FORTIFICATION OF BISCUIT WITH SOYBEAN AND SUNFLOWER PROTEIN CONCENTRATES LOW IN ANTINUTRITIONAL FACTORS

M.A.H.Sorour [1]; S.H.Abou-El-Hawa [2]; M.A.H.El-Geddawy [2] and E.M.M Abdel-Zaher[1]

[1]Food & Dairy Sci. Dept., Fac. of Agric. (Sohag), South Valley Univ.

[2]Food Sci. & Tech. Dept., Fac. of Agric., Assiut Univ.

Abstract: The present study was carried out to evaluate the protein concentrate-low in some antinutrients prepared from defatted soybean and sunflower meals. Extractions of protein concentrate by different methods led to reducing some antinutrients in the final product according to preparing methods. Using of various solvent extraction techniques decreased phytic acid content by 39-42 and 78.5% in soybean and sunflower protein concentrate, respectively. Trypsin inhibitor, phenolic compounds and tannins were reduced in soybean and sunflower protein concentrate compared with its contents in the defatted meals. The reduction percentage of tannin content in the final protein concentrate reached 84% of its value in defatted sunflower meals. Addition soybean and sunflower protein concentrates low in antinutritional factors to wheat flour(72% extraction) by 5 and 10% levels in making biscuit improved protein percentage from 7 to 14%. The sensory evaluation of biscuit supplemented with 5 and 10% soybean protein concentrate low in antinutritional factors revealed that, no significant differences between all biscuit samples and the control in their texture. Biscuit fortified by low-antinutrients sunflower protein concentrate by 5 and 10% had good sensory scores of all characteristics. The sensory evaluation indicated that, biscuit could be fortified by soybean or sunflower protein concentrate up to 10% of wheat flour without affecting their quality.

Key words : biscuit, soybean, sunflower protein, antinutritional

Introduction

Oilseeds are considered a good source of vegetable oils, protein, crude fiber, phosphorus, vitamins and the energy values and could be adequate for supplying useful amounts of calories (Enujiugha and Ayodele-oni, 2003). Due to the high percentage of good quality protein in oilseeds, it can make a significant contribution to the nutritional value. Thus both the quality and quantity of protein are raised in cereal-based food to which oil seed flours has been added (Kaushik, 1989 and NRC 1993). Oilseeds use is usually restricted by the presence of one or more endogenous antinutritional factors (Kaushik, 1989 and NRC

1993). The major groups of antinutritional factors present in oilseeds are protease inhibitors, phytic acid, lectins, glucosinolates, tannin, etc (Jansman and Poel, 1993). Phytic acid forms very stable complexes with minerals ions rendering them to become unavailable for intestinal uptake (Lopez *et al*., 2002). It could also form complexes with proteins, proteases and amylases of intestinal tract, thus inhibiting proteolysis (Tabekhia and Luh, 1980). Phytate can also bind with starch directly by hydrogen bonding with a phosphate group and indirectly through the proteins (Raboy and Dickinson, 1984). Trypsin inhibitors are widely distributed within oilseeds which have the ability to inhibit the activity of trypsin enzymes within the gastrointestinal tract of animals. In fact trypsin inhibitors are believed to be the most important antinutrient present in soybean (Krogdahl *et al*., 1994). These are mainly responsible of the poor bioavailability of protein, such poor susceptibility to protein may be due to the presence of disulfide bonds in the formation of protein complex (Clemente *et al*., 2000). Polyphenols constitute one of the most numerous and ubiquitous groups of plant metabolites and are integral part of both human and animal diets (Bravo, 1998). Polyphenols are described as astringent, and it is generally held their most important characteristic is their propensity to form complexes with proteins, polysaccharides and alkaloids (Spencer *et al*., 1988). The presence of polyphenols in foods produces a sensation of astringency. This is thought to arise from the precipitation of oral proteins and muco polysaccharides. Polyphenols in the digestive tract display many detrimental effects, including the inhibition of iron absorption, and esophageal cancer (Warner and Azen, 1988).

The objective of the present investigation was an attempt to produce low-antinutrients soybean and sunflower protein concentrates with the aim of producing a high quality biscuits for school children by replacement of wheat flour by either one of them.

Materials and Methods

Materials: The seeds of soybean (*Glycine max* L. Merr.) Clark variety were obtained from Agronomy Department, Faculty of Agriculture, Assiut University (Summer) 1999. Sunflower seeds (*Helianthus annuus* L.) miack variety were obtained from Department of Oil Crops, Agriculture Research Center, Giza, Egypt. Five Kg of each were brought and packed in polyethylene bags and kept at 4 ºC until tested.

Preparation of samples:

Raw seeds: Whole dry seeds were manually cleaned from broken seeds, dust and other foreign

materials and ground to 60 mesh flour size in electric blender.

Dehulling: Clean seeds were soaked in distilled water (1:5 w/v seeds to water ratio) for 12 hours at room temperature, seed coats and germs were removed manually. The dehulled seeds were dried at 55°C for 30 hours.

Preparation of low-antinutrients protein concentrates:

Defatted soybean and sunflower meals: Dehulled seeds were ground at 4ºC in a Waring Blender. Oil was extracted by shaking the flour with n-hexane using a flour –to –hexane ratio of 1: 3 w/v for 16 h at room temperature. The suspension was filtered, the residue was dried and ground to pass through 60-mesh sieve, according to the method described by Saeed and Cheryan (1988).

Preparation of low-antinutrients soybean protein concentrate:

There are three basic methods for preparation of soybean protein concentrate consisting of acid leach, hot-water leach and alcohol leach methods.

Acid leach method: Acid leach soybean protein concentrate was prepared according to Pandjaitan *et al*. (2000). Finely ground defatted soy flour was dispersed in 1:10 distilled water (w/v) and stirred for 1 hour at ambient temperature. The dispersion was adjusted to pH 4.5 with 1N hydrochloric acid, the precipitated protein was removed by centrifugation, washed once, neutralized to pH 7 and dried.

Hot-water leach method: Hot-water leach soybean protein concentrate was prepared according to the method of Pandjaitan *et al*. (2000).

Alcohol leach method: Alcohol leach soy protein concentrate was prepared according to the method of Campbell (1985).

Preparation of low-antinutrients sunflower protein concentrate:

Phytate was removed by aqueous extraction and separation at acidic and /or alkaline conditions, depending on the relative solubility of the protein and phytate according to the method described by Saeed and Cheryan (1988). The final residue designated the reduced phytate protein concentrate. In order to reduce the phenolic compounds, the method of Sodini and Canella (1977) was used. The previous reduced phytate protein concentrate was suspended in acidified butanol in ratio of 1:20(w/v). Acidified butanol was prepared by mixing 1-butanol with 0.005N HCl(92:8 w/v) and the pH was adjusted to 5.0 with 0.5 N HCl. The extraction was carried out for 15 minute at room temperature under constant stirring with a magnetic stirrer. At the end of the extraction, the suspension was filtered through filter paper Whatman No. 3 under suction. The

residue was extracted several times more under identical experimental conditions. The final residue was the protein concentrate, which was dried at room temperature. In order to minimize Trypsin inhibitor contents, the reduced Phytate and Polyphenols protein concentrate was autoclaved at 1.05 kg cm^{-2} pressure for 15 minutes. The samples were dried at 70°C. The final residue was reduced phytate-polyphenols protein concentrate, which was dried at at 70°C as well as milled in electric mill to pass through a 0.5 nm sieve and stored in plastic containers until required for further analysis.

Chemical analysis:

Moisture, crude oil, ash, crude protein, and crude fiber contents were determined according to A.O.A.C. (1990) Standard methods, while**,** total carbohydrates were calculated by difference.

Determination of phytate: phytate content was determined in the samples as described by Mohamed *et al*. (1986) and modified method by Sorour (1997).

Trypsin inhibitors activity: Trypsin inhibitors activity was assayed according to A.A.C.C**.** method (1980).

Determination of phenolic compounds: The evaluation of phenolic compounds in samples was carried out according to Ermakova (1972).

Determination of tannins: Tannins were assayed according to modified vanillin-HCl method of Price *et al*. (1978) as described by Babiker and El-Tinay (1992).

Biscuit manufacture*:*

Biscuit was manufactured according to the method of Asenjo *et al*. (1985). Biscuits were supplemented with 5 and 10% of low-antinutrients soybean and sunflower protein concentrates. The basic ingredients were wheat flour, hydrogenated oil, sugar solution (54% sucrose + 10% glucose + 36% water), sodium bicarbonate and vanilla essence. The biscuit prepared by dry-mixing flour and additives, addition of low-antinutrients protein concentrates, then kneading the mixture to a homogeneous consistency. The dough was shaped manually and baked in an oven at 180-190ºC for 10-15 min. and evaluated.

Sensory evaluation: Sensory evaluations of the prepared biscuit products were performed by a panel of ten judges and included two replications. ***Statistical analysis:*** The analysis of variance (ANOVA) was performed on all values using the statistical analysis system (SAS) program version 6.12 (SAS, 1997). The level of significance was set at 0.05.

Results and Discussions

Chemical composition and antinutrients of raw samples**.** The

proximate chemical analysis of selected underutilized oilseeds is presented in Table 1. Soybean and sunflower seeds considered a rich source of oils and proteins. The crude protein values for the respective oil seeds were 35.8 and 20.0%, wheareas the crude oil values were 23.2 and 45.0%, respectively. Data in Table 2 showed that phytic acid contents in oilseeds were 1.40%, and 1.17% on dry weight basis in soybean, and sunflower seeds, respectively. The results are in the line with those reported by Yao *et al*. (1983). Soybean seeds contained the high level of trypsin inhibtors compared with sunflower seeds. Kheir (1990) indicated that, the trypsin inhibitors activity as antinutritional factor were 40.79 and 44.32 TIU/g in two soybean varieties namely, Clark and Crawford, respectively, while, El-Shemy (1996) reported that, trypsin inhibitor activity in soybean was 27.5 TIU/g. The level of phenolic compounds was higher for sunflower compared with its content present in soybean seeds. Pedrosa *et al*. (2000) revealed that, total phenolics content in entire sunflower seeds were 1.04-1.30 g/kg. Saeed and Cheryan (1988) found that, the values of phenolic compounds in two cultivars of sunflower were 1.55 and 1.49% in Hybrid 7000 and Hybrid 7111, respectively.

Table(1): proximate chemical composition of soybean and sunflower seeds (on dry weight basis)*.

Samples	Ash (%)	Crude oil (%)	Crude Protein (%)	Crude fiber (%)	Carbohydrate (%)**
Soybean	5.3	23.2	35.8	4.4	31.3
Sunflower	3.6	45.0	20.0	7.0	24.4

* Average of three replicates. ** Calculated by difference

Table(2): Antinutiritional factors content in some raw soybean and sunflower lseeds (on dry weight basis)*.

Samples	Phytic acid (mg/100g)	Trypsin inhibitors (TIU/g)	Phenolic compounds (mg/100g)	Tannins (mg/100g)
Soybean	1413.4	26.30	862.75	457.00
Sunflower	1177.0	6.50	1063.40	440.00

Average of three replicates.

Tannins are major phenolic compounds in oilseeds. The percentage of tannins from phenolic compounds were 53% and 41% for soybean and sunflower, respectively. These results are in good agreement with those mentioned by Brooker (1999), who found that, tannins are one of the most common secondary products found in plant species. These compounds are complex phenolic molecules produced in plants.

Chemical composition and antinutrients of protein concentrates: The proximate chemical composition of protein concentrate prepared from soybean and sunflower seeds are presented in Table 3. The results indicated that soybean protein concentrate contained high levels of protein compared with its defatted meals. There are three methods used in preparation of protein concentrate of soybean which gave lower crude fiber and high protein content in the final product. The level of protein in soybean protein concentrate was affected by the preparation method, where alcohol leach method was the highest protein yield. The results in the same table demonstrated that the extraction process of sunflower appreciably increased protein content from 60.3% in defatted meal to 73.8 in the final protein concentrate.

Table(3): Chemical composition of soybean and sunflower protein concentrates (on dry weight basis)*.

Samples	Moisture	% on dry weight basis				
		Crude oil	Crude Protein	Ash	Crude fiber	Carbo-hydrates**
Defatted soybean meal	7.5	1.7	48.0	7.0	5.0	38.3
Soybean protein concentrate						
-Acid leach	5.0	1.2	64.0	4.1	3.0	27.7
-Hot water leach	6.4	1.5	63.5	4.5	3.2	27.3
-Alcohol leach	5.7	0.7	67.0	4.3	3.6	24.4
Defatted sunflower meal	6.4	1.5	60.3	7.6	4.5	26.1
Sunflower protein concentrate	3.3	0.9	73.8	4.0	2.8	18.5

* Average of three replicates.

** Calculated by difference

Table(4): Antinutrients content of soybean and sunflower protein concentrates*.

Samples	Phytic acid (mg/100 g)	Trypsin inhibitor (TIU/g)	Phenolic compounds (mg/100 g)	Tannin (mg/100 g)
Defatted soybean meal	2146.7	10.60	685.8	340.0
Soybean protein concentrate:				
-Acid leach method	855.6	5.20	363.1	320.0
-Hot-water leach method	842.9	1.06	367.6	330.0
-Alcohol leach method	825.2	3.50	360.4	300.0
Defatted sunflower meal	3476.9	3.60	2178.6	380.0
Sunflower protein concentrate	252.5	ND**	94.6	60.0

* All values are expressed on dry weight basis. **ND = Not detected.

The removal of oil from seeds increased phytic acid by about 51.9% and 78.5% in both defatted soybean and sunflower meals, respectively. Abdel-Gawad(1991) found that, extraction of oil from sunflower seeds caused an increase in phytic acid content by 147% over the whole seeds. In soybean protein concentrates, the use of various solvents for extraction techniques decreased phytic acid content by 39.5-41.6% in soybean protein concentrate (Table 4). Extraction and purification of protein by alcohol leach method reduced Phytate, Phenolic compounds, and tannins compared with other extraction methods. The sunflower protein concentrate-low in antinutrients prepared from defatted meal by adjusting a suspension to the isolectric point reduced the phytate content by 78.5%. These results are in agreement with those reported by Saeed and Cheryan (1988). The results in Table 4 showed also that trypsin inhibitor and tannins content decreased in final sunflower protein concentrate due to removal of coats and oil which caused such reduction. Tannins content was lower (60 mg/100 g) in sunflower protein concentrate compared with its value in defatted sunflower meals (380 mg/100 g). Similar finding was reported by El-Shemy (1996**)** who found that, antinutritional factors decreased after dehulling of soybean and faba bean seeds. The data in Table 4 indicated that, phenolic compounds were lower in sunflower protein concentrate fractions compared with defatted sunflower meals. These results are very close to that reported by Pawar *et al*. (2001)**,** who found that, the removal

of oil from sunflower meal further increased polyphenols by about 120%. Similar findings are reported by El-Shemy (1996).

Biscuits supplemented with protein concentrates: The results in Table 5 indicated that, addition of soybean (alcohol leach method) or sunflower protein concentrates-low in antinutritional factors to wheat flour (72% extraction) lead to an increases in protein content by increasing the levels of replacement due to the high content of protein in the supplemented ingredients. Faheid and Hegazi (1991) found that, the addition of legume flours to wheat flour increased protein content of cookies to 14.6%. Hussein *et al*., (2002) revealed that, the addition of soy flour at different levels to wheat flour increased protein, fat, ash and crude fiber in bread compared with the control.

Table(5): Protein and some antinutrients content in biscuits supplemented with soybean and sunflower protein concentrate*.

Biscuit samples	Crude protein %	Phytic acid (mg/100g)	Trypsin inhibitor (TIU/g)	Phenolic compounds (mg/100 g)	Tannin (mg/100g)
Control	7.30	202.8	0.46	359.3	270.0
Soybean protein concentrate:					
-5%	12.00	395.0	0.71	359.3	280.0
-10%	12.50	455.7	0.87	359.3	280.0
Sunflower protein concentrate					
-5%	12.70	242.2	0.14	350.4	210.0
-10%	13.50	219.1	0.10	339.2	193.0

* All values are expressed on dry weight basis.

Phytate and tannin contents increased in manufactured biscuits by increasing the levels of soybean and sunflower protein concentrates compared with its value in control. Trypsin inhibitor was reduced in manufactured biscuits due to its a reduced amount of wheat flour. No changes of phenolic compounds in final biscuits with added soybean protein concentrate were detected, while it was decreased in biscuit supplemented with sunflower protein concentrate.

Sensory evaluation of biscuit products: Table 6 shows the organoleptic evaluation of biscuit made from wheat flour (72% extraction) supplemented with 5% and 10% soybean protein concentrate prepared by alcohol leach methods and 5%, 10% sunflower protein concentrate. The

statistical analysis of data indicated that there were no significant differences between all biscuit samples and the control in their texture. Biscuit samples containing 5% soybean protein concentrate (alcohol leach method) recorded significant differences between their taste and odor compared with control and other samples. On the other hand, biscuit containing 5% soybean protein concentrate prepared by alcohol leach method recorded the highest score in color evaluation compared with other samples. Likewise, fortification of biscuit with 5% soybean protein concentrate enhanced the over all acceptability scores than control and other samples. In addition, Biscuit fortified with sunflower protein concentrate by 5% and 10% had a good sensory score.

From the organoleptic evaluation, it could be concluded that, biscuit could be fortified by soybean or sunflower protein concentrate up to 10% of wheat flour without affecting their quality. Furthermore, it would also enhance some properties and nutritive value of the produced biscuit.

Abbreviation for symbols a and b, any formulas during the same symbol, HAVE no significant difference in between.

Table(6): Statistical analysis of organoleptic evaluation of biscuit products containing 5 and 10% of soybean and sunflower protein concentrate.

Biscuit samples	Taste	Color	Texture	Odor	Over all acceptability
Control	7.1^{ab}	6.7^{b}	7.4^{a}	7.1^{ab}	7.8^{ab}
Soybean protein concentrate (alcohol method):					
- 5%	8.1^{a}	7.8^{ab}	8.0^{a}	7.8^{a}	8.6^{a}
- 10%	7.2^{ab}	6.8^{b}	7.2^{a}	6.2^{b}	7.1^{b}
Sunflower protein concentrate:					
-5%	6.4^{b}	7.1^{ab}	7.1^{a}	6.9^{ab}	7.9^{ab}
-10%	7.4^{ab}	7.2^{ab}	7.4^{a}	6.9^{ab}	7.4^{ab}
L.S.D.	1.078	1.059	NS.	1.076	1.146

NS = Non significant.

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تقوية البسكويت بمركزات بروتينية لفول الصويا وعباد الشمس والمنخفضة في محتواها من مضادات التغذية

محمد عبد الحميد حسن سرور[1]، صلاح حسنين أبو الهوى[2] ، محمد الأنور حسن الجداوى[2] ، وإيمان محمد ممدوح عبد الظاهر[1]

[1] قسم علوم الأغذية والألبان- كلية الزراعة بسوهاج جامعة جنوب الوادي

[2] قسم علوم وتكنولوجيا الأغذية- كلية الزراعة- جامعة أسيوط

تهدف هذه الدراسة إلى تقييم مركزات البروتين المنخفضة في محتواها من مضادات التغذية والمجهزة من دقيق فول الصويا وعباد الشمس ، وقد أظهرت الدراسة أن استخلاص المركز البروتيني ومعاملته بطرق مختلفة(الغسيل بالحامض و الماء الساخن والكحول) أدى إلى خفض تركيز بعض مضادات التغذية في المنتج النهائي طبقا لطريقة التحضير المتبعة ، حيث انخفض حمض الفيتيك بنسبة تراوحت بين 39 – 42% فى مركز الصويا ، 78.5% فى مركز بروتين عباد الشمس من قيمتها فى مطحون البذور منزوع الدهن ، كما انخفض تركيز مثبطات التربسين والمركبات الفينولية والتانينات فى المركزات البروتينية للصويا وعباد الشمس وكانت نسبة الإنخفاض أعلى فى محتوى التانينات حيث وصلت الى 84% فى المركز البروتيني النهائي لعباد الشمس من قيمتها فى دقيق عباد الشمس منزوع الدهن قبل الاستخلاص. وعند اضافة مركزات بروتين الصويا وعباد الشمس المنخفضة فى محتواها من مضادات التغذية الى دقيق القمح (استخلاص 72%) بنسبة 5 ، 10 % لعمل بسكويت مدعم بالبروتين أدى ذلك الى زيادة نسبة البروتين فى المنتج من 7 الى 14% كما أظهر التقييم الحسي للبسكويت المدعم بـ 5 ، 10% من المركزات البروتينية المنخفضة فى محتواها من مضادات التغذية أنه لا توجد فروق معنوية بين عينات البسكويت تحت الدراسة والكنترول فى درجة القوام فى حين أدى إضافة المركزات البروتينية بنسبة 5 ، 10% الى تحسن فى جميع الخواص الحسية كما أظهر التقييم الحسي أيضا أنه يمكن تدعيم البسكويت بهذه المركزات بنسبة تصل الى 10% دون أن يؤثر ذلك على جودة المنتج النهائى.