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ORIGINAL ARTICLES

Partial Substitution of Eggs by Lupin Flour and its Protein Isolates in Cakes Manufacturing

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ABSTRACT

The effects of lupin flour substitution on physical and chemical properties were studied. Wheat flour was partially substituted in F1 and F2 formulas with lupin flour (LF) at a ratio of 5 and 10%. At the same time, eggs amount was involved in the formula by substituting 25 and 50%, respectively, of their original amount that found in the control sample (F). F3 and F4 formulas contained lupin protein isolate in ratios of 2 and 4% of wheat flour, respectively, with 25 and 50% of the egg amounts substitution, respectively. The lupin protein isolates (PI) had higher significantly ($p \leq 0.5$) chemical compositions and physical properties. Protein isolates (PI) showed higher water absorption (WAC), oil absorption capacity (OAC) and foam capacity (FC) higher than that of their corresponding flours. A decrease in cake hardness and moisture content was obtained when the lupin proportion was increased by substituting of 25 and50% of eggs compared with control. On the other hand, an opposite pattern was observed in case of the volume and specific volume when compared with control. The sensory evaluation shows that 5% lupin flour and 2% PI substitution could be the best substitution in cakes. The chemical compositions of different formula cakes showed a significant higher level in protein content and a significant level lower level in carbohydrates and fat. It could be suggested that the cakes prepared by 25% egg and 5% LF or 2% PI substitution could result in high acceptable cakes.

Kew words: Partial Substitution, Lupin Flour, Eggs, Cakes Manufacturing

Introduction

Eggs are the most costly ingredients in some cake formulas preparing. In yellow cakes, eggs are a significant source of cholesterol (Gilbertson and Porter, 2001). The use of vegetable proteins for partial or total substitution of eggs in cake formulations appears, therefore, to be an interesting objective and especially so for the people with specific dietary needs or restrictions (vegans, vegetarians, hypercholesterolemia cases, etc,…). The almost unique foaming, emulsifying and heat coagulating properties of egg proteins confer them a very important functional role in the definition of cake characteristic, namely volume and texture. This makes it is extremely difficult to successfully substitute eggs by different source of proteins, even by the use of several types of additives, such as hydrocolloid, in cakes (Arozarena *et al.,* 2001). The utilization of plant proteins as food hydrocolloids is based on their functional properties, such as emulsification, solubility, foaming properties, water and oil absorption capacities and gelling properties (Kinsella and Phillips, 1989 and Kinsella 1979). The utilization of lupin, a positive protein profile, flour in bakery products can be achieved and can improve the rheological properties of dough (Pozani *et al.,* 2002). Main fields of lupine utilization are in vegan and vegetarian tofu-like products as well as in gluten free foods such as bread, cookies, noodles and sauces. Lupin seed proteins have been proposed as an alternative to proteins extracted from soy bean due to its similar protein content (30 – 40 % of dry weight) and its functionality satisfactions in number of food systems (Torres *et al.,* 2005). Lupin is a good source of nutrients, not only for proteins but also for lipid, dietary fiber, minerals and vitamins (Martinez-Villaluenga *et al.,* 2006). Furthermore, lupins contain some phytochemical compounds with antioxidant capacity such as polyphenols, mainly tannins, and flavonoids (Oomah *et al*., 2006 and Martinez-Villaluenga *et al.,* 2009). In general, defatted protein isolates improve sensory profiles, thus could be considered as a good consumer acceptance, beside unchanged the high functionality of the final products (Bader *et al.,* 2011).

The aim of this work is to estimate the possibilities of partial substitution of egg protein in yellow cake by lupine flour and its protein isolate.

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Materials And Methods

Materials:

Raw materials:

Sweet lupin seeds (*Lupinus albus* L) were obtained from the Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. Wheat flour, fresh whole eggs, sugar, butter, salt, fresh milk, vanilla and baking powder were obtained from the local market at Giza.

Preparation of the lupin flour:

Foreign materials in lupin seeds were inspected and removed by picking and then the lupin seeds were ground by flour miller to pass through 60 mesh screens.

Methods:

Analytical methods:

Determination of moisture, protein, fat, fiber and ash contents as dry weight basis were carried out according to AOAC (1990), while the carbohydrate contents were calculated by difference.

Preparation of protein isolates:

Protein isolate was prepared by isoelectric precipitation method described else where (Alamanous and Doxastakis, 1997 and 1995). The pH of the alkaline extraction was 8.5 and the precipitation of protein process was done at pH 4.5 followed by centrifugation at 4000 RCF for 20 min. The precipitated residue was several times washed with deionized water and kept frozen tell uses.

Determination of functional properties:

Foam capacity (FC) and stability (FS) were determined by the method of Lin *et al*., (1974). The FC could be calculated as follows:

FC (%) $=\{(V_2-V_1)/V_1\}*100$ Where,

V_1 is the volume of solution (one g in 100 ml) and

V_2 is the volume of solution (one g in 10 ml) after whipping in a blender set to speed 2 for 2 min (Lawal *et al*., 2005).

On the other hand, Foam stability (FS) is the recorded foam volume in the graduated cylinder after whipped and at intervals of 20, 40, 60 and 120 min of setting at ambient temperature (Lawal *et al*., 2005).

Water and oil absorption capacities:

The water absorption capacity (WAC) was measured using the method of Sosulski, (1962). It is the volume of supernatant of the centrifuged (at 5000 g for 30 min.) solution (one g in 10 ml distilled water) after allowed to stand at room temperature for 30 min (Lawal *et al*., 2005).

The oil absorption was determined by the method of Lin *et al.,* (1974). It is the volume of supernatant of the centrifuged (at 5000 g for 30 min.) solution (one g in 10 ml oil) after allowed to stand at room temperature for 30 min (Lawal *et al*., 2005).

Emulsion activity and stability:

The emulsion activity (EA) and stability (ES) were determined according to the method described by Naczk *et al*., (1985) with modification by Lqari *et al*., (2002) as follows:

The EA could be calculated as follows:

EA (%) =(height of emulsified layer/ height of total content) *100 Where LF or PI (10 mg/ml) were homogenized with 5 ml oil for 1 min, centrifuged the resulted emulsion at 1100g for 5 min and the heights of the emulsified layer and that of the total contents in the tube were measured.

The ES (%) could be calculated by substituting the corresponding values of the same parameters of EA (%) after heating of those emulsions at 80° c for min. before centrifuging (Lawal *et al*., 2005).

Cake Preparation:

Lupin flour (LF) and protein isolated (PI) was used to prepare of 16 % protein content emulsion through substituting egg (its estimated protein content reached about 15 %) contents. Cup cakes were prepared according to the standard formula method (AACC, 1983) with some modification to produce good cup cake. Cake formula consisted of wheat flour (200 g), sugar (160 g), butter (100 g), fresh whole eggs (182 g), salt (3 g), fresh milk (24 g), baking powder (9 g) and vanilla (2 g). The mixing and baking procedures were holding constant over the experiment designs. A multi-stage mixing method was selected. This consisted of creaming the sugar with butter by mixing for 5 min followed by addition of milk (30 sec) followed by addition all the other ingredient, i.e., flour, baking power and eggs for the control sample and blended for 5 min. The lupin flour or lupin protein isolate were used instead of the eggs in the tested formulas. The ingredients variability of the tested formulas was found in Table (1).

The ingredients were thoroughly mixed as previously describing and the mixture was then poured into small aluminum cup (100 g each) and baked for 20 min at 170 ^{o}C. After baking, the cakes were cooled for 30 min at room temperature packaged and frozen stored till used.

Table 1: Partial substitution of eggs by lupin flour or its protein isolate in the tested formulas

Formula	Amounts (in g) in formula				
	F	F1	F 2	F 3	F 4
Wheat flour	200.0	190.0	180.0	196.0	192.0
Fresh eggs	180.0	136.5	91.0	136.0	91.0
Lupin flour	00.0	10.0	20.0	4.0	8.0
Sugar	160.0	160.0	160.0	160.0	160.0
Butter	100.0	100.0	100.0	100.0	100.0
Salt	3.0	3.0	3.0	3.0	3.0
Fresh milk	24.0	24.0	24.0	24.0	24.0
Baking powder	9.0	9.0	9.0	9.0	9.0
Vanilla	2.0	2.0	2.0	2.0	2.0

F= Control formulae
F1= Containing 25% of the egg amounts and 5% of wheat flour was substituted by 5% LF
F2= Containing 50% of the egg amounts and 5% of wheat flour was substituted by 5% LF
F3= Containing 25% of the egg amounts and 2% of wheat flour was substituted by 2% PI
F4= Containing 50% of the egg amounts and 4% of wheat flour was substituted by 4% PI

Cake analysis:

Moisture, volume and hardness were determined according to Arozarena *et al.,* (2001). Cake samples were, also, sensory evaluated by 10 well trained panelists for various quality attributes such as overall acceptability, texture, mouthfeel, crust color, crumb color and aroma according to the method described by AACC (1983).

Statistical analysis:

All determinations were carried out in triplicates and their mean values and standard deviation were reported. Analyses of variance (ANOVA) were achieved to calculate the significant differences toward the different treatment means at 0.05 level.

Results and Discussion

Chemical compositions of sweet lupin flour (LF) and its protein isolate (PI) are shown in Table (2). Significant ($p < 0.05$) difference higher levels were found in ash, fiber, fat and carbohydrate contents of lupin flour compared with its protein isolate and concerning protein content the relation was vice versa. Such results were concurrent with Martinez-Villaluenga *et al*., (2009) who reported that lupin protein isolate (by alkaline water extraction/isoelectric precipitation) had 92.50 % protein. The obtained data of PI were, also, in agreed with that found by Sujak *et al.,* (2006) who reported that PI was lower in ash, fiber, fat and carbohydrate and due to the higher amounts of protein contents and their properties as well as the presence of lower amount of other components contents, PI can represent an effective source of valuable protein ingredients for a number of food formulations.

Functional properties of LF and PI:-

The oil (OAC) and water (WAC) absorption capacity, highly desirable characteristics in products, are of great importance from an industrial viewpoint, since it reflects the emulsifying capacity (Escamilla-Silva *et al.,* 2003).

Table 2: Chemical composition of sweet lupin flour (LF) and protein isolates (PI) (% as dry weight basis).

Materials	Protein	Ash	Fiber	Fat	Carbohydrate
Sweet lupine flour (LF)	39.37 ± 0.77	2.20 ± 0.15	3.92 ± 0.11	3.08 ± 0.10	51.43 ± 0.45
Sweet lupine protein isolates (PI)	92.20 ± 0.91	1.09 ± 0.52	1.10 ± 0.10	1.03 ± 0.05	4.59 ± 0.56
LSD at (0.05)	1.46	0.68	0.21	0.15	0.68

- Each value is mean of three replicates and followed by ± SD.

Functional properties (WAC, OAC, EC, ES, FC and FS) of lupin flour and protein isolate are shown in Table (3). Protein isolates (PI) showed a higher water absorption (WAC) and a higher level of oil absorption capacity (OAC) than lupine flour (2.85 ± 0.20 and 1.81± 0.20 versus 1.56± 0.61 and 1.42±0.30, respectively).

Doxastakis *et al.,* (2007) found that a higher OAC value was reported for proteins of lupin than that found in case of the lupin flour. Laqari *et al.,* (2002), also, reported a higher OAC for proteins of *lupinus angustifolius* than that of the lupin flour, probably this is due to the albumin and globulin amount fractions presence in both tested materials.

Table 3: Functional properties of sweet lupin flour (LF) and protein isolate (LPI)

Functional properties	Lupin Flour (LF)	Lupin protein isolates (PI)	LSD (0.05)
WAC	1.81 ± 0.20	2.845 ± 0.20	0.24
OAC	1.42 ± 0.30	1.56 ± 0.61	0.06
EC	80.00 ± 2.40	82.2 ± 1.82	1.23
EC	73.8 ± 1.32	74.9 ± 0.40	0.86
FC	121.60 ± 0.15	129.90 ± 0.15	1.46
Foaming stability % (FS)			
After	LF	PI	LSD (0.05)
Zero min	96.09 ± 0.10	97.30 ± 0.10	0.88
20 min	96.00 ± 0.11	97.00 ± 0.11	0.04
40 min	96.00 ± 0.12	96.50 ± 0.05	0.01
60 min	92.00 ± 0.11	95.00 ± 0.15	0.64
120 min	90.00 ± 0.10	94.00 ± 0.11	0.92

WAC is Water absorption (in ml/g) OAC is Oil absorption (in ml/g)
EC is Emulsion capacity (in %) EC is Emulsion stability (in %)
FC is Foaming capacity (in %) FS is Foaming stability (in %)
- Each value is mean of three replicates and followed by ± SD.

Emulsifying capacities are important properties for application of lupine protein isolates in different food systems.

Emulsion capacity and its stability of lupine flour and its isolates showed on significant difference, where they were 80.0 ± 2.4 and 82.2 ± 1.82 versus 73.8 ± 1.32 and 74.9 ± 0.40, respectively. It simply meaning that a slight significantly variation was found between the corresponding samples. Such results are in agreement with that found by Sathe *et al.,* (1982a and b). The lupin protein isolates showed a very high potential for the application as emulsifiers in different food products. On contrary, (Laqri *et al*., 2002) observed that emulsion capacity property of flour and protein isolates were non-significance different. Proteins showed a foam property when whipped because of their surface active properties.

However, emulsion capacity and stability of flour and protein isolates had no significant difference.

Foam capacity increment in the current study in the PI than in LF, as the concentration of protein content increased, was confirmed by Lawal *et al.,* (2005).

Protein isolates showed a higher FS value (94.0 %) after 120 min than that of the corresponding LF (90.0 %). From the pervious results, FC showed an increase value as the protein content presence increased which was in agreement with that reported by Lawal *et al*., (2005). The creation foam capacity could be due to that the proteins in dispersions lowering the surface tension at the water air interface (Surowka and Fik, 1994 and Gonzalez -Perez *et al.,* 2005).

Chemical compositions of the tested cakes:-

The chemical compositions of different lupin cakes formula are shown in Table (4). The protein content was significantly higher ($p \leq 0.05$) and the carbohydrate content was significantly decreased as a result of using LF or PI. The differences were more pronounced in case of PI than in case of LF. While there was a little or no change was occurred in fat. Ash and fiber contents had no significant difference ($p \leq 0.05$) in all lupin cake.

Table 4: Chemical compositions (% as dry weight basis) of cake manufacturing by using a partial substitution of eggs by lupin flour and protein isolated

Cake samples	Protein	Carbohydrate*	Fat	Ash	Fiber
F	12.11±0.45	65.47±1.08	18.92±1.22	1.50±0.12	2.00±0.17
F1	14.82±0.30	62.74±0.88	18.32±1.00	1.62±0.20	2.50±0.11
F2	16.00±0.40	60.84±1.16	18.52±1.15	1.80±0.22	2.84±0.33
F3	15.82±0.34	62.04±1.43	18.22±0.92	1.60±0.09	2.32±0.16
F4	16.92±0.20	60.88±0.46	18.00±0.84	1.70±0.17	2.50±0.25
LSD	1.20	1.62	0.32	0.72	1.00

* Calculated by difference F= Control formulae
F1= Containing 25% of the egg amounts and 5% of wheat flour was substituted by 5% LF
F2= Containing 50% of the egg amounts and 5% of wheat flour was substituted by 5% LF
F3= Containing 25% of the egg amounts and 2% of wheat flour was substituted by 2% PI
F4= Containing 50% of the egg amounts and 4% of wheat flour was substituted by 4% PI
- Each value is mean of three replicates and followed by ± SD.

Therefore, lupin has an attracted interest, worldwide, as a potential of high protein food ingredient suitable for human consumption (Johnson and Gray, 1993). The cakes produced with this vegetable protein, are very rich (>10 %) in the amino acid (arginine) and can be useful for children or athletes, who need this supplement, as well as for vegetarians, vegans and people with high cholesterol levels and for other obvious reasons (Petterson and Crosbie 1990).

Physical properties of the tested cakes:-

Volume, hardness, specific volume and moisture content of the tested cakes are presented in Table (5). A gradient decrease in cake hardness and moisture content was found when the egg amount in the cake formulas was decreased by 25 and 50 % and substituted by 5 and 10 % lupin flour (LF).

Table 5: Physical properties of cake manufacturing by using a partial substitution of eggs by lupin flour and protein isolated

Cake samples	Moisture %	Volume (cm^3)	Hardness (g)	Specific volume (cm^3/g)
F	23.00±1.12	175.75±14.33	713 ±32.16	1.401±0.18
F1	22.85±1.26	180.00±12.64	682±44.26	1.420±0.09
F2	22.72±2.11	185.40±9.88	669±42.14	1.458±0.11
F3	22.52±2.24	189.20±10.26	681±36.22	1.465±0.08
F4	22.11±1.88	192.30±11.42	670±32.62	1.493±0.10
LSD	0.63	2.82	2.86	0.04

F= Control formulae
F1= Containing 25% of the egg amounts and 5% of wheat flour was substituted by 5% LF
F2= Containing 50% of the egg amounts and 5% of wheat flour was substituted by 5% LF
F3= Containing 25% of the egg amounts and 2% of wheat flour was substituted by 2% PI
F4= Containing 50% of the egg amounts and 4% of wheat flour was substituted by 4% PI
- Each value is mean of three replicates and followed by ± SD.

Such slightly gradient decrement was insignificant in between the cake samples and in relative to the control cake in case of moisture content, but it was highly significant different in case of hardness. It could be, also, noticed that the decrement was more obvious in case of the PI than in the LF cake samples. On the other hand, an opposite trend was found in case of the volume and specific volume. Wherein, the cake samples volume and specific volume were gradient significantly increased in case of dimension of the egg amounts or increase the LF or PI amounts. The gradient significantly increments were more detectable in case of PI than in case of the corresponding LF cake samples. It could be, also, noticed that the volume and specific volume of both LF and PI samples were completely significantly differed than the control sample.

Arozarena *et al*., (2001) illustrated that the volume of cakes was directly related with leavening action and foam capacity of lupin. A higher volume implies a less compact cake structure, therefore a lower value for cake hardness. On the other hand, a more intense leavening process should cause an increase in water loss during baking resulting in a lower final moisture content.

Sensory evaluation of the tested cakes:-

The sensory evaluation testes were carried out to confirm and estimate the degree of enhancement in the physical properties of the tested cake as a result of egg substitution by LF or PI. Therefore, data presented in Table (6) show the sensory evaluation scores of the cake samples panel testing. The tested lupin cakes were submitted to a series of qualitative measures (including elements such as overall acceptability, texture, crust and crumb color, flavor and mouthfeel).

Table 6: Sensory evaluation of cake manufacturing by using a partial substitution of eggs by lupin flour and protein isolated

Cake samples	Overall-	Aroma	Crust color	Crumb color	Texture	Mouthfeel
F	9.76±0.84	9.70±0.72	9.80±0.79	9.70±0.65	9.80±0.74	9.80±1.04
F1	9.57±1.15	9.60±0.87	9.55±1.11	9.45±0.71	9.55±1.07	9.65±0.92
F2	9.47±0.66	9.65±1.02	9.45±0.86	9.35±0.81	9.35±0.78	9.55±0.59
F3	9.44±1.07	9.20±0.66	9.60±0.74	9.50±1.04	9.60±0.92	9.30±0.78
F4	9.20±1.15	9.00±0.90	9.00±1.11	8.90±0.68	9.00±1.12	9.20±0.82
LSD	0.77	0.92	0.95	0.84	0.76	0.82

F= Control formulae
F1= Containing 25% of the egg amounts and 5% of wheat flour was substituted by 5% LF
F2= Containing 50% of the egg amounts and 5% of wheat flour was substituted by 5% LF
F3= Containing 25% of the egg amounts and 2% of wheat flour was substituted by 2% PI
F4= Containing 50% of the egg amounts and 4% of wheat flour was substituted by 4% PI
- Each value is mean of three replicates and followed by ± SD.

Overall acceptability score for cake made using 5 and 10% of lupin flour was 9.57 and 9.47, respectively, compared with 9.76 for the control caked sample. The corresponding values in case of PI were closed to that of in LF case samples and were insignificant differed. The same pattern was found in all the tested attributes (aroma, crust and crumb color, texture and monthfeel. In general, it was found that all the tested attributes of both LF and PI cake samples were insignificant differed either in between or in relative to the control samples. These results are concurrent and confirmed by Schindler *et al*., (2011).

Consequently, due to its nutritional composition and satisfactory functional properties, lupin flour and protein isolate can be used in cakes manufacturing. The addition of up to 10 % lupine flour improves water-binding texture, shelf-life, aroma and nutritive values. It, also, appears that 5 % LF and 2 % PI could be used for the partial substitution and a successful alternative of egg us a preferred process in egg free cakes.

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