Effects of Blending Ratio and Processing of Lupine Bean On Nutritional Quality and Sensory Evaluation of Wheat-Lupine Bread

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Abstract

Background

Bread is one of the most popular and widespread baked products in the world. It is an important staple food made of wheat flour, salt, and yeast. However, wheat protein is lower than that of proteins from pulses. White lupine is a good source of protein, fiber, minerals, and vitamins. It has some anti-nutritional factors which inhibit its consumption. The use of lupine as human food, specifically in baked products has been limited.

Objective

This study was conducted to investigate the effect of blending ratio and processing of lupine bean on nutritional quality and sensory evaluations of wheat-lupine bread.

Methods

The study was done by factorial design. All white lupine bean was prepared in a randomized completely block design (RCBD). The quality characteristics of bread were analyzed by three-way ANOVA (Analysis of Variance) was carried out to determine the F-value and the level of significance, Duncan’s multiple range tests at 5% probability were used for the comparison between means using statistical tools of SPSS version 25.

Result

The effect of processing and blending ratio (140:10:15) had a high value in protein, fat, and mineral content. Sensory acceptance of wheat-lupine bread was affected by the interaction of blending ratio and processing. On a 5 point hedonic scale, the composite sample wheat flour with 5, 10, and 15% white lupine flour and supplementation had the highest scores were 4.60, 3.80, 3.80, 4.40, and 3.95 in color, taste, flavor, appearance, and overall acceptability respectively.

1. Background

Bread is one of the most popular and widespread baked products in the world. It is an important staple food made of wheat flour, salt, and yeast. In developing functional bakery products, it is important to develop a product with physiological effectiveness and consumer acceptance in terms of appearance, taste, and texture. Nowadays consumers prefer to eat healthier foods to prevent non-communicable diseases. Thus, industries and researchers are involved in optimizing bread-making technology to improve the variety, quality, taste, and availability of food products (1). Wheat bread represents the main
source of carbohydrates, minerals, and vitamins for most people. However, wheat protein is lower than that of proteins from pulses and oilseeds due to its lower levels of lysine, methionine, and threonine (2).

Lupine bean is an economically and agriculturally valuable plant that can grow in different soils and climates. Interest in lupine production is increasing, not only because of its strong capacity to fix nitrogen, making accessible macro and micro-elements elute to the soil sub-layer (3). It is a good source of nutrients, not only proteins but lipids, dietary fiber, minerals and vitamins (4), phytochemicals with antioxidant capacities, such as polyphenols, mainly tannins and flavonoids (5) which are responsible for the health benefits.

Lupine like other legumes has some anti-nutritional factors (ANFs) which inhibit its consumption. The main anti-nutritional substances found in lupine are alkaloids of the quinolizidine group. These bitter compounds make the bean unpalatable and sometimes toxic. Furthermore, the presence of phytates, tannins are hinders its consumption without processing. The task of plant breeders is to produce an alkaloid-free lupine that can be consumed by humans after soaking in running water, which can be easily converted into protein-rich food (6, 7). Therefore, the potential of lupine in human nutrition has generally been underestimated worldwide. So there is a need for designing studies to develop lupine-based convenient food products (8). To make Lupinus albus edible various modern and traditional processing methods like soaking after roasting, germination, fermentation, alkaline, and thermal treatments are some of the processing methods (8). When lupine flour and wheat flour are used together in food formulations, a supplemented and complementary effect is achieved due to the low lysine and high sulfur-containing amino content of wheat flour proteins (9, 10). The other limitation is also Celiac Disease (CD) and which is an effective treatment for celiac disease is a gluten-free diet that excludes cereals that contain gluten (11). The use of lupine as human food, specifically in baked products has been limited, due mainly to the poor sensory quality of resulting products (12). Therefore this study was done on the effect of white lupine bean processing and blends with wheat flour to improve nutritional quality and sensory evaluation of wheat-lupine bread.

2. Materials And Methods

2.1. Research design

The study was done 2x3x3 factorial design comprising white lupine bean from 2 soaking conditions, 3 roasting temperatures, and blended with 3 categories of supplementation of wheat flour. All white lupine bean was prepared in duplicate/triplicate following a randomized completely block design (RCBD). Using this design the effect of blending ratios and processing were the interaction of factors on proximate compositions, minerals content, and sensory evaluation of bread was investigated. Upper and lower levels of variables were selected based on the different composite to wheat flours (13, 14).

2.2. Study area
This study was carried out for breadmaking was done at Debre Markos University where the whole apparatuses and chemicals available there, to get a good product.

2.3. Wheat flour preparation

Wheat grain sample was purchased from the local market and the sample was graded, sorted, and cleaned manually to remove foreign matters, immature and damaged. They cleaned 15 kg of whole wheat grain was milled (Retsch, Cyclon Miller-Twister) and sieved to pass a 60µm sieve. The flour samples were packed in a sack and stored at room temperature.

2.4. White lupine bean preparation

A white lupine bean sample was purchased from the local market and the sample was graded, sorted, and cleaned manually to remove foreign matters, immature and damaged. They cleaned 5kg of the sample was roasted at 120,130 and 140 ºC by using an electric oven and lasted for about 12 minutes. Then it was allowed to cool for about 10minute at room temperature and then soaked in a bucket of tap water (1:10, lupine: tap water) for 10 and 15days, after each 8hours changed the water. The hull of beans was taken off by pressing on the bean with hands finger, then washed with tap water, and dried at 60ºC by using an electric oven. They dried, milled with (Retsch, Cyclon Miller-Twister) grinder and sieved to pass a 60µm sieve, stored in sealable polyethylene bags in plastic, and stored at room temperature before analysis and making baked food (15).

2.5. Flour blending

Wheat and processed white lupine flour were blended in the percentage of 95:5, 90:10, and 85:15, and 100% of wheat flour as control with each sample weighed out into different places using a weighing balance. The blends were homogenized manually.

2.6. Breadmaking

The bread samples were produced in batches by mixing and kneading manually each of the above flour blends with the ingredients using a stainless-steel bowl. Ingredients used for making control bread samples were included 500gm of wheat flour with 4gm of salt, 4gm of yeast, and 300ml of water. The supplemented bread was prepared using white lupine flour at 5, 10, and 15%. After thorough kneading in each case, the dough was allowed to ferment and develop for 15min before being knocked back and then molded into a cylindrical shape. After molding in each case, the dough was then placed in a well-oiled baking pan where it was proofed for 40minutes at room temperature before it was baked in a cabinet oven pre-heated. All different bread formulas were baked at 250°C for 10-15min then cooled and stored at room temperature for chemical analysis after organoleptic evaluation.

2.7. Proximate analysis

The American Association of Cereal Chemists (16) procedure was used to determine the proximate compositions (crude protein, crude fat, crude fiber, and total ash) of the bread samples made from wheat-lupine flour. Protein concentration was determined by Kjeldahl method by Digester, Automatic Distillation
and Titration System (VELP- SCIENTIFICA, UDK-119), ash was quantified by incineration of the samples in a muffle furnace, total fat was extracted with petroleum ether using a Soxhlet apparatus (LOBA CHME PVT.LTD), and crude fiber was quantified by the enzyme-gravimetric method. All analyses were carried out thrice.

2.8. Mineral analysis

For mineral determination, the American Association of Cereal Chemists (16) procedure was used to determine minerals content. Calcium, zinc, and iron were determined using a UV-VIS spectrophotometer.

2.9. Anti-nutritional factor analysis (total alkaloids)

The alkaloid content was determined gravimetrically by the method of (17). A sample (0.5g) was dispersed in 10mL of 10% acetic acid solution in ethanol. The dispersed solution was well shaken and then allowed to stand for about 4hrs before it was filtered. The filtrate was evaporated to one-quarter of its original volume. Concentrated NH4OH was added dropwise to precipitate the alkaloids. The precipitate was filtered into pre-weight filter paper and washed with 1% NH4OH solution. The precipitate in the filter paper was dried in the oven at 60 ºC for 30 min and reweight until it attains constant mass. Alkaloid was expressed in percentage.

2.10. Sensory evaluation

Sensory evaluation of white lupine bread taste, color, flavor, appearance, and overall acceptance as the method described by using a 5-points hedonic scale method (18). Bread samples were evaluated organoleptically by a panel of 20 panelists. On the day of evaluation, bread was placed on small plates and labeled with three digital random codes. Panelists were provided with distilled water and unsalted crackers to clean their palates between samples. The bread samples were presented in random order and panelists were asked to rate their assessment of taste, color flavor, appearance, and overall acceptance on a 5-point hedonic scale (ranging from 5 = like very much to 1 = disliked very much). The raw scores were assembled and statistically analyzed using appropriate software.

2.11. Statistical analysis

All experiments were replicated at least thrice. All data were statistically analyzed by three-way ANOVA (Analysis of Variance) using SPSS version 25 and expressed as mean values ± standard deviations. Duncan's multiple range tests were used to determine significant differences. Differences were regarded as significant at 95% (p < 0.05).

3. Results And Discussion

This research was conducted on the effect of blending ratio and processing of white lupine with wheat flour bread quality (proximate composition, mineral content, and sensory evaluation).
3.1. Proximate analysis

The bread was prepared by the effect of blending ratio and white lupine processing within wheat flour. The results obtained for proximate analysis were given in “Table 1”. White lupine flour had significantly different (p < 0.05) higher levels of protein. The supplementation of wheat flour with 5, 10, and 15% lupine flour can result in the improved nutritional quality of bread. However, with this inclusion lupine has been recognized as potentially allergenic, and the risk for sensitive consumers should therefore be minimized.

We have previously demonstrated that thermal and steeping conditions such as roasting and soaking resulted in a considerable reduction of the alkaloid content of lupine. More precisely, roasting at 140°C and soaked for 15 days almost abolished the alkaloids. It was the aim of the present study to elucidate if these hypoallergenic lupine flours (obtained by roasting and soaking) can be used to produce palatable mixed wheat-lupine bread of high quality. Lupine has a high protein content (Lupinus albus L. 38g/100g), which makes it a valuable supplement for wheat flour and the production of high protein bread. However, white lupine flour is deficient in sulfur-containing amino acids, methionine, and cysteine, which are present in significant quantities in wheat flour. Therefore, a mixture of wheat and lupine flour should have a higher nutritional value than the individual ingredients. The substitution of 10% of wheat flour with processed (roasted at 140°C and soaked for 15 days) lupine resulted in a significant increase in the total protein content of the flour mixture. These are almost to the literature reported values (19–21). White lupine flour had significantly different (p < 0.05) higher levels of crude fat. In past studies, the crude fat content of lupine was identified and quantified (19, 20, 22, and 23). The above reported crude fat content was detected in this study. The addition of sweet lupine flour to wheat flour increased since legumes are generally higher in fiber content cereals (19, 23). Lupine flour had a high content of crude fiber which has many desirable properties, including white color, high water capacity, and beneficial effects on human health (2, 22, and 24). Therefore, lupine bean flour can be supplemented into many foods to make healthier dietary products, such as bakery, dairy, and meat products. But, there is no significant difference (p < 0.05) ash content of wheat-lupine bread. These are almost similar to the literature reported values (2, 20, and 23).
### Table 1
Proximate compositions of wheat-lupine bread samples.

| Soaking Condition (day) | Roasting Temperature (°C) | Blending Ratio (%) | Protein (%) | Fat (%) | Fiber (%) | Ash (%) |
|-------------------------|---------------------------|--------------------|-------------|---------|-----------|---------|
| 10                      | 120                       | 5                  | 13.39 ± 0.24<sup>d</sup> | 3.51 ± 0.00<sup>d</sup> | 3.16 ± 0.00<sup>a</sup> | 4.83 ± 1.03<sup>a</sup> |
| 10                      | 15.24 ± 0.26<sup>c</sup>  | 4.82 ± 0.00<sup>c</sup> | 2.59 ± 0.00<sup>c</sup> | 3.66 ± 0.00cd |
| 15                      | 15.05 ± 3.64<sup>c</sup>  | 5.95 ± 0.00ab<sup>b</sup> | 2.65 ± 0.00<sup>c</sup> | 3.57 ± 0.10cd |
| 130                     | 5                         | 13.84 ± 0.31<sup>d</sup> | 4.24 ± 0.05cd<sup>b</sup> | 3.16 ± 0.01a  | 4.73 ± 0.58<sup>ab</sup> |
| 10                      | 18.30 ± 0.00<sup>ab</sup>| 4.77 ± 0.00<sup>c</sup> | 2.65 ± 0.00<sup>c</sup> | 3.85 ± 0.36bc |
| 15                      | 18.82 ± 0.19<sup>ab</sup>| 5.33 ± 0.00<sup>bc</sup> | 2.36 ± 0.30d<sup>d</sup> | 3.71 ± 1.45c  |
| 140                     | 5                         | 14.67 ± 0.11cd<sup>d</sup> | 4.85 ± 0.00<sup>c</sup> | 3.21 ± 0.27a  | 4.31 ± 1.15b  |
| 10                      | 18.26 ± 0.13<sup>ab</sup>| 5.25 ± 0.00<sup>bc</sup> | 2.99 ± 0.00ab<sup>b</sup> | 3.64 ± 0.40cd |
| 15                      | 19.72 ± 0.45<sup>a</sup> | 5.64 ± 0.00<sup>b</sup> | 3.14 ± 0.00a<sup>a</sup> | 3.67 ± 1.39cd |
| 15                      | 120                       | 5                  | 13.58 ± 1.02<sup>d</sup> | 4.28 ± 0.03cd<sup>d</sup> | 3.18 ± 0.90a<sup>a</sup> | 4.17 ± 0.61bc |
| 10                      | 14.24 ± 1.17cd<sup>d</sup>| 4.58 ± 0.00<sup>c</sup> | 3.05 ± 0.32ab<sup>b</sup> | 3.39 ± 0.46d  |
| 15                      | 17.57 ± 0.59b<sup>b</sup>| 5.59 ± 0.06b<sup>b</sup> | 3.15 ± 0.00a<sup>a</sup> | 3.96 ± 1.59bc |
| 130                     | 5                         | 13.51 ± 0.13<sup>d</sup> | 5.66 ± 0.11<sup>b</sup> | 3.29 ± 0.00a<sup>a</sup> | 4.52 ± 1.71b  |
| 10                      | 15.82 ± 0.18c<sup>c</sup>| 5.69 ± 0.00b<sup>b</sup> | 2.78 ± 0.30bc<sup>d</sup> | 3.01 ± 0.85ed |

**, *** and ns represent significant at 5%, significant at 1% and non-significant at 5% probability level respectively. Note: °C = white lupine bean roasting temperature at 120, 130 and 140°C respectively and Day = number of soaking days for 10 & 15th days were white lupine bean takeout from water. %: blending ratios of white lupine bean flour to wheat flour (5, 10 and 15%). CV = coefficient of variation and DMRT = Duncan’s multiple range test at α equal to 0.05. Mn = grand mean.
| Soaking Condition (day) | Roasting Temperature (°C) | Blending Ratio (%) | Protein (%) | Fat (%) | Fiber (%) | Ash (%) |
|------------------------|---------------------------|-------------------|-------------|---------|-----------|---------|
| 15                     | 140                       | 5                 | 16.20 ± 0.10<sup>bc</sup> | 5.92 ± 0.01<sup>ab</sup> | 2.86 ± 0.30<sup>b</sup> | 3.09 ± 1.07<sup>ed</sup> |
| 10                     | 140                       | 5                 | 14.79 ± 0.40<sup>cd</sup> | 5.79 ± 0.00<sup>ab</sup> | 2.97 ± 0.00<sup>ab</sup> | 2.88 ± 0.61<sup>e</sup> |
| 15                     | 140                       | 5                 | 20.84 ± 0.01<sup>a</sup> | 5.85 ± 0.00<sup>ab</sup> | 2.51 ± 0.00<sup>cd</sup> | 2.24 ± 1.00<sup>ef</sup> |
| Wheat flour (Control) 100% | 140                       | 5                 | 11.04 ± 0.19<sup>e</sup> | 1.94 ± 0.00<sup>e</sup> | 2.26 ± 0.30<sup>e</sup> | 3.55 ± 0.30<sup>cd</sup> |
| Mn                     |                            |                   | 16.233       | 5.133   | 2.820     | 3.578   |
| CV (%)                 |                            |                   | 0.15         | 0.37    | 0.21      | 0.52    |
| DMRT (p < 0.05)        |                            |                   | <0.00***     | <0.00***| <0.00***  | <0.24<sup>ns</sup> |

**, *** and ns represent significant at 5%, significant at 1% and non-significant at 5% probability level respectively. Note: °C = white lupine bean roasting temperature at 120, 130 and 140°C respectively and Day = number of soaking days for 10 & 15th days were white lupine bean takeout from water. %: blending ratios of white lupine bean our to wheat our (5, 10 and 15%). CV = coefficient of variation and DMRT = Duncan’s multiple range test at α equal to 0.05. Mn = grand mean.

### 3.2. Minerals content

The result obtained for minerals was given “Table 2”. The mineral content of wheat and lupine flour especially the calcium content of white lupine higher compared to wheat flour as well as the concentration of lupine flour increase the calcium content of the blend. Treatment (10:140:5) were a higher zinc concentration compared to other treatments. Lupine flour has higher amounts of Ca, Zn, K, and P when compared to wheat flour. Similar results for the mineral content of lupine flour have been reported in previous works (19, 20). The mineral content of wheat and sweet lupine flour especially the calcium content of sweet lupine higher compared to wheat flour (20, 25). However, iron is decreased due to processing (cooking, fermentation, germination, and soaking) (20). Soaking reduces phytic acid, freeing iron and resulting in higher HCl extractability (26, 27).
### Table 2
Minerals content of wheat-lupine bread samples.

| Soaking Condition (day) | Roasting Temperature (°C) | Blending Ratios (%) | Iron (mg/100gm) | Zinc (mg/100gm) | Calcium (mg/100gm) |
|-------------------------|---------------------------|---------------------|-----------------|-----------------|--------------------|
| 10                      | 120                       | 5                   | 0.61 ± 0.02<sup>ab</sup> | 0.58 ± 0.61<sup>c</sup> | 23.61 ± 0.51<sup>c</sup> |
|                         |                            |                     |                 |                 |                    |
| 10                      | 130                       | 5                   | 0.60 ± 0.04<sup>ab</sup> | 0.53 ± 0.61<sup>cde</sup> | 15.41 ± 0.42<sup>e</sup> |
|                         |                            |                     |                 |                 |                    |
| 15                      | 130                       | 5                   | 0.58 ± 0.02<sup>b</sup> | 0.59 ± 0.81<sup>c</sup> | 24.11 ± 0.03<sup>abc</sup> |
| 130                     | 5                          | 0.37 ± 0.06<sup>d</sup> | 0.52 ± 0.64<sup>cde</sup> | 12.28 ± 0.31<sup>f</sup> |
| 140                     | 5                          | 0.61 ± 0.00<sup>ab</sup> | 0.69 ± 0.05<sup>a</sup> | 25.43 ± 0.25<sup>ab</sup> |
|                         |                            |                     |                 |                 |                    |
| 15                      | 120                       | 5                   | 0.21 ± 0.00<sup>d</sup> | 0.61 ± 0.37<sup>bc</sup> | 26.81 ± 0.23<sup>a</sup> |
|                         |                            |                     |                 |                 |                    |
| 130                     | 5                          | 0.35 ± 0.00<sup>c</sup> | 0.66 ± 0.00<sup>ab</sup> | 12.92 ± 0.10<sup>f</sup> |

****, *** and ns represent significant at 5%, significant at 1% and non-significant at 5% probability level respectively. Note: °C = white lupine bean roasting temperature at 120, 130 and 140 °C respectively and Day = number of soaking days for 10 & 15th days were white lupine bean takeout from water. %: blending ratio of white lupine bean flour to wheat flour (5, 10 and 15%). CV = coefficient of variation and DMRT = Duncan’s multiple range test at α equal to 0.05. Mn = grand mean.
### 3.3. Anti-nutritional factor content (total alkaloid)

The determination of alkaloids in the lupine bean samples was carried out by employing previously reported (28) techniques. Alkaloids found in the studied roasting and then soaking white lupine bean samples are given in “Table 3”. The range of percentage alkaloids present in the unprocessed and processed white lupine bean was from 2.591–0.064%. This result shows that the alkaloid content of the bean decreased 40 times after processing. The alkaloid content of bitter lupine bean also shows a decreasing trend to each processing method which ranges from 0.889 % to 0.064%. The result was in agreement with previous literature report that tubers and plant leaves contain a substantial proportion of alkaloids (29). These are almost similar to the literature reported values (6) a reduction of total alkaloids follow roasting, boiling, and germination and (30) TTM (Traditional Turkish Method) was more effective than extrusion technique for the elimination of lupine alkaloids.
Table 3
Alkaloid content of wheat-lupine bread samples.

| Roasting Temperature (°C) | Soaking Condition (day) | Alkaloid Content (gm) |
|---------------------------|-------------------------|-----------------------|
| Raw                       | -                       | 2.591 ± 0.06^a        |
| 120                       | 10                      | 0.899 ± 0.11^b        |
| 120                       | 15                      | 0.499 ± 0.11^d        |
| 130                       | 10                      | 0.699 ± 0.11^c        |
| 130                       | 15                      | 0.399 ± 0.01^f        |
| 140                       | 10                      | 0.399 ± 0.02^f        |
| 140                       | 15                      | 0.064 ± 0.02^g        |
| CV (%)                    |                         | 0.046                 |
| DMRT (p < 0.05)           |                         | < 0.0001***           |

**,** *** and ns represent significant at 5%, significant at 1% and non-significant at 5% probability level respectively. Note: °C = white lupine bean roasting temperature at 120, 130 and 140°C respectively and Day = number of soaking days for 10 & 15th days were white lupine bean takeout from water. CV = coefficient of variation and DMRT = Duncan’s multiple range test at α equal to 0.05.

3.4. Sensory evaluation of wheat-lupine bread

The sensory evaluation profiles of wheat-lupine bread are shown “Table 4” The mean value had greater than 3 at five levels of hedonic scales; this implies that they were accepted by panelists. The quality of bread is determined by organoleptic characteristics. In this study, descriptive qualitative analysis was used to evaluate the organoleptic characteristics. According to the descriptive sensory evaluation, the bread made with wheat flour had less flavor than those made from a mixture of wheat and white lupine flour. The inclusion of white lupine produces a slight lupine flavor. When processed white lupine bean was used it was observed that the flavor and the smell of the bread increased in roasted and soaked. The color of bread is an important character that affects consumer preferences (19). The browning of the crust is due to Maillard reactions, which occur as a result of the increased content of protein, added when using lupine flour (21, 22). It has been shown that lupine flour can also change the color of the product obtained bread with lupine addition, leading to a more yellow crumb and brown crust (21, 31, and 32). The addition of (10%) lupine flour to shortcakes, gingerbreads, and pancakes enables the preparation of good quality foodstuffs in terms of sensory properties (33). The color of the crust and crumb darkens with the increasing content of lupine flour in the dough. For some consumers, the yellow color of the crumb would seem attractive because it resembles cakes that have been elaborated with eggs (10, 34). But, there is no significant difference in taste, aroma, texture, and appearance (except color) between
bread made with wheat flour and those made with up to 12% lupine flour (35). The observations made by participants in the tasting session were related to taste, smell, and aroma (20, 31). The flavor difference of the sample was the highest (3.80) and the lowest (2.20) value was found. This is maybe due to the high protein contents of lupine flour with a bland flavor (20). The flavorings compounds which found in bread are aldehydes, ketones, ester, and alcohol and sugar especially ketones functional groups are abundant flavoring compound in fresh bread. The difference in appearance was the highest (4.40) and lowest (2.40) value observed (21). Therefore, the overall acceptance of wheat-lupine bread due to blending ratio and processing effect was the highest (3.95) and lowest (2.70) was observed.
Table 4
Sensory characteristics of wheat-lupine bread samples.

| Soaking Condition (day) | Roasting Temperature (°C) | Blending Ratio (%) | Color | Taste | Flavor | Appearance | Overall A |
|-------------------------|---------------------------|--------------------|-------|-------|--------|------------|-----------|
| 10                      | 120                       | 5                  | 4.00 ± 1.22bc | 3.80 ± 0.45a | 3.60 ± 0.55ab | 3.80 ± 1.09b | 3.80 ± 0.59a |
| 10                      | 3.20 ± 1.30cd            | 2.00 ± 1.23g       | 2.80 ± 1.79cd | 3.80 ± 1.30b | 2.95 ± 0.85c |
| 15                      | 3.00 ± 1.41cd            | 3.20 ± 1.64c       | 3.00 ± 1.58c | 4.40 ± 0.55a | 2.90 ± 1.00c |
| 130                     | 5                         | 4.40 ± 0.55b       | 3.20 ± 0.84c | 3.80 ± 0.45a | 3.60 ± 0.55bc | 3.95 ± 0.41a |
| 10                      | 4.40 ± 0.55b             | 3.20 ± 1.30c       | 3.60 ± 1.14ab | 3.60 ± 1.14bc | 3.70 ± 0.96ab |
| 15                      | 3.60 ± 0.89c             | 3.60 ± 1.52ab      | 3.20 ± 1.30bc | 3.80 ± 1.30b | 3.55 ± 1.20b |
| 140                     | 5                         | 3.40 ± 1.52c       | 3.60 ± 1.52ab | 3.00 ± 1.00c | 4.00 ± 0.00ab | 3.50 ± 0.86b |
| 10                      | 3.60 ± 1.14c             | 2.60 ± 1.34e       | 3.20 ± 1.09bc | 3.60 ± 1.67bc | 3.25 ± 0.94bc |
| 15                      | 3.60 ± 1.95c             | 3.40 ± 1.14b       | 3.20 ± 1.64bc | 2.80 ± 1.79cd | 3.25 ± 0.47bc |
| 15                      | 120                       | 5                  | 2.60 ± 1.14d  | 2.80 ± 1.09d  | 3.20 ± 0.84bc | 3.40 ± 0.55c | 3.00 ± 0.73c |
| 10                      | 3.00 ± 1.41cd            | 3.20 ± 1.64c       | 2.80 ± 1.30cd | 2.80 ± 1.30cd | 2.95 ± 1.08c |

**, *** and ns represent significant at 5%, significant at 1% and non-significant at 5% probability level respectively.
### Table 2: Effects of Blending Ratio and Processing of Lupine Bean on Nutritional Quality and Sensory Evaluation of Wheat-Lupine Bread Prepared by Different Methods

| Soaking Condition (day) | Roasting Temperature (°C) | Blending Ratio (%) | Color | Taste | Flavor | Appearance | Over All A |
|-------------------------|---------------------------|--------------------|-------|-------|--------|------------|------------|
|                         |                           | 15                 |       |       |        |            |            |
|                         |                           | 130                |       |       |        |            |            |
|                         |                           | 10                 |       |       |        |            |            |
|                         |                           | 15                 |       |       |        |            |            |
|                         |                           | 140                |       |       |        |            |            |
|                         |                           | 10                 |       |       |        |            |            |
|                         |                           | 15                 |       |       |        |            |            |
| Control (100%)          |                           |                    |       |       |        |            |            |
| Mn                      |                           |                    |       |       |        |            |            |
| CV (%)                  |                           |                    |       |       |        |            |            |
| DMRT (p < 0.05)         |                           |                    |       |       |        |            |            |

****, *** and ns represent significant at 5%, significant at 1% and non-significant at 5% probability level respectively.

### 4. Conclusion

The effects of blending ratio and processing of lupine bean on nutritional quality and sensory evaluation of wheat-lupine bread prepared by different methods were investigated. Finally, we conclude that the use of white lupine flour from thermally-treated and soaked beans in bread is feasible and that the functional tolerance is good. Roasted and soaked condition lupine beans maintain the bread-making properties of...
raw lupine flour, allowing their use in baking. The effect of the processing and blending ratio was different. Results of processing and blending ratio show that the combination of roasting temperature, soaking condition, and blending ratio had a positive effect on the nutritional (protein, fat, fiber, and minerals) content of bread. However, the combination of roasting temperature and soaking condition play a negative role on alkaloid content was very low than raw lupine flour. In addition, processing and supplementation had good color, taste, flavor, appearance, and overall acceptability of bread. Wheat-lupine bread had good quality after roasting temperature (140°C) and soaking condition (10th day), which was more conducive to the formation of blending-processed products.

5. Abbreviations

AACC: American Association Cereal Chemistry; ANFs: Anti-nutritional Factors; ANOVA: Analysis of Variance; Ca: Calcium; CD: Celiac Disease; CV: Coefficient of Variation; DMRT: Duncan's Multiple Range Test; Fe: Iron; HCl: Hydrochloric; NH₄OH: Ammonium hydroxide; K: Potassium; P: phosphorus; RCBD: Randomized Completely Block Design; SPSS: Statistical Software of Social Package; TTM: Traditional Turkish Method; UV-VIS: Ultra Violet Spectrophotometer; Zn: Zinc

Declarations

Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable

Availability of data and material
The datasets used and/or analyzed during the current study are available from the principal author on reasonable request.

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Authors’ Contributions
Conceptualization: TA: designed the study, performed the statistical analysis, wrote the protocol, managed the analyses of the study, managed the literature searches, and wrote the first draft manuscript.
TA, WW, HT, and SA: Entering data into computer software, Supervision, Validation, Writing review & editing and all authors read and approved the final manuscript.

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