

# Physicochemical and Nutritional Properties of Plant-Based Burger Patties: The Role of Hydrocolloids and Pea Protein

## Propiedades fisicoquímicas y nutricionales de las hamburguesas vegetales: el papel de los hidrocoloides y la proteína de guisante

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### Resumen

Uno de los principales desafíos en el desarrollo de análogos cárnicos de origen vegetal es reproducir las propiedades fisicoquímicas y texturales de los productos cárnicos procesados convencionales. Este reto ha cobrado relevancia debido al creciente interés global por fuentes alternativas de proteína. Entre ellas, las proteínas vegetales han logrado una amplia aceptación gracias a su disponibilidad, versatilidad funcional y aptitud para incorporarse en diversas matrices alimentarias, como las hamburguesas vegetales. El contenido y la retención de humedad son parámetros críticos de calidad en la formulación de hamburguesas vegetales, ya que influyen directamente en la percepción del consumidor sobre la frescura y la calidad global del producto. Un nivel elevado de humedad se asocia con propiedades texturales deseables, en particular una sensación en boca suave y jugosa, característica de las hamburguesas de carne tradicionales. No obstante, la adición de humedad debe evaluarse cuidadosamente, pues un desequilibrio con la estructura del producto puede afectar negativamente la percepción sensorial. La incorporación de ingredientes con capacidad de retención de agua es una de las estrategias más efectivas para reducir la pérdida de humedad durante el procesamiento de hamburguesas vegetales. Estos ingredientes —principalmente hidrocoloides y aislados proteicos de origen vegetal— presentan una alta afinidad tanto por el agua libre como por los componentes estructurales de la matriz alimentaria. Sin embargo, la proporción e interacción de estos agentes retenedores de agua deben evaluarse con precisión, ya que también pueden generar retos en la formulación o comprometer otras propiedades funcionales y nutricionales del producto final. Para analizar estos efectos, el presente estudio evaluó formulaciones compuestas por agua (85 % – 90 %), aislado de proteína de arveja (5 % – 10 %) y un hidrocoloide comercial (5 % – 10 %), utilizando un diseño de mezclas de vértices extremos. Los resultados muestran que la concentración de hidrocoloide influye significativamente en el contenido de agua de las hamburguesas vegetales fritas, alcanzando valores superiores al 60 %. Un mayor contenido de agua se asocia con una disminución de la fuerza de corte/compresión (de 300 N a aproximadamente 100 N), lo que se traduce en una textura más suave. Asimismo, el rendimiento de cocción mejora, superando el 100 %, mientras que la contracción del producto se reduce, con disminuciones de diámetro inferiores al 2 %. Sin embargo, este mayor contenido de humedad también se vincula con un aumento en la absorción de grasa durante la fritura, elevando los niveles de lípidos en más de un 15 %. Además, la incorporación de hidrocoloides reduce el contenido total de fitatos (<2 mg/g de muestra) como resultado tanto del efecto térmico como de la dilución por un ingrediente libre de fitatos (como el hidrocoloide), lo que podría mejorar la biodisponibilidad de minerales. Por otro lado, un mayor nivel de aislado de proteína de arveja incrementa la digestibilidad proteica in vitro (superior a 5 mg GE/g de muestra), tal como se anticipó en pruebas preliminares, contribuyendo así a un mejor perfil nutricional. No obstante, un incremento en la cantidad de proteína también eleva los valores de fuerza de corte/compresión (>300 N), lo que impacta negativamente la textura. Estos hallazgos ponen de relieve el doble efecto de los ingredientes con capacidad de retención de agua sobre las propiedades fisicoquímicas y nutricionales de las hamburguesas vegetales. Por lo tanto, el ajuste preciso de las concentraciones de hidrocoloide y aislado proteico resulta esencial para optimizar el equilibrio entre textura, retención de humedad, contenido de grasa y disponibilidad de nutrientes.

**Palabras claves:** Lenteja común; arveja; hidrocoloide; reología; fuente de proteína vegetariana.

### Abstract

One of the main challenges in the development of plant-based meat analogs is replicating the physicochemical and textural properties of conventional processed meat products. This issue has gained relevance due to the increasing global demand for alternative protein sources. Among these, plant proteins have achieved broad acceptance owing to their availability, functional versatility, and suitability for incorporation into diverse food matrices, such as plant-based burger patties. Moisture content and retention are critical quality parameters in the formulation of plant-based burger patties, as they directly influence consumer perception of freshness and overall product quality. High moisture levels are associated with desirable textural properties, particularly soft and juicy mouthfeel, which is characteristic of traditional meat-based patties. Nevertheless, moisture addition should be carefully evaluated, as an imbalance with the product's structural texture can negatively affect sensory perception. The incorporation of water-holding ingredients is one of the most effective strategies to reduce water loss during the processing of plant-based burger patties. These ingredients—mainly hydrocolloids and plant-based protein isolates—exhibit a strong affinity for both free water and the structural components of the food matrix. However, the proportion and interaction of these water-holding agents must be carefully evaluated, as they may also introduce formulation challenges or compromise other functional and nutritional attributes of the final product. To investigate these effects, the present study evaluated formulations containing water (85% – 90%), pea protein isolate (5% - 10%), and a commercial hydrocolloid (5% - 10%) using an extreme vertices mixture design. The results indicate that hydrocolloid concentration significantly influences the water content of fried plant-based burger patties, with values exceeding 60%. Increased water content is associated with a decrease in shear/compression force (from 300 N to approximately 100 N), resulting in a softer texture. Cooking yield also improves, reaching values above 100%, while product shrinkage is reduced, as evidenced by diameter reductions below 2%. However, this higher moisture content is also linked to an increased fat absorption during frying, with fat levels rising more than 15%. Additionally, the incorporation of hydrocolloids reduces total phytate content (<2 mg/g of sample) as a result of both thermal effects and dilution by phytate-free ingredient (such as hydrocolloid), which may further enhance mineral bioavailability. Furthermore, increasing level of added pea protein isolate improves in-vitro protein digestibility (above 5 mg GE/g of sample) as expected based on preliminary trials, thereby contributing to an enhanced nutritional profile. However, increased protein levels also lead to higher shear/compression force values (>300 N), negatively affecting texture. These findings highlight the dual impact of water-holding ingredients on the physicochemical and nutritional attributes of the plant-based burger patties. Therefore, precise adjustment of hydrocolloid and protein isolate concentrations is essential to optimize the balance between texture, moisture retention, fat content, and nutrient availability.

**Keywords:** Lens culinaris; pea; hydrocolloid; rheology; vegetarian protein source.



## INTRODUCTION

The global meat industry has experienced significant growth over the past decade. According to the Food and Agriculture Organization (FAO), global meat production (beef, pork, poultry, pigmeat, and ovine) has increased fivefold since 1960, reaching approximately 363.9 million tons in 2023 [1]. In 2022, the largest producers were Asian countries, accounting for 43.6% of total production, followed by North America (18%) and South American (13.5%) [2], [3]. One of the main drivers of this growth is the rising global demand for meat, primarily fueled by global population expansion. However, this trend comes at the expense of high resource consumption, extensive land use, greenhouse gas emissions, and the generation of byproduct - all of which contribute significantly to climate change. Furthermore, several studies have associated red meat consumption with increased risks of cardiovascular diseases, colorectal cancer, and type 2 diabetes [4].

In response to these concerns, organizations such as the World Health Organization (WHO) have advocated for a shift toward plant-based diets [5]. Similarly, increasing awareness of sustainability, health, and ethical considerations has contributed to changes in dietary habits, with flexitarian customers being a major driver of market trends in plant-based products, particularly those seeking meat-like textures. Another important factor influencing these trends is the age profile of customers, as younger generations such as Millennials and Generation Z are having a notable impact [6], [7], [8]. Plant based meat analogs (PBMA) have emerged as a protein-rich food alternative that is well accepted by these type of consumer groups, characterized by distinct sources and production methods. PBMA have attracted considerable attention due to their potential nutritional advantages over red meat, and the plant-based protein market is projected to surpass \$27 billion by 2030 [4], [8]. However, the growing consumption of PBMA presents challenges to the meat industry, as these products must be carefully developed to closely replicate the physicochemical, nutritional and sensory properties of conventional meat products.

The raising popularity of plant-based proteins (PBPs) is largely driven by ongoing innovation in PBMA development. A variety of PBPs have been identified for use in the food industry, including soy protein, legume-based proteins, wheat gluten, and seed-based proteins [9]. The selection of raw materials for meat analog development depends on several factors, such as the type of final product (e.g., minced products like burger patties and nuggets, muscle-type products such as chicken or steak analogs, or emulsified products like sausages), raw materials availability, technological capabilities, and economic feasibility [10].

Consumer research indicates that meat-eaters are more likely to adopt PBMA when these products closely mimic meat in terms of texture, flavor and sensory experience, and when they can be easily integrated into familiar meals. It is important to highlight that sensory attributes – particularly texture - remain one of the main technological challenges in the development of PBMA. However, achieving desirable sensory alone is insufficient, nutritional adequacy must also be addressed.

From a technological perspective, moisture content plays a critical role in the development of PBMA, as it directly affects texture and consumer perception [4], [11]. To improve the soft and hydrated texture typical of conventional meat, food engineers commonly incorporate hydrocolloids or moisture-retaining agents PBMA formulations, especially in plant-based burger patties. Hydrocolloids-including polysaccharides, gums, and cellulose derivatives - perform multiple functions in food systems, such as increasing viscosity, acting as binding, thickening, or gelling agents, as well as enhancing palatability, mouthfeel perception, and product stability [12]. During frying, their primary function is to form a surface barrier that limits oil uptake and reduces water loss [13].

In parallel, the addition of isolated PBPs enhances both quality and functionality in plant-based burger patties formulations, making their inclusion essential when aiming to simulate animal-based protein characteristics [8]. The functional properties of PBPs depend on their structure and composition. Key nutritional characteristics include protein digestibility, typically assessed through in-vitro protein digestion assays. Important physical and technological properties include moisture retention content, oil uptake, reduction of antinutritional factors, and structural modifications during thermal processing-particularly during frying.

Although various hydrocolloids and PBPs are available for the development of plant-based burger patties, understanding their behavior within the formulation and during thermal processing is essential for optimizing product performance. Therefore, the objective of this study was to investigate the effects of hydrocolloids and pea protein incorporation on the physicochemical and nutritional properties of a plant-based burger formulation.

## MATERIALS AND METHODS

### Materials

Whole dry store-bought lentils were used as the primary protein source for the development of a plant-based burger patty formulation. The lentils were purchased in a local market.

A commercial hydrocolloid mix (HM), SUPRAMEAT PBT5468, was supplied by IPF (Medellin, Colombia). This mixture consists of wheat starch, wheat protein, and methylcellulose. The manufacturer recommends a dosage exceeding 10% of the total sample weight. Pea protein isolate (PPI), PISANE C9, was also supplied by IPF (Medellin, Colombia). According to the manufacturer, the protein isolate contains 93% dry matter and 86% protein content.

Commercial transglutaminase was obtained from Molecular Cuisine. This enzyme, commonly used in the meat industry, catalyzes the cross-linking of lysine and glutamine residues present in protein sources. The dosage applied in this study was 5 g of enzyme per 1 kg of protein (dry basis) [t].

### Plant-based burger patties formulation and processing

The plant-based burger patty formulation included ingredients such as fresh onion, garlic, paprika, parsley leaves, and seasoning including cumin, thyme, celery, oregano and salt. All ingredients were pre-mixed before making the patty formation. Lentil grains were pre-cooked in water at 70 °C for 30 seconds. This thermal treatment facilitated partial starch pre-gelatinization and softened the grain testa in agreement with preliminary trials. The pretreated lentils were then combined with the previous mixed vegetables and seasoning, along with the hydrocolloid mix, pea protein isolate, transglutaminase enzyme, and water, according to the proportion established in the experimental design (Table 1). The complete mixture was homogenized and shaped into 100 g portions. Each patty was pan-fried in sunflower oil at 190 °C for 3 minutes on each side. After frying, the samples were left to rest at room temperature for 1 hour and then stored at 4 °C for 12 hours prior to analysis. All the evaluations described in the following sections were performed using these stored and fried samples.

### Experimental design

To evaluate the effect of water-holding components on the properties of plant-based burger patties, a mixture design response surface methodology (MDRSM) with an extreme vertices design was applied using Minitab 22 software (Minitab Inc., Pennsylvania, USA). The experimental region was defined using three pseudo-components as independent variables: water (X1), hydrocolloid (X2), and protein (X3). The variables range are shown in Table 1, and the factor intervals were established based on preliminary trials.

The MDRSM included 9 experimental formulations and 5 central point replicates, for a total of 13 treatments conducted in randomized order to minimize systematic error. Treatments 1 through 4 represent vertex points and midpoints between vertices in the factor space. Treatments 5 to 8 correspond to second-degree centroids, located at the midpoints of lines connecting two adjacent vertices. Treatments 9 to 13 are replicates at the central point of the mixture space, representing third-degree centroids (ternary mixtures of the three components).

TABLE 1. MIXTURE DESIGN APPLIED TO EVALUATE THE WATER HOLDING COMPONENTS IN PLANT-BASED BURGER PATTIES FORMULATION.

Treatment	Statistical order	Components		
		Water (%) X1	Hydrocolloid (%) X2	Pea Protein (%) X3
1	8	80.0	10.0	10.0
2	1	85.0	5.0	10.0

3	7	90.0	5.0	5.0
4	5	85.0	10.0	5.0
5	10	82.5	8.75	8.75
6	3	85.0	6.25	8.75
7	2	87.5	6.25	6.25
8	9	85.0	8.75	6.25
9	4	85.0	7.5	7.5
10	6	85.0	7.5	7.5
11	13	85.0	7.5	7.5
12	12	85.0	7.5	7.5
13	11	85.0	7.5	7.5

A second-order polynomial model was fitted to describe the relationships between the dependent variables and the mixture components. The general form of the model is shown in Equation (1).

$$Y = \beta_0 + \sum_{i=0}^k \beta_i x_i + \sum_{i=0}^k \beta_{ii} x_i^2 + \sum_{i<j}^k \beta_{ij} x_i x_j + \varepsilon_i \quad (1)$$

Where Y is the predicted response, and are mixture components, is the interception, are the lineal coefficients, are the interaction coefficients, and is the random error.

Statistical analysis and construction of contour plots were conducted using Minitab version 22 (Minitab Inc., Pennsylvania, USA). Regression analysis and analysis of variance (ANOVA) were performed to identify significant model terms. Each experimental response was measured in replicates. Optimization of the response was carried out using a graphical overlay technique, which enables the simultaneous optimization of multiple variables. All mixture components were constrained within their specified ranges, while the responses were either maximized or minimized according to the desired formulation objectives.

### Cooking properties

#### *Cooking yield (%)*

The weight of each plant-based burger patty was recorded before and after the frying using an analytical balance. For each treatment, three patties were measured under identical conditions. Cooking yield was calculated according to Equation (2), as described by Presenza et al. (2022).

$$\text{Cooking yield (\%)} = \frac{\text{Cooked weight}}{\text{Raw weight}} * 100 \quad (2)$$

#### *Diameter reduction (%)*

The diameter of the plant-based burger patties was measured before and after frying using a digital caliper. For each treatment, three patties were measured under identical conditions. Diameter reduction was calculated using Equation (3), as described by Presenza et al. (2022).

$$\text{Diameter reduction (\%)} = \left( \frac{\text{Raw diameter} - \text{Cooked diameter}}{\text{Raw diameter}} \right) * 100 \quad (3)$$

### Moisture content (%)

Moisture content was determined according to the standard method described by AOAC 950.46 [16]. Petri dishes were pre-dried at 105 °C for 3 hours, cooled in a desiccator, and then weighed. Three grams of each sample were placed into the pre-dried petri dish (W1) and dried in an oven (Cole-Parmer Bpg-7052) at 105 °C for 3 hours. After drying, samples were cooled in a desiccator and weighed again (W2). Moisture content was calculated using Equation (4).

$$\text{Moisture (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad (4)$$

Where W1 is the initial weight of the samples before drying and W2 is the final weight after drying. Each measurement was performed in triplicate for every treatment.

### **Fat content (%)**

Fat content determination was determined by petroleum ether extraction following AOAC 920.39 standard method [17]. Two grams of dried and milled sample were placed into a cellulose extraction thimble (Fisherbrand Filtration Extraction Thimbles - Grade 645 Cellulose) and fitted into a Soxhlet equipment. Petroleum ether was used as the extraction solvent, and the process was conducted for 4 hours. After extraction, the solvent was recovered in rotary evaporation (Labrota 4001, Heidolph). The flask containing the extract was subsequently dried in an oven at 75 °C for 2 hours. Once dried, the flask was weighed, and the ether extract was quantified using Equation (5) and expressed as fat content (%).

$$\text{Fat content (\%)} = \frac{W_1 - W_2}{W_3} \times 100 \quad (5)$$

Where W1 is the weight (g) of the flask containing the extracted fat, W2 is the weight (g) of the empty flask, and W3 is the weight (g) of the sample. Each treatment was analyzed in triplicate.

### **Shear/compression force**

The shear/compression force test was adapted from Martín et al. (2023), with minor modifications. The analysis was performed using a Kramer five-blade probe. Samples were obtained from pre-cooked plant-based burger patties and cut into pieces measuring 60 mm (width) x 60 mm (length) x 20 mm (depth). All dimensions were verified using a digital Vernier caliper with 0.1 mm accuracy. The tests were conducted on a CAM-MUE-2kN texture analyzer (Lloyd, UK) with a trigger load capacity of 2 kN. Parameter settings were configured using SISCAL LAB software. Test conditions included a 0.5 N preload, a crosshead speed of 8 mm/min, and a penetration deep of 70%. All measurements were carried out under controlled ambient conditions: 19 °C, 1 atm pressure, and 55% relative humidity. Each treatment was evaluated in ten replicates, and the mean force value was reported for each treatment.

### **In-vitro Protein digestion**

In-vitro Protein digestion was quantified following the Megazyme (2019) method, with modifications. The protocol was adjusted by reducing the reagent amount by half and, the results were compared with the positive confirmation control samples provided in the kit. A five hundred mg sample was weighed and placed into a 50 mL conical tube for simulated gastric and intestinal digestion. The sample was first incubated at 37 °C and pH 2.2 for 30 minutes. Then, 1 mL of pepsin solution was added, and gastric digestion continued for an additional 60 minutes. After the gastric phase, the pH was adjusted to 7.4, and 200 µL of a trypsin/chymotrypsin solution were added. Intestinal digestion proceeded for 4 hours at 30 °C. Enzymatic activity was finished by placing the samples in a boiling water bath for 10 minutes.

Protein precipitation in the digested samples was performed by adding 1 mL of 40 % trichloroacetic acid (TCA), followed by incubation at 4 °C for 16 hours. The resulting supernatant - representing the digested protein fraction - was collected and quantified. For quantification, 1.75 mL of the supernatant were centrifuging at 15000 g for 10 minutes at room temperature using a MICROCL 21R (Thermo Scientific) microcentrifuge. The resulting protein solution was diluted (1:10) in acetate buffer, and a colorimetric reaction was carried out by mixing 400 µL of the diluted sample with 200 µL of 2% ethanol – based ninhydrin solution. The mixture was incubated in a MULTITHERM H5000-H for 35 minutes at 70°C and 100 rpm. After cooling for 10 min, 600 µL of 50% (v/v) ethanol were added. Absorbance was measured at 570 nm using a Varioskan Lux spectrophotometer (Thermo Scientific, USA). A standard calibration curve was prepared from a 10 mM L-glycine stock solution and fitted across the range 0 - 1 mM. The R2 of the standard curve ranged from 0.9935 to 0.9991. In-vitro protein digestion was expressed as L-Glycine Equivalent per gram of sample.

### **Total Phytate Content**

Total Phytate content was determined following the procedure described by Agostinho et al. (2016). Sample preparation involved the acid extraction of 1 g of dry, previously defatted material, mixed with 20 mL of 0.65 M HCL and stirred for 2 hours. The mixture was centrifuged

at 3500 rpm for 15 minutes, and the resulting supernatant (extract) was collected and stored at -20 °C overnight, as described Latta et al. (1980). For phytate separation, the extract was passed through a Poly-Prep AG 1X8 ion-exchange resin cartridge (Bio-Rad). Cartridge preparation was carried out as follows: the was pre-treated with 5 mL of 0.2 M HCL, followed by shaking and elution with 8 mL of ultrapure Type 1 water (18 MΩ). Next, 10 mL of 1 M NaCl was added, followed by shaking and elution with 8 mL of ultrapure Type 1 water. The cartridge was then activated with 5 mL of 0.2 M HCL, followed again by shaking and elution [22]. For phytate recovery, 8 mL of the extract was loaded onto the resin and eluted using 30 mL of 0.07 M NaCl. The collected eluted was used for phytate quantification[22], [23]. Phytate content was quantified using a colorimetric assay with Wade reagent. A 0.9 ml of the elute was mixed with 0.3 mL of Wade reagent (0.03% FeCl<sub>3</sub>-6H<sub>2</sub>O and 0.3% sulfosalicylic acid in distilled water). The reaction mixture was thoroughly mixed, and absorbance was measured at 500 nm using a Varioskan Lux (Thermo Scientific, USA), as described by Agostinho et al. (2016). Phytate concentration was calculated based on a calibration curve prepared with phytic acid standards and expressed using Equation (6).

$$TP(\text{mg/L}) = \left( \frac{\text{Abs}_{\text{sample}} - b}{m} \right) \times \left( \frac{D}{S} \right) \times \left( \frac{M}{A} \right) \quad (6)$$

Where TP represents the total phytate content, b and m are the intercept (mg) and slope (L) of the phytic acid standard curve, respectively; D is the volume (mL) of 0.65 M HCl used during the extraction stage; S is the weight (g) of the dry, defatted sample; M is the total volume (mL) of 0.07 M NaCl used for phytate elution (30 mL); and A is the volume (mL) of the extract aliquot loaded onto the resin (8 mL).

### Sensory analysis

The study was conducted with a panel of 45 selected assessors from Bogotá, Colombia, all characterized by their habitual consumption of plant-based burger products (S2). Participants were between 18 and 55 years old and included both genders (23 female and 22 male). The sensory evaluation was carried out at the ALS Global sensory laboratory in accordance with ISO 8589[24]. The facility consisted of separate rooms for sample preparation and evaluation, as well as individual booths equipped with controlled temperature and relative humidity.

Frozen samples (-18 ± 2 °C) were unpackaged and pan-fried prior to presentation to the panelists. Both target and commercial reference samples were anonymized using three-digit random codes generated by the GesAnl ALSFull5en5e software. Sample were presented monadically and in sequential order. The quantity of each was standardized and sufficient for evaluation.

Panelists were informed about the purpose of the study and instructed to provide honest and comprehensive responses (S3). To minimize carryover effects, they were asked to cleanse their palate with crackers and water between samples. Prior to the test, assessors were trained on the use of the evaluation scales and instructed on how to rate the intensity of the sensory attributes.

The sensory evaluation was structured into four categories: (i) overall acceptance and perception of the product, (ii) attribute-specific sensory perception, (iii) packaging and visual appeal, and (iv) open comments.

Data collection was performed using the GesAnl ALSFull5en5e software. Frequency distributions were calculated for each attribute and sample. Descriptive statistics - including mean, mode, median, first and third quartiles, minimum, and maximum - were computed to support interpretation of sensory data.

## RESULTS

### Cooking Yield (%)

The significant effects of the formulation variables on cooking yield are summarized in Table 2. The results indicate a synergistic effect between water-hydrocolloid (X1 x X2) and hydrocolloid-protein (X2 x X3) interactions, with the X1 x X2 combination exerting a stronger influence on cooking yield with significant effect (p-Value < 0.05). The response surface in Figure 1a confirms that the samples were highly affected by the proportion of hydrocolloid

and water in the mixture. When a high amount of hydrocolloid was added, the mixture exhibited the highest cooking yield. This phenomenon can be attributed to the limited release of water vapor and controlled oil uptake during the frying process. This behavior will be discussed in the following sections. The incorporation of hydrocolloids promotes the formation of a polymeric network between the components of the plant-based burger patty – mainly starches and methylcellulose - which are capable of retaining water due to their hydration capacity [25], [26]. When the samples are immersed in hot oil during frying, it is possible that a small fraction of water content is evaporated and released upon contact with the oil at high temperature. During frying process, plant-based burger patties tend to develop micro-channels or small cavities within their structure, which facilitate the partial release water vapor and the simultaneous oil absorption [27]. This sequence of events contributes to the final cooking yield.

Methylcellulose is the primary component of the hydrocolloid used in this study and acts as a controller of mass transfer between water and oil in the system. It is important to note that the functional behavior of methylcellulose depends on its concentration in the mixture, frying time, and interaction with lipids. The results reported in this study provides evidence supporting these observations. Due to its thermal gelation properties, methylcellulose creates a protective layer during frying that limits moisture loss and controlling oil uptake, thereby improving the quality of the final fried product [13].

Similar results have been reported by Bakhsh et al. (2021), who observed a relationship between reduced water - holding capacity and increasing cooking loss due to a decrease in methylcellulose concentration in plant-based meat analogues. These findings are supported by Arora et al. (2017), who found a positive correlation between the concentration of agglutinant agents and cooking yield in the production of plant-based sausages. In their study, cooking yield increased, and processing losses decreased as higher concentration of binding agents were incorporated into the formulations.

TABLE 2. REGRESSION COEFFICIENTS AND ANALYSIS OF VARIANCE OF MATHEMATIC MODELS.

Dependent variables	Independent Variables						R2	P (lack of fit)
	Water (%) X1	Hydrocolloid (%) X2	Protein (%) X3	X1 x X2	X1 x X3	X2 x X3		
Cooking Yield (g)	136.80	3912.00	-199.50	-4671.00*	-	92.00	87.58	0.37
Diameter Reduction (%)	-2.63	-1422	8.8	1700.00*	-	1507.00	44.03	0.06
Shear/Compression force (N)	-199.00	-83833.00	1706.00	100449*	-	66602.00	53.83	0.08
Moisture Content (%)	83.20	5744.00	108.00	-6660.00*	-	-6512.00*	50.68	0.08
Fat Content (%)	32.70	4146.00	36.00	-4865.00*	-	-4018.00	56.75	0.09
In-vitro Protein Digestion (mg GE/g)	1.80	-1737.00	-102.40	1934.00	-	3546.00*	57.10	0.14
Total Phytate Content (mg/g)	-0.35	-550.00	23.60	659.00*	-	352.00	68.68	0.26

Cooking yield = Y1, Diameter reduction = Y2, Shear/Compression Force = Y3, Moisture content = Y4, Fat content = Y5, In-vitro Protein Ddigestion = Y6, Total Phytate Content = Y7. \* Significant at p-Value < 0.05

### Diameter Reduction (%)

The most relevant dimensional parameter affected in this study was diameter, which exhibited shrinkage in all evaluated samples. The results indicate a statistical effect of the interactions (p-Value < 0.05) between X1 x X2 (Table 2). However, the addition of hydrocolloids exerted the most pronounced inverse effect, as illustrated in the contour surface (Figure 1b), where diameter reduction was less substantial compared to the effects of water or protein addition. This shrinkage can be attributed to the release of fluids during the cooking process. Such behavior may result from the limited moisture retention capacity of pea proteins and the competitive interaction for water between pea protein and hydrocolloids. It is important to emphasize that the fluid retention or release depends on the composition, structure and interactions among the components present in the plant-based burger patties [30]. Hydrocolloids are water-soluble polysaccharides that act as cross-linking and binding agents

between proteins or fibrous elements, thereby enhancing moisture content and improving textural properties in plant-based analogues [31]. The results consistent with the findings of Cornet et al. (2021); Van Der Sman et al. (2013), and Vu et al. (2022), who identified three primary mechanisms responsible of water holding capacity in polymeric foods systems: (i) water-polymer mixing effects, (ii) salt ion distribution effects, and (iii) elastic deformation effects. In the present study, the combined influence of these three factors may explain the observed reduction in shrinkage, as reflected in the diameter measurements. During mixing, water interacts with other components influenced by the changes in van der Waals forces, hydrogen bonding, and electrostatic interactions. The high hydrocolloid content in the plant-based burger patties formulation contributes to elevated moisture content in the product due to the abundance and accessibility of hydroxyl groups, which interact effectively with water. In contrast, proteins possess fewer functional groups, limiting their contribution to water retention. Additionally, plant proteins undergo conformational modifications in response to ion presence within the formulation, altering their net charge, structure, and hydration behavior. Moreover, exposure to high frying temperatures induces protein denaturation, leading to aggregation and the formation of a compact network, which further limits the matrix's capacity of retain water [13]. However, this effect is influenced by the concentration of hydrocolloids and their interactions with other components in the formulation, which can modify the extent of protein network formation and moisture retention. The present findings reinforce the conclusions drawn by Dinani et al. (2023), confirming results observed in experiments involving mixtures of wheat gluten, pea protein, and hydrocolloids.

### Shear/Compression Force

Statistical analysis of shear/compression force revealed a significant effect (p-Value < 0.05) for the water-hydrocolloid interaction X1 x X2, compared to other variables. However, the individual effect of hydrocolloid exhibited a notable negative influence on this parameter (Table 2). The contour surface in Figure 1c illustrates the relationship between hydrocolloid concentration and the applied force, with values ranging from 300 N to 490 N.

These results suggest that hydrocolloids can modify the textural properties of plant-based burger patties [4], [35]. Their inclusion in formulation influences both moisture content and oil uptake during frying, contributing to increased fat content. As shown in Figure 1c, a high hydrocolloid concentration resulted in a softer texture, reflected in the reduction of shear/compression force values.

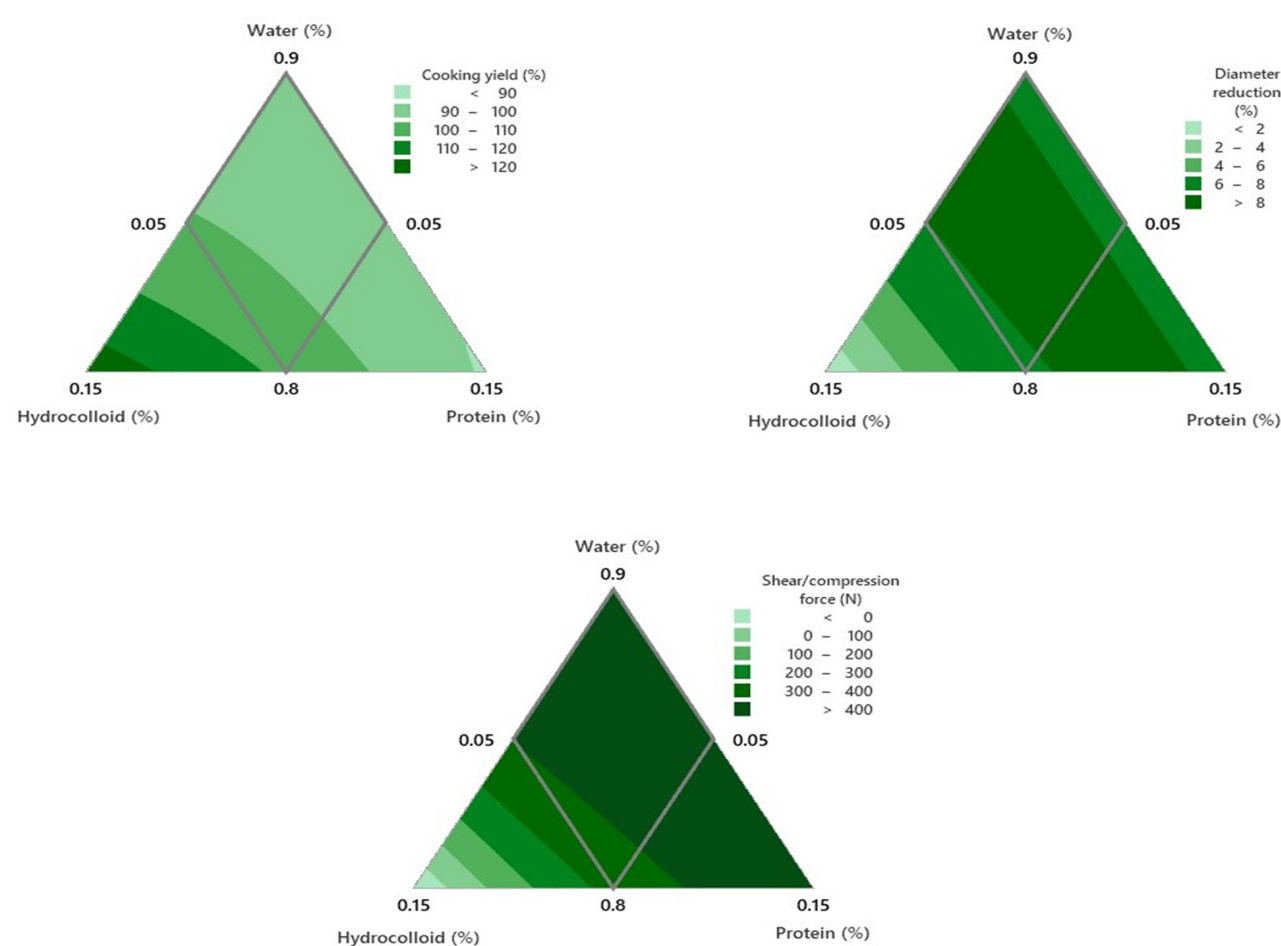


Figure 1. Contour surfaces of physical properties of plant-based burger patties (a) Cooking Yield (%); (b) Diameter Reduction (%); (c) Shear/Compression Force (N)

Some authors report that during frying, hydrocolloids contribute to the formation of an external crust, which acts as a barrier to water loss [13]. However, it is essential to emphasize that hydrocolloids are also commonly employed as fat replacers or texture modifiers in deep-fried products. The type and concentration of hydrocolloid must be carefully optimized to achieve the desirable texture properties without inducing excessive water loss, which could compromise adhesion, cohesion and overall structural integrity of the product [36], [37], [38].

The findings of the present study are consistent with those reported by Peñaranda et al. (2024), who confirmed that methylcellulose and fructo-oligosaccharides (FOS) play a key role in water retention, thereby enhancing the cohesiveness of the plant-based burger patty matrix. Improved water retention also contributes to a decrease in hardness, resulting in a softer texture perception. A similar trend was observed in a study evaluating the addition of  $\beta$ -glucans as hydrocolloid in low-fat beef burger formulations, where increased water-holding capacity led to reduction in hardness [40]. However, contrasting findings have been reported by Tunnarut et al. (2022) and Han et al. (2023), who observed that hydrocolloids such as methylcellulose, carrageenan, xanthan gum can also increase hardness and shear force in plant-based patty formulations.

### Moisture Content

The significant effect of the formulation variables on moisture content was evident across the tested samples, with values ranging from 45.92% to 55.08% across the experimental surface (Figure 2a). The results indicate a synergistic effect between water-hydrocolloid (X1 x X2) and hydrocolloid-protein (X2 x X3) interactions, both exhibiting a similar magnitude of influence. However, when compared to the individual effects of each component, hydrocolloids demonstrated the most pronounced impact (Table 2).

The contour surface (Figure 2a) shows a marked reduction in moisture content in fried plant-based burger patties when hydrocolloid concentration in the formulation was low. These findings underscore the critical role of hydrocolloids in retaining water within the final product. This behavior is particularly important, as it contributes to a higher cooking yield after frying [43], while minimizing product shrinkage.

Both hydrocolloid and water content play essential roles in moisture retention. Hydrocolloids act as a thickening agents with high gelling capacity and strong water – binding potential, thereby limiting moisture loss during thermal process [43]. In addition, hydrocolloids provide structural integrity to plant-based burger patties, both before and after frying. Their gelling capacity enhances cohesion and adhesion within the patty matrix, contributing to formulation stability [13]. The degree of structural integrity is influenced by hydrocolloid concentration, whereas adhesive properties are closely associated with cooking yield [43]. Given the strong interaction between hydrocolloids and the available moisture in the formulation, it is important to expand the discussion regarding the role of hydrocolloids in entrapping water to form gel-like networks. This phenomenon is commonly addressed in studies on water states in deep-fried products, which describe how hydrocolloids alter the mobility and state of water. The increase in bound water prevents water losses and reduces oil absorption during frying. Bound water is characterized by its restricted molecular mobility due to interactions with the hydrophilic functional groups present in hydrocolloids [44], [45].

The moisture content values observed in this study are consistent with those reported for soy-based vegan burgers (41.17% to 56.97%) and pea-based vegan burgers (40.76% to 62.09%) [46]. Similarly, formulations containing hydrocolloid additions have demonstrated retained moisture levels ranging from 43.74% to 57.47% in plant-based burger patties[47].

### Fat Content

Statistical analysis of fat content (d.b.) revealed values ranging from 6.3 % to 15.7 % across the experimental design (Figure 2b). The primary interaction with a significant effect ( $p$ -Value < 0.05) was water-hydrocolloid (X1 x X2), which exhibit the greatest magnitude compared to other interaction terms. Similarly, among the individual components, hydrocolloids had the most substantial influence on the fat content (Table 2).

Figure 2b suggests that the fat content in plant-based burger patties is mainly influenced by oil uptake during the frying process. Previous studies have attributed this phenomenon to mass transfer mechanisms - particularly diffusional process - in which moisture loss is

accompanied by oil absorption [13]. In contrast to previous reports, the present study found that fat content increased with higher hydrocolloid concentrations. This outcome may be attributed to the excess hydrocolloid binding free water prior to frying, as evidenced by moisture retention values of approximately 55%. The high water-binding capacity of hydrophilic hydrocolloids, combined with their ability to interact with starch and proteins, promotes the formation of a cohesive barrier network within the patty matrix. While this network effectively entraps water, it also generates structural channels during frying, facilitating oil penetration and ultimately increasing oil uptake.

Conversely, the free water in the matrix escapes as vapor, modifying the patty's microstructure and increasing its surface permeability[48]. The unbound water undergoes a phase transition from liquid to vapor due to the elevated frying temperatures. This diffusional process leads to a reduction in free moisture - defined as water not associated with hydrocolloids or proteins. As the vapor exits through microchannels in the matrix, it generates capillaries and voids that facilitate oil up take by capillarity - driven filtration[13]. Consequently, fat content is directly related to oil uptake in fried patties, and its variation may depend on changes in hydrocolloid concentration or thermal process stability[49].

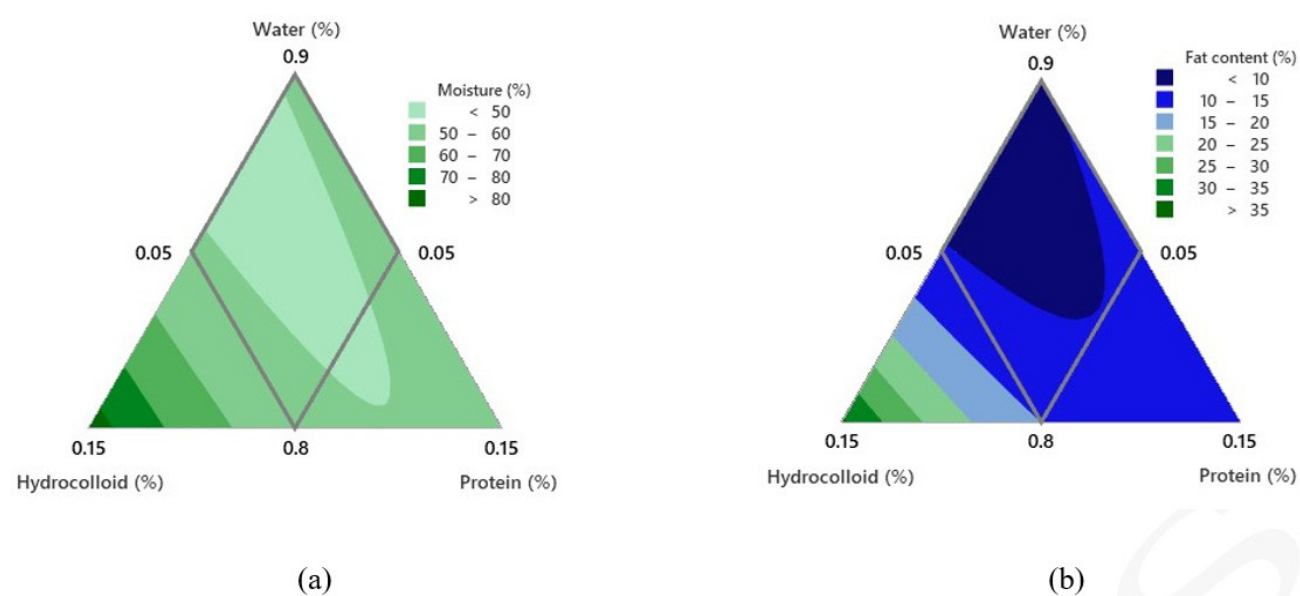


Figure 2. Contour surfaces of chemical properties of plant-based burger patties (a) Moisture content (%); (b) Fat Content (%)

Similar findings were reported by Bakhsh, et al. (2021), who observed that high concentration of hydrocolloids (e.g., methylcellulose) in plant-based burger patties lead to increased fat content alongside reduced moisture. Other studies suggest that hydrophilic hydrocolloids reduce water loss due to their strong water - binding capacity and, in some cases, serve as physical barriers to fat absorption[13]. Kyriakopoulou et al. (2021) also confirmed that certain hydrophilic hydrocolloids—such as methylcellulose, hydroxypropyl methylcellulose (HPMC), long-fiber cellulose, corn zein, and alginates—can enhance oil encapsulation and reduce oil uptake in textured meat analogues. These findings are consistent with earlier studies by Gazmuri et al. (2009), and Varela et al. (2011), which demonstrated that hydrocolloids form moisture-retaining barriers and modify the microstructure of fried products, thereby limiting fat absorption. For instance, the Colombian brand PIETRAN® includes various hydrocolloids in its veggie burger formulation, such as E461 (methylcellulose), E410 (guar gum), and E407 (carrageenan), illustrating the industry's adoption of these ingredients to improve product quality. Given these insights, further evaluation under the specific formulation and processing conditions used in this study is warranted to fully understand the impact of the hydrocolloids on oil uptake and moisture retention in plant-based burger patties.

Figure 3 illustrates the structural behavior of plant-based burger patties before and after frying. The selected samples correspond to treatments from the experimental design that differ in hydrocolloid and protein proportions. Figure 3a and 3b represent a formulation with higher proportion of pea protein (85% water, 5% hydrocolloid, 10% pea protein), where evident structural changes include a high number of channels that facilitate water loss. In contrast, Figure 3c and 3d show a formulation with increased hydrocolloid content (85% water, 10% hydrocolloid, 5% pea protein), which develops a more regular structure with uniform layers, contributing to the structural integrity of the samples. Notably, structural

differences between treatments are more pronounced in the fried samples (Figures 3b and 3d), whereas in the raw samples (Figure 3a and 3c), these differences are less perceptible.

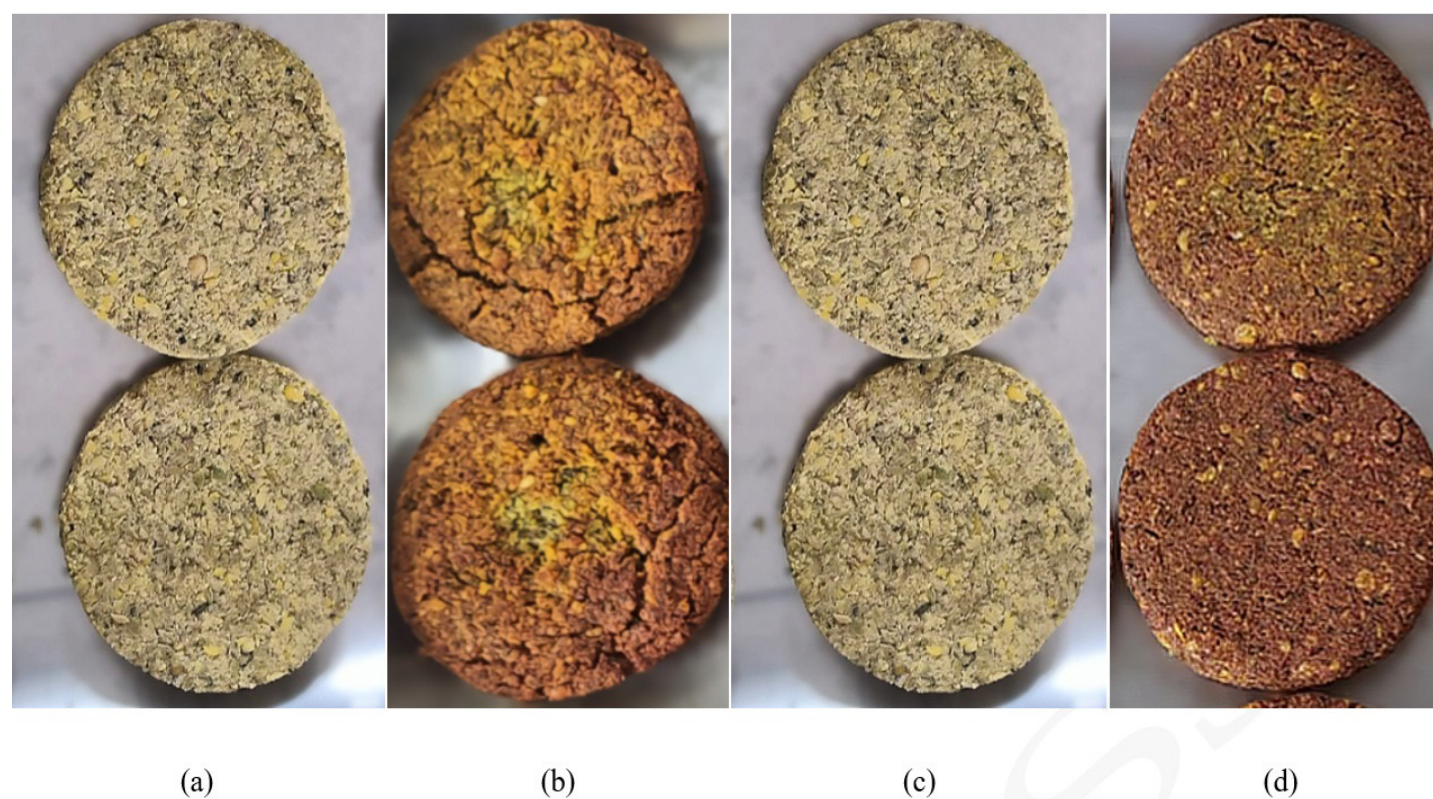


Figure 3. Plant-based burger patty samples, (a) Raw sample 10% pea protein addition; (b) Fried sample 10% pea protein addition; (c) Raw sample 10% hydrocolloid addition; (d) Fried sample 10% hydrocolloid addition

The mechanisms governing water loss and oil uptake during frying in plant-based burger patties are significantly influenced by the type and properties of the hydrocolloids used. Different hydrocolloids exhibit varying capacities to reduce oil uptake and loss of moisture due to differences in their film-forming abilities and interactions with water. For instance, Sakhale et al. (2011) evaluated the incorporation of different hydrocolloids (1.5% inclusion level) into samosa casings and reported significant reductions in oil content. Xanthan gum reduced oil uptake by 53.32% followed by carboxymethylcellulose (CMC, 48.85%), guar gum (43.72%), and hydroxypropyl methylcellulose (HPMC, 30.69%). These findings provide an interesting information to understand the hydrocolloid performance in fried systems, in this context the behavior observed in the present study may differ due to the composition of the commercial hydrocolloid employed – methylcellulose, wheat protein, and starch – which can interact synergistically to form moisture-retaining networks with unique effects on oil uptake.

These reductions are likely attributed to the formation of more effective moisture barriers during frying, particularly at higher hydrocolloid concentrations, as evidenced in Figure 3. In summary, the selection and application of specific hydrocolloids can be strategically used to modulate oil absorption and moisture retention in fried plant-based products. The effectiveness of each hydrocolloid depends on its unique physicochemical properties. In the present study, the commercial hydrocolloid used was a blend of methylcellulose, wheat protein, and starch. This hydrocolloid was incorporated into the formulation of plant-based burger patties to enhance moisture retention and reduce oil uptake during frying. The synergistic effect of its components contributes to water retention through starch gelatinization, which leads to the formation of encapsulating water and reducing its evaporation during frying. Wheat protein enhances structural integrity and interacts with starch to form a cohesive network, while methylcellulose creates a surface barrier that limits both moisture loss and oil absorption[44]. Moreover, the efficacy of the hydrocolloid used in this study was shown to depend on its concentration, as discussed in previous sections.

### In-vitro Protein Digestion

Digestibility is a biological process in which food components are broken down and fragmented into smaller molecules that are absorbed through different sections of the gastrointestinal tract[52], [53]. Statistical analysis of in-vitro protein digestion revealed a significant effect of the hydrocolloid-protein interaction (X2 x X3) (Table 2). Figure 4a indicates that in-vitro protein digestion increases with pea protein addition but decreases with higher hydrocolloid content.

The inclusion of pea protein in this study had two main objectives. First, it was intended to balance the overall protein content of the formulation (Table S1), considering the diluting effect of water and hydrocolloids. Second, it aimed to evaluate the specific impact of pea protein on the in-vitro digestibility of the plant-based burger patties. The contour surface in Figure 4a demonstrates a clear positive effect of pea protein on in-vitro protein digestion. These findings support previous reports by Kyriakopoulou et al. (2021), who observed that pea protein contributes to the formation of firmer textures after thermal processing, thereby acting as a structural enhancer. However, if pea protein concentration is not carefully controlled, it may lead to increased shear / compression force, resulting in a harder product and negatively affecting textural quality. Conversely, the results indicate that high concentrations of hydrocolloids negatively impact in-vitro protein digestion (Figure 4a). This reduction may be attributed to the ability of hydrocolloids to form a gel network that physically hinders enzymatic activity. The gel structure can act as a barrier, limiting access of digestive enzymes to protein substrates and thereby impairing digestion. This inhibitory effect is likely related to the formation of complex gel matrices that reduce the efficiency of enzymatic hydrolysis of plant protein [54], [55].

The findings of this study confirm and extend the conclusions previously reported by Zhi et al. (2024), who reported that high concentration of methylcellulose (MC) in plant-based fish cake analogues significantly reduced in-vitro protein digestibility. In the present study, the inhibition of protein digestion appears to result from both physical and chemical mechanisms. Physically, high MC concentrations increase product hardness and induce matrix compaction, forming a three-dimensional gel-like network that acts as a diffusional barrier and limits the accessibility of digestive enzymes such as pepsin to protein substrates. Chemically, MC can interact with proteins through hydrophobic active sites, potentially masking cleavage sites and interfering with enzymatic hydrolysis. The three-dimensional configuration generated by MC further enhances this effect by restricting enzyme penetration within the compact protein–hydrocolloid matrix. Additionally, elevated MC levels may increase the viscosity of gastrointestinal fluids, further slowing enzyme diffusion and activity. Together, these effects explain the observed reduction in digestibility. [55], [57].

### Total Phytate Content

Phytates are naturally occurring compounds found in lentils and serve as the primary storage form of phosphorus. However, they are classified as antinutritional factors due to their ability to chelate essential minerals such as iron, calcium, zinc and magnesium, reducing their bioavailability and absorption in the gastrointestinal tract [58]. Thermal treatment plays a critical role in the degradation or inactivation of the phytates[59]. Thereof, the quantification of phytate content in food products is widely used to estimate mineral bio-accessibility, particularly in systems where the absorption inhibitors are present [60], [61]. In the present study the phytate content ranged from 3.17 mg/g to 4.38 mg/g. Statistical analysis revealed a significant effect of the water-hydrocolloid (X1 x X2) (Table 2). The contour surface in Figure 4b shows a reduction in the phytate levels with increasing hydrocolloid concentrations.

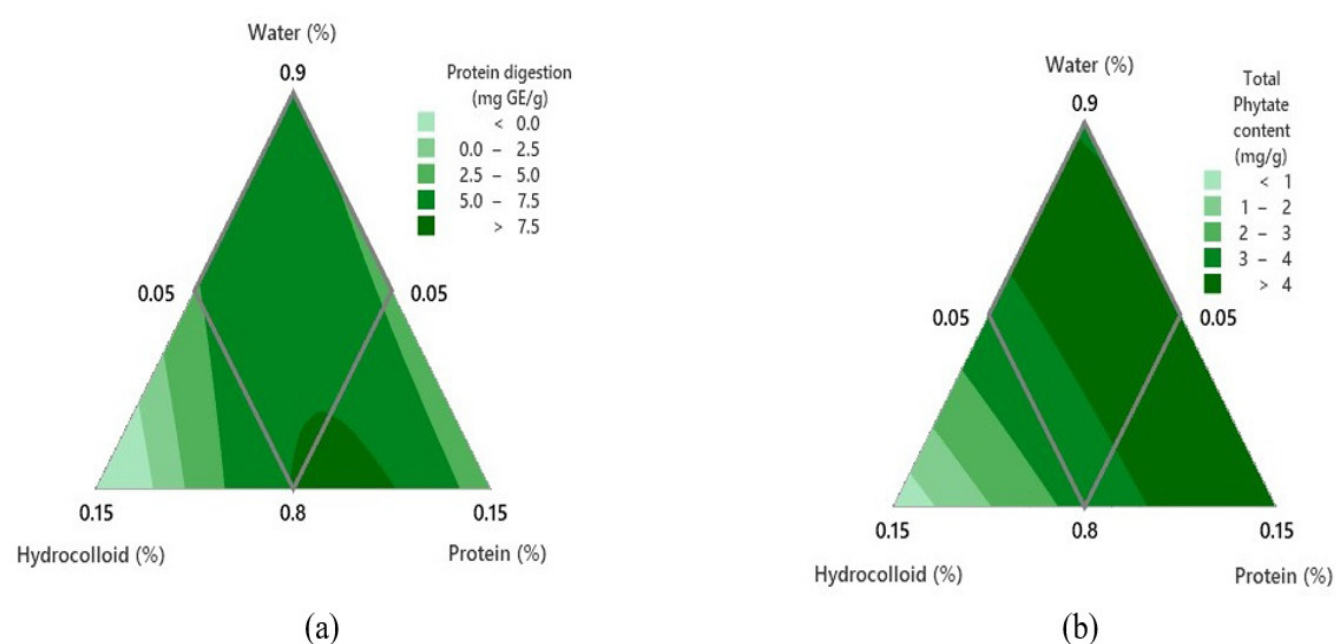


Figure 4. Contour surfaces of nutritional properties of plant-based burger patties (a) In-vitro Protein Digestion; (b) Total Phytate Content

Phytates are inherently present in vegetable proteins sources such as lentils – the main protein content in this formulation – and to lesser extent in pea protein, which was incorporated for its water-holding properties. The observed reduction in phytate content may be attributed to a dilution effect resulting for the addition of hydrocolloids and their ability to retain water [62]. Moreover, the proportion of water in the formulations may enhance heat transfer during frying, promoting partial phytate degradation at high temperatures. Previous studies support this interpretation. For instance, Dewan et al. (2024) reported a decline in phytate content as processing temperatures increased.

Similarly, the results of this study are consistent with the findings by Pospiech et al. (2025), who investigated the relationship between residual phytate content and mineral bio-accessibility. Their study demonstrated partial inactivation of phytase during frying at 200 °C, though it was not sufficient for complete phytase inactivation in burger patties based on pea protein, soy protein, and sunflower protein.

### Optimization

The optimal formulation region for producing a plant-based burger patty with high moisture content and in-vitro protein digestion, along with low shear/compression force and reduced fat content, was identified by superimposition the contour plots corresponding to the selected response variables, based on the desired product quality specifications (Figure 5a). The formulation with the highest overall desirability score of 0.87 (Figure 5b) consisted of 83.8% water, 9.4% hydrocolloid, and 6.8% pea protein.

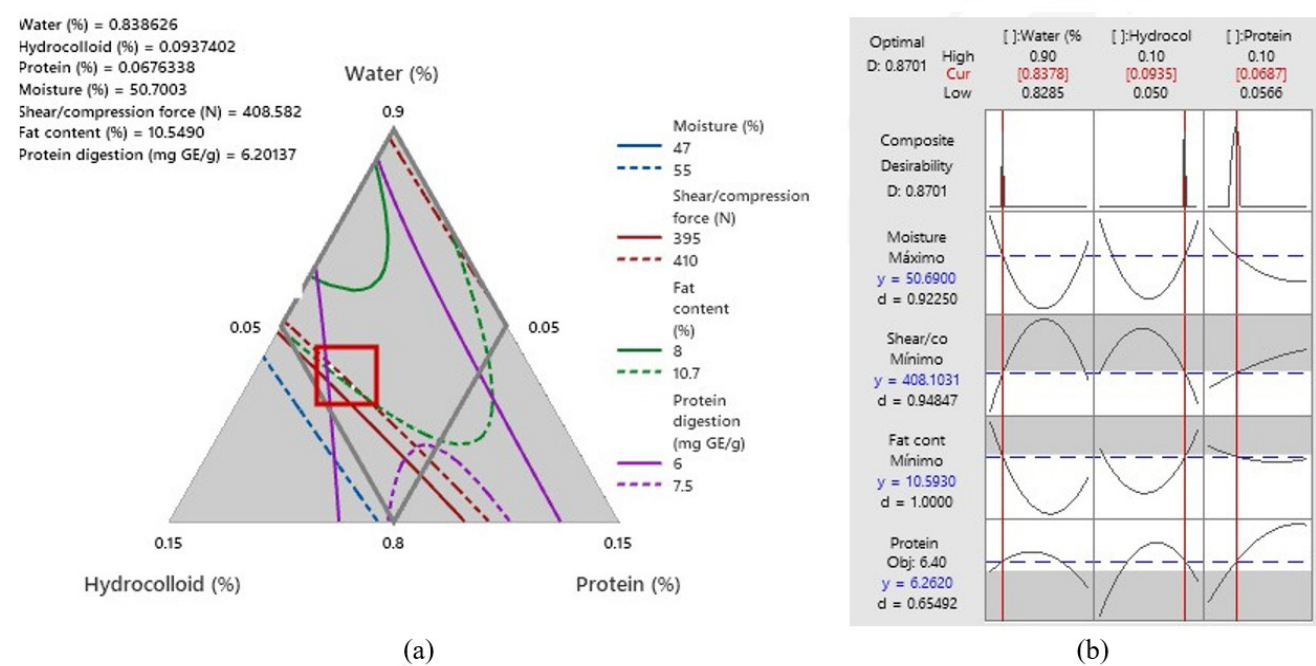


Figure 5. Optimization (a) Contour surfaces superposition for optimization; (b) Response optimization by D-optimal

The optimal formulation was developed and subsequently evaluated through sensory analysis. It was then compared against various commercial alternatives, and the results of this comparison are discussed in the following section.

### Sensory analysis

The sensory evaluation results indicate favorable acceptance and overall perception of the plant-based burger patty (Figure 6). All panelists (100%) stated they would consume the product again. In addition, 89% reported they would recommend it to family and friends, 71% expressed positive overall satisfaction, and 82% preferred the plant-based burger patty developed in this study over commercial alternatives. Despite these encouraging results, certain improvements are necessary to enhance consumer acceptance levels.

Regarding specific sensory attributes, 100% of the panelists recognize a pronounced spiced flavor in the product, however, perceptions of this characteristic were mixed, with only 34% rating it positively. While 62% described the chewing experience as pleasant, 49% suggested that the texture needed improvement, citing descriptors such as dry, gritty, and lumpy. Cutting performance was positively evaluated - 100% of the participants confirmed uniform slicing with a knife. Both flavor and visual appearance were well received, and all panelists correctly identified the product as plant based.

In terms of packaging and presentation, all participants (100%) expressed a preference for individual packing in transparent containers, highlighting a strong consumer desire for full product visibility.

Open-ended comments revealed that many panelists found the texture to be dry and somewhat difficult to swallow, and perceived the flavor as being overly dominated by spices such as lentil, cumin, garlic, and rosemary. Common suggestions included increasing the product's juiciness, reducing the intensity of the spices in the formulation, and improving the overall softness of the texture.

Although the results demonstrate generally positive acceptance, further product development trials are recommended to refine the formulation and better align with consumer expectations.

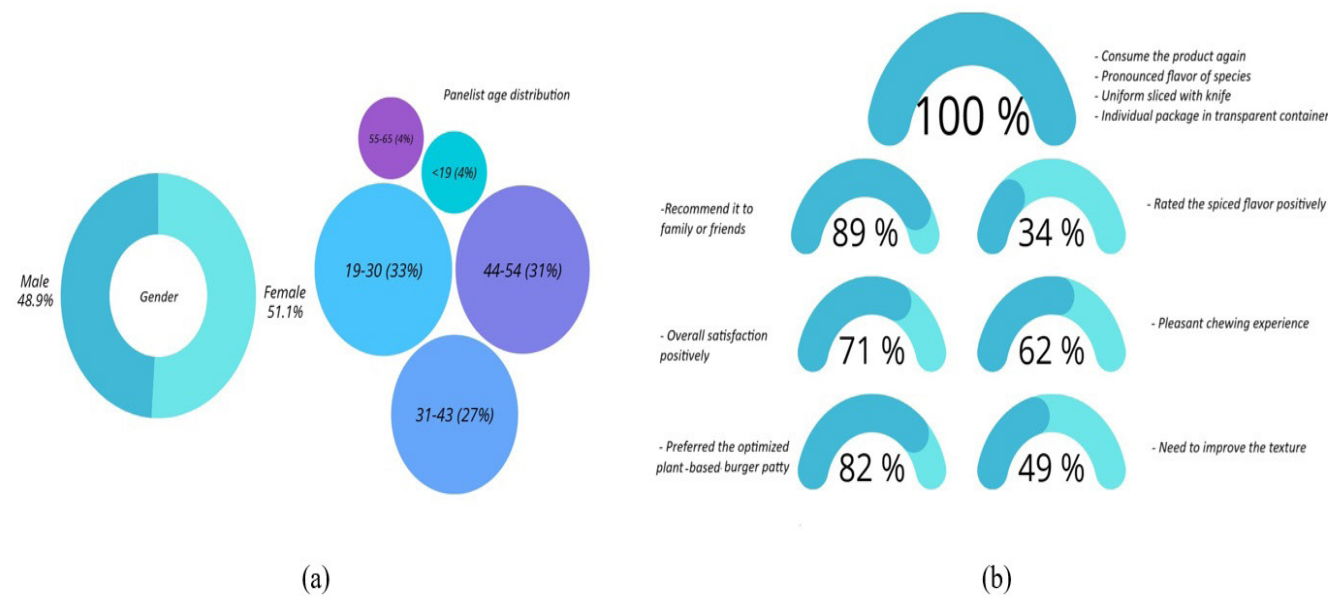


Figure 6. Sensory analysis (a) Panelist distribution; (b) Summary of sensory perception of plant-based burger patty

## CONCLUSION

The incorporation of commercial water-holding agents in plant-based burger patty formulations significantly ( $p < 0.05$ ) influenced their physicochemical and nutritional attributes in a concentration-dependent manner. At optimal levels, hydrocolloids enhanced water retention by forming a network structure capable of effectively entrapping moisture, which contributed to higher cooking yields and reduced shrinkage. However, excessive concentrations negatively affected in-vitro protein digestibility and increased fat content, indicating the importance of balancing hydrocolloid levels to achieve the desired quality. . However, during the frying, elevated temperatures caused partial water evaporation, and diffusion mechanisms facilitated oil absorption into the final product. An increased inclusion of hydrocolloids in the patties enhanced water retention and fat content, which directly influenced the physical properties by increasing cooking yield and reducing shrinkage, as evidenced by minimal diameter reduction. While these characteristics may be perceived as desirable in terms of product size and shape, a comparative assessment with similar commercial products reveals that lower fat content is often combined with a pronounced juiciness and a softer texture. This observation suggests that, in the present formulation, hydrocolloid levels should be carefully optimized to maintain structural advantages without compromising sensory attributes critical for consumer acceptance.

Conversely, a reduction in in-vitro protein digestion was observed under conditions of high hydrocolloid concentration. Nevertheless, the inclusion of pea protein also played a key role in modifying product functionality. While it contributed to balancing the protein content and improving in-vitro protein digestibility, excessive amounts led to firmer textures, as reflected by increased shear/compression force values. High hydrocolloid content may negatively impact the nutritional functionality of the product if protein digestibility falls below desirable levels. Although a precise critical threshold was not fully established in this study, the results suggest that maintaining pea protein levels below the point where texture becomes excessively firm is essential to preserve both sensory quality and nutritional performance.

An increase in hydrocolloid content was associated with a reduction in phytate levels; however, this decrease in the antinutritional factor does not inherently indicate an

improvement in mineral bioavailability. Such an effect would require specific evaluation to be confirmed.

These results emphasize the importance of optimizing the ratio between hydrocolloids and proteins to achieve desirable physicochemical properties and improved nutritional quality in plant-based burger patties. Furthermore, sensory analysis supported the instrumental findings. The formulation optimized for hydrocolloid and pea protein levels was more favorably rated in terms of overall acceptability. Nevertheless, excessive firmness and dryness – often associated with high protein content – negatively affected consumer perception. These outcomes highlight the crucial role of sensory evaluation in guiding formulation development and refining plant-based meat alternatives to better meet consumer expectations.

#### CRedit AUTHORSHIP CONTRIBUTION STATEMENT

R. Yamid-Pismag: Conceptualization, methodology, software, formal analysis, data curation, original draft writing, writing revision, and editing. J. Peña-Camargo: Validation, formal analysis, research, original draft writing, writing revision, and editing. M. Chavarro-Córdoba: Research P. Gómez-Buitrago: Conceptualization. J. Luis-Hoyos: Conceptualization, methodology, validation, resources, project management, fundraising, supervision.

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#### DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author due to (specify the reason for the restriction).

#### CONFLICTS OF INTEREST

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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