EFFECTS OF INCORPORATION OF LUPIN FLOUR ON THE QUALITY ATTRIBUTES OF BEEF BURGER

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Supporting Information

ABSTRACT: Lupin flour could have the potential to be an alternative to meat products due to its nutritional, health, and functional properties. A factorial experiment was performed to investigate the effect of lupin seed flour treatment (without, steaming, and roasting), meat substitution level with lupin seed flour (0, 5, 10, and 15%), and the interaction between them on the quality attributes of cooked beef burger by measuring CIELAB color, texture profile analysis (TPA), chemical composition (before and after cooking), and cooking properties (cooking loss, fat and moisture retention, and shrinkage). Based on the results of the factorial experiment, a completely randomized design was used to evaluate the sensory attributes of selected treatments. The different substitution levels mainly affected CIELAB color values, chemical composition, and cooking properties. On the other hand, the interaction effect between substitution level and treatment affected TPA. Considering all results, steaming treatment and a substitution level of 10% were selected as the best treatment to produce beef burgers. In comparison to the control burger, the developed burger had higher values of L* (increased by 21.26%), b* (increased by 32.94%), and moisture retention (increased by 37.85%); lower values of fat (decreased by 16.11%), protein (decreased by 6.37%), and moisture content (decreased by 2.64%); and nonsignificantly different values with other tests performed. This study demonstrated that the incorporation of lupin flour in beef burgers could have the potential to substitute meat, create an alternative burger with a high percentage of plant protein, and expand the application of lupin flour in the food industry.

Keywords: Beef, Chemical composition, Cooking loss, Physical properties, Sensory properties, Texture.

INTRODUCTION

There is an increasing trend globally for replacing meat with plant-based products (Estell et al., 2021; Smetana et al., 2021; Bryant et al., 2022); it is among the top three trends (Estell et al., 2021). This trend comes as a response to the increased problems encountered in meat production. Pollution, high carbon footprint, the spread of animal diseases (Zhang et al., 2022), negative impacts on the environment (Smetana et al., 2021), increased social cost, animal welfare (Siegrist and Hartmann, 2023), and adverse health effects (Marinova and Bogueva, 2019) are examples of problems of meat production.

Different meat alternatives are investigated in the literature, such as cultured meat, microbial proteins, insect proteins, and plant-based proteins (Zhang et al., 2022), where the last type is the most promising one in terms of acceptability by consumers (Siegrist and Hartmann, 2023). Plant-based meat alternatives are mostly centered on pulses. Lupin is one of the candidates to replace meat due to its nutritional and health benefits. Lupin contains 30-42% proteins, 30-41% fibers (mainly insoluble), fat in the form of mono poly-unsaturated fatty acids, minerals, vitamins, and antioxidants. Another nutritional benefit of lupin compared to other legumes is the low antinutritional factors (Abreu et al., 2023). Several review papers reviewed lupin’s health benefits, with the most recent being conducted by Bryant et al. (2022), which reported strong indicators that lupin consumption improves satiety, reduces blood pressure, and lower degree indicators of decreasing serum lipids and improves glycemic index. Lupin addition was investigated in different food products; Abreu et al. (2023) recently reviewed papers that investigated adding lupin to different food products. However, it has been reported that lupin protein lacks gelling and thickening properties, which may limit its use as a food ingredient (Abreu et al., 2023).

One of the most popular meat products is the beef burger, whose market size in 2020 was 862 billion USD, representing 36% of the global fast food market (Petrat-Melin and Dam, 2023). Therefore, reducing the meat in burgers by replacing it with plant protein will participate in the global reduction of meat production. To the best of our knowledge, limited studies evaluated lupin seed flour as a meat alternative in burgers. These studies investigated the final products using chemical and sensory methods with no information regarding the effect of using lupin on instrumental color and texture values (El-Sayed, 2009; Dalain et al., 2023). In addition, little information is available about the use of different treatments on the functionality of lupin flour. Therefore, the current study aimed to investigate the effects of different treatments of lupin seed flour and different meat substitution levels on the quality attributes of cooked beef burgers.
MATERIALS AND METHODS

Materials
To conduct the current study, the following ingredients were used: frozen beef meat (18.34% protein, 10.66% fat, and 67.67% moisture as tested by Food scan) was imported from Brazil and obtained from the national poultry company: sweet lupin seeds (Egypt) obtained from Al-Sufara’a bakery, refined salt (Amra, Jordan), and burger spices were obtained from Al-Jada’el company (Amman, Jordan).

Experiment design
A 3*4 factorial experiment with two replicates was performed to study the effects of lupin seed treatment (without, steamed, and roasted) and different levels of lupin seed flour used to replace meat (0, 5, 10, and 15%) on the quality attributes of beef burgers. Based on the results of the factorial experiment, selected samples from different treatments were sensory evaluated using a completely randomized design.

The basic formula for the preparation of the meat burger
A commercial beef burger recipe was adopted and modified from one of the local meat suppliers (national poultry company, meat processing plant, Al Karak-Jordan). The components of the formula were as follows: Brazilian beef meat (89%), salt (0.40%), spices (0.1%), and water (10.5%).

Treatment of lupin seeds and preparation of lupin flour
The roasting and steaming treatments were used to modify the functional properties of lupin seeds. 200 g of lupin seeds were utilized, with two replicates per treatment. In roasting treatment, the lupin seeds were milled (high-speed multifunction comminutor, WM-500, China), and then the flour passed a sieve with a diameter of 1 micron. Flour was roasted in an oven at 160 °C for 10 mins (JE IO TECH (OV12), Korea). The steaming treatment was done on lupin seeds before grinding using a flow cook (CFS, Denmark) with 500 rpm fan speed with 40% wet steam (160 °C). Steamed seeds were dried in a drier for 24 hours at 50 °C. Finally, lupin was ground on a mill (high-speed multifunction comminutor, WM-500, China) and allowed to pass through a 1-micron sieve.

Preparation of burgers with different levels of treated lupin flour to replace meat
This experiment used ten meat burgers’ formulas (Table 1). The first formula was the original formula -without lupin (control), which was described previously in the section entitled "the basic formula for the preparation of the meat burger." In the other formula, different levels of treated lupin flour were used to replace meat.

The first step was weighing all the needed ingredients. After that, the frozen beef meat (-7 to -9 °C) was ground using a commercial frozen meat cutter (auto-grind machine, CFS, Denmark) equipped with a 20 mm grinding plate. After grinding, the meat temperature rose to -4 °C. The next step was mincing the meat, which was done with a meat mincer (K&G Watter, 419/E130, Germany) equipped with a 3 mm mince plate. Minced meat was portioned according to the required amount for each formula. At this step, other ingredients in the formula were added and mixed manually for three minutes. A detailed description of treated lupin seed flour preparation was described in the section entitled "treatment of lupin seeds and preparation of lupin flour." A portion of the meat mixture (70 g) was formed using a manual circular plastic mold (9.5 cm in diameter). The temperature of the meat mixture was monitored to be at 0 ± 0 °C during the forming step. Burger pieces were placed in a shock freezer (Blitzer, S6F-30.2Y-40P, Germany) at a temperature of -33 to -36 °C for 30 mins until the product’s core temperature reached (-15 to -18 °C). The burgers were then vacuum-sealed in plastic bags, placed in cardboard boxes, and frozen at -18 °C for 18 days before being grilled and evaluated.

Table 1 - Developed formulas used in the study to prepare beef burgers

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>0% (control)</th>
<th>5%</th>
<th>10%</th>
<th>15%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef Meat (10% Fat)</td>
<td>89</td>
<td>84.55</td>
<td>80.1</td>
<td>75.65</td>
</tr>
<tr>
<td>Salt</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>Spices</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Water</td>
<td>10.50</td>
<td>10.50</td>
<td>10.50</td>
<td>10.50</td>
</tr>
<tr>
<td>Lupin flour*</td>
<td>0</td>
<td>5% of meat (4.45% of total mix)</td>
<td>10% of meat (8.9% of total mix)</td>
<td>15% of meat (13.35% of total mix)</td>
</tr>
<tr>
<td>Total</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

*untreated or steamed or roasted

Cooking of burger pieces
Frozen burger pieces were removed from the freezer and grilled directly using a commercial grill (Electric Grill, Sonifer, China). The grilling was performed at 185 to 215 °C and continued for 10.5 mins. The burger was placed for two minutes on the first side and two minutes on the other side. After that, the burger piece was flipped every minute until the core temperature of the bean reached 75 °C, knowing that the diameter and weight measurements were taken for each

burger piece before and after grilling. After that, the burger pieces were cooled to room temperature and packed in plastic bags for further evaluation.

**Color evaluation**
The color of cooked samples was assessed using a non-contact spectrophotometer (X-rite VS-450, UK) and Oncolor software (CyberSoft, UK). The International Commission on Illumination (CIE) Lab color values and differences were determined for each sample. Three burgers were evaluated for each treatment, and the average was calculated to perform the statistical analysis.

**Cooking measurements**

**Cooking loss**
Burger pieces were weighed before and after cooking. After cooking, the burger pieces were allowed to cool at room temperature for 15 mins. Cooking loss was determined according to the following formula:

\[
\text{Cooking loss (}) = \frac{\text{Raw burger weight} - \text{Cooked burger weight}}{\text{Raw burger weight}} \times 100
\]

**Moisture retention and fat retention**
According to the formulas presented by Romero et al. (2019), the moisture retention and fat retention values were determined.

\[
\text{Fat retention (}) = \frac{\text{Cooked weight} \times \text{Percent fat in cooked samples}}{\text{Raw weight} \times \text{Percent fat in raw samples}} \times 100
\]

\[
\text{Moisture retention (}) = \frac{\text{Cooked weight} \times \text{Percent moisture in cooked samples}}{\text{Raw weight} \times \text{Percent moisture in raw samples}} \times 100
\]

**Shrinkage**
The dimensional shrinkage was calculated according to Ismail et al. (2021) as follows:

\[
\text{Diameter shrinkage (}) = \frac{(\text{Raw diameter} - \text{Cooked diameter})}{\text{Raw diameter}} \times 100
\]

**Chemical analysis**
Before and after cooking, the protein, fat, and moisture percentages of beef meat burgers were measured using FOSS FoodScan™ Meat Analyzer (Scanco, Costa Rica, San José).

**TPA**
TPA parameters (hardness, resilience, springiness, cohesiveness, and chewiness) were determined using a texture analyzer (TVT, Perten, Sweden) according to the method described by Alrawashdeh and Abu-Alruz (2022).

**Sensory evaluation**
Based on the results of the previous test, five burger mixes - with different levels of meat substitution with lupin flour - were selected (control "without lupin," 5% and 10% with untreated lupin flour, 5% and 10% with steamed lupin flour). Roasted lupin flour was excluded in this part due to its negative impact on the cooked beef burger's TPA compared to the steamed lupin seed flour. From the National Poultry Company, ten skilled panelists were chosen after asserting that they usually consume lupin without being allergic. Members of the committee were requested to assess the samples and report their findings on a sensory evaluation form. The grilled samples were evaluated on a nine-point hedonic scale, with one signifying severe hate and nine denoting intensity. Three sensory parameters (color, taste, and texture) were assessed for each sample. Three-digit codes were used to number each sample. Burgers were grilled according to the procedure described in the section "cooking of burger pieces."

**Statistical analysis**
Using Minitab® 19.20.20, the data were analyzed using a fully randomized factorial design (CRD) with two replicates. For sensory evaluation, data was analyzed according to the completely randomized design. All the data are presented as mean values with their standard deviations. The statistically significant differences between the means were determined using Tukey's test, and the significance level was set at \( p \leq 0.05 \).

**RESULTS**
A 3*4 factorial design was used to assess the main effects of two factors (Type of treatment of lupin seed flour and substitution level of meat with lupin flour) and their interaction on the quality of a developed meat burger. Three levels of treatment type (Lupin without treatment, steamed lupin, and roasted lupin) and four substitution levels (0, 5, 10, and...
15%) were applied. In the following sections, only significant results were reported. Only the results of the interaction effects were reported when they were significant; if not, the significant main effects were only reported.

**CIELAB color values of cooked beef burger**

**L* values**

L* values of cooked beef burgers were significantly affected (P≤0.05) only by substitution levels; therefore, only the results of the effect of substitution level were presented. Substitution levels above 5% significantly increased the L* values of meat burgers compared to 0 and 5% substitution levels (Figure 1).

**a* values**

a* values of meat burgers were not significantly affected by substitution levels, treatment type, and interaction between them. The a* values for different samples of burger meat ranged between 7.96 and 8.71 (Table 2).

**b* value**

b* values of meat burgers were significantly affected (P≤0.05) only by substitution levels. Up to a 10% substitution level, there was a significant increase in b* values with every increase in substitution level (Figure 2).

**ΔE*ab values**

ΔE*ab values of meat burgers were significantly affected (P≤0.05) only by substitution levels. There was a significant increase in ΔE*ab with every increment in the substitution level (Figure 3).

### Table 2 - Effect of substitution levels on CIELAB color values of cooked burger

<table>
<thead>
<tr>
<th>Substitution level</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>ΔE*ab</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>35.52±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.51±0.035&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.21±0.61&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.43±0.76&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>5%</td>
<td>36.82±4.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.71±0.49&lt;sup&gt;c&lt;/sup&gt;</td>
<td>19.13±1.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.85±1.57&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>10%</td>
<td>43.07±1.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.40±0.44&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21.55±0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.08±1.29&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>15%</td>
<td>45.72±1.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.96±0.85&lt;sup&gt;c&lt;/sup&gt;</td>
<td>22.35±0.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.39±1.51&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*M±Std.D, the values in the same column followed by the same letter are not significantly different at the 0.05 probability level as determined by Tukey’s test.

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**Figure 1** - Effect of using different substitution levels of lupin flour on the L* values of cooked meat burgers. Values followed by the same letter are not significantly different.

**Figure 2** - Effect of using different substitution levels with lupin flour on the b* values of cooked meat burgers. Values followed by the same letter are not significantly different.

**Figure 3** - Effect of using different substitution levels with lupin flour on the ΔE*ab values of cooked meat burgers. Values followed by the same letter are not significantly different.

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Texture profile analysis (TPA)

Hardness values were significantly affected by the treatment type of lupin seed flour, substitution levels, and their interaction; accordingly, only the results of the interaction effect were presented (Figure 4). The hardness values were not significantly affected in the roasting and steaming treatments with varying lupin flour substitution levels compared to the control sample (0% substitution level). In the lupin without treatment, the hardness values were significantly affected by increased substitution levels of more than 5%, and the meat burger with a 10% substitution level had the lowest hardness value.

Cohesiveness values were significantly affected by the treatment type of lupin seed flour, the substitution levels of lupin flour, and the interaction between them; accordingly, only the results of the interaction effect will be presented (Figure 5). In roasting treatment with increased substitution levels of more than 5%, the cohesiveness significantly decreased compared to a 0% substitution level. The control treatment significantly decreased the cohesiveness values at a 15% substitution level, but it had no significant effect at the different levels of substitution. In contrast, the cohesiveness values were not significantly affected in the steaming treatment with varying substitution levels.

Resilience values of meat burgers were significantly affected (P<0.05) by substitution levels, treatment type of lupin seed flour, and interaction between them; consequently, only the results of the interaction effect were displayed (Figure 6). The resilience values significantly decreased as the substitution level increased to 15% in the lupin without treatment and steamed lupin. In roasting treatment, the resilience values significantly decreased with increased substitution levels of lupin flour of more than 5% compared to a 0% substitution level.

Springiness values of meat burgers were not significantly affected by the treatment type of lupin seed flour, substitution levels, and interaction between them. The results of interaction effects are shown in Table 3.

The treatment type of lupin seed flour, the substitution levels, and the interaction between them significantly affected chewiness values; therefore, only the results of the interaction effect were shown (Figure 7). Steaming and roasting treatments with different levels of substitution of lupin flour did not significantly affect the chewiness values. In contrast, as the substitution level increased from 5% to 15% in lupin without treatment, the values of chewiness significantly decreased compared to a 0% substitution level, with no significant differences between substitution levels above 0%.

![Figure 4](https://example.com/Figure4.png)

**Figure 4** - Effect of the interaction between treatment type and substitution levels on hardness values on cooked meat burgers. Values followed by the same letter are not significantly different.

![Figure 5](https://example.com/Figure5.png)

**Figure 5** - Effect of the interaction between treatment type and substitution levels on cohesiveness values of cooked meat burger. Values followed by the same letter are not significantly different.

![Figure 6](https://example.com/Figure6.png)

**Figure 6** - Effect of the interaction between treatment type and substitution levels on resilience values of cooked meat burger. Values followed by the same letter are not significantly different.

![Figure 7](https://example.com/Figure7.png)

**Figure 7** - Effect of the interaction between treatment type and substitution levels on chewiness values of the cooked meat burger. Values followed by the same letter are not significantly different.

The burgers made with a 5% substitution level did not significantly differ from the control. The protein content of uncooked burgers increased significantly with increasing substitution levels above 5%, and the lowest fat content (10.78% ± 0.582) was recorded for burgers formulated with a 15% substitution level (Figure 9). Regarding the effect of flour substitution level, the protein content of uncooked burgers significantly decreased with each 5% increase in the substitution level, while its values ranged between 62.55 and 72.07%, with significant differences between them.

The fat content of cooked burgers was significantly affected only by substitution levels, but the fat content of uncooked burgers was not significantly affected by substitution levels, treatment type, and interactions between them. The fat content of cooked burgers significantly decreased with increasing substitution levels above 5%, and the lowest fat content (7.05% ± 0.191) was recorded for burges formulated with a 15% substitution level (Figure 9). The protein content of uncooked burgers was significantly affected by substitution levels, treatment type, and interactions between them; therefore, the results of the interaction effect will be presented. The protein content of cooked burgers was significantly affected by the substitution level; therefore, the results of the substitution level will be presented

### Chemical analysis

The moisture content of cooked and uncooked burgers was significantly affected only by substitution levels (Figure 8). Only cooked burgers made with 10% and 15% substitution levels had significantly less moisture content than the control treatment, while the burgers made with a 5% substitution level did not significantly differ from the control. In contrast, the moisture content of uncooked burgers significantly decreased with each 5% increase in the substitution level, and its values ranged between 62.55 and 71.07%, with significant differences between them.

The fat content of cooked burgers was significantly affected only by substitution levels, but the fat content of uncooked burgers was not significantly affected by substitution levels, treatment type, and interactions between them. The fat content of cooked burgers significantly decreased with increasing substitution levels above 5%, and the lowest fat content (10.78%) was recorded for burgers formulated with a 15% substitution level (Figure 9).

The protein content of uncooked burgers was significantly affected by substitution levels, treatment type, and interactions between them; therefore, the results of the interaction effect will be presented. The protein content of cooked burgers was significantly affected by the substitution level; therefore, the results of the substitution level will be presented (Figure 10). Regarding the effect of flour substitution level, the protein content of uncooked burgers increased significantly with increasing substitution levels. All the treatments of uncooked burgers made with a 15% substitution level had the highest effect in increasing the protein content. Samples of cooked burgers formulated with substitution levels of 5, 10, and 15% had the highest effect in decreasing the protein content with no significant differences between them, which was significantly different from the control treatment.

### Table 3 - The effect of the interaction between treatment type of lupin seed flour and substitution on TPA of cooked meat burger

<table>
<thead>
<tr>
<th>Interaction effect</th>
<th>Texture Profile Analysis TPA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment type</td>
<td>Hardness (g)</td>
</tr>
<tr>
<td>-------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>0% (control)</td>
<td>9239±310a</td>
</tr>
<tr>
<td>5%</td>
<td>6771±297a</td>
</tr>
<tr>
<td>10%</td>
<td>4795±212a</td>
</tr>
<tr>
<td>15%</td>
<td>6253±323c</td>
</tr>
<tr>
<td>Steaming treatment</td>
<td>0% (control)</td>
</tr>
<tr>
<td>5%</td>
<td>9432±198a</td>
</tr>
<tr>
<td>10%</td>
<td>9123±270a</td>
</tr>
<tr>
<td>15%</td>
<td>9724±608a</td>
</tr>
<tr>
<td>Roasting treatment</td>
<td>0% (control)</td>
</tr>
<tr>
<td>5%</td>
<td>8831±528a</td>
</tr>
<tr>
<td>10%</td>
<td>8862±754a</td>
</tr>
<tr>
<td>15%</td>
<td>9511±183a</td>
</tr>
</tbody>
</table>

*M±Std.D, the values in the same column followed by the same letter are not statistically significantly different at the 0.05 probability level as determined by Tukey’s test.

Figure 8 - Effect of using different levels of substitution of lupin flour on the moisture content of meat burgers before and after cooking. Values followed by the same letter are not significantly different.
Cooking measurements

The values of cooking loss were significantly affected only by substitution levels. The values of cooking loss significantly decreased with each 5% increase in substitution level, with significant differences between them. On the other hand, as substitution levels increased from 0% to 15%, cooking loss values decreased significantly from 32.14% to 13.09% (Figure 11). Fat retention was not significantly affected by substitution levels, treatment type, and interaction between them. The substitution levels significantly affected moisture retention (Figure 12). A significant increase in moisture retention values was obtained when substitution levels were increased, with significant differences between them. The burgers made with a 15% substitution level had the highest effect in increasing moisture retention values. The values of shrinkage were significantly affected by substitution levels of lupin flour (Figure 13). The results showed significant differences between values of shrinkage when increasing substitution levels. The lowest significant shrinkage values were for burgers with a 15% substitution level, which was followed by a 10% substitution level. The impact of using selected different treatments and different levels of lupin seed flour on sensory analysis is shown in Table 4. The steaming treatment with 10% lupin seed flour had the highest sensory score, whereas the control treatment with 0% lupin seed flour received the lowest sensory score. However, these differences were not significantly different.

Citation
DISCUSSION

Formulation of beef burgers

High meat consumption worldwide is a growing danger to human health (Caputo et al., 2023). Red and processed meat intake has been associated with elevated risks of (CVD) (Bechthold et al., 2017) and a high prevalence of malignancies (Bouvard et al., 2015). Due to their high protein content (Sujak et al., 2006), low-fat content (Annicchiarico et al., 2014), and significant beneficial effects on the physiological state of the human body, in particular for those suffering from diabetes, hypertension, obesity, and (CVD) (Ahmed, 2014; Prusinski, 2017), this study sought to use lupin flour as a meat alternative in the preparation of beef burgers by substituting a portion of the meat with lupin flour. To achieve this purpose, the effects of the treatment of lupin seeds (without treatment, steaming, and roasting) and the substitution level of meat with lupin flour (0, 5, 10, and 15%). To the best of our knowledge, limited studies investigated the incorporation of lupin flour into meat products; for instance, El-Sayed (2013) studied the effect of replacing meat with 5 and 7.5% of lupin seed flour on the quality characteristics of beef burger patties. Dalain et al. (2023) studied the impact of three different levels of sweet lupin flour (10, 20, and 30%) on the quality of chicken burgers. Regarding previous studies on the effect of the treatments of lupin flour on the quality of beef sausage, Leonard et al. (2019) studied the impact of roasted lupin flour on beef sausage's physicochemical and sensory characteristics.

Instrumental color analysis

The CIELAB color parameters (L*, a*, and ΔE*ab) were significantly affected (P≤0.05) only by substitution levels. In general, the developed beef burgers had higher L*, b*, and ΔE*ab with increasing substitution levels regardless of the treatment type. It is expected for the color of beef burgers to become lighter when using vegetables as an alternative to meat. This result is consistent with the results of Lee et al. (2021), who found that by replacing meat with lupin flour, the concentration of this pigment decreased, resulting in a product with a lighter color. In another study, L* values of chicken patties increased due to replacing chicken meat with oat flour (Serdaroglu, 2006). Shokry (2016) found that as the meat substitution with quinoa flour increased, the meat burger's L* and b* values increased. Similarly, Al-Juhaimi et al. (2017) reported that as the Moringa oleifera seed flour level increased, the beef burger patties had high L* and b* values.

TPA

Textural properties of meat burgers are among the most influential factors in consumer acceptance (Fiorentini et al., 2020). The hardness, cohesiveness, resilience, and chewiness values were significantly affected by the interaction...
between the substitution levels of lupin flour and the type of treatment, whereas different treatments did not affect springiness. This means that the texture profile parameters changed in a manner dependent on the type of treatment and the substitution level used. It is evident from the results the importance of the treatment of lupin flour in maintaining the texture integrity of cooked beef burgers – compared to the control with zero substitution level – with increasing the substitution level. From the results, using steamed lupin seeds flour with a 10% substitution level resulted in a texture profile that did not significantly differ from the control beef burger (0% substitution level); this indicates the role of lupin seeds treatment with steaming in improving the functional properties of lupin flour. Using untreated lupin seed flour to substitute meat in beef burgers significantly decreased hardness, chewiness, cohesiveness, and resilience. The results of the untreated lupin seeds flour are consistent with the results of the previous study; for instance, Vu et al. (2022) reported that the cooked plant-based patties had softer (lower hardness, cohesiveness, resilience, and chewiness) than the cooked beef patties. The changes in the structural properties might be attributed to the changes in the chemical composition and structure of different formulas; it has been reported that animal protein's heterogeneity with vegetable protein adversely affects its structural properties (Godschalk-Broers et al., 2022). Bakhsh et al. (2021) reported significant differences in the textural properties of meat and meat substitutes after cooking.

Steamed lupin flour is an excellent choice for incorporating lupin flour into beef burgers. A previous study highlighted that protein isolates derived from legume grains subjected to steaming exhibited improved foaming properties (Naiker et al., 2020). This indicates that using steamed lupin flour can enhance the foaming capacity and stability of the burger mixture. Proteins with good foaming properties, characterized by flexible surfactant molecules that form cohesive visco-elastic films at the air-water interface, can contribute to the desired texture of the burgers. The steaming treatment enhances the ability of lupin proteins to rapidly adsorb at the air-water interface, leading to increased foamability and improved cohesion in the burger mixture. Additionally, the higher ratio of acidic to basic amino acids in steamed lupin flour enhances protein solubility and flexibility, allowing for better spreading on the air-water interface and improved foam formation. Therefore, steamed lupin flour in beef burgers can enhance their textural properties by improving foaming capacity, stability, and cohesion (Adebowale and Maliki, 2011; Naiker et al., 2020).

**Chemical analysis**

The chemical parameters tested were mainly affected by the substitution level. This result is consistent with the findings of Serdaroğlu et al. (2018), who found that the incorporation of various levels of dried pumpkin pulp and seed mixture (0, 2, 3, and 5%) led to significant alterations in the majority of chemical parameters (protein, moisture, and fat) for both raw and cooked beef patties. The moisture content of uncooked (62.55 to 71.07%) and cooked burgers (56.34 – 60.2%) was significantly affected only by substitution levels. Logically, the moisture content decreased with an increased substitution level because we removed beef meat and added dry lupin flour instead. The moisture content range in uncooked burgers was 8.52%, whereas in cooked burgers, it was 3.68%. The decreased range in cooked burgers was related to the improved water retention properties of burgers made with lupin flour, which increased with increasing substitution levels. Because the moisture contents of cooked burgers ranged from (50.78–60.86%), this result is close to what Abbas (2009) discovered in his study on the concurrent manufacturing of burgers from veal and legumes (peas and beans). Results were in contrast to the findings of Devatkal et al. (2011), who reported that the moisture content of cooked nuggets made with 5% sorghum flour instead of wheat flour was greater than that of the control group.

The fat content of uncooked burgers was not significantly affected by substitution levels, treatment type, and interactions between them. However, the fat content of cooked burgers ranged between 10.78 and 13.72, significantly decreasing with each increment of lupin flour addition (Figure 9). This can be explained by the improved water retention with increasing the substitution level, considering that the fat content was expressed as a wet matter basis. The protein content of uncooked burgers increased significantly with increasing substitution levels due to the higher amount of protein in lupin flour (30–40%) Prusinski (2017) compared to that in beef meat used in this study (18.34%). However, the protein values after cooking were significantly lower in samples containing lupin flour (25.74-25.89%) compared to the control sample (27.63%). The justification of the contradictory results between protein content in uncooked and cooked might be explained by the increased water-holding capacity of burgers with the increased substitution levels and the low gelling power of lupin proteins (Abreu et al., 2023), which could cause loss of protein upon cooking.

**Cooking measurements**

The meat industry relies heavily on cooking properties such as cooking yield, cooking loss, and shrinkage to predict the behavior of products during cooking (Ahmed and Abdel-Rahman, 2022). It was evident from the results that the cooking loss values were significantly decreased by increasing the substitution levels. Regarding cooking shrinkage, it could be noticed that the values decreased as the level of substitution lupin flour increased from 5 to 15%. The lowest significant cooking loss and shrinkage values were for burgers with a 15% substitution level. These results may be attributed to the content of total fibers in lupin seeds, averaging 101 g/kg (Tizazu and Emire, 2010), and illustrated the role of high fiber content in the enhancement of cooking yield, water holding capacity, cooking loss, and shrinkage. This result was in line with a previous study by Shokry (2016) on the cooking yield in meat burgers formulated with quinoa flour, who illustrated that the cooking yield increased with increasing quinoa flour incorporated in beef burgers. Tabarestani and Tehrani (2014) found that combining soy flour with starch increased cooking yield, and splitting pea flour...
in the mixed formula improved textural properties. As the levels of substitution of lupin seed flour increased, the moisture retention values were significantly affected and increased. This result is in accordance with those found by El-Sayed (2013), in which the moisture retention values were increased with increasing levels of lupin flour in beef burger samples. Tabarestani and Tehrani (2014) also documented improved moisture retention in low-fat hamburger patties with starch added.

**Sensory Evaluation**

A sensory evaluation test was conducted to evaluate consumers’ acceptability and satisfaction toward beef burgers treated by steaming compared to the control sample (Table 4). The results showed no significant difference between steaming treatments with varying substitution levels compared to the control. This result is in agreement with the work of Devatkal et al. (2011), who reported that adding 5% sorghum flour to gluten-free chicken nuggets had the same flavor and texture as the control group (5% wheat flour), regarding all the sensorial attributes. Ramadan et al. (2016) found no significant differences in color, flavor, odor, appearance, or general acceptability between chicken burgers made with other grains such as wheat, sorghum, and maize. Dalain et al. (2023) studied the formulation of chicken burgers with three various levels of substitution of sweet lupin (10, 20, and 30%) and reported that chicken burgers containing 20% sweet lupin flour had the greatest sensory qualities.

**CONCLUSION**

Due to what we have reviewed of the results, the use of lupin flour had an apparent effect on improving the properties of beef burgers, as the most important results were reducing the shrinkage rate and the percentage of losses after cooking, noting that the shrinkage rate was in the control sample at (26.45%) and reached (18.8%), and the cooking loss percentage was in the control sample at (32.14%) and reached (13.09%). However, the results demonstrated the important effect of treatment, particularly steaming, in improving the functional properties of lupin seed flour, which were evident in maintaining the texture properties of the cooked beef burger while increasing the substitution levels. Lupin flour can be recommended as a potential new functional material for meat manufacturers that can replace soy proteins, which will be reflected in the quality of the final product.

**DECLARATIONS**

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**Authors’ contribution**

The first author performed the experiments and analysis and participated in writing. The second author designed the experiments, statistically analyzed the results, and participated in writing.

**Conflict of interests**

The authors have not declared any conflict of interest.

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