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Sweet Lupin and Whey Protein Concentrate as Supplementants for Utilizing in Semi–Hard Biscuit and Its Chemical Properties

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Abstract

This study aimed to supplement soft wheat flour with different levels of sweet lupin powder and/or whey protein concentrate for producing a semi hard sweet biscuit as a functional product and their effects on the chemical and physical properties of produced biscuit were carried out. The results indicate that lupin and whey protein had 3.94 and 7.5 times higher than those of wheat flour. Fat content was higher in lupin with 2 and 4 folds compared with wheat flour, respectively. Lupin had higher ash content with 1.3 and 8.30 folds than its corresponding value in whey protein and wheat flour. Regarding to fibers content, lupin represented 4.44 and 1.33 times higher than that of wheat flour and whey protein, respectively. Generally, the higher the supplementation level of lupin flour and whey protein, the higher the protein, lipid, fiber and ash contents, while the lower the carbohydrate one. The water activity (aw) values have been decreased with increasing supplementation levels of lupin flour and whey protein, which prove the ability of lupin flour and whey protein to capture water which limit the available water for reactions and will affect the shelf life of the products, The farinogram parameters showed an increase in development time by increasing the ratios of lupin and whey protein, The degree of softening increases in samples supplemented with lupin and whey protein. Gelatinization temperature was increased ranged between 58.5˚C in wheat flour to 66˚C in higher supplemented samples. While, the maximum gelatinization decreases by increase supplementation levels of lupin flour and whey protein. Hardness of biscuit increased by increasing supplementing wheat with levels of lupin powder and whey protein. The same trend was found in Fracturability which increased by increasing the supplementation. Resilience values ranged between 0.01 and 0.10, while adhesiveness values ranged between 0.01 and 0.80. Biscuit samples supplemented with 15% lupin powder and 7% whey protein had high score of color, crust appearance, texture, aroma, taste and overall acceptability. The lowest score of crust appearance, texture, aroma, taste and overall acceptability were in supplemented samples with 45% wheat flour, 40% lupin flour and 15% whey protein. The amino acids profile of biscuit sample supplemented with (15%) lupin flour and (7%) whey protein which demonstrates rising in all of amino acids compared to control one. Leucine came as a predominant essential amino acid (0.65%) in control sample raised with 1.75 fold to be 1.14% in supplemented one. Similar finding was also detected when total EAA was compared (raised with 1.8fold). Only cysteine and methionine were less than 0.5% in treated sample rather than those of control. Regarding to the NEAA, glutamic acid is the major NEAA in both of treated or untreated samples (3.3%). The total NEAA was raised by 1.37 fold owing to supplementation process with 15% lupin and 7% whey protein. Similar increasing fold was recorded when total EAA/ NEAA ratio was considered.

***Keywords**:* Sweet Lupin, Whey Protein Concentrate, Biscuit, Chemical Composition, Amino Acids.

1. Introduction

Legumes are an important source of protein, including essential amino acids, vitamins, minerals, dietary fiber, oligosaccharides, bioactive phenolic compounds and minerals **(1,2)**. The protein content in legume seeds is around 17- 40%, for comparison, the range of their contents in meat is (18-25%) **(3)**. Lupins distinguish by a high protein and dietary fiber content **(4)**. In addition, lupin seeds characterized by high level of total unsaturated fatty acids; i.e., over 80% of fatty acid **(5)**. In recent years, sweet lupin seeds (*Lupinus albus* L.) were often the object of research, in which its nutritional value was confirmed. Frequent consumption of legumes, in Mediterranean diet, as well as in many Asian countries reduces the risk of civilization diseases. A significant relationship was found between the diet

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rich in legumes and the lower risk of coronary heart disease **(4)**, type 2 diabetes **(6)**, obesity **(7)**. The intake of legumes is also associated with a lower risk of osteoporosis **(7)**. The high dietary fiber content of lupin is typically associated with cholesterol-lowering activity **(8)**, fiber content improve the backing quality **(9)**. To use lupin as food ingredient its alkaloid content must be maintained as low as possible **(2)**. All lupin flours are also very low in starch, unlike wheat flour in which starch is the major component **(10)**.

Lupin seeds contain vitamins such as thiamine, niacin, riboflavin and tocopherols, as well as minerals including iron, zinc and manganese **(11)**. Minerals very important and play an important role in elimination of some food contaminants especially in nano scale such as nano silver **(12)**. In addition, Lupin flour and protein isolates are containing antioxidants **(13)** in the form of the polyphenolic tannins and flavonoids **(14)**. Antioxidants very important for health to its basic role in balance of oxidative stress **(15, 16)**. Comparing with soybean and other legumes, lupin have lower level of anti-nutritional Components such as phytate and saponins **(11)**. Lupin proteins and dietary fiber have the potential to increase the nutritional quality and modify the technological properties of bread and other baked products when wheat flour is supplemented by lupin flour. The main limiting amino acids in lupin are S-amino acids (methionine and cysteine), valine and tryptophan **(17)**. The amounts of lysine, isoleucine, leucine, phenylalanine and tyrosine in lupin are comparable to the Food and Agricultural Organization (FAO) standards for amino acids of ideal reference protein appropriate for adult **(18)**. The amino acid profile of lupin complements that of wheat, which is higher in sulphur-containing amino acids but lower in lysine. The advantages of using legume flours, including lupin, in combination with wheat flour in baked products are increased protein content and improved amino acid balance of the final product. Legumes including lupin lack the gluten protein required for desired dough and bread quality **(19)**. This lack of gluten limits the incorporation rate of lupin in bread and hence limits the improvement in nutritional and health benefits that can be gained through its incorporation **(20)**.

In study on other baked products such as biscuits, gluten-free cakes, and muffins **(21)** demonstrated that lupin flour incorporation rates of 20-30% can be achieved without reducing sensory quality and acceptability. It was reported that lupin flour can be used to formulate acceptable baked products **(10)**, as well as other foods such as pasta **(22)**, meat products **(23)** and dairy products **(24)**.

A- part from being highly nutritious, the functional properties for example: high solubility, foaming capability, water-holding capacity and emulsifying properties in whey proteins have been remarkable **(25)**. These properties are appreciated for bakery products, such as cakes, essentially when used as whey protein concentrate (WPC). In fact, whey proteins' functionality has been described by several studies in cakes, indicating an improvement in the dough texture **(26, 27)**. Whey proteins are the best quality proteins available and have high digestibility and a complete amino acid profile. Whey proteins are also recommended due to their beneficial effects on enhancing immunity and reducing the risk of heart disease and the incidence of cancer **(28)**. There were several reports of the effect of whey on the properties of wheat dough and wheat-based bakery products **(29, 30)**. For example, the ability of whey proteins to thicken and form a gel upon heating might be advantageous for some applications; however, this ability might also be disadvantageous for others **(31)**. Therefore, the selection of the protein source with appropriate functionality might play an important role in certain application. In addition, if a continuous protein phase could be created when amount of foreign proteins was higher than gluten in wheat flour, they might confer a protective effect on dough structure for obtaining an improvement in bakery products quality.

This study aimed to supplement wheat flour by different levels of powdered sweet lupin and whey protein concentrate for producing a semi hard sweet biscuits afunctional product.

2. Materials and Methods

2.1. Materials

Soft Wheat Flour 72% extraction was obtained from Arabian Milling & Food Industries Company in New Bourg El Arab, 3rd Zone, Alexandria governorate, Egypt. Whey protein (WP) concentrate produced by Agropur Company, Canada. *Lupin (Lupinus albus)* seed was obtained from Legume Research Department, Field Crops Research Institute, Agriculture Research Center, Giza, Egypt. Other materials (margarine, baking powder, sugar, milk, egg and salt) were obtained from local market, Giza, Egypt.

2.2 Methods:

2.2.1. Preparation of Lupin flour

Lupin seeds were powdered using custom electric mill available at Bread and Pastries Department, Food Technology Research Institute, Agriculture Research Center, Giza, Egypt.

2.2.2. Chemical analysis

Moisture, ash, ether extract, fiber and crude protein were determined according to the methods described in the **(32)**. Moreover, total carbohydrates were determined by difference. Energy value (KCal. /100gm) of baked samples was calculated using the following equation: Energy value = [(carbohydrate x 4) + (fat x 9) + (protein x 4)]. All results were recorded as mean value of three replicates.

2.2.3. Rheological properties:

2.2.3.1. Farinograph test

The farinograph (Brabender Farinograph, Germany) was used to study the hydration and mixing characteristics of the dough according to **(33)**.

2.2.3.2. Amylograph test

Amylograph test was carried out according to the method described in the **(33)** using an Amylograph (Brabender. Amylograph Germany).

2.2.4. Preparation of biscuits

Biscuits were prepared according to the standard procedure for semi-hard sweet biscuits in Bread and Pastries Department, Food Technology Institute, Agriculture Research Center. Ingredients were mixed by gradually adding the wheat flour, and kneading until forming a homogeneous mass. The dough was stretched with a kitchen roll and the cookies were shaped using appropriate molds. The cookies were distributed into pre-greased and floured trays and then oven baked at a temperature of about 180°C (180 ± 2ºC) for 20 minutes, in an experimental electrical oven (Brand Teimarmor). were prepared according to the method described by **(34)**.

2.2.5. Sensory evaluation of biscuits:

The biscuit were evaluated by ten trained panelists at Bread and Pastries Department, Food Technology Research Institute, Agriculture Research Center, Giza, Egypt. Biscuit sensorial tested for general appearance 20, taste 20, odor 20, color 20, texture 20 and over all acceptability 100 degrees according to **(35)**.

2.2.6 Physical characteristics

Diameter (mm): The diameter (D) was measured by placing six biscuit edge to edge to get an average diameter in millimeters. Thickness, expansion factor (D/T), weight (g) and volume (cm3) of biscuit was measured according to the standard methods **(33)**.

Table 1. Blends of soft wheat flour (72% extraction) supplemented with different levels of lupin flour and whey protein used for producing biscuits.

Treatments No.	Blends
1(control)	100% soft wheat flour 72% extraction rate.
2	85% soft wheat flour + 15% Lupin flour (LF)
3	85% soft wheat flour + 15% whey protein (WP)
4	78% soft wheat flour + 15% Lupin flour+ 7% whey protein
5	45% soft wheat flour + 40% Lupin flour+15% whey protein
6	30% soft wheat flour + 50% Lupin flour+ 20% whey protein

Table 2. Formula of prepared biscuits

Ingredients	Weight (g)
Soft wheat flour	100
Sugar	30.0
Batter Milk	15.0
Skimmed milk powder	0.5
Ammonium bicarbonate	0.6
Sodium bicarbonate	0.33
Fresh whole egg	24.00
Vanillin	0.3

2.2.7. Determination of water activity (aw) of biscuits

The water activity was measured with a Rotronic (model Hy-grolab3 - Switzerland). The ground sample of biscuit was filled with the plastic cups approach the top and the Hygroplam probe was inserted in these cups. After almost three to four minutes, the display showed the water activity, along with temperature **(36)**.

2.2.8. Texture analysis

The texture profile analysis was performed using a texturometer TA-XT2 Plus from Stable Microsystems. The test involved two compression cycles using a flat probe with 75 mm diameter (P/75), separated by 5 seconds. The parameters used for the test were: 30 kg force load cell, pre-test, test and post-test speeds equal to 1.0 mm/s, distance 4 mm and trigger force 0.1 N. The textural properties: hardness, resilience, springiness, cohesiveness and chewiness were calculated after equations **(37)**.

2.2.9. Amino acids profile

Amino acids were determined using a Mikrotechna AAA881 automatic amino acid analyzer according to the method of **(38)** as described by **(39)** where samples were hydrolyzed in the presence of 6 M HCl at 110oC for 24 h under a nitrogen atmosphere. Sulfur-containing amino acids were determined after performic acid oxidation.

2.2.9.1. Protein quality measurements

The quality of biscuit's protein was determined by using the following measurements:

2.2.9.2. Essential amino acid index (EAAI)

Essential amino acid index (EAAI) was calculated using the equation of **(40)**. Whole egg protein was used as a standard reference protein as follows:

$$EAAI = \sqrt[n]{\frac{100a \times 100 \ldots 100j \times 100}{av \times bv \ldots jv}}$$

Where: n = number of essential amino acids, a, b …..j = represent the concentration of essential amino acids (lysine, tryptophan, isoleucine, valine, arginine, threonine, leucine, and phenylalanine, the sum of methionine and cystine plus histidine) in tested sample and av, bv ….. jv = content of the same amino acids in standard protein (%), respectively.

2.2.9.3. Protein efficiency ratio (PER)

PER of biscuit's protein was calculated according to the equation described by (41)as follows:

$$PER = 0.06320\ [X10] - 0.1539$$

Where X10 = threonine + valine + methionine + isoleucine + leucine + phenylalanine + lysine + histidine + arginine + tyrosine.

2.2.9.4. Biological value (BV)

Biological value of biscuit's protein was computed according to the method of (40) as follows: BV = 1.09 (EAAI) – 11.7.

2.2.8.5. Chemical Score (CS)

Chemical score (CS) of biscuit's protein was performed according to (42) by dividing the content of EAA of the tested protein, between the amino acid content in the reference protein.

2.2.10. Statistical analysis:

Data were analyzed using Co Stat, version 3.03 for personal computer according to **(43)**. The tests used were ANOVA and student-Neuman-Keuls test. A treatment effect was assumed to be statistically significant at $P < 0.05$.

3. Results and Discussion

Chemical composition of raw materials was presented in **Table 3**. Moisture content was low in both lupin powder (6.00%) and whey protein (7.60%), while it was higher in soft wheat flour (72% ext. rate) (14.20%). The protein content in whey protein was superior values which was 78.00%, followed by lupin powder 41.00%, the lowest percentage of protein which was 10.40 presented in wheat flour 72%. Lupin powder had the higher fat, ash and crude fibers compare to other raw materials which was 4.00, 3.90 and 2.00%. The fat in whey protein and wheat flour was (1.00 and 2.00), ash (2.86 and 0.47) and fiber content were (1.50 and 0.45). On the other side, carbohydrate content in wheat flour was 85.68 % higher than whey protein 15.64 and 49.1%. Such findings indicate the followings:

Lupin and whey protein had 3.94 and 7.5 times higher than those of wheat flour. Fat content was higher in powdered lupin with 2 and 4 compared with wheat flour respectively.

Lupin had higher ash content with 1.3 and 8.30 than corresponding value in whey protein and whey protein and wheat flour. regarding to fibers content, lupin represented 4.44 and 1.33 times higher than that of soft wheat flour and whey protein, respectively, similar finding were found in lupin powder **(1)**.

Presented data in **Table 4** showed that protein content of suggested biscuit was gradually increased by increasing supplementation level, high protein and high dietary fibre lupin flour can be successfully incorporated into biscuits by replacing up to 20% of wheat flour. By replacing wheat flour with lupin flour at 20% level substantial improvement in the protein and fibre contents can be achieved without adversely affecting physical and sensory properties of biscuits, the obvious increase in protein content was found by increasing the level of either whey protein or lupin powder substitution. There is a little significant difference in fat content between biscuit samples **(44)**. Studied that the higher protein, fat content was found in biscuit supplemented with high percentages of lupin flour and whey protein which showed similar results in wheat replacement with lupin.

The ash content increased in biscuit which had high substitution regarding to carbohydrates content, the increase in substitution caused a decremental trend in carbohydrate content. There is no significant difference in food energy between different samples. Generally, the higher the supplementation level of lupin flour and whey protein the higher the protein, lipid, fiber and ash content, while the lowest the carbohydrate one.

The water activity (aw) values in **Table 5** has been decreased with increasing supplemented level of lupin flour and whey protein, which prove the ability of lupin flour and whey protein to capture water which limit the available water for reactions and will affect the shelf life of the products, The available water is used to solubilise the added sucrose, to form the foam structure and subsequent gelatinisation of starch, but in supplemented samples, whey protein competes with the added sucrose for the available water. Hence, as whey protein increases, sucrose´s solubility decreases, causing it going to crystalise when exposed to heat, the results agree with **(44)**.

Table 3. Chemical composition of raw materials used in biscuits.

(%)	Wheat flour	Whey protein (WP)	Lupin flour powder (LF)
Moisture (%)	14.20	7.60	6.00
Total protein (%)	10.40	78.00	41.00
Ether extract (%)	1.00	2.00	4.00
Ash (%)	0.47	2.86	3.90
Crude Fiber (%)	0.45	1.50	2.00
Total carbohydrate (%)	87.68	15.64	49.1

The physical analysis of biscuit supplemented with different levels of lupin powder and whey protein were demonstrated in **Table 6**. There is no significant differences in diameters and volume. However, there were a little significant difference in thickness, weight and specific volume between biscuit samples and its control, which match with **(45)** who proved that biscuits prepared by replacing soft wheat flour with lupin flour at 20% demonstrated a significant increase in thickness.

The farinograph parameters showed increasing in development time by increasing the ratio of lupin powder and whey protein **(Table 7)**. The development time in control sample (wheat 72% ext.) was 80 second and reaches to 4.45 min in samples supplemented with high lupin powder and whey protein ratio. According to **(29)** dough stability decreased when whey protein was added similar to our findings, dough stability gradually decreases by increasing wheat flour by lupin powder and whey protein. The degree of softening increase in samples supplemented with lupin powder and whey protein. The degree of softening after 12min in samples of soft wheat flour (72% ext. rat) was (55 B.U), while in biscuit samples with lupin powder and whey protein substitution ranged between 76 and 186 B.U.

Supplementing by lupin powder and whey protein and its effect on amylograph parameters was given in **Table 8**. Result showed that investigate that gelatinization temperature was increased ranged between 58.5˚C in wheat flour to 66˚C in highest supplemented samples. While, the maximum gelatinization decreases by increase the substitution of lupin powder and whey protein. Supplemented samples decreased from 820 B.U in wheat flour to 484 B.U. The maximum gelatinization temperature increased in all supplemented. The time of maximum gelatinization gradually increased in biscuit samples supplemented with lupin powder and whey protein and its control. Time of maximum gelatinization increased from 34 min to 39.29 min in biscuit samples.

(34) Discussed improving effect of adding whey protein to cookies comparing to basic cookies, and **(45)** who proved that all of the textural properties, except for cohesiveness, demonstrated significant changes due to incorporation of lupin flour in the formulation. The texture profile analysis of biscuit prepared by supplementing wheat with lupin powder and whey protein was given in **Table 9**. Hardness of biscuit increased by increasing supplementing wheat with levels of lupin powder and whey protein. The same trend was found in Fracturability which increased by increasing the supplementation. Resilience values ranged between 0.01 and 0.10, while adhesiveness values ranged between 0.01 and 0.80.

(46) Noticed that adding lupin flour showed remarkable effect to sensory properties of cookies. The sensory evaluation of supplemented biscuit showed high color score was high in supplemented biscuit samples with 15% lupin powder and biscuit samples with (15% lupin powder + 7% whey protein) **(Table 10)**. Biscuit samples supplemented with 15% lupin powder and 7% whey protein had high score of color, crust appearance, texture, aroma, taste and overall, acceptably. The lowest score of crust appearance, texture, aroma, taste and overall, acceptably was in supplemented samples with 45% wheat flour, 40% Lupin flour and 15% whey protein.

Table (11) showed the amino acids profile of biscuit samples supplemented with (15%) lupin flour and (7%) whey protein, and (15%) whey protein, the essential amino acid (i.e. lysine, leucine, phenylalanine, threonine, isoleucine, valine, methionine, tyrosine and cysteine) and non-essential amino acid (i.e. glutamic, aspartic, proline, arginine, glycine, alanine, serine and histidine) profile of the produced biscuit is presented in **Table 11**. In general, non-essential amino acids were decreased when legume flours were incorporated into the formulation with the exception of glutamic acid which was higher in case of legume supplemented biscuit than that of control ones. Moreover, the total non-essential acid (sum of those amino acids) was decreased by its turn.

Results showed that incorporation of legume flours into biscuits formulation led to an increase in essential amino acid contents of the produced biscuits. Obvious increase was noticed in case of lysine (the first limiting amino acid in cereals) by up to 2.10 and 1.70 times in case of formulations with 7% why and 15% lupin flour and 15% lupin flour and biscuit flour than that of control biscuit, respectively.

On the other hand, the sulfur- containing amino acid, methionine was decreased as the portion of added legume flour increased. This could be attributed to the lower methionine content of legume flours when compared to flour (cereal) flour as previously reported by **(47, 48)** who reported that the nutritional value of cereal proteins is limited by the low amount of lysine and tryptophan, while legume protines are low in sulfur – containing amino acids such as methionine and cysteine. However, total essential amino acid content was increased by 36.97 and 35.02 in case of treatment respectively than that of control biscuits protein.

Protein quality of biscuits

To determine the quality of biscuits protein as a result of incorporating lupin flour and why protein in the different biscuits formulations under investigation, different parameters were used including TEAA/TNEAA (%), Essential Amino Acid Index (EAAI), Protein Efficiency Ratio (PER), Biological Value (BV) and Chemical Score (CS) and the results are shown in Table 12. Incorporation of legumes flour was found to increase the ratio of TEAA/ TNEAA in all biscuits treatments. This increase was found to be positively correlated with the higher percentage of legume flour and why protein in the biscuits formulations. EAAI is an important parameter, which is used to evaluate the quality **(49)**. Biscuit containing legume flours and why protein had achieved higher EAAI values than control ones. The EAAI ranged from75.36 in case of biscuit containing 7%W+15 % L. to 70.91 in those containing 15%Why protein. Biscuits containing 7%W+15 % L. had relatively higher EAAI than those with 15%W. This could be attributed to the higher EAA profile of legume flour when compared to why protein one.

BV represents the absorbed proportion of protein from a food to be incorporated into the proteins of the body **(50)**. From **Table 12**, it could be observed that the values of BV in biscuits formulations with legume flours was doubled by a 1.50 and 1.40 times in the treatments number 1 and 2 compared to the control biscuits. This result is due to the higher essential amino acid content of legume flours and why protein.

PER can be defined as the gain of a test subject weight divided by the unit intake of a particular food protein, and has been extensively used as a parameter in protein quality evaluation **(51)**. Biscuits with legume flours have achieved PER values higher than that of control biscuits ranging from 2.71 to 3.36 with a positive correlation with the addition level of legume flours. The same trend was observed with regard to CS which ranged from 78.97 in case of 7%W+15 % L to74.81 in case of 15%W ones.

Table 4. Chemical composition of biscuit supplemented with different levels of lupin flour and whey protein.

Treatments	Moisture%	Protein%	Fat %	Ash %	Crude fiber %	Carbohydrates%	Food energy
1	5.32[c]	8.09[e]	11.74[c]	1.07[c]	0.24[c]	78.86[a]	453.46[a]
2	5.50[b]	11.18[d]	12.48[b]	2.16[bc]	0.31[b]	73.87[a]	452.52[a]
3	6.38[a]	13.60[c]	12.82[b]	2.16[bc]	0.33a[b]	71.09[b]	454.14[a]
4	5.75[b]	13.20[c]	13.03[b]	2.34[b]	0.28[bc]	71.15[b]	454.67[a]
5	5.44[c]	20.30[b]	13.48[a]	2.91[a]	0.34[b]	62.97[c]	454.4[a]
6	5.37[c]	23.90[a]	13.68[a]	3.17[a]	0.37[a]	58.88[d]	454.24[a]
LSD at 0.05	0.280	0.53	0.42	0.26	0.04	5.63	9.11

* See Table 1.

Table 5. Water activity (aw) of supplemented biscuit with different levels of Lupin flour powder and whey protein.

Treatments	a_W	C°
1	0.518	21.91
2	0.516	21.98
3	0.5145	22.01
4	0.507	22.19
5	0.4975	22.385
6	0.501	22.445

* See Table 1.

Table 6. Physical analysis of biscuit with different supplemented levels of lupin flour powder and whey protein

Treatments	Diameter	Thickness	Expansion Factor	Weight	Volume	Specific Volume
1	3.93[a]	0.79[a]	4.97 [b]	4.73[a]	8.11[a]	1.71[b]
2	3.78[a]	0.47[b]	8.04 [a]	3.98[b]	9.80[a]	2.48[a]
3	4.04[a]	0.80[a]	5.05 [a]	4.45[ab]	8.22[a]	1.86[ab]
4	3.76[a]	0.47[b]	8.00 [a]	3.98[b]	9.86[a]	2.48[a]
5	4.01[a]	0.68[a]	5.89 [a]	4.85[a]	9.55[a]	1.96[ab]
6	3.73[b]	0.67[a]	5.56 [a]	4.60[a]	8.66[a]	1.88[ab]
LSD at 0.05	0.29	0.12	2.41	0.48	1.90	0.52

* See Table 1, Weight: W(g); Diameter: D (mm); Thickness: T (mm); Expansion factor: (D/T); biscuit Volume: B. V(cm^3); Specific volume: S V(cm^3)/(g).

Table 7. Effect of supplementing with different levels of lupin flour and whey protein with soft wheat flour (72% ext. rate) in Farinograph parameters.

Treatments	Water absorption (%)	Development time (min.)	Stability time (min.)	Degree of Softening after (10min)	Degree of Softening after (12min) B.U
1	58.5	1:20	4:55	44	55
2	60	1:45	4:50	93	108
3	58	4:43	2:50	12	90
4	61	4:05	3:48	10	76
5	57.4	4:00	2:32	53	120
6	56	4:45	1:00	177	186

* See Table 1.

Table 8. Effect of supplementing with different levels of lupin flour and whey protein with soft wheat flour (72% ext. rate) in Amylograph parameters.

Treatments	Gelatinization temperature (˚C)	Gelatinization maximum	Maximum Gelatinization Temperature (˚C)	Time of maximum gelatinization (min)
1	58.50	820	83.10	34:00
2	59.80	750	84.50	35:65
3	61.00	744	85.10	36:20
4	61.00	714	85.00	36:11
5	64.40	557	89.00	37:45
6	66.00	484	90.00	39:29

* See Table 1.

Table 9. Texture Analysis of biscuit produced from soft wheat flour 72% ext. rate supplemented with different levels of lupin flour and whey protein.

Treatments	Hardness (N)	Adhesiveness (mj)	Resilience	Fracturability (N)
1	35.20	0.20	0.02	13.08
2	34.02	0.01	0.02	33.62
3	36.05	0.80	0.01	36.05
4	48.26	0.10	0.02	69.54
5	84.81	0.01	0.10	70.32
6	94.76	0.40	0.01	84.53

* See Table 1.

Table 10. Sensory evaluation of biscuit produced from soft wheat flour (72% ext. rate) supplemented with different levels of lupin flour and whey protein.

*Treatments	Color 20	Crust appearance 20	Texture 20	Aroma 20	Taste 20	Overall, acceptably 100
1	15.66[b]	17.00[a]	17.33[ab]	16.66 [b]	16.33[b]	83.00[b]
2	19.33[a]	18.00[a]	18.33[a]	16.33[b]	16.66[ab]	88.65[a]
3	18.33[a]	17.00[a]	17.33[a]	17.33 [ab]	17.66[a]	87.66[ab]
4	19.33[a]	18.66[a]	18.33[a]	19.00[a]	18.33[a]	93.66[a]
5	16.00 [ab]	12.00[c]	15.66[a]	15.00[b]	14.66[c]	73.33[c]
6	15.33[b]	16.33[b]	15.66[b]	15.33[bc]	14.66[c]	77.33[c]
LSD at 0.05	2.85	2.25	2.10	1.99	1.32	5.59

* See Table 1.

Table 11. Amino Acid composition (g amino acid / 100g Protein) of Biscuit produced from wheat flour 72% extraction supplemented with different levels of lupin flour and whey protein.

Amino Acid	Control	Treatment (1)7%W+15L	Treatment (2)15%W	Egg
Essential amino acids (EAA)				
Lysine	2.10	4.4	3.53	7.24
Leucine	5.56	5.91	5.66	8.60
Phenylalanine	2.20	4.7	5.68	5.41
Threonine	3.21	4.47	3.23	4.37
Isoleucine	3.60	4.77	3.95	5.33
Valine	3.67	5.00	4.69	6.76
Methionine	1.37	1.26	1.73	5.41
Tyrosine	3.97	4.62	4.08	3.90
Cysteine	1.54	1.74	2.47	2.15
Total EAA	27.02	36.97	35.02	46.81
Non-Essential amino acid (NEAA)				
Glutamine	25.00	25.66	29.42	13.29
ASP+ASN	4.78	5.530	4.82	10.51
Proline	7.57	7.87	8.03	4.06
Arginine	4.45	6.06	5.14	6.52
Glicina	3.01	4.09	4.20	3.42
Alanine	3.23	4.16	3.46	5.81
Serine	5.51	6.21	5.81	7.72
Histadine	2.20	2.42	2.34	2.39
Total NEAA	56.41	61.34	63.22	53.74

Table 12. Protein Quality Parameters of biscuits

	Control	Treatment (1)7%W+15 %L	Treatment (2)15%W
TEAA/TNEAA (%)	47.89	60.27	55.39
EAAI[a]	54.81	75.36	70.91
PER[b]	1.87	2.61	2.37
BV[c]	48.04	70.44	65.60
CS[d]	57.72	78.97	74.81

a: Essential Amino Acid Index, b: Protein Efficiency Ratio, c: Biological Value, d: Chemical Score

4. Conclusion

Legumes could be an important protein source, especially when supplementing cereal-based diets. In terms of overall cost efficiency, legumes represent an advantageous alternative to animal proteins. Legume protein is the natural low-cost complementary protein to the cereal one. Cereals are poor in the essential amino acid lysine which is their first limiting amino acid, and on the other hand legumes are low in sulfur containing amino acid such as methionine which cereals are rich in. Thus, incorporation of legume flours into cereal products formulations has the ability to improve the different parameters of protein quality

5. Conflict of Interests

There are no conflicts of interest declared by the Authors.

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