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NUTRITIONAL AND SENSORY QUALITIES OF ENRICHED PROTEIN BISCUITS

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ABSTRACT

The main objective of this study was to enhance the nutritional value of biscuits by substitution wheat flour by different levels of sweet lupine flour and whey protein concentrate (WPC) as a source of good quality proteins. The study was conducted in two experiments. At the first experiment, biscuits were prepared by substituting wheat flour with different levels of sweet lupine flour (10, 15, 20, 25, 30 and 35%). The biscuits were evaluated for nutritional and sensory qualities. Biscuits containing sweet lupine flour showed significant increase ($p<0.05$) in protein content and this increase was associated with the level of substitution. Sensory evaluation showed that biscuit containing sweet lupine flour at level of 20% was the most acceptable, therefore, it was used as a control biscuit in the second experiment of study. During this experiment, biscuits were prepared by replacing wheat flour by 20% sweet lupine flour and different levels of WPC. Protein content of resultant biscuits increased significantly ($p<0.05$) with the increase of WPC as compared with control biscuit containing 20% sweet lupine flour only. Biscuit prepared by replacing wheat flour with sweet lupine flour at the level of 20% and 10% of WPC enhanced protein quality by improving essential amino acids content and it was considered as the most acceptable biscuit. It contained 15.51g protein and 465.55Kcal energy per100g biscuit. Furthermore, 50 g of this biscuits could provide 50% of the daily protein requirements for 3-10 years old children and 24.3-28.7% of their daily energy requirements. Therefore, this study recommended intake the resultant enriched protein biscuits for patients who suffer from Protein-Energy Malnutrition (PEM).

Key words: Biscuits, wheat flour, sweet lupine flour, whey protein concentrate, nutritional value, sensory evaluation.

INTRODUCTION

Protein-energy malnutrition is common in developing countries, especially rural areas (Meite *et al.*, 2008). This protein malnutrition, which may cause diseases such as kwashiorkor, marasmus, and immune deficiencies, can be explained by several factors, the main one being the lack of quantitative and qualitative protein intake (Burgess, 2005).

Healthy foods have been met with marked success in the last two decades. These healthy foods termed "functional foods" are growing rapidly all over the world due to the increased awareness of the consumers on the impact of food on health (Stoon, 2002).

In many countries of the world, biscuits are one of the most important popular bakery products for children and adults. Biscuits are the most popular bakery items consumed nearly by all sections of the society in Egypt. Among ready-to-eat snacks, biscuits possess several attractive features including low cost in comparison with other processed foods, availability in different forms, varied taste, relatively long shelf-life, more convenience and good eating quality (Iwegbue, 2012; Baljeet *et al.*, 2014). Therefore, biscuits are considered as a good product for fortification and nutritional improvement. Biscuits consist of three major components; flour, sugar and fat. The main ingredients of any bakery products is wheat

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flour. Wheat flour is low in protein (7–14%) and is deficient in essential amino acids such as lysine (Jideani and Onwubali, 2009) and certain other useful food components like dietary fiber.

Protein fortification of biscuits is of current interest, because of increasing awareness of consumers towards health. Protein-fortified biscuits contains nutrients in concentrated forms. Various types of protein-fortified biscuits have been prepared by fortifying the wheat flour with soy flour (Aleem *et al.,* 2012), chickpea flour (Baljeet *et al.,* 2014), mustard flour (Desayi, 2012), Fenugreek (Mahmoud *et al.,* 2012) and corn germ flour (Mridula *et al.,* 2007).

Sweet lupine seeds (*Lupinus albus* L.) is a species in the family Leguminosae. Lupine is a good source of nutrients, not only proteins but lipids, dietary fiber, minerals and vitamins (Torres *et al.*, 2005). Lupine flour contains 40–45% protein (Evans *et al.,* 1993). Lupines contain phytochemicals with antioxidant capacity, such as polyphenols, mainly tannins and flavonoids (Oomah *et al.*, 2006). Therefore, frequent consumption of lupine could help protect against cardiovascular disease as well as in reduction of blood glucose and cholesterol levels (Sirtori *et al.*, 2004; Hall *et al.*, 2005; Duranti, 2006). Considering its nutritional and functional properties, lupine flour has a high potential to be used in different foods such as fermented foods, pasta, crisps, bread, biscuits and cakes (Pollard *et al.*, 2002; Abdelrahman, 2014). Lupine flour is lower in cost compared with other similar legume flours such as soybean (Jayasena and Quail, 2004). Therefore, with the substitution of Lupine flour, it can be produced products with improved nutritional and functional qualities at a comparatively lower cost.

Legume proteins are rich in lysine and deficient in sulphur-containing amino acids, whereas cereal proteins are deficient in lysine (Chastain *et al.,* 1994). Lupine seeds represent a good balance of essential amino acids (Drakos *et al.*, 2007). They are considered to be a good source of lysine, and are generally poor in the sulfur-containing amino acids (methionine and cysteine) (Gulewicz *et al.,* 2008) and threonine (Pisariková *et al.*, 2008).

Whey is defined as the watery component removed after the setting of the curd in cheese manufacture. Whey proteins are extracted from whey, the liquid material created as a by-product of cheese production. Whey protein concentrate is one of the most cheaply available rich sources of high quality proteins offering many health benefits, and it has the ability to improve the food products due to its various functional properties. Whey protein concentrate (WPC) have high protein levels and overall are very nutritious. Whey proteins are a good source of sulfur-containing amino acids which are proven to maintain antioxidant levels in the body (Pasin and Miller, 2000). The whey protein ß-lactoglobulin is an excellent source of essential and branched chain amino acids which are required in some individuals with liver conditions (USDEC, 2003). Incorporating whey products into the diet of those who suffer from cirrhosis may have a positive effect and overall health benefit. Benefits of whey protein concentrate (WPC) in food applications include antioxidant activity due to its ability to elevate cellular glutathione levels (Solak and Akin, 2012).

Biscuits have become a popular food source in many feeding programs. A good example of a school feeding programs in Egypt is the Basic Education programs. Where a meal be offered to primary school pupils and junior school students during school hours in the day. Protein-rich biscuits will also be beneficial to the low-income groups and institutions. Therefore, this study aimed at produce enriched protein biscuits from wheat-lupine-WPC composite flour.

MATERIALS AND METHODS

Materials

Local Egyptian sweet lupine seeds (*Lupinus albus* L.), wheat flour (72% extract), sugar, salt, shortening, sodium bicarbonate, ammonium bicarbonate and baking powder were purchased from local markets in Sharkia Governorate, Egypt. Lupine flour was obtained after grinding lupine using an electrical mill (Braun, Germany). The milled flour was stored in air-tight polyethylene bags at 4°C until used. Whey protein concentrate (WPC) was purchased from

Greenland Company, Tenth of Ramadan City, Sharkia Governorate, Egypt. All other chemical reagents used in the experimental analysis were of analytical grade.

Methods

Preparation of Biscuits

The conducted study included two experiments as follows;

First experiment

Biscuits were prepared from different blends of wheat and sweet lupine flours. These blends were prepared by replacing 10% (T_1), 15% (T_2), 20% (T_3), 25% (T_4), 30% (T_5) and 35% (T_6) of wheat flour by sweet lupine flour. Control (1) (T_0) sample was prepared without addition of sweet lupine flour. All the other ingredients were added at the same ratio percentages as shown in Table 1.

Second experiment

Best treatment (T_3) according sensory evaluation in the first experiment (biscuits contained 20% sweet lupine flour) was used as a control biscuit for the second experiment (Control 2). Then four treatments of biscuits were prepared by substitution of wheat flour by WPC at levels of 4, 7, 10 and 13%. All the other ingredients were added at the same ratio percentages as in control (2) as shown in Table 2.

Processing and baking of biscuit

Biscuits were prepared according to the method of Manohar and Rao, (1999). Sugar and fat were creamed at room temperature for 3-4 min in a mixer, sodium and ammonium bicarbonate and salt were dissolved in (20-22 ml water), added to the above cream and mixed for 5 min to obtain a homogenous cream. Wheat flour or blends of wheat flour with sweet lupine flour only or blends of wheat flour with sweet lupine flour and WPC sieved twice with baking powder and added to above cream and mixed for 3 min. Biscuits dough was sheeted to a thickness of 3.5 mm, sheets were cut using circular shape (45 mm diameter), placed on a tray and baked at 160°C for 15 min, then allowed to cool for 1 hr. The resultant biscuits were packaged in polyethylene bags and stored in air-tight containers at room temperature for further analyses.

Chemical analyses

Biscuits were analyzed for protein, fat, fiber and ash contents according to their respective methods as described in AACC (2000). Carbohydrate was calculated by difference.

Calculation of energy density (Kcal/100g sample)

The energy densities of different biscuit treatments were calculated according to the methods described by Insel *et al.* (2002) as follows:

E=4 (protein %+ carbohydrate %)+9 (fat %)

Where:

E=Energy density per 100g of product.

Amino acids

Amino acids profile were analyzed using amino acid analyzer (LC3000 Eppendorf, Germany), according to the method of the AOAC (2006).

Colour measurements

The colour of the biscuit was measured using a Hunter Lab colour analyzer (Hunter Lab Color Flex EZ, USA). The colour of a sample is denoted by the three dimensions, L^* (brightness or whiteness), a^* (redness and greenness) and b^* (yellowness and blueness). The total colour difference (ΔE) was calculated according to Shrestha *et al.* (2012).

Sensory evaluation

Sensory attributes being colour, taste, odour, crispness and appearance were evaluated by 20 students and staff of the Department of Food Science, Faculty of Agriculture, Zagazig University, Egypt according to the method described by Saleh *et al.* (2012).

Statistical Analysis

All biscuit treatments were carried out in triplicate. Results were expressed as mean±standard deviations. Data were analyzed under ANOVA and the Duncan test was applied to compare mean values. Statistical significant was set at $p<0.05$.

Table 1. Various ingredients of biscuits incorporated with different levels of sweet lupine flour

Ingredients (g)	Treatments						
	T_0	T_1	T_2	T_3	T_4	T_5	T_6
Wheat flour	100	90	85	80	75	70	65
Sweet lupine flour	-	10	15	20	25	30	35
Sugar	30	30	30	30	30	30	30
Shortening	20	20	20	20	20	20	20
Salt	1	1	1	1	1	1	1
Ammonium bicarbonate	1	1	1	1	1	1	1
Sodium bicarbonate	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Baking powder	0.3	0.3	0.3	0.3	0.3	0.3	0.3

T_0: Contained 100% wheat flour (Control 1) T_1: Contained 90% wheat flour+10% sweet lupine flour

T_2: Contained 85% wheat flour+15% sweet lupine flour T_3: Contained 80% wheat flour+20% sweet lupine flour

T_4: Contained 75% wheat flour+25% sweet lupine flour T_5: Contained 70% wheat flour+30% sweet lupine flour

T_6: Contained 65% wheat flour+35% sweet lupine flour

Table 2. Various ingredients of biscuits incorporated with sweet lupine flour and different levels of WPC

Ingredients (g)	Treatments				
	T_3	T_7	T_8	T_9	T_{10}
Wheat flour	80	76	73	70	67
Sweet lupine flour	20	20	20	20	20
WPC	-	4	7	10	13
Sugar	30	30	30	30	30
Shortening	20	20	20	20	20
Salt	1	1	1	1	1
Ammonium bicarbonate	1	1	1	1	1
Sodium bicarbonate	0.4	0.4	0.4	0.4	0.4
Baking powder	0.3	0.3	0.3	0.3	0.3

T_3: Contained 80% wheat flour+20% sweet lupine flour (Control 2)

T_7: Contained 76% wheat flour+20% sweet lupine flour +4% WPC

T_8: Contained 73% wheat flour+20% sweet lupine flour +7% WPC

T_9: Contained 70% wheat flour+20% sweet lupine flour +10% WPC

T_{10}: Contained 67% wheat flour+20% sweet lupine flour +13% WPC

RESULTS AND DISCUSSION

First Experiment

Effect of Substitution of Wheat Flour by Different Levels of Sweet Lupine Flour on Nutritive Value and Sensory Quality of Biscuits

Chemical composition of flours and WPC

The chemical composition of wheat flour, sweet lupine flour and WPC are given in Table 3. Results indicated that the highest value of protein was recorded for sweet lupine flour (40.25%) followed by WPC (35%), while wheat flour had the lowest protein value being 11.54%. It was observed that WPC contained the highest ash level (7%) followed by sweet lupine flour (4%), while the lowest value of ash was observed in wheat flour (0.58%). Results are in agreement with Ahmad *et al.* (2001) who reported that protein content of wheat flour was 8.23 to 12.71 % and ash content valued 0.42 to 0.66%. In addition, these results are in accordance with findings of Tizazu and Emire (2010) who reported that protein content of lupine flour was (40.22%). Also, the present findings are in accordance with the data reported by Savita *et al.* (2013) who showed that the protein and ash content of WPC were 34.3% and 6.6%, respectively. Furthermore, similar results were obtained by Mohamed and Rayas-Duarte, (1995) who found that ash content of lupine flour was 4%.

The fat content of sweet lupine flour and WPC were found to be higher (8.64 and 6%, respectively) than that of wheat flour (0.86%). Similar results was reported by Tizazu and Emire (2010) who found that fat content of lupine flour was 8.92%. Sweet lupine flour had the highest crude fiber content (12.25%) being in agreement with the value reported by Mahfouz *et al.* (2012) who reported that white lupine contained 12.4% crude fiber. Zdunczyk *et al.* (1996) also reported that lupine flour had 12.9 % crude fiber. Carbohydrate content varied from 34.86% to 86.01%, being the lowest for sweet lupine flour and highest for wheat flour.

Chemical composition and nutritional value of biscuits prepared from different levels of sweet lupine flour

Data presented in Table 4 illustrate the chemical composition and nutritional value percentages of biscuits prepared from different levels of sweet lupine flour. Substitution of wheat flour by sweet lupine flour increased significantly ($P<0.05$) protein, fat, ash and crude fiber contents and decreased significantly ($P<0.05$) carbohydrate content of the biscuits in compare with the control biscuit (T_0).

The fat content of the biscuits increased significantly ($P<0.05$) from 15 to 16.98% with increase in sweet lupine flour from 0 to 35% as compare with control biscuit (T_0). The increase in the fat content could be due to the increase in the proportion of sweet lupine flour in the flour blend. This could be due to the fact that sweet lupine flour contain higher percentage of fat than wheat flour (Table 3). Results are in agreement with Rayas-Duarte *et al.* (1996) who found that as the per cent of substitution of lupine flour increased, the fat content of lupine-containing spaghetti samples increased.

The protein content of biscuits increased significantly ($P<0.05$) as the substitution level was increased and it was the highest (16.4%) at 35% substitution level. The protein content of the biscuits increased by 24.9, 39.8, 56.2, 69.9, 84.8 and 95.2% in compare with control biscuit (T_0) with the increase in sweet lupine flour at levels of 10, 15, 20, 25, 30 and 35%, respectively. The increase was probably due to the high protein content of sweet lupine flour (Table 3). Similar trend was reported by Rayas-Duarte *et al.* (1996) who showed that the addition of lupine flour to spaghetti significantly increased the protein content.

However, the carbohydrate content of the biscuits decreased significantly ($P<0.05$) with the increase in substitution level of sweet lupine flour as compare with control biscuit (T_0). This decrease can be explained by the low carbohydrate content of sweet lupine flour (Table 3).

Table 3. Chemical composition of raw materials used in biscuits preparation (on dry weight basis) (Mean ± SD)

Components (%)	Raw Materials		
	Wheat flour	Sweet lupine flour	WPC
Protein	11.54±0.30^{c}	40.25 ± 0.20^{a}	35.00±0.50^{b}
Fat	0.86±0.08^{c}	8.64 + 0.19^{a}	6.00±0.14^{b}
Ash	0.58±0.08^{c}	4.00 ± 0.03^{b}	7.00±0.30^{a}
Fiber	1.01±0.04^{b}	12.25 ± 0.11^{a}	-
Carbohydrate	86.01±0.16^{a}	34.86±0.12^{c}	52.00±0.18^{b}

Means having different letters in the same column are significantly differed (P<0.05).

Table 4. Effect of substation of wheat flour by sweet lupine flour on the chemical composition and nutritional value of biscuit samples (on dry weight basis %) (Mean ± SD)

Treatments	Fat	Protein	Carbohydrate	Ash	Fiber	Energy value (Kcal/100g)
T_0 (Control)	15.00±0.21^{f}	8.40±0.45^{g}	74.59±0.22^{a}	1.31±0.24^{e}	0.70±0.24^{g}	466.96±0.35^{a}
T_1	15.57±0.11^{e}	10.49±0.32^{f}	70.95±0.11^{b}	1.47±0.21^{cd}	1.52±0.21^{f}	465.89±0.42^{b}
T_2	15.85±0.21^{de}	11.74±0.21^{e}	68.93±0.27^{c}	1.55±0.41^{bcd}	1.93±0.41^{e}	465.33±0.28^{bc}
T_3	16.16±0.41^{cd}	13.12±0.15^{d}	66.70±0.12^{d}	1.65±0.11^{abc}	2.37±0.11^{d}	464.72±0.38^{cd}
T_4	16.47 ±0.91^{bc}	14.27±0.13^{c}	64.74±0.27^{f}	1.72±0.32^{abc}	2.80±0.32^{c}	464.27±0.28^{de}
T_5	16.77±0.12^{ab}	15.52±0.16^{b}	62.65±0.18^{f}	1.81±0.18^{ab}	3.23±0.18^{b}	463.61±0.33^{e}
T_6	16.98±0.21^{a}	16.40±0.41^{a}	61.04±0.44^{g}	1.90±0.44^{a}	3.68±0.41^{a}	462.58±0.54^{f}

Means having different letters in the same column are significantly differed (P<0.05).

The ash content increased significantly (P<0.05) from 1.31 to 1.9% with increase in the percentage of sweet lupine flour from 0 to 35% as compare with control biscuit (T_0). It was found that the ash content was the lowest in control biscuit and the highest value was recorded for the biscuit made from flour blends containing 35% of sweet lupine flour. These results may be due to higher content of ash in sweet lupine flour than wheat flour (Table 3). These results are in accordance with data reported by Bugis (2009) who indicated that supplementation of wheat flour with lupine flour increased the protein, fiber and ash contents in kaizer bread.

The crude fiber content in resultant biscuits increased significantly (p<0.05) as the substitution level of sweet lupine flour increased as compare with control biscuit (T_0) and it ranged from 0.7 to 3.68%. The increase in crude fiber content may be due to higher content of crude fiber in sweet lupine flour than wheat flour (Table 3). Results are in agreement with Abdelrahman (2012) who reported that mean protein and dietary fiber increased with increasing substituting wheat flour with lupine flour in cake.

In addition, the calorie value of the biscuits decreased significantly (p<0.05) as the substitution level of sweet lupine flour increased as compared with control biscuit (T_0). The total

calorific value of control sample was observed to be high (466.96 Kcal/100g) compared to sweet lupine flour biscuits. These results are in accordance with findings of Lee *et al.* (2006) who estimated that lupine-enriched bread had lower calorie value than white bread.

Colour measurement of biscuits prepared from different levels of sweet lupine flour

The colour data of biscuits prepared from different levels of sweet lupine flour are shown in Table 5. The *L** values that represent lightness showed no significant change up to 20% sweet lupine flour substitution in biscuits while, over this level of sweet lupine flour the biscuits became darker (lower *L**) than control biscuit (T_0) as the sweet lupine flour quantity increased. This could be due to the Maillard browning reactions which produce darker brown pigments, which occur as a result of the increased content of protein, when using lupine flour (El-Dash and Campos, 1980). Doxastakis *et al.* (2002) reported that breads prepared with lupine flour had dark crust colour.

The *a** values (greenness or redness) increased significantly ($P<0.05$) at 30% and 35% substitution levels of sweet lupine flour. In addition, the *b** (yellowness) and ΔE (reflect total colour difference between control and treated samples) values increased significantly ($P<0.05$) with increasing the sweet lupine flour level in biscuits as compare with control biscuit (T_0). Similar results were obtained by Nasar-Abbas and Jayasena, (2012) who reported that as the percentage of lupine flour in muffins increased, the yellowness (*b**) was increased. Also, results are in agreement with Abdelrahman (2014) who reported that all cake containing lupine flour gave higher *b** values than the control (wheat flour).

Sensory evaluation of biscuits prepared from different levels of sweet lupine flour

Results of sensory evaluation in terms of colour, taste, odour, crispness, appearance and overall acceptability of biscuits with different percentages of sweet lupine flour are presented in Table 6. Substitution wheat flour by sweet lupine flour showed substantial effect on the sensory properties of biscuits. Significant differences ($P<0.05$) were found between biscuits prepared from substitution of wheat flour by different levels of sweet lupine flour in compare with the control biscuit for colour, taste, odour, crispness and appearance. Results showed that the sensory scores for colour increased significantly ($P<0.05$) of biscuits containing sweet lupine flour at different levels in comparison with the control biscuit. Sweet lupine flour causes a yellowing due to the presence of fat-soluble pigments and it was attractive to the judges. Similar trend was reported by Rayas-Duarte *et al.* (1996) who achieved that colour scores of spaghetti containing from15 to 30% lupine flour were much higher than those prepared by adding from15 to 30% light buck wheat or amaranth. Also, Jayasena *et al.* (2008) found that instant noodle samples containing up to 30% lupine flour had higher colour scores than those prepared solely with wheat flour.

Taste, odour, crispness and appearance scores of the biscuit samples were not affected by the sweet lupine flour addition up to 25% level. However, more than 25% sweet lupine flour addition, the taste, odour, crispness and appearance scores decreased significantly ($P<0.05$) as compare with control biscuit (T_0). The decrease in taste and odour was probably due to a beany flavour and/or an aftertaste while, for crispness probably due to texture hardness caused by high protein and dietary fiber contents of sweet lupine flour when added at high levels. Results are in agreement with Jayasena *et al.* (2008) who demonstrated that taste and flavour scores were affected by lupine flour addition at higher levels (>30% levels) in instant noodle. Also, results indicated that there were significant differences ($P<0.05$) in overall acceptability of biscuits containing sweet lupine flour at different levels. Biscuit containing 20% of sweet lupine flour had the highest mean maximum overall acceptability of 93.0 compared to 86.5 for the control biscuit. From the obtained results biscuit containing 20% of sweet lupine flour have been taken as the basis to the second experiment for improving protein quality and acceptability of biscuits with WPC.

Table 5. Effect of wheat flour substituted by different levels of sweet lupine flour on colour measurements of biscuit samples (Mean ± SD)

Treatments	*L**	*a**	*b**	ΔE
T_0 (Control)	73.00±0.43^{a}	1.14±0.55^{c}	25.27±0.19^{g}	0.00±0.00^{g}
T_1	72.87±0.56^{a}	1.50±0.56^{c}	30.60±0.64^{f}	5.34±0.32^{f}
T_2	72.25±0.41^{a}	2.00±0.32^{c}	33.20±0.33^{e}	8.01±0.54^{e}
T_3	72.00±0.62^{a}	2.46±0.43^{cb}	35.40±0.23^{d}	10.26±0.61^{d}
T_4	69.21±0.72^{b}	3.00±0.41^{bc}	37.20±0.42^{c}	12.65±0.52^{c}
T_5	68.84±0.24^{bc}	4.20±0.29^{ab}	39.80±0.52^{b}	15.42±0.54^{b}
T_6	67.91±0.52^{c}	5.50±0.61^{a}	42.50±0.52^{a}	18.49±0.76^{a}

Means having different letters in the same column are significantly differed (P<0.05).

Table 6. Effect of different level of sweet lupine flour on sensory characteristics of biscuit samples (Mean ± SD)

Treatments	Colour (20)	Taste (20)	Odour (20)	Crispness (20)	Appearance (20)	Overall acceptability (100)
T_0 (Control)	11.2±0.66^{d}	19.0±0.43^{a}	18.3±0.42^{a}	19.0±0.40^{a}	19.0±0.61^{a}	86.5±0.96^{e}
T_1	14.1±0.51^{c}	18.0±0.56^{a}	18.0±0.54^{a}	19.0±0.72^{a}	19.0±0.54^{a}	88.1±0.65^{d}
T_2	16.3±0.41^{b}	18.0±0.75^{a}	18.0±0.36^{a}	19.0±0.57^{a}	19.0±0.72^{a}	90.3±0.74^{b}
T_3	19.0±0.82^{a}	18.0±0.43^{a}	18.0±0.73^{a}	19.0±0.41^{a}	19.0±0.51^{a}	93.0±0.60^{a}
T_4	19.0±0.72^{a}	17.1±0.42^{a}	18.0±0.41^{a}	18.0±0.74^{ab}	18.0±0.26^{a}	90.1±0.53^{c}
T_5	19.0±0.54^{a}	14.3±0.49^{b}	16.0±0.82^{b}	16.5±0.81^{b}	16.0±0.61^{b}	81.8±0.81^{f}
T_6	19.0±0.72^{a}	12.5±0.63^{b}	15.5±0.50^{b}	14.2±0.64^{c}	16.0±0.82^{b}	77.2±0.63^{g}

Means having different letters in the same column are significantly differed (P<0.05).

Second Experiment

Effect of Different Levels of WPC on the Quality and Nutritive Value of Biscuits Made From Wheat and Sweet Lupine Flours

Chemical composition and nutritional value of biscuits prepared from wheat and sweet lupine flours with different levels of WPC

Table 7 presented, the chemical composition and nutritional value of biscuits prepared from wheat and 20% sweet lupine flours with different levels of WPC. The data revealed that substitution of wheat flour by 4, 7, 10 and 13% of WPC increased significantly (P<0.05) protein contents in compare with control biscuit (T_3). However, such substitution decreased significantly (P<0.05) carbohydrate contents in resultant biscuits as compared with control biscuit containing 20% sweet lupine flour only. The protein content of biscuits increased as the substitution levels of WPC increased and it was the highest (16.3%) at 13% substitution level. Biscuits contained WPC at levels of 4, 7, 10 and 13% increased protein contents by 6.7, 12.7, 18.2 and 24.2%, respectively, than control biscuit (T_3). The increase in protein content was probably due to higher protein content of WPC than wheat flour (Table 3). Such data are in good agreement with that previously reported by Parate *et al.* (2011) who showed that incorporation of WPC in the formulation of

Table 7. Chemical composition and nutritional value of biscuits prepared from wheat and sweet lupine flours with different levels of WPC (on dry weight basis %) (Mean ± SD)

Treatments	Fat	Protein	Carbohydrate	Ash	Fiber	Energy value (Kcal/100g)
T_3 (control)	16.16±0.41^a	13.12±0.25^e	66.70±0.12^a	1.65±0.11^a	2.37±0.11^a	464.72±0.38^a
T_7	16.29±0.23^a	14.00±0.12^d	65.59±0.21^{ab}	1.80±0.16^a	2.32±0.2^a	464.97±0.32^a
T_8	16.40±0.25^a	14.78±0.26^c	64.61±0.23^{bc}	1.92±0.35^a	2.29±0.21^a	465.16±0.21^a
T_9	16.55±0.14^a	15.51±0.11^b	63.64±0.42^{cd}	2.03±0.11^a	2.27±0.14^a	465.55±0.28^a
T_{10}	16.73 ±0.2^a	16.30±0.22^a	62.57±0.25^d	2.15±0.31^a	2.25±0.12^a	466.05±0.11^a

Means having different letters in the same column are significantly differed (P<0.05).

biscuits increased the protein content proportionately with increasing level of incorporated WPC. Also, Constandache, (2005) showed that incorporation of 10% WPC increased protein content of bread up to 20%.

However, there were no significant differences in fat, ash, fiber and calorie contents for all biscuits contained sweet lupine flour and different levels of whey protein concentrate as compared with control biscuit (T_3). These results are in accordance with findings of Munaza *et al.* (2012).

Colour measurement of biscuits prepared from wheat and sweet lupine flours with different levels of WPC

The L^* values that represent lightness showed no significant change up to 10% WPC substitution level in biscuits prepared from wheat and sweet lupine flours (Table 8). At 13% substitution level there was a significant decrease (P<0.05) in lightness of biscuits as compared with control biscuit (T_3). Similarly, up to 10% substitution of wheat flour with WPC did not affect in a^* and b^* values of biscuits in compare with control biscuit (T_3). However, at 13% substitution level a^* and b^* values scores were significantly higher than that of control biscuit (T_3).This could be due to the Maillard reactions between sugars and amino acids. However, ΔE value increased significantly (P<0.05) with increase the WPC levels in biscuits as compared with control biscuit (T_3).

Sensory evaluation of biscuits prepared from wheat and sweet lupine flours with different levels of WPC

Data presented in Table 9 show the effect of whey protein concentrate on sensory characteristics of biscuits prepared from wheat and sweet lupine flours. The sensory scores for colour, taste, crispness and appearance were not affected significantly by the WPC addition up to 10% level. However, at 13% WPC substitution level the mean colour, taste, crispness and appearance scores decreased significantly (P<0.05) as compared with control biscuit (T_3). The decrease in crispness score may be due to texture hardness caused by high protein content of biscuit. The sensory scores for odour of biscuits were improved significantly (P<0.05) by the addition of WPC at levels of 7 and 10% as compared with control biscuit (T_3).

Results demonstrated that there were significant differences (P<0.05) in overall acceptability of biscuits containing sweet lupine flour and WPC at different levels. Biscuit containing 20% sweet lupine flour and 10% of WPC had the highest mean maximum overall acceptability of 95.7 compared to 93.0 for the control biscuit (T_3). These results are in accordance with data reported by Savita *et al.* (2013) who reported that addition of 10% whey protein concentrate yielded the best quality pasta.

Amino acid contents

The amino acids profile of biscuits were presented in Table 10. Lupine seeds has a good source of lysine, and are generally poor in the sulfur-containing amino acids methionine, cysteine and threonine. Whey proteins are a good source of sulfur-containing amino acids. The results indicated that the substitution of wheat flour with sweet lupine flour and WPC elevated protein content as well as protein quality (essential amino acids profile). Biscuit containing 20% sweet lupine flour increased the

Table 8. Colour measurements of biscuits prepared from wheat and sweet lupine flours with different levels of WPC (Mean ± SD)

Treatments	*L**	*a**	*b**	ΔE
T_3 (Control)	72.00±0.61[a]	2.46±0.33[b]	35.40±0.42[b]	0.00±0.00[e]
T_7	71.50±0.32[a]	2.70±0.43[b]	35.70±0.31[b]	0.63±0.40[d]
T_8	71.00±0.11[a]	3.00±0.16[b]	36.00±0.24[b]	1.29±0.22[c]
T_9	70.50±0.41[a]	3.40±0.35[b]	36.20±0.61[b]	1.94±0.54[b]
T_{10}	69.00±0.32[b]	4.70±0.23[a]	37.50±0.53[a]	4.29±0.31[a]

Means having different letters in the same column are significantly differed (P<0.05).

Table 9. Sensory characteristics of biscuits prepared from wheat and sweet lupine flours with different levels of WPC (Mean ± SD)

Treatments	Colour (20)	Taste (20)	Odour (20)	Crispness (20)	Appearance (20)	Overall acceptability (100)
T_3 (Control)	19.0±0.42[a]	18.0±0.83[a]	18.0±0.63[b]	19.0±0.41[a]	19.0±0.31[a]	93.0±0.40[c]
T_7	19.0±0.79[a]	18.2±0.66[a]	18.0±0.54[b]	19.0±0.72[a]	19.0±0.54[a]	93.2±0.75[c]
T_8	19.2±0.71[a]	18.3±0.55[a]	19.0±0.66[a]	19.0±0.87[a]	19.0±0.72[a]	94.5±0.84[b]
T_9	19.5±0.42[a]	19.0±0.43[a]	19.2±0.73[a]	19.0±0.41[a]	19.0±0.31[a]	95.7±0.40[a]
T_{10}	16.0±0.82[b]	15.6±0.72[b]	17.2±0.41[b]	17.0±0.64[b]	15.1±0.56[b]	80.9±0.73[d]

Means having different letters in the same column are significantly differed (P<0.05).

most of essential amino acids profile in compare with control biscuit (T_0) including isoleucine, leucine, lysine, methionine, threonine and valine. The total lysine content in biscuit containing 20% sweet lupine flour increased from 1.94 to 3.62g/100 g protein. The increase was found to be 86.6%. This might be due to the high content of total lysine in sweet lupine flour. The present findings are in accordance with data reported by Rayas-Duarte *et al.* (1996) who reported that the lysine content of spaghetti substituted with lupine flour at 20% substitution was 3.5 g/l00g protein.

Furthermore, biscuit containing 20% sweet lupine flour in combination with 10% WPC (T_9) had the highest essential amino acids content in compare with biscuit containing 20% sweet lupine flour only (T_3) and control biscuit made from wheat flour only (T_0). The most of essential amino acids profile increased including isoleucine, leucine, lysine, methionine, threonine and valine. Also, sulfur-containing amino acids increased. The total methionine and threonine contents in biscuit containing sweet lupine flour and WPC (T_9) increased from 0.66 and 2.97 in (T_3) to 0.8 and 3.72g/100 g protein in (T_9), respectively. The obtained results are in agreement with Constandache (2005) who reported that incorporation of 10% WPC increased the proportion of essential amino acids, including lysine, threonine, isoleucine, leucine, methionine, and valine in bread.

According to the WHO (2007) expert consultation, the protein requirements of children aged 3-10 and 10-18 years are 0.73 and 0.7 g/kg/day, respectively. Based FAO (2004) weight for age values, the daily protein requirements for these children to 11-22 and 24-40 g/day for 3-10 and 10-18 years old, respectively. According to the FAO/ WHO/ UNU (2001) the energy requirements of children aged 7-8, 8-9, 9-10, 10-11 and 11-12 years are 1623, 1764, 1916, 2078 and 2245 Kcal/day in the mean, respectively. The biscuit containing 20% sweet lupine flour and 10% WPC met the target of providing 15.51g protein and 465.55Kcal energy per 100g biscuit weight. Therefore, consumptions of 50 and 100g of biscuits would provide 50% of the daily protein intake for children aged 3-10 and 10-18 years, respectively, and provide 28.7, 26.4, 24.3, 22.4 and 20.7%, respectively, from the daily energy requirements for children aged 7-8, 8-9, 9-10, 10-11 and 11-12 years, respectively.

Table 10. Amino acids content of biscuit samples (g/100g protein)

Amino acids	Treatments		
	T_0	T_3	T_9
Isoleucine	4.28	4.55	5.32
Leucine	8.01	8.35	9.35
Lysine	1.94	3.62	3.80
Methionine	0.64	0.66	0.80
Phenylalanine	5.28	5.26	4.95
Threonine	2.93	2.97	3.72
Valine	5.01	5.20	5.45
Tyrosine	0.14	1.51	1.87
Cysteine	0.39	0.41	0.66
Histidine	2.91	2.92	2.87
Alanine	4.05	3.69	4.00
Arginine	7.38	7.45	6.81
Aspartic acid	8.13	9.25	9.67
Glutamic acid	35.2	24.71	24.13
Glycine	4.41	4.00	3.73
Proline	11.65	7.65	6.87
Serine	4. 00	4.65	4.90

Conclusions

It could be concluded that biscuit prepared from the substitution of wheat flour with 20% sweet lupine flour and 10% WPC as animal protein improved the protein content and quality of biscuit and it was considered as the most acceptable biscuit. Additionally, 50 g of biscuits could provide 50% of the daily protein requirements for 3-10 year old children and 24.3-28.7% of their daily energy requirements.

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الجودة التغذوية والحسية للبسكويت الغني بالبروتين

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الهدف الرئيس لهذه الدراسة هو تعزيز القيمة الغذائية للبسكويت عن طريق استبدال دقيق القمح بمستويات مختلفة من دقيق الترمس الحلو ومركز بروتينات الشرش كمصدر للبروتينات ذات الجودة المرتفعة، وقد أجريت تجربتين في هذه الدراسة حيث تم إعداد البسكويت في التجربة الأولى باستبدال دقيق القمح بمستويات مختلفة من دقيق الترمس الحلو (١٠% و ١٥% و٢٠% و ٢٥% و ٣٠% و٣٥%)، وقد تم تقييم البسكويت من حيث الجودة التغذوية والحسية، وقد أظهرت النتائج زيادة معنوية في نسبه البروتين عند مستوى معنوية ٠,٠٥ في البسكويت الذى يحتوى على دقيق الترمس الحلو وكانت هذه الزيادة مرتبطة بنسب الاستبدال، وأظهر التقييم الحسي أن البسكويت الذي يحتوى على ٢٠% دقيق ترمس حلو كان الأكثر قابلية، لذلك أستخدم كعينة قياسية للتجربة الثانية، وتم إعداد البسكويت في التجربة الثانية عن طريق استبدال دقيق القمح بـ ٢٠% دقيق ترمس حلو ومستويات مختلفة من مركز بروتينات الشرش، وأظهرت النتائج أن هناك زيادة معنوية في نسبه البروتين عند مستوى معنوية ٠,٠٥ في البسكويت الناتج بزيادة نسبه مركز بروتينات الشرش بالمقارنة بالعينة القياسية المحتوية على ٢٠% دقيق ترمس حلو فقط، والبسكويت الذى تم إعداده باستبدال دقيق القمح بـ ٢٠% دقيق ترمس حلو و١٠% مركز بروتينات الشرش عزز جودة البروتين عن طريق زيادة نسبه الأحماض الأمينية الأساسية وكان الأكثر قابلية حيث أحتوى على ١٥,٥١جم بروتين و ٤٦٥,٥٥ كيلو كالورى طاقة لكل ١٠٠جم بسكويت، بالإضافة إلى ذلك فإن ٥٠جم من هذا البسكويت يمكن أن يمد الأطفال في عمر من ٣-١٠ سنوات بـ ٥٠% من احتياجاتهم اليومية من البروتين و٢٤,٣-٢٨,٧% من احتياجاتهم اليومية من الطاقة، لذلك توصى الدراسة بتناول المرضى الذين يعانون من سوء التغذية الناتج عن نقص البروتين والطاقة هذا البسكويت الغني بالبروتين.

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