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EFFECT OF FORTIFICATION PAN BREAD WITH LUPINE FLOUR ON THE CHEMICAL, RHEOLOGICAL AND NUTRITIONAL PROPERTIES

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ABSTRACT

The aim of this research was to study the effect of fortified pan bread with Lupine flour (5, 10 and 15 %) on the chemical, rheological and nutritional properties. Lupine flour showed higher levels of crude protein, ash, crude fat and fiber than the wheat flour. Conversely, wheat flour showed higher contents in carbohydrates. Rheological studies of dough showed clear differences between wheat flour, Lupine flour, with increasing addition of Lupine flour. Farinograph show that increased water required for the optimum bread making. Water absorption, dough development time and stability time increased as fortification with Lupine flour increased. Extensographs showed that, extensibility of dough decreased as the fortification level increased from 5 to 15 %. The essential amino acids in bread fortified with Lupine flour were higher than that of control. Also, pan bread fortified with Lupine flour had higher chemical score (CS) values, Protein efficiency ratio PER and essential amino acid index (EAAI) than control. Biological value (BV) and in vitro protein digestibly (IVPD) increase with increased addition Lupine of flour. Sensory evaluation indicate that substitution with 5 and 10 % Lupine flour gives parameter values at least as good as the control sample and produces acceptable bread.

INTRODUCTION

Searching for new and valuable sources of protein to nutritionally supplement traditional food has led to an increasing interest in the use of legume seeds (Martinez-Villaluenge *et al.,* 2009). Many nutritional studies on using Lupine seeds (*lupinus albus*) in animal and human nutrition reported that Lupine can replace soy bean successfully. Lupine is widely used in food production particularly as a valuable and technologically desirable additive mainly in bakery products as well as in dietary and function food products (Loza and Lampart Szczapa, 2008). Lupine seed is mentioned in the ancient and traditional pharmacopoeia books as an ant diabetic product (Bertoglio *et al.,* 2011) they demonstrated that the active protein responsible for the claimed anti-diabetic effect of the Lupine seed is effective in man, in addition to animal models. Also another authors report on the glucose lowering effect of Lupine - gamma-conglutin in human subjects (Capraro *et al*., 2011). Fontanari *et al.*, (2012) demonstrated that protein isolate from Lupine has a metabolic effect on endogenous cholesterol metabolism and a protector effect on development of hepatic statuses. The presence of phenolic acids and flavonoids in Lupine seeds as antioxidant activity has been reported by (Siger *et al.,* 2012).Lupine does not contain gluten, thus it is sometimes used as a functional ingredient in gluten-free foods (Scarafoni *et al.,* 2009). Lupine kernel fiber has also a potential as a human food ingredient and it has been used in production of fiber-enriched baked goods and pasta (Smith *et al*., 2006).

The purpose of this investigation was to study the effect of fortification pan bread with Lupine flour in ratios of 5, 10 and 15 % on the dough rheological and the quality of protein of pan bread.

MATERIALS AND METHODS

Materials

Raw material

Wheat flour (72%) and sweet Lupine seeds were obtained from a local market. the lupine was milled using a hummer mill 1400 perten) and passed through a 10 mm sieve

Methods:-

Chemical analysis:

Chemical analysis of raw material and samples were analyzed in triplicate by the methods described in (AOAC, 2000) for moisture, cured protein, ether extract, crude fiber, and ash contents. Carbohydrate was calculated by difference.

Baking methods:

Wheat flour was fortified with 5, 10 or 15 % Lupine flour and pan bread was baked according to the AACC, (2000) method.

Rheological properties:

Farinograph and Extinograph were carried out according to the method of AACC, (2000).

Amino acids:

Amino acid content was determined as described by Moore *et al.,* (1958). Amino acids were determined using an AAA 400 automatic amino acid analyzer (INGOS, Czech Republic).Prior to analysis; samples were subjected to acid hydrolysis in the presence of 6 M HCl at 105 °C for 24 hours. Sulphur-containing amino acids were determined separately in 6 M HCl after oxidative hydrolysis (formic acid + hydrogen peroxide, 9:1 v/v, 20 h at 4 °C)

Estimation of nutritional values of lupine protein flour:

The quality of protein was estimated by determination of total amino acids (AA), as well as the fraction of the exogenous amino acids (EAA). The nitrogen content in human food and fodder varies between 16 and 18 g/100 g of protein isolate (16 g/100 g for Leguminous plants; FAO/WHO/UNU, (1985); FAO/WHO, (1991). The chemical score (CS) was calculated on the basis of the procedure described previously by Rakowska, *et al*., (1978). The exogenous amino acids (EAA) were estimated according to Oser, (1959) in terms of geometric mean of all the concentrations of participating exogenous amino acids compared to a concentration of corresponding standard (in g/16 gN):

In the classical method of Oser, (1951, 1959), concentrations of Lys, sum of Met + Cys, Thr, Ile, Trp, Val, Leu, His and Phe + Tyr were considered,

whereas the standard for mature human (MH) excludes histidine. The essential amino acid index (EAAI) was calculated as follows:
$EAAI = 10^{logEAA}$

Protein efficiency ratio (PER) was expressed traditionally as the ratio of the weight gain to the amount of the protein consumed in rat. According to Alsmeyer *et al.,* (1974), this method cannot be applied to humans, mainly because it measures organism growth but not maintenance. These authors proposed an equation predicting protein usability which is expressed in terms of concentrations of only two amino acids – leucine and tyrosine, based on experiments on their availability/digestibility:

$$\mathrm{PER} = -0.468 + 0.454\mathrm{Leu} - 0.105\mathrm{Tyr},$$

Where Leu and Tyr are concentrations of these amino acids expressed in g/16 gN.

Sensory Evaluation:

Sensory evaluation of the baked loaves quality characteristics was carried out following cooling to room temperature for 2 h. Sensory evaluation was performed by ten panelists in Institute of Food Technology research Center. Loaves were randomly assigned to each panelist. The panelists were asked to evaluate each loaf for appearance, crumb texture, crumb grain, crust color, taste, odor and overall acceptability. A 10 point scale was used where 10 "excellent and 1 "extremely unsatisfactory (Matz, 1972).

Biological Value:

Biological value was calculated using the equation suggested Mitchell and Block, (1946) as follow:-
B.V=49.9+10.53 PER

In Vitro protein digestibility:

In vitro protein digestibility was determined by the method described by Santosh and Chanhan, (1986).

Statistical analysis:

Analysis of variance (ANOVA) was carried out using SAS program (Statistical Analysis Sys Ion. 9.1) SAS Institute Inc. (SAS, 2004). Bread characteristics of wheat dough with or without Lupine flour or bread were analyzed using ANOVA effect was found significant, indicated by a significant ($p < 0.05$), differences between the respective means were determined using least significant difference (LSD) and considered significant when $p < 0.05$. Mean ± standard deviation of mean was used.

RESULTS AND DISCUSSION

The data in Table (1) shows the chemical composition of the Lupine flour (LF) and wheat flour 72 % extraction. The results of Lupine flour show that Protein, ash, fiber and fat contents higher than in wheat flour 72 % extraction. From these results it could be noticed that protein of (LF) is higher significant difference at $p \leq 0.05$ than (WF), this result agree with (Sujak *et al.,* 2006 and Erbas *et al.,* 2005).

Table (1): Chemical composition of the Lupine flour (LF) and wheat flour 72 % extraction

Samples	Protein %	Ash %	Fiber %	Fat %	Carbohydrate %
Sweet lupine flour (Lf)	39.37[a]± 0.77	2.20[a]±0.15	3.92[a]±0.11	3.08[a]±0.10	51.43[b]±0.45
Wheat flour 72% (WF)	10.05[b]±0.25	0.6[b]±0.06	0.45[b]±0.14	10.0[b]±1.0	78.9[a]±0.56
LSD at 0.05 %	1.46	0.68	0.21	0.15	2.68

Farinograph and Extensograph parameters:

The results of the farinogram and extensogram studied are shown in table (2). The amount of water (absorption) required to reach the farinogram curve on the 5oo B.U (Brabender units) line increased steadily with every increment of (LF), the presence of Lupine flour increased the water required for the optimum bread making absorption.

Table (2): Farinograph and Extinsograph data of dough made from wheat flour (WF) and Lupine flour (LF).

Samples	Farinograph					Extinsograph			
	Water absorption (%)	Arrival time (min)	Development time (min)	Dough stability (min)	Dough softening (B.U)	Elasticity (B.U)	Extensibility (mm)	P.N	Energy (Cm)
WF	61.2	0.5	1.0	2.0	40.0	215.0	115.0	1.9	18.0
LF with 5 %	65.1	1.5	1.5	3.0	20.0	255.0	105.0	2.4	23.0
LF with 10 %	69.8	2.0	2.0	6.0	-	345.0	100.0	3.5	44.0
LF with 15 %	72.9	4.0	4.0	3.5	-	380.0	80.0	4.8	38.0

The addition of Lupine flour to wheat flour brought about some significant changes in its dough mixing behavior as measured by the farinograph. Farinograph data of wheat flour (control) and those of the fortified with Lupine flour, at a 5 %, 10 % and 15 % level, are shown in Table (2). fortification of wheat flour with Lupine flour increased the water required for optimum bread making absorption (from 61.2 % for wheat flour to 65.1 %, 69.8 % and 72.9 % when 5 %, 10 %,15 % Lupine flour was added respectively). An increase in water absorption, following incorporation of various vegetable protein concentrates or isolates to wheat flour, has also been reported by other researchers who attributed the water absorbing capacity of these protein preparations to their ability to compete for water with other constituents in the dough system. According to these authors the ability of these proteins to absorb high quantities of water results in dough which exhibit increased farinograph water absorption values (Doxastakis *et al.,* 2002). The quantity of added water is considered to be very important for the distribution of the dough materials, their hydration and the gluten protein network development. These results confirmed by Sudha *et al.,* (2011) who studied the effects of wheat bran and oat bran as sources rich in insoluble dietary fiber and soluble dietary fiber in the formulation of instant vermicelli and study its influence on the rheological characteristics and product quality.

The incorporation of wheat bran and oat bran from (0 to 20 %) in the blends increased the water absorption significantly from 58.3 to 64.1 %. Rosell *et al.,* (2001) reported that the differences in water absorption is mainly caused by the greater number of hydroxyl group that exist in the fiber structure and allow more water interaction through hydrogen bonding. It could be noticed that water absorption increased with increasing amount of Lupine fiber. The observed effect agrees with the increased water absorption found by Sosulski and Wu, (1988) when they added field pea hulls, wheat, corn and wild oat barns to the bread dough. The time required for the control dough to reach 500 BU consistencies was also modified by Lupine flour addition. During this phase of mixing, the water hydrates the flour components and the dough is developed.

Dough development time (DDT) was higher for all wheat-Lupine flour blends than control (1 min), also between Lupine samples was observed at different concentration (Table 2). The increase in dough development time resulting from Lupine flour or fiber addition could have been due to the differences in the physicochemical properties between the constituents of the Lupine and those of the wheat flour, as has been previously reported by Paraskevopoulou *et al.,* (2010) who studied the incorporation of Lupine protein in wheat flour. The time required for the dough development or time necessary to reach 500 BU of dough consistency was modified in a different by each cereal bran. Highest development time values were obtained in dough's with Lupine fiber (5, 10 and 15 %). Similar results were expressed by

Daglioglu and Gundogdu, (1999) who studied with stabilized rice bran in bread making.

Regarding dough stability, it appears that the dough sample containing 5 % Lupine exhibited higher stability and resistance to mechanical mixing values than the control, while it decreased as the substitute level increases from 10 % to 15 %. In general, the stability value is an index of the dough strength, with higher values indicating stronger dough. The increase in the stability time was related to the amount of substitution. Thus, stability times of 6.0 and 3.5 min are observed for the dough fortified with 10 and 15 % Lupine, respectively.

Dough softening degree increased significantly with increasing amount of Lupine flour in blends. Similar dominant viscoelastic behavior in dough characteristics on blending with cowpea flour and chickpea flour were observed by Sharma *et al.,* (1999). The changes in dough characteristics upon addition of Lupine flour may be attributed to dilution of gluten-forming proteins causing weakening of dough’s. Variation in hydration behavior of two proteins may be another reason for differences in dough characteristics.

Table (2) also showed that extensibility required breaking the strength of dough decreased as the substituted level increased from 5 to 15 %. The ratio P/N (proportional number) increased as the proportion of wheat substitution (LF). The lower values of the above parameters could be attributed to the dilution of the wheat gluten structure by the added protein (Dervas *et al.,* 1999).

This conclusion is consistent with the results of studies by Roccia *et al.,* (2009) who found that the substitution of wheat protein by soy protein decreased mixture elasticity, indicating dough network weakening. One other reason for the weakening of dough strength resulting from vegetable protein addition could stem from the fact that the substitution of gluten proteins by the non-gluten-forming vegetable proteins causes a dilution effect and consequently weakens the dough. This confirms the data from literature that the both protein fractions (gliadin and glutenin) must be present for optimal gluten network development in a specific ratio. Trend to viscoelastic behavior is given.

Chemical composition of the pan bread fortified with lupine flour:

Chemical composition of the produced bread is presented in Table (3). It’s observed that increasing the (LF) content will increasing crude protein, ash, fat and fiber conteants in bread. This means that as (LF) increase, the nutritional quality of bread improve. Crude protein has show higher statistical difference ($p \leq 0.05$). The samples show 12.46, 14.33, 15.43 and 17.00 for control, 5 %, 10 % and 15 % respectively. These results show the positive relation between percent of wheat flour fortified with lupine flour. Lupine flours can be an excellent choice for improving the nutritional value of bread (Dervas *et al,.* 1999).

Table (3): Chemical composition of bread fortified with Lupine flour on dry weight

Samples	Protein%	Ash%	Fiber%	Fat%	Carbohydrate%
Control bread	12.46	2.60	0.75	5.36	78.83
Bread with 5 %	14.33	3.00	1.06	5.43	76.18
Bread with10 %	15.43	3.23	1.90	5.56	73.88
Bread with15 %	17.00	4.00	1.95	5.66	71.39
LSD	0.66	0.25	0.30	0.12	0.70

Amino acid contents:

The results in Table (4) shows the amino acid composition of the Lupin flour and bread fortified with Lupine flour (LF). The produced bread fortified with 15 % (LF) have higher amount of total amino acid (AA) than did control bread and other produced bread. The essential amino acids content (EAA) was calculated, on the basis of nature human (NH) and whole egg standards (WE) examined. On the other hand, EAA were higher in the fortified bread than control bread. Nutritional values of Lupin flour and bread were estimated the chemical protein scores (CS) were calculated from the comparison of concentrations of less abound. 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 cysteín. (Gulewicz *et al.,* 2008) and threonine Pisariková *et al.,* (2008). Fortified with Lupine flower showed higher CS values, PER and essential amino acid index (EAAI) than control bread. It was also found have a better and nutritionally more beneficial amino acid composition than had the control bread Favier *et al.,* (1995).

Table (4): Amino acid composition of Lupine flour and bread fortified with (5%, 10% and 15%) Lupine flour (g/100g protein)

Amino acid	FAO*	Lupine flour	Bread supplemented with lupine flour			
			Control (o)	5 %	10 %	15 %
Essential Amino acid						
Lysin	7.0	6.43	6.72	8.74	9.20	9.32
Leucine	8.6	8.62	3.81	4.51	4.99	5.66
Phenyl alanin	9.3	2.4	4.82	3.88	4.00	4.32
Threonine	4.7	3.31	2.68	2.35	2.42	2.48
Iso leucine	5.4	3.36	3.60	2.60	2.65	2.77
Valine	6.6	3.34	3.90	3.40	3.54	3.68
Methionine	3.5	2.19	1.60	1.59	1.55	1.50
Histidine	2.2	3.00	1.91	1.77	1.68	1.51
Tyrosin	-	3.53	1.85	2.61	2.94	3.33
Total Essenial A.A.		36.18	30.89	31.45	32.97	34.57
Non-Essential Amino acid						
Glutamic		16.94	32.38	29.54	27.42	27.05
Aspartic		14.35	4.59	7.50	10.08	10.34
Proline		4.78	10.26	9.02	8.11	7.20
Arginine		9.14	3.52	3.50	3.72	4.76
Glycine		4.85	3.52	3.24	3.10	3.11
Alanine		3.30	2.90	2.92	2.76	3.35
Serine		6.37	4.00	4.00	3.99	4.81
Total non Essential A.A.		59.73	61.17	59.72	59.18	60.62
Total A.A.		95.91	92.06	93.44	94.04	95.19
Chemical score		76.49	65.31	66.94	69.70	73.08
EAAI (Essential A.A.)		62.68	54.41	59.52	60.27	64.38
PER		3.08	1.07	1.30	1.49	1.75

***FAO/WHO/UNU (1985) and FAO/WHO (1991)**

Biological value (BV) and In vitro protein digestibility (IVPD):

Result in Table (5) showed increase in (BV) and (IVPD) with increased Lupine flour in bread, this due to the highest protein content of lupine. This result agrees *with* (Dervas *et al.,* 1999). (Mubarak, 2001) reported the addition Lupine flour improved in-vitro protein digestibility.

Table (5): In vitro protein digestibility (IVPD) and biological value (PV) Lupine flour and bread fortified with (5%, 10% and 15%) Lupine flour

Samples	In vitro protein digestibility %	biological value %
Lupine flour	60.12	82.33
Bread (Control)	55.40	61.17
Bread with 5 % (LF)	63.20	63.59
Bread with 10 % (LF)	66.53	65.59
Bread with 15 % (LF)	68.06	68.33

Sensory evaluation:

Sensory evaluation data presented in Table (6). The results indicate that various forms of Lupine can be used satisfactorily as a food ingredient in a wide range of foods but that many recipes need modification because of the unique properties of Lupine. Lupine in various forms was judged to be acceptable to consumers as a base for bread and other bakery product. Typical loaves are obtained with or without substitution of LF. Loaf volumes of pan breads fortified with 10 % and 15 % (LF) decreased. Crust, color, crumb color became more yellow and texture showed evidence of thickened cells. Although, crumb structures were not drastically impaired upon fortification of wheat flour by 10 or 15 % of the title compounds. Most people who have tried Lupine-wheat flour mixes have found the texture, taste and frequently the color to be appealing (Kyle, 1994).

Table (6): Sensory evaluation of pan bread fortified with Lupin flour.

Samples	Volume	Appearance	Crumb texture	Crumb grain	Crust color	Taste	Odor	Overall acceptability
Control	9.6 ± 0.69	9.6 ± 0.63	9.3 ± 0.64	9.3 ± 0.82	9.6 ± 0.65	8.73 ± 1.04	8.9 ± 0.98	9.23 ± 0.65
Bread with 5 % LF	9.5 ± 0.71	9.6 ± 0.9	9.5 ± 0.71	9.3 ± 0.76	9.6 ± 0.59	8.8 ± 1.15	9.0 ± 1.07	9.3 ± 0.9
Bread with 10 % LF	9.3 ± 0.70	9.0 ± 0.83	8.8 ± 0.7	9.0 ± 0.87	9.2 ± 0.81	8.5 ± 1.42	8.8 ± 1.11	8.9 ± 0.78
Bread with 15 % LF	9.00 ± 0.71	8.6 ± 0.81	8.3 ± 0.71	8.5 ± 0.87	8.2 ± 0.56	8.3 ± 0.94	8.8 ± 0.86	8.45 ± 0.83
LSD at 0.05 %	0.50	0.90	0.99	1.0	1.1	0.55	0.11	0.15

It appears, therefore, that the decrease in bread volume resulting from Lupine flour or fiber addition is most likely due to the combined effects of gluten dilution and mechanical disruption of the gluten network structure by the Lupine particles. In addition, examination of the loaf internal structure revealed that the crumb of the Lupine flour or fiber containing bread contained a small number of gas cells compared to the control fortification with (LF) 5 %, 10 % and 15 % loaf to a acceptable of bread. The acceptability of these pan products was high (Petterson and Crosbie, 1989). Lupine flour can be used at up to 10 % inclusion in breads without affecting baking quality. Most reduced bread making potential degree of reduction depends on the people who have bread from Lupine wheat flour mixes have found texture, taste and color to be appealing.

Conclusion

Pan bread fortified with Lupine was found to be nutritional most valuable bread as it had high amino acid. The white Lupine was found to be suitable for human nutrition and also the production of protein supplements and high-protein concentrates for further food processing flour gives parameter values at least as good as the control sample produces acceptable bread .

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تـأثير تـدعيم خبـز القوالـب بـدقيق التـرمس علـى الصـفات الكيميائيـة والريولوجيـه والتغذويه

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اجريت هذة الدراسة بغرض تأثير التدعيم بالترمس بنسب ٥ ، ١٠ ، ١٥ % على الصفات الكيمائية والتغذوية والصفات الريولوجية لخبز القوالب أشارت النتائج أن البروتين والرماد والألياف الخام نسبتة أعلى فى دقيق الترمس عن دقيق القمح وعلى العكس دقيق القمح أعلى فى الكربوهيدرات . أظهرت النتائج أن للصفات الريولوجية فروق واضحة لدقيق الترمس مقارنة بدقيق القمح .أوضحت نتائج الفارينوجراف زيادة فى كمية امتصاص الماء ووقت تطور العجينة والثبات بزيادة نسبة اضافة دقيق الترمس للحصول على خبز جيد الصناعة .وأكدت نتائج الأكستنسوجراف أن المطاطية تقل كلما زادت اضافة نسبة دقيق الترمس من ٥-١٥%. تشير النتائج أن دقيق الترمس والخبز المدعم عالى فى نسبة الأحماض الأمينية اللازمة بينما تقل كلما قلت نسبة اضافه دقيق الترمس عن ١٥ % دلت النتائج على ان دقيق الترمس عالى فى الاحماض الامينية الاساسية وكذلك الخبز المدعم بنسبة ٥ ، ١٠ ، ١٥ % مقارنة بخبز الكنترول (الغير مدعم). اظهر الخبز المدعم انه عالى فى قيمة chemical score ونسبة البروتين اللازمة PER ودليل الاحماض الامينية الاساسية (EAAI) مقارنة بالخبز الكنترول. شوهدت زيادة فى هضم البروتين (IVPD) والبروتين الحيوى (BV) كلما زادت نسبة التدعيم بدقيق الترمس ودلت نتائج التقيم الحسى عن أن نسبة الاضافة ٥ ، ١٠ % لدقيق الترمس يعطى قيم جيدة وخبز مقبول كخبز الكنترول. ونستطيع ان نستنتج ان خبز القوالب المدعم بدقيق الترمس بنسب حتى ١٠ % يحسن جودة البروتين.

بتحكيم البحث

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