

# Mixtures development, physicochemical characterization, and consumer acceptance for high-protein vegan burgers based on pre-gelatinized grains flours without gluten

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## Abstract

Veganism is on the rise as a major trend in today's world. Plant proteins are gaining popularity as substitutes for animal proteins, given their versatility across various sources. It is believed that blending complementary protein sources in modern analog products can offer a balanced nutrient profile. To provide vegan consumers with a convenient and nutritious plant-based protein option, three different powder formulations of vegan burgers were developed using gluten-free pre-gelatinized flours (MIX 1, MIX 2, and MIX 3), carrying out physicochemical characterization and consumer acceptance. Samples 1 and 2 showed notably high levels of crude protein (17.99 and 18.09), surpassing previous studies. Hardness (N) ranged from 6.66 to 8.41, with no significant differences observed, suggesting that the stiffness of grains used did not impact this parameter. While sensory analysis did not yield significantly different results across attributes like color, taste, flavor, and texture, overall preference favored sample 3 (49%). The formulation containing sorghum (*Sorghum bicolor*) and pea isolate (*Pisum sativum*), unlike others, likely contributed to its higher acceptance.

**Keywords:** rice (*Oryza sativa*); pea (*Pisum sativum*); lentil (*Lens culinaris*); sorghum (*Sorghum bicolor*); oat (*Avena L.*); meat analogs; extrusion process; market trend.

**Practical Application:** Developed vegan burger mixes offer nutritious options for plant-based diets.

## 1 INTRODUCTION

The consumption of plant-based proteins derived from grains has emerged as a significant dietary trend. Casalvara et al. (2024) emphasized the importance of grains such as quinoa, amaranth, and millet as excellent sources of protein, offering a wide range of essential amino acids vital for human health. Lee et al. (2009) suggested that regular consumption of grain-based proteins may contribute to reducing the risk of chronic diseases such as cardiovascular and type 2 diabetes due to their favorable impact on lipid profiles and glycemic control.

The demographic growth of vegans and vegetarians has emerged as a prominent trend in the global market, supported by recent studies and consumer behavior analyses. Research conducted after 2020 indicates a substantial increase in the number of individuals adopting plant-based diets, driven by concerns regarding animal welfare, environmental sustainability, and personal health benefits. Bryant et al. (2022) showed a significant rise in the prevalence of vegetarian and vegan lifestyles across diverse demographics, with millennials and Generation Z leading this cultural shift toward plant-based eating patterns.

Moreover, market reports from Euromonitor International (2024) and Masterson (2023) underscore the growing demand for plant-based products, ranging from meat alternatives to dairy substitutes, reflecting a fundamental shift in consumer preferences toward more sustainable and ethical consumption choices. As the

vegan and vegetarian population continues to expand worldwide, businesses across various industries are adapting their strategies to cater to this burgeoning market segment, signaling a profound transformation in the global food landscape.

The incorporation of rice (*Oryza sativa*) as a protein source in the human diet has garnered attention for its numerous health benefits, such as promoting muscle growth and repair due to its rich amino acid profile, particularly high in essential amino acids like leucine, isoleucine, and valine. Liu et al. (2021) and Wang et al. (2023) demonstrated rice protein's potential in aiding weight management and improving metabolic health by enhancing satiety levels and regulating blood sugar levels. Furthermore, rice protein is hypoallergenic, making it a suitable alternative for individuals with food sensitivities or allergies to other protein sources.

The incorporation of pea (*Pisum sativum*) protein into the diets of vegan and vegetarian individuals has emerged as a significant dietary trend, including its high protein content, rich amino acid profile, and bioavailability comparable to animal-based protein sources. Research conducted by Gorissen et al. (2018) and Tang and Moore (2023) underscores pea protein's effectiveness in supporting muscle growth and maintenance, making it a valuable option for individuals following plant-based diets. Furthermore, pea protein has been associated with various health benefits, including improved satiety, blood sugar regulation, and cardiovascular health.

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The utilization of lentils (*Lens culinaris*) as a protein source in the human diet also offers numerous health benefits such as the high protein content, dietary fiber, and essential micro-nutrients such as iron, folate, and potassium. Papanikolaou (2023) and Vasanthi et al. (2021) emphasized lentils' role in promoting heart health by reducing cholesterol levels and improving blood pressure regulation due to their soluble fiber and bioactive compounds. Additionally, lentils have been associated with enhanced satiety and weight management, attributed to their low glycemic index and ability to prolong feelings of fullness. As a versatile and sustainable protein source, lentils offer a valuable dietary option for individuals seeking to improve their nutritional intake and support overall health and well-being.

Despite its numerous health benefits and low cost, sorghum (*Sorghum bicolor*) remains underutilized in the human diet. Recent studies have highlighted sorghum as a nutritious grain rich in fiber, protein, antioxidants, and essential minerals. Awika et al. (2017) and Taylor et al. (2022) underscored sorghum's potential to reduce the risk of chronic diseases like diabetes and cardiovascular ailments due to its low glycemic index and high phytochemical content. The authors affirm that sorghum's resilience to drought and its ability to grow in marginal lands make it an environmentally sustainable crop with the potential to address food security challenges. Despite these advantages, sorghum consumption in human diets remains limited, primarily due to a lack of awareness, limited availability, and unfamiliarity with its culinary uses.

On the contrary, oat (*Avena L.*) enjoys high utilization in the human diet, owing to their widespread popularity, mild flavor profile, versatility, numerous health benefits, and affordability. Renowned for its creamy texture and neutral taste, oat is a staple ingredient in various dishes, including breakfast cereals, baked goods, and savory dishes. Maki et al. (2023) and Rebello et al. (2021) consistently highlighted oat's health-promoting properties, including its role in lowering cholesterol levels, regulating blood sugar, and promoting gut health due to its soluble fiber content and unique bioactive compounds. Moreover, oat's low cost and accessibility make it an attractive option for consumers seeking nutritious and budget-friendly food choices.

Considering this entire context, the hamburger has solidified its status as a staple in modern dietary culture, renowned for its convenience, versatility, and widespread appeal. However, efforts to enhance the healthfulness of this beloved food item have sparked interest in plant-based alternatives. As consumers increasingly prioritize health and sustainability, plant-based burger options have emerged as viable alternatives, offering comparable taste and texture while significantly reducing saturated fat and cholesterol content. Hall et al. (2019) and Van Oldenborgh et al. (2022) demonstrated the nutritional advantages of plant-based burgers, highlighting their potential to improve cardiovascular health and support weight management. Moreover, the growing market for plant-based burgers reflects shifting consumer preferences toward more sustainable and ethical food choices, with companies investing in innovation to meet the rising demand.

In the production of pre-gelatinized flours from grains, the extrusion process subjects raw grains to high temperature and pressure, leading to the partial gelatinization of starches. This partial gelatinization enhances the functional properties of the flours, such as improved water absorption, thickening capacity, and enhanced stability. The extrusion process offers numerous advantages, including increased digestibility, reduced levels of antinutritional factors, and prolonged shelf life of resultant products. It is also possible to affirm that extrusion technology is highly versatile, enabling the production of a diverse range of textured, fortified, and functional food products tailored to meet various consumer preferences and nutritional requirements (Singh & Sarkar, 2021; Maskey et al., 2020).

Considering all the topics and benefits already mentioned, to offer vegan consumers a nutritious and practical source of vegetal protein, three different formulations of vegan burgers were developed from pre-gelatinized flours of rice (*O. sativa*), pea (*P. sativum*), lentil (*L. culinaris*), sorghum (*S. bicolor*), and oat (*Avena L.*). For this, physicochemical characterization and consumer acceptance were also carried out.

## 2 MATERIALS AND METHODS

### 2.1 Raw materials

Rice (*O. sativa*), pea (*P. sativum*), lentil (*L. culinaris*), sorghum (*S. bicolor*), oat (*Avena L.*), and spices were obtained from suppliers of Naturalmente Produtos Naturais (Maringá, Paraná, Brazil).

### 2.2 Preparation of the pre-gelatinized flours

All the grains were ground in a knife mill (ACB Labor) and mixed in the pre-established proportions for the study (MIX 1, MIX 2, and MIX 3) according to Table 1. Posteriorly, they were humidified by adding 4% water (m/m) and the extrusion cooking was performed in a single screw (50 mm in diameter and 200 mm longer) extruder (Inbramaq®, IB-50) without a die plate. A single batch was prepared for each grain mixture based on a reference methodology (Graça et al., 2020) and milled a second time to obtain flour characteristics.

**Table 1.** Formulations of developed vegan burger mixtures (%).

Ingredients	MIX 1	MIX 2	MIX 3
Rice ( <i>Oryza sativa</i> )	35.00	35.00	35.00
Pea ( <i>Pisum sativum</i> )	22.00	13.00	00.00
Lentil ( <i>Lens culinaris</i> )	13.00	22.00	17.50
Sorghum ( <i>Sorghum bicolor</i> )	00.00	00.00	17.50
Oat ( <i>Avena L.</i> )	14.00	14.00	14.00
Sunflower oil	05.49	05.49	05.49
Smoked paprika	05.21	05.21	05.21
Beetroot flour	01.74	01.74	01.74
Mustard powder	01.74	01.74	01.74
Salt	01.04	01.04	01.04
Dehydrated parsley	00.52	00.52	00.52
Chilli powder	00.17	00.17	00.17
Citric acid	00.09	00.09	00.09

**2.3 Mixture formulations**

After the preparation of pre-gelatinized flours, oil and spices were added to each formulation in the same proportion for each mixture, as shown in Table 1.

**2.4 Instant burger preparation**

As one of the objectives of developing this new product was practicality, the formulations were developed for consumers to only mix water, stir until a dough forms, mold it most conveniently, and cook. Then, the proportion of water was calculated similar to the hamburger powder (1:1), resulting in a 100-g burger with 1 cm high and 7 cm in diameter, as shown in Figure 1.

**2.5 Texture analysis**

Texture analysis (hardness, cohesiveness, and gumminess) was conducted at room temperature (25°C) using a texture analyzer (TAXT Plus, Stable Microsystems®) equipped with a 1/2" (12.7 mm) diameter stainless steel cylindrical probe (P/0.5) (Software Texture Exponent Lite® version 6.1.4). The conditions for analysis were carried out according to the modified method of Ganhão et al. (2010): penetration distance = 20% of the thickness of the burger (2 mm), force = 1.0 g, probe speed before and during penetration = 1 mm/s, and probe speed post penetration

= 10.0 mm/s. Texture profile parameters were evaluated following the descriptions suggested by Bourne (1978). All analyses were performed with five samples for each treatment.

**2.6 Physicochemical analysis**

The formulations were evaluated by water activity, pH, compacted density, moisture, ash content, crude protein, crude fiber, fiber in neutral detergent (FDN), fiber in acid detergent (FDA), and color parameters (L\* = luminosity, a\* = redness, b\* = yellowness), always in triplicate.

The water activity was determined using an Aqualab Series 4TE digital refractometer after equilibration of the samples at 25°C. The pH was determined using a calibrated digital pH meter (Hanna-Instruments HI 3221®). The compacted density was determined with a previously weighed 50 mL beaker filled with the powder and beaten 50 times on the bench from a pre-established height of 2.5 cm, calculating the mass/compressed volume ratio (Tonon et al., 2009).

The National Forage Testing Association method 2.2.2.5 was used for moisture (Ileleji et al., 2010). The Association of Official Agricultural Chemists methods were used for crude fiber and ash content, 978.10 and 923.03, respectively (AOAC, 2006). Crude proteins were also determined using the AOAC method (2001.11) (Cunniff, 1995). FDN and FDA were measured according to Van Soest et al. (1991). Color was measured with a Minolta CR-400 colorimeter (Minolta®).

**2.7 Sensory analysis**

For sensory evaluation, the hamburgers were prepared from each formulation with the proposed water ratio (1:1) and cooked in a hot air convection oven (180°C) for 20 min. The samples were coded and presented to 103 tasters at the Sensorial Laboratory of Universidade Estadual de Maringá at an approximate temperature of 60°C. For all the samples, tasters scored color, taste, flavor, texture, and overall acceptability with the help of a 9-point hedonic scale, with 9 indicating extreme like and 1 indicating extreme dislike (Wichchukit & O'Mahony, 2015).

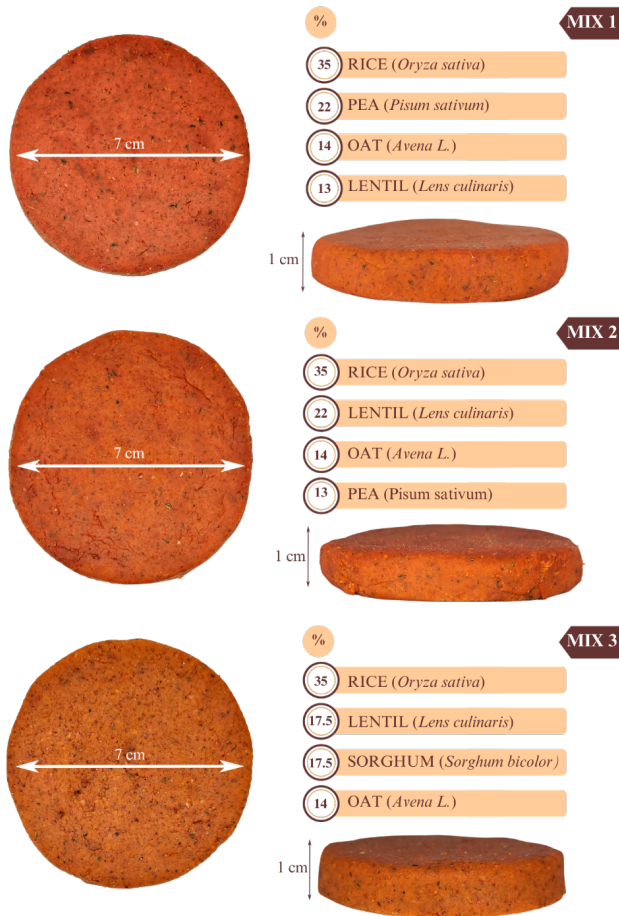
**2.8 Statistical analysis**

The complete experiment was replicated two times using a completely randomized design. The chemical composition and physical and chemical characteristics were performed in triplicates in each experiment repetition. In acceptability (sensory analysis), the experimental design consisted of randomized complete blocks (the treatments were the formulations and the blocks were the tasters). Data were submitted to analysis of variance at 5% probability significance. The SAS Inst computer system evaluated the differences between the hamburger formulations and consumer acceptance (SAS Institute, 2010).

**3 RESULTS AND DISCUSSION**

**3.1 Physicochemical characteristics**

Table 2 presents the results of the physicochemical characteristics of the mixtures (MIX 1, MIX 2, and MIX 3).



**Figure 1.** Burgers prepared from each formulation (MIX 1, MIX 2, and MIX 3) with the proposed water ratio (1:1).

Significant values were not observed between MIX 2 and MIX 3 samples for water activity. On the contrary, MIX 1 (0.46) was significantly higher in comparison because this formulation had a higher concentration of pea (*P. sativum*). For pH parameters, the values were significantly lower in MIX 3 (5.84) compared with MIX 1 and MIX 2 (5.98 and 5.97). According to Lima et al. (2018), in their work on vegetal burgers of cashew fiber and cowpea, the pH was 5.77, corresponding to values similar to those found in our work.

Compacted densities were in the range 0.67–0.70, demonstrating no significant differences between the developed formulations and homogeneity in this aspect. The moisture content of mixtures ranged from 5.40 to 5.76, showing no significant differences between them ( $p > 0.05$ ). Wichchukit et al. (2013) found similar values in their work with enriched pea protein texturing as a substitute for meat in hamburgers (5.32–6.10).

A similar situation was observed in ash parameter that had no significant differences between the samples (3.05–4.02), characteristics also found by Chilón-Llico et al. (2022) in their research about protein, quality, and sensory perception of hamburgers based on quinoa, lupin, and corn, with values ranging between 2.81 and 5.88.

Significant differences were also not found between samples 1 and 2 (17.99 and 18.09) in the crude protein analysis performed. However, sample 3 presented a lower quantity of crude proteins (11.90) in comparison, which is probably due to the different formulations created during the research, especially the presence of sorghum (*S. bicolor*) and the absence of pea (*P. sativum*) in formulation 3, which does not occur in the first two. When our results are compared with similar studies, a positive point is the most significant amount of proteins found in our formulations (11.90 to 18.09). For example, Benevides et al. (2023) found 5.66 and Lima et al. (2018) detected only 4.86.

Considering the results found for fiber analysis, there were no observed significant differences between the samples (4.68–5.00). In their work with a hamburger analog with cashew fiber, lentil, and spirulina, Benevides et al. (2023) found 10.25 for

crude fiber. This point shows us the possibility of adding greater sources of fiber ingredients in future studies. The same situation happened with FDN and FDA analysis when no significant differences were detected. The range for FDN was 10.38–10.89 and for FDA, it was 5.50–6.66.

$L^*$  color parameter was in the range 57.29–60.30, without significant differences. For  $a^*$  color parameter, there were significant differences between all mixture samples, probably because of the different colors of the grains used to elaborate the pre-gelatinized flours of formulations. On the contrary, for the  $b^*$  color parameter (yellowness), significant differences between samples were not identified.

Considering that the variation in color parameters between the three different mixtures was not so significant, we can consider that the extruded grains presented homogeneity in this aspect. Peñaranda et al. (2023) detected for color parameters in powder,  $L^*$  values between 76.19 and 79.42,  $a^*$  values between 2.71 and 4.51, and  $b^*$  values between 19.10 and 21.75. Also, for texturized protein with different formulations,  $L^*$  ranged between 54.80 and 58.20,  $a^*$  between 1.51 and 10.71, and  $b^*$  between 21.2 and 28.5.

### 3.2 Texture analysis

Table 3 presents the texture analysis results for the hamburgers prepared from each formulation with the proposed water ratio (1:1) without cooking as shown in Figure 1.

Considering the results found for hardness (N), significant differences between the samples were not observed (6.66–8.41), indicating that the stiffness of the different grains used in the formulations does not affect this parameter. Kim et al. (2021) found a range between 3.9 and 8.3 for hardness (N) during their work with beef flavor vegetable hamburgers, demonstrating a large range of difference between their samples for hardness (N), which suggests that the different formulations of extruded grains developed in our work do not vary so much in this sense.

It is possible to observe a similar situation in cohesiveness analysis, in which values are not significantly different between formulations ( $p < 0.05$ ), ranging from 0.90 to 0.92. Monego et al. (2018) found a range of 0.68–0.70 for hamburgers made with meat from lambs fed on whole cottonseed, demonstrating a lower value for this parameter compared with meat hamburgers.

For gumminess, the values also had no significant differences, ranging from 6.13 to 7.59. Considering that this indicator should be used to describe the taste of semi-solid foods, we can affirm that the taste of the samples does not change enough to be detected. In this way, other characteristics can be considered for a possible release of this product on the market, for example, the mixture that presents the best nutritional values.

**Table 2.** Physicochemical parameters of hamburger mixes.

	MIX 1	MIX 2	MIX 3
Water activity (g/ml)	00.46 <sup>a</sup> ± 00.00	00.45 <sup>b</sup> ± 00.00	00.45 <sup>b</sup> ± 00.00
pH	05.98 <sup>a</sup> ± 00.00	05.97 <sup>a</sup> ± 00.00	05.84 <sup>b</sup> ± 00.00
Compacted density (g/ml)	00.69 <sup>a</sup> ± 00.00	00.70 <sup>a</sup> ± 00.00	00.67 <sup>a</sup> ± 00.00
Moisture (%)	05.76 <sup>a</sup> ± 00.06	05.40 <sup>a</sup> ± 00.01	05.56 <sup>a</sup> ± 00.01
Ash (%)	04.02 <sup>a</sup> ± 01.32	03.87 <sup>a</sup> ± 00.33	03.05 <sup>a</sup> ± 00.40
Crude protein (%)	17.99 <sup>a</sup> ± 00.01	18.09 <sup>a</sup> ± 00.02	11.90 <sup>b</sup> ± 00.06
Crude fiber (%)	04.78 <sup>a</sup> ± 00.13	05.00 <sup>a</sup> ± 00.36	04.68 <sup>a</sup> ± 00.02
FDN (%)	10.38 <sup>a</sup> ± 00.39	10.89 <sup>a</sup> ± 00.02	10.70 <sup>a</sup> ± 01.92
FDA (%)	06.61 <sup>a</sup> ± 00.09	06.66 <sup>a</sup> ± 00.17	05.50 <sup>a</sup> ± 00.50
$L^*$	60.30 <sup>a</sup> ± 05.46	58.38 <sup>a</sup> ± 03.80	57.29 <sup>a</sup> ± 04.07
$a^*$	11.41 <sup>a</sup> ± 00.11	13.99 <sup>b</sup> ± 00.36	16.64 <sup>c</sup> ± 00.24
$b^*$	39.67 <sup>a</sup> ± 00.10	40.58 <sup>a</sup> ± 01.69	38.77 <sup>a</sup> ± 00.13

\*Results are expressed as mean ± SD; Values with different letters in the same line are significantly different ( $p < 0.05$ ) by Tukey's test.; Colorimeter parameters mean  $L^*$  = luminosity,  $a^*$  = redness,  $b^*$  = yellowness.

**Table 3.** Texture parameters of hamburgers prepared without cooking.

	MIX 1	MIX 2	MIX 3
Hardness (N)	08.41 <sup>a</sup> ± 00.80	07.49 <sup>a</sup> ± 00.17	06.66 <sup>a</sup> ± 00.49
Cohesiveness	00.90 <sup>a</sup> ± 00.00	00.91 <sup>a</sup> ± 00.00	00.92 <sup>a</sup> ± 00.00
Gumminess	07.59 <sup>a</sup> ± 00.68	06.78 <sup>a</sup> ± 00.12	06.13 <sup>a</sup> ± 00.38

\*Results are expressed as mean ± SD; Values with different letters in the same column are significantly different ( $p < 0.05$ ) by Tukey's test.

### 3.3 Sensorial analysis

Table 4 shows the sensory evaluation results and the testers' overall preference among the hamburger samples (1, 2, and 3).

No significant differences between all the samples for color, taste, flavor, or texture can be observed. This behavior could be a positive point considering the possibility of the development of high-protein vegan burgers with different grains and proportions without affecting consumer acceptance.

For colors, the results ranged from 6.18 to 6.42, for taste from 5.69 to 6.43, for flavor from 6.12 to 6.20, and for texture from 6.16 to 6.44, indicating that the scores flowed through "neither like nor dislike" and "like slightly."

Although none of the attributes presented significantly different values between the samples, tasters had an overall preference over sample 3, with 49% of the choices. This formulation contains sorghum (*S. bicolor*) and is absent of peas (*P. sativum*), unlike the others, which may contribute to a better acceptance considering the strong flavor that peas can impart to the final product.

## 5 CONCLUSIONS

The growing rise of the vegetable protein market has become a driving force in the search for meat analogs, even though the challenges are due to the technological properties of isolated plant protein. Improving its sensory attributes requires more research but, on the contrary, it is possible to affirm that proteins obtained by the extrusion process improve these properties in texturization.

In this work, we detected a rise of 49% in preference through 103 tasters at MIX 3. A formulation with pre-gelatinized sorghum (*S. bicolor*) flour presented 11.90 of crude protein (%) and 04.68 of crude fiber (%). This result cannot cancel out the properties of the other samples (MIX 1 and MIX 2), which presented significantly higher amounts of proteins (17.99 and 18.09), leaving the possibility of improving the sensory properties of these formulations to future work and adding ingredients that also provide a higher concentration of fiber.

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**Table 4.** Results of sensory analysis and overall preference.

	MIX 1	MIX 2	MIX 3
Color	06.37 <sup>a</sup> ± 00.06	06.18 <sup>a</sup> ± 00.41	06.42 <sup>a</sup> ± 00.36
Taste	05.69 <sup>a</sup> ± 00.90	06.20 <sup>a</sup> ± 00.57	06.43 <sup>a</sup> ± 00.17
Flavor	06.12 <sup>a</sup> ± 00.16	06.14 <sup>a</sup> ± 00.51	06.20 <sup>a</sup> ± 00.93
Texture	06.16 <sup>a</sup> ± 00.56	06.31 <sup>a</sup> ± 00.82	06.44 <sup>a</sup> ± 00.23
Overall preference (%)	16	38	49

\*Results are expressed as mean ± SD; Values with different letters in the same column are significantly different ( $p < 0.05$ ) by Tukey's test.

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