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Chemical and Nutritional Aspects of Some Safflower Seed Varieties

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Abstract

In this study, three Safflower varieties (*Carthamus tinctorius* L.) Malawi, Giza1, Ethiopia were obtained from Egypt; of which the Ethiopian variety was analyzed for content of moisture, crude fiber, proteins, oils, carbohydrates, and ash. In addition, detailed studies were conducted on amino acid profile and minerals. Total phenols and fraction of phenolic compound were studied. The moisture content ranged from 5.24% to 6.23% and the protein content ranged from 14.70% to 16.21%, crude fiber 21.34% to 22.51 %,total lipid 32.47% to 35.12%, nitrogen free extract ranged from 22.47% to 26.11%, and ash 3.45% to 4.21% (on wet weight basis). Amino acid analyses revealed that Malawi, Giza1 and Ethiopian have higher level of arginine 5.28, 4.76, and 3.94 (g/100g) respectively. The total polyphenols content of defatted safflower meal was ranged from 452.52 mg to 677.27 mg (GAE /100g).

Keywords: *Carthamus tinctorius*; Safflower; Chemical composition; Amino acid; Minerals; Phenolic

Introduction

Safflower is a very ancient crop which originated in the Middle East [1]. Safflower (*Carthamus tinctorius* L.) is commonly known as khortom in Egypt, kusum in India and Pakistan and honghua (red flower) in China [2,3]. It is oil seed crops, contain about 80% oleic and linoleic acid, iodine value (148) and saponification value (190) [4], cultivated mostly for its high-quality oil, cut flowers, vegetables and medicinal plant. Safflower oils were used as a source of oil in the paint industry and edible oil for cooking, margarine production, and salad oil [2]. Therefore, it needed to be developed the oils produced from Safflower to be as a commercial product for edible oil, medicinal uses and pharmaceuticals, source of α-tocopherol, paint, varnishes and soap manufacturing industries. Safflower is a minor crop with a world production of about 834,000 tons in 2013 [5]. Several safflower seed products can be used as animal feeds: the seeds, the by-product of the oil extraction (safflower meal) and the hulls [6].

Materials and Methods

Safflower *Carthamus tinctorius L.* seeds of two Egyptian varieties namely: Malawi and Giza 1 were obtained from the farm of Agricultural Faculty, Cairo University during 2011/2012 season, while the imported variety from Ethiopian was obtained from local market of Cairo City during 2012 were used in this study.

Gross chemical composition

Moisture, crude oil, total nitrogen, ash content and crude fiber of safflower seed kernel were determined as outlined in AOAC [7]. Nitrogen free extract was calculated by difference.

Determination of minerals

Sodium and potassium were determined by Flame Photometer 410, whereas Calcium, magnesium, manganese, copper, iron and zinc were determined using Perkin-Elmer Atomic Absorption Spectrophotometer 2380, at Agricultural Research Center in Giza, as described in AOAC [8].

Amino acids composition

Amino acids were determined according to the method described by Pellett and Young [9] with some modifications, which could be summarized as follows: 200 mg of dried, defatted sample was hydrolyzed with 5 ml of 6 N HCl, in sealed tube at 110°C for 24 hours and the hydrolysate was filtered. The residue was washed with distilled water and the filtrate was evaporated on water bath at 50°C. The residue was dissolved in 5 ml/loading buffer (0.2 N sodium citrate buffer of pH 2.2). Amino acids were determined chromatographically using Beckmen Amino Acid Analyzer Model 119 CL, at National Research Center, Giza-Cairo.

Tryptophan determination: Tryptophan was determined calorimetrically using the method described by Sastry and Tummuru [10].

Determination of total phenolic compounds

The Folin-Denis method was used for total-phenolic (TP) analysis with tannic acid as a standard. Folin-Denis reagent is mixture of 10 g of sodium thungstate, 2 g of phosphomolybdic acid and 5 ml of phosphoric acid in 75 ml of distilled water that was refluxed for 2 h, cooled, and diluted to 100 ml with distilled water.

To use the tannic acid as a standard, the procedure described by Makkar [11] was applied: A sodium carbonate saturated-solution was obtained by adding 40 g of sodium carbonate to 150 ml of distilled water, then dissolved for 1h at dark and adjusted to 200 ml. A standard-solution of tannic acid was obtained by dissolving 50 mg of tannic acid in 100 ml of distilled water. Aliquots of 0, 20, 40, 60, 80 and 100 µl of the standard solution were dispensed into tubes containing 0.5 ml of Folin-Denis reagent and 2.5 ml of sodium carbonate saturated-solution. Finally, standards were diluted to 4 ml with distilled water and quickly shaken. Their absorbance was determined after 35 min in dark at 750 nm [8].

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Determination of total polyphenol was prepared by adding 0.5 ml of Folin-Denis and 2.5 ml sodium carbonate reagents to 1 ml of each safflower meal extracts. Using absorbance standard curve, TP content was estimated. Units of TP were expressed as µg of tannic acid equivalents per ml of safflower meal extracts. For safflower meal extracts, tannic acid equivalents were multiplied by 20, based on an extraction ratio of 1:20 (w/w).

Determination of phenolic compounds by high performance liquid chromatography (HPLC): Phenolic compounds of safflower cake were determined by using Hewlett Packared HPLC (Model 1100) at National Research Center, Cairo using a hypresil C18 reversed phase column (250 × 4.6 mm) with 5µm particle size-injection by means of Rheadyne injection value (Model 7125) with 50 µl fixed loop as used. A constant flow rate of 1 ml min-1 was used with two mobile phase (A) 0.5% acetic acid in distilled water at pH 2.65 and solvent(B) 0.5% acetic acid in 99.5% acetonitrile. The elution gradient was linear starting with (A) and ending with (B) over 35 min, using UV detector set at wavelength 245 nm. Phenolic compound of each sample were identified by comparing their relative retention time with those of the standards mixture chromatography. The concentration of an individual compounds was calculated based on peak area measurement, then converted to µ phenolic g-1dry weight.

Fatty acids composition

Preparation of methyl ester of fatty acids: The methyl esters of fatty acids were prepared from aliquots total lipids using 5 ml 3% H_2SO_4 in absolute methanol and 2 ml benzene as mentioned by Rossell et al. [12]. The contents were heated for methanolysis at 90°C for 90 minute. After cooling, phase separation was performed by addition of 2 drops distilled water and methyl esters were extracted with 3 aliquots of 2 ml hexane each. The organic phase was removed, and filtered through anhydrous sodium sulfate.

Gas liquid chromatographic of methyl esters of fatty acids: The methyl esters of fatty acids were separated using HP 6890 GC capillary column gas liquid chromatography with a dual flame ionization were carried out on (30 m × 0.32 mm × 0.25 µm) DB-225 capillary column, stationary phase (50% cyanopropyl phenyl + 50% dimethyl polysiloxane). Column temperature: initial temperature was 150°C, the temperature was programmed by increasing the temperature from 150-170°C at the rate of 10°C/minute, then increased from 170°C-192°C at the rate of 5°C/minute, holding for five minutes and then increased from 192-220°C during 10 minutes, holding three minutes. The injector and detector temperatures were 230°C and 250°C, respectively. Carrier gas: Hydrogen flow rate 40 ml/minute, nitrogen at the rate 3 ml/minute, and air flow rate was 450 ml/minute. Peak identifications were established by comparing the retention times obtained with standard methyl ester. The areas under the chromatographic peak were measured with electronic integrator. It was carried out in Agricultural Research Center in Giza–Cairo.

Results

Gross chemical composition

The proximate chemical composition of safflower seeds are presented in Table 1. Moisture content was ranged from 5.24 to 6.23% and found to be in agreement with the results of Bozan and Temelli [13], Vorpsi et al. [14], Ingale and Shrivastava [15] and Yu et al. [16] who stated that, the moisture content was 6.10, 6.26, 6.33 and 5.58%, respectively. However, the local Egyptian Malawi varieties recorded slight low moisture content compared to the imported Ethiopian one. In addition, the lower moisture content is an important factor for keeping quality during storage. The protein content of the studied safflower seeds was ranged from 14.70% to 16.21% (on dry weight basis). The obtained protein content values were in agreement with that reported by Zazueta [17], kim [18], Rahmatalla et al. [19] and Kim [20]. However Nagaraj [21] mentioned that, the protein content of safflower seeds was ranged from 14 to 19%. Oil content of safflower seeds is a very important economic trait for safflower varieties and considered one of the most important factors affecting the success of safflower insert in new areas. The crude oil content of the studied safflower seeds was ranged from 32.47 to 35.12% as shown in Table 1. The studied safflower varieties seed consider a good source of oil. Similar results were reported by Vorpsi et al. [14] and Mariod et al. [22]. Beside, among the Egyptian varieties, Giza 1 variety contained higher oil content (35.62%) compared by Malawi variety which contained (32.47%) while the imported Ethiopian variety contained intermediate level of oil (33.89%). Data in Table 1 revealed that Malawi safflower varieties seeds have the highest value of crude fiber (22.51%) followed by Giza 1 varieties (22.33%) then, Ethiopian varieties (21.31%). Similar research for crude fibers content in five varieties of safflower seeds (25.01% to 29.24%) was reported by Bardhi et al. [23]. On other hand, the data revealed that, the nitrogen free extract was ranged from 22.47 to 26.11% in the studied safflower seeds. The ash content was ranged from 3.45 to 4.21% as indicated in Table 2. Similar results for ash content of various varieties of safflower seeds were reported by Kim [18], Ingale and Shrivastava [15] and Yu et al. [16].

Minerals composition

Minerals analyses are essential to guarantee the quality of any food product. There are many minerals that are considered nutrients and are vital for the proper functioning of the body. Equally, there are a number of minerals that are toxic to the human body and interfere with its functioning and undermine health [22].

The results indicated that, safflower seeds are good source of phosphor which ranged from 663.00 to 770.40 mg/100g (on dry weight), while potassium (K) content was ranged from 156.15 to 203.60 mg/100g and calcium (Ca) was ranged from 59.00 to 101.50 mg/100g (dry weight). Similar results were recorded by Rahmatalla et al. [24] and Mckevith [25]. Potassium plays an important role in human physiology, and sufficient amounts of it reduce the risk of heart stroke,

Varieties	Moisture	Characteristics*				
		Crude protein	Crude oil	Fiber	Ash	Nitrogen free extract**
Malawi	5.24^{a}	14.7^{a}	32.47^{a}	22.51^{a}	4.21^{c}	26.11^{b}
Giza1	6.28^{b}	16.21^{a}	35.12^{c}	22.33^{a}	3.87^{b}	22.47^{a}
Ethiopian	5.67^{a}	15.62^{a}	33.89^{b}	21.34^{a}	3.45^{a}	25.7^{b}

$^{a.b.c}$Values with different subscripts on the same column are significant ($p<0.05$)
Calculated by differences on dry weight basis (%)
**Calculated by differences

Table 1: Gross chemical composition of safflower seed varieties.

while calcium plays an important role in building stronger, denser bones early in life and keeping bones strong and healthy later in life [26]. However, the sodium content of safflower seeds was low and ranged from 12.22 to 39.32 mg /100g. With relatively high content in Giza 1 varieties compared to Malawi and Ethiopian varieties. Iron (Fe) the important element for blood building, content was ranged from 3.53 to 3.98 mg/100g and zinc (Zn) content was ranged from 1.49 to 2.06 mg/100g. However higher results for iron content (4.90 mg/100g) and zinc content (5.10mg/100g) was reported by Mckevith [27]. Magnesium (Mg) content of the studied safflower seeds was ranged from 30.55 to 61.10 mg/100g Moreover, Giza 1 varieties contained duplicate magnesium content compared with Malawi varieties, while, the imported Ethiopian variety contained intermediate level (40.84 mg/100g) as shown in Table 2. Copper (Cu) and Manganese (Mn) content of safflower seeds was similar to the results of Kim [27] and Yu et al. [13]. In addition, Cadmium (Cd) a toxic element was absent in the all studied safflower seeds

Amino acids composition of safflower seeds

Amino acids compositions of the three studied safflower seeds are presented in Table 3. The Data indicated that all essential amino acids were presented in safflower seeds and their content value were 14.03,14.93 and 11.48 g /100g protein of Malawi, Giza 1 and Ethiopian safflower seeds; respectively. As it is well known, the human bodies do not synthesis the essential amino acid, but can be obtained by from food. Eight amino acids are generally regarded as essential for human; phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, leucine and lysine [27]. Besides, the other, non-essential amino acids, except cysteine, which was not detected, constituted 28.65, 23.77 g/100g and 21.47 g/100g protein of Malawi, Giza 1 and Ethiopian safflower seeds; respectively. Moreover, among essential amino acids, leucine, phenylalanine and valine recorded the highest content in safflower seeds protein, while glutamic acid, arginine and aspartic acid recorded the highest values among the nonessential amino acids as shown in Table 3. However, the antioxidant activity of these amino acids suggests a disease preventive role as exemplifies by arginine which is beneficial for prevent of cardiovascular disease [28].

Arginine is a factor for maintaining the nitrogen balance in muscles and can enhance the lean tissue to fat tissue body fat ratio; a great factor for weight management [29]. Aspartic acid deficiency decrease cellular energy and may like be a factor in chronic fatigue. Adequate methionine prevents disorder of hair, skin and nail; reduce liver fat and protect the kidney. On other hand, essential amino acid contributes to good health and wellbeing. Deficiency of lycine leads to physical and mental handicap [30]. However, the essential amino acids represent 32.87, 38.61 and 34.84% of Malawi, Giza 1 and Ethiopian safflower seed protein; respectively. Similar results was reported by Mariod et al. [22] who found that, the essential amino acids represented 36% of total amino acids in safflower seeds protein. Moreover, the obtained amino acids composition of safflower seeds protein was found to be similar with reported values of Rahmatalla et al. [24], Ingale and Shrivastava [15] and Mariod et al. [22] for different varieties of safflower seeds.

Minerals	Safflower Varieties		
	Malawi	Giza 1	Ethiopian
K	194.95	203.60	156.15
P	770.40	673.80	663.00
Mg	30.55	61.10	40.84
Ca	101.50	99.00	59.00
Na	12.22	39.32	18.56
Fe	3.98	3.56	3.53
Zn	2.06	1.66	1.49
Cu	0.46	0.46	0.31
Mn	0.42	0.41	0.26
Cd	0.00	0.00	0.00

Table 2: Minerals content of safflower seeds (mg/100g on dry weight).

Amino acids	Safflower seed varieties		
(g/100 g protein)	Malawi	Giza1	Ethiopian
Essential amino acids			
Threonine	0.93	0.81	1.03
Valine	2.05	1.83	2.47
Methionine	0.2	1.08	0.47
Isoleucine	1.16	2.81	1.41
Leucine	2.93	1.43	3.92
Phenylalanine	2.67	2.05	3.15
Lysine	1.27	4.69	1.36
Tryptophan	0.27	0.23	0.22
Total essential amino acids	11.48	14.93	14.03
Non-essential amino acids			
Histidine	1.01	1.23	1.22
Arginine	3.94	4.76	5.28
Aspartic acid	2.59	2.59	3.23
Serine	1.19	1.3	1.61
Glutamic acid	6.35	6.73	8.82
Proline	1.32	1.92	2.95
Alanine	2.3	2.05	2.6
Cysteine	ND	ND	ND
Tyrosine	1.76	2.32	1.82
Glycine	1.01	0.87	1.12
Total non-essential amino acids	21.47	23.77	28.65
E.AA./non-E.AA. ratio	0.54	0.63	0.49
*ND: Not detected.			

Table 3: Amino acids composition of safflower seed varieties.

Phenolic compounds of safflower seeds

There are many evidences that, natural products and their derivatives have efficient anti-oxidative characteristics, consequently linked to anti-cancer, hypolipidemic, anti-aging and anti–inflammatory activities. Phenols and polyphenols are stronger antioxidants than the vitamins. Several epidemiological studies showed a lower risk with increasing intakes plant foods and protection against DNA damage [31]. Anti-oxidative capacity of *Carthamus tincotorius* were evaluated by Koyama et al. [32] and they attributed their strong anti-oxidative activity to the major phenolic compounds serotonin and their glucoside derivatives.

Total polyphenols content of safflower seeds meal

Total polyphenols content of defatted safflower meal was tabulated in Table 4. The data revealed that the total polyphenols contents of the studied safflower seed meals were 452.52, 677.27 and 604.04 mg (GAE /100g) for Malawi, Giza 1 and Ethiopian varieties; respectively. The obtained results are in agreement with the results of Sreeramulu and Raghunath [33] who found that, total phenols in safflower seed was 599(mg GAE /100 g) but, it was lower than 1526 (mg /GAE 100g)which reported by Bozan and Temelli [13] and higher than 55.52 (mg GAE /100g) which reported by Yu et al. [16]. However as shown in Table 5 among the Egyptian safflower varieties, Giza 1 safflower seeds contained more total polyphenols compared with Malawi variety, but its phenols content was nearly similar to the imported Ethiopian variety.

Varieties	Malawi	Giza1	Ethiopian
Total phenol(mg/100g of safflower defatted meal)	452.52[a]	677.27[c]	604.04[b]
[a,b,c] Values with different subscripts on the same row are significant (p<0.05)			

Table 4: Total phenol compound content in safflower meal (mg GAE/100g).

Phenolic compound	Safflower seed varieties		
	Malawi	Giza1	Ethiopian
N-(p-Coumaroyl) serotonin 7-O-β-D-glucoside	22.82	0.9	3.57
N-Feruloyl serotonin 7-O-β-D-glucoside	7.03	2.93	19.23
N-(p-Coumaroyl) serotonin	11.15	31.14	32.99
N-Feruloylserotonin	23.1	22.64	24.99
Matairesinoside	3.67	7.1	7.6
Acacetin 7-O-β-D-glucuronide	5.8	17.32	3.06
Acacetin	3.11	7.62	1.05
Luteolin	4.99	2.92	1.34
Unknown	3.1	0.65	2.55

Table 5: Phenolic compound content of safflower seed varieties (% of the total phenolic compound).

Determination of phenolic compounds of safflower seeds meal

By using of HPLC, phenolic compounds of safflower seeds were fractionated to eight fractions namely: N-(p-Coumaroyl) serotonin 7-O-β-D-glucoside, N-Feruloylserotonin 7-O-β-D-glucoside, N-(p-Coumaroyl) serotonin, N-Feruloylserotonin, Matairesinoside, Acacetin 7-O-β-D-glucuronide, Acacetin, Luteolin as well as unknown fraction as shown in Table 5. However, Kim et al. [34] isolated the polyphenols of safflower seed also, to nine phenolic compounds. The obtained results indicated that serotonin and its derivatives constituted the major content of polyphenols of safflower seeds. Similar observation was reported by Kim et al. [34], Katsuda et al. [35] Seo and Choi [36]. Moreover, as early reported by Zhang et al. [37] Serotonin derivatives in the safflower seeds are family of molecules containing seven to ten members featuring a serotonin moiety bound to a phenyl propanoid moiety via an amide bond. However, N-(p-Coumaroyl) serotonin and N-Feruloyl serotonin represented about 34% and 58% of total phenolic compound as indicated in Table 5. Beside, Malawi variety seeds contained more N-(p-Coumaroyl) serotonin 7-O-β-D-glucoside (22.82%) compared with Giza 1 variety (0.90%) and Ethiopian variety (3.57%)while Ethiopian variety contained more of N-Feruloylserotonin 7-O-β-D-glucoside (19.23%) compared with Malawi variety (7.03%) and Giza 1 variety (2.03%) of total phenolic compound. Moreover, Giza 1 variety contained more acacetin 7-O-β-D-glucuronide and acacetin compared with the other two varieties.

Fatty acids composition of safflower oils

As known, the fatty acid composition of vegetable oil is a main factor affecting its commercial uses and it influenced by a lot of factors such as genotype of the variety, environmental conditions, etc. [38]. The fatty acids composition of total lipids extracted from Ethiopian, Malawi and Giza1 safflower seeds are shown in Table 6 the results of analysis for fatty acid showed that the unsaturated fatty acid linoleic (74.60, 78.24 and 77.90%) and oleic (14.19, 11.22 and 11.39%) and the saturated fatty acid palmitic (6.03, 6.57 and 6.66%) and stearic (2.61, 2.01 and 2.06%) were the most abundant fatty acids in respecting decreasing order, which together composed about 97.43, 98.04 and 98.01% of the total fatty acids of Malawi, Giza 1 and Ethiopian varieties; respectively. A negligible amount of linolenic acid was detected (0.07 - 0.08%) and minor amount of ecosenoic (C 20:1), palmitoleic (C16:1), arachidic (C 20:0) and behenic (C 22: 0) were present and the values of them did not exceed 0.89% of the total fatty acids. These results are comparable to data reported by Sabzalian et al. [39].

Besides compared to most other common edible oils, safflower oil contains the highest level of the linoleic acid, an essential fatty acid, which is make it as premium edible oil, because of its nutritional advantages and potential therapeutically properties in the prevention of coronary heart disease and cancer but the presence of the large amounts of linoleic acid makes the oil quite sensitive to oxidation [40,41]. Moreover, the essential fatty acid is not easily synthesized in the human system and must be supplied externally through the diet [42]. Smith indicated that, the importance of safflower seed oil is in its linoleic acid content, which is a required product with high polyunsaturated fatty acid clime. However, the total saturated fatty acid content of safflower oil was low (9.24% to 9.40%) of total fatty acids content while, the total unsaturated fatty acid was about 90% of total fatty acids content as shown in Table 6. Generally, high intakes of saturated fatty acids have been associated with raised blood cholesterol levels, one of the risk factors associated with heart diseases. In comparison mono unsaturated fatty acids decrease the bad cholesterol, (LDL-C), moreover polyunsaturated fatty acids also decrease LDL-C, intake of n-6 PUFA above 10% energy may have adverse effects on good cholesterol, (HDL- C) as mentioned by Clarke et al. [32]. On other hand, the nutritional quality index (linoleic/saturated fatty acids) is very high and ranged from 7.99 to 8.47 compared with that of groundnut oil which ranged from 1.8 to 2.4 as reported by Nagaraj [21]. In opposite the ratio of oleic to linoleic acid being a measure of oil keeping quality (oil stability index) was low and ranged from 0.14 to 0.19 as presented in Table 6. Besides, the all studied safflower seed oils contained long chain fatty acids (C_{20} - C_{24}) with minor mounts. The same observation was detected by Bozan and Temelli [13] when studied flax, safflower and poppy seed oils.

Conclusion

Total phenols and fraction of phenolic compound were studied. The moisture content ranged from 5.24 to 6.23% and the protein

Fatty acids	Carbon chain	Safflower seed varieties		
		Malawi	Giza1	Ethiopian
Palmetic	C16:0	6.03	6.57	6.66
Palmetolic	C16:1	0.06	0.09	0.09
Margaric acid	C17	0.02	0.03	0.02
Margaoliec acid	C17:1	0.00	0.01	0.01
Stearic	C18:0	2.61	2.01	2.06
Oleic	C18:1	14.19	11.22	11.39
Linoleic	C18:2	74.60	78.24	77.90
Linolenic	C18:3	0.07	0.08	0.08
Arachidic	C20:0	0.34	0.29	0.30
Ecosenoic	C20:1	0.19	0.17	0.17
Behenic	C22:0	0.24	0.24	0.25
Unknown	Unknown	1.51	0.91	0.93
Lignoceric acid	C24:0	0.10	0.10	0.11
Total saturated fatty acid		9.34	9.24	9.40
Total unsaturated fatty acid		89.11	89.81	89.64
Nutritional quality index (linoleic/ saturated fatty acid)		7.99	8.47	8.29
Oil stability index (oleic/linoleic)		0.19	0.15	0.15

Table 6: Fatty acids composition of total lipids of safflower seed varieties (% of total fatty acids).

content ranged from 14.70 to 16.21%, crude fiber 21.34 to 22.51%, total lipid 32.47% to 35.12%, nitrogen free extract ranged from 22.47% to 26.11%, and ash 3.45 to 4.21% (on wet weight basis). Amino acid analyses revealed that Malawi, Giza1 and Ethiopian have higher level of arginine 5.28, 4.76, and 3.94 (g/100g) respectively. The total polyphenols content of defatted safflower meal was ranged from 452.52 mg to 677.27 mg (GAE /100g).

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