

COMPARATIVE ANALYSIS OF CHICKEN AND MEAT ANALOGUE PATTIES: EVALUATING PHYSICOCHEMICAL, COOKING, TEXTURAL, MICROBIAL, AND SENSORY ATTRIBUTES

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Abstract

This study aimed to develop and evaluate meat analogue patties using soy protein isolate (SPI) and whey protein isolate (WPI) in varying ratios (0%, 5%, 10%, 15% WPI), comparing them to a traditional chicken patty (T_X). Enzymatic crosslinking with microbial transglutaminase and high-moisture extrusion were employed to develop textured non-meat patties. The physicochemical, cooking, textural, microbial, and sensory attributes of the patties were assessed. Results indicated that increasing WPI content significantly influenced moisture retention, cooking loss, and textural properties. The chicken patty (T_X) exhibited the highest initial moisture content (73.52%) and final moisture retention (63.14%), along with the highest cooking yield (78.50%). The pure soy-based patty (T_0) showed the greatest cooking loss (27.70%) and diameter change (8.50%). WPI incorporation increased hardness, ranging from 13.20N (T_0) to 15.90N (T_3), and cohesiveness, from 0.44 (T_X) to 0.48 (T_3). Final findings revealed that cooked patties with 15% WPI (T_3) had the highest hardness (16.50N) and cohesiveness (0.38), which was attributed to a denser cross-linked structure in patties containing WPI. Color analysis showed that chicken patties exhibited the highest lightness (L^*), while increasing WPI led to greater redness (a^*). pH values were slightly acidic for all patties, with chicken patties showing the lowest (most acidic) pH. Sensory analysis revealed that patties with optimized WPI content (T_2 and T_3) achieved higher overall acceptability than both pure soy (T_0) and chicken (T_X) patties, particularly in appearance and color. Microbial analysis showed a decrease in total plate count (TPC) from 3.32 log CFU/g (T_X) to 3.03 log CFU/g (T_3) initially, with a further reduction after cooking to 2.86 log CFU/g (T_X) and 2.48 log CFU/g (T_3). This study demonstrates the potential of utilizing soy and whey protein blends to create meat analogues with comparable and, in some aspects, superior qualities to chicken patties, offering a sustainable and nutritious alternative.

Keywords: Soy protein isolate, whey protein isolate, cooking loss, cooking yield, cohesiveness, superior qualities

INTRODUCTION

Global population is continuously increasing, and it is anticipated that by the year 2050 it will rise to 9.7 to 10 billion (Quintieri et al 2023; Fukase and Martin, 2020). This exponential growth will have a significant impact on the demand of food worldwide (Fukase and Martin, 2020). Food insecurity remains a serious challenge in the twenty-first century. According to the UN, approximately one in three people—totaling 2.3 billion individuals worldwide—experienced moderate to severe food insecurity in the year 2021. (Dawood and Van Vuuren, 2023). While the present situation is already pathetic, feeding the future population is another menace, as it presents a major global societal challenge. (Van Dijk et al., 2021). Protein deficiency is a major concern in food insecurity and malnutrition. As the population grows, caloric intake will rise, necessitating increased protein production (FAO, 2012). Demand for protein is rising rapidly, and sustainability may become unmanageable. By 2050, protein needs are projected to increase by 78%, reaching 361 million tons annually (Van Peteghem et al., 2022). Proteins are essential macronutrients involved in structural, mechanical, and cellular functions, also providing energy when needed. Composed of amino acids, they are found in meat, dairy, legumes, vegetables, and grains. Each gram provides 4 kcal of energy, with a recommended intake of 0.8–1 g per kg of body weight daily. Protein should constitute 5–20% of energy intake for children (1–3 years), 10–30% for those aged 4–18, and 10–35% for adults to maintain nitrogen balance (Morris and Mohiuddin, 2020). Two major sources of protein are plants and animals, with plants fulfilling 80% of global protein needs and animal-based sources fulfilling 20% of the total requirements (Aschemann-Witzte et al., 2021). Recent research suggests that both plant and animal proteins have distinct advantages and disadvantages, supporting a balanced approach to protein consumption. Plant-based proteins are associated with reduced risks of cardiovascular diseases, certain cancers, and mortality, especially compared to red and processed meats (Visioli, 2024; Mariotti, 2019). They also have significantly lower environmental impacts in terms of greenhouse gas emissions, water use, and land requirements (Visioli, 2024; Ferrari et al., 2022). However, animal proteins contribute to nutrient adequacy in diets, albeit with high variability depending on the specific source (Mariotti, 2019). Certain studies have linked animal proteins to increased cardiovascular disease and diabetes risk (Mariotti, 2019). As knowledge about the complex interplay between protein sources, health outcomes, and environmental impacts increases, it is suggested that future dietary guidance should emphasize a shift toward more plant-based protein sources (Visioli, 2024; Ferrari et al., 2022). However, animal protein sources cannot be completely neglected, as they are nutritionally superior due to their better profile of essential amino acids (Reid-McCann et al., 2022). Thus, a balanced approach that ensures both sustainability and good nutrition can be achieved by optimizing the use of plant and animal proteins. Hence, this study focused on developing a meat analogue patty primarily based on soy protein (plant-based) while incorporating whey protein as an animal protein source to enhance its nutritional profile. This patty is sustainable, nutritious, and healthy for consumption. The selection of these two proteins among various plant and animal options was based on their compatible Protein Digestibility-Corrected Amino Acid Score (PDCAAS) (Sui et al., 2021; Hertzler, 2020; Rizzo and Baroni, 2018). Initially, both proteins were enzymatically crosslinked using microbial transglutaminase in various ratios to create hybrid proteins with enhanced functional properties, including apparent viscosity, strength, water-holding capacity, hardness, and emulsifying stability etc. (Liang et al., 2020; Zhang and Zhao, 2013). Later, high moisture extrusion was done to form texturized structure. The concept was inspired by Plant-Based Meat Alternatives (PBMA) and Textured Vegetable Proteins (TVP), which are designed to replicate the texture, taste, appearance, scent, and nutritional attributes of real meat products (Indriani et al., 2022). After rehydration and the addition of spices and condiments, meat analogue burger patties were created and compared with actual chicken patties before and after cooking. The objective was to develop a healthier, nutritious, tasty, and sustainable meat alternative for public acceptance and commercialization.

2. MATERIALS AND METHODS

The study was conducted at the Department of Food Science and the Hi-Tech Laboratory, Faculty of Life Sciences, Government College University Faisalabad. Four crosslinked protein combinations were formed and subjected to high-moisture extrusion to produce texturized hybrid proteins. These proteins were then combined with spices and condiments to develop four meat analogue burger patties, which were compared to a real chicken patty.

2.1. Procurement of raw materials

Whey protein isolates (WPI) (90% pure) were sourced from Protein Factory, while soy protein isolates (SPI) (90% pure) were obtained from Nutrena. Microbial transglutaminase enzyme (MTG) was purchased from Sunson Industry Group Co., Ltd., China, and stored in a cool, dry place. Other analytical-grade reagents, including PBS solution (pH 7), distilled water, spices, and condiments, along with essential lab equipment, were procured locally. Fresh boneless chicken breast meat was obtained from a local butcher for meat-based burger patty preparation.

2.2. Enzymatic crosslinking

2.2.1. Enzymatic crosslinking of the proteins

The entire methodology of Cui et al. (2020) which included novel ultrasonication technique for better occurrence of crosslinking among the glutamine and lysine amino acids was followed (Cui et al., 2020; Sun and Arntfield, 2012).

For this purpose, initially soy protein isolates were dissolved in phosphate buffered saline solution (0.2 mol/L and pH 07). The ratio of solute to solvent was 100 mg protein per 100 ml. Then the same procedure was repeated with whey protein in a separate beaker. Later four small beakers were taken and in the first beaker only soy protein solution was taken, in second beaker five percent whey protein solution was also added along with the soy protein, the ratio of whey kept on increasing in other two beakers (10 % and 15%). All four solutions were stirred on a SCILOGEX MS7-H550-S magnetic stirrer for a duration of 02 hours. During this procedure of stirring the temperature was set to room temperature. Later, all the solutions were refrigerated for a duration of 12 hours at 4°C. For the process of ultrasonication 30 ml of each sample was placed within a 50 ml Erlenmeyer flask. Then all the protein mixtures were ultrasonicated on the VCX-750 probe type ultrasonic processor at 300 W. The duration of this process was 45 minutes. In order to maintain the temperature between 25 to maximum 35°C. This entire process was carried out within an ice bath. Later, the microbial transglutaminase enzyme (MTG) was added to all the solutions at the rate of 50 units per gram of protein. After that all protein solutions were placed in a water bath and were heated at 45°C for a duration of 02 hours. Finally, after the occurrence of cross-linking the enzyme was deactivated. For the deactivation of enzyme, little modification was made in the original methodology, while in the original methodology the enzyme was heated at 90°C for 10 minutes, but in the current study heating was done at 80°C. The reason for deactivation at relatively lower temperature was that even though this temperature is sufficient for deactivation, secondly, exposure to too high temperature results in the deterioration of the functional properties of proteins. After deactivation of the enzyme the protein solutions were initially cooled to the room temperature. After that protein was separated from the solution, which is not mentioned in the original methodology, however, in the current study muslin clothes were used to entrap the cross-linked proteins and then the proteins, captured or clung within the clothes were hanged in the refrigerator at 4°C for the duration of 12 hours. After that the little moisture that was still present within the proteins was removed by doing lyophilization in a VaCo 5 laboratory freeze dryer following the methodology of Simoni et al. (2017). The specifications of the conditions were -50°C temperature and 150 µmHg for the duration of 8 hours.

2.2.2. Development of textured crosslinked protein via high moisture extrusion

The methodology of Samard et al. (2021) with slight modifications was followed. Extrusion was performed using a locally manufactured and customized a single barrel twin-screw extruder installed at Haider Foods Industry, located on Samundari Road, Faisalabad. The extruder had a length-to-diameter (L/D) ratio of 27:1. The feed material had a moisture content of approximately 55%. The die diameter was about 1 cm and temperature was maintained at 150°C, the feeding rate was set to 1000 g per minute, and the screw speed was adjusted to 200 rpm. Finally, the extruded proteins were dried in a hot air oven at 50°C for 1 complete day.

2.2.3. Burger patty preparation

Meat analogue burger patties were prepared following Samard et al. (2021) with modifications. Dried hybrid extrudates (1 × 1 × 5 cm) were cut into pellets, hydrated (1:12 water ratio) for 1 hour, drained, and minced using a meat grinder. Ground texturized proteins were mixed with canola oil for 2 minutes, followed by powdered additives for 10 minutes, and kneaded for 3 minutes. A control chicken patty (Tx) was prepared using minced chicken breast, mixed with the same spices and condiments. The ball paste was then shaped into patties using a burger patty press maker. After being placed in plastic bags, the meatless patties were allowed to rest for an hour at room temperature. The patties were cooked for seven minutes at 160°C in an electric pan covered with a glass lid. The cooked patties' internal endpoint temperature varied between 75°C and 80°C. The patties were cooked and then let to cool for an hour at room temperature. The detailed recipe of the burger patty is depicted in table 1.

Table 1. Recipes of burger patties

Ingredients	Burger Patty				
	Tx	T0	T1	T2	T3
Protein source	90 g Chicken breast	90 g 100% crosslinked & textured SPI	90 g Textured hybrid protein (95% SPI crosslinked to 5% WPI)	90 g Textured hybrid protein (90% SPI crosslinked to 10% WPI)	90 g Textured hybrid protein (85% SPI crosslinked to 15% WPI)
Canola Oil	5 ml	5 ml	5 ml	5 ml	5 ml
Salt	1 g	1 g	1 g	1 g	1 g
Black Pepper	1 g	1 g	1 g	1 g	1 g
Garlic powder	1 g	1 g	1 g	1 g	1 g
Onion powder	1 g	1 g	1 g	1 g	1 g
Net Weight	100 g	100 g	100 g	100 g	100 g

2.2.4. Proximate analysis: Moisture

All the cooked and uncooked samples were dried in a hot air oven at 105°C until the constant weight was gained (AOAC, 2020). The calculations were done according to the formula given below.

$$\text{Moisture (\%)} = \frac{\text{weight before drying (g)} - \text{weight after drying (g)}}{\text{weight before drying (g)}} \times 100$$

2.2.5. Cooking characteristics

2.2.5.1. Cooking loss

For determining the cooking loss of the methodology of Rajaretnam and Malik. (2023) was followed. Three samples from different formulations of burger patties were weighed immediately after development. The samples were then weighed again after cooking to record the loss of cooking value. The test was conducted in triplicate. Cooking loss (%) was calculated using the formula:

$$\text{Cooking loss (\%)} = \frac{\text{raw weight} - \text{cooked weight}}{\text{raw weight}} \times 100$$

2.2.5.2. Moisture retention

The moisture content of cooked patties was determined by drying each sample at 105 °C until constant weight was achieved (Samard et al., 2021).

$$\text{Moisture retention (\%)} = \frac{\text{cooking yield (\%)} \times \text{moisture of cooked patty (\%)}}{100}$$

2.2.5.3. Cooking yield

The cooking yield was determined by measuring the weight of raw and cooked burger patty. The analysis was performed in the triplicate (Samard et al., 2021).

$$\text{Cooking yield (\%)} = \frac{\text{cooked patty weight (g)}}{\text{raw patty weight (g)}} \times 100$$

2.2.5.4. Diameter change

The diameter of raw and cooked patties was initially measured (Samard et al., 2021). For that purpose, a vernier caliper was used. Later the percentage of these parameters was obtained using the equation:

$$\text{Diameter change (\%)} = \frac{\text{raw patty diameter (mm)} - \text{cooked patty diameter (mm)}}{\text{raw patty diameter (mm)}} \times 100$$

2.2.5.5. Thickness change

The thickness of raw and cooked patties was measured according to the methodology of Samard et al. (2021). The percentage of these parameters was obtained using the equation:

$$\text{Thickness change (\%)} = \frac{\text{raw patty thickness (mm)} - \text{cooked patty thickness (mm)}}{\text{raw patty thickness (mm)}} \times 100$$

2.2.6. Physicochemical analysis

2.2.6.1. Color

For doing the color analysis to find out the values of L*, a* and b* the methodology of Rajaretnam and Malik. (2023) was used. However, in order to find the value of surface color, the methodology of Wu et al. (2019) that has been mentioned in the previous sections was used. These values were determined for both cooked and raw samples.

2.2.6.2. pH

In order to do the analysis of pH of both cooked and raw, uncooked burger patties the methodology of Rajaretnam and Malik. (2023) was used.

2.2.7. Textural analysis

The texture of raw and cooked burger patties was analyzed using a Texture Analyzer equipped with a 35 mm aluminum cylinder probe. The testing parameters included a test speed of 2.0 mm/s, a compression strain of 10%, and a recovery period of 5 seconds between the two compressions (Rajaretnam and Malik, 2023). The measured properties included Hardness, Springiness, Cohesiveness, and Chewiness.

2.2.8. Microbial analysis: (Total plate count/total viable count)

The methodology of Butt et al. (2024) followed to determine the total plate count (TPC). The agar used for pour-plate method was Nutrient agar (OXOID CM0003). Initially, the texturized proteins were finely grounded and completely pulverized then for each treatment 10 g of the extruded protein sample was homogenized in 90 ml of sterile normal saline solution (0.89w/v) to create a main 10-fold dilution, which was then serially diluted (10⁻¹ to 10⁻⁵) in the same diluents. Petri dishes containing agar culture media were inoculated with these sequential dilutions in order to count or detect the total number of bacteria.

2.2.9. Sensory analysis

The methodology of doing the sensory analysis by a 9-point hedonic scale was inspired by the methodology of Butt et al. (2024) and Rajaretnam and Malik. (2023). Trained panelists, who had prior experience of sensory evaluation were selected for this analysis. These people included university professors and senior students. Their parameters that were studied included Appearance, Texture, Flavor, Taste, color and overall acceptability.

2.2.10. Statistical analysis

All analyses of the crosslinked proteins, rat groups studied in efficacy study, extruded and texturized proteins, and finally the meat analogue burger patties were performed in triplicate, and the results were presented as means. The acquired data was statistically analyzed using IBM SPSS Statistics 25 software. Montgomery. (2019) described the methodology for performing one-way and multimode analysis of variance (ANOVA) in a completely randomized design (CRD). Same methodology was followed. The means were interpreted using Tukey's HSD test.

3. RESULTS AND DISCUSSION

3.1. Proximate Composition: Comparison of moisture content before and after cooking

The mean values are depicted in table 2. It can be seen that the highest moisture content before as well as after cooking was shown by chicken meat burger patty, followed by the pure texturized crosslinked soy protein. It best mimicked the texture of real fibrous texture of chicken meat. This trend was supported by a study according to which textured vegetable proteins (TVPs) made from soy protein, wheat gluten, and starch at 50% moisture and 130°C die temperature showed the closest similarity to commercial meat patties in terms of texture and cooking properties (Samard et al., 2021).

However, as the content of whey protein increased, the texture began to change a little and so did the properties, hence it can be seen that the lowest moisture content at both stages was shown by T₄ burger patty. It had highest content of whey protein incorporated into soy protein through the procedure of crosslinking. The reason for lower moisture content in patties having higher content of whey protein can be understood through the study of Zhang et al. (2024), who demonstrated that adding whey protein to vegetable protein isolate (pea protein) lead to a denser cross-linked structure. Another study also depicted that whey protein results in denser products (Kreger et al., 2012). This increase in density reduces porosity and hence the water holding capacity and total moisture retention is reduced. According to another study, the addition of whey protein to even non-protein sources such as potato flakes, dose shows the similar trend of decreasing the water absorption index (Dushkova et al., 2012), suggesting that whey protein may reduce moisture content in burger patties.

3.2. Cooking characteristics

3.2.1. Cooked patty weight

The mean values are depicted in table 2. Though the difference was not so huge, still then it can be seen that the highest cooked weight was reported in the real chicken patty, followed by the patty having the highest content of whey protein in soy protein and the lowest cooked patty weight was reported in the pure soy protein-based patty. Increasing the amount of whey protein in the soy protein gave characteristics similar to the properties of chicken patty to the analogue patties. The reason for reduction in the cooked weight of all the patties, despite having similar weight prior to cooking, was moisture removal, since the highest amount of moisture was retained by pure soy patty, the highest amount of moisture reduced during cooking was also observed in this patty and hence the weight was reduced. Research on ground meat patties reveals that higher moisture content leads to greater weight loss during cooking. Patties with higher fat and water content experienced more shrinkage and moisture loss (Velioglu et al., 2010). The current study on meat analogues also follows a similar trend.

3.2.2. Cooking loss

The mean values are depicted in table 2. It can be seen that the lowest cooking loss was reported in the real chicken patty, followed by the patty having the highest content of whey protein in soy protein and the highest cooking loss was reported in the pure soy protein-based patty. Increasing the amount of whey protein in the soy protein gave characteristics similar to the properties of chicken patty to the analogue patties. The reason for cooking loss in all patties was moisture removal, since the highest amount of moisture was retained by pure soy patty, the highest amount of moisture reduced during cooking was also observed in this patty and hence the cooking loss was increased. Research on patties reveals that higher moisture content leads to greater weight loss during cooking. Patties with higher water content experienced more shrinkage and moisture loss (Velioglu et al., 2010). The current study on meat analogues also follows a similar trend.

3.2.3. Moisture retention

The mean values are depicted in table 2. It is evident that the chicken meat burger patty retained the most moisture, followed by the pure texturized crosslinked soy protein. It best simulated the actual fibrous texture of chicken meat. According to a study that supported this trend, the texture and cooking characteristics of textured vegetable proteins (TVPs) made from soy protein and some other ingredients were most similar to those of commercial meat patties (Samard et al., 2021). However, the texture and properties changed slightly as the amount of whey protein increased. As a result, the T₄ burger patty, which had the highest amount of whey protein incorporated into soy protein through the process of crosslinking, was found to have the lowest moisture content. Zhang et al. (2024) showed that adding whey protein to vegetable protein isolate results in a denser cross-linked structure, which helps explain why patties with higher whey protein content had lower moisture content. Whey protein also produces denser products, according to another study (Kreger et al., 2012). Porosity is decreased by this density increase, which lowers the water-holding

capacity and overall moisture retention. Another study suggests that whey protein may lower the moisture content of burger patties by showing a similar trend of decreasing the water absorption index when added to even non-protein sources like potato flakes (Dushkova et al., 2012).

3.2.4. Cooking yield

The mean values are depicted in table 2. It can be seen that the highest cooking yield was reported in the real chicken patty, followed by the patty having the highest content of whey protein in soy protein and the lowest cooking yield was reported in the pure soy protein-based patty. Increasing the amount of whey protein in the soy protein gave characteristics similar to the properties of chicken patty to the analogue patties. The reason for increased cooking yield in real meat patty and high whey patty was the low cooking loss of these patties. As per many studies cooking yield is inversely proportional to the cooking loss. In shrimp, a mathematical model was developed to predict yield loss based on cooking temperature and time (ERDOĞDU et al., 1999). For pork ham, cooking shrinkage in volume was found to be significantly correlated with cooking loss (Du and Sun, 2005). In fatty duck liver, dry matter content was the best predictor of technological yield, which is the opposite of fat loss during cooking (Theron et al., 2012).

3.2.5. Diameter change

The mean values are depicted in table 2. It can be seen that the lowest diameter change was reported in the real chicken patty, followed by the patty having the highest content of whey protein in soy protein and the highest diameter change was reported in the pure soy protein-based patty. Increasing the amount of whey protein in the soy protein gave characteristics similar to the properties of chicken patty to the analogue patties. The reason for the reduced diameter changes in real meat patty and high whey patty was the low cooking loss of these patties. As per many studies, cooking loss has direct relation to the diameter change or other form of shrinkages. Research shows that cooking shrinkage, particularly in volume, is significantly correlated with cooking loss ($r = 0.91$) (Du and Sun, 2005). Isolated endomysial sheaths can shrink up to 70% in diameter when heated, potentially accounting for fluid expulsion during cooking (Champion et al., 1988). Muscle fiber length changes occur in three temperature zones during cooking, with cross-sectional area decreases being greater in contracted samples (Bouton et al., 1976). According to another study, higher cooking temperatures generally result in greater cooking loss and simultaneously causing shrinkage (Vaskoska et al., 2020). As per Vaskoska et al. (2020) as well, the diameter change, and cooking loss have direct relationship.

3.2.5.1. Thickness change

The mean values are depicted in table 2. It can be seen that the lowest thickness change was reported in the real chicken patty, followed by the patty having the highest content of whey protein in soy protein and the highest thickness change was reported in the pure soy protein-based patty. Increasing the amount of whey protein in the soy protein gave characteristics similar to the properties of chicken patty to the analogue patties. The reason for the reduced thickness changes in real meat patty and high whey patty was the low cooking loss of these patties. As per many studies, cooking loss has direct relation to the thickness change or other form of shrinkages. Research shows that cooking shrinkage, particularly in volume, is significantly correlated with cooking loss ($r = 0.91$) (Du and Sun, 2005). Isolated endomysial sheaths can shrink up to 70% when heated, potentially accounting for fluid expulsion during cooking (Champion et al., 1988). Muscle fiber length changes occur in three temperature zones during cooking, with cross-sectional area decreases being greater in contracted samples (Bouton et al., 1976). According to another study, higher cooking temperatures generally result in greater cooking loss and simultaneously causing shrinkage (Vaskoska et al., 2020). As per Vaskoska et al. (2020) as well, the thickness change, and cooking loss have direct relationship.

3.2.6. Physicochemical analysis

3.2.6.1. Color analysis

The mean values are depicted in table 3. It can be seen that the highest L^* value before as well as after cooking was shown by chicken meat burger patty, followed by the pure texturized cross-linked soy protein. And the lowest value of L^* was shown by that meat analogue patty that had highest content of whey protein incorporated into the soy protein. The reason could be that whey protein tends to change color more rapidly when exposed to high temperature and hence the lightness is decreased. As per one study, the addition of non-plant additive to plant-based meat analogues may reduce lightness (L^*) after cooking (Bakhsh et al., 2021). Another research has also investigated non plant protein (insect protein) as a component in meat analogue burgers, finding that its addition can brightness (L^*) (Krawczyk et al., 2024). The findings of these studies support the current trend. It can be seen that the lowest a^* value before as well as after cooking was shown by chicken meat burger patty, followed by the pure texturized crosslinked soy protein. And the highest value of a^* was shown by that meat analogue patty that had highest content of whey protein incorporated into the soy protein. The reason could be that whey protein tends to change color more rapidly when exposed to high temperature and hence the lightness is decreased while redness is increased. Across studies, cooking generally reduced redness (a^*) values in both animal and plant-based products (Bakhsh et al., 2021; Youssef and Barbut, 2011; Han et al., 2023), with non-meat protein additions resulting in lighter and less red products compared to all-meat controls (Youssef and Barbut, 2011). It can be seen that the highest b^* value before as well as after cooking was shown by chicken meat burger patty, followed by the pure texturized crosslinked soy protein. And the lowest value of b^* was shown by that meat analogue patty that had highest content of whey protein incorporated into the soy protein. As per literature, the color of meat and meat analog patties is influenced by various factors. Chicken meat

burger patties and pure soy or other plant proteins tend to have higher b^* values (yellowness) compared to other formulations (Bakhsh et al., 2021). It can be seen that the lowest surface color (ΔE) value before as well as after cooking was shown by chicken meat burger patty, followed by the pure texturized crosslinked soy protein. And the highest value of ΔE was shown by that meat analogue patty that had highest content of whey protein incorporated into the soy protein. As per one study, Soy and whey protein isolates impacted color in emulsified meat batters (Youssef and Barbut, 2011).

3.2.6.2. pH analysis

The mean values are depicted in table 3. It can be seen that before and after cooking pH remained weak acidic in all patties, but after cooking the pH became slightly more acidic. However, in both cases, the most acidic pH was in the actual chicken patty, followed by that burger patty that had highest content of whey protein incorporated into the soy protein. As the content of whey protein increased in soy protein the pH tended to become more acidic, which was compatible with the pH of real meat patty and the least acidic pH was observed in pure soy protein based batty. Research suggests that soy protein isolates (SPI) generally have a more alkaline pH profile compared to whey protein isolates (WPI). Rats fed a WPI diet exhibited more acidic urinary pH than those on an SPI diet (Aparicio et al., 2014).

Table 2. Results of moisture content, cooking and physiochemical characteristics of chicken patty and meat analogue burger patties

Analysis	Burger Patty				
	Tx	T0	T1	T2	T3
Initial Moisture content (%)	73.52 ± 0.34 ^a	73.52 ± 0.34 ^a	68.47 ± 0.28 ^c	67.12 ± 0.31 ^d	65.30 ± 0.29 ^c
Final Moisture content (%)	63.14 ± 0.45 ^a	61.73 ± 0.40 ^{ab}	59.60 ± 0.38 ^b	57.85 ± 0.41 ^c	55.48 ± 0.37 ^d
Cooked weight (g)	78.5 ± 1.25 ^a	72.3 ± 3.31 ^a	74.8 ± 3.28 ^a	76.4 ± 1.26 ^a	77.2 ± 2.24 ^a
Cooking loss (%)	21.50 ± 0.31 ^d	27.70 ± 0.40 ^a	25.20 ± 0.35 ^b	23.60 ± 0.32 ^c	22.80 ± 0.29 ^{cd}
Moisture retention (%)	47.23 ± 0.28 ^a	46.32 ± 0.29 ^{ab}	45.89 ± 0.30 ^b	44.72 ± 0.31 ^c	42.28 ± 0.33 ^d
Cooking yield (%)	78.50 ± 0.32 ^a	72.30 ± 0.40 ^c	74.80 ± 0.35 ^d	76.40 ± 0.31 ^c	77.20 ± 0.29 ^b
Diameter change (%)	7.20 ± 0.35 ^c	8.50 ± 0.40 ^a	8.00 ± 0.42 ^{ab}	7.50 ± 0.28 ^{bc}	7.00 ± 0.29 ^c
Thickness change (%)	7.18 ± 0.31 ^{bc}	8.39 ± 0.40 ^a	7.98 ± 0.35 ^{ab}	7.29 ± 0.32 ^{bc}	6.97 ± 0.29 ^c
Initial L* value	78.54 ± 1.18 ^a	68.91 ± 1.24 ^b	67.45 ± 1.11 ^c	66.18 ± 1.05 ^{cd}	65.02 ± 1.09 ^d
Final L* value	72.35 ± 1.24 ^a	72.35 ± 1.24 ^a	64.12 ± 1.15 ^c	63.09 ± 1.07 ^{cd}	61.85 ± 1.21 ^d
Initial a* value	3.12 ± 0.16 ^c	6.28 ± 0.19 ^d	7.02 ± 0.21 ^c	7.85 ± 0.23 ^b	8.34 ± 0.26 ^a
Final a* value	6.24 ± 0.18 ^c	8.36 ± 0.22 ^d	9.22 ± 0.25 ^c	9.97 ± 0.28 ^b	10.45 ± 0.31 ^a
Initial b* value	16.85 ± 0.34 ^a	15.47 ± 0.29 ^b	14.85 ± 0.31 ^c	14.12 ± 0.28 ^d	13.65 ± 0.27 ^c
Final b* value	14.56 ± 0.37 ^a	13.21 ± 0.44 ^b	12.74 ± 0.41 ^c	12.02 ± 0.39 ^d	11.48 ± 0.35 ^c
Initial ΔE value	41.46 ± 0.88 ^a	34.27 ± 0.82 ^b	32.85 ± 0.75 ^c	31.84 ± 0.72 ^c	31.05 ± 0.70 ^d
Final ΔE value	37.43 ± 0.85 ^a	30.90 ± 0.74 ^b	29.91 ± 0.69 ^c	29.91 ± 0.69 ^c	27.82 ± 0.61 ^d
Initial pH	6.15 ± 0.05 ^d	6.50 ± 0.04 ^a	6.45 ± 0.04 ^{ab}	6.40 ± 0.05 ^{bc}	6.32 ± 0.06 ^c
Final pH	5.92 ± 0.04 ^d	6.28 ± 0.03 ^a	6.22 ± 0.03 ^{ab}	6.18 ± 0.04 ^b	6.10 ± 0.05 ^c

3.2.7. Textural analysis

3.2.7.1. Hardness

The mean values are depicted in table 3. From the results it is visible that as the content of whey protein continued to increase, the hardness also tended to increase. Not only in the T₄ patty (that had highest content of whey protein) was the hardness more than that of other combinations of meat analogue patties, but it was also more than the hardness of the chicken patty. The results of the current study were similar to the findings of Zhang et al. (2024), who demonstrated that adding whey protein to vegetable protein enhanced hardness and cohesiveness.

3.2.7.2. Springiness

The mean values are depicted in table 3. From the results it is visible that as the content of whey protein continued to increase, the springiness tended to decrease. Not only in the T₄ patty (that had highest content of whey protein) the springiness was less than that of other combinations of meat analogue patties, but it was also less than the springiness of the chicken patty. Springiness showed a trend that was exactly opposite to the trend of hardness. And literature also

shows that with the increase in hardness the springiness is decreased. Increasing compression generally leads to higher hardness, gumminess, and chewiness values in texture profile analysis (TPA) (Bourne and Comstock, 1981; Schubring, 2000). However, springiness may decrease with increased compression (Schubring, 2000).

3.2.7.3. Cohesiveness

The mean values are depicted in table 3. Through the results it can be observed that the cohesiveness before and after cooking remained lowest in the case of chicken patty, followed by the pure soy protein based patty, as the content of whey protein continued to increase the cohesiveness also continued to increase, hence the highest value was observed in the T₃ burger patty, This patty had highest concentration of whey protein incorporated within soy protein. The results of the current study were similar to the findings of Zhang et al. (2024), who demonstrated that adding whey protein to protein isolate enhanced cohesiveness. Another trend that was visible was that after cooking, the cohesiveness of all patties, including the chicken patty and meat analogue patties was decreased. A study showed that cooking, especially roasting and grilling, decreases the cohesiveness in the burger patties (Dreeling et al., 2000). Another study showed that the cooking process generally decreases cohesiveness, as evidenced by the lower cohesiveness values in cooked patties compared to raw ones (Zorrilla et al., 2000).

3.2.7.4. Chewiness

The mean values are depicted in table 3. Through the results it can be observed that the chewiness before and after cooking remained lowest in the case of chicken patty, followed by the pure soy protein based patty, as the content of whey protein continued to increase the chewiness also continued to increase, hence the highest value was observed in the T₃ burger patty, This patty had highest concentration of whey protein incorporated within soy. Another trend that was visible was that after cooking, chewiness also increased in all the samples, including the real chicken patty. The trend of chewiness was exactly similar to the trend of hardness, as the samples having more hardness also had higher chewiness. Moreover, as in the case of hardness the increasing trend was seen after cooking, similar phenomena were observed in the case of chewiness as well. And literature also shows that the increase in hardness leads to higher chewiness values in texture profile analysis (TPA) (Bourne and Comstock, 1981; Schubring, 2000).

3.2.8. Microbial analysis (Total plate count)

The mean values are depicted in table 3. From the results it can be seen that the highest microbial load, both before and after cooking was shown by the chicken patty, followed by the soy protein burger patty. However, as the concentration of whey protein increased in the soy protein isolates, the microbial growth continued to decrease. This could be because of the reason that these burger patties had relatively lower amount of moisture and least acidic pH. Both of which conditions are comparatively unfavorable for microbial growth (Butt et al., 2024; Preetha et al., 2020).

Table 3. Results of textural and microbial characteristics of chicken patty and meat analogue burger patties

Analysis	Burger Patty				
	Tx	T0	T1	T2	T3
Initial hardness (N)	12.35 ± 0.45 ^a	13.20 ± 0.50 ^{cd}	14.60 ± 0.52 ^{bc}	14.75 ± 0.54 ^b	15.90 ± 0.58 ^a
Final hardness (N)	14.80 ± 0.51 ^c	14.45 ± 0.53 ^c	15.10 ± 0.56 ^{bc}	15.80 ± 0.58 ^b	16.50 ± 0.62 ^a
Initial springiness (mm)	3.10 ± 0.09 ^a	2.91 ± 0.08 ^b	2.82 ± 0.07 ^c	2.73 ± 0.06 ^d	2.65 ± 0.05 ^c
Final springiness (mm)	2.75 ± 0.07 ^a	2.85 ± 0.08 ^a	2.65 ± 0.06 ^b	2.55 ± 0.05 ^c	2.45 ± 0.05 ^d
Initial cohesiveness	0.44 ± 0.01 ^d	0.45 ± 0.01 ^c	0.46 ± 0.01 ^{bc}	0.47 ± 0.02 ^{ab}	0.48 ± 0.02 ^a
Final cohesiveness	0.35 ± 0.01 ^b	0.34 ± 0.01 ^c	0.36 ± 0.01 ^{ab}	0.37 ± 0.01 ^a	0.38 ± 0.01 ^a
Initial chewiness (N·mm)	5.65 ± 0.18 ^c	6.35 ± 0.19 ^d	6.95 ± 0.20 ^c	7.50 ± 0.21 ^b	8.05 ± 0.23 ^a
Final chewiness (N·mm)	6.40 ± 0.18 ^c	6.70 ± 0.19 ^d	7.15 ± 0.20 ^c	7.95 ± 0.21 ^b	8.66 ± 0.21 ^a
Initial TPC (log cfu/g)	3.32 ± 0.04 ^a	3.27 ± 0.03 ^b	3.20 ± 0.03 ^c	3.14 ± 0.03 ^d	3.03 ± 0.02 ^c
Final TPC (log cfu/g)	2.86 ± 0.05 ^a	2.79 ± 0.05 ^b	2.68 ± 0.04 ^c	2.62 ± 0.04 ^d	2.48 ± 0.03 ^c

3.2.9. Sensory analysis

3.2.9.1. Appearance

The mean values are depicted in figure-1. From the results it can be seen that, although almost all the burger patties had compatible score in the appearance but the best scores of appearance were shown by T₂ and T₃ burger patties, these two patties had high amount of whey protein, the T₀ burger patty, that was pure soy protein based burger patty had the lowest score for the appearance among all the meat analogue burger patty, interestingly, the T_x patty, that was real chicken patty had the worst appearance. These results were supported by the findings of various studies of literature. Whey protein concentrate has been successfully used to create textured whey protein (TWP) patties, which were found to be as acceptable as commercial textured vegetable protein (TVP) patties (Taylor and Walsh, 2002). Additionally, non-plant-based protein has also been investigated as a component in soy-protein-based burgers, with varying concentrations affecting sensory acceptance (Krawczyk et al., 2024). In the current study also, it was found that the addition of whey protein, a non-plant protein, improved the appearance in a positive way, by increasing the acceptability to the extent that it even surpassed the appearance of actual meat patty.

3.2.9.2. Texture

The mean values are depicted in figure-1. From the results it can be seen that, although almost all the burger patties had compatible score in the texture, the best score was shown by the real chicken patty (T_x), followed by the T₂ burger patty, this patty had second highest concentration of whey protein incorporated into the soy protein, the third best score was shown by the T₀ burger patty that was pure soy protein burger patty, interestingly it was found that increasing too much whey protein as in the case of T₃ patty, the texture became less appealing and palatable. Literature has shown that the usage of whey protein in chicken patties minimally affected the sensory properties, with some variations in color and texture (Mah and Brannan, 2009). Sensory evaluations in another study revealed that TWP patties were as acceptable as commercial textured vegetable protein patties (Taylor and Walsh, 2002). In the current study also, it was observed that at the optimized rate whey protein dose enhances the texture of the vegetable-based meat analogue burger patty, however, too much usage results in the adverse results.

3.2.9.3. Flavor

The mean values are depicted in figure-1. From the results it can be seen that, although almost all the burger patties had compatible score in the flavor, the best score was shown by the real chicken patty (T_x), followed by the T₃ burger patty this had the highest amount of whey protein incorporated into the soy protein, and the worst score was shown by the pure soy protein based burger patty (T₀). The findings of the literature also supported the current trend. The incorporation of textured vegetable protein (TVP) in meat patties can significantly alter their taste characteristics (Bakhsh et al., 2021). The incorporation of Whey protein isolate (WPI) though affects the color and texture but does not significantly impact the flavor (Mah & Brannan, 2009). On the other hand, the addition of textured soy granules or vegetables can influence sensory attributes, with soy-extended burgers generally scoring lower in acceptability compared to control or vegetable-extended versions (Kassem and Emara, 2010). In the current study also, the pure soy protein-based patty had lower score and as the concentration of the whey protein increased the flavor started enhancing.

3.2.9.4. Taste

The mean values are depicted in figure-1. From the results it can be seen that, although almost all the burger patties had compatible score in the taste, the best score was shown by the real chicken patty (T_x), followed by the T₃ burger patty this had the highest amount of whey protein incorporated into the soy protein, and the worst score was shown by the pure soy protein based burger patty (T₀). The findings of Mah and Brannan. (2009) and Kassem and Emara. (2010) supported the trend of the current study.

3.2.9.5. Color

The mean values are depicted in figure-1. From the results it can be seen that, although almost all the burger patties had compatible score in the color, the best score was shown by T₃ burger patty, this patty had highest content of whey protein within the soy protein, real chicken patty also showed the score for color that was inferior to most of the meat analogue burger patty. The worst score was shown by the pure soy protein-based burger patty. In the literature it has been observed that the addition of whey protein isolate (WPI) in the patty dose changes color (Mah and Brannan, 2009). However, In the current study it was found that the color was improved to that extent that it became even better than real meat patty.

3.2.9.6. Overall acceptability

The mean values are depicted in figure-1. From the results, although almost all the burger patties had compatible score in the color, the meat analogue patties having higher content of whey protein (T₂ and T₃) had overall acceptability even better than real chicken patty, and the patty having only soy protein had worst score. Studies showed that the incorporation of whey protein enhanced the sensory properties (Mah and Brannan, 2009). On the other hand, the addition of textured soy granules decreased the sensory score in acceptability (Kassem and Emara, 2010).

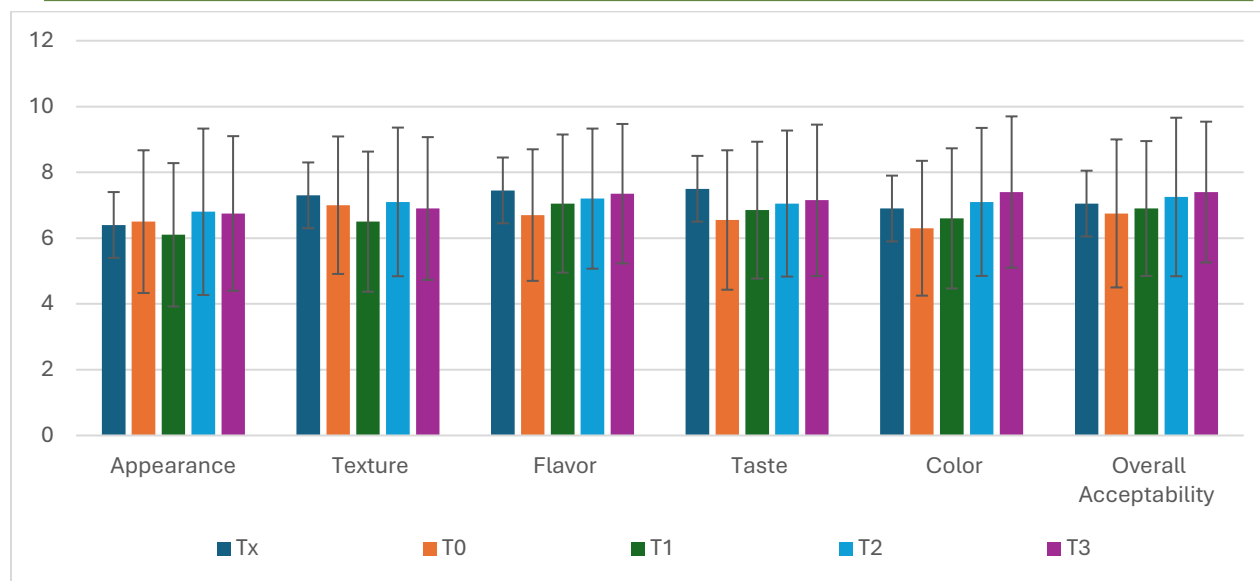


Figure 1. Pictorial depiction of the sensory evaluation of different burger patties

CONCLUSION

The development of meat analogue patties using soy and whey proteins demonstrated the feasibility of developing a product that closely mimics, and in certain aspects surpasses, the qualities of traditional chicken patties. The incorporation of whey protein significantly influenced the physicochemical, cooking, and textural properties, leading to a product with enhanced hardness, cohesiveness, and sensory appeal. Notably, patties with optimized whey protein content achieved higher overall acceptability than both pure soy and chicken patties, suggesting that a balanced blend of plant and animal proteins can effectively meet consumer preferences for taste, texture, and appearance. The reduced microbial load in patties with higher whey protein content indicates improved food safety. This study highlights the potential of utilizing protein blends and advanced processing techniques to produce sustainable and nutritious meat alternatives, addressing the growing global demand for protein while considering environmental and health impacts. Future research should focus on further optimizing the formulation and processing techniques to enhance specific attributes and explore wider applications of these meat analogues in various food products.

REFERENCES

1. AOAC. Official Methods of Analysis of Association of Official Analytical Chemist, 2020, AOAC International, Virginia USA.
2. Aparicio, V. A., Nebot, E., Tassi, M., Camiletti-Moiron, D., Sanchez-Gonzalez, C., Porres, J. M., & Aranda, P. (2014). Whey versus soy protein diets and renal status in rats. *Journal of medicinal food*, 17(9), 1011-1016.
3. Aschemann-Witzel, J., Gantriis, R. F., Fraga, P., & Perez-Cueto, F. J. (2021). Plant-based food and protein trend from a business perspective: Markets, consumers, and the challenges and opportunities in the future. *Critical Reviews in Food Science and Nutrition*, 61(18), 3119-3128.
4. Bakhsh, A., Lee, S. J., Lee, E. Y., Hwang, Y. H., & Joo, S. T. (2021). Characteristics of beef patties substituted by different levels of textured vegetable protein and taste traits assessed by electronic tongue system. *Foods*, 10(11), 2811.
5. BOURNE, M. C., & COMSTOCK, S. H. (1981). Effect of degree of compression on texture profile parameters. *Journal of Texture Studies*, 12(2), 201-216.
6. Bouton, P. E., Harris, P. V., & Shorthose, W. R. (1976). Dimensional changes in meat during cooking. *Journal of Texture Studies*, 7(2), 179-192.
7. Butt, M. A., Shukat, R., Afzaal, M., Saeed, F., Imran, A., Ahmed, A., ... & Shah, M. A. (2024). Comparative evaluation of the quality and safety attributes of local and branded beef seekh kabab. *Cogent Food & Agriculture*, 10(1), 2360769.
8. Champion, A. E., Purslow, P. P., & Duance, V. C. (1988). Dimensional changes of isolated endomysia on heating. *Meat Science*, 24(4), 261-273.
9. Cui, Q., Wang, G., Gao, D., Wang, L., Zhang, A., Wang, X., ... & Jiang, L. (2020). Improving the gel properties of transgenic microbial transglutaminase cross-linked soybean-whey mixed protein by ultrasonic pretreatment. *Process Biochemistry*, 91, 104-112.

10. Dawood, F., & van Vuuren, J. H. (2023). A multi-dimensional spatial index for the quantification of food insecurity. *Journal of Agriculture and Food Research*, 14, 100768.
11. Dreeling, N., Allen, P., & Butler, F. (2000). Effect of cooking method on sensory and instrumental texture attributes of low-fat beefburgers. *LWT-Food Science and Technology*, 33(3), 234-238.
12. DU, C. J., & SUN, D. W. (2005). Correlating shrinkage with yield, water content and texture of pork ham by computer vision. *Journal of Food Process Engineering*, 28(3), 219-232.
13. Dushkova, M. A., Menkov, N. D., & Toshkov, N. G. (2012). Functional characteristics of extruded blends of potato flakes and whey protein isolate. *International Journal of Food Engineering*, 7(6).
14. ERDOĞDU, F., BALABAN, M. O., & CHAU, K. V. (1999). Mathematical model to predict yield loss of medium and large tiger shrimp (*Penaeus monodon*) during cooking. *Journal of food process engineering*, 22(5), 383-394.
15. FAO (Food and Agriculture Organisation). 2012. World agriculture: towards 2030/2050 - 2012 revised version. In ESA Working paper No. 12-03, 160.
16. Ferrari, L., Panaite, S. A., Bertazzo, A., & Visioli, F. (2022). Animal-and plant-based protein sources: a scoping review of human health outcomes and environmental impact. *Nutrients*, 14(23), 5115.
17. Fukase, E., & Martin, W. (2020). Economic growth, convergence, and world food demand and supply. *World Development*, 132, 104954.
18. Han, J. H., Keum, D. H., Hong, S. J., Kim, Y. J., & Han, S. G. (2023). Comparative evaluation of polysaccharide binders on the quality characteristics of plant-based patties. *Foods*, 12(20), 3731.
19. Hertzler, S. R., Lieblein-Boff, J. C., Weiler, M., & Allgeier, C. (2020). Plant proteins: assessing their nutritional quality and effects on health and physical function. *Nutrients*, 12(12), 3704.
20. Indriani, W., Crespo, I., Dewi, D. P. A. P., & Insani, R. M. S. (2022). Effect of substitution of texturized vegetable protein using tempeh towards nutrional and textural quality of plant-based nuggets. *Indonesian Journal of Life Sciences*, 77-88.
21. Kassem, M. G., & Emara, M. M. T. (2010). Quality and acceptability of value-added beef burger.
22. Krawczyk, A., Fernández-López, J., & Zimoch-Korzycka, A. (2024). Insect Protein as a Component of Meat Analogue Burger. *Foods*, 13(12), 1806.
23. Kreger, J. W., Lee, Y., & Lee, S. Y. (2012). Perceptual changes and drivers of liking in high protein extruded snacks. *Journal of food science*, 77(4), S161-S169.
24. Liang, X., Ma, C., Yan, X., Zeng, H., McClements, D. J., Liu, X., & Liu, F. (2020). Structure, rheology and functionality of whey protein emulsion gels: Effects of double cross-linking with transglutaminase and calcium ions. *Food Hydrocolloids*, 102, 105569.
25. Mah, E., & Brannan, R. G. (2009). Reduction of oil absorption in deep-fried, battered, and breaded chicken patties using whey protein isolate as a postbreeding dip: Effect on flavor, color, and texture. *Journal of Food Science*, 74(1), S9-S16.
26. Mariotti, F. (2019). Animal and plant protein sources and cardiometabolic health. *Advances in nutrition*, 10, S351-S366.
27. Montgomery, Douglas C. *Introduction to statistical quality control*. John Wiley & sons, 2019.
28. Morris, A. L., & Mohiuddin, S. S. (2020). Biochemistry, nutrients.
29. Preetha, S. S., & Narayanan, R. (2020). Factors influencing the development of microbes in food. *Shanlax International Journal of Arts, Science and Humanities*, 7(3), 57-77.
30. Quintieri, L., Nitride, C., De Angelis, E., Lamonaca, A., Pilolli, R., Russo, F., & Monaci, L. (2023). Alternative protein sources and novel foods: benefits, food applications and safety issues. *Nutrients*, 15(6), 1509.
31. Rajaretnam, S., & Malik, N. (2023). Physical and Sensory Properties of Vegetarian Burger Patty. *Enhanced Knowledge in Sciences and Technology*, 3(2), 422-431.
32. Reid-McCann, R. J., Brennan, S. F., McKinley, M. C., & McEvoy, C. T. (2022). The effect of animal versus plant protein on muscle mass, muscle strength, physical performance and sarcopenia in adults: protocol for a systematic review. *Systematic Reviews*, 11(1), 1-9.
33. Rizzo G, Baroni L. Soy, soy foods and their role in vegetarian diets. *Nutrients*. 2018 Jan 5;10(1):43.
34. Samard, S., Maung, T. T., Gu, B. Y., Kim, M. H., & Ryu, G. H. (2021). Influences of extrusion parameters on physicochemical properties of textured vegetable proteins and its meatless burger patty. *Food Science and Biotechnology*, 30, 395-403.
35. Schubring, R. (2000). Untersuchung von Einflussfaktoren auf die instrumentelle Texturprofilanalyse (TPA) von Fischerzeugnissen. 2. Einfluss von Messgeschwindigkeit und Kompression. *Deutsche Lebensmittel-Rundschau*, 96(2), 45-50.
36. Simoni, R. C., Lemes, G. F., Fialho, S., Gonçalves, O. H., Gozzo, A. M., Chiaradia, V., ... & Leimann, F. V. (2017). Effect of drying method on mechanical, thermal and water absorption properties of enzymatically crosslinked gelatin hydrogels. *Anais da Academia Brasileira de Ciências*, 89, 745-755.

37. Simoni, R. C., Lemes, G. F., Fialho, S., Gonçalves, O. H., Gozzo, A. M., Chiaradia, V., ... & Leimann, F. V. (2017). Effect of drying method on mechanical, thermal and water absorption properties of enzymatically crosslinked gelatin hydrogels. *Anais da Academia Brasileira de Ciências*, 89, 745-755.
38. Sui, X., Zhang, T., & Jiang, L. (2021). Soy protein: Molecular structure revisited and recent advances in processing technologies. *Annual Review of Food Science and Technology*, 12, 119-147.
39. Sun, X. D., & Arntfield, S. D. (2012). Gelation properties of myofibrillar/pea protein mixtures induced by transglutaminase crosslinking. *Food Hydrocolloids*, 27(2), 394-400.
40. Taylor, B. J., & Walsh, M. K. (2002). Development and sensory analysis of a textured whey protein meatless patty. *Journal of food science*, 67(4), 1555-1558.
41. Theron, L., Cullere, M., Bouillier-Oudot, M., Manse, H., Dalle Zotte, A., Molette, C., ... & Vitezica, Z. G. (2012). Modeling the relationships between quality and biochemical composition of fatty liver in mule ducks. *Journal of Animal Science*, 90(9), 3312-3317.
42. Van Dijk, M., Morley, T., Rau, M. L., & Saghai, Y. (2021). A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. *Nature Food*, 2(7), 494-501.
43. Van Peteghem, L., Sakarika, M., Matassa, S., & Rabaey, K. (2022). The role of microorganisms and carbon-to-nitrogen ratios for microbial protein production from bioethanol. *Applied and Environmental Microbiology*, 88(22), e01188-22.
44. Vaskoska, R., Ha, M., Naqvi, Z. B., White, J. D., & Warner, R. D. (2020). Muscle, ageing and temperature influence the changes in texture, cooking loss and shrinkage of cooked beef. *Foods*, 9(9), 1289.
45. Velioğlu, H. M., Velioğlu, S. D., Boyacı, İ. H., Yılmaz, İ., & Kurultay, Ş. (2010). Investigating the effects of ingredient levels on physical quality properties of cooked hamburger patties using response surface methodology and image processing technology. *Meat Science*, 84(3), 477-483.
46. Visioli, F. (2024). Redefining Protein Quality: Integrating Health Outcomes and Environmental Impacts in the Plant-Animal Protein Debate. *Foods*, 13(24), 4128.
47. Wu, S., Fitzpatrick, J., Cronin, K., & Miao, S. (2019). The effect of pH on the wetting and dissolution of milk protein isolate powder. *Journal of Food Engineering*, 240, 114-119.
48. Youssef, M. K., & Barbut, S. (2011). Effects of two types of soy protein isolates, native and preheated whey protein isolates on emulsified meat batters prepared at different protein levels. *Meat science*, 87(1), 54-60.
49. Zhang, J., Yuan, J., Han, X., Li, Q., Liao, X., & Zhao, J. (2024). Development and characterization of fibrous high moisture extrudates based on pea protein isolate and whey protein. *Journal of the Science of Food and Agriculture*.
50. Zhang, Y. N., & Zhao, X. H. (2013). Study on the functional properties of soybean protein isolate cross-linked with gelatin by microbial transglutaminase. *International Journal of Food Properties*, 16(6), 1257-1270.