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Effect of Wheat Gluten on The Quality Attributes of Plant-Based Meat Analogue Formulated with Pea, Soy and Pumpkin Seed Flours

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ABSTRACT

This study explores the effect of wheat gluten on the quality attributes of plant-based meat analogues (PBMAs) formulated with pea, soy, and pumpkin seed flours. PBMAs were developed with varying gluten levels (0–40 parts), and analyzed for moisture, protein, fat, fiber, ash content, pH, cooking loss, water holding capacity (WHC), swelling capacity (SC), and sensory attributes. WHC is defined as the maximum amount of water a given quantity of material can retain, making it a critical quality attribute associated with freshness and the proteins' ability to form a cohesive gel network. SC is the volume occupied by a known weight of a food after it has absorbed water and reached equilibrium without dissolution. Protein content significantly ($p<0.05$) increased with higher gluten levels, from 33.95% in the control to 55.28% in the highest gluten formulation ($p < 0.05$). Fat content decreased from 28.00% to 6.36% ($p < 0.05$), and cooking loss reduced from 7.3% to 3.14% ($p < 0.05$). The WHC increased from 73.25% to 92.00% ($p < 0.05$), and swelling capacity increased from 0.5123 to 2.8680 ($p < 0.05$). Sensory evaluations (texture and flavor) showed that a moderate gluten concentration (20 parts) optimized sensory parameters by balancing texture, flavor, and overall acceptance. These findings suggest that wheat gluten enhances both the functional and sensory qualities of PBMAs, improving their potential as sustainable meat alternatives.

1. INTRODUCTION

As global food demands rise, the need for sustainable protein sources becomes increasingly urgent. Traditional livestock production, while efficient in delivering essential nutrients such as protein, also poses considerable challenges, including environmental impacts and economic strain. Research indicates that the demand for meat protein is anticipated to nearly double by 2050 due to population growth and dietary shifts (Kyriakopoulou *et al.*, 2021). This increasing demand would typically require an expansion of animal agriculture, yet resources such as land and water remain limited. Furthermore, livestock farming contributes significantly to greenhouse gas emissions, including carbon dioxide, methane, and nitrous oxide, exacerbating climate change (Siddiqui *et al.*, 2022). These environmental and resource limitations, combined with the rising cost of meat products, affect accessibility, especially for lower-income populations, leading researchers and industry professionals to explore plant-based alternatives (Bryngelsson *et al.*, 2022). Plant-based meat analogues have emerged as a viable option that can help bridge the nutritional gap while minimizing environmental strain. These products, derived from plant sources such as soy, wheat gluten, legumes, pumpkin seeds and peas, are engineered to replicate the sensory qualities of animal meat—texture, appearance, and taste—while offering distinct health benefits, including high fiber, vitamins, antioxidants, and lower saturated fats (P. Singh *et al.*, 2021).The development of meat like texture relies on the formation of three dimensional protein networks particularly by gluten which consists of gliadins and glutenins sub-units. Gliadins contribute the viscous properties whereas glutenins are responsible for elasticity and strength. The combination of these two sub-units during hydration and mechanical processing (eg.extrusion) allows he formation of disulpide cross-linked networks, creating an anisotropic, fibrous structure that mimics the muscle fibers of meat, helping create a chewy, juicy texture. (Wieser, 2007). Wheat protein is ideal for products requiring dough formation whereas soya and pea protein is suitable for meat analogs, dairy substitutes protein rich snacks and plant-based beverages. Soy flour rich in lysine offers complementary amino acid profile to wheat flour. Incorporating peas flour can boost the mineral (Fe, Zn Mg, Mn) content of the products. Peas flour inclusion increases the water absorption and reduces sough stability leading to challenges in maintaining product structure during backing. Substitution wheat flour with legumes dilutes gluten network. Processing advancements, particularly extrusion technology, allow for the development of PBMAs that closely mimic meat's fibrous structure, enhancing their appeal as sustainable substitutes (Joshi and Kumar, 2015). PBMAs are often classified by their moisture content into low and high-moisture varieties; low-moisture PBMAs, also known as textured vegetable proteins, are typically used in conjunction with real meat for texture enhancement, while high-moisture varieties are fully plant-based and designed as complete meat replacements. Importantly, PBMAs also address health concerns associated with red and processed meats, which are high in saturated fats and linked to cardiovascular issues and other health risks (Van Vliet *et al.*, 2020). Additionally, while zoonotic diseases remain a risk with animal-derived foods, PBMAs reduce this risk significantly due to their plant-based composition, making them a safer option in pandemic-sensitive times (P. Singh *et al.*, 2021). Thus, PBMAs not only represent a healthier, more sustainable alternative but also align with ethical and environmental goals, positioning them as essential to future food security.

Meat analogues, or plant-based meats, are crafted to replicate the sensory qualities of animal meat—such as flavor, texture, and appearance—while being made from alternative sources like soy, wheat gluten, and pulses (A. Singh and Sit, 2022). These products have gained popularity as sustainable substitutes for animal proteins amid rising concerns over the environmental impact of traditional meat production. With consumer preferences, especially in Western countries, shifting toward more sustainable food options, these ingredients are selected for their ability to closely mimic the sensory and nutritional properties of meat, providing comparable taste, texture, and protein content (Kyriakopoulou *et al.*, 2021). Among various plant flours, full-fat soy flour, pea flour and pumpkin seed flours offers unique properties that improve protein content, texture, moisture retention, and overall stability, meeting consumer demand for sustainable, nutritious, and affordable plant-based meat alternatives (Bryngelsson *et al.*, 2022). Full-fat soy flour is rich in protein, with levels reaching up to 50%, making it an excellent replacement for animal protein in meat analogues (Lusas and Riaz, 1995). Its moisture retention properties and water-holding capacity significantly enhance the texture and juiciness of products like chicken nuggets and beef patties, mimicking the sensory qualities of real meat (Riaz, 2005). Soy flour's high protein quality also ensures that meat analogues maintain nutritional value similar to that of lean meat, while being cost-effective (Ng, 2011). Pea flour is a non-GMO, clean-label ingredient with high protein content, low fat, and a balanced amino acid profile. The primary pea proteins, vicilin and legumin, contribute to strong emulsification and foaming abilities, which aid in the textural stabilization of meat analogues (Yuliarti *et al.*, 2021). Despite its weaker gelling properties compared to soy, adjustments in pH, temperature, and salt addition can enhance its textural performance, enabling its effective use in high-moisture extrusion processes for structured meat analogues (Osen *et al.*, 2014). Extrusion is a common technique in PBMA production, involving high temperature, pressure, and mechanical shear. This process has been shown to reduce ANFs in legumes and other plant proteins. For instance, extrusion at temperatures exceeding 140°C and moisture contents between 12–25% can effectively eliminate trypsin inhibitors and other ANFs, thereby enhancing protein digestibility and bioavailabilit. Pumpkin seed flour, derived primarily from *Cucurbita maxima*, has gained recognition for its protein and oil content, as well as functional attributes like water and fat absorption, protein solubility, and foam stability (Roy and Datta, 2015). These properties support moisture retention and texture consistency, essential for sensory appeal in meat analogues. Moreover, its high unsaturated fatty acid content, particularly linoleic and oleic acids, provides additional nutritional value and contributes to shelf stability, making pumpkin seed flour suitable for long-lasting food products (Hleap-Zapata *et al.*,).

Wheat gluten is integral to plant-based meat analogues due to its distinctive viscoelastic properties, which enable it to replicate the texture, structure, and mouthfeel of animal meat. Comprised primarily of the protein gliadin and glutenin, wheat gluten imparts elasticity and extensibility, essential for achieving the fibrous, meat-like texture in these products (Aguilera, 2019). The presence of disulfide linkages further enhances gluten’s strength and flexibility, making it an effective binder that reduces cooking losses and preserves product integrity during preparation (Boukid, 2021). Additionally, gluten’s protein network traps gases during processing, promoting volume retention and improving the structure of the final product (Flambeau *et al.*, 2024). When paired with other proteins, such as soy or pea, gluten enhances both the sensory and nutritional appeal of meat analogues, supporting consumer acceptability (Kumar *et al.*, 2017). While its Protein Digestibility Corrected Amino Acid Score is lower than soy protein, gluten's high protein content and functional properties make it invaluable in meat analogue formulations, establishing it as a sustainable and cost-effective substitute for animal proteins (C. Sun *et al.*, 2021). Despite these promising attributes, there is a need for in-depth research on the optimal inclusion of wheat gluten in formulations that combine multiple plant protein bases, such as pea, soy, and pumpkin seed flours, to maximize textural, structural, and sensory qualities. Limited studies explore how wheat gluten can specifically improve the binding, moisture retention, and sensory appeal in such composite formulations (Wen *et al.*, 2020).

This study aims to develop an innovative PBMA by incorporating soy flour, wheat gluten, pea flour, and pumpkin seed flour to enhance both nutritional quality and sensory appeal. Comprehensive analyses of physical properties and proximate composition—including moisture, protein, fat, fiber, and ash content—will be undertaken to evaluate the nutritional profile and structural stability of the product. Sensory evaluations will further assess consumer acceptability based on key attributes such as texture, flavor, color, taste, and overall appeal. Central to this research is an investigation into the specific effects of wheat gluten on critical quality parameters, providing insights into optimizing plant-based meat formulations. Findings from this study are expected to advance the development of high-quality, sustainable meat alternatives that successfully integrate functional attributes with consumer-preferred sensory characteristics.

2. MATERIALS AND METHODS

2.1 Collection of Raw Materials

White soybeans ("Gaurav" variety) and " pea seeds ("Rajmash" variety) from Dharan, Nepal contributed moisture (4.34% and 5.12%), protein (39.10% and 24.23%), fat (24.69% and 0.42%), fiber (2.15% and 2.18%), ash (3.22% and 3.38%), and carbohydrates (31.22% and 69.75%) respectively. Pumpkin seeds from Dhankuta provided moisture (10.23%), protein (29.47%), fat (35.94%), fiber (5.09%), ash (7.50%), and carbohydrates (21.39%). Seasonings comprised "Aayo" iodized salt and a local spice mixture.

2.2 Preparation of Flours

The soybeans were sorted, washed, and soaked in a 1% sodium bicarbonate solution for 10 hours. Afterward, the beans were precooked at 100°C for 15 minutes, dehulled, dried at 100°C for 5 hours and milled into fine flour (250 µm) and stored in a LDEP bag. Green pea grains were cleaned, soaked in distilled water at a 1:5 ratio for 24 hours, dried in a hot-air oven (100°C for 5 hours), milled into fine flour (250 µm) and packaged in LDEP bad. Pumpkin seeds, extracted from ripe pumpkin fruit were washed, dried in oven-dried at 50°C overnight, ground into a fine powder (250 µm), and packaged in an airtight container.

2.3 Recipe Formulation and Preparation of Plant Based Meat Analogue (PBMA)

Five Various recipes (Table 1) for PBMAs were formulated using a mixture version of Design Expert® 13 (Stat-Ease 2023), variations in the proportions of soy flour and wheat gluten while maintaining constant quantities of other ingredients (Mazumder *et al.*, 2023). Wheat protein is ideal for products requiring dough formation whereas soya and pea protein is suitable for meat analogs. Soy flour rich in lysine offers complementary amino acid profile to wheat flour. Incorporating peas flour can boost the mineral (Fe, Zn Mg, Mn) content of the products. Peas flour inclusion increases the water absorption and reduces sough stability leading to challenges in maintaining product structure during backing. Substitution wheat flour with legumes dilutes gluten network.(Kyriakopoulou *et al.*, 2021).

Soy, pea and pumpkin seed flour, were mixed with wheat gluten in a bowl chopper at RT(27-32°C) for 5-7 minutes, added with water and a spice mixture (including black pepper, ginger, cinnamon, cardamom, bay leaves, and cloves), continues mixed for another 5-7 minutes to create a consistent batter which was then stuffed into artificial casings (Plastic made from PE), dried on conventional tray drier at 50°C (air flow rate 1.5 - 3.0 m^3/min per kg products, air velocity 1.5- 3.0 m/s, RH < 20 %)) until the moisture content below 40%, cooled and packaged in LDPE bag.

Table 1

Formulation of Meat Analogues with Varying Wheat Gluten and Soy Flour Levels

S. N	Code	Wheat Gluten (parts)	Soy Flour (parts)	Water (%)	Pea Flour (%)	Pumpkin Seed Flour (%)	Salt (%)	Mixture of Spice (%)
1	**A**	0	50	35	10	4	0.5	0.5
2	**B**	10	40	35	10	4	o.5	0.5
3	**C**	20	30	35	10	4	o.5	0.5
4	**D**	30	20	35	10	4	0.5	0.5
5	**E**	40	10	35	10	4	0.5	0.5

2.4 Proximate analysis of PBMAs

Moisture content was determined through gravimetric analysis, and crude protein was measured using the micro-Kjeldahl method. Crude fat was assessed by solvent extraction, while ash content was obtained through incineration. Fiber content was analyzed via acid-alkali digestion, and carbohydrate content was calculated by difference. Additionally, pH was measured to complete the nutritional profile of the meat analogue. These methods provided a precise composition analysis, crucial for assessing the product's quality and nutritional value (AOAC, 2005).

2.5 Functional Properties Analysis of PBMAs

2.5.1 Cooking Loss

Cooking loss was determined by heating a weighed sample at 80 ± 2°C for 20 minutes in a polyethylene bag, cooled and weighted. The cooking loss was calculated as a percentage of the initial weight described by (Hur *et al.*, 2008).

$$\text{Cooking Loss} = \frac{\text{Wt. of raw product(g)-Wt. of cooked product(g)}}{\text{Wt. of raw product(g)}} \times 100\%$$

2.5.2 Water-Holding Capacity (WHC)

Five -gram sample was placed in a pre-weighed centrifuge tube with 30 mL of water, vortexed for 1 minute, then held at 25±2°C for 30 minutes before being centrifuged at 3500 rpm for 15 minutes. Non-absorbed water was removed, and WHC was calculated as a percentage described by (Mesías and Morales, 2017).

$$\text{WHC}(\%)=\frac{\text{Wt. before centrifugation(g)-Wt. after centrifugation(g)}}{\text{Wt. before centrifugation(g)}}\times 100(\%)$$

2.5.3 Swelling Capacity (SC)

Ten-gram dry sample was soaked in water at 37±2°C until equilibrium swelling was reached. The volume of the swollen sample was then recorded. SC was calculated based on the initial and final volumes. (Singh et al., 2006).

$$\text{SC}=\frac{\text{Final volume-Initial volume}}{\text{Weight of sample}}$$

2.5.4 Sensory Evaluation

The sensory evaluation of the boiled and warmed meat analogue samples were conducted using a hedonic rating test. Panelist (semi-trained students from the B.Tech 4th year and faculty members from the Central Campus of Technology) assessed various parameters, including appearance, flavor, texture, juiciness, taste, and overall palatability, employing a 9-point hedonic scale as defined by (Ranganna, 1986), where score 9 indicated "extremely liked" and score 1 indicated "extremely disliked." The Samples were presented randomly, and panelists were instructed to assign higher scores to the meat analogue's purple-brown color, while evaluations of texture and taste were based on their individual preferences.

2.5.6 Statistical Analysis

All physical and chemical analyses were performed in triplicate, with results presented as mean ± standard deviation, using Microsoft Excel. All the data were tested for normality assumption (Shapiro-Wilk test). The impact of wheat gluten addition was assessed through analysis of variance (ANOVA) followed by the Duncan test (for identify which pairs of groups differ, to reduce Type I error) for all parameter, utilizing SPSS software (SPSS Inc., Chicago). Means were further separated using Tukey's HSD post hoc test (for to reduce Type I error), with a significance level (p < 0.05).

3. RESULT AND DISCUSSION

3.1 Proximate Composition of Plant-Based Meat Analogue (PBMA)

The proximate composition of the formulated PBMAs is summarized in Table 2. Significantly (P<0.05) differences in protein, fat, and fiber content were observed across the products, while no significant difference in moisture, ash, and carbohydrate levels showed.

Table 2
Proximate Composition of PBMAs

Parameters	Product A	Product B	Product C	Product D	Product E
Moisture%(wb)	$38.04^{a} \pm 1.50$	$38.04^{a} \pm 1.50$	$38.21^{a} \pm 1.22$	$38.21^{a} \pm 1.22$	$38.27^{a} \pm 1.23$
Protein%(db)	$33.95^{a} \pm 1.26$	$39.54^{ab} \pm 1.47$	$45.60^{bc} \pm 0.07$	$51.51^{cd} \pm 0.07$	$55.28^{d} \pm 0.06$
Fat%(db)	$28.00^{a} \pm 1.08$	$24.55^{a} \pm 0.95$	$19.65^{b} \pm 0.16$	$10.57^{c} \pm 0.09$	$6.36^{d} \pm 0.05$
Ash%(db)	$4.10^{a} \pm 0.56$	$2.99^{a} \pm 0.41$	$2.75^{a} \pm 0.15$	$2.14^{a} \pm 0.12$	$2.00^{a} \pm 0.11$
Fiber%(db)	$4.09^{a} \pm 0.21$	$3.60^{ab} \pm 0.19$	$3.40^{ab} \pm 0.17$	$2.97^{b} \pm 0.15$	$1.99^{c} \pm 0.11$
Carbohydrate%(db)	$29.86^{a} \pm 1.10$	$32.00^{a} \pm 1.18$	$28.60^{a} \pm 1.74$	$34.22^{a} \pm 2.06$	$32.75^{a} \pm 1.92$
P^{H}	$6.52^{a} \pm 0.04$	$6.41^{a} \pm 0.02$	$6.51^{a} \pm 0.02$	$6.25^{a} \pm 0.03$	$6.18^{a} \pm 0.01$

(Mean ± SD, values with different superscripts within the same row indicate significant differences at p < 0.05)

The moisture content of the PBMAs ranged from 38.04% to 38.27%, suggests that variations in wheat gluten levels do not substantially impact moisture retention, thereby supporting consistent texture and juiciness, which are essential for consumer appeal (Mishal *et al.*, 2022). Similar stability in moisture content has been reported in gluten-enriched formulations (P. Singh *et al.*, 2021).The inclusion of wheat gluten significantly enhanced protein content in the PBMAs, with E displaying the highest protein level at 55.28% ± 0.06, while A recorded the lowest at 33.95% ± 1.26. This underscores gluten's role in protein incorporation within plant-based formulations, consistent with the findings of (Z. Chen *et al.*, 2020). Mishal *et al.*, 2022), observed similar protein enhancements in comparable with A, clear decreasing trend in fat content was observed with increased gluten concentration; product A exhibited the highest fat level at 28.00% ± 1.08, while E had the lowest at 6.36% ± 0.05 (p < 0.05). This reduction is likely due to the binding properties of gluten, which enhance moisture retention and decrease fat absorption (Rojas *et al.*, 2021). The ash content across all products showed no significant differences, ranging from 2.00% ± 0.11 in E to 4.10% ± 0.56 in A (p > 0.05), indicating that the incorporation of wheat gluten does not significantly affect the mineral composition of the PBMAs (Hamid *et al.*, 2020). Fiber content in the PBMAs significantly decreased with increasing levels of wheat gluten, with A exhibiting the highest fiber content at 4.09% ± 0.21 and E the lowest at 1.99% ± 0.11 (p < 0.05). This suggests that the incorporation of gluten may displace fiber-rich ingredients (Atwan, 2023). Carbohydrate content among the PBMAs varied from 28.60% ± 1.74 in C to 34.22% ± 2.06 in D, though these differences were not statistically significant (p > 0.05). Lastly, the pH levels of the PBMAs remained stable across all products, ranging from 6.18 ± 0.01 in E to 6.52 ± 0.04 in A, with no significant differences observed ($p > 0.05$). This stability is crucial for microbial safety and product quality (Mishal *et al.*, 2022).While gluten itself is low in fiber, its combination with high fiber ingredients in specific ratio can enhance the fiber content, structural properties and water retention of plant based meat analogues.

3.2 Functional Properties of PBMAs

Cooking loss refers to the reduction in weight of food products resulting from thermal processing, influenced by factors such as pH levels, texture, and the quantity of water lost during cooking. Meat that exhibits lower cooking losses is typically regarded as higher quality, indicating minimal degradation of meat components (Dinani *et al.*, 2023;), with normal cooking losses generally ranging from 1.5% to 54.5% (Azhari *et al.*, 2019). Water holding capacity (WHC) is defined as the maximum amount of water a given quantity of material can retain, making it a critical quality attribute associated with freshness and the proteins' ability to form a cohesive gel network. Swelling capacity (SC), which measures a material's ability to absorb water and expand in volume during hydration, is essential for the texture, juiciness, and overall quality of meat and meat analogues; SC can vary significantly, with beef muscle proteins ranging from 50% to 150% (Akhter et al., 2016), chicken between 70% and 120% (Dhama *et al.*, 2023), and fish displaying SC of 60% to 100% (Selvamuthukumaran and Maqsood, 2023), while meat analogues with higher gluten content demonstrate SC of 30% to 90% (Keyata *et al.*, 2021). Five formulations of meat analogues (A–E) were developed with gluten levels varying from 0 parts in A to 40 parts in E, and were analyzed for cooking loss, WHC, and SC. The results are summarized in Table 3.

Table 3
Functional Properties of PBMAs

Parameters	**Product A**	**Product B**	**Product C**	**Product D**	**Product E**
Cooking Loss	$7.305^{a} \pm 0.027$	$6.383^{b} \pm 0.038$	$5.380^{c} \pm 0.136$	$4.348\ ^{d}\pm 0.056$	$3.140^{e} \pm 0.053$
Water Holding	$2.102^{a} \pm 0.058$	$30.253^{b} \pm 0.095$	$32.465^{c} \pm 0.121$	$34.447^{d} \pm 0.062$	$36.040^{e} \pm 0.020$
Swelling Capacity	$0.5123^{a} \pm 0.0090$	$0.6102^{a} \pm 0.0020$	$1.1570^{b} \pm 0.0533$	$2.1560^{c} \pm 0.0377$	$2.8680^{d} \pm 0.0963$

(*Mean ± SD, values with different superscripts within the same row indicate significant differences at $p < 0.05$*)

Cooking loss significantly ($p < 0.05$) decreased with increasing gluten content, ranging from 7.305% in A to 3.140% in E, with analysis of variance (ANOVA) and Tukey's post-hoc test confirming statistically significant differences among all products, each belonging to a distinct significance group. This reduction suggests that gluten contributes to a robust protein network that effectively retains moisture during cooking, aligning with previous research indicating that gluten proteins form a cohesive matrix that traps water, fat, and other components, thereby minimizing moisture loss (Mascrez, 2024). Such moisture retention is essential for plant-based meat analogues, which rely on juiciness to replicate the sensory qualities of conventional meat. Lower cooking loss enhances juiceness, improve texture (more tender) and gives more satisfactory bites (Kyriakopoulou *et al.*, 2021) Water holding capacity (WHC) also increased significantly ($p < 0.05$) with higher gluten levels, rising from 2.102 in A to 36.040 in E. The high water-binding capacity of gluten enhances WHC, critical for moisture retention and texture in food products (Mascrez, 2024), as the gluten protein matrix allows for effective water retention (Lappi *et al.*, 2022). The improved WHC in high-gluten formulations contributes to a juicier texture, beneficial for applications like plant-based meats where moist, tender textures are desired (Shanthakumar *et al.*, 2022). Additionally, SC increased significantly ($p < 0.05$) with higher gluten content, from 0.5123 in A to 2.8680 in E. The increased SC observed in gluten-rich products signifies a greater potential for expansion during hydration, resulting in a bulkier texture that closely mimics the characteristics of animal-derived products. This attribute is particularly beneficial for achieving the desired texture and chewiness in meat alternatives (Kyriakopoulou *et al.*, 2021). In contrast, the lower SC in gluten-free formulations results in a denser structure less suitable for applications requiring volume and chewiness. Overall, this study underscores the importance of optimizing gluten levels in soy-based meat analogues to enhance their functional and sensory qualities, with findings indicating that higher gluten content leads to reduced cooking loss, increased WHC, and elevated SC, ultimately improving moisture retention, texture, and chewiness, thus enhancing the quality and consumer appeal of plant-based meat analogues in response to the growing demand for sustainable alternatives (Shanthakumar *et al.*, 2022). Chemical agents cleave di-sulphide bonds, leading to de-polymerization of gluten, decreased the network structure integrity, resulting in increased water release and more viscous but less elastic dough. This unfolding of the protein network, thereby enhancing its SC (Antonio et al., 2020). Similarly native pumpkin seed protein isolate exhibits higher gel strength compared to soy and peas isolates- demonstrating stronger networks under heat due to its hydrophobicity and insoluble particulate structure. (Chen, *et al.,* 2022)

3.3 Sensory Evaluation of PBMAs

To support the shift from conventional meat to plant-based alternatives, consumer research highlights the need for PBMA that closely mimic the sensory experience of real meat, with an emphasis on achieving a fibrous, meat-like texture to enhance consumer acceptance (Kaleda *et al.*, 2021). Essential sensory qualities for high-quality meat analogues include texture, appearance, flavor, taste, and overall acceptability, with particular attention to minimizing any off-flavors or undesirable aftertastes. This study assessed the impact of varying wheat gluten concentrations on the sensory attributes of PBMAs formulated with pea, soy, and pumpkin seed flours. Five formulations (A–E) were developed, each containing different wheat gluten levels (0–40 parts), to investigate how ingredient ratios affect overall sensory quality. The sensory score (Table 4), include ratings for texture, color, flavor, taste, and overall acceptance, illustrating the influence of wheat gluten on the sensory characteristics of these PBMAs

Table 4

Sensory Evaluation of Plant BMAs

Attribute	Product A	Product B	Product C	Product D	Product E
Texture	$5.8^{ab} \pm 1.0$	$6.5^{b} \pm 0.9$	$8.0^{c} \pm 0.9$	$6.0^{ab} \pm 0.8$	$5.1^{a} \pm 0.9$
Color	$6.5^{bc} \pm 0.9$	$7.1^{bc} \pm 0.7$	$7.7^{c} \pm 0.7$	$6.3^{ab} \pm 0.8$	$5.3^{a} \pm 1.0$
Flavor	$6.5^{bc} \pm 0.8$	$6.1^{ab} \pm 0.8$	$7.4^{c} \pm 0.7$	$5.6^{ab} \pm 0.8$	$4.7^{a} \pm 0.8$
Taste	$6.4^{c} \pm 1.0$	$6.0^{bc} \pm 0.9$	$7.2^{c} \pm 0.8$	$5.3^{ab} \pm 0.8$	$4.5^{a} \pm 0.9$
Overall acceptance	$6.3^{b} \pm 0.8$	$6.3^{b} \pm 0.8$	$7.8^{c} \pm 0.7$	$5.4^{ab} \pm 0.9$	$4.7^{a} \pm 0.8$

(Mean ± SD, values with different superscripts within the same row indicate significant differences at $p < 0.05$)

The results demonstrated significant differences ($p < 0.05$) in texture scores confirming the substantial impact of gluten concentration on the firmness and cohesiveness of PBMAs. Product C, which contained 20 parts gluten, achieved the highest texture score (8.0 ± 0.9), suggesting that this gluten concentration provides the optimal balance for a desirable texture. Products A with lower gluten content (0 parts gluten), and E with higher gluten content (40 parts gluten), scored significantly lower. Specifically, E showed signs of excessive toughness, indicating that beyond a certain concentration, the addition of gluten negatively affects texture, making it less appealing. Supporting studies reinforce these findings, as (Rizvi, 1976) observed that increased gluten levels improve particle quality and texture appeal. (Hamid *et al.*, 2020) similarly reported that 20% wheat gluten incorporation significantly enhanced texture, while (Nivetha *et al.*, 2019) found optimal texture with a 50:30:20 blend of mushroom, paneer, and wheat gluten. Additionally, (Egbert and Borders, 2006) demonstrated that powdered wheat gluten improves binding and texture in meat analogues, further supporting the positive effects of gluten on texture. There were significant differences ($p < 0.05$) in colour among formulations, confirming that gluten concentration plays a key role in the color of PBMAs, a critical attribute for consumer appeal. Product C (20 parts gluten), achieved the highest color score (7.7 ± 0.7), suggesting that this gluten concentration results in a color most similar to traditional meat. However, increasing the gluten concentration beyond this level, as seen in E (40 parts gluten), resulted in a lower color score (5.3 ± 1.0), possibly due to darker or less uniform coloration. This effect may be attributed to excess gluten causing an undesirable surface color. These findings align with the work of (Hamid *et al.*, 2020), who observed improved color attributes with 20% gluten incorporation. (Rizvi, 1976) also noted that higher gluten levels enhance the quality of brown particles, contributing to a more visually appealing product. (Olavarria, 1981) found that gluten levels between 10-20% led to improved color scores, further supporting the positive effect of gluten on the visual characteristics of plant-based meat. Additionally, (Nivetha *et al.*, 2019) reported superior color in a wheat gluten blend, and (Nova, 2023) highlighted that combining gluten with jackfruit enhances product brightness, making it more visually appealing. The results of flavor revealed significant differences ($p < 0.05$) across formulations, confirming that gluten concentration plays a critical role in influencing the flavor of plant-based meat analogues. Flavor scores showed a positive correlation with moderate gluten levels, with C (20 parts gluten) achieving the highest rating (7.4 ± 0.7). In contrast, both lower and higher gluten levels resulted in decreased flavor acceptance, with E (40 parts gluten) receiving the lowest score (4.7 ± 0.8), likely due to excess gluten overpowering other flavor components. These findings are consistent with those of (Olavarria, 1981), who observed improved flavor scores at 10-20% gluten levels. (Hamid *et al.*, 2020) also reported enhanced flavor in meat analogues with 20% gluten incorporation. Additionally, (Egbert and Borders, 2006) noted that gluten enhances binding, contributing to flavor retention. Further support for these findings comes from (Nivetha *et al.*, 2019) and (Nova, 2023), both of whom reported flavor enhancements when gluten was included in formulations, reinforcing the positive influence of gluten on flavor in plant-based meat analogue. There were significant differences ($p < 0.05$) in taste among formulations, confirming that gluten concentration has a substantial effect on taste. Taste scores were highest in C (7.2 ± 0.8), which featured an optimal balance of gluten and soy, resulting in a more palatable experience for consumers. In contrast, formulations with lower gluten content as A (0 parts), lacked the desired taste profile typically associated with traditional meat analogues. Furthermore, higher gluten levels in E negatively impacted taste, likely due to the overly dense and chewy texture, which reduced overall palatability. Similar findings were reported by (Hamid *et al.*, 2020) and (Kumar *et al.*, 2012), who observed improvements in taste with 20% and 18% gluten incorporation, respectively, emphasizing gluten's role in enhancing mouthfeel and palatability. The binding properties of gluten, as noted by (Egbert and Borders, 2006), further suggest that gluten may contribute to improved flavor perception. Nova, 2023 found that a gluten-jackfruit blend enhanced mouthfeel, ultimately contributing to a more enjoyable taste experience. Overall acceptance revealed significant ($p < 0.05$) among formulations, confirming that gluten concentration notably influences the overall acceptance of plant-based meat analogues. Product C (20 parts gluten) achieved the highest overall acceptance score (7.8 ± 0.7), indicating that a moderate gluten level best aligns with consumer expectations for texture, flavor, color, and taste. In contrast, A with no gluten and those with excessively high gluten content (E) received significantly lower acceptance scores, suggesting that extreme variations in gluten concentration, either too low or too high, detract from sensory quality. This indicates that while gluten enhances sensory attributes up to a certain threshold, excessive amounts may result in diminishing returns, were texture and flavor deviate from consumer preferences. These findings are consistent with previous studies; (Hamid *et al.*, 2020) and (Nivetha *et al.*, 2019) observed improved overall acceptance with increasing gluten ratios, while (Kumar *et al.*, 2012) found that an 18% gluten concentration maximized consumer acceptance in meat analogues. Furthermore, (Egbert and Borders, 2006). Incorporating the gluten with 30 % exhibited improved textural characteristic, including chewiness and hardness. Therefore 20% gluten might offer a balance between enhancing textural properties and maintaining consumer acceptability. Egbert and Borders highlighted that gluten incorporation enhances binding and texture, thereby improving overall product quality. (Nova, 2023) reinforced these results, noting that the combination of gluten with jackfruit enhanced mouthfeel and other sensory attributes.

4. CONCLUSION

In conclusion, the inclusion of wheat gluten significantly enhances the quality attributes of plant-based meat analogues formulated with pea, soy, and pumpkin seed flours. The study demonstrates that moderate concentrations of gluten, particularly 20 parts, optimize the texture, flavor, color, and overall sensory appeal of these formulations, closely resembling the characteristics of traditional meat. However, higher gluten levels (40 parts) resulted in undesirable toughness and a reduction in flavor. A balanced incorporation of gluten, on the other hand, improved moisture retention, reduced cooking loss, and increased water-holding capacity—critical factors in achieving a juicy, tender, and palatable product. Plant-based meat analogues have emerged as a viable option that can help bridge the nutritional gap while minimizing environmental strain. These products, from plant sources such as soy, wheat gluten, legumes, pumpkin seeds and peas, are engineered to replicate the sensory qualities of animal meat—texture, appearance, and taste—while offering distinct health benefits, including high fiber, vitamins, antioxidants, and lower saturated fats (P. Singh *et al.*, 2021).Furthermore, gluten enhanced the protein content and reduced fat levels, aligning with consumer demand for healthier, more nutritious meat alternatives. Soy flour rich in lysine offers complementary amino acid profile to wheat flour. Incorporating peas flour can boost the mineral (Fe, Zn Mg, Mn) content of the products. Peas flour inclusion increases the water absorption and reduces sough stability leading to challenges in maintaining product structure during backing. Substitution wheat flour with legumes dilutes gluten network.

This research study optimizing multi-protein formulation by reducing the reliance on soya protein and underscores the essential role of gluten in enhancing both the functional and sensory qualities of plant-based meat analogues, providing valuable insights into optimizing formulations for increased consumer acceptance and sustainability within the expanding market of meat alternatives.

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CONFLICTS OF INTEREST

The authors report no conflicts of interest for this research work.

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