TECHNOLOGICAL AND BIOLOGICAL STUDIES ON BISCUIT FORTIFIED WITH PROTEIN ISOLATE FROM IVY SEEDS

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ABSTRACT

In this study wheat flour 72% extraction was fortified with protein isolate from ivy seeds at levels of 3, 6 and 9% for biscuit production. Amino acids content, solubility of nitrogen and in-vitro digestibility of protein isolate were studied. Biological evaluation using hyperlipidemic rats fed on biscuit containing 3,6 and 9% protein isolate was investigated as well. The results indicated that protein content in the isolate from ivy seeds reached to 82.98%. The maximum nitrogen solubility of protein isolate was found to be 94% at pH 8. The limiting amino acids in protein isolate were methionine and cystine. The in-vitro digestibility of protein isolate were ranked as follow. Pepsin-pancreatin > pancreatin > pepsin > trypsin. The farinograph test showed that fortification of wheat flour with 3, 6 and 9% protein isolate caused remarkable increasing in water absorption, arrival time, dough development time and degree of weakening. At the same time dough stability was decreased compared to control. Sensory evaluation revealed that biscuit containing protein isolate up to 6% was acceptable. Biological evaluation of rats declared that after 8 weeks, the values of triglycerides, total lipids, total cholesterol, low density lipoprotein (LDL), aspartate amino transferase (AST) and alanine amnio transferase (ALT) were decreased while high density lipoprotein (HDL), total protein, albumin and globulin were increased in hyperlipidemic group of rats fed on biscuits contain 3, 6 and 9% protein isolate compared to hyperlipidemic control group fed on biscuit free of protein isolate.

Key words: *Ivy seeds, protein isolate, in-vitro digestibility, biological evaluation and biscuit.*

INTRODUCTION

In developing and underdeveloped countries, there is an urgent need of nutritious foods to meet the nutritional requirement of the ever-increasing populations. Since legume seeds are important sources of protein, complex carbohydrate and dietary fiber in the diet. There has been a worldwide interest in searching for potential utilization of unconventional legumes (Morrow, 1991).

Because of inadequate supplies and shortage of food proteins, there has been a constant search of unconventional legumes as new protein sources for use as both functional food ingredients and nutritional supplements (Onweluzo et al., 1994).

The Ivy seed: had classified as legumes and it is less exploited as protein source. This seeds are traditionally used as a soup ingredient for therapeutic purposes such as ameliorating symptoms of dropsy, relieving diarrhea, and tonic to the viscera (Li, 1973).

Cholesterol is an important constituent of living tissues by virtue of its dual role both as a structural component of biological membranes, and as a precursor of cholecalciferol, steroid hormones and bile acids. Cholesterol also acts as a risk factor for adverse conditions such as cardiovascular diseases and cholelithiasis. Several studies have demonstrated that lowering LDL-cholesterol diminishes both cardiovascular and overall mortality (Dwyer, 1995). It is well known that diet plays an important role in the control of cholesterol homeostasis. In this context, it has been reported that legumes lower serum LDL-cholesterol (Duane, 1997).

It is known that legumes are an important source of protein for countries having short supplies of animal proteins, hence, the main aims of this intervention trial were to study in-vitro digestibility and in-vivo properties of protein isolate from ivy seeds.

MATERIALS AND METHODS

Materials:

Ivy seeds *(Dolichos lablab L.)* were obtained from Medicinal Plants and Agricultural Seeds Harraz Company, Cairo, Egypt.

Wheat flour (72% extraction) was obtained from Middle and West El-Delta Mills Company.

Kits (triglycerides, lipids, cholesterol, high density lipoprotein, low density lipoprotein, total protein, albumin, aspartate amino transferase and alanine amino transferase) were obtained from Randox Laboratories Ltd., Diamond Road, Crumlin, Co., Antrim, United kingdom, BT294QY.

Methods:

Protein isolate from ivy seeds were prepared according to the method of Thompson (1977) as follows: dehulled seeds were ground to obtain a flour. One K.gm.of flour was suspended in 15 liters distilled water the pH was adjusted to pH 9 by 1 N NaOH. The suspension was shaked for 20min. at room temperature, then centrifuged at 1000 xG for 20min, the extract was adjusted to pH 4 by 1N HCL and centrifuged at 1000 xG for 20min. The precipitate was washed with distilled water then neutralized by 1N NaOH to pH 7. The neutralized precipitate was dried under vacuum then ground to obtain protein isolate.

Nitrogen solubility was determined according to the method of Thompson (1977). The methods described in AOAC (1995) were used to determine Moisture, protein, fat, fiber and ash.

The in-vitro digestibility by pepsin-pancreatin system was determined according to the method of Akeson and Stahmann (1964), while digestibility by pepsin, trypsin and pancreatin system was determined according to the method of Abd El-Aal et al. (1986).

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Amino acids were estimated by using Amino Acid Analyzer (LC 3000 eppendorf- Germany) according to the method of Rubin et al. (1975). Amino acids score was calculated according to the FAO/WHO/UNU (1985).

Farinograph test was carried out according to the method out lined in AACC (1990).

Biscuit samples were prepared according to the method of Wade (1988) as follows: the creamed mass consisting of 160gm shortening, 160gm sugar, 1gm vanilla, 5gm skim milk powder and 10gm baking powder were mixed for 10min. Then 1000gm flour and water was added. The dough was placed on the baking sheets and cutted with the biscuits cutter. Dough outside the stainless steel cutter was removed withe spatula before raising the cutter. The dough was baked in the laboratory oven at 180°c for 12 minutes.

Color, flavor, appearance, taste and texture of biscuit samples were sensory evaluated by ten panelists according to the method of Smith (1972).

Biological evaluation:

Male Sprague-Dawley strain rats (40 rats) weight rated between 90-100gm were divided as follow one group (8 rats) fed on basal diet until the end of experimental period and considered as normal control. The basal diet consisted of 10% casein as protein, 10% corn oil, 4% salts mixture, 1% vitamins mixture, 5% cellulose and 70% starch as recommended by AIN (1977). Other rats (32 rats) were fed on hyperlipidemic diet containing (10% corn oil + 5% sheep tail fat + 1% cholesterol) and 0.5% bile salt for two weeks in order to induce hyperlipidemic. One group (8 rats) from hyperlipidemic rats were fed on hyperlipidemic diet until the end of experimental period and considered as hyperlipidemic control. Other hyperlipidemic rats (24 rats) were divided into 3 groups (8 rats each) and fed on diet containing 3, 6 and 9% protein isolate for 8 weeks. Distilled water and diets were offered *ad libitum*. After 8 weeks, blood was collected from all groups of rats by withdrawning from vein plexus eye by a heparinized capillary tube containing EDTA as anticoagulant, then the blood was centrifuged at 3000 r.p.m to obtain the serum which was strored at (-20°C) for biochemical analysis.

Biochemical analysis:

Total cholesterol, triglycerides and total lipids in blood serum samples were determined according to the method of Allain et al. (1974), Lowell et al. (1973) and Knight et al. (1972) respectively. Total protein, albumin and globulin were determined by the method of Henry (1964).

Total cholesterol, triglycerides and total lipids in heart were extracted as described by Fernandez et al. (1997) as follows: heart sample (1g) was suspended in chloroform: methanol (2:1) for lipid extraction overnight and homogenized, then filtrated through filter paper Watman 45 and the aliquots were evaporated, resuspended in ethanol and determined as mentioned before.

High density lipoprotein cholesterol was determined according to the method of Warnick et al. (1982). Low density lipoprotein cholesterol was calculated according to the equation of Fniedwald et al. (1972).

Aspartate amino transferase (AST) and alanine amino transferase (ALT) were determined using the method of Varley et al. (1980).

The data obtained were statistically analyzed using the Statistical Analysis System SAS (1996).

RESULTS AND DISCUSSION

Chemical and amino acids composition :

With respect to chemical composition of ivy seeds and its protein isolate, the results in Table (1) demonstrated that the protein content of ivy seeds was 26.30%, while reached to 82.98% as for protein isolate. From these results it can be said that ivy seeds seemed to be a potential source of protein. This merit can be useful for production of good quality food containing protein isolate from ivy seeds.

Table (1): Chemical composition (%) of ivy seeds and its protein isolate (on dry weight basis).

	Moisture	Protein	Fat	Fiber	Ash	Carbohydrates*
Ivy seeds	11.29	26.30	2.32	7.26	3.64	60.48
Protein isolate	3.72	82.98	1.21	2.58	1.45	11.78

* Calculated by difference.

Regarding nitrogen solubility of ivy seeds protein isolate, the data in Table (2) showed the variations of nitrogen solubility on different pH conditions. It could be noticed that the minimum nitrogen solubility of protein isolate was 5% at pH 4. on the contrary the maximum nitrogen solubility of protein isolate was 94% at pH8. In this concept Idouraine et al. (1991) reported that the legume protein studied all showed good solubility in both the acidic and alkaline pH regions which was important characteristic in food formulations.

Table (2): Effect of pH on the nitrogen solubility (%) of protein isolate

pH values	2	4	6	8	10
Nitrogen solubility (%)	81	5	65	94	92

In respect amino acids content of protein isolate were tabulated in Table (3) and chemical score in Table (4). The data revealed that methionine and cystine were the limiting amino acids. These results are in accordance with those mentioned by Laurena et al. (1991) who reported that sulphur-containing amino acids (methionine and cystine) of legumes had been to be the first limiting amino acids. In this concept Carroll (1991) reported that hypocholesterolemic effect is thought to be at least partially attributable to the amino acid profile of the plant protein itself. In addition to providing essential amino acids, the ultimate success of utilizing any seed protein as food ingredients depends largely upon its desirable functional properties (Aluko and Yada, 1995).

Table (3): Amino acids profiles of protein isolate as compared to FAO pattern (g/100g protein).

Essential amino acids		FAO/WHO/UNU (1985)	Non essential amino acids	
Isoleucine	4.16	4.0	Alanine	4.10
Leucine	6.11	7.0	Arginine	6.02
Lysine	5.15	5.5	Aspartic acid	9.25
Methionine + Cystine	1.27 + 1.24	3.5	Glutamic acid	11.10
Phenylalanine + Tyrosine	4.51 + 3.13	6.1	Glycine	3.22
Threonine	3.18	4.0	Histidine	3.04
Valine	5.09	5.0	Proline	4.14
Tryptophan *	ND		Serine	3.16

* Not determined

Table (4): Chemical score.

Essential amino acids	%
Isoleucine	104
Leucine	87.28
Lysine	93.63
Methionine + Cystine*	71.71
Phenylalanine + Tyrosine	125.24
Threonine	79.5
Valine	101.8

* Limiting amino acids.

The in-vitro digestibility of protein isolate by different enzyme systems were presented in Table (5). The digestibility of protein isolate from ivy seeds could be ranked as follows: pepsin-pancreatin > pancreatin > pepsin > trypsin. In this context Sarwar (1990) reported that poor digestibility of protein in diets of developing countries could be related to the use of less-refined cereals only as major source of protein.

Table (5): In-vitro digestibility of protein isolate from ivy seeds by different enzyme systems*

Enzyme system	Digestibility (%) of protein
Pepsin	64.25
Trypsin	59.30
Pancreatin	72.12
Pepsin-pancreatin	87.31

* Average of two determinations.

Farinograph properties and sensory evaluation of biscuit :

In regard to farinograph test the data in Table (6) showed that replacement of protein isolate at levels 3,6 and 9% led to a remarkable increase in water absorption compared to that of contorl wheat flour. The increase in water absorption was probably as a direct result of the higher protein content of the blends

causing increase in water holding capacity as mentioned by Chou and Morr (1979). As well as, arrival time, dough development time and degree of weakening increased when different levels of protein had added compared to only wheat flour. The increment in dough development time reflected the expected differences in the physical and chemical properties of the legume protein products (Smith and Muller, 1965). At the same time dough stability showed decreasing in blends containing different levels of protein isolate as could be seen in Table (6) compared to only wheat flour.

Table (6): Farinograph properties of blends containing wheat flour and different levels of protein isolate.

Farinograph parameters	Replacement levels (%)			
	0	3	6	9
Water absorption (%)	54.43	60.35	64.12	67.37
Arrival time (min)	2	3	4.5	5.5
Dough development time (min)	3	4.5	5.5	6.5
Dough stability (min)	9	8	6	5.5
Degree of weakening (BU)	40	45	50	60

Concerning sensory evaluation of biscuit, the obtained data in Table (7) revealed that values of appearance, color, flavor, taste and texture were significantly different compared to the control when protein isolate had added at level 9%. In this concept it can be said that bakery products are consumed worldwide. Therefore fortification with high protein legume provides a good apportunity to improve the nutritional quality of protein consumed by many people .

Table (7): Sensory evaluation of biscuit*.

Samples	Appearance (20)	Color (20)	Flavor (20)	Taste (20)	Texture (20)
Biscuit (Control)	18.84a	18.72a	18.95a	19.30a	19.43a
Biscuit (3% PI)**	18.41a	18.53a	18.82a	19.11a	18.90a
Biscuit (6% PI)	18.10a	18.14a	18.64a	18.61a	18.71a
Biscuit (9% PI)	17.00b	17.12b	17.13b	17.28b	17.58b

* Values within each column with the same superscript are not significantly different.
** PI = Protein isolate.

Biological evaluation:

As regards in-vivo evaluation using rats, the obtained results are shown in Table (8), in comparison normal control with hyperlipidemic control groups of rat the data referred that hyperlipidemic control fed on hyperlipidemic diet led to remarkable increment in blood values of triglycerides, total lipids, total cholesterol, LDL, AST and ALT while HDL, total protein, globulin and albumin were remarkable decrement. From the smae table, it was noticed that after 8 weeks, values of triglycerides, total lipids, total cholesterol, LDL, AST and ALT were decreased, while HDL, total protein, globulin and albumin were increased in hyperlipidemic rats fed on biscuit containing 3,6 and 9% protein isolate compared to hyperlipidemic control group of rats fed on biscuit free of protein isolate. The reduction in LDL was in agreement with the data obtained by Kingman et al. (1993) who found that the limiting amino acid of legumes is methionine, an essential amino acid which is required, among other things for the formation of the methyl donor S-adenosly methionine (SAM). SAM is functional in the synthesis of choline for phosphatidyl choline (lecithin), which is the principal phospholipid associated with LDL. This led to propose that the cholesterol-lowering effects of vegetable proteins could be the result of a relative deficiency of methionine or choline, or both, leading to limited numbers of LDL particles being available for the transport of cholesterol in the plasma. Similar results were obtained by Sharma (1987), Tanaka & Sugano (1989) and Morita et al. (1997) they found that the methionine content in dietary protein was considered to be the responsible factors in lowering the serum cholesterol levels in rats.

Table (8): Values* of triglycerides, total lipids, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), total protein, albumin, globulin, aspartate amino transferase (AST) and alanine amnio transferase (ALT) for groups of rats after 8 weeks.

Serum analysis	Component groups of rats**				
	Normal control	Hyperlipidemic control	Group No. 1	Group No. 2	Group No. 3
Triglycerides (mg/dl)	81e	194a	141b	122c	95d
Lipids (mg/dl)	287e	425a	372b	351c	296d
Cholesterol (mg/dl)	102d	214a	163b	137c	105d
HDL (mg/dl)	52.50a	35.18e	39.11d	45.14c	48.00b
LDL (mg/dl)	33.30e	140.02a	95.69b	67.46c	38.0d
Protein (g/dl)	7.10a	4.85c	5.23bc	5.91b	6.53b
Albumin (g/dl)	4.22a	2.73b	2.98b	3.20b	3.70ab
Globulin (g/dl)	2.88a	2.12a	2.55a	2.71a	2.83a
AST (Iu/l)	31.22c	36.40a	34.26b	33.76b	32.17c
ALT (Iu/l)	22.73b	25.35a	25.10a	24.36ab	23.88b

* Values within each row with the same superscript letters are not significantly different.
** Groups No. 1, 2 and 3 were fed on biscuit containing 3,6 and 9% protein isolate respectively.

In respect to values of triglycerides, total lipids and total cholesterol of heart for groups of rats, the data in Table (9) by comparison normal control with hyperlipidemic control group of rat the data referred that hyperlipidemic control fed on hyperlipidemic diet led to remarkable increase in heart values of triglycerides, total lipids, total cholesterol. From the same table, it can be seen that after 8 weeks total lipids, triglycerides and total cholesterol were decreased in heart of rats fed on biscuit contained 3,6 and 9% protein isolate compared to hyperlipidemic control rats fed on biscuit without protein isolate. These results are in accordance with data reported by some authors. Malaspina et al. (1981) who reported that a high HDL/total cholesterol ratio was considered to reduce the risk of coronary heart disease in humans, even when the total cholesterol level was elevated. In addition, human studies indicated that a higher proportion of HDL cholesterol in serum was antiatherogenic (Barter and Rye, 1996). Some recent clinical studies have supported the concept that a decreased plasma triglyceride level was associated with a lower risk of coronary heart disease (Davignon and Cohn, 1996). In general, a combined reduction in the levels of both the total cholesterol and LDL cholesterol would reduce the risk of coronary heart disease in humans (Schaefer et al., 1995).

Table (9): Values of triglycerides, total lipids and total cholesterol of heart for groups of rats after 8 weeks.

Groups of rats*	Triglycerides (mg/g)	Lipids (mg/g)	Cholesterol (mg/g)
Normal control	3.14d	31.53d	3.76e
Hyperlipidemic control	8.18a	40.27a	9.94a
Group No. 1	6.74b	37.45b	8.02b
Group No. 2	4.35c	34.10c	6.32c
Group No. 3	3.88cd	34.49c	5.01d

* Groups No. 1, 2 and 3 were fed on biscuit containing 3,6 and 9% protein isolate respectively.
** Values within each column with the same superscript letters are not significantly different.

At last, it can be concluded that the ivy seeds seemed to be a potential source of protein, useful for the production of good quality food.

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الملخص العربى

دراسات تكنولوجية وحيوية على البسكويت المدعم بالبروتين المعزول من بذور اللبلاب

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فى هذه الدراسة تم تدعيم دقيق القمح استخلاص ٧٢% بالبروتين المعزول من بذور اللبلاب بنسب ٣، ٦، ٩% لإنتاج بسكويت تم دراسة التقييم الحسى للبسكويت الناتج. كذلك تم دراسة المحتوى من الأحماض الأمينية وأقصى إتاحة للنتروجين والقيمة الهضمية للبروتين المعزول. كما تم أيضا دراسة التقييم البيولوجي لفئران التجارب المغذاه على وجبة عالية الدهون ثم تغذت على بسكويت مدعم بالبروتين بنسب ٣، ٦، ٩% ومقارنة النتائج بالكنترول. أوضحت النتائج أن نسبة البروتين المعزول كانت ٨٢,٩٨% وأن أقصى إتاحة للبروتين كانت ٩٤% عند أس هيدروجيني ٨ وأن الحمضان الأمينيان الميثونين والسستين هما الحمضان المحددان فى البروتين المعزول. القيمة الهضمية للبروتين المعزول بواسطة الإنزيمات كانت فى الترتيب التنازلى التالى: البيسين المتبوع بالبنكرياتين > البنكرياتين > البيسين > التربسين. أوضحت نتائج اختبار الفارينوجراف أنه كلما زادت نسبة استبدال البروتين المعزول أدى ذلك إلى زيادة الماء الممتص وزمن الوصول ومدة تكوين العجينة ودرجة ضعف العجينة وفى نفس الوقت انخفضت مدة ثبات العجينة. التقييم الحسى أوضح أن البسكويت المحتوى على البروتين المعزول كان مقبولا حتى نسبة استبدال ٦%. التقييم البيولوجي لفئران التجارب اوضح أن الفئران المغذاه على وجبة عالية الدهون والتى تغذت على بسكويت يحتوى على ٣، ٦، ٩% بروتين معزول لمدة ٨ أسابيع انخفضت بها قيم كل من الجلسريدات الثلاثية والفوسفوليبيدات الكلية والكولسترول والليبوبروتين منخفض الكثافة فى حين زاد كل من الليبوبروتين عالى الكثافة والبروتين الكلى والألبيومين والجلوبيولين وذلك بمقارنتها بمجموعة الفئران (الضابطه) المغذاه على وجبة عالية الدهون محتوية على بسكويت خالى من البروتين المعزول.