Development and evaluation of extruded protein snacks added lentil and texturized soy protein

Karolynne Sousa GOMES¹ ², Gabrielle Fusiger BERWIAN² ², Caroline Balesiefer Vicenzi TIEPO¹, Luciane Maria COLLA¹ ³

Abstract
This study aimed to develop an extruded protein snack added to lentil flour (L) and textured soy protein (S) and to evaluate the developed formulations sensorially, physicochemically, and physically. Five formulations were prepared: C (100% flour corn (C)), S40% (60% flour corn and 40% soybean), LS20% (80% flour corn, 15% soybean, and 5% lentil), LS15% (85% corn, 7.5% soybean, and 7.5% lentil), and LS10% (90% corn, 7% soybean, and 3% lentil). The research was approved by the Research Ethics Committee of the Universidade de Passo Fundo (55484622.2.0000.5342). Seventy untrained tasters participated in the acceptance test, where the LS15% and LS10% formulations were the most accepted (83.5 and 80.2%, respectively). In the physicochemical characterization, it was observed that all formulations improve nutritional quality, with a significant increase in protein content (11–23%) in all formulations. Physically, the formulations with corn replacement rates showed higher resistance and lower crunchiness. Furthermore, all formulations showed higher water activity (0.55–0.61 aw) than the standard sample (0.34 aw). Therefore, the incorporation of lentils and soy flours benefited the nutritional quality of the snacks but negatively affected their physical parameters and sensory acceptability.

Keywords: extruded snacks; protein; soy; lentil.

Practical Application: Development of snacks with better nutritional quality with the addition of vegetable raw materials to increase protein content. Textured soy and lentil proteins are economically and nutritionally viable raw materials for improving the nutritional quality of snacks. It is possible to perform up to a 40% substitution in extruded snacks.

1. Introduction
Extruded snacks are expanded foods with a crunchy texture and salty flavor produced through extrusion cooking using different temperature ranges and high pressure. The popularity of snacks has grown a lot in recent years due to their practicality, wide availability, variety of flavors and formats, and crunchy texture (Karun et al., 2023). However, there is a growing concern about the nutritional composition of this food, which generally has high percentages of fat, simple carbohydrates, and sodium but few macronutrients such as protein and fiber (Brennan et al., 2013). Consumption of foods with a high glycemic index and a high percentage of fat has been associated with the development of food addiction, type 2 diabetes, and cholesterol (Belik, 2020; Lennerz & Lennerz, 2018; Roberts, 2000).

Because of this, several studies have been carried out incorporating legume flours such as lentils and soy in the production of extruded snacks since legumes are excellent protein sources and have a good profile of essential amino acids (Martin et al., 2022). Legumes, when combined with cereals such as corn, improve considerably the nutritional value of the food and increase the protein contents of these foods, which in some cases can even be considered a source of protein and an alternative to the lack of this nutrient (Mosibo et al., 2022).

Given this scenario, lentils, as they are a source of iron, fiber, minerals, vitamins, and proteins, have been used in the preparation of various foods, including extruded snacks (Perez de la Vega et al., 2011). However, legumes are low in amino acids containing sulfur and lysine, so they are often combined with cereals such as corn and oilseeds such as soybeans, which have a good content of these amino acids (Li et al., 2022). Therefore, this present work sought to develop an extruded protein snack with lentil and texturized soy protein.

2. Materials and methods
2.1. Raw material
The raw materials used in the production of extruded snacks were lentil and corn flour obtained from Cisbra (Rio Grande do Sul, Brazil) and textured soy protein obtained from Bremil S/A
Protein extruded snacks added from vegetable raw materials (Rio Grande do Sul, Brazil). The control snack was provided by the partner company, Temabi.

2.2. Experimental design

The study was divided into three main stages. In the first stage, the formulations were prepared based on the physical-chemical composition of the previously characterized flours following the methodology described in AOAC 960.52 and preliminary tests (Supplementary Material 1). The extruded snacks were produced in stick format and flavored with cheese. The format and flavor were defined together with the partner company, which has corn snacks in stick format and cheese flavor as one of its most commercialized products.

In the second stage, the extruded snacks were analyzed for nutritional composition to assess whether there was a significant increase in protein. Analyses of physical parameters were performed to evaluate the effect of incorporating textured soy and lentil protein on texture, density, porosity, water absorption, water solubility, and water activity.

In the third stage, the snacks were sensorially analyzed with the acceptability test.

2.3. Production of snacks

The extrusion snacks were produced on an industrial scale in an automated twin-screw extruder line (RX 200, series 3586, Rafamáquinas, 2018), with a pressure of 30 bar and a flow rate of 180 kg/h. The extrusion conditions and the composition of the formulations were defined based on preliminary tests. The composition and extrusion conditions were not kept fixed, as can be seen in Table 1.

The composition of the formulations was defined according to the amount of protein present in the raw materials used. The extrusion conditions were the optimal conditions found for each formulation during the continuous process (Table 1). The snacks produced were stick-shaped with cheese and barbecue flavors (Figure 1).

2.4. Chemical analysis

Proteins were quantified using the Kjeldahl method using a conversion factor of 6.25 (AOAC 960.52). Lipids were determined by the Soxhlet method using hexane as a solvent (AOAC 920.39). Humidity was measured in an oven at 105 °C until constant weight (AOAC 925.10). The estimate of the amount of minerals was made in a Bunsen burner for complete incineration of the samples and subsequently muffled at 550 °C (AOAC 945.38). The determination of crude fiber used a sample degreased by acid digestion followed by basic digestion; after this step, the samples were taken to an oven at 105 °C and subsequently to the muffle at 550 °C (AOAC 945.38). What was left over was quantified as carbohydrates (AOAC, 1995, 2005).

2.5. Physical analysis

2.5.1. Density

The density was calculated according to Stojceska et al. (2009).

2.5.2. Water activity

To analyze water activity, the samples of snacks were placed in a metallic chamber with a probe. The section was hermetically closed and placed at temperature equilibrium (25±1 °C) in an electric thermohygrometer (testo 650, apex control automation, Brazil) for direct measurement.

![Figure 1](image-url) Appearance of the developed formulations (*A*) S40% formulation; (*B*) LS20% formulation; (*C*) LS15% formulation; (*D*) LS10% formulation. S40% (corn 60% and soybean 40%), LS20% (corn 80%, soybean 15%, and lentil 5%), LS15% (corn 85%, soybean 7.5%, and lentil 7.5%), and LS10% (corn 90%, soybean 7%, and lentil 3%).

<table>
<thead>
<tr>
<th>COMPOSITION</th>
<th>EXTRUSION CONDITIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>COMPOSITION</strong></td>
<td><strong>Temperature I (°C)</strong></td>
</tr>
<tr>
<td>Corn (C)</td>
<td>Soybean (S)</td>
</tr>
<tr>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>LS10%</td>
<td>90</td>
</tr>
<tr>
<td>LS15%</td>
<td>85</td>
</tr>
<tr>
<td>LS20%</td>
<td>80</td>
</tr>
<tr>
<td>S40%</td>
<td>60</td>
</tr>
</tbody>
</table>
2.5.3. Texture

Hardness and crispness parameters were determined using a texture analyzer (TA-XT Plus, Stable Micro System) with a 50 kg load cell. The test parameters were set at a distance of 10 mm between the probe and the base, a speed of 1 mm/s, and a distance between the two supports of the base of 1.5 mm. The result was expressed as the average of ten repetitions.

2.5.4. Water absorption index and water solubility index

The water solubility index (WSI) and the water absorption index (WAI) were determined using the method of Singh and Smith (1997).

2.5.5. Porosity

Porosity was determined as a function of particle density and apparent density, as described by Wang et al. (1999). Two determinations were made for each sample.

2.6. Sensory analysis

The research was approved by the research ethics committee of the Universidade de Passo Fundo with approval number 55484622.2.0000.5342. The acceptability test was performed following the procedure described in ISO 11136 (ISO, 2014). For the test to be meaningful, a total of 70 untrained tasters who consume extruded snacks from the city of Passo Fundo-RS, with age groups ranging from 18 to 65 years, participated in the research voluntarily.

The acceptability test of the extruded snacks was conducted in a sensory laboratory with individual booths, artificial lighting, and air circulation. The samples were presented monadically in plastic containers randomly coded with three numerical digits. The snacks delivered to the tasters were cheese-flavored, and water was also provided so tasters could clean their palates between tastings. The test to be meaningful, a total of 70 untrained tasters who consume extruded snacks from the city of Passo Fundo-RS, with age groups ranging from 18 to 65 years, participated in the research voluntarily.

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2.7. Statistical analysis

Data were analyzed with statistical software (Statistica 7.0), applying the variance test (ANOVA); when there was a difference, Tukey’s test was performed with a confidence interval of 95%.

3. Result and discussion

The flours used in the present study showed average values of crude protein of 19% for lentils, 6.84% for corn grits, and 38% for textured soy protein. The incorporation of lentil flour (L) and textured soy protein (S) in the production of snacks resulted in a marked improvement in the protein component of interest. The results are shown in Table 2. All formulations showed a significant increase in protein percentage when compared to the control. According to European legislation n. 1924/2006, formulations with 40% substitution can be classified as “high protein” foods, and formulations with 30, 20, and 15% substitution can be classified as protein sources. In this way, the incorporation of TPS and lentil flours fulfilled the objective of increasing the percentage of proteins in the extruded snacks.

The increase in proteins and the reduction in carbohydrates in the S40% formulation are mainly due to the greater substitution of corn in the formulation for soy only. Soy is a flour with a high protein content but a low carbohydrate content, which affects the expansion of the snack.

The formulations with S40% and LS15% replacement showed a significant difference in expansion when compared to the control sample, which indicates that the addition of oil during the cooking of the mixtures to improve the expansion of the snacks increased the final lipid content. Adding oil to the dough during extrusion is a method used to improve expansion when flours have components such as proteins and fibers in high amounts that compromise extrusion. Oil acts as a lubricant during the rotational movement of the screws, allowing the dough to cook and expand better. There is also the formation of starch-lipid complexes that end up making the structure more stable and controlling the formation of gas bubbles (Table 2).

The most accepted formulations were those with the lowest replacement rate (Table 2) because they were the ones that did not show major changes in texture and appearance, in addition to having a taste closer to that of corn snacks, referring to the tasters of the commercial snack.

On the contrary, the formulations with the highest replacement rate were not well accepted due to their compact appearance and darker color (Figure 1). Additionally, tasters typically described the samples as having a soggy texture and a mild salty taste that is uncharacteristic of snack foods.

In Figure 1, it is possible to observe the appearance of the developed formulations. The influence of the addition of flour on the expansion of snacks is clear in Figures 1A and 1B, as the snacks are less expanded and have a darker color and oily appearance.

### Table 2. Centesimal composition of formulations and standard sample.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Prot ±sd</th>
<th>Fib ±sd</th>
<th>Lip ±sd</th>
<th>Ash ±sd</th>
<th>Carb ±sd</th>
<th>Accep ±sd</th>
<th>IA (%) ±sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>7.99±0.46</td>
<td>1.31±0.06</td>
<td>9.65±0.44</td>
<td>3.27±0.10</td>
<td>80.81±1.29</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>S40%</td>
<td>22.91±0.3</td>
<td>1.26±0.16</td>
<td>20.09±0.5</td>
<td>3.79±0.16</td>
<td>56.89±0.67</td>
<td>5.90</td>
<td>65.70</td>
</tr>
<tr>
<td>LS20%</td>
<td>14.50±1.0</td>
<td>0.84±0.30</td>
<td>11.23±0.38</td>
<td>2.96±0.64</td>
<td>72.71±0.97</td>
<td>6.00</td>
<td>66.70</td>
</tr>
<tr>
<td>LS15%</td>
<td>11.99±0.8</td>
<td>0.76±0.29</td>
<td>15.52±1.01</td>
<td>3.74±1.11</td>
<td>70.39±1.41</td>
<td>7.50</td>
<td>83.50</td>
</tr>
<tr>
<td>LS10%</td>
<td>11.10±0.8</td>
<td>0.36±0.03</td>
<td>8.39±0.09</td>
<td>1.32±0.43</td>
<td>79.91±1.21</td>
<td>7.20</td>
<td>80.20</td>
</tr>
</tbody>
</table>

Prot: Protein; Fib: Crude Fiber; Lip: Lipids; Ash: Ashes; Carb: Carbohydrates; Accep: Acceptability. The same letters in the same column do not differ statistically (p<0.05). C (100% corn (C)), S40% (corn 60% and soybean 40%), LS20% (corn 80%, soybean 15%, and lentil 5%), LS15% (corn 85%, soybean 7.5%, and lentil 7.5%), and LS10% (corn 90%, soybean 7%, and lentil 3%).
Figures 1C and 1D did not show major visual and expansion changes, appearing very similar to commercial corn snacks. Therefore, the low replacement rate of corn flour in these formulations and the higher concentration of carbohydrates suggest a higher percentage of starch, which guarantees better expansion.

The formulations with higher substitution rates showed a greater impairment in the visual aspect, as observed in Figure 1, which shows a compact appearance when compared to the control sample. In formulations with low substitution, the snacks presented an appearance closer to the control sample, with greater expansion and homogeneous size.

Color is an important visual parameter that showed alteration when compared to the control sample showing a reddish-brown hue, indicating a possible occurrence of the Maillard reaction. In addition, the coloring of the flours, especially the TSP, may have contributed to the color change observed in the formulations with the highest substitution (Danbaba et al., 2019).

Similar observations were made by Kreger et al. (2012) who incorporated soy protein and whey into the production of extruded corn meal-based snacks and reported that the level of inclusion and the type of protein influenced the appearance of the snacks, showing non-uniformity in terms of size, expansion, and porosity.

The physical parameters of the snacks were also evaluated, and the results can be seen in Figure 2.

Texture is one of the most important attributes in determining the quality of an extruded snack. Extruded snacks are characterized as having a crunchy texture with little hardness (Karun et al., 2023). All formulations demonstrate a significant increase in hardness. The high replacement rate of corn grits in these formulations caused a reduction in an expansion that is directly related to hardness and crispness, as the proteins interfere with the distribution of water in the matrix due to their conformation and hygroscopic properties, making it difficult for the starch to absorb water during cooking (Chakraborty et al., 2019). In addition, the lower molecular weight of the substituted flour proteins added to a higher proportion of amylose and amyllopectin may have compromised the melt viscosity and consequently the formation of gas bubbles that help in the expansion and texture of the snacks (Monnet et al., 2019).

The formulations with S40% and LS20% replacements showed reduced crispness. The high replacement rates may have led to a decrease in starch in the formulations and an increase in protein, which compromised the expansion and affected the texture (Tadesse et al., 2019). However, the SL15% and SL10% samples presented higher crispness than the control sample (Figure 2). The high percentage of carbohydrates and the low

![Figure 2](image-url)
percentage of fibers and proteins (Table 2) in both formulations may have contributed to the high crispness observed. Similar results were observed in the study by Aussanasuwannakul et al. (2022), who developed extruded snacks with soy residue from soy milk processing. It was observed that as the replacement percentage increased, the hardness of the snacks increased and the crispness decreased. The authors attributed the increase observed to the increase in protein and fiber content in the formulation and the reduction in the percentage of starch.

Density is an excellent indicator of snack expansion, as the denser the snack, the smaller the expansion. The S40% and LS20% formulations differed significantly when compared to the control sample (Figure 2), indicating that the high substitution rates adopted in the study increased the density. Fibers and proteins in high concentrations dilute the starch present and establish bonds with water and starch molecules, leading to a reduction in the extensibility of the melt during the exit from the matrix, compromising the expansion and leading to an increase in density (Korkerd et al., 2016; Selani et al., 2014). Similar results were observed by Seth and Rajamanickam (2012), who included soy, sorghum, and millet in the production of extruded snacks and observed an increase in hardness with the incorporation of flours due to the increase in macronutrients such as proteins and fibers during extrusion.

The LS15% and LS10% formulations did not differ significantly from the control sample in terms of density (Figure 2), indicating that replacing up to 20% of the corn gritz does not significantly affect the density of the snacks. The results observed for this parameter corroborate those mentioned for texture and visual appearance (Figure 1), where snacks with more discreet substitutions had greater expansion and a more crunchy texture.

The water activity, as well as the previous parameters, is very important for the quality of the extruded snack. Snacks are foods that have low water activity, and, according to the literature, they must be within the range of 0.4–0.5 to avoid changing the texture (Dewidar & El Ghandour, 2020). All samples differed statistically from the control, ranging from 0.34 to 0.61 for water activity. A high water activity favors microbial development in addition to enabling the occurrence of physical and chemical reactions such as hydrolytic rancidity, loss of texture, and loss of sensory acceptance that will alter the characteristics of the food and affect the sensory acceptance of the product (Brasil, 2019; Kumar et al., 2018). In addition, the amorphous areas present in the extruded snacks favor the gain of water and the retention of water molecules in the melt even after leaving the matrix, increasing the water activity (Shah et al., 2017).

Similar results were observed by Igual et al. (2020), who incorporated pea flour and insects in the production of extruded snacks and observed an increase in water activity due to the increase in the percentage of protein that interferes with gelatinization and compromises the distribution of water in the matrix.

Figure 3 presents the results for the analysis of absorption and solubility in water and for porosity.

*Same letters in the same column do not differ statistically (p<0.05). C (100% corn), S40% (corn 60% and soybean 40%), LS20% (corn 80%, soybean 15%, and lentil 5%), LS15% (corn 85%, soybean 7.5%, and lentil 7.5%), and LS10% (corn 90%, soybean 7%, and lentil 3%).

**Figure 3.** Result for water absorption, water solubility, and porosity analyses*. 
Water absorption is a good indicator of starch gelatinization during extrusion (Ding et al., 2005). As can be seen in Figure 3, the samples did not differ statistically when compared to the standard sample, indicating that although they had a lower percentage of starch and a higher percentage of protein in their composition, this was not enough to compromise starch gelatinization in the formulations. In addition, the high speed of the screw applied during the production of snacks favored greater starch gelatinization, as shown in Figure 3. The present result is similar to those of Sahu et al. (2022), who also observed the positive influence of screw speed on starch gelatinization.

Water solubility is an important indicator of the integrity of starch molecules during extrusion (Yağci et al., 2022). Figure 3 demonstrates that all formulations showed significantly lower results than the control sample for the water solubility parameter. The greater presence of proteins and lipids in the extruded dough interferes with the elasticity of the dough and makes it difficult to break down and solubilize the molecules. Furthermore, the action of shear during extrusion may not have been sufficient to degrade the starch molecules and make them more soluble. The use of temperatures close to or lower than those of the control may also not have been high enough to break the amylase walls and increase the solubility (Jakkannwar et al., 2018). This result is similar to that of Yadav et al. (2021), who also observed a reduction in the solubility value with higher replacement rates.

Porosity is an important attribute for extruded snacks, as the greater the porosity, the lower the resistance of the snack to breakage (Blandino et al., 2022). Figure 3 shows that the LS20% and LS40% samples differed significantly from the control sample. This result indicates that these formulations, due to their more compact walls, had greater resistance to breakage. Figure 1 shows that the LS20% and LS40% formulations are more compact than the others, corroborating the results. The reduction in porosity observed in the formulations indicates that the higher proportion of components such as proteins, lipids, and fibers may have affected the distribution of water in the matrix, compromising starch plastification and the formation of alveolar structures, leading to the observed drop in porosity (Peksa et al., 2016). Similar results were described by Deepika et al. (2022), who incorporated fish flour into the composition of extruded snacks and observed a reduction in the porosity of the snacks with an increase in protein.

One of the extrusion parameters that most influence porosity is temperature. Low temperatures associated with high humidity directly influence the expansion since starch gelatinization does not occur correctly, leading to the formation of thicker cell walls and fewer gas bubbles (Panyakamma et al., 2014).

4. Conclusion

Extruded snacks showed a significant increase in protein in all formulations, indicating that replacing corn with textured soy and lentil protein was efficient and has great potential for use in the production of value-added foods. Physically, the formulations with the highest substitution rates suffer the greatest changes in physical parameters and appearance. Sensorially, the LS15% and LS10% formulations showed high acceptability.

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References


