Research Article

Development and Characterization of Multigrain Pan Bread Prepared Using Quinoa, Lupin, and Fenugreek Seeds with Yellow Maize as a Gluten-Free Diet

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Celiac disease causes serious health problems for humans. Therefore, the consumption of gluten-free diets (GFDs) is the only therapy to prevent patients from developing the disease. The objective of the current study was to investigate the proximate analysis, mineral compositions, and antioxidant activities of the quinoa, germinated sweet lupin, fenugreek, and yellow maize, and they were used to develop gluten-free multigrain pan breads. A total of four different grain blend formulations were used to develop the pan bread. The textural properties, color, and sensory evaluation of the developed multigrain pan bread were also determined. The results of the present study showed a significantly higher fat content was found in germinated lupin (13.56%) and quinoa (12.76%), followed by germinated fenugreek and yellow maize (9.68% and 4.67%, respectively). The results indicated that the development of multigrain pan bread with fortification of quinoa, germinated lupin, germinated fenugreek, and yellow maize imparted significant improvement in the nutritional content. Therefore, it could be recommended that the addition of up to 15% of germinated lupin and fenugreek, 60% quinoa, and 10% yellow maize does not negatively affect the sensory characteristics and quality attributes of pan bread.

1. Introduction

In the past few decades, the consumer has given more attention to gluten-free (GF) food products due to their health benefits and reduced the risk of celiac disease [1]. Celiac disease (CD) is an immune-mediated disease that harms the villi that are responsible for nutrient absorption [2]. Celiac disease is caused by consumption of gluten-based products such as wheat, barley, and oats [3–6]. There are a very few gluten-free food products available on the market with a higher cost. Thus, the consumption of gluten-free products provides both nutritional health benefits and economic benefits [7]. Therefore, the consumption of lupin as a legume which contains excellent amounts of protein, fiber, and bioactive compounds helps in improving health by reducing the risk of chronic diseases [7, 8]. It can be an excellent alternative to wheat to develop a pan bread with higher nutritional value. This might be possible due to cross-linking of lupine protein, which helps to improve volume and high water-binding capacity (WBC) and delays sluggishness in bread due to the presence of lupine fiber [9]. Fenugreek (Trigonella foenum-graecum) and quinoa flour also contain good amounts of nutritional and bioactive compounds, which helps in the reducing blood sugar, cholesterol and is anticarcinogenic and antioxidant in activity [10]. Moreover, lupin, fenugreek, and quinoa flour are being utilized in the development of bakery based food products by improving the nutritional, sensorial, and functional properties of food.
products [11, 12]. The yellow maize (Zea mays L.) is also considered an energy food source for health, which contains rich amounts of carotenes, xanthophylls, phenolic compounds, and other phytochemicals [9, 13]. In the food industry, psyllium is used as an alternative to gluten as well as a thickening agent [14–16]. It is a rich source of nutritional composition and soluble fiber [17, 18].

The objective of the current study was to investigate the proximate analysis, mineral compositions, and antioxidant activities of quinoa, germinated sweet lupin, fenugreek, and yellow maize and how they were used to develop gluten-free and multigrain pan breads and their consumer acceptability.

2. Materials and Methods

2.1. Materials and Samples Preparation. The raw materials and ingredients such as quinoa, sweet lupin, fenugreek, and yellow maize were purchased from the Grain Soils and Flour Mills Organization in Saudi Arabia. The standard and analytical grade of chemicals and reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

The whole grains of quinoa, sweet lupin, fenugreek, and yellow maize were cleaned to remove foreign particles, then dried at 50–60°C to obtain fine flour, and stored at 5°C for further analysis [19]. Figure 1 presents the raw materials, preparations, and measurements.

2.2. Preparation of Germinating Sweet Lupine and Fenugreek Seeds. Lupine seeds were soaked in water for 12 h to remove bitterness, and then the lupin and fenugreek seeds were germinated for 3 days in an incubator at 25°C. Afterwards, the seeds were dried to obtain a fine powder and stored at 5°C for further analysis [20].

2.3. Proximate Analysis of Raw Materials. Proximate composition such as protein, fat, crude fibers, ash content, and carbohydrate content in milled material quinoa, lupin, fenugreek seeds, and yellow maize were determined using standard [21]. The dietary fiber in the samples was estimated according to the methodology followed by [22].

2.4. Determination of Minerals Content of Raw Materials. Minerals content such as magnesium, sodium, potassium, phosphorus, iron, and calcium were determined in raw materials according to the standard method. 2 g of each raw material was ashed and dissolved in 5 mL of 2% HNO₃. The mixture was filtered for mineral determinations by the atomic absorption spectrometer [23].

2.5. Extract Preparation. The extraction of the different flours was prepared according to the procedure reported by using 70% of ethanol [24]. The extract was filtered using filter paper No. 1 to obtain a transparent extract to determine phenols, flavonoids, and antioxidant activity.

2.6. Estimation of Total Phenolic and Flavonoid Content from Raw Material Extracts. The phenolic content (TPC) was measured by the standard Folin–Ciocalteu (FC) reagent assay and absorbance was calculated as mg of gallic acid Equi/g DW dry weight [25]. Total flavonoid content (TFC) was estimated according to the procedure followed by Alkaltham et al. (2021) and calculated as mg of quercetin Equi/g DW.

2.7. Determination of Antioxidant Activity. The antioxidant activity of the raw materials was evaluated using different assays such as DPPH (2,2′-diphenyl-1-picryl-hydrazyl-hydrate) assay at 517 nm, ABTS (2,2′-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) assay at 734 nm, and FRAP (free radical reducing power) assay at 593 nm according to [26, 27].

2.8. Formulation and Preparation of Multigrain Pan Bread. The different pan bread blend formulation dough was prepared by varying the amounts of raw materials with the addition of 3% psyllium as alternative gluten (Table 1). The dough was placed in a warm place until it had risen just over the top of the loaf pan for 1 h. The prepared different blend formulations of pan bread were baked in an oven at 450–500°C for 2.5–3 min, and they were aerated at room temperature according to the method described by [21].

2.9. Sensory Evaluation. The sensory evaluation of the prepared multigrain pan bread was assessed using a nine-point hedonic scale system. A total of 10 trained judges from the members of the Food Science and Nutrition Department and Faculty Science-Taif University were selected for the sensory evolution of pan breads based on the color, texture, odor, appearance, and overall acceptability of pan breads.

2.10. Color Measurements. The color scales of the pan bread in terms of (L*) lightness, (a*) redness, and (b*) yellowness were measured by using a colorimeter (ZE-6000, Nippon, Japan). The average value of the triplicated reading is reported as the result.

2.11. Texture Profiles of Pan Breads

2.11.1. Instrumental Analyses of Different Pan Breads. Textural properties such as hardness, resilience, cohesiveness, springiness, and chewiness of the multigrain pan bread were determined according to the methodology using a texture analyzer (Brookfield Engineering Laboratories, Inc., MA 02346–1031, USA) [28, 29].

2.12. Statistical Analysis. The average value of the results with standard deviation (SD) is reported as the results. The data was analyzed using the ANOVA statistical tool of SPSS software. A Duncan multiple range test with a (p<0.05) significance level was conducted to determine the significance difference between the samples.
3. Results and Discussion

3.1. Proximate Composition of Raw Materials. The results of proximate analysis of the raw materials indicated that the germinated lupin and fenugreek had significantly ($p < 0.05$) higher protein 25.67% and 24.94%, followed by quinoa and yellow maize with 14.38% and 9.56%, respectively (Table 2). In addition, the germinated lupin (13.56%) and quinoa (12.76%) also showed the highest amount of crude fiber, followed by germinated fenugreek and yellow maize (9.68% and 4.67%, respectively). Legumes contain high amounts of chemical nutritional compounds that do not contain gluten and are added from 10% to 30% to bakery products to give a product with a high nutrition value and quality [30]. Moreover, the results showed that both germinated fenugreek and lupin had the highest total dietary fiber, at 28.86% and 19.28%, respectively, followed by quinoa and yellow maize, at 15.21% and 7.72%, respectively. Furthermore, soluble and insoluble dietary fibers were parallel to the results of total dietary fiber for raw materials. Dietary fibers consist of insoluble carbohydrates and lignin, which are resistant to the digestive system [31]. Moreover, it was reported that a sufficient amount of fiber in a meal gives more health benefits, involving body mass reduction, reducing hypertension, and lowering glucose in the blood and chronic heart diseases [32]. Quinoa flour compared to wheat flour has a higher protein and fiber content with a low value of carbohydrate contents [33]. Phytochemicals are attained by the maize, which is very helpful and healthy for human beings. Many proteins, vitamins, ashes, minerals, and amino acids are isolated from the maize crop which is very helpful in human health sustainability [13, 34].

3.2. Minerals Content of Raw Materials. Table 3 shows the results for the mineral compositions of magnesium (Mg), sodium (Na), potassium (K), phosphorus (P), iron (Fe), and calcium (Ca) present in different seed flours. The results indicated that the mineral content in quinoa seed flour was Mg (40.38 mg/100 g), Na (210.35 mg/100 g), K (533.12 mg/100 g), P (11.76 mg/100 g), Fe (12.83 mg/100 g), and Ca (70.56 mg/100 g) [11].

The mineral content in germinated lupin seeds was recorded as 68.54 mg/100 g, 117.27 mg/100 g, 240.19 mg/100 g, 25.86 mg/100 g, 10.49 mg/100 g, and 204.16 mg/100 g, respectively. Lupin seeds contain high levels of vitamins, as well as rich amounts of minerals including iron, zinc, and manganese [10]. Moreover, the mineral contents of the germinated fenugreek were 75.84 mg/100 g, 120.46 mg/100 g, 280.27 mg/100 g, 94.29 mg/100 g, 14.85 mg/100 g, and 240.12 mg/100 g, respectively. Fenugreek seeds can be
considered as a significant source of micronutrients, phenols, and flavonoids with great health-elevating effects [11].

The mineral content of yellow maize seeds were 5.12 mg/100 g, 25.53 mg/100 g, 215.28 mg/100 g, 4.17 mg/100 g, 2.26 mg/100 g, and 20.49 mg/100 g, respectively. They analyzed the white and yellow maize flours, which showed that the yellow maize flour has a higher value of mineral content than the white maize flour [35]. The results observed that the quinoa had the highest amounts of sodium and potassium, whilst, germinated lupin and germinated fenugreek had rich amounts of sodium, potassium, and calcium. In addition, yellow maize had a rich source of potassium.

3.3. Total Phenolic and Flavonoid Content of Raw Materials.

The results of the present study indicated that quinoa has significantly higher levels of total phenolic (2.26 mg GAE/g DW) and flavonoid compounds (0.43 mg QE/g DW) compared to other ones. The current results are in line with findings that reported the flavonoids and phenol content at 490.2 and 2239 mg/kg, respectively, in quinoa seeds [36]. Specific quinoa seeds are a rich source of bioactive compounds such as phenols and flavonoids that can prevent oxidative stress [37].

The germinated lupin seeds showed 0.15 mg GAE/g DW and 0.29 mg QE/g DW of total phenolic and flavonoid compounds, respectively. Lupin seeds are rich sources of polyphenolic compounds and other antioxidants (Ben et al., 2021). Moreover, they found that the seeds of the lupin varieties contained high amounts of phenolic compounds [38]. Meanwhile, the germination of fenugreek seeds had the highest source of phenols and flavonoids content with 1.75 mg GAE/g DW and 4.21 mg QE/g DW, respectively. Fenugreek varieties had contained total phenolic content from 127.8 to 139.2 mg GAE/100 g [39]. Flavonoids have to scavenge free radical, anti-inflammatory, and act as antimicroorganism agents [40]. Total phenols and flavonoids in yellow maize were recorded at 2.01 mg GAE/g DW and 0.75 mg QE/g DW, respectively. Phenolic compounds that are present in the maize bran are very helpful for human health.

3.4. Antioxidant Activity of Raw Materials. The results indicated that the antioxidant activity (ABTS, DPPH, and FRAP) in quinoa was 2.02, 5.67, and 5.49 Mmol TEACg-1, respectively. On the other hand, germinated lupin had 4.67, 3.59, and 4.94 Mmol TEACg-1, respectively, and on the other hand, germinated fenugreek reported 4.08, 4.39, and 5.62 Mmol TEACg-1, respectively. The antioxidant activity in yellow maize was reported at 3.98, 3.81, and 5.12 Mmol TEACg-1, respectively. The results of this investigation showed that the germinated sweet lupin showed significantly (p < 0.05) higher total antioxidant activity (ABTS) followed by germinated fenugreek and yellow maize (Figure 2). On the other hand, significantly higher free radical scavenging activity (DPPH) was recorded in quinoa, followed by germinated fenugreek and yellow maize. The free radical scavenging activity (FRAP) was recorded statically at par in all the samples; therefore, the higher FRAP activity was associated with germinated fenugreek. The primary and secondary antioxidant activities of the plant extracts are enhanced due to the presence of the phenolic and flavonoid contents; they readily donate a hydrogen atom to DPPH to give DPPH-H, which is considered a required mechanism of antioxidants [41]. Furthermore, they found a relationship between phenolic content and FRAP [42, 43]. Moreover, they indicated that the phenolic content was linked with ABTS activity [44]. Therefore, it could be noticed that the significance of the phenolic acid content as a reducing agent may be due to the donation of an electron.
3.5. Chemical Analysis, Dietary Fiber, and Antioxidant Activity for Different Blends of Pan Bread. Table 4 indicates the chemical composition, total dietary fibers, and insoluble and soluble dietary fiber, as well as antioxidant quantity (total phenolic and flavonoids) and antioxidant activity that were determined in different blends of pan bread. The results pointed out that crude protein was 13.91 g/100 g DW in the control pan bread made from quinoa and yellow maize, whereas the different blends were increased gradually from 14.96 g/100 g DW in the blend (1) to 18.28 g/100 g DW in the blend (4) pan bread. These gradual increases in blends may be due to the germinated sweet lupin and germinated fenugreek containing the highest protein content of 25.67 and 24.94 g/100 g DW, respectively. Whilst, total lipids, crude fiber, and ash content were slightly increased in different blends of pan bread, which may be the reason the quinoa, germinated sweet lupin, and germinated fenugreek were nearly equal in these parameters. The total dietary, insoluble, and soluble dietary fiber elevated in blend (4) pan bread was 15.39, 10.47, and 4.92 g/100 g DW, respectively, and in the control pan bread was 14.52, 10.2, and 5.50 g/100 g DW, respectively. This slight increase in blended pan bread (4) compared to the control pan bread causes the quinoa, germinated sweet lupin, and germinated fenugreek to be higher in total dietary fiber than the yellow maize. Total phenols and flavonoids in addition to antioxidant activity were found to not significantly vary between the control pan bread and different blends of pan bread for the reason that the ingredients for preparing pan bread contained nearly the same amounts of total phenols and flavonoids.

3.6. Sensory Properties for Different Blends of Pan Bread. Sensory evaluation is one aspect of the greatest importance since consumer acceptance is usually encouraged in the marketing process of any food product. The results showed that the acceptability of pan bread for the blend (3) made from 60% quinoa and 15% from both germinated lupin and fenugreek and 10% yellow maize, followed by blend (2) made from 70% quinoa and 10% from both germinated lupin and fenugreek and 10% yellow maize, and blend (1), which was prepared from 80% quinoa and 5% from both germinated lupin, fenugreek, and 10% yellow maize (Figure 3). The taste and odor of the blends were acceptable. This may be due to the addition of both germinated lupin and fenugreek, which improved the taste and texture by 15%, thus the pan bread became more delicious. The increased 20% addition of both germinated lupin and fenugreek was found in pan bread in the blend (4) and was unacceptable for the taste and odor. Moreover, yellow maize improved the pan bread texture and palatability by gelatinization of the starch. The disappearance of the bitterness of produced pan bread might be due to the germination of both lupin and fenugreek [11]. The fortification of pan bread with quinoa, germinated lupin, germinated fenugreek, and yellow maize flour might be an effect of some sensory properties like color, crust, and crumbs caused by the presence of germinated fenugreek (deep color) and yellow maize. Likewise, it was observed that the deep color of pan bread may be due to Maillard reaction through baking, which may be caused by higher lysine content [45]. The fortification of pan bread with up to 60% quinoa, 15% of both germinated lupin and germinated fenugreek, and 10% yellow maize slightly influenced the texture and appearance of pan bread and was accepted by judges in sensory evaluation.

3.7. Color Analysis for Different Blends of Pan Bread. The color analysis as (L*) lightness, (a*) redness, and (b*) yellowness was estimated in pan bread, and the results are depicted in Figure 4. The results showed that the increasing amount of both germinated lupin and fenugreek flour in the ingredient is up to 20% when making pan bread. Therefore, the pan bread turned brown and red due to the high content of protein in quinoa germinated lupin and fenugreek flour compared with blend (1), which contained 80% quinoa, 20% of both germinated lupin and fenugreek, and 10% yellow maize, followed by blend (2), which contained 70% quinoa, 10% of both germinated lupin and fenugreek, and 10% yellow maize, and blend (3), prepared from 60% quinoa, 15% of both germinated lupin and fenugreek, and 10% yellow maize. The brown color of the pan bread may be caused by the Maillard reaction that occurs among sugars and amino acids through the baking process [46]. The (L*) and (b*) values increased, while the (a*) value decreased in the pan bread blend (4). This may be due to elevation of the natural pigments. Besides, the deep color of the pan bread can be due to the high content of protein and crude fiber of fenugreek and yellow maize flour [11].

3.8. Texture Profile Analysis for Different Blends of Pan Bread. Figure 5 indicates the results of the textural properties of multigrain pan bread. The results indicated that the hardness
of the pan breads gradually increased with the increasing substitution levels of quinoa with germinated lupin and germinated fenugreek from 1.43 g in control, which contained 90% quinoa and 10% yellow maize, to 3.52 g in the blend (4), which contained 50% quinoa, 20% of each germinated lupin and fenugreek, and 10% yellow maize. The results of the present study are in agreement with the previous findings. They reported that bread fortified with seeds and grains may be harder due to the higher protein content, which makes the bread harder [47]. As well as, different blends of gluten-free bread (gluten is responsible for the softness of bread) have shown that the combination (4) pan bread is more crunchy than the control. Moreover, gumminess showed an increase from 0.45 N in the control to 0.76 N in the blend (4), and also, chewiness was increased from 4.62 N in the control pan bread to 8.98 N in the blend (4), whilst springiness was increased from 4.62 in the control to 8.38 in the blend (4). These increases in gumminess, chewiness, and springiness may be due to an increase in dietary fibers in the blends when increasing both germinated

Table 4: Nutrition values of different blends of pan bread on DW.

<table>
<thead>
<tr>
<th>Nutrition values</th>
<th>Control</th>
<th>Blend (1)</th>
<th>Blend (2)</th>
<th>Blend (3)</th>
<th>Blend (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein (g/100 g DW)</td>
<td>13.91 ± 0.94e</td>
<td>14.96 ± 0.97d</td>
<td>16.11 ± 1.05c</td>
<td>17.84 ± 1.13b</td>
<td>18.28 ± 1.34a</td>
</tr>
<tr>
<td>Total lipids (g/100 g DW)</td>
<td>6.05 ± 0.32e</td>
<td>6.15 ± 0.41d</td>
<td>6.29 ± 0.38e</td>
<td>6.38 ± 0.36b</td>
<td>6.51 ± 0.41a</td>
</tr>
<tr>
<td>Crude fiber (g/100 g DW)</td>
<td>11.96 ± 0.71a</td>
<td>12.07 ± 0.83b</td>
<td>12.20 ± 0.86c</td>
<td>12.32 ± 0.84d</td>
<td>12.43 ± 0.96c</td>
</tr>
<tr>
<td>Ash content (g/100 g DW)</td>
<td>4.01 ± 0.12a</td>
<td>4.12 ± 0.23b</td>
<td>4.23 ± 0.13c</td>
<td>4.35 ± 0.19d</td>
<td>4.52 ± 0.13c</td>
</tr>
<tr>
<td>Total carbohydrates (g/100 g DW)</td>
<td>64.07 ± 4.36a</td>
<td>62.70 ± 3.49b</td>
<td>61.17 ± 4.21c</td>
<td>59.11 ± 3.29d</td>
<td>58.26 ± 3.68e</td>
</tr>
<tr>
<td>Total dietary fiber (g/100 g DW)</td>
<td>14.52 ± 0.89c</td>
<td>14.70 ± 1.04b</td>
<td>14.95 ± 1.12a</td>
<td>15.19 ± 1.17e</td>
<td>15.39 ± 1.04d</td>
</tr>
<tr>
<td>Insoluble dietary fiber (g/100 g DW)</td>
<td>10.02 ± 0.82e</td>
<td>10.13 ± 0.86d</td>
<td>10.25 ± 0.91c</td>
<td>10.34 ± 0.76b</td>
<td>10.47 ± 0.76a</td>
</tr>
<tr>
<td>Soluble dietary fiber (g/100 g DW)</td>
<td>4.50 ± 0.14e</td>
<td>4.61 ± 0.15d</td>
<td>4.70 ± 0.18c</td>
<td>4.85 ± 0.24b</td>
<td>4.92 ± 0.17a</td>
</tr>
<tr>
<td>Total phenolic acids mg GAE/g</td>
<td>2.23 ± 0.04e</td>
<td>2.26 ± 0.03d</td>
<td>2.29 ± 0.04c</td>
<td>2.32 ± 0.04b</td>
<td>2.36 ± 0.07a</td>
</tr>
<tr>
<td>Total flavonoid mg QE/g</td>
<td>0.47 ± 0.01e</td>
<td>0.66 ± 0.03d</td>
<td>0.85 ± 0.05c</td>
<td>1.04 ± 0.02b</td>
<td>1.23 ± 0.05c</td>
</tr>
<tr>
<td>ABTS m mol TEAC/g</td>
<td>2.21 ± 0.01e</td>
<td>2.45 ± 0.02d</td>
<td>2.69 ± 0.04c</td>
<td>2.93 ± 0.05b</td>
<td>3.17 ± 0.06a</td>
</tr>
<tr>
<td>DPPH m mol TEAC/g</td>
<td>5.48 ± 0.02e</td>
<td>5.71 ± 0.03d</td>
<td>5.94 ± 0.03c</td>
<td>6.17 ± 0.07b</td>
<td>6.40 ± 0.08a</td>
</tr>
<tr>
<td>FRAP m mol TEAC/g</td>
<td>5.45 ± 0.02e</td>
<td>5.65 ± 0.02d</td>
<td>5.95 ± 0.03c</td>
<td>6.18 ± 0.05b</td>
<td>6.45 ± 0.07a</td>
</tr>
</tbody>
</table>

Figure 3: Sensory properties of pan bread blends.

Figure 4: Color analysis of multigrain pan bread. (L*: lightness & darkness, (a*): redness & greenness, and (b*): yellowness & blueness.)

Figure 5: Texture profile analysis of pan bread blends.
lupin and fenugreek. It showed that gumminess, springiness, and chewiness increased when the usage level of vegetable fibers increased [48]. Whereas the adhesiveness increased gradually in the pan from 14.25 in the control pan bread to 35.57 in pan bread blend (4). The adhesiveness increased by increasing the values of protein and fiber content and also increased by the adhesiveness of pan bread due to the blends of pan bread being gluten-free [49].

4. Conclusion

In conclusion of the present study, the raw materials such as quinoa, germinated lupin, germinated fenugreek, and yellow maize contained high amounts of protein, fiber, minerals, antioxidants, polyphenols, and antioxidant capacity. The results confirmed that the sensory evaluation, color analysis, and texture profile analysis showed that the best pan bread was found by adding 60% quinoa, 20% of both germinated lupin and fenugreek, and 10% yellow maize to prepare functional and gluten-free pan bread for celiac disease patients. This preliminary work brings novel information about the health benefits of pan bread prepared with quinoa, lupin, and fenugreek germinations, which could be very useful not only for the scientific community and celiac patients but also for pharmaceutical and food industries in developing innovative and functional food products to enhance human health.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare no conflicts of interest.

References


