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NUTRITIONAL AND FUNCTIONAL PROPERTIES OF FLAXSEED PROTEIN PRODUCTS

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ABSTRACT

Chemical compositions, functional and nutritional quality of flaxseed flour (FF), flaxseed protein isolate (FPI) and flaxseed protein concentrate (FPC) were chemically studied. Globulin was found to be the major fraction in FF (47.68%). The protein solubility pattern of FF at different relatively pH's exhibited abroad pH range from 3 to 6 meanwhile solubility increases in alkaline pH than in acidic pH. FPI had higher ($p \leq 0.05$) protein content than both FPC and FF; while total fat, fiber and total ash contents in both FPI and FPC were nearly similar. The FF is a good source of Ca, Mg and of high content of K and Fe than both FBI and FPC. All flaxseed protein products had highest levels of isoleusine, total aromatic amino acids, valine and tryptophan comparing to that reported in FAO/WHO (1973) profile. In general lysine and leucine were the first and second limiting amino acids, respectively. Both FPI and FPC had the highest fat and water absorption capacities, emulsification capacity and foam capacity than FF. Panelists accepted cakes containing up to 15% of FF and 10% of both FPI and FPC. Therefore Flaxseed protein products could be useful to use as a good quality nutrients substitution or supplementation and as a functional agent in food system.

Keywords: flaxseed- flaxseed protein- chemical compositions- nutritional quality- functional properties

INTRODUCTION

Flaxseed, also known as linseed, is enjoying an upsurge in popularity as a result of reports on its benefits to human health and its potential to reduce the risk of certain diseases (Oomah and Mazza, 2000). However, the current market for edible flaxseed, as well as recent research on its role in human health, has focused on the whole intact flaxseed and its oil components (Oomah, 2001). The defatted meal is presently being used as livestock feed, and limited attention has been given to the physicochemical and functional properties of the constituent proteins. This information is essential for increasing the utilization of flaxseed proteins, which have the potential to become important value-added products from the edible oil industry, as evidenced by the successful entry of soy proteins into the functional food ingredient market. Only a few studies have been conducted on the characteristics and functionality of the protein components fractionated from flaxseed.

The functional properties are the intrinsic physico-chemical, that affects the behavior of food system during processing and storage, e.g. solubility, foamability, and emulsification properties (Oshodi and Ekperigin, 1989).

Vassel and Nesbitt (1945) reported that Osborn in 1892 was the first investigator isolated flaxseed protein fractions of a globulin with 18.6% nitrogen and an albumin-like protein with 17.7% nitrogen. Thereafter, research on flaxseed proteins has been concerned primarily with methods for the extraction of protein from the oil-crushed meal (Dev *et al.*, 1986a; Oomah and Mazza 1993; Oomah *et al.*, 1994; Smith *et al.*, 1946; and Sosulski and Bakal, 1969 and Wanasundara and Shahidi, 1996 and 1997).

Only a few studies have been conducted on the characteristics and functionality of the protein components fractionated from flaxseed. These studies reported that flaxseed consists of two major storage proteins, a predominant salt-soluble fraction with high molecular weight (11– 12 S), and a water-soluble basic component with low molecular weight (1.6– 2 S) (Dev *et al.*, 1986b; Dev and Sienkiewicz, 1987; Madhusudhan and Singh, 1985a and 1985b; Marcone *et al.*, 1998a; and Youle and Huang, 1981), and suggest similarity between the properties of the major storage protein of flaxseed and those of other important oilseeds (Madhusudhan and Singh, 1985c and 1985d; Marcone *et al.*, 1998b and 1998c; and Oomah and Mazza, 1993). However, most of the properties of the flaxseed major protein fractions are still awaiting investigation (Marcone, 1999 and Oomah, 2001).

The main objective of this study was to prepare flaxseed flour (FF), protein isolate (FPI) and concentrate (FPC), the nutritional and functional properties were also evalulated to obtain basic information for potential applications of flaxseed proteins in foods.

MATERIALS AND METHODS

Materials

Flaxseed

Flaxseed (*Linum usitatissimum*) variety Sakha-1 was obtained from Field crop Research Institute, Agriculture Research Center, Cairo, Egypt.

Flaxseed flour (FF):

flaxseed was crushed and then defatted by soaking in n-hexane (*BP*.40 - 60°C) at room temperature for 18 hr with change the solvent several times. The defatted flour was ground again to pass through a 60 mesh sieve and reffered as flaxseed flour (FF).

Flaxseed protein isolate (FPI):

The protein was extracted twice by 0.1 N NaOH (pH 10.0) at room temperature using 1: 15 (w/ v) flour to solvent ratio after shacking for 1 hr and then centrifuged at 4000 rpm for 20 min. The extracts were combined and acidified to pH 4.5 using 1 N HCl. The precipitate was recovered by centrifugation at 4000 rpm for 20 min and then washed by distilled water and drying at 50°C for 16 hr in a vacuum oven.

Flaxseed protein concentrate (FPC):

The protein was extracted twice by 0.5% Na_2CO_3 as mentioned above. The obtained supernatant was first dialyzed against distilled water for 72 hr with change the distilled water every 4 hr and then dried as mentioned above for FPI. Both FPI and FPC were ground to pass through a 60-mesh sieve.

Methods

1- Chemical composition:

The contents of moisture, crude oil, crude protein (N x 6.25) and total ash of flaxseed protein products were determined as described in AOAC (1995).

2-Protein classification:

The Osborn classification of protein was done according to the method of Abd El-Aal *et al.* (1986) using distilled water, 1M sodiumchloride, 70% ethanol and 0.2 M sodium hydroxide solutions for albumins, globulins, prolamins and glutelins, respectively. The remaining residue after the successive extractions was quantitatively transferred to Kjeldahl digestion flask and digested with concentrated H_2SO_4 to determine the residual protein.

3- Protein- pH solubility profile:

One gram of FF was dispersed in 20 ml of aqueous solvent and the pH adjusted in the range of 1 to 12 with 0.5 M HCl or NaOH. The suspension was shaken at room temperature (~ 25°C) for 1 hr, centrifuged at 4000 rpm for 20 min, and the clear supernatant pH was recorded. The clear supernatant was used for nitrogen determination.

4- Protein solubility index:

It was estimated in distilled water at pH 9, 5% NaCl and 0.2% NaOH as described by Smith *et al.* (1959).

5- Functional properties methods:

Water and fat absorption capacities were determined according to the procedure of Sosulski (1962) and Sosulski *et al.* (1976), respectively. Emulsification capacity (ml oil/ gm protein) was determined as described by Beuchat *et al.* (1975). The method of Lawhon *et al.* (1972) was used to determine the foaming capacity and stability. The percentage increase in volume after 30 sec was recorded as foam capacity. The change in volume of foam volume was recorded after 15, 30, 45, 60 and 90 min of standing at room temperature (~ 25°C) as foam stability.

6- Determination of amino acids content and biological value:

Amino acids composition of flaxseed protein products were determined using a Mikrotechna AAA 881 automatic amino acid analyzer according to the method described by Moore and Stein (1963). Meanwhile, tryptophan content was chemically determined by the method of Miller (1967). Available lysine content was determined according to the method described by Carpenter (1960).

Biological value of flaxseed protein products was determined based on its amino acids composition. Chemical score of amino acids was calculated using the FAO/WHO (1973) reference pattern. Essential Amino Acid Index (EAAI) was calculated according to Oser (1959) using the amino acid composition of the whole egg protein published by Hidvégi and Békés (1984). Protein efficiency ratio (PER) was estimated according to the following regression equation proposed by Alsmeyer *et al.* (1974) as follow: PER = - 0.468 + 0.454 (leucine) - 0.105 (tyrosine). Biological value (BV) was calculated according to MØrup and Olesen Index (1976).

7- In-vitro protein digestibility (IVPD):

It was determined as described by Salgó *et al.* (1985). A two digestive enzymes (trypsin-pancreatin) system were used in pH-drop method.

8- Preparation of cake:

Cakes were prepared according to the formula of Khalil (1998) using the following recipe: 28 gm wheat flour, 24 gm margarine, 24 gm sugar, 13.55 gm whole egg, 0.45 gm baking powder and 10 ml defatted milk to prepare the control cake. To prepare the replacer cakes, the formula was replaced with 5, 10 and 15% of FF, FPI and FPC. Cake batters were baked at 180 °C for 45 min. Cakes were allowed to cool at ambient temperature and then sensory properties were performed by a ten- member panel to measure appearance, crust and crumb colour, flavour, texture and overall acceptability. A hedonic scale of 1 to 10 was used, 1= very poor and 10= excellent (Johnson *et al.* 1989).

9- Statistical analysis.

Results are expressed as the mean value ± standard deviation (SD) of three separate determinations, except for the minerals and amino acid contents, which were determined in duplicate. Data were statistically analyzed using analysis of variance and least significant difference using SAS (1985). Significant differences were determined at the $p < 0.05$ level.

RESULTS AND DISCUSSION

Protein classification:

Table (1) shows the protein fractions of flaxseed separated according to their solubility in different solvents. Significant ($p<0.05$) differences were observed among the protein fractions of FF. Globulin represented the major protein fraction (47.68%) followed by albumin (31.95%) and glutelin (13.94%). Panford (1989) observed that water extracted only 25% of total meal nitrogen, while 5% NaCl, 70% ethanol and 0.1 NaOH extracted 29%, 4% and 42% of total meal nitrogen, respectively. However, Sosulski and Bakai (1969) found that 42% of the total meal nitrogen is to be water-soluble, 47% soluble in NaCl, 2% soluble in 70% ethanol and 3.5% soluble in NaOH for flaxseed meal.

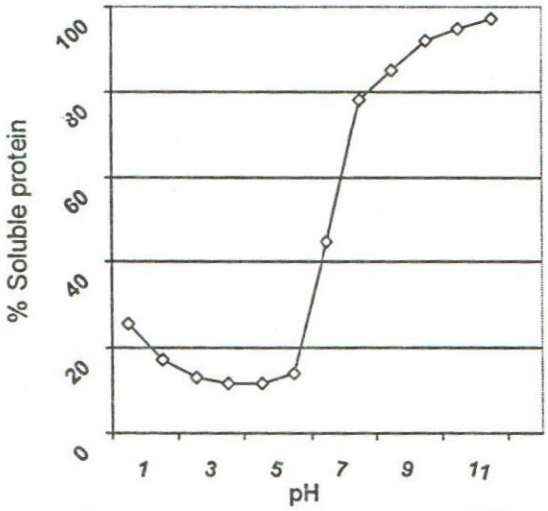
Table (1): Protein fractions of flaxseed flour according to their solubility.

Protein fraction	Protein (%)	Protein yield (%)
Water- soluble (Albumin)	$14.83^{b} \pm 1.18$	31.95
Salt- soluble (Globulin)	$22.14^{a} \pm 1.95$	47.68
Alcohol- soluble (Prolamine)	$1.25^{d} \pm 0.23$	2.69
Alkaline- soluble (Glutelin)	$6.47^{c} \pm 0.29$	13.94
Residual protein	$1.74^{d} \pm 0.17$	3.74

Means in the same column with no common superscript letter are significantly different ($p \leq 0.05$).

Protein solubility at different pH:

The effect of pH on protein solubility of FF is presented in Fig (1). The protein solubility *vs.* pH gave a broad minimum solubility between pH 3.0 and 6.0, where the protein solubility was lower than 15%. Protein solubility was increased above pH 6 and 92% at pH 10. Below pH 3.0, the protein solubility was increased, and makes 28% the total protein at pH 1.0. Dev *et al.* (1986a) reported that about 20- 24% of the total protein is soluble at the isoelectric point. Therefore, the pH 10.0 was chosen as the suitable pH for future protein extraction.



Fig(1): Effect of pH on protein solubility of flaxseed flour.

Chemical composition of flaxseed protein products:

The chemical composition of flaxseed protein products is shown in Table (2). The protein content of FPI was significantly ($p< 0.05$) higher than both FF and FPC. Meanwhile, FF had higher content of carbohydrate, fat and crude fiber than both FPI and FPC. The lowest fat content in both FPI and FPC may be due to the protein- lipid interaction during processing leading to non extractable complexes. Madhusudhan and Singh. (1985c) found that the protein content of defatted flaxseed flour was 52%. Dev and Quensel (1988) reported that the protein content of flaxseed protein prepared at isoelectric precipitation ranged from 56 to 86%. On the other hand, our results were in agreement with those reported by Sosulski and Bakai (1969).

Mineral composition:

The minerals composition of flaxseed protein products are show in Table (3). The FF had highest content in all minerals than both FPI and FPC except sodium. All products of flaxseed protein had a quite level of trace

elements such as zinc, copper and iron; therefore, these products can be used to cover the limitation in nutrition. The high content of K can be utilized beneficially in the diets of people who take diuretics to control hypertension and suffer from excessive excretion of K through body fluid. Wiesenfeld *et al.* (2003) reported that flaxseed meal was rich in copper, potassium and magnesium, but had less zinc.

Table (2): Chemical composition of flaxseed protein products. (on dry weight)

Components	FF	FPI	FPC
Total protein	$46.43^{c} \pm 0.37$	$90.25^{a} \pm 0.82$	$82.37^{b} \pm 0.75$
Total fat	$3.18^{a} \pm 0.37$	$0.58^{b} \pm 0.09$	$0.76^{b} \pm 0.12$
Crude fiber	$10.35^{a} \pm 0.28$	$0.26^{b} \pm 0.03$	$0.49^{b} \pm 0.07$
Total ash	$6.64^{a} \pm 0.27$	$3.97^{b} \pm 0.32$	$3.16^{b} \pm 0.35$
Total Carbohydrates*	$33.40^{a} \pm 1.97$	$2.92^{c} \pm 0.39$	$8.69^{b} \pm 0.65$

Means in the same column with no common superscript letter are significantly different ($p \leq 0.05$)

*** By difference**

FF= flaxseed flour

FPI= flaxseed protein isolate

FPC= flaxseed protein concentrate

Table (3) Minerals content of flaxseed protein products (mg/ 100 gm sample).

Minerals content	FF	FPI	FPC
Calcium	165	86	92
Iron	585	426	384
Magnesium	347	184	257
Phosphorus	435	165	255
Potassium	639	421	443
Sodium	31	63	58
Zinc	3.51	2.34	2.14
Copper	1.24	0.76	0.65
Manganese	3.13	1.28	2.01

Amino acids:

Table 4 shows the amino acids composition of flaxseed protein products. All flaxseed protein products had high contents of isoleusine, valine, total aromatic amino acids and tryptophan than those reported in FAO/ WHO (1973) profile. Both FPI and FPC had high contents from total sulfur amino acids, threonine and valine than FF. Relatively to the FAO/ WHO (1973) pattern; all flaxseed protein products are rich in the most essential amino acids and poor in leucine and lysine. Generally, Sosulski and Sarwar (1973) reported similar results to those presented in Table 4. The lysine/ arginine ratio was 0.33 for FF, 0.41 for FPI and 0.38 for FPC, compared to 0.88 for both soybean and canola proteins as reported by Oomah and Mazza (2000), suggesting that flaxseed protein may be less lipidemic and atherogenic than either soybean or canola proteins (Oomah, 2001).

All flaxseed protein products had a high content of arginine, glutamic acid and aspartic acid like the other oilseed proteins. Madhusudhan and Singh (1985a)

reported same trend for arginine, glutamic acid and aspartic acid of flaxseed meal. Youle and Huang (1981) reported that amino acids composition with high nitrogen content was to be important for supplying nitrogen for germination. Protein sources rich in arginine and glutamine have recently gained popularity because of the potential preventative functions of arginine against heart disease (Pszczola, 2002) and of glutamine in supporting the immune system (Oomah, 2001) and improving athletic performance (Blenford, 1996).

Table (4): Amino acids composition of flaxseed protein products (gm/ 100 gm protein).

Amino acids	FF	FPI	FPC	FAO/ WHO (1973)
Isoleucine	4.58	4.27	4.21	4.0
Leucine	5.49	5.83	5.37	7.0
Lysine	3.24	3.49	3.46	5.5
Cystine	1.42	1.38	1.59	3.5
Methionine	1.75	2.01	1.94	
Tyrosine	2.46	2.65	2.62	6.0
Phenylalanine	5.86	5.96	5.76	
Theronine	3.87	4.07	4.24	4.0
Tryptophan	4.32	4.52	4.05	1.0
Valine	5.49	5.57	5.73	5.0
Total essential amino acid	38.48	39.75	38.97	36.0
Aspartic acid	10.80	10.52	10.16	
Glutamic acid	19.46	18.44	17.87	
Serine	5.08	4.58	5.49	
Proline	4.13	5.37	4.83	
Histidine	3.18	3.04	2.94	
Arginine	9.46	8.45	9.02	
Glycine	4.77	4.58	5.15	
Alanine	4.64	5.27	5.57	
Total non-essential amino acid	61.52	60.25	61.03	

Nutritional quality:

Table (5) shows the nutritional quality of flaxseed protein products. Chemical score of FF was lower than that both FPI and FPC.

Table (5): Nutritional quality of flaxseed protein products.

Nutritional parameter	FF	FPI	FPC
Chemical score (CS)%	58.9	63.5	62.9
First limiting amino acid	Lysine	lysine	lysine
Second limiting amino acid	Leucine	leucine	Leucine
Essential amino acids index	65.84	67.16	66.05
Protein efficiency ratio	1.63	1.72	1.54
Biological value	67.06	68.01	65.17
Available lysine gm/ 100 gm protein	2.15	2.58	2.45
In-vitro protein digestibility	64.25^{b}±1.07	76.54^{a}±1.54	74.36^{a}±1.25

Means in the same column with no common superscript letter are significantly different (p≤0.05)

Meanwhile, the chemical score of FPI and FPC were nearly similar. Lysine and leucine were the first and second limiting amino acids, respectively in all flaxseed protein products

The PER, BV, available lysine and values of FPI were higher than those both FF and FPC. In-vitro protein digestibility of both FPI and FPC was significantly higher than FF. The main contributing factors for such poor digestibility are their structural characteristics, the relative proportion of the globulin fraction and the presence of other antinutratients.

Functional properties:

Functional properties of flaxseed protein products are shown in Table (6). However, protein solubility index of both FPI and FPC was significantly ($p<0.05$) high than FF in distilled water at pH 9.0 and 0.2% NaOH, it was significantly ($p<0.05$) low in 5% NaCl than FF. Both FPI and FPC had significantly ($p<0.05$) low of water and fat absorption capacities than FF. Several factors affect water binding by food proteins, viz. amino acid composition, protein conformation, surface hydrophobicity, etc. (Kinsella, 1982). Madhusudhan and Singh (1985b). Reported that flaxseed flour had a greater fat absorption capacity than soy flour; suggesting that flaxseed proteins are possibly more lipophilic. Both FPI and FPC performed better in emulsification capacity than FF. Generally, flaxseed-protein products were have emulsion-stabilizing effect comparable with those of gelatin, those extending meat emulsion with flaxseed-protein products may lead to a reduction in fat losses during cooking and a reduction in the firmness of cooked emulsion.

Foam capacity and stability

Foam capacity and stability of flaxseed protein products are shown in Fig (2). Both FF and FPC had low foaming capacity than that of FPI. The foam capacity of FF was 66 ml, compared to 68 ml and 98 ml for FPC and FPI, respectively. Foam stability of all flaxseed protein products was decreased markedly at room temperature within the first 15 min and then decreased gradually up to 90 min. Generally, this decrease may be due to collapsing and bursting of the formed air bubbles (Kinsella, 1976).

Table (6): Functional properties of flaxseed protein products.

Functional properties	FF	FPI	FPC
Protein solubility index in:			
Distilled water at pH9	$85.27^{b}\pm1.30$	$90.37^{a}\pm1.42$	$91.41^{a}\pm1.27$
5% NaCl	$82.54^{a}\pm1.79$	$10.67^{b}\pm0.65$	$11.28^{b}\pm0.84$
0.2% NaOH	$92.64^{b}\pm1.63$	$96.54^{a}\pm1.57$	$95.91^{a}\pm1.86$
Water absorption (gm/100gm protein)	$619.5^{a}\pm4.64$	$586^{b}.7\pm3.98$	$433.3^{c}\pm4.17$
Fat absorption (gm/100gm protein)	$508.6^{a}\pm3.58$	$322.5^{b}\pm2.54$	$193.9^{c}\pm2.04$
Emulsification capacity (ml oil/gm protein)	$41.4^{b}\pm0.60$	$88.6^{a}\pm0.9$	$92.5^{a}\pm1.0$

Means in the same column with no common superscript letter are significantly different ($p\leq0.05$)

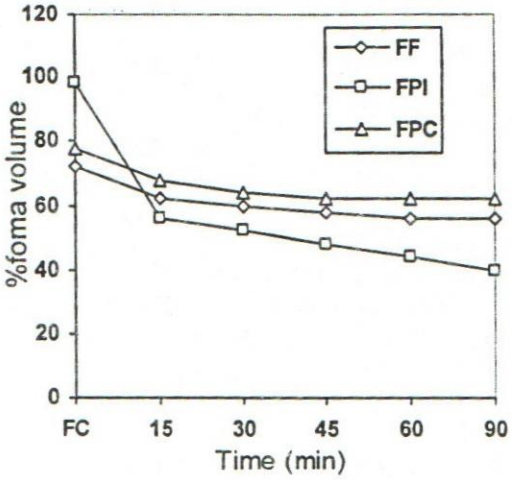


Fig (2): Foam capacity and stability of flaxseed flour, protein isolate and concentrate.

Sensory properties of cakes:

The results of sensory properties of cakes prepared with an addition of FF, FPI and FPC at levels 5, 10 and 15% are shown in Table (7). No significant ($p<0.05$) differences were observed among different cakes fortified with FF at levels, both FPI and FPC (at 5 and 10% levels) and cake free from flaxseed protein products in their appearance, crust and crumb colour, flavour and texture. According the overall acceptability, the panelists accepted cakes containing up to 15% for FF and 10% for both FPI and FPC.

Table (7): Sensory properties of cakes prepared with flaxseed protein products levels.

Parameters	Control (0.0%)	FF			FPI			FPC		
		5%	10%	15%	5%	10%	15%	5%	10%	15%
Appearance	8.30^{a}	8.28^{a}	8.29^{a}	8.28^{a}	8.29^{a}	8.29^{a}	8.00^{b}	8.28^{a}	8.30^{a}	8.00^{b}
Crust colour	8.55^{a}	8.45^{a}	8.55^{a}	8.54^{a}	8.55^{a}	8.52^{a}	8.21^{b}	8.53^{a}	8.54^{a}	8.20^{b}
Crumb colour	8.55^{a}	8.50^{a}	8.53^{a}	8.53^{a}	8.54^{a}	8.55^{a}	8.00^{b}	8.55^{a}	8.55^{a}	7.92^{b}
Flavour	8.64^{a}	8.63^{a}	8.64^{a}	8.60^{a}	8.60^{a}	8.65^{a}	7.83^{b}	8.50^{a}	8.50^{a}	7.75^{b}
Texture	8.76^{a}	8.72^{a}	8.75^{a}	8.75^{a}	8.72^{a}	8.71^{a}	7.14^{b}	8.75^{a}	8.75^{a}	7.40^{b}
Overall acceptability	8.56^{a}	8.52^{a}	8.55^{a}	8.54^{a}	8.54^{a}	8.54^{a}	7.84^{b}	8.52^{a}	8.53^{a}	7.85^{b}

Means in the same row with no common superscript letter are significantly different ($p\leq 0.05$)

In conclusion: Flaxseed flour, flaxseed protein isolate and concentrate are rich in protein, had a well balanced amino acid composition, mineral contents and more nitrogen solubility at neutral pH. It could be utilized as a good quality nutrient either as substitution or supplementation or both and as a functional agent in food system.

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القيمة الغذائية و الخواص الوظيفية لبروتينات بذور الكتان

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تم دراسة التركيب الكيماوي، الخواص الوظيفية والغذائية لدقيق بذور الكتان (FF)، البروتين المعزول (FPI) وكذلك البروتين المركز (FPC). وجد أن الجلوبيولين كان المركب الرئيسي لبروتين دقيق بذور الكتان (FF) ويمثل ٤٧,٦٨% من البروتين الكلي. كما اظهر منحنى ذائبية بروتين دقيق بذور الكتان (FF) على درجات حموضة مختلفة انخفاضا واضحا عند pH في حدود ٣-٦، كما كان معدل الذوبان في الوسط القلوي أعلى منه في الوسط الحامض. كما أظهرت النتائج ارتفاع محتوي البروتين المعزول (FPI) من البروتين الكلي عن محتوي كل من البروتين المركز (FPC) ودقيق بذور الكتان (FF). في حين كانت نسبة الدهن، الألياف و الرماد متقاربة في كل من البروتين المعزول (FPI) والمركز (FPC). كما لوحظ أن البروتين المعزول (FPI) والمركز (FPC) أعلى امتصاصا للماء والدهن عن دقيق بذور الكتان (FF). كما وجد أن دقيق بذور الكتان (FF) غني بأملاح الكالسيوم و الحديد، كذلك لوحظ أن منتجات بذور الكتان غنية في الأيزوليوسين، الأحماض الأمينية الكبريتية، الأحماض الأمينية الحلقية و التربتوفان مقارنا بالـ FAO/WHO (1973). كان اللايسين الحمض الأميني المحدد الأول والليوسين الحامض الأميني المحدد الثاني في كل منتجات بذور الكتان. أوضح التقييم الحسي للكيك المدعم بمنتجات بذور الكتان أوضحت انه يمكن استخدم دقيق الكتان (FF) حتى نسبة ١٥% بينما استخدم كل من البروتين المعزول (FPI) والمركز (FPC) حتى نسبة ١٠%. لذا فان مُنتَجات بروتين الكتان يُمكنُ الاستفادة منها كبدائل غذائيةٍ جيدةٍ أو إضافات وظيفية في نظام الغذاء.