH/g oil), respectively, while acid value were 0.87, 0.27 and 0.79 (mg KOH/g oil), respectively, and iodine value were 175, 128 and 145 (g I_2/100 g oil), respectively, finally, peroxide value were 1.95, 0.77 and 1.31 (Meq oxygen/Kg), respectively. Alpha linolenic acid was the major fatty acid in linseed oil (60.21%), while linoleic acid was the main fatty acid in both, soybean oil (52.83%) and walnut oil (61.85%). β-sitosterol was the main sterol in the linseed, soybean or walnut oils (54.72, 40.13 and 76.85%, respectively). As shown by the results, the three studied oils showed high content of essential fatty acids, (both omega-3 and omega-6), making them promising oils to support diets with essential fatty acids.*

Key words: *Alpha- linolenic acid, linoleic acid, linseed oil, soybean oil, walnut oil, Physical properties and chemical properties of oils, sterols.*

INTRODUCTION

National Heart Association has recommended increasing consumption of fatty fish or omega-3 polyunsaturated fatty acids (PUFAs) supplements to prevent CVD. Non- fish sources of omega-3 PUFAs vary in their capacity to regulate blood levels of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) (C20, 22, omega-3 PUFAs) and CVD risk factors (Jump *et al.,* 2012). Therefore, many studies have been conducted to verify the effectiveness of certain foods in the prevention of these cardiovascular diseases (De Miranda *et al.,* 2014). On the other hand, high omega-3/omega-6 ratio, promote the pathogensis of cardiovascular diseases (Simopoulos, 2008). Meanwhile, omega-3 fatty acids can improve arterial and endothelial function and decrease the risk of thrombosis (Kris-Etherton *et al.,* 2003). Effects of different types of PUFAs on body adiposity are controversial. However, the issues remain to be elucidated regarding the optimal dosage of omega-3 fatty acids and the ratio of omega-3 to omega-6 fatty acids (Kromhout *et al.,* 2012).

Linseed oil is the only oil of plant origin known to have the highest concentration of alpha-linolenic acid

(ALA). Linseed oil has been tested in clinical trials that have described its potential beneficial effect against specific disorders, such as dyslipidemia and cardiovascular diseases (Lemos *et al.,* 2012 and Makni *et al.,* 2008). Soybean is the world's most important legume crop, and the most widely commercialized oilseed growing in different climates worldwide (Pavlova, 1989). Soybean oil is one of the main oils that contains high amounts of monounsaturated and polyunsaturated fatty acids (MUFAs and PUFAs). This specific fatty acid composition helps to reduce blood cholesterol fractions, thus lowering the risk of heart disease. However, soybean oil is highly susceptible to oxidative process (Naz *et al.,* 2005). Among nut oils, walnut oil contains the highest amount of PUFAs (Amaral *et al.,* 2003). The fatty acid composition of walnut oil is unique compared to other nuts oil because walnut oil contains predominantly, linoleic acid (49 to 63 %), and a considerable amount of ALA (8 to 15.5%) (Moigradean *et al.,* 2013). Therefore, this study aims to identify the oils of linseed, soyabean and walnut by studying their physical and chemical properties in addition to studying their contents of fatty acids and sterols to discuss the possibility of adding them to the food oils used to improve their properties and health effects.

MATERIALS AND METHODS
Materials:
Linseed (*Linum usitatissimum* L.), Golden cultivar was obtained from Research Station of Gemiza, Gharbia, Egypt. Soybean (*Glycine max* L.), Gemiza 111 cultivar was obtained from agriculture research centre, Giza, Egypt. Walnut (*Juglans regia* L.), was obtained from local market and identified by horticulture department faculty of agriculture, Menoufia University.

Methods:
Extraction of linseed and soybean oils:
The seeds of linseed (*Linum usitatissimum L.*) and soybean (*Glycine max L.*) were cleaned and render them free of dust, then oil was obtained by hydraulic piston (model no: 6Y) and subsequently filtrated (filter press).

Extraction of walnut oil:
Seeds of walnut (*Juglans regia L.*) were manually cracked and shelled, then milled into a fine powder in an electric mill (Braun, model 1021), and then soaked in pure n-hexane for 24 hours. The mixture were collected and filtered. This process was repeated three times using new solvent each time. The solvent was evaporated under vacuum at 40-45ºC in rotary evaporator (HAHN SHIN) model-HS-2005-N, under vacuum, made in Korea.

Physical Characteristics of oils:
1- Determination of refractive index:
Refractive index of oil samples was measured using Abbè refractometer (Carl Zeiss JENA, GDR, made in China) at 25ºC according to the Method of (A.O.A.C. 2000).

2- Determination of color:
A lovibond tintometer (Model F, Visual, made in China) was applied to measure the color using 5.25 inch cell according to the method of the (A.O. C. S. 1989).

3- Determination of Specific Gravity:
Specific Gravity of oil samples was measured using a 10-ml automatic gas pycnometer at 30ºC according to the Method of (A.O.A.C. 2000).

Chemical Characteristics of oils:
1- Determination of Acid value (AV):
Acid value was determined according to (A.O.A.C. 2003). 3 g of oil were dissolved in 25 ml ethanol; the mixture

was titrated by 0.1 N alcoholic potassium hydroxide using phenolphthalein indicator and `acid value was calculated as follows:

Acid value (mg KOH/g oil) = $\frac{56.1 \times N \times V}{W}$

Where: N = normality of KOH.   W = weight of oil in g.

V = volume (ml) of KOH. 56.1 = equivalent weight of KOH.

2- Determination of Saponification value (SV):

Saponification value was determined according to (A.O.A.C. 1995). 5 g filtered oil was boiled with alcoholic potassium hydroxide solution (100 ml, 0.5%) for about 30 min. The reaction mixture was cooled down and then titrated with hydrochloric acid solution (0.5 N) using phenolphthalein as an indicator. Another experiment was carried out as a blank without the addition of the oil sample. The calculation was made according to the following equation:

Saponification Value (mg KOH/g oil) = $\frac{(B-S) \times N \times 56.1}{W}$

Where:

B = volume of hydrochloric acid solution 0.5 N required by blank.

S = volume of hydrochloric acid solution 0.5 N required by oil.

N = normality of hydrochloric acid solution.

56.1= equivalent weight of KOH.   W = weight of oil in g.

3- Determination of Ester Value (EV):

The ester value was calculated by the following equation according to (A.O.A.C. 2003).

Ester value = (Saponification value - Acid value).

4- Determination of Iodine value (IV) (Wijs Method):

The iodine value was determined according to (Singh et al., 1981). 0.25g of the oil sample is treated with an excess of iodine bromide (IBr) in glacial acetic acid. Unreacted iodine bromide is reacted with potassium iodide, which converts it to iodine. The iodine concentration is then determined by titration with standard sodium thiosulphate (0.1N). Iodine value was calculated by using the following equation:

Iodine Value (gl$_2$/100g oil) = $\frac{(B-S) \times N \times 127 \times 100}{W \times 1000}$

Where: B = volume in ml of $Na_2S_2O_3$ used in blank.

S = volume in ml of $Na_2S_2O_3$ used in sample.

W = weight of oil in g.

127 = equivalent weight of iodine.

5- Determination of Peroxide value (PV):

Peroxide value was determined according to (A.O.A.C. 1984). 4g of oil sample was dissolved in a mixture of acetic acid and chloroform (3:2; v/v) and saturated KI solution is added to the sample and the amount of iodine liberated from KI by the oxidative action of peroxides present in the oil is determined by titration with standard sodium thiosulphate using starch solution as an indicator. Titration was also performed for blank's peroxide value. Peroxide value was calculated by using the following equation:

Peroxide value (Meq oxygen/kg) = $\frac{(S-B) \times N \times 1000}{W}$

Where: B: volume of sodium thiosulphate used in blank.

W: weight of oil in g.

N: normality of standard sodium thiosulphate.

6- Determination of Oxidative Stability Index:

The oxidative stability index is measured by an accelerated oxidation test using the well established rancimate method. Stability is expressed as the oxidation induction time (hour) which is measured with a Rancimat 679 apparatus (Metrohm Co., Switzerland), using an oil sample of 5 g warmed to 120ºC, and 20 liters/ hour air flow. The time taken to reach a fixed level of conductivity was measured (Laubli and Bruttel., 1986). This assay was conducted in Oils & Fats Research Dept., Food Technology Research Institute, Agriculture Research Center, Egypt.

7- Determination of Unsaponifiable Matter (%):

Unsaponifiable matter was determined according to the method described in (A.O.A.C. 2000). A known weight of the oil (ca, 2-2.5g) was dissolved in ethanol (25 ml), and then KOH solution (10% w/v) was added. The oil was saponified on water bath for 30 min under reflux air condenser. The alcoholic soap solution was quantitatively transferred into separator funnel using a total volume of 50 ml of distilled water and 50 ml of petroleum ether. The unsaponifiable matter was extracted three

times with petroleum ether, washed several times with distilled water and dried over anhydrous sodium sulphate and then filtered into a weighted flask. The solvent was evaporated using a boiling water bath and the flask was dried at 105°C until constant weight was reached. The percentage of unsaponifiable matter was calculated according to the following equation:

% unsaponifiable matter = weight of the residue/weight of oil ×100

8- Determination and identification of sterols:

The GC-MS analysis of the sterols samples was carried out according to (Soupas et al., 2004) by using gas chromatography– mass spectrometry instrument stands at the Laboratory of medicinal and aromatic plants, National Research Center with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TR-5MS column (30 m x 0.32

mm i.d., 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 0.8 mL/min at a split ratio of 1:10 and the following temperature program: 50 C for 3 min; rising at 5 C/min to 300°C and held for 15 min. The injector and detector were held at 220° and 200°C, respectively. Diluted samples (1:10 hexane, v/v) of 1 µL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. The compounds were identified using mass spectra (authentic chemicals and Wiley spectral library collection).

9- Determination and identification of fatty acids:

Fatty acids analyses of oils were performed by gas chromatography (GC). Samples were transformed into fatty acid methyl esters (FAME) with methanolic boron trifluoride (12%) methanolic solution and were determined on a gas chromatograph with FID detector (Perkin Elmer Auto System XL) with auto sampler and Ezchrom integration system. Carrier

gas (He); ca.25 Psi – air 450 ml / min – Hydrogen 45 ml – split 100 ml/min. Oven temperature was 200 °C while for injector and detector it was 250 °C according to (A.O.A.C. 2012).

Results and Discussion

1- Physical characteristics of linseed, soybean or walnut oils:

Data in Table (1) showed that linseed oil recorded the highest value in both parameters i.e. refractive index and specific gravity (1.49 and 0.93, respectively) followed by walnut oil (1.48 and 0.92, respectively) while soybean oil showed the lowest values (1.47 and 0.92, respectively). On the other hand, color (red) showed different order; linseed oil was the highest value (9.5) followed by soybean oil (3.5) and finally walnut oil was (2.6).

These results are similar to that of several previous studies conducted on the three types of oils (Rabrenovic et al., 2011; Tenva et al., 2014; and Uzunovaet al., 2015), which may be explained by the fact that these values reflect the characteristic of physical properties for these types of oils, which do not vary by place of study, but may vary depending on the plant variety used in the experiment.

Table (1): Physical characteristics of linseed, soybean and walnut oils:

Physical characteristics	Type of oil		
	Linseed	Soybean	Walnut
Refractive index	1.49	1.47	1.48
Color(red)	9.50	3.50	2.60
Specific gravity	0.93	0.92	0.92

2- Chemical characteristics of linseed, soybean or walnut oils:

Determination of the chemical characteristics of oils gives us an idea about the chemical composition of oils, and so their efficacy to storage process and their validity for use.

Data in Table (2) revealed that linseed oil recorded the highest values for acid value, iodine value and peroxide value (0.87mg KOH/g oil, 175gI$_2$/100g oil and 1.95Meq oxygen/kg, respectively) followed by walnut oil (0.79mg KOH/g oil,

145gI$_2$/100g oil and 1.3Meq oxygen/kg, respectively), whilst for soybean oil they were (0.27mg KOH/g oil,128gI$_2$/100g oil and 0.77Meq oxygen/kg, respectively).

On the other hand, unsaponifiablematter (%) showed different order: linseed oil was the highest value 1.03%followed by soybean oil 0.78% and the lowest one was for walnut oil 0.48%.

Walnut oil also recorded the highest amounts for saponification value and ester value (194mg KOH/g oil and 193.21 respectively) followed by linseed oil (192mg KOH/g oil and 191.13 respectively) whereas for soybean oil they were (191mg KOH/g oil and 190.73 respectively).

Finally soybean oil recorded the highest value of stability induction period at 100°C, (10.7 hour) followed by walnut oil (4.86 hour) whilst it was for linseed oil (3.94 hour).

These results are in agreement with those of Rabrenovicet al., (2011) and Viorica-Mirela et al., (2012). The reason of the rapprochement of these values is that they are normal values for these oils at ideal conditions of storage especially acid value, peroxide value and stability induction.

Table (2): Chemical characteristics of linseed,soybean or walnut oils:

Chemical Characteristics	Type of Oil		
	Linseed	Soybean	Walnut
Acid value (mgKOH/g oil)	0.87	0.27	0.79
Saponification value (mg KOH/g oil)	192	191	194
Ester Value	191.13	190.73	193.21
Iodine value (gI$_2$/100g oil)	175	128	145
Peroxide value(Meq oxygen/kg)	1.95	0.77	1.31
Unsaponifiable Matter (%)	1.03	0.78	0.48
Stabilityinduction period at 100°C in hour	3.94	10.7	4.86

3- Identification of sterols composition in linseed, soybean or walnut oils (% of total sterols):

Data in Table (3) indicated that sitosterol was the major sterol in the investigated linseed, soybean or walnut oils, (54.72%, 40.13% and 76.85%, respectively) followed by campesterol (22.60%, 24.22% and 16.11%, respectively) while

the lowest values were for stigmasterol (16.01%, 21.28% and 7.04%, respectively), and lanosterol in linseed and soybean oils was, (6.66% and 14.36%, respectively) while it was not detected in walnut oil. These results agree with those of Gunstone *et al.,*(1994); Martinez *et al.,*(2010) and Tenva *et al.,*(2014).

Table (3): Identification of sterols composition in linseed, soybean or walnut oils (% of total sterols)

Sterols	Type of Oil		
	Linseed	Soybean	Walnut
β-Sitosterol	54.72	40.13	76.85
Campesterol	22.60	24.22	16.11
Stigmasterol	16.01	21.28	7.04
Lanosterol	6.66	14.36	ND

4.4. Identification of fatty acids composition in linseed, soybean or walnut oils (% of total fatty acids):

Data in Table (4) stated that linseed oil revealed the highest amount of alpha-linolenic acid (C18:3, omega-3), (60.21%) followed by walnut oil (12.93%) and soybean oil (7.12%) while walnut oil exhibited large amount of linoleic acid (C18:2, omega6), (61.85%) followed by soybean oil (52.83%)and linseed oil (14.35%).

On the other hand, soybean oil recorded the highest percentage of oleic acid (C18:1, omega-9), (22.66%) followed by walnut oil (14.71%) and linseed oil (13.49%).

Data also showed that total saturated / total unsaturated fatty acids ratio was (1:8.93) for walnut oil followed by linseed oil (1:7.70) then it was for soybean oil (1:4.87).

On the other hand the ratio of omega-3 to omega-6 fatty acids showed the highest level for linseed oil (4.14:1) followed by walnut oil (0.21:1) and soybean oil (0.14:1) at the same trend omega-3: omega-9 ratio recorded the highest level for linseed oil (4.39:1) followed by walnut oil (0.87:1) and soybean oil (0.31:1).

Linseed, soybean or walnut oils showed a high level of total unsaturated fatty acids, reached to over than 80%, also walnut and soybean oils presented a high levels of linoleic

acid, reached to over than 50%,while linseed oil exhibited over than 60% of alpha-linolenic acid.

Table (4): Identification of fatty acids composition in linseed,soybean or walnut oils (% of total fatty acids):

Fatty acids	Type of oil		
	Linseed	Soybean	Walnut
C16:0	5.71	11.88	7.09
C18:0	5.39	4.44	2.88
C20:0	0.17	0.36	0.09
C22:0	0.19	0.34	ND
Total saturated F.A	11.5	17.04	10.07
C16:1	0.07	0.05	0.08
C18:1	13.49	22.66	14.71
C18:2 omega-6 F.A	14.35	52.83	61.85
C18:3 omega-6 F.A	0.20	ND	0.11
C18:3 omega-3 F.A	60.21	7.12	12.93
C20:1	0.14	0.25	0.24
Total unsaturated F.A	88.5	82.96	89.93
Total saturated/Total unsaturated F.As	1:7.70	1:4.87	1:8.93
$\sum$ omega-3fatty acids	60.21	7.12	12.93
$\sum$ omega-6fatty acids	14.55	52.84	61.96
$\sum$ ω-9 fatty acids	13.73	22.96	15.03
omega-3: omega-6 ratio	4.14:1	0.14:1	0.21:1
omega-3: omega-9 ratio	4.39:1	0.31:1	0.87:1

These results are parallel with those of Dubois et al., (2007); Harper et al., (2006); Simpolous, (2004); and Tolkachev and Zhuchenko, (2000).

It can be concluded from such results that the three oils are promising sources for essential fatty acids because of the high proportion of essential fatty acids in general (linoleic acid C18:2, omega 6 and alpha-linolenic acid C18:3, omega-3), where these fatty acids play an important role in the synthesis of many vital compounds needed by humans, which requires

eating with food, In particular, flaxseed oil had a very high content of alpha-linolenic acid (the precursor of other acids in omega-3 family), which necessitates feeding experiments on experimental animals to study its effect on the synthesis of EPA and DHA, which are considered essential steps in the synthesis of Eicosanoids as a prelude to the synthesis of prostaglandins and leukotrienes.

Conclusion

It is possible to deduce the importance of flaxseed, soybean, or walnut oils from the results obtained through this study, whether the physiochemical properties or their content of unsaturated fatty acids and essential fatty acids as well as sterols, which requires further work to evaluate their effect in improving the contents of essential fatty acids especially omega-3 fatty acids when added to diets in experimental animals.

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الملخص العربي

تعتبر الزيوت عنصرًا مهمًا جدًا في الأنظمة الغذائية المختلفة ، مما جعل من المهم لدي الباحثين استمرار البحث عن مجموعة متنوعة من مصادر هذه الزيوت المقبولة للمستهلكين من جهة وتتميز بالقيمة الغذائية العالية وخصائص التخزين من جهة أخرى. أجريت هذه الدراسة لتحديد الخواص الفيزيائية لزيوت الكتان ، فول الصويا ، وعين الجمل (معامل الانكسار ، اللون ، الكثافة النوعية) وكذلك الخواص الكيميائية (رقم الحامض، رقم التصبن، رقم الإستر ، الرقم اليودي ورقم البيروكسيد). كما تم تقدير النسبة المئوية للمادة الغير متصبنة وفترة الثبات علي 100 درجة مئوية. ولتحديد القيمة الغذائية لهذه الزيوت تم تقدير محتواها من الأحماض الدهنية (بواسطة جهاز كروماتوجراف الغاز) وكذلك محتواها من الإستيرولات (بوسطة جهاز GC-MS). وقد أظهرت الخصائص الفيزيائية لزيوت بذر الكتان و فول الصويا وعين الجمل أن معامل الانكسار كان (1,48 ، 1,47 , 1,84) ، على التوالي ، في حين كانت كثافة اللون (9,5, 3,5 , 2,6) , على التوالي وكانت الكثافة النوعية (0,93 , 0,92 , 0,92) , على التوالي. من ناحية أخرى ، أظهرت الخصائص الكيميائية لزيوت بذر الكتان و فول الصويا و عين الجمل أن رقم التصبن كان 192 , 191 , 190,73 (مللجرام KOH / جرام زيت), على التوالي ، في حين كان رقم الحامض 0,87 , 0,27 , 0,79 (مللجرام KOH / جرام زيت), على التوالي ، وكانت قيمة الرقم اليودي 175 , 128 , 145 (جرام يود/ 100جرام زيت), على التوالي ، وأخيرا ، كانت قيمة رقم البيروكسيد 1,95 ، 0,77 , 1,31 (مللميكافئ أوكسجين / كجم زيت), على التوالي. كان حمض ألفا لينولينيك هو الحمض الدهني الرئيسي في زيت بذر الكتان (60,21 ٪) ، في حين كان حمض اللينوليك هو الحمض الدهني الرئيسي في كل من زيت فول الصويا (52,83 ٪) وزيت عين الجمل (61,85 ٪). كان بيتا سيتوستيرول هو الإستيرول الرئيسي في زيوت بذر الكتان وفول الصويا وعن الجمل (54,72 ، 40,13 , 76,85٪), على التوالي. وبناء علي ما أظهرته النتائج فإن الزيوت الثلاثة تحت الدراسة تحتوي علي نسبة عالية من الأحماض الدهنية الأساسية (كلا من أوميجا-3 وأوميجا-6) ، مما يجعلها زيوت واعدة لدعم الأنظمة الغذائية التي تحتوي على الأحماض الدهنية الأساسية.