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Germinated lupin (*Lupinus albus*) flour improves Arabic flat bread properties

D.Z. Al Omari[1], S.S. Abdul-Hussain[2] and R.Y. Ajo[3*]

[1]Nutrition and Food Technology Department, College of Agriculture, Jordan University of Science and Technology (JUST), P.O. Box 3030, 22110 Irbid, Jordan; [2]Food Sciences and Biotechnology Department, College of Agriculture, University of Baghdad, Baghdad, Iraq; [3]Al-Huson University College, Al-Balqa Applied University, P.O. Box 50, 21510 Al-Huson, Jordan; radwan_ajo@bau.edu.jo

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RESEARCH ARTICLE

Abstract

Arabic flat bread (AFB) is one type of bread that is consumed in most of the Middle East countries. It is usually prepared from wheat (*Triticum aestivum*) flour. However, it is low in its nutritive value. Whereas lupin (*Lupinus albus*) is known as a potential source in increasing food nutritional value, which could be increased by germination. Thus, this study was conducted to substitute 5, 10, 15, or 20% of wheat flour (WF) in AFB making with different levels of germinated lupin flour (GLF), and to determine the effect of these substitutions on different properties of the resulted germinated lupin bread (GLB), considering lupin flour free bread as control bread (CB). GLB and CB were analysed chemically, physically and organoleptically. Chemical analysis showed that the rate of protein, lipid, ash and fibre contents were significantly increased in GLF compared to WF. Conversely, chemical components were significantly increased in their corresponding GLB samples. However, the organoleptic evaluation indicated that there were no significant differences between CB and the different level samples of GLB in all bread characteristics except in loaf face colour. While the specific volume of all treated AFB decreased as the level of GLF incorporation increased. All GLB samples exhibited a significant increase in bread yellowness and redness; whereas lightness was decreased particularly at a high amount of incorporation. Softness of bread crumb was adversely affected with storage time in AFB; that means that CB was significantly softer than GLB. It is concluded that nutritive values of AFB could be improved by using GLF, since the chemical components were increased in bread without affecting their organoleptic properties. Also, this study could be encouraging to develop new healthy food products that are useful for feeding programmes and dieting.

Keywords: Arabic flat bread, germination, lupin, organoleptic, wheat flour

1. Introduction

Arabic flat bread (AFB) or pita bread is one of popular type of flat bread prepared mainly from wheat flour (WF) (Amr, 1988; Al-Khafagi *et al.*, 1994; Alomari and Abdul-hussain 2013; Quail, 1996). The nutritional quality of wheat proteins is lower than legume proteins because it has low levels of lysine, methionine, and threonine (Kulp, 1988). On the other hand, demand for wheat-based bakery products especially in developing countries is increasing, where the major grain is wheat (Quail, 1996). These baked products could be improved nutritionally by supplementation with non-wheat proteins such as those from legumes, including lupins (Abu-Ghoush, *et al.*, 2008; Quail, 1996).

Lupin (*Lupinus albus*) contain high amount of proteins, fibres, bioactive compounds (isoflavons and phytosterols), with little starch and fat contents (Obeidat *et al.*, 2013; Rumiyati *et al.*, 2013). It is known to be used as complementary protein to supply certain amino acids such as lysine and tryptophan which have a potential for influencing the nutritional profile of foods, in which it will affect its physical and chemical characteristics (Barneveld, 1999; Erbas *et al.* 2005; Lampart-Szczapa *et al.* 2003; Mohamed and Rayas-Duarte, 1995).

Several studies have mentioned that lupin flours are considered to be beneficial in the control of various diseases, such as diabetes, because of the high content of indigestible fibres and it can reduce the risk of cardiovascular disease because of their effect as hypoglycemic and hypolipidemic agents (Duranti, 2006; Rumiyati *et al.*, 2012; Wolever, 1990).

However, the potential benefits of lupin can be limited by the presences of alkaloids, which could produce a bitter taste and yellow colour when incorporated in foods. It was observed that seed germination reduces antinutrational compounds such as phytate, trypsin inhibitors and alkaloid levels through physiological changes (Mohamed and Rayas-Duarte, 1995); in addition to its role in increasing the concentration and activity of phenolic and phytosterol compounds (Rumiyati *et al.*, 2013).

Recent studies indicate that adding ungerminated and germinated lupin flour (GLF) at a level of 30-50% of wheat flour to a cookie recipe produced acceptable products, however, in general 30% is the most desirable level for flavour and texture (Obeidat *et al.*, 2013). Lupin flour was also incorporated into flat bread (Alomari and Abdul-Hussain 2013; Ballester *et al.*, 1988; Lampart-Szczapa *et al.*2003). Despite the good results obtained from these investigations, limited studies were focused on AFB. The current work was conducted to study the effect of substituting WF with different levels of GLF in producing AFB and to determine their nutritive values, i.e. chemical, physical, organoleptic characteristics. Also, to find how GLF incorporation in AFB could affect the bread crumb softness and colour.

2. Materials and methods

Lupin seeds germination and milling

Sweet lupin seeds were purchased from the local market of Amman, Jordan. Lupin seeds were cleaned by discarding small, broken and damaged seeds. Then the seeds were washed three times with distilled water (DW) and disinfected with 0.3% sodium hypochlorite solution to limit microbial growth, then again rinsed two times with DW to get a neutral pH. The lupin seeds were then soaked in DW for 20 h. The soaked lupin seeds were dried in a ventilated oven at 50 °C for 2 h and then germinated by spreading them on cotton that was frequently moistened with DW, at 25 °C for 72 h until the sprouts were visible. Next, the dry seeds were discarded and sprouted seeds were dried at 50 °C for 10 days in a ventilated oven (drying oven, model 1212, serial no. 13267; Vindon Scientific Ltd., Rochdale, UK). The dried sprouted lupin seeds were ground in an electric mill (model 1021; Braun, Kronberg, Germany) to pass through a 60 mesh sieve (British standard screen). The milled flour samples (GLF) were placed in 50 kg air-tight polyethylene bags, sealed and stored in a refrigerator (4 °C) until required (Obeidat *et al.*, 2013).

Bread making

Straight grade (Muwahad) wheat flour was obtained from Modern Flour Mills and Macaroni Factories (Amman, Jordan) with 77 to 80% extraction rate according to method No. 293 of JISM (2005). In this study, GLF was incorporated in AFB at five substitution levels: 0 (100% WF), 5, 10, 15 and 20%. The formula used in the preparation of experimental breads was adopted from Maleki and Daghir (1967) including 1000 g WF (14% moisture basis), 3 g dried yeast, 2 g salt, and water as determined by farinograph. For all levels, the ingredients were mixed with the determined amount of water until a cohesive dough mass was obtained. The resulting bulk dough was fermented for 30 min then divided into balls of 100 g each. The dough pieces were dusted with flour of the same formulation (~10 g) and rounded into ball shapes. The balls were covered with a wet cloth and fermented for 10 min (intermediate proofing) and then flattened into elliptical sheets and baked in automated oven (model CD61; Rational, Landsberg am Lech, Germany) at 470 °C to optimum crust colour which was determined by a professional baker. After baking, bread samples were cooled for 15 min, placed in polyethylene bags, sealed until evaluation.

Evaluation of Arabic flat bread quality

Arabic flat bread specific volume

Specific volume of the bread (cm^3/g) was defined as the quotient between loaf volume and loaf weight. Breads were weighed (g) and then their loaf volumes (ml) were determined by sesame seed displacement. Specific volume was calculated (ml/g) (Amr, 1988).

Arabic flat bread colour measurement

Crust colour was measured with a Colour Tech-PCM (Cole-Parmer International, Vernon Hills, IL, USA). This defines colour numerically in terms of lightness (L^*), redness (a^*), and yellowness (b^*) values. The colour of bread crust was measured using the Hunter colour method. Colour values of each AFB sample were determined at three different points (Jooyandeh, 2009).

Arabic flat bread crumb softness value

The staling rate of AFB was determined by measuring the compressibility with a penetrometer (PNR-10 Penetrometer; Petrotest Instruments Gmbh & Co. KG, Dahlewitz, Germany) (Maleki *et al.*, 1980). Softness of AFB levels was measured when they were: (1) fresh; (2) after 24 h; and (3) 48 h of storage in sealed polyethylene bags at room temperature (20 °C). For crumb softness, two slices of 23 mm were taken from the centre of the bread and each sample was compressed in five spots by a weigh of 54.6 g for 5 s. The compression spots were marked by holes on the four corners and centre of a 6 × 6 cm cardboard template placed on the cut surface of each sample. Data for five points from each AFB were averaged to give the compressibility, measured with a penetrometer unit (1 penetration unit = 0.1 mm) according to Maleki *et al.* (1980).

Chemical analysis of flour and Arabic flat bread

The chemical composition of all samples was determined according to methods of AOAC (1997) as following: moisture (No. 925.10), proteins (No. 920.152), lipids (No. 922.06), ash (No. 923.03) and fibre (No. 985.29). The carbohydrate content was calculated by deducting the total moisture, ash, protein and lipid contents from 100.

Sensory evaluation

All bread samples were evaluated within 1 h of baking by twelve randomly selected panellists (graduate students and staff members of the nutrition science and food technology department at JUST, Irbid, Jordan) who evaluated the loaves randomly based on standard criteria. They were asked to evaluate each bread sample upon on its loaf surface colour, homogeneity of colour, taste and flavour, odour, chewiness, tearing quality and overall acceptability. A 9 point scale was used where 1 = extremely dislike and 9 = extremely like. The evaluation was carried out at room temperature (around 20 °C), and distilled water was provided for mouth washing. A bread scoring sheet for quality characteristics was adapted from Quail (1996).

Statistical analysis

The data were analysed by using SPSS (version 15.0, 2007; SPSS, Chicago, IL, USA). One-way analysis of variance (ANOVA) test was performed to test differences between blends followed by mean separation using Duncan's analysis test. Findings with a P-value ≤ 0.05 were considered to be statistically significant. The experiments were conducted under laboratory conditions with three replicates for each level. Bread quality test was measured by calculated the overall bread quality scores together then analysed using SPSS.

3. Results and discussion

Chemical analysis of flours and bread

The chemical compositions of WF and GLF are shown in Table 1. Moisture and carbohydrate contents of GLF were significantly (P≤0.05) lower than WF content, which could be attributed to the GLF composition which contains oligosaccharides and polysaccharides that are characterised with high water holding capacity (Hallén *et al.* 2004; Köksel *et al.*, 2000). However, the ash, fibre, protein and lipid contents were significantly (P≤0.05) higher in GLF than that of WF in a ratio of 9.23, 3.9, 3.35, and 7.30%, respectively. This finding could be attributed partly to the germinated lupin seeds used in this study, which were not exposed to hulling or decortications before milling and also to the effect of germination on increasing the nutrients value (Asp *et al.* 1996; Hallén *et al.* 2004). These results indicated that, the GLF is more nutritionally valuable than WF and its worthwhile to start using GLF in production of bread.

AFB chemical analysis results, revealed that as GLF was increased, moisture, ash, fibre, protein and lipid contents in AFB were increased significantly (P≤0.05) to the ratio of 1.3, 5.8, 7.96, 1.46 and 2.6%, respectively, in comparison to CB as shown in Table 2. However, carbohydrates in all levels of AFB were significantly (P≤0.05) decreased compared to CB in a ratio of 1.38% when WF with 20% GLF was used. Some studies showed that the nutritional value was increased by including germinated cowpea flour, chickpea and broad bean flour to WF in bread and cookie production which agreed with the study results (Abdul-Hussain *et al.* 2009; Hallén *et al.*, 2004; Rababah *et al.*, 2006). Moreover, it was found that lupin has a role in reducting the reduction of glycemic index, hypocholesterolemic action, protective effects against colourectal cancer, reduction of constipation problems, increasing satiety in addition increasing vitamins and minerals absorption (Asp *et al.*, 1996). Thus the study results demonstrated that using GLF to produce GLB is promising since it is considered to be a high nutritionally and healthy bread that could be used in special feeding programmes targeting young children in developing countries and school children who need more protein per unit of body weight (Manley, 2000).

Table 1. Chemical composition (%) of wheat flour and germinated lupin flour.[1,2]

Flour type[3]	Moisture (%)	Ash (%)	Fibre (%)	Protein (%)	Lipid (%)	Carbohydrate (%)
WF	12.00±0.04[a]	0.46±0.01[b]	1.53±0.03[b]	9.90±0.02[b]	1.13±0.03[b]	76.51[a]
GLF	4.25±0.09[b]	4.25±0.05[a]	6.04±0.02[a]	33.18±0.02[a]	8.26±0.02[a]	50.06[b]

[1] Values are means of replicates ± standard deviation (n=3).

[2] Means with different letters in the same column are significantly differed at P≤0.05.

[3] WF = wheat flour; GLF= germinated lupin flour.

Table 2. Analysis of flat Arabic bread with different substitution levels of germinated lupin flour.[1,2]

Flour type[3]	Moisture (%)	Ash (%)	Fibre (%)	Protein (%)	Lipid (%)	Carbohydrate (%)
CB	19.97±0.09[e]	0.76±0.03[e]	0.33±0.06[e]	10.76±0.05[e]	0.95±0.08[e]	67.23[a]
GLB5	21.60±0.05[d]	2.88±0.06[d]	0.58±0.01[d]	11.03±0.08[d]	1.44±0.05[d]	62.47[b]
GLB10	22.20±0.04[c]	4.04±0.07[c]	1.50±0.05[c]	13.48±0.06[c]	1.85±0.07[c]	56.93[c]
GLB15	24.1±0.07[b]	4.26±0.07[b]	1.54±0.04[b]	14.58±0.01[b]	2.08±0.05[b]	53.44[d]
GLB20	26.16±0.03[a]	4.42±0.01[a]	2.63±0.02[a]	15.80±0.09[a]	2.5±0.08[a]	48.49[e]

[1] Values are means of replicates ± standard deviation (n=3).
[2] Means with different letters in the same column are significantly differed at $P \leq 0.05$.
[3] CB = control bread; GLB = germinated lupin bread (5, 10, 15, and 20 refer to the substitution level of wheat flour by germinated lupin flour).

Sensory evaluation

Table 3 presents the mean sensory quality of AFB samples for the four levels of GLF used in this study in comparison to CB. There were no significant ($P \leq 0.05$) differences in homogeneity of colour, taste and flavour, odour, chewiness, tearing quality, and acceptability. However, CB had a significantly ($P \leq 0.05$) higher score for loaf surface colour compared with other AFB samples, with the yellow colour of lupin contributing to this result. Guillamón *et al.* (2010) reported that lupin flour caused a yellowing due to the presence of fat-soluble pigments, primarily lutein and zeaxanthin. The study results were inconsistent with those obtained by Zacarias *et al.* (1985). They reported that there were no significant differences in taste, aroma, texture and appearance (except colour) between breads made with wheat flour and those made with up to 12% lupin flour.

Bread specific volume

The results shown in Table 4 indicate that the specific volume of AFB decreased as the level of GLF incorporation increased without any significant differences between bread samples. It has been observed that CB and GLB 5 gave the highest volume, while the GLB 15 and GLB 20 gave the lowest bread volume of loaf but not significantly different form the other bread samples. These results are in agreement with other studies that used composite flour in bread making, e.g. cowpea flour (Hallén *et al.*, 2004), lupin and soya flours (Doxastakis *et al.*, 2002) and lupin flour (Alomari and Abdul-Hussain, 2013).

Colour characteristic of bread

L*, a*, and b* values of AFB are shown in Table 5. All samples contained GLF, exhibited a white-yellowish colour and differences among the samples were already visible without instrumental aid, particularly at higher levels of

Table 3. Panellist organoleptic evaluation of Arabic flat bread with different substitution levels of germinated lupin flour.[1,2]

Bread treatment[3]	Loaf face colour[4]	Homogeneity of colour[4]	Taste and flavour[4]	Odour[4]	Chewiness[4]	Tearing quality[4]	Acceptability[4]
CB	4.25±0.13[a]	4.08±0.20[a]	4.00±0.11[a]	4.41±0.11[a]	4.00±0.21[a]	4.16±0.25[a]	6.25±0.22[a]
GLB5	3.08±0.12[b]	3.66±0.28[a]	3.83±0.19[a]	3.91±0.26[a]	3.91±0.32[a]	4.08±0.20[a]	5.91±0.23[a]
GLB10	2.83±0.10[b]	3.41±0.27[a]	3.75±0.10[a]	3.75±0.23[a]	3.83±0.31[a]	4.00±0.11[a]	5.75±0.32[a]
GLB15	2.66±0.22[b]	3.41±0.25[a]	3.66±0.29[a]	3.75±0.14[a]	3.58±0.18[a]	3.83±0.12[a]	5.33±0.29[a]
GLB20	2.50±0.32[b]	3.33±0.31[a]	3.50±0.23[a]	3.33±0.11[a]	3.41±0.10[a]	3.83±0.15[a]	5.00±0.17[a]

[1] Values are means of replicates ± standard deviation (n=3).
[2] Means with different letters in the same column are significantly differed at $P \leq 0.05$.
[3] CB = control bread; GLB = germinated lupin bread (5, 10, 15, and 20 refer to the substitution level of wheat flour by germinated lupin flour).
[4] Hedonic scales: 1 = dislike extremely; 2 = dislike very much; 3 = dislike moderately; 4 = dislike slightly; 5 = neither like nor dislike; 6 = like slightly; 7 = like moderately; 8 = like very much; 9 = like extremely.

Table 4. Arabic flat bread specific volume at different levels of germinated lupin flour as substitution of wheat flour.[1,2]

Bread treatment[3]	Specific volume
CB	2.12±0.27[a]
GLB5	1.83±0.18[a]
GLB10	1.79±0.40[a]
GLB15	1.67±0.15[a]
GLB20	1.60±0.28[a]

[1] Values are means of replicates ± standard deviation (n=3).
[2] Means with different letters in the same column are significantly differed at $P\leq0.05$.
[3] CB = control bread; GLB = germinated lupin bread (5, 10, 15, and 20 refer to the substitution level of wheat flour by germinated lupin flour).

incorporation with significant increases in the b* and a* value in AFB samples; whereas lightness (L*) was adversely affected by these factors. The results showed that the colour changes were significantly higher in samples contain 15 and 20% wheat flour substitutions. This can be explained mainly by the availability of carotene pigment in GLF and also to the Maillard reaction that occurs when the bread is exposed to heat. Also, GLF contains a high amount of lysine, which is the most reactive amino acid in the context of Maillard reactions, in addition to the availability of reducing sugars in GLF. Therefore, the increase in GLF incorporation in AFB samples led to increases in the Maillard reaction (Obeidat *et al.*, 2013).

Bread crumb softness value

The most widely used indicator of bread staling is a measurement of the increase in crumb firmness or crumb softness, since it has a close association with human perception of freshness. Table 6 shows that CB had significantly ($P<0.05$) higher crumb softness value than other GLB levels with three different storage times, followed by the bread substituted with 5 and 10% GLF. However, the bread with 15 and 20% of GLF substitution showed lower crumb softness values. These results were

Table 5. Arabic flat bread colour at different levels of germinated lupin flour as substitution of wheat flour.[1,2,3]

Bread treatment	L*	a*	b*
CB	83.53±0.17[a]	2.07±0.16[b]	2.63±0.14[d]
GLB5	79.49±0.15[b]	2.19±0.21[b]	2.87±0.10[c]
GLB10	77.95±0.22[bc]	2.64±0.30[b]	2.99±0.22[b]
GLB15	77.42±0.23[bc]	2.85±0.33[b]	3.05±0.22[b]
GLB20	75.65±0.25[c]	5.08±0.30[a]	3.25±0.21[a]

[1] Values are means of replicates ± standard deviation (n=3).
[2] Means with different letters in the same column are significantly differed at $P\leq0.05$.
[3] CB = control bread; GLB = germinated lupin bread (5, 10, 15, and 20 refer to the substitution level of wheat flour by germinated lupin flour); L* = lightness; b* = yellowness; a* = redness.

Table 6. Effect of germinated lupin flour substitution and storage time on Arabic flat bread crumb softness.[1,2,3]

Bread treatment	Crumb softness (PU) of fresh AFB	Crumb softness (PU) of AFB after 24 h	Crumb softness (PU) of AFB after 48 h
CB	8.58±0.22[a]	7.93±0.23[a]	6.90±0.25[a]
GLB5	7.44±0.24[b]	7.12±0.28[ab]	5.17±0.27[b]
GLB10	7.19± 0.12[b]	6.72±0.30[b]	4.96±0.10[b]
GLB15	5.67±0.31[c]	5.53±0.24[c]	4.31±0.11[bc]
GLB20	5.60±0.15[c]	4.48±0.14[d]	3.64±0.34[c]

[1] Values are means of replicates ± standard deviation (n=3).
[2] Means with different letters in the same column are significantly differed at $P\leq0.05$.
[3] CB = control bread; GLB = germinated lupin bread (5, 10, 15, and 20 refer to the substitution level of wheat flour by germinated lupin flour); PU = penetration unit.

expected since the use of non-glutenous legume flours with wheat flour results in adverse effects on physical dough properties and bread quality (Abdul Hussain *et al.*, 2009). Adding improvers can resolve these rheological problem and help strengthen the dough to get better bread quality (Obeidat *et al.*, 2013).

It was also noticed that the longer the storage times of AFB the lower the crumb softness value. This result was compatible with other studies (Abdul Hussain *et al.*, 2009, Flander *et al.*, 2007; Izydorczyk, *et al.*, 2008).

4. Conclusions

The present study revealed that using germinated lupin flour in Arabic flat bread significantly increased its protein, lipid, ash and fibre contents and reduced the carbohydrate level. Thus, the resulting bread was considered to be more nutritionally functional. Increased consumption of such functional foods not only improves the nutritional status of the general population, but also helps those suffering from chronic diseases such as cardiovascular disease and diabetes type 2, which are associated with today's changing life-style and environments.

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