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NUTRITIONAL AND FUNCTIONAL PROPERTIES OF JOJOBA, HIBISCUS AND PUMPKIN SEED KERNAL FLOURS

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ABSTRACT

The, present study was carried out to maximizing the available and valuable sources of proteins of the agriculture plant`s waste and industry duties . So this research focusing on the nutritional and functional properties of defatted hibiscus, jojoba and pumpkin seeds flour. Analysis were carried out on defatted seeds, to determine proximate chemical composition , such as crude protein , ether extract, ash, crude fiber, amino acids and carbohydrate content.The latter was calculated by difference. Protein content ranged of 23.4, 28.0 and 53.5 % of dry mater base (DM) of jojoba seeds flour (JF), hibiscus seeds flour (HF), and pumpkin seeds flours (PF) respectively. In addition, fat content ranged from 13.9 % to 33.8 % of HF and PF, respectively, while the highest fat content 54.9% obtained from JF. Antinutritional compounds, such as phytates detected in all flours, ranged from 0.23 to 0.5 g/100g DM. Functional properties of all flours were assessed, such as water absorption index (WAI), water soluble index(WSI), acid number (pH), foam capacity (FC) and foam stability (FS). All flours were excellent in their functional properties except FC and FS of PF was inferior good. Amino acids compositions of these flours were also determined. The protein of these flour had abundant amount of total essential amino acids and higher than recommended by FAO/ WHO (1973). This research classified the amino acids of all seeds flour into groups to identify the flour potential for food supplementation in bakery and meat processed formulation. The nutritional value of the protein and its quality methods based on *in vitro* (chemical) and animal bioassays *in vivo* for assessment of protein quality have been developed by predicting protein quality depending on the determination of amino acids and calculated chemical scores, essential amino acid index (EAAI), amino acid / essential amino acids (A/E) ratio, amino acids score (AAS), estimated biological (BV) and calculated protein efficiency ratio (PER). An *in vivo* of protein quality depending on biological assessment using rats which depending on true digestibility percentage (TD), BV and net protein utilization (NPU) were investigated. By comparison, between their chemical estimation and bioassay, it's clearly view that, both of HF and PF had good quality protein; as expressed by their high TD, BV and NPU when compared to poorly JF. Both of these flours were significantly a quite similar to standard protein of casein. Meanwhile, jojoba flour had a considerable amount of antinutritional factors of simmondsin, which act as satiety inducers and impede bioavailability of protein. All of these nutritional values of the different flours HF and PF concluded those have an excellent protein and nourished sources. These flours could be potentially added to food system, such as bakery products, complementary foods and meat products, moreover JF have been promising not only their nutritive value but also for their responsibility to diminish satiety and reducing food intake for dietetic persons.

Keywords: amino acid , antinutritional, A/E ratio , BV, chemical composition, chemical scores, EAAI , EAAS, functional properties, , flour , hibiscus, food supplementation, jojoba , PER, phytates, protein quality , pumpkin, nutritional value, , seeds , simmondsin

INTRODUCTION

The seeds of jojoba (*Simmondsia*) are a source of stable long chain wax-esters used primarily in cosmetics for their emollient properties, and sparingly as an industrial lubricant additive Wisniak, (1987). The wax esters constitute was ≈ 50% of the seed, whereas the remainder, defatted jojoba meal (JM) has only a few industrial applications. Defatted JM contains relatively large amounts of the unique cyanoglucosides, simmondsin, simmondsin ferrulate and other analogs. Several studies have attributed decrease food intake and weight loss to these compounds when ~ 10% defatted JM was included in the diet of rats (Cokelaere *et al.,* 1992) this reduction between weight and appetite suggested that lower intake is the principle diver of weight. Another work, at jojoba meal concentrations including approximately 20% reduction in food intake (FI), no major toxic effects were seen in rats treated for several months Cokelaere *et al., (* 1993). The rich residue remaining after oil extraction referred to as jojoba meal, is hardly used at all valorization of the jojoba meal could be an important marketing impulse for the jojoba industry. The major composition of jojoba protein was albumin (79%) and globulin (21%) which have similar amino acid pattern Shresth *et al.,* (2002). JM supplementation of the diet could be reducing the satiation; also it was intended to reduction the calorie intake and expansion of adipose tissue. Shresth *et al.,* (2002) reported that increase protein digestibility achieved by boiling may be attributed to inactive of protease inhibitors and denaturation of protein Hence, according to these results defatted jojoba flour can be safely to perceived to human diet and reducing the prevalence of obesity. In addition, compounds other than simmondsins are present in meals, including polyphenols, phytic acid, trypsin inhibitors and bitter substance Vermaut, (1998), that may contribute to an impaired food intake and energy balance of jojoba meal diet. Aliza Benziani (1997) and Cokelaere *et al.,* (1998) reported that simmondsin as glycoside in jojoba meal was elevated and nopathological toxicological influence on liver, pancreas and kidney could be happened from fed experimental animal on diet containing simmondsin and or jojoba meal, also there is no pathological changes in internal organs of rats and no negative effect on fertility and reproductive system. Jojoba meal supplementation, their is no sign of teratogenic effects on broiler fetuses, or delayed negative effects on reproductive performance were seen Cokelaere *et al.,* (2001).

Hibiscus sabdariffa (Reselle or karkade) is apopular plant (prolonged to the family Malvceace) in some Middle East countries. It is used for its fiber and calyxes of the flower, which are use in cold and warm beverages, jellies, sauces and beverages. The karkade calyxes have nutritional requirements and contain a valuable source of Fe. Advanced reports, stated that roselle have a medical effects as antimutagenic and chemoperventive activity in colon cancer Chewonarin *et al.,* (1999).

Hibiscus seeds are a secondary waste produced from roselle plant after used karkade in both mainly uses in fibrous and calyces of flowers. Karkade seeds contain considerable amounts of protein (25%) and oil (21%) AL-

Wandawi *et al.,* (1984) and Abu-Tarboush, (1995). The chemical composition of hibiscus seed was moisture (8.205), dry matter (91.8%), crude protein (21.35%), Crude fiber (11.98), and ash (5.34%) according to EL-Adawy and Khalil (1994). Seed oil is containing a primarily polyunsaturated and amino acids as renewable source of industrial material and nutritive matter also. Nutritional properties of oil and protein of whole seeds have been studied by AL-Wandawi *et al.,* (1984) and Abu Tarboush *et al.,* (1997).

Pumpkin seeds utilized directly for human consumption and snacks after salting and roasting in Arabian countries. AL-Khalifa, (1996). These seeds are excellent sources of protein (25.2 – 37%) Lazos, (1986). The kernels of pumpkin seed have been utilized as additive to some food dishes Nwokolo and Sim, (1987). Several reporters exist on the nutritive value of proteins and oils of pumpkin seeds. The nutritional quality of pumpkin seed flour had a higher value of chemical score, essential amino acid index. Functional properties of seeds were excellent in pumpkin seed kernel flours, this seeds promise to intended in the excellent bakery product and ground meat products, not only as a nutrient supplement EL-Adawy and Taha, (2001). However, very little information has been reported on physicochemical and functional properties of pumpkin seed protein. Pumpkin seeds have been used in traditional medicine North American and Mexico Science long as an antihelmintic agent and for supportive treatment in functional disorders of the bladder and for difficulties in urination Srivastava and Singh (1967).

The objectives of this study were to investigate the nutritional and functional properties of these three non-conventional uses of these seeds.

MATERIALS AND METHODS

1- Preparation of different seed extracts:

Jojoba seeds were commercially obtained from Misr Company for Natural Oils, Al-Obeor Buildings, Cairo, Egypt. Jojoba oil was extracted by using Soxtec extractor (Foss), to obtain defatted jojoba flour (JF) by using method according to AOAC, (2005).

Pumpkin seeds (*Cucurbita*) were purchased from different sources of local market (without roasting and salting). Preparing flour by dehulling, drying, grinding and defatting by using solvent extraction by Soxtec extractor (FOSS) and petroleum ether (40-60) using AOAC, (2005). Defatted pumpkin seeds were crushed and finely ground to obtain defatted pumpkin flour (PF). The color of pumpkin flour was greenish.

Hibiscus seeds (*Hibiscus sabdariffa*) were obtained from Agricultural Research Center, Field Crop Research Institute. Ministry of Agriculture, Giza, Egypt. The seeds were cleaned; crushed and grinding to obtain meals then perceived to oil extraction, the residue was defatted hibiscus flour (HF). Hibiscus seeds flour have a brownish creamy color and finely grounded to obtain hibiscus flour (HF). All seeds flours were preserve at refrigerator temperature (5 °C) until used.

2- Proximate chemical composition:

The following AOAC methods (2005) were used to determine proximate composition: drying at 105 ° C for 3 hr for moisture methods; incineration at 550 ° C for ash in muffle. Defatting in soxtec apparatus (Foss, Switzerland) with petroleum ether (40-60) for lipids extraction and micro-kjeldahl for protein (N x 6.25). Crude fiber and phytates were determined by using AOAC (2005). Carbohydrate content was calculated by differences.

3- Physicochemical parameters:

a- Water absorption index (WAI) and water solubility index (WSI):

WAI and WSI were assessed as method described by Anderson *et al.,* (1969) and Sosulski *et al.,* (1976). Each flour sample (2.5 gm) was mixed with 30 ml of distilled water in a tare 60 ml centrifuge tube. The slurry was stirred with glass rods for 1 min at room temperature and centrifuged at 3000 xg for 10 min. The supernatant was then poured carefully into tared evaporating dish. The WAI was calculated from the weight of the remaining gel and expressed in gram of solids / gram of original solids was calculated from the weight of dry solids recovered by evaporating the supernatant overnight at 110 °C.

b- Foam capacity and foam stability (FC) and (FS)

Foam capacity and foam stability (FC) and (FS) were assessed according to method of Lawhon *et al.,* (1972) using 1% flour solution in bream blander at 1600 rpm for 5 min. The percentage increase in foam volume was recorded as foam capacity (FC). The change in volume of foam after 15, 30, 40, 45 and 60 min of standing at room temperature (≈ 30 °C) was recorded as foam stability.

c- pH determination:

The pH of flour samples was recorded using a pH meter. Each flour sample (10 g) was suspended in 100 ml distilled water. After cooling, the slurry was shaken (1500 rpm, 25 ° C, 20 min) using orbital shaker.(Cherry,1981)

4- Amino acid analysis:

Amino acid determination was performed according to method of the method of the AOAC, (2005). Oxidation with performic acid, to protect methionine and cystine from distraction during acid hydrolysis with 6 M HCl) were carried out in closed conical flash for determine all amino acid0 other than tryptophan. Sample of 20-30 mg weighted in conical flask and 5 ml of performic acid was added. The flask was closed and placed in ice water bath for 16 hr. Sodium metabisulfate and 25 ml HCl 6 N were added to the oxidized mixture. The flask was placed in an oven at 110 ° C for 24 hr. The flask was then opened and all removed by evaporating samples to dryness in rotary evaporator. A suitable volume of sodium citrate puffer (pH 2.20) was added to the dried film of hydrolyzed sample. After all soluble material completely dissolved, the samples analyzed for amino acids using Eppendorf LC 3000 (EZ Chrom , software used for data collection and processing). The results were calculated as percentage of total crude protein. Determinate tryptophan was carried out using method described by Miller (1967) after hydrolysis of samples with barium hydroxide.

5- Chemical prediction of protein and nutritional quality:

a- Chemical estimation:

Protein and nutritional quality assessment of the hibiscus, jojoba and pumpkin kernel seeds flour were performed using amino acids profile and using egg amino acid pattern as reference protein (Mitchel and Block 1946), Sarwar *et al.,* (1985) and Human pattern of amino acid requirements (FAO/ WHO 1973) suggested pattern of amino acid requirements for human.

b- Calculation of protein and nutritional quality indexes:

b-1- Chemical score of amino acids was calculated according to (FAO/ WHO 1973) reference pattern to estimate chemical score % and first, second and third limiting amino acids. Calculation of amino acid score as follows:

$$\text{Amino acid score} = \frac{\text{mg of amino acid in 1 gm tested protein}}{\text{mg of amino acid in requirement pattern}} \times 100$$

b-2- Calculation of A/E Ratio: the relationship between the content of individual essential amino acid in the food protein (A) and the total essential amino acid contents (E) was conducted by according to FAO (1965) as follows:

$$\text{A / E Ratio} = \frac{\text{mg of the individual essential amino acid}}{\text{gm of total essential amino acids}}$$

b-3- Essential amino acids index (EAAI) for flour seeds were determined from the formula expressed by Mente *et al.,* (2002): The amino acids results were expressed as µmoles of amino acid per gram of flour samples (µmole / g) and as grams per 100 g determined amino acid for reference egg protein.

$$\text{EAAI} = \sqrt[n]{\alpha\alpha 1/AA1 \times \alpha\alpha 2/AA2 .... \times \alpha\alpha 11/AA11}$$

Where: aa1 is the A/E ratio in the flour samples (EAA/ Total EAA + tyrosine x 100), AA1 is the A/ E ratio in the composition amino acid of egg as standard, (EAA/ Total EAA + tyrosine x 100).

b- 4- Predicted biological values (BV): the predicted BV values were calculated by using the regression equation of Mørup and Olesen (1976).

$$BV = 10^{2.15} \times q_{Try}^{0.41} \times q_{Met+Cys}^{0.60} \times q_{Thr}^{0.77} \times q_{Lys}^{2.4} \times q_{Phy+Tyr}^{0.21}$$

Where: q_i = ai sample / ai reference for ai sample ≤ ai reference and q_i = ai reference / ai sample for ai sample ≥ ai reference; ai represented mg of amino acid / g of total essential amino acids.

b-5- Protein efficiency ratio (PER): was estimated according to the following equation purposed by Alsmeyer *et al.,* (1974), equation NO (3).

$$\text{PER} = -1.816+0.435\ (\text{Met}) + 0.780\ (\text{Leu}) + 0.211\ (\text{His}) - 0.944\ (\text{Tyr})$$

***In vivo* protein quality and nutritive value:**

The protein quality and nutritive value for protein of studied seeds flour were carried out by experiment based on the nitrogen balance using albino rats. The experimental protocol has been followed as approved by Eggum

(1973). male albino, age 28 days old rats with an average weight 70 ± 5.0 g were selected for experimental trials. The rats were housed into four groups each of five rats, kept individually in polypropylene metabolic cages receiving on barley for one day. The cages fitted with steel screen bottom were housed in a room temperature at 20 ± 1°C and 55 % relative humidity, light cycle 12hr light off / on. The animals were weighed at the beginning of the study. Basal diet was prepared using casein as reference protein and given to control group. Mean while, the tested diets were formulated with HF, JF and PFaccording to Eggum (1973) formula. All the diets were prepared and stored in refrigerator. The diet groups were given into the animals about 10 grams per day. The nitrogen of collected faces and urine were determined using micro-kjeldahl method AOAC, (2005).

True Digestibility (TD %), Biological value % (BV) and Net protein utilization (NPU) were determined according to Eggum, (1973) to asses the nutritional performance of diets based on weight gain and nitrogen balance. The calculation also was carried out according to Eggum (1973) as follows:

$$\mathbf{TD = [N_i - (NF_1 - NF_2)] \div [N_i] \times 100}$$

$$\mathbf{BV = [N_i - (NF_1 - NF_2)] - [(NU_1 - NU_2)] \div [N_i - (NF_1 - NF_2)] \times 100}$$

Where: N_i, Nitrogen intake of animal fed test diet; NF_1, Nitrogen excreted in faeces of animals fed test diet; NF_2, Nitrogen excreted in faeces of animal fed protein-free diet; NU, Nitrogen excreted in urine of animals fed test diet; NU_2, Nitrogen excreted in urine of animals fed protein-free diet.

Net protein utilization (NPU) was calculated according to Eggum (1973) as follows:

$$\mathbf{NPU = [BV \times TD] \div 100}$$

Statistical analysis:

All the data obtained were subjected to the principle of analysis of variance statistical analysis was performed by one-way analysis of variance (ANOVA), using SAS session is executing on the WIN-98 platform (SAS software, version 8th Edition Cary, NC, 1999) computer package and followed by Duncan's Multiple Range comparison for the different treatments. Differences were considered significant when $p<0.05$.

RESULS AND DISCUSSION

Chemical composition:

Chemical composition of defatted hibiscus, jojoba and pumpkin seeds flours were presented in Table (1), a wide variation were clearly shown among hibiscus, jojoba and pumpkin seeds flour in their crude protein, fat, ash, crude fiber and carbohydrate. PF, HF and JF have a high ratio of protein ranked descending 53.2, 28.0 and 23.4 %, respectively.

The appreciable amount of fat ratio obtained from jojoba (54.9%) and followed by pumpkin 33.8 % and hibiscus seeds 13.9%. This result is agreed by Idouraine *et al.,* (1996) who found that the oil content of pumpkin seed (*Cucurbita pepo*) was 34.5-53.6%. From this results may be suggested that pumpkin and jojoba were contained a appreciable amount of protein and fat using in some food formulation and used to prepare food for children , pregnant , lactating mother and aid to fight against malnutrition. Another research by Cokeleare *et al.,* (1993) reported that, the seeds flour remaining after extracted of the jojoba oil, it contains about 30 % protein , dietary fiber and carbohydrates could be serve as an animal and human ingredient. However when used as an animal feed ingredient, the meal causes food intake reduction and growth retardation. Hibiscus and pumpkin seed flours were contained a considerable amount of crude fiber 7.2 and 5.6%, respectively.

Antinutritional factor of phytates shown in Table (2), low total quantity of phytates can be shown in pumpkin and jojoba which were 0.23 and 0.32 g/ 100 gm DM, respectively. This results also indicated by Akwaowa *et al.,* (2000) who found that seeds of pumpkin contain about 0.13 g/100 gm DM of phytic acid which was considered to our study , the pumpkin seed flour was ratio 0.23 g/100 g DM.

Phytates are known as capture for minerals elements especially Ca, Mg, Fe and Zn, and there by causing the ability of there minerals to be absorbed during metabolism (Forbes and Erdman 1983).

Generally , phytic acid intake of 4-9 mg/ 100g DM able to decrease iron absorption by 4-5 fold in human according to Herrel *et al.,* (1992). Meanwhile, jojoba and Hibiscus seed flours had a moderate ratio of phytates 0.32 and 0.3 g / 100 g DM, respectively, and remain at low level of previous eliminate absorption ratio of iron absorption.

Table (1): Proximate composition (%)dry mater base(DM) of defatted hibiscus, jojoba and pumpkin seeds flours.

Proximate composition matter (DM %)	HF	JF	PF
Moisture	8.9	6.3	4.0
Crude protein	28.0	23.4	53.2
Crude fat	13.9	54.9	33.8
Ash	6.6	2.6	4.3
Crude fiber	7.2	5.5	5.6
Carbohydrate	44.3	13.6	3.1
Antinutritional factor			
Phytates (g / 100 g DM)	0.5	0.32	0.23

Oil seeds protein and modified or processed oil seeds proteins incorporated into foods to
improve nutritive value and functional properties function properties (water absorption index, foaming capacity and stability) were influenced by protein behavior.

Functional properties:

Table (2), illustrates that the functional properties of different types of seeds flour, both hibiscus and pumpkin seeds Flour had the highest ratio of WAI (1.97 and 1.92 Kg gel / Kg solid) for their protein content. Increasing the amount of protein percentage is consequently led to a corresponding increase in WAI. This property may give an advantage to these flours in some

Table (2): Functional properties of defatted hibiscus, jojoba and pumpkin seeds flours.

Functional properties	HF	JF	PF
pH	6.97	6.43	7.40
Water absorption index (kg gel / kg solid) WAI	1.92 ± 0.06	1.62 ± 0.01	1.97 ± 0.30
Water solid index (kg solid / kg original solid) WSI	32.21 ± 1.9	51.66 ± 2.13	28.66 ± 0.76
Foam capacity (% volume increase)	45.0	45.0	20.0
Foam capacity (ml) at			
15 min	31.0	31.0	16.7
30 min	25.0	28.0	12.0
45 min	20.0	25.9	9.01
60 min	20.0	20.0	4.80

Mean ± SD

bakery products, as meat replaces and thickening agent Briskey (1976) and Del-Rosario and Flores (1981*).*

As shown in table (2) , Foam capacity percentage and foam stability properties of the flour were similar among HF and JF flours apparently shown higher stable than PF . In addition, both jojoba and hibiscus seeds flour had a high amount of hydrophobic amino acids of protein (41.41-37.22 g/16g N) as obvious later at Table (4) which suggested that JF and HF attributed to using as meat replaces and hydrophilic agent.

This data suggested that HF and JF were desirable as whipping, topping where foaming is an important property Kinsella (1979).

pH of flour soluble in water of HF JF and PF was 6.97 , 6.43 and 7.40, respectively, in fact that flours was stable at alkaline and acidic region meant that the three flours might be useful in formulation of an acidic foods such as protein rich carbonate beverage and milk analogue products Cherry (1981).

Amino acid pattern:

Protein is the one of the essential nutrients in the human food. Both the quantity and quality of protein provided by a food are important. The protein quality, also known as the nutritional or nutritive value of food, depends on its amino acids content and the physiological utilization of specific amino acids after digestion, absorption and minimal obligatory rates of oxidation. The ratio of individual essential amino acids in tested protein to the total amino acids, content of the same protein, (A/E), amino acid score, limiting amino acids, calculated PER, EAAI and estimated BV of different protein of studied seeds are tabulated in tables 3, 4 , 5, 6 and 7.

Amino acids composition of HF, JF and PF were analyzed and the results are shown in Table (3). It is clear from these results that, the amino acids divided into two groups, the first one is essential and the second is non-essential , so the three proteins of flours had appreciable amount of total essential amino acids ratio which is higher than the pattern of essential AA that recommended by FAO/ WHO (1973) except PF . Therefore, the total essential amino acids of PF,HF and JF arranged ascending 38.11,40.63 and 42.40 g/16gN respectively .

Table (3) shows that, hibiscus and jojoba seeds flour had a lowest value of methionine, which was 1.68 and 0.73g/16g N, respectively. While pumpkin seeds flour had a higher, content of methionine 2.18 g/16gN by comparing HF and JF. Moreover, total sulfur essential amino acids (methionine +cystine) in HF was recorded a high content (3.97g/16gN) compared with FAO/ WHO (1973) (3.50g/16gN). In addition, the highest levels of total aromatic essential amino acids (tyrosine + phenylalanine + tryptophan) detected in HF and JF compared with FAO/WHO (1973) or egg amino acids reference especially tryptophan. Therefore, PF contained a higher value of total aromatic essential amino acid (10.13g/16gN), compared with FAO/WHO (1973) (7.0g/16gN). Pumpkin seeds flour contained an appreciable amount of essential amino acids such as isoleucine, leucine, methionine, tryptophan and phenylalanine, those were 3.42, 6.98, 2.18, 3.42 and 5.03g/16gN ,respectively, than by hibiscus and jojoba flours. Otherwise, tryptophan level of pumpkin seeds flour was 1.68g/16gN. These results are in agreement with those reported by Zdunczyk *et al.,* (1999) whom found that protein of pumpkin seed cake contained considerable quantities of tryptophan 1.54g/16gN.

Table (3): Amino acids composition of hibiscus, jojoba and pumpkin kernel seeds flour (g/16gN).

Amino acid	HF	JF	***	PF FAO/ WHO (1973) provisional pattern	Egg amino acids reference
Isoleucine	3.14	3.13	3.42	4.0	6.30
Leucine	6.39	6.42	6.98	7.0	8.80
Lysine	4.54	5.57	3.48	5.5	7.00
Cystine	2.29	2.14	1.13	--	2.40
Methionine	1.68	0.73	2.18	--	3.40
Cys + Met	**3.97**	**2.87**	**3.31**	**3.5**	**5.80**
Phenylalanine	4.64	4.24	5.03	6.0	5.70
Tyrosine	2.97	2.57	3.42	--	4.20
Tryptophan	3.95	4.80	1.68	1.0	1.50
Phy+Tyr+Try	**11.56**	**11.61**	**10.13**	**7.0**	**11.4**
Threionine	3.14	4.84	3.02	4.0	5.10
Valine	4.21	5.05	4.40	5.0	6.80
Histidine	3.68	2.91	3.37	2.4*	**--**
Σ Essential A. A.	**40.63**	**42.4**	**38.11**	**38.4**	**51.2**
Aspartic	9.71	9.45	8.76		
Glutamic	17.54	11.48	17.98		
Serine	3.71	4.24	4.97		
Glycine	5.00	7.88	5.03		
Alanine	4.18	3.59	4.23		
Proline	4.03	5.57	3.29		
Arganine	8.86	7.54	10.54	2.0**	
Σ Non essential A. A.	**53.03**	**49.75**	**54.80**		

A.A.: amino acid
*** Histidine (Food & Nut. Board of the Acad. Of Sci., 1963)**
**** Arginine (Rose, 1937)**
***** This pattern is agreed with Zdunczyk *et al.,* (1999).**

Jojoba flour AA pattern was shown in table (3) , the highest ratio of total essential amino acids 42.4g /16gN was higher than reference FAO/WHO (1973) provisional pattern by 10% approximately .Total aromatic amino acids

, leucine , lysine and valine are the most predominant essential amino acids in jojoba flour .Moreover, JF had a higher level of leucine (6.42g/16gN) when compared to HF only . However, jojoba seeds flour were higher in lysine, tryptophan, threionine and total essential AA, when compared to FAO/WHO (1973) reference pattern. Otherwise, total sulfur AA concentration of JF is lower than their concentration in pumpkin and hibiscus seeds flour and as compared with FAO/WHO (1973) by range 5 –18%.

Humans must include adequate amounts of nine amino acids in their diet; these essential amino acids cannot be synthesized from other precourses. Essential amino acids of human must be contains of histidine as recommended by Marian, (1981).

As shown in table (3), hibiscus seeds flour had a highest content of essential amino acid histidine (3.68g/16gN) followed by pumpkin flour and jojoba flour, which recorded 3.37 and 2.91g/16gN, respectively. Morover, all proteins of HF, JF, and PF contained a high concentration of histidine compared with FAO/WHO (1973).

Non-essential amino acids of hibiscus, jojoba and pumpkin flour were tabulated in table (3). It's clear from the results that, total non essential amino acids of hibiscus flour and pumpkin flour are quite similar 53.03 and 54.80g/16gN, respectively. Glutamic acid is the most predominant non-essential amino acid in HF, JF and PF that was 17.54, 11.48 and 17.98g/16gN, respectively. Aspartic acid was the highest concentration in HF and JF (9.71 and 9.45 g/16gN respectively) and 8.76g/16gN for PF. While arginine recorded high values (8.86 ,7.54 and 10.54g/16gN)for HF ,JF ,and PF , respectively, as compared with FAO/WHO (1973) (2.0g/16gN).This result is agreed with AL-Fawaz (2004) who found that the arginine, glutamic acid and aspartic acid are showed the highest concentration in pumpkin seed kernel . Moreover JF had the highest concentration of glycine (7.88 g/16gN) compared with it's concentration in the other flours HF and PF (5.0 and 5.05 g/16gN respectively). In addition, JF contained the highest concentration of proline (5.57 g/16gN) compared with it's concentration in HF and PF (4.03 and 3.29g/16gN respectively).

Classification of amino acids:

Classification of AA for different groups of amino acids according to chemical properties is demonstrated in Table (4). Among of three seed flours, hibiscus contained appreciable amount of sulfur containing AA and acidic AA, whereas uncharged polar content was lower than JF. Pumpkin seed flour containing the highest basic AA and moderate in hydrophobic and uncharged polar content AA compared to both AA groups of both HF and JF. Generally , the highest ratio of sulfur containing AA such as methionine and cystine are suggested not only require an appropriate amount for human nutrition but also precipitate the deficiency in methionine and detoxify the toxins in the diet Maner and Gomez (1973). Jojoba seeds flour

Table (4): Distribution of amino acid classified according to similar properties of defatted hibiscus, pumpkin and jojoba kernel seeds flour.

Group of amino acids	HF	JF	PF
Hydrophobic (nonpolar)[a]	37.22	41.41	36.24
Uncharged polar [b]	12.11	13.79	12.54
Basic [c]	17.08	16.02	17.39
Acidic [d]	27.25	20.93	26.74
Sulfur- containing [e]	3.97	2.87	3.31
Aromatic [f]	11.56	11.61	10.13

a: Gly, Alan, Val , Leu, Pro, Met, Phe, Try and Ileu b: Ser, Thr, Cys and Tyr
c: Lys , Arg and His d: Asp and Glu e: Cys and Met f: Phe , Tyr and Trp

This table designed and grouped amino acids according to Zhu *et al.,* (2006).

contained a highest amount of hydrophobic and uncharged AA and polar AA, meanwhile, the sulfur containing AA and basic AA were the lowest compared to their containing of HF and PF.

From these results, it could be concluded that , the hydrophobic, acidic and basic AA containing are raked as the highest level content the flours exaggerated from the HF, JF and PF. Meanwhile, containing of uncharged is the lowest containing as general among these seed kernel flours.

Table (5): Comparison between the essential amino acids content of hibiscus (HF), Jojoba (JF) and pumpkin (PF) seeds flour with those of FAO reference protein (mg / gm protein).

Essential amino acids	FAO reference protein (1973)	HF		JF		PF	
		AA*	AAS**	AA*	AAS**	AA*	AAS**
Lysine	55.0	45.4	82.5[c]	55.7	101.2	34.8	63.2[a]
Isoleucine	40.0	31.4	78.5[b]	31.3	78.2[a]	34.2	85.5[c]
Leucine	70.0	63.9	91.2	64.2	91.7[c]	69.8	99.7
Cys + Met	35.0	39.7	113.4	28.7	82.0[b]	33.1	94.5
Phenylalanine + Tyrosine	60.0	76.1	126.8	68.1	113.5	84.5	140.8
Threionine	41.0	31.4	76.5[a]	48.4	118.0	30.2	73.6[b]
Tryptophan	10.0	39.5	395.0	48.0	480.0	16.8	168.0
Valine	50.0	42.0	84.0	50.5	101.0	44.0	88.0

AA*: Amino acid (mg / gm protein). AAS: Amino acids score.**
a: The first limiting amino acid b: The second limiting amino acid c: the third limiting amino acid

Calculation of nutritional value and protein quality:

Predicting protein and nutritional qualities:

Chemical score and limiting amino acids:

Table (5) and (6) shown that the essential amino acids of HF, JF and PF by comparison with those of FAO (1973) reference protein to obtain amino acids score. It is clear from the data illustrated in table (5) and (6) that lysine is the first limiting amino acids in PF while it is third limiting amino acid in HF. Isoleucine is the first limiting amino acid in JF, meanwhile it's the second and third limiting A.A in HF and PF , respectively. However, Meth +Cys are the second limiting amino acids in JF, but leucine is the third one in JF. Therionine is performed as the first limiting one in HF, while, it is the second one in PF.

Estimated EAAI, PER , BV and A/E indices:

Table (6), illustrates that the higher calculated EAAI and predicted PER for PF 81.4 % and 2.06 respectively, however implies the protein of PF has a higher in biological value (BV) 80.08 % in comparison to HF and JF. Our results indicated that, the PF is quite similar resembling casein corresponding on its EAAI and PER. Therefore, it could be proved that, pumpkin seed flour higher in biological value and protein quality than either HF or JF. Predicted PER value of HF, PF and JF are not exceeding 2.00 except pumpkin seeds flour, which describes that the protein of pumpkin seeds is good quality Friedman (1996). Also this result is closely agrees by EL-Adawy and Taha (2001) who estimated that PER of pumpkin seeds kernel flour was 2.15. Hibiscus and jojoba flour had moderated EAAI % (70.8 and 61.3%) and PER (1.88 and 1.70), respectively. In contrast, reported by Bryant *et al.,* (1988) that the calculated PER was 2.17 for (*Hibiscus sabdariffa*) protein isolate. Therefore, the estimated BV of JF is lesser than 43.58 % while HF was 68.91%. Depending on BV, PER and EAAI and computing to casein nutritive value of subjected seeds flours under this research, concluded that the pumpkin seeds flour is the excellent source of nutritive value and followed by hibiscus and jojoba. Therefore, these seeds flour can be used as supplemented for complimentary food protein, improving their nutritional values, usually by supplying lysine which is abundant in these flours proteins but limiting in cereal proteins. Examples of supplementation included bread and other baked goods with these flour proteins.

Table (6): Protein quality and nutritional value computations amino acids of hibiscus, jojoba and pumpkin kernel seeds flour.

Protein quality and nutritional value	HF	JF	PF	Casein as* standard protein
EAAI (%)*	70.8	61.3	81.4	90.2
Chemical score (%)	76.5	78.2	63.2	58.4
First limiting amino acid	Threionine	Isoleucine	Lysine	Meth + Cys
Second limiting amino acid	Isoleucine	Meth + Cys	Thrieonine	Isoleucine
Third limiting amino acid	Lysine	Leucine	Isoleucine	---
BV (Estimated biological value)	68.91	43.58	80.08	
PER (Protein Efficiency Ratio)	1.88	1.70	2.06	2.14 (2..50)***

*** EAAI: Calculated Essential Amino Acids Index.**
**** Casein as standard protein is obtained from Zdunczyk *et al.,* (1999)**
***** calculated PER as estimated by Angulo-Bejarano *et al.,* (2008).**

Table (6) shown that the therionine , isoleucine and lysine are the first and/or second limiting amino acids in HF , J F and PF except Meth +Cys is the second limiting amino acid, in JF with chemical score 76.5 % , 78.2 %

and 63.2 , respectively , based on the FAO/WHO (1973) provisional pattern.
Similar finding by Kamel *et al.,* (1985) and EL-Soukkary (2001) who found that, the lysine is the first limiting amino acid in raw pumpkin seeds flour. The third limiting amino acids was lysine in HF, but isoleucine was the third limiting amino acid in PF. Table (7) shows the A/E ratio (an individual essential amino acid content/total essential amino acid content) of HF, JF and PF, and selected protein source materials by comparison with FAO pattern (1965).

Table (7): A/ E ratio (mg essential amino acid of total essential amino acids) of HF, JF and PF compound with FAO reference protein (1965).

Essential amino acids	FAO* reference protein	HF	JF	PF
Lysine	152	111	131	91
Isoleucine	111	77	73	89
Leucine	194	157	151	183
Cys + Met	97	97	67	86
Phenylalanine + Tyrosine	166	187	160	221
Threionine	114	77	114	79
Tryptophan	28	97	113	44
Valine	139	103	119	115

16 FAO: Protein requirements. (FAO, Rome, 1965)

Hibiscus, jojoba and pumpkin seeds flour could be considered as rich sources for either aromatic amino acids of phenylalanine + tyrosine (187, 160 and 221), , or tryptophan (97,113 and 44), respectively. In addition, the HF and PF are used as excellent sources for either leucine (157 and 183) or sulfur amino acids such as Meth+ Cys (97 and 86), respectively. Moreover, JF could be considered as the rich source in either lysine (131) or threoinine (114) and valine (119). Pumpkin seeds flour is used as rich source for either isoleucine (89) or valine (115).

***In vivo* protein quality and nutritional assessment :**

The protein quality and nutritional value such as TD, BV and NPU for utilized hibiscus, jojoba and pumpkin seeds flour and casein were shown in Table (8), the true digestibility was significantly (p<0.05) different for all flours. Pumpkin seeds kernel flour had the highest TD (81.68%) and follows by hibiscus flour (76.86%), these nearest to TD of casein as control (reference control diet) 97.06 %. As expected, rats fed with the standard casein used dietary protein more efficiency when compared to counterparts fed with the HF and PF. These results were agreed with EL-Adawy and Taha (2001) who reported that, PF had the highest *in vitro* protein digestibility (90.0%).

In addition, the JF diet groups exhibit a significantly lower ratio (46.11%) of TD when computation than obtained by casein and either HF or PF. Significantly, JF as protein rich source, can be supplemented to animal foods but it contain appreciable amount of antinutitional factor as a glucoside act as nutritional and food intake improvement after animal administration

(Lievens *et al.,* (2003). The ability of simmondsin to reduce satiety molecule Chole Cytokinine (CCK) at first act, CCK as hormone reduce meal size by decreasing meal duration and eating rate and increases the latency to eat Lievens *et al.,* (2003). This also agree with Cokelaere *et al.,* (2000) who concluded that by feeding rats for 10 week on 0.25% simmondsin was induced a transit time of diet compared to pair fed rats. However, the study did not reveal any biochemical signal toxicity.

Table (8): Biological assessment for defatted hibiscus, jojoba and pumpkin kernel seeds flour.

Biological parameters	Casein	HF	JF	PF
True digestibility (TD) %	$97.44^{a} \pm 0.38$	$76.86^{b} \pm 1.23$	$49.93^{c} \pm 3.82$	$81.68^{b} \pm 5.32$
Biological value (BV) %	$90.82^{a} \pm 0.25$	$64.50^{c} \pm 0.89$	$42.24^{d} \pm 1.35$	$78.63^{b} \pm 1.82$
Net protein utilization (NPU)	$88.49^{a} \pm 0.60$	$49.57^{b} \pm 1.54$	$21.10^{c} \pm 2.01$	$62.30^{b} \pm 6.89$

Values are mean ± SD
Mean values in vertical row bearing same letter are significantly (p<0.05) different and others are nonsignificant.

As stated before, JF had a significantly lowest true digestibility (49.93%) which attributed to antinutritive value of simmondsin. This is also agreed with Nestares *et al.,* (1966) who appreciated, that various antinutritional substances including trypsin inhibitors and different antnutritional factors impeded the complete digestion of protein and increasesd the endogenous fecal excretion of nitrogen, that partially responsible for decrease in protein digestibility value of such raw hibiscus, jojoba and showed a moderate increase in TD of pumpkin seeds flour.

Table (8) shown, that the biological value of JF was significantly (p<0.05) lowered when compared to control diet and those HF and PF. These results are also in agreement with those obtained by estimated biological value in Table (6), jojoba flour has estimated BV 43.5 % depend on chemical investigation , while *in vivo* biological value was 42.24%. Both HF and PF were shown a moderate biological value when compared to casein. From these results, it could be appreciated that, PF had a good quality followed by HF, that intended these seeds particularly attractive for countries where the high prevalence of protein energy malnutrition is due to the poor nutritional quality of the diet.

Significantly, low ratio of NPU (21.10 %) being performed from JF and followed by HF. Meanwhile both HF and PF were significantly equal when compared to casein. Science, Shermer and Perkins (1975) indicated that the decrease bioavailability of amino acids, especially methionine lowered net protein utilization. This statement is agreed with previous obtained results, while JF had the lowest sulfur containing amino acids (2.87 g / 16 g N) and considered as a second limiting amino acids as obviously in Tables (4) and (6). Moreover, the protein digestibility and BV of HF and PF were illustrated in Tables (6) and (8) both of these flours had an appreciable amount of sulfur containing amino acids when compared to recommended pattern according FAO / WHO (1973), that permits to meet nitrogen and amino acids requirements for human. Depending on the TD and BV of HF and PF, it could be finalize those flours of hibiscus and pumpkin seeds were an alternative source of protein to supplement poor protein source which are lacking in essential amino acids .

From these previous results can be concluded that pumpkin and hibiscus seed flours are good sources for used in a supplementation and complementary foods. In addition, jojoba flour is promised as especial flour and protein for dietetic foods with low energy intake and appetite reduction as well.

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الخواص الغذائية والوظـيفــية للـدقيق الناتج من بذور الكــركدية و الجــوجوبا و القــرع

وائل حلمي موسي الرفاعي، سناء عبد الحميد محفوظ، ، محمـد سيد مسعود و شــيرين عبـاس صادق نـــدا .

المركز الاقليميي للأغذية والأعـلاف –مركز البحوث الزراعيــة –الجيزه –مصـر .

أستهدفت هذة الدراسة زيادة الأستفادة من المصادر المتاحة والمتعددة للبروتين الناتج من مخلفات النباتات الزراعية وأيضا النواتج المتخلفة عن التصنيع . لهذا تم تقييم الخواص الوظيفية والغذائية للدقيق الناتج من ثلاث أنواع من البذور النباتية بعد نزع الدهن وهى بذور الكركدية , الجوجوبا و القرع . كما تم تقدير التركيب الكيماوى لها (البروتين – المستخلص الإثيرى(الدهن) – الألياف – الأحماض الأمينية و الكربوهيدرات). وقداحتوت تلك الأنواع الثلاث من الدقيق على نسبة من البروتين تراوحت بين ٢٣,٤ , ٢٨,٠ , ٥٣,٥% لكل من دقيق الجوجوبا , الكركدية و القرع على التوالى . كما احتوت تلك البذور على نسب مختلفة من الزيت (٥٤,٩ , ١٣,٩ , ٣٣,٨ %) على التوالى . أيضا تم تقدير محتوى المواد المانعة من الاستفادة غذائيا وأهمها الفيتات وتراوحت نسبتة فى الدقيق من ٠,٢٣ إلى ٠,٥ جم / ١٠٠ جم مادة جافة .

كما تم تقييم الخواص الوظيفية للدقيق الناتج من تلك البذور واشتملت على قدرة امتصاص

الماء , قدرة ذوبان الماء , حجم الرغوة وثباتها ,ووجد ان لدقيق هذة البذور خواص جيدة ماعدا حجم وثبات الرغوة لدقيق بذور القرع .

ووجد أن الدقيق امنزوع الدهن لتلك البذور أحتوي علي أعلي نسبة من الأحماض الأمينية الكلية الأساسية مقارنة بمرجعية الFAO/WHO (1973)

وقد تم تقسيم الأحماض الأمينية الي مجموعاتها المختلفة أعتمادا علي المجاميع الدالة على التركيب الكيماوى للأحماض الأمينية وهذا لتحديد أمكانية أضافتها لتدعيم العجائن و أستخدامها لخلطات اللحوم المصنعة .وتعرضت الدراسة للمقارنة ما بين القيمة الغذائية والحيوية للبروتين الموجود في دقيق تلك البذور وذلك بالتقييم الكيماوي و الحيوى .

أشتمل التقييم الكيماوي علي تقدير الأحماض الأمينية المحددة و المعامل الكيماوي و معامل الأحماض الأمينية ألأساسية والنسبة مابين الأحماض ألأمينية الكلية والأساسية والقيمة البيولوجية المحسوبة و معامل كفاءة البروتينن

أما التقييم الحيوى الذي تم إجراؤه علي فئران التجارب فأعتمد علي المهضوم من البروتين الفعلي و القيمة الحيوية

والبروتين الكلي المهضوم و إتضح من المقارنة أن دقيق الكركدية والقرع ذات قيمة حيوية وجودة بروتين عالية أعتمادا علي ما سبق توضيحة .

وبينما أظهرت الدراسة أن الجوجوبا أقلهم وأفقرهم غذائيا بينما أقترب كلا من دقيق الكركدية والقرع من الكازين القياسي .وقد وجد أن الجوجوبا تحتوي علي مواد تقلل الأستفادة الغذائية تتمثل في وجود الثيمودنزين المسؤول عن ضعف القابلية للتغذية وضعف الشهية مما قد يقلل من الأستفادة الحيوية ، في حين أن القيمة المقدرة كيماويا في بعض التقييمات عالية .

وبناء علي ما سبق فأن الدقيق الناتج عن هذه البذور محل الدراسة يمكن أستخدامة في تدعيم المخبوزات و الأغذية التكميلية و اللحوم أعتمادا علي الخواص الوظيفية والقيمة الغذائية .مع الأخذ في الإعتبار أن دقيق الجوجوبا قد يكون محتوي علي نسب مقبولة من الأحماض الأمينية الغذائية إلا أنه خافض للشهية وبالتالي يقلل مستويات الطاقة المأخوذة لدي الأشخاص متبعي الحمية الغذائية.

5083 5084 5085 5086 5087 5088 5089 5090 5091 5092

5093 5094 5095 5096 5097 5098 5099 5100 5101

5083 5084 5085 5086 5087 5088 5089 5090 5091 5092

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