Article

# Development and Characterization of High-Fiber, Gluten-Free Pasta for Celiac Disease Patients 

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#### Abstract

Celiac disease (CD) is a multi-organ complex autoimmune disorder triggered by a glutencontaining diet in genetically predisposed individuals. The only effective treatment for people with CD is strict, lifelong adherence to a gluten-free diet to reduce severe disease outcomes. Therefore, this study aimed to produce high-nutrition gluten-free pasta by substituting wheat flour with lupin flour, flaxseed flour, rice flour, and corn starch. For this purpose, six gluten-free pasta treatments (T1-T6) were produced with different flour compositions. In addition, inulin, xanthan gum, beta-glucan, and Moringa leaf powder in fixed amounts were added to all treatments. For the proximate analysis, color measurements and sensory evaluation were determined for all treatments. Proximate analysis of our results showed that substituting wheat flour with composite flour blends was satisfactory for producing nutritious pasta products without affecting their quality. Compared to the control group, T6 had a significant increase in fiber ( $4.68 \pm 0.25 \mathrm{vs} .1 .24 \pm 0.28$ ), lipid ( $21.99 \pm 0.38 \mathrm{vs} .9 .32 \pm 0.25$ ), protein $(13.84 \pm 0.30$ vs. $13.45 \pm 0.51)$, and ash content $(1.65 \pm 0.07$ vs. $1.28 \pm 0.06)$ of gluten-free pasta. However, the carbohydrate content decreased compared to the control treatment $(46.10 \pm 0.69$ vs. $60.84 \pm 0.75)$. The color measurement evaluation found a significant difference in the lightness $\left(L^{*}\right)$, redness $\left(a^{*}\right)$, and yellowness $\left(b^{*}\right)$ values between the control and all gluten-free pasta treatments. The sensory evaluation of the finished gluten-free pasta treatments and control sample indicated that the quality score for overall acceptability varied widely for different treatments due to individual preferences. Our study concluded that gluten-free pasta with high nutritional value from gluten-free flour is a good alternative product for celiac patients.


Keywords: lupine flour; flaxseed flour; inulin; xanthan gums; Moringa leaf powder

## 1. Introduction

Celiac disease (CD) is a complex autoimmune disease leading to damage to the small intestine, villous atrophy, and, consequently, abnormal absorption of nutrients. Classical symptoms include intestinal disorders such as vomiting, abdominal distension, and chronic diarrhea [1]. On the other hand, about $50 \%$ of CD patients present extra intestinal symptoms, including dermatitis herpetiformis, osteoporosis, anemia, and neurological problems [2]. The prevalence of CD is about 0.6 to $1.0 \%$ worldwide [3], and around 275,818 people suffer from CD at the global level [4]. However, the disease prevalence is underestimated in many countries due to a lack of awareness of the atypical presentation of the disease symptoms [5]. CD is one of the most common diseases, resulting from both environmental
and genetic factors [2]. The determinants of genetic susceptibility include human leukocyte antigen (HLA) and non-HLA genes [6], while environmental factors are mainly related to dietary gluten consumption. Gluten is a major storage protein present in wheat, barley, and rye and directly related to CD severity and disease outcomes [3]. Oats can be considered a gluten-free cereal product, as reported by several studies. However, there are some issues regarding the claims that oats are a gluten-free cereal; oats are described as one of the gluten-containing cereals by the Codex Alimentarius, and the presence of symptoms of exacerbation and malabsorption is believed to be connected to the inclusion of oats in gluten-free diets. Gluten intake causes serious damage to the small intestine mucosa, distinguished by crypt hyperplasia, lymphocytic infiltration, inflammation, and villous flattening, leading to gastrointestinal and abdominal pain, weight loss, and diarrhea [7].

The only effective treatment for CD is strict, lifelong adherence to the gluten- free diet [7]. Nevertheless, the replacement of gluten is a major technological challenge because it is one of the most important structure-building proteins that is responsible for enhancing the quality, elasticity, extensibility, and cohesiveness that contribute to the rheological properties of wheat-based products [8]. Even a small amount of $50 \mathrm{mg} /$ day or less can cause adverse mucosal deterioration in CD patients, leading to a risk of anemia, osteoporosis, secondary inflammation, autoimmunity, malignancies, and even death [9]. However, an alternative approach can be adopted to develop gluten-free products with similar quality and structural properties as cereal products [10].

Numerous gluten-free food products, such as rice, corn, sorghum, millets, and potato/ pea starch, and various gluten replacers, including xanthan gum, have been known as suitable alternatives while maintaining the physical-sensorial properties of gluten-free, cereal-based products [7]. Considering that, lupin is considered an excellent replacement (gluten-free crop) due to its unique nutritional and functional properties in bakery and pastry products [11]. Moreover, lupin contains a range of bioactive antioxidants, carotenoids, and phytoestrogen compounds beneficial to reducing low-density lipoprotein levels, blood pressure, plasma cholesterol, and triglyceride levels [12]. Rice is another alternate type of cereal used in the manufacturing of gluten-free products and is characterized by several health benefits, including hypoallergenic activity and high digestible carbohydrate contents [13]. Concomitantly, corn contains storage proteins called zeins, which are different from gluten and are a rich source of vitamins, minerals, dietary fiber, and functional elements such as phenolics, anthocyanins, $\beta$-carotene, lutein, and flavonoids [13]. In addition, flaxseed is a very good source of soluble and insoluble fiber, which helps to prevent irritation, constipation, bowel syndrome, and diverticular disease [14]. It also has antioxidant, anti-diabetic, and anti-inflammatory functions, which in turn reduce the risk of cardiovascular disorders, renal disorders, bone disorders, and cancer [15]. Considering the health benefits of gluten-free products, and since cereal products that are designed to meet the requirements of celiac disease patients, especially gluten-free pasta, are scant, limited, low-quality, and provide poor mouthfeel and flavor, in addition to their high cost in Jordan and neighboring countries, this study is designed to formulate gluten-free pasta that is high quality, nutritious, and affordable by substituting wheat flour with lupine, flaxseed, rice flour, and corn starch suitable for CD patients.

## 2. Materials and Methods

### 2.1. Pasta Ingredients

### 2.1.1. Flour

Milled lupin flour, rice flour, and flaxseed flour were obtained from the local supplier (Modern Flour Mills and Macaroni Factories Company, Amman, Jordan) and kept at room temperature (from $24^{\circ} \mathrm{C}$ to $28^{\circ} \mathrm{C}$ ) until analysis.

### 2.1.2. Pasta Ingredients

Corn starch, eggs, salt, and vegetable oil were obtained from the local market in Irbid City, Jordan. Moringa leaf powder was obtained from Durrat Almanal for Development
and Training, while inulin, xanthan gum, and beta-glucan powder were obtained from the health company for natural and organic food products (Jothoor, Amman, Jordan).

### 2.1.3. Mixing

First, dry ingredients were weighed and premixed by using an electrical mixer (Kenwood ${ }^{\circledR}$, Hampshire, UK) for 1 min at speed 4 , then eggs and oil were added and mixed for one more min. Later, the water was added, and the dough was mixed for 5 more minutes to combine the ingredients. The dough was then briefly kneaded by hand, covered with plastic wrap, and left to rest for about 30 min . The dough was divided into three pieces, and each piece was rolled out with a rolling pin (20-25 passes) over a flat board until a final thickness of dough (approximately 2 mm ). The dough was then formed into the desired shape (fettuccine) by using a pasta-making machine (Marcato Atlas 150 Stainless Steel Manual Pasta Maker Machine, Campodarsego, Italy). Finally, the pasta samples were left uncovered in a dry area for 24 h at $25^{\circ} \mathrm{C}$ and $52 \%$ relative humidity to dry in the open air and carefully turned at regular intervals to dry and prepare for cooking [16].

### 2.1.4. Cooking

For cooking, 300 mL of water was heated in a small cooking pot until boiling. Twenty-five $\pm 0.5 \mathrm{~g}$ of pasta sample were added to the boiling water, boiled for 10 min , and then drained through a colander.

### 2.2. Experimental Design

### 2.2.1. Control Sample

The basic raw ingredients that were used in pasta production are illustrated in Table 1.
Table 1. Raw ingredients of the pasta.

| Ingredient | Amount |
| :--- | :--- |
| Wheat Flour $(\mathrm{g})$ | 240 |
| Salt $(\mathrm{g})$ | 1.0 |
| Egg $(\mathrm{g})$ | 60 |
| Water $(\mathrm{mL})$ | 150 |
| Oil $(\mathrm{g})$ | 4.67 |

The formulation of the control sample is shown in Table 2, which consists of basic raw ingredients and a fixed amount of xanthan gum ( $0.4 \%$ ), inulin ( $2 \%$ ), beta-glucan ( $2 \%$ ), and Moringa leaf powder ( $2 \%$ ) that were determined through preliminary experiments.

Table 2. Control pasta formula.

| Ingredient | Amount |
| :--- | :---: |
| Wheat Flour $(\mathrm{g})$ | 240 |
| Salt $(\mathrm{g})$ | 1.0 |
| Egg $(\mathrm{g})$ | 60 |
| Water $(\mathrm{mL})$ | 150 |
| Oil $(\mathrm{g})$ | 4.67 |
| Xanthan gum $(\mathrm{g})$ | 1.0 |
| Inulin $(\mathrm{g})$ | 4.8 |
| Beta-glucan $(\mathrm{g})$ | 4.8 |
| Moringa oleifera leaves $(\mathrm{g})$ | 4.8 |

### 2.2.2. Preliminary Analysis

Preliminary work was carried out to determine the appropriate amount of xanthan gum, inulin, beta-glucan, and Moringa oleifera leaves by preparing three samples of pasta using wheat flour ( $100 \%$ ) in order to find the optimal formula in terms of taste, texture, color, and aroma of pasta (Tables 3 and 4).

Table 3. The ingredient percentage levels of xanthan gum, inulin, beta-glucan, and Moringa in preliminary work (\%).

| Ingredients | Sample 1 | Sample 2 | Sample 3 |
| :--- | :--- | :--- | :--- |
| Xanthan gum (\%) | 0.20 | 0.4 | 0.6 |
| Inulin (\%) | 1 | 2 | 3 |
| Beta-glucan (\%) | 1 | 2 | 3 |
| Moringa oleifera leaves (\%) | 1 | 2 | 3 |

Table 4. Ingredients and amounts of the preliminary analysis.

| Ingredient | Sample 1 | Sample 2 | Sample 3 |
| :--- | :--- | :--- | :--- |
| Flour $(\mathrm{g})$ | 240 | 240 | 240 |
| Salt $(\mathrm{g})$ | 1.0 | 1.0 | 1.0 |
| Egg $(\mathrm{g})$ | 60 | 60 | 60 |
| Water $(\mathrm{mL})$ | 150 | 150 | 150 |
| Oil $(\mathrm{g})$ | 4.67 | 4.67 | 4.67 |
| Xanthan gum $(\mathrm{g})$ | 0.5 | 1 | 1.5 |
| Inulin $(\mathrm{g})$ | 2.4 | 4.8 | 7.2 |
| Beta-glucan $(\mathrm{g})$ | 2.4 | 7.8 | 7.2 |
| Moringa leaf powder $(\mathrm{g})$ | 2.4 |  |  |

Seven trained panelists participated in the initial analysis of the three-pasta samples (preliminary work) to determine the appropriate amount of xanthan gum, inulin, betaglucan, and Moringa oleifera leaves to be added to the final product. Xanthan gum in the amounts of $0.5 \mathrm{~g}, 1 \mathrm{~g}, 1.5 \mathrm{~g}$ and inulin, $\beta$-glucan, and Moringa oleifera in the amounts of $2.4 \mathrm{~g}, 4.8 \mathrm{~g}, 7.2 \mathrm{~g}$ were added to the three-pasta samples, respectively, and panelists decided to add 1 g of xanthan and 4.8 g of inulin, $\beta$-glucan, and Moringa oleifera due to their suitable firmness and mouthfeel and to overcome the structural problems of gluten-free pasta designed for CD patients. Furthermore, pasta dough containing 4.8 g of inulin, $\beta$-glucan, and Moringa oleifera was accepted after consensus, considering the best suitable nutritional, physical, and chemical properties, as well as its stabilizing and thickening properties, as well as its gelation and emulsification properties. Finally, sample 2 was decided to be a suitable composition to meet the research objectives.

### 2.2.3. Treatment Formulation

For this study, six gluten-free pasta treatments (T1-T6), including lupin, flaxseed, rice flour, and corn starch, were developed with different flour compositions. Different levels of lupin flour, flaxseed flour, corn starch, and rice flour were used in the pasta formulation based on the ratio of lupine flour to flaxseed flour (4:1) and the ratio of corn starch to rice flour (1:1), as shown in Table 5.

Table 5. Composition of different pasta treatments.

|  | Treatment |
| :--- | :--- |
|  |  |
|  | 0\% Lupin flour |
| Treatment 1 (T1) | $0 \%$ Flaxseed flour |
|  | $50 \%$ Corn starch |
|  | $50 \%$ Rice flour |
|  | $8 \%$ Lupin flour |
| Treatment 2 (T2) | $2 \%$ Flaxseed flour |
|  | $45 \%$ Corn starch |
|  | $45 \%$ Rice flour |
|  | $16 \%$ Lupin flour |
| Treatment 3 (T3) | $4 \%$ Flaxseed flour |
|  | $40 \%$ Corn starch |
|  | $40 \%$ Rice flour |
|  | $24 \%$ Lupin flour |
| Treatment 4 (T4) | $6 \%$ Flaxseed flour |
|  | $35 \%$ Corn starch |
|  | $35 \%$ Rice flour |
|  | $32 \%$ Lupin flour |
| Treatment 5 (T5) | $8 \%$ Flaxseed flour |
|  | $30 \%$ Corn starch |
|  | $30 \%$ Rice flour |
|  | $40 \%$ Lupin flour |
| Treatment 6 (T6) | $10 \%$ Flaxseed flour |
|  | $25 \%$ Corn starch |
|  |  |

In addition, inulin, xanthan gum, beta-glucan, and Moringa leaf powder in fixed amounts were added to all treatments. A fixed amount of xanthan gum ( $0.4 \%$ ), inulin ( $2 \%$ ), beta-glucan ( $2 \%$ ), and Moringa leaf powder ( $2 \%$ ) were determined through preliminary experiments (Table 6).

Table 6. Formulations of gluten-free pasta treatments.

| Treatment | Lupin <br> Flour $\mathbf{( g )}$ | Flaxseed <br> Flour $(\mathbf{g})$ | Corn <br> Starch $\mathbf{( g )}$ | Rice Flour <br> $\mathbf{( g )}$ | Xanthan <br> Gum $(\mathbf{g})$ | Inulin <br> $\mathbf{( g )}$ | Beta-Glucan <br> $\mathbf{( g )}$ | Moringa Leaf <br> Powder (g) |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :---: |
| T1 | 0 | 0 | 120 | 120 | 1 | 4.8 | 4.8 | 4.8 |
| T2 | 19.2 | 4.8 | 108 | 108 | 1 | 4.8 | 4.8 | 4.8 |
| T3 | 38.4 | 9.6 | 96 | 96 | 1 | 4.8 | 4.8 | 4.8 |
| T4 | 57.6 | 14.4 | 84 | 84 | 1 | 4.8 | 4.8 | 4.8 |
| T5 | 76.8 | 19.2 | 72 | 72 | 1 | 4.8 | 4.8 | 4.8 |
| T6 | 96 | 24 | 60 | 60 | 1 | 4.8 | 4.8 | 4.8 |

### 2.2.4. Proximate Analysis

The proximate analyses (moisture content, protein content, crude fiber content, fat content, and carbohydrate content) were determined according to the Association of Official Analytical Chemists (AOAC 978.04) methods [17]. The carbohydrate contents were calculated by difference. Measurements of the proximate analyses were replicated four times for accuracy, and the results were expressed as the average value.

### 2.2.5. Physical Analysis

Physical properties, including color measurement, were performed for the pasta sample, control, and treatment groups. The color measurements were replicated six times
for accuracy, and then the average value was determined for the final analysis. The color of the pasta samples, control, and treatments was measured by a Minolta colorimeter CR-300 (Ramsey, NJ, USA) and recorded in the L $\mathrm{L}^{*} \mathrm{a}^{*} \mathrm{~b}$ color system. The $\mathrm{L}^{*} \mathrm{a}^{*} \mathrm{~b}$ color system consists of a luminance or lightness component ( $\mathrm{L}^{*}$ ) and 2 chromatic components: the ( $\mathrm{a}^{*}$ ) component for green $(-a)$ to red $(+a)$ and the $\left(b^{*}\right)$ component from $(-b)$ to yellow $(+b)$ colors. The colorimeter was calibrated using a standard white plate. The values of the white standard were $\mathrm{L}=97.1 \mathrm{a}=+0.13, \mathrm{~b}=+1.88$. The color was measured at two dissimilar positions, and the external color of both sides was measured.

### 2.2.6. Sensory Evaluation

A sensory evaluation test was performed to evaluate the quality and acceptability of the control and treatment groups. The sensory evaluation test was conducted at Jordan University of Science and Technology laboratories. A total of 40 consumers of different ages and genders from Jordan University of Science and Technology were selected and involved in the sensory evaluation test. All participants received information about the test, and all participants provided their informed consent to participate in the study. All consumers were trained by the researcher using standard product evaluation criteria. The sensory evaluation was carried out by using the following five-point hedonic scale: 5 : like extremely 4 : slightly 3: neither like nor dislike; 2: dislike slightly; and 1: dislike extremely. The pasta was cooked without any pasta sauce provided to consumers. Each pasta sample was analyzed for color, taste, aroma, texture, appearance, and overall acceptability. The consumers scored the pasta samples according to their preferences for each sensory attribute, and $p<0.05$ was used to determine the statistical significance of the sensory attribute. The hedonic scale test was used for color, taste, aroma, texture, appearance, and overall acceptance [18].

### 2.2.7. Statistical Analysis

Data were analyzed using the general linear model procedure with the SAS Version 8.2 software package (SAS 2002, SAS Institute Inc., Cary, NC, USA). Means were separated by least significant difference (LSD) analysis at an LSD of $0.05 p$ value.

## 3. Results and Discussion

### 3.1. Chemical Composition of the Pasta Treatments

The results of the proximate analysis for raw flours (wheat flour, lupine flour, rice flour, flaxseed flour, and corn starch) are shown in Table 7.

Table 7. Chemical composition of different gluten-free pasta treatments and the control.

| Flours | Moisture \% * | Ash \% * | Protein \% * | Fiber \% * | Lipid \% * | Carbohydrate \% * |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Control | $13.89 \pm 0.38{ }^{\text {a }}$ | $1.28 \pm 0.06^{\text {c }}$ | $13.45 \pm 0.51{ }^{\text {a }}$ | $1.24 \pm 0.28{ }^{\text {f }}$ | $9.32 \pm 0.25{ }^{\text {f }}$ | $60.84 \pm 0.75{ }^{\text {b }}$ |
| T1 | $13.26 \pm 0.34{ }^{\text {b }}$ | $1.17 \pm 0.08^{c}$ | $6.70 \pm 0.65{ }^{\text {e }}$ | $2.19 \pm 0.30{ }^{\text {e }}$ | $14.29 \pm 0.60{ }^{\text {e }}$ | $62.38 \pm 1.28{ }^{\text {a }}$ |
| T2 | $13.24 \pm 0.45{ }^{\text {b }}$ | $1.41 \pm 0.10^{\text {b }}$ | $9.56 \pm 0.41^{\text {d }}$ | $1.83 \pm 0.24{ }^{\text {e }}$ | $14.0 \pm 0.08^{\text {e }}$ | $59.68 \pm 0.46{ }^{\text {b }}$ |
| T3 | $12.90 \pm 0.22^{\text {b }}$ | $1.49 \pm 0.07^{\text {b }}$ | $10.79 \pm 0.24^{\text {d }}$ | $2.79 \pm 0.23{ }^{\text {d }}$ | $15.33 \pm 1.05^{\text {d }}$ | $57.93 \pm 1.23{ }^{\text {c }}$ |
| T4 | $11.84 \pm 0.38^{\text {c }}$ | $1.50 \pm 0.04{ }^{\text {b }}$ | $12.79 \pm 0.22^{\text {c }}$ | $3.33 \pm 0.28^{\text {c }}$ | $16.64 \pm 0.56^{\text {c }}$ | $55.90 \pm 0.88{ }^{\text {d }}$ |
| T5 | $12.90 \pm 0.33{ }^{\text {b }}$ | $1.47 \pm 0.10^{\text {b }}$ | $12.07 \pm 0.19^{\text {b }}$ | $3.95 \pm 0.14{ }^{\text {b }}$ | $19.87 \pm 0.48^{\text {b }}$ | $49.56 \pm 0.50{ }^{\text {e }}$ |
| T6 | $11.92 \pm 0.48^{\text {c }}$ | $1.65 \pm 0.07{ }^{\text {a }}$ | $13.84 \pm 0.30^{\text {a }}$ | $4.68 \pm 0.25{ }^{\text {a }}$ | $21.99 \pm 0.38{ }^{\text {a }}$ | $46.10 \pm 0.69{ }^{\text {f }}$ |

Column values with the same letters were not significantly different ( $p \leq 0.05$ ). Column values with different letters were significantly different ( $p \leq 0.05$ ). * These values are the average of four replicates $\pm$ the standard deviation. Control sample: $100 \%$ wheat flour.

### 3.1.1. Moisture Content

The results of the moisture content percent indicate the percentages of moisture content of the control sample (13.89) and pasta treatments (T1: 13.26), (T2: 13.24), (T3: 12.9), (T4: 11.84), (T5: 12.9), and (T6: 11.92), respectively. This variation in the moisture content might be due to the various levels of water holding capacity of the components of different treatments. According to the Afifah (2017) study, moisture content generally decreased
as the level of lupin and flaxseed composite flour increased [19]. This could be due to the protein content, which appears to be a critical factor in the ability of the flours to absorb more water. Accordingly, the reduction in the ability of wheat flour to bind water may be attributed to its low protein content [20]. Therefore, this could explain the reason that water absorption increased with an increase in protein content because lupin flour contains more protein than wheat flour [21]. In addition, the higher water absorption capacity of wheat flour could be attributed to the presence of a higher amount of carbohydrates (starch) in the flour [22]. The control sample moisture content was significantly higher ( $p<0.05$ ) compared to other treatments, while T4 and T6 had the lowest moisture content, and there was no significant difference between them.

### 3.1.2. Ash Content

The percentages of ash content in the control sample and pasta treatments were 1.28 for the control and $1.17,1.41,1.49,1.50,1.47$, and 1.65 for $\mathrm{T} 1, \mathrm{~T} 2, \mathrm{~T} 3, \mathrm{~T} 4, \mathrm{~T} 5$, and T 6 , respectively. Compositional differences between the flour blends resulted in differences in pasta ash content [22]. The results of the ash content indicated that there are significant differences between the control and gluten-free pasta treatments, except for T1. The ash contents of the gluten-free pasta treatments were generally higher than those of the control group. It is shown that T6 showed the highest ash content and was significantly higher ( $p<0.05$ ) compared to other treatments and the control sample. The ash content of the pasta treatments increased with the addition of lupin flour and flaxseed, since the average ash content in lupin was $2.65 \%$ [23] and 3-4\% in flaxseed [24].

### 3.1.3. Protein Content

The percentage of protein content of the control sample and pasta treatments for the control (13.45), T1 (6.70), T2 (9.56), T3 (10.79), T4 (12.79), T5 (12.07), and T6 (13.84), respectively. The percentage of protein content in the gluten-free pasta treatments varied significantly ( $p<0.05$ ) from each other. The differences in crude protein content might be due to compositional differences in the flour blends [25]. Gluten refers to a family of proteins, mainly glutenin and gliadin (prolamins), a storage protein in the endosperm of cereal grains such as wheat, rye, and barley and found in different amounts in each cereal [26]. As can be seen, T1 contains the least protein content compared to all other treatments because corn starches have a smaller amount of protein as compared to rice [27]. The percentage of protein increased significantly as the percentage of flaxseed and lupin flour incorporated into the samples increased. This could be due to the lupin and flaxseed flour protein content, which contains about 40-45\% protein in lupin [28] and about 28-30\% protein in flaxseed [29]. Our results showed that control pasta ( $100 \%$ wheat) was high in protein due to the protein content in wheat. There was no significant difference in the protein content between T6 and the control, while T6 was significantly higher in protein content compared to other treatments.

### 3.1.4. Crude Fiber Content

The percentages of fiber content in control sample 1.24 and pasta treatments were $2.19,1.83,2.79,3.33,3.95$, and 4.68 , respectively. The results of the fiber content indicated that there was a significant difference between the control sample and different glutenfree pasta treatments. The crude fiber content of gluten-free pasta treatments was higher than the control sample because of the incorporation of fiber-rich ingredients (lupin and flaxseed flour). The fiber content of flaxseed is about 20-28\% of total dietary fiber [24], and lupin flour is about $25-30 \%$ fiber [28]. The percentage of crude fiber content in the gluten-free pasta treatments varied significantly ( $p<0.05$ ) from each other except for T1 and T2, which were insignificantly different ( $p>0.05$ ) from each other. On the other hand, T6 was significantly higher in fiber content compared to other treatments and the control, which had the lowest fiber content.

### 3.1.5. Lipid Content

The results of the lipid content percent are shown in Table 7. The percentages of lipid content of the control sample and pasta treatments were 9.32 for the control group, 14.29 for T1, 14.0 for T2, 15.33 for T3, 16.64 for T4, 19.87 for T5, and 21.99 for T6, respectively. The results of the lipid contents indicate that there was a significant difference between the control and different gluten-free pasta treatments. It is shown that the lipid content of the gluten-free pasta formulations was higher than the control because of the incorporation of flaxseed and lupin flour into the samples, since the average fat content is $30-40 \%$ in flaxseed [24] and $15 \%$ in lupin [30]. The control sample showed the least lipid content since wheat flour contains about $2.10 \%$ fat. The percentage of lipid content in gluten-free pasta treatments varied significantly $(p<0.05)$ from each other except for T 1 and T 2 .

### 3.1.6. Carbohydrate Content (NFE)

The percentages of carbohydrate content of the control sample and pasta treatments were $62.38,59.68,57.93,55.90,49.56$, and 46.10 for T1, T2, T3, T4, T5, and T6, respectively, as compared to the control group (60.84). The percentage of carbohydrate content in the gluten-free pasta treatments varied significantly ( $p<0.05$ ) from each other. It is shown that T1 has the highest carbohydrate content compared to all other treatments since it contains $50 \%$ rice flour, $50 \%$ corn starch, and no flaxseed or lupin flour. Table 7 illustrates that the carbohydrate content was also high in the control sample ( $100 \%$ wheat flour), since wheat flour contains $78.10 \%$ carbohydrates [31]. It is shown that the percentage of carbohydrates in gluten-free pasta treatments decreased as the percentage of flaxseed and lupin flour incorporated into the samples increased. The results may be attributed to flaxseed's low carbohydrates (sugars and starch) [32] as well as lupin flour, which contains negligible amounts of sugar and starch [28].

### 3.1.7. Color Properties of Pasta

Color is one of the most important quality attributes influencing consumer food choices, perceptions, and purchase behavior. The effects of the gluten-free pasta formulation on color measurements were assessed and are presented in Table 8.

Table 8. Color evaluation of different gluten-free pasta treatments and the control.

| Treatment | L (Lightness) * |  | a (Redness) * |  | B (Yellowness) * |  | $\Delta \mathrm{E}$ |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Mean | $\pm$ SE | Mean | $\pm$ SE | Mean | $\pm$ SE | Mean | $\pm$ SE |
| Control | $42.17{ }^{\text {a }}$ | $\pm 1.57$ | $-6.86{ }^{\text {c }}$ | $\pm 0.62$ | $26.66{ }^{\text {d }}$ | $\pm 0.53$ | - | - |
| T1 | $33.42{ }^{\text {c }}$ | $\pm 1.67$ | $-6.46{ }^{\text {bc }}$ | $\pm 0.54$ | $19.08{ }^{\text {f }}$ | $\pm 0.58$ | $17.30{ }^{\text {bc }}$ | $\pm 1.73$ |
| T2 | $34.43{ }^{\text {bc }}$ | $\pm 1.58$ | $-6.46{ }^{\text {bc }}$ | $\pm 0.68$ | $23.04{ }^{\text {e }}$ | $\pm 1.21$ | $12.89{ }^{\text {c }}$ | $\pm 1.69$ |
| T3 | $31.22{ }^{\text {c }}$ | $\pm 1.73$ | $-5.15{ }^{\text {bc }}$ | $\pm 0.59$ | $26.55{ }^{\text {d }}$ | $\pm 1.68$ | $15.38{ }^{\text {bc }}$ | $\pm 2.19$ |
| T4 | $31.54{ }^{\text {c }}$ | $\pm 2.08$ | $-4.92{ }^{\text {b }}$ | $\pm 1.24$ | $30.94{ }^{\text {c }}$ | $\pm 0.42$ | $17.72{ }^{\text {b }}$ | $\pm 2.04$ |
| T5 | 38.52 ab | $\pm 0.56$ | $-0.78{ }^{\text {a }}$ | $\pm 0.25$ | $35.43{ }^{\text {b }}$ | $\pm 0.67$ | $18.54{ }^{\text {b }}$ | $\pm 0.99$ |
| T6 | $31.33{ }^{\text {c }}$ | $\pm 1.41$ | $-0.76{ }^{\text {a }}$ | $\pm 0.28$ | $39.52^{\text {a }}$ | $\pm 1.68$ | $29.82{ }^{\text {a }}$ | $\pm 0.84$ |
| LSD | 4.52 |  | 3.13 |  | 3.13 |  | 4.79 |  |
|  | Column values with the same letters were not significantly different ( $p \leq 0.05$ ). Column values with different letters were significantly different $(p \leq 0.05)$. * These values are the average of four replicates $\pm$ the standard deviation (SD). Control sample: $100 \%$ wheat flour. Standard Errors (SE). |  |  |  |  |  |  |  |

Changes in the color of the pasta $(\Delta \mathrm{E})$ were determined relative to the control sample, which is taken as a reference. The $L^{*}, a^{*}$, and $b^{*}$ color coordinates represent lightness/darkness, redness/greenness, and yellowness/blueness, respectively. The $L^{*}, a^{*}$, and $b^{*}$ values of the control and gluten-free pasta treatments showed that the ranges of pasta color for $\mathrm{L}^{*}, \mathrm{a}^{*}$, and $\mathrm{b}^{*}$ were 42.17 to $31.22,-0.78$ to -6.86 , and 39.52 to 19.08 , respectively.

Our results found that color parameters $\left(L^{*}, a^{*}, b^{*}\right)$ significantly changed between the control and gluten-free pasta treatments. The difference in color characteristics may be attributed to the differences in colored pigment in the flours, which in turn depend on the biological origin of the plant [33]. The differences in color values among the pasta treatments may be due to the variation in percentage of the composite flour for each treatment due to the natural coloring component of composite flour [11].

The color of wheat pasta (control) differs from all the gluten-free pasta treatments with higher brightness ( $\mathrm{L}^{*}=42.17$ ) and higher greenness $(a=-6.86)$. This could be due to the high levels of naturally occurring carotenoids (mainly b-carotene and zeaxanthin) in the lupin flour content [12], which resulted in a darker product (with lower $L^{*}$ values) as compared with the control. In addition, flaxseed flour had a significant effect on the color of the gluten-free pasta treatments. The lightness ( $L^{*}$ value) of the gluten-free pasta decreased as the flaxseed flour concentration increased. The darker color of the gluten-free pasta treatments may be attributed to the higher content of ash and the specific color of the legume flour [11].

Regarding the $\mathrm{a}^{*}$ values, which are associated with redness/greenness, the control pasta sample and gluten-free pasta treatments tend to have a greenish color because of the addition of dried Moringa leaf powder, which reflects its natural green color, which could be due to the mixture of chlorophyll [34]. Meanwhile, the yellowish color ( $\mathrm{b}^{*}$ ) of the control pasta sample and gluten-free pasta treatments diminished slightly with the addition of dried Moringa leaf powder.

Total color differences $(\Delta \mathrm{E})$, which represent the magnitude of the color difference between the reference pasta (control) and each pasta treatment, were in the range of 12.89 to 29.82 ( $p<0.05$ ). T6 had the most intense color and was significantly different ( $p<0.05$ ) from other treatments since it received the highest $\Delta \mathrm{E}$ value of 29.82 , whereas T 2 was close to the reference sample (control) with a $\Delta \mathrm{E}$ value of 12.89 .

### 3.1.8. Sensory Evaluation of Pasta

Based on the results of the sensory analysis, it was shown that the values of the color score generally increased as the level of lupin and flaxseed composite flour increased, except for T5. This could be due to the specific color of the lupin and flaxseed flour. The color of the pasta is slightly greenish because of the addition of dried Moringa leaf powder, which reflects its natural green color. Color score values were significantly different between control and gluten-free pasta treatments and among gluten-free pasta treatments.

Colored pasta in recent years has achieved market success and is well accepted by consumers. T6 had the most intense color and was also visually pleasing among testers in terms of color. T2 and T6 received the highest taste score, while the sensory score for taste generally decreased in T3, T4, and T5 in comparison with the control sample, which might be because of the beany flavor of grain legumes [12].

The sensory score for aroma generally decreased with increasing levels of lupin and flaxseed flour in comparison with the control pasta ( $100 \%$ wheat flour), which received the highest aroma score. This could be due to the high protein contents of the lupin and flaxseed flours and the beany flavor of the grain legumes [12]. The texture score generally decreased in comparison with the control pasta, except for T4. This could be because lupine flour is not capable of forming a gluten matrix that affects the overall structural characteristics [12]. Even though the texture of gluten-free pasta treatments is not the same as that of the control ( $100 \%$ wheat pasta), it is acceptable for consumers. This acceptance among consumers could be due to the addition of inulin, which can enhance the textural and rheological properties. In addition, xanthan gum was added in order to imitate the gluten network and improve food texture [35]. Moreover, the lipids of the flaxseed flour could contribute to the lubrication of the gluten network, minimize the discontinuity that occurs in the protein network, and favor the texture of the product [12,32]. In addition, as shown in Table 9, the values of the texture scores were not significantly different between
the control and gluten-free pasta treatments, except for T3, which was significantly different from the control and other gluten-free pasta treatments.

Table 9. Sensory evaluation of different gluten-free pasta treatments and the control *.

| Treatments | Color | Taste | Aroma | Texture | Appearance | Acceptability | Average |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Control | $3.30^{\mathrm{ab}}$ | $3.28^{\mathrm{bc}}$ | 3.70 | $3.45^{\mathrm{a}}$ | 3.10 | $3.00^{\mathrm{bc}}$ | $3.30^{\mathrm{bc}}$ |
| T1 | $2.98^{\mathrm{b}}$ | $3.23^{\mathrm{bc}}$ | 3.55 | $3.18^{\mathrm{a}}$ | 2.68 | $2.45^{\mathrm{c}}$ | $3.01^{\mathrm{dc}}$ |
| T2 | $3.65^{\mathrm{a}}$ | $3.95^{\mathrm{a}}$ | 3.58 | $3.28^{\mathrm{a}}$ | 3.28 | $3.55^{\mathrm{ab}}$ | $3.55^{\mathrm{a}}$ |
| T3 | $3.35^{\mathrm{ab}}$ | $2.83^{\mathrm{c}}$ | 2.93 | $2.38^{\mathrm{b}}$ | 2.83 | $2.98^{\mathrm{c}}$ | $2.88^{\mathrm{e}}$ |
| T4 | $3.75^{\mathrm{a}}$ | $2.98^{\mathrm{c}}$ | 3.38 | $3.53^{\mathrm{a}}$ | 2.88 | $2.80^{\mathrm{c}}$ | $3.22^{\mathrm{dc}}$ |
| T5 | $2.88^{\mathrm{b}}$ | $3.08^{\mathrm{bc}}$ | 3.48 | $3.15^{\mathrm{a}}$ | 2.98 | $2.88^{\mathrm{c}}$ | $3.07^{\mathrm{de}}$ |
| T6 | $3.43^{\mathrm{ab}}$ | $3.55^{\mathrm{ab}}$ | 3.63 | $3.28^{\mathrm{a}}$ | 3.28 | $3.75^{\mathrm{a}}$ | $3.48^{\mathrm{ab}}$ |
| LSD | 0.57 | 0.57 | NS | 0.56 | NS | 0.56 | 0.23 |

Column values with the same letters were not significantly different ( $p \leq 0.05$ ). Column values with different letters were significantly different $(p \leq 0.05)$. * These values are the average of four replicates $\pm$ the standard deviation (SD). Control sample: $100 \%$ wheat flour.

## Appearance and Overall Acceptability Score

There were no significant differences in appearance between the control and glutenfree pasta treatments. T2 and T6 received the highest appearance scores compared to the control and other gluten-free pasta testaments. Regarding the overall acceptability score, it was shown that T2 and T6 received the highest overall acceptability scores compared to the control and other gluten-free pasta treatments. The overall acceptability score varied for different treatments based on individual preference, and changes in general trend and acceptability criteria were also reported in previous studies [14].

## 4. Conclusions

Based on the study, we conclude that gluten-free pasta comprised of lupin flour, flaxseed flour, corn starch, and rice flour is a good alternative product for celiac patients. Regarding the different treatments, T6 had the greatest increase in fiber, lipid, protein, and ash content and a decreased carbohydrate content when compared to the control treatment. All treatments showed a high nutritional value, were convenient for consumption, and had a good texture that was palatable to the consumer. Also, replacing wheat flour with composite flour blends can be a suitable alternative for producing nutritious pasta without affecting its quality. Therefore, this product can also be used for patients with diabetes, cardiovascular disease, and hypertension, in addition to CD. However, further research is needed on the improvement of new gluten-free pasta formulas and their nutritional quality.

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