

Quality Attributes of Hibiscus Seed Oil Compared with Soybean Oil

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ABSTRACT

The hibiscus and soybean seeds were studied in terms of some chemical composition including protein, oil, carbohydrates, fiber, ash and mineral contents, also physical and chemical properties, fatty acid composition, triacylglycerol (TAG), some bioactive compounds and some parameters as indicator of oxidative stability [predict oxidizability (O.S), calculated oxidizability (COX) and atherogenic index (AI)] of these oils extracted from previous these seeds. Results showed that soybean seed had higher values of oil, protein, ash and mineral contents, (i.e, NPK) compared to hibiscus seed. While, hibiscus seed had the highest level of total carbohydrates and crude fiber. Also data indicated that the values of parameters of physical and chemical properties and fatty acids composition of hibiscus oil parallel with those of soybean oil, also these parameters were corresponded coincided within the range of other edible vegetable oils. The results showed that, the total polyphenols and pigment contents of hibiscus oil were higher than those of the soybean oil, and vice versa for (O.S) and (COX). Also results indicated that the levels of TAG [LLL, LLO and (LOO+ LLS)] with ECN42, 44 and 46 of soybean oils were (7.31, 18.37 and 16.8%, respectively.) being higher than that in hibiscus oil (1.27, 11.09 and 13.26%, respectively.).

Keyworded: Chemical composition of hibiscus and soybean seeds physicochemical properties, fatty acid composition, bioactive components, stability and triacylglycerol of oils.

INTRODUCTION

One of the possible alternative crop is Hibiscus Sabdariffa, also known as Sorrelor Roselle. It is an herb belonging to the malvaceae family which is grown in Nigeria, India and west indies, and to some extent in tropical America. (Mohamed *et al*, 2007 and Nakpong and Woothikanokkhan 2010).

Roselle can be found in almost all warm countries such as India, Saudi Arabia, Malaysia, Indonesia, Thailand, Philippines, Vietnam, Sudan, Egypt and Mexico. Roselle plants are cultivated for their sepals, which are used as hot or cold drink. In Egypt, roselle produce 900Kg seeds / Feddan as an added value product. The total area in old and new land is 12000 Feddans (MOA,2018). This mean that, there is about 10800 tons of seeds that are produced. The roselle seeds contain about 17% of fixed oil, in another word, about 1836 tons of fixed oil that could be produced / year. This amount of oil can reduce the gap between production and consumption in Egypt. Roselle seed oil is of unsaturated type and contains mainly fatty acid oleic (47.8%) and linoleic (30%), the physicochemical properties, refractive index, acid value, iodine value and peroxide value were found in the range set by FAO/WHO and SSMO, and the roselle seed oil is considered an edible oil (Ali Abdella and Abd Elaziz, 2014).

There are published reports indicating that the roselle seeds are eaten in some parts of Africa and also have been roasted as a coffee substitute material. Its powder could also be used in cookies production as a flour substitute till 20% to enhance the protein and dietary fibers content. Also roselle seed are a good source of oil and protein (Nady A. Elneairy, 2014).

The hibiscus seeds are somewhat bitter. However, in Africa, they are ground into meal for human food due to their high protein content and contain a substantial amount of oil that resembles that of cotton seed. Roselle seeds were found to be rich in protein (27.745%), carbohydrates (40.45%) and oil (20.83%). The total phenolic content 1.66% (Abdoulaye *et al.*, 2013).

Oil seed crops are vital sources of oils of nutritional, pharmaceutical and industrial importance. The characteristics of oils from different sources depend mainly on their composition and oil from single source can be

suitable for all purposes (Ramadan and Morsel, 2003). The fatty acid profile of the oil revealed that it is highly unsaturated (76.45%) with linoleic acid the highest (44.39%) (Betiku and Adepoju, 2013). The oil of Roselle can be classified in the oleic- linoleic acid group. High unsaponifiable matters content (1.55%) is related to use the oils in cosmetics industry (Nzikon *et al.*, 2011).

Hibiscus seeds are a potential source of TG, phytosterols, phospholipids, protein and other natural products that remain largely under utilized (Holser and Bost, 2004).

The aim of this research is to study the quality and characteristics of hibiscus oil and compared it with soybean oil.

MATERIALS AND METHODS

Materials :

- Hibiscus Sabdariffa L. seeds were obtained from south Tahreer Horticultural Research station.
- Soybean seeds were obtained from Food Tec. Res. Ins. Agric. Rec. center, Giza, Egypt.

Methods :

Extraction of oils from hibiscus Sabdariffa and soybean seeds: Hibiscus Sabdariffa and soybean seeds were crushed twice using grinder model (MF10 microfine grinder drive), soaked in pure n-hexane for 24 hours. The miscella were collected and filtered. This process was repeated three times using fresh solvent each time. The solvent was evaporated under vacuum at 40-45°C in rotary- evaporator, the oil was dried over anhydrous sodium sulfate, filtered, stored in dark brown bottles without any further purification and then kept at 5°C until analysis (A.O.C.S 1981):

- 1- Chemical composition of hibiscus sabdariffa and soybean seeds:- Moisture, oil , crude protein, ash and total carbohydrates contents were determined according to the methods of A.O.A.C (2000). But fiber content was estimated by deference.
- 2- Minerals in defatted meal of hibiscus and soybean seeds were determined according to the methods of the A.O.A.C. (2000)
- 3- Physical and chemical characteristics of hibiscus and soybean seed oils:

- * Refractive index of oils, was determined at 25°C according to A.O.A.C. (2012) by using refractometer (NXRL-3 poland).
- * Color was determined according to the methods described by the A.O.A.C. (2012) using a lovibond tintometer model F, 5.25 inch cell.
- * Free fatty acids (%) and peroxide values (Meq.O₂/kg oil) were determined according to the methods of the A.O.A.C. (2012).
- * Iodine and saponification values: Calculated from fatty acids percentages by equation according to Susana Nelson (1995).
- * Absorbency in ultraviolet at 232 and 270 nm. :
Ultraviolet and visible spectra were conducted using a pye unicum double beam recording spectrophotometer Model SP 1600, as described by Kates (1972). The oil samples were dissolved in freshly distilled cyclohexane and the absorption were measured at 232 and 270 nm.
- 4- Determination of fatty acid composition: The fatty acids methyl esters were prepared using trans-esterification with cold methanolic solution of potassium hydroxide. The fatty acids methyl esters were identified by GC-capillary column according to the method of IOOC (2001).
- 5- Determination of some bioactive compounds in oil samples:-
* Total polyphenols are determined in oil samples according to the method of Gutfinger (1981).
* Identification of phenolic and flavonoid compounds was done by HPLC according to the methods of Goupy *et al.* (1999).
* Determination of total pigments (chlorophyll and β – carotene): The chlorophylls of oil samples were determined according to the method of Mosquera *et al.* (1991) , while the β carotene was determined as described by Pupin *et al.* (1999).
- 6- Pure TAGs were prepared from oils by solid phase extraction chromatography described by Neff *et al.*, 1992.

TAG composition was identified according IOC, method (1998) using HPLC- refractive index detector.

- 7- Stability of oils was evaluated by the Rancimat method (Mendez, 1997). Stability was expressed as the oxidation induction time (hours), measured with the Rancimat 679 apparatus (Metrohm Co., Switzerland), using an oil sample of 5g heated to 100°C with air flow of 20 L/h.

RESULTS AND DISCUSSION

Chemical composition:-

Chemical composition of hibiscus and soybean seeds are presented in Table (1). As considered in this Table, differences were observed between these seeds in their content of oil, crude protein, fiber, carbohydrate and ash. Crude carbohydrate and moisture content were higher in hibiscus seeds (43.14 and 11.3% respectively) than in soybean seeds (34.61 and 5 %respectively) . The oil and protein contents of soybean seed (19 and 26.31%, respectively.) were higher than those for hibiscus seed (16.98 and 10.14%, respectively.). There were also differences in crude fiber and ash contents of the hibiscus and soybean seeds. With regarding the results in the same

table, there were also differences in mineral contents (N, P, K) of hibiscus and soybean seeds. Nitrogen (N) was the highest value followed by phosphorus content(P) then potassium content (K) in both hibiscus and soybean seeds. The NPK contents of soybean seeds recorded an increased value (4.21, 1.24 and 0.89%, respectively.) compared with those for hibiscus seed (1.62, 1.04 and 0.69%, respectively.). Finally, hibiscus seeds contained a considerable amounts of lipid, protein, carbohydrates and essential minerals (NPK).

Table 1. Chemical composition of hibiscus and soybean seeds and mineral content in their meals on dry weight :

Constituents (%)	Seeds	
	Hibiscus	Soybean
Moisture	11.3	5
Oil	16.98	19
Protein	10.14	26.31
Crude fiber	13.7	10.0
Ash	4.74	5.08
Total carbohydrates	43.14	34.61
Mineral content in meal (%):-		
N	1.62	4.21
P	1.04	1.24
K	0.69	0.89

Physical and chemical properties of hibiscus and soybean oils :

The physical and chemical properties of hibiscus seed and soybean oils are shown in Table (2). The statue of hibiscus seed oil was liquid at room temperature, the color redness units were 8.9 and blue units were 6 at yellow 50 and refractive index (RI) was 1.4720 at 25°C. Regarding the data in this table the RI of hibiscus seed oil was almost the same as soybean oil. Also results indicated, low acidity (0.6%) and peroxide value was 8.55(Meq.O₂/kg oil) of hibiscus oil , these values indicated that the good resistance of this oil to hydrolysis . On the other hand, saponification and iodine values of hibiscus oil recorded high values, being 202.71 (mg KOH/g oil), 126.12 I₂/100g oil respectively. A high saponification value of hibiscus oil indicated, a high concentration of triglyceriedes of this oil, and also it has a high iodine value, because it contains a high amount of unsaturated fatty acids. Values of conjugated diene and triene (K232 and 270 nm.) of hibiscus seed oil, were 2.75 and 0.62 nm. respectively, these values were higher than those of soybean oil under study. Finally, the previous values of hibiscus were within the range of soybean oil and other vegetable oils.

Table 2. Physical and chemical properties of hibiscus and soybean oils :-

Physical and chemical properties	Oils	
	Hibiscus	Soybean
Statue of oil	Liquid	Liquid
Refractive index at 25°C	1.4720	1.4698
Color (redness units):		
Yellow	50	35
Red	8.9	20
Blue	6	-
Acidity (%)as a oleic acid	0.6	0.37
Peroxide value (Meq.O ₂ /kg oil)	8.55	2.1
Iodine value (I ₂ /100g oil)	126.12	135.08
Saponification value (mg KOH/goil)	202.71	203.08
Conjugated diene at 232nm.	2.75	1.99
Conjugated triene at 270nm.	0.62	0.31

Fatty acids composition of hibiscus and soybean oils:

GLC analysis of fatty acids present in hibiscus and soybean oils are shown in Table (3). The results indicate that the oils were highly unsaturated, 77.61 and 83.27 % in hibiscus and soybean oils respectively. The major saturated fatty acids in previous oils were palmitic (16.52 and 11.39%) and stearic (5.36 and 4.51% acids respectively, and the main unsaturated fatty acids were linoleic (40.14 and 52%) and oleic (25.27 and 22.23%) respectively. The ratio of saturated fatty acids to unsaturated fatty acids in hibiscus oil recorded a slight increase compared with in soybean oil. Commonly, there are difference in amounts of the major fatty acids in both hibiscus and soybean oils. This difference in percentages of fatty acids composition of hibiscus and soybean oils may be due to different environmental condition, and also as a result of differences in characteristic and composition of different oils .

Table 3. Fatty acids composition of hibiscus and soybean oils

Fatty acids composition (%)	Oils	
	Hibiscus	Soy bean
Palmitic acid C16:0	16.52	11.39
Palmitoleic acid C16:1	0.52	0.11
Stearic acid C18:0	5.36	4.51
oleic acid C18:1	25.27	22.23
Linoleic acid C18:2	40.14	52.00
Linolenic acid C18:3	11.53	8.08
Arachidic acid C20:0	0.51	0.83
Eicosenoic acid C20:1	0.15	0.85
TSFA.	22.39	16.73
TUNSSFA.	77.61	83.27
TSFA./TUNSSFA.	0.29	0.2

Natural antioxidants and oxidative stability of hibiscus and soybean oils :-

Many researcher found that plant polyphenols compounds scavenge free radicals, thereby protect humans against oxidative damage and disease (Abdoulaye *et al.*,2013). Carotenes protect cells against the effect of light, air and sensitizer pigments having the ability to quench excited sensitizer molecules as well as singlet oxygen and can also serve as antioxidant under conditions other than photosensitization (Pamela *et al.*, 1998). Atherogenic Index (AI) takes into account monounsaturated fats and also distinguishes between different saturated fats in calculating atherogenic potential of diet (De Lorenzo *et al.*, 2001), also this factor (AI) is important for the human health point of view, the lower value is in the favour of good health (Radwan *et al.*, 2010). Table (4) show some natural antioxidants (T.polyphenols and pigment contents), stability, O.S, COX and AI of hibiscus and soybean oils. From the results it could be noticed that, the total polyphenols, pigments contents (Carotenoides and chlorophylls), stability and AI of hibiscus seed oil recorded a higher value compared with those of the soybean oil. On the contrary, the predict oxidizability and calculated oxidizability value of soybean oil recorded an increased value compared with hibiscus seed oil . Finally, the stability of hibiscus oil was higher compared with soybean oil, this may be due to higher amount of T.polyphenols, carotenoids and chlorophylls.

Table 4. Some natural antioxidants in hibiscus and soybean oils

Natural antioxidants (ppm)	Oils	
	Hibiscus	Soybean
Total polyphenols	196.0	22.0
Carotenoides	1.5	0.40
Chlorophylls	1.7	0.9
Stability (hr.)	16.5	11.66
Predict oxidizability (O.S)	0.64	0.69
COX	6.88	7.33
AI	0.214	0.13

whearas : O.S: indicates the oxidative stability (predict oxidizability) and calculated as reported by Cosgrove *et.al.*, (1987).

$$O.S = [(0.02 \times C18:1\% + C18:2\% + C18:3\% \times 2)] / 100$$

COX: refer to calculated oxidizability value as reported by Fatemi and Hammond (1980).

$$COX = 1(16:1\% + 18:1\% + 20:1\% + 22:1\%) + 10.3 (18:2\%) + 21.6(18:3\%) / 100 .$$

AI: indicates the atherogenic index calculated as outlined by De Lorenzo *et. al.*(2001).

$$AI = (12:0\% + 14:0\% + 16:0\%) / (\omega\text{-3PUFA} + \omega\text{-6PUFA} + MUFA).$$

Phenolic and flavonoids compounds in hibiscus and soybean oils:-

Phenolic compounds make a major contribution to the nutritional properties and oxidative stability of any oils. Phenolic and flavonoid compounds were determined for both hibiscus and soy bean oils and obtained results are presented in Table (5). The results in this table revealed that, there were twenty two and fifteen phenolic compounds in hibiscus and soybean oils respectively. Also the obtained data in the same table showed that, there were eleven and three flavonoid compounds in hibiscus and soybean oils respectively. The illustrated data, revealed that, pyrogallol compound gave the highest value in soybean oil (15.09 ppm.) followed by 4-Aminbenzoic compound (10.02 ppm.) , but the first compound recorded a lower value in hibiscus oil (2.256 ppm.), while 4-Aminbenzoic compound was not detected in hibiscus oil . On the other hand salycilic compound recorded a higher value (1.709 ppm.) in hibiscus oil compared with that in soybean oil (0.14 ppm.). Regarding flavonoid compounds, hesperidin and hespertin compounds recorded higher values in soybean oil, (2.25 and 0.28 ppm., respectively.) compared to those of hibiscus oil, while narengin compound was the highest (0.223 ppm.) compared with other flavonoid compounds in hibiscus oil, followed by hesperidin compound (0.180 ppm.) and quercetrin compound (0.121 ppm.).

Triacylglycerol composition of hibiscus and soybean oils :

Triacylglycerol (TAG) composition of hibiscus and soybean oils were determined by HPLC-RI detector. From the results in Table (6), it could be noticed that, the retention time of both hibiscus and soybean oils increased with increasing saturation degree. The results revealed that, TAC (LnLnLn) with ECN 36 was 0.08% in hibiscus oil, while it was not detected in soybean oil. Also, data showed that, the amount of TAG (LnLLn) and (LnLL) with ECN 38 and 40 respectively in hibiscus oil was (5.28 and 4.31%, repectively) higher than that in soybean oil (0.14 and 1.28%, respectively.).

Table 5. Phenolic and flavonoids compounds in hibiscus and soybean oils :-

Phenols (ppm)	Oils		Flavonoid (ppm)	Oils	
	Hibiscus	Soybean		Hibiscus	Soybean
Syringin	0.819	-	Rutin	0.028	-
Gallic	-	0.07	Luteolin	0.033	-
Feulic	0.028	0.03	Naringin	0.223	-
Iso-ferulic	0.017	-	Apegenin	0.024	-
Alpha-coumaric	0.019	-	Hesperidin	0.180	2.25
Pyrogallol	2.256	15.09	Rosmarinic	0.018	-
Ellagic	0.002	0.30	Quercetin	0.014	-
Oleuroein	0.612	-	Quercetrin	0.121	0.08
Benzoic	0.076	0.39	Naringinin	0.004	-
Salicylic	1.709	0.14	Kompferol	0.002	-
Catechein	0.118	-	7-hydroxy flavone	0.004	-
Protocatechuic	0.013	0.09	Hesperitin	-	0.28
3-hyd-tyrosol	0.382	-			
Chlorogenic	0.054	0.04			
Catechol	0.063	-			
Epi-catechin	0.036	0.173			
Caffeine	0.080	0.072			
p-hydro benzoic	0.104	0.141			
Vanilic	0.083	0.130			
Cinamic	0.012	0.010			
Coumarin	0.047	0.040			
p-coumaric	0.050	-			
4-Amino-benzoic	-	10.02			
3,4,5methoxy cinnamic	0.0041	-			

TAG (LLL) with ECN 42 recorded a higher value (7.31%) in soybean oil compared to (1.27) in hibiscus, while the values of TAG (LnLO) and (LnLP) with ECN 42 were 2.76 and 1.33 in hibiscus oil respectively but were found 0.40 and 0.23%, in soybean oil respectively. According to data in the same table, soybean oil contains higher amounts of TAG (LLO), (LnOO) and (LLP) with ECN 44 (18.37, 4.01 and 3.02%, respectively) than those in hibiscus oil (11.09, 1.44 and 0.76%, respectively), but TAG (LnOP) and (LnPP) were 0.99 and 0.3% in hibiscus oil, and were not detected in soybean oil. TAG (LOO+LLS) with ECN 46 of soybean oil was (16.8%) which is higher than in hibiscus oil (13.26%), but LOP and LnPP with ECN 46 were nearly the same in two studied oils. On the other hand hibiscus oil contained a higher values of TAG (OOO+LOS), (POO+SLP) and (POP+PPP) with ECN 48, being 8.56, 15.29 and 5.21%, respectively, and 8.14, 12.59 and 2.2% respectively in soybean oil. Also TAG (SOO), (SLS), (SOP) and (PPS) with ECN 50 of hibiscus oil recorded higher values (0.63, 2.03, 7.08 and 3.95%, respectively) compared with soybean oil that gave (0.00, 2.00, 0.00 and 1.94%, respectively). The group of TAG (OOO+LOS) + (POO+SLP) + (POP+PPP) in hibiscus seed oil was (29.06) higher than that in soybean oil (22.93%) and the same observation was found in TAG group with ECN 50, it was 13.69% in hibiscus seed oil and 3.94% in soybean oil. These results emphasized the higher stability of hibiscus seed oil than in soybean oil. This results are in agreement with (Neff *et al.*, 1994) reported that increase TAG POO and POP content increased oxidative stability. Also, (Neff W. and M El Agaimy 1996) reported that oxidative stability of soybean and canola oil was increased with increasing oleic acid and trioleoylglycerol (OOO) content.

Table 6. Triacylglycerols (TAG) of hibiscus and soybean oils

ECN value	RT	TAG	Hibiscus oil (%)	Soybean oil (%)
36	9.29	LnLnLn	0.08	-
38	13.47	LnLLn	5.28	0.14
40	17.42	LnLL	4.31	1.28
42	22.2- 24.89	LLL	1.27	7.31
		LnLO	2.76	0.40
44	28- 36.19	LnLP	1.33	0.23
		LLO	11.09	18.37
		LnOO	1.44	4.01
		LLP	0.76	3.02
		LnOP	0.99	-
46	38.8- 45.1	LnPP	0.3	-
		(LOO+LLS)	13.26	16.8
		LOP	14.35	13.98
		LnPP	0.91	1.63
48	52.4- 63.0	(OOO+LOS)	8.56	8.14
		(POO+SLP)	15.29	12.59
		(POP+PPP)	5.21	2.2
50	76.6- 883	SOO	0.63	-
		SLS	2.03	2.00
		SOP	7.08	-
		PPS	3.95	1.94

whereas: S= stearic acid, P= palmitic acid, O= oleic acid, L= lenoleic acid, Ln= lenolenic ECN = Equivalent carbon number

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صفات الجودة لزيت بذور الكركدية مقارنة بزيت الصويا

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أجريت هذه الدراسة لتقدير التركيب الكيميائي لبذور الكركدية والصويا مشتملة على البروتين والزيوت والكربوهيدرات والالياف والرماد ومكونات المعادن وأيضا لتقييم الخواص الطبيعية والكيميائية وتركيب الاحماض الدهنية والتراى أسيل جليسرول وأيضا بعض المركبات النشطة حيويًا وبعض القياسات الدالة على الثبات الاوكسيدي للزيوت المستخلصة من هذه البذور. وقد أوضحت النتائج احتواء بذور الصويا على قيم عالية من الزيت والبروتين والرماد ومكونات المعادن مثل النتروجين والفوسفور والبوتاسيوم مقارنة ببذور الكركدية وبالعكس احتوت بذور الكركدية على قيم عالية من الكربوهيدرات والالياف الخام. كما أشارت النتائج أيضا الى أن قيم قياسات الخواص الطبيعية والكيميائية وتركيب الاحماض الدهنية لزيت الكركدية تتطابق مع زيت الصويا وأيضا في داخل المدى لقياسات الزيوت النباتية الاخرى. كما أوضحت النتائج أيضا أن قيم المركبات الفينولية الكلية والصبغات (الكاروتين والكلوروفيل) والثبات (AI) لزيت الكركدية أكبر من الموجودة في زيت الصويا والعكس بالعكس بالنسبة (O.S) و (COX). كما تشير النتائج الى أن مستويات التراى أسيل جليسرول (لينوليك لينوليك لينوليك) و (لينوليك لينوليك أولين) و (لينوليك أوليك أوليك + لينوليك لينوليك أستيريك) عند ECN 46, 44, 42 13.26, 11.9, 1.27% على التوالي). أكبر من زيت الكركدية (16.8, 18.37, 7.31% على التوالي).