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Effect of soy and pea protein isolate addition on the physicochemical properties of mung bean protein-based burger patties

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ABSTRACT

The incorporation of protein isolated from legumes, such as soy and pea protein isolates, into meat analogs has been reported to enhance their textures and overall quality. This study aims to examine the impact of including soy and pea protein isolates on the physicochemical properties of mung bean protein-based burger patties. Nine combination treatments were used in this study: MBP (4,0), MBP-SP (3:1), MBP-SP (2:2), MBP-SP (1:3), SP (0:4), MBP-PP (3:1), MBP-PP (2:2), MBP-PP (1:3), and PP (0:4). The findings indicate that the addition of soy protein isolate alone or pea protein isolate alone increases the protein content of the patties up to 41% on a dry basis, along with enhancing mineral content, including calcium, magnesium, and zinc. The addition of soy protein isolates reduces the brightness of the patties, with values decreasing up to 40.74, whereas the integration of pea protein isolate maintains brightness at 51.17 and increases redness up to 15.27 and yellowness up to 11.19. The inclusion of soy and pea protein isolates reduces the hardness, with values reaching up to 0.91 kgf and 0.94 kgf, respectively, and decreases chewiness, with values up to 0.084 kgf and 0.171 kgf, respectively. Using pea protein isolates alone in burger patties made from mung bean protein results in enhanced coloration compared to soy protein isolates. Microstructure analysis indicates that patties with soy protein isolate exhibit a more consistent lipid distribution than those made with pea protein isolate.

Contribution/Originality: This study describes the effect of soy and pea protein isolate addition on the physicochemical properties of mung bean protein-based burger patties. It investigates the impact of combining mung bean protein with soy and pea protein isolates on the properties of the patties, rather than other aspects.

1. INTRODUCTION

The use of plant-based protein as a substitute for animal protein has increased recently. This is driven by the escalating demand for protein due to the growing human population. However, animal protein production is increasingly limited. Besides that, there is a growing perception that animal protein production triggers several environmental issues, including exacerbating global warming through increased greenhouse gas emissions from livestock farming (Berardy, Costello, & Seager, 2015). Furthermore, there are issues related to the extensive use of agricultural land, fresh water, and feed, as well as the efficiency of converting plants to animal protein during the animal

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protein production process. There is also an increase in chronic diseases resulting from excessive consumption of animal protein (Huang et al., 2024).

The growing public awareness of the importance of health and environmental impacts is driving the rise of plant-based protein as an alternative to meet protein needs. This is because plant-based protein is considered healthier, reducing the risk of chronic diseases associated with excessive meat (animal protein) consumption, and is more affordable and sustainable. This growing awareness has also driven the growth of the plant-based processed food industry, such as meat analogs. Meat analogs are promoted as a low-cholesterol, healthier, and more sustainable source of protein. The meat analogs industry is expanding, offering significant benefits for food security and animal welfare, although challenges remain in reducing nutritional deficits (Shi, Wang, & Fang, 2024) and improving the texture of meat analogs.

Mung bean protein is a plant-based protein that offers several nutritional advantages and can be used as a promising ingredient for processing into meat analogs (De Angelis, Opaluwa, Pasqualone, Karbstein, & Summo, 2023). MBP is particularly rich in lysine and leucine, essential amino acids often limited in other plant proteins (Huang, Li, Fan, Qian, & Wang, 2024; Tarahi, 2024). It was reported to be highly digestible, making it suitable for vegetarian diets and plant-based protein formulations (Huang et al., 2024) and enhancing nutrient absorption, making it a superior choice for plant-based diets (Li et al., 2023). Mung bean protein can be extracted from mung bean seeds, which contain around 20-32% protein, and isolates reaching up to 99% purity depending on the extraction method (Wintersohle, Kracke, Ignatzy, Etzbach, & Schweiggert-Weisz, 2023). It can also be extracted from a by-product of the mung bean starch extraction process (Ratnaningsih & Songsermpong, 2021). MBP has functional properties – such as excellent gelling, emulsifying, foaming, water- and oil-holding capacity, and fibrous texture formation, which make it suitable for developing meat analogs (Baig, Ajayi, Mostafa, Sivapragasam, & Maqsood, 2023; De Angelis et al., 2023) and essential for creating desirable textures in meat analogs (Li et al., 2023; Tarahi, 2024). MBP can be developed into many kinds of meat analogs, such as extruded meat pieces (Brishti et al., 2021; Seetapan et al., 2023), burger patties (Baig et al., 2023; Ratnaningsih, Songsermpong, Jittanit, & Rumpagaporn, 2024), sausages (Baig et al., 2024), and tuna analogs (Charoenthaikit et al., 2025). It can be developed to be plant-based burger patties utilizing tapioca starch and hydrocolloid as a binding agent (Ratnaningsih et al., 2024). However, the texture and nutritional properties of the burger patties still need to be improved to mimic real meat. Therefore, further research in developing meat analogs using mung bean protein is still necessary.

Previous research reported that incorporating soy protein isolate (SPI) in meat analogs offers several nutritional benefits that make it a compelling alternative to traditional protein sources. Soy protein is recognized for its high-quality protein content, containing all essential amino acids except for sulfur-containing ones, making it a complete protein source, similar to animal proteins, and is associated with various health benefits (Michelfelder, 2009). Meat analogs made from soy typically have lower saturated fat levels and higher polyunsaturated fatty acids compared to traditional meat (Hegarty & Ahn, 1976). Soy protein isolate is also a good source of minerals such as potassium, calcium, and phosphorus, which are beneficial for bone health (Hegarty & Ahn, 1976). Regular consumption of soy protein is linked to lower cholesterol levels and reduced risk of heart disease (Michelfelder, 2009). Soy protein may alleviate menopausal symptoms in women, contributing to overall well-being (Michelfelder, 2009). It has been shown to help maintain bone density, particularly in postmenopausal women (Michelfelder, 2009). The incorporation of soy protein isolate (SPI) in meat analogs significantly influences their texture and sensory properties, enhancing their appeal as plant-based alternatives. SPI enhances the gel network within textured plant-based protein matrices, leading to improved hardness and water-holding capacity (WHC) (Wi, Bae, Kim, Cho, & Choi, 2020). When combined with wheat gluten, SPI can create a fibrous texture similar to that of meat, particularly in high-moisture extruded products (Dubey, Kumar, & Singh, 2024). The addition of hydrocolloids and salts can further enhance the fibrous structure and mechanical properties of SPI-based products, making them more appealing to consumers (Dinani, Zhang, Vardhanabhuti, & van der Goot, 2023).

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Besides that, the incorporation of pea protein isolate (PPI) in meat analogs significantly alters their nutritional profile compared to traditional protein sources. PPI is increasingly utilized in plant-based meat alternatives due to its high protein content and favorable health benefits, making it a viable substitute for animal proteins. PPI can provide up to 55 g of protein per 100 g in meat analogs, which is comparable to traditional meat sources (De Angelis et al., 2020). Plant-based meat analogs containing PPI often exhibit higher fat content (11.2 g/100g) and lower carbohydrates (10.4 g/100g) compared to traditional plant proteins like tofu (Lindberg, Woodside, Kelly, Robinson, & Nugent, 2024). PPI is associated with various health benefits, including improved satiety and potential blood pressure regulation (Stilling, 2020). The use of PPI enhanced the texture of meat analogs, allowing them to better mimic the fibrous structure of animal meat (Plattner et al., 2024). Increasing PPI content generally enhances hardness and chewiness, with optimal textural properties observed at 30% to 60% PPI inclusion (Plattner et al., 2024). The combination of PPI with other proteins, such as oats, can improve fibrousness, which is a desirable trait in meat analogs (Kaleda et al., 2021). Reformulated products with PPI have shown favorable sensory attributes, such as improved texture and spreadability, leading to higher consumer acceptance (Trindade et al., 2023). Sensory evaluations indicated that formulations with approximately 10% PPI yield high overall liking scores, suggesting optimal consumer acceptance (Weenuttranon, Hirunyophat, Saeiam, Bunnak, & Saelee, 2023).

Improving the texture and nutrients of mung bean protein-based meat analogs is still required to mimic real meat. Previous research has shown that the addition of soy protein isolate or pea protein isolate can improve the textures and nutritional properties of meat analogs, even providing a fibrous texture that resembles real meat. There is no research reported yet on the impact of soy and pea protein isolates incorporation into mung bean protein-based burger patties. This addition is expected to improve the texture profile and enhance the nutritional properties of the burger patties for better quality. Therefore, the objective of this research is to investigate the effect of the incorporation of soy protein isolate and pea protein isolate in the mung bean protein-based burger patties.

2. MATERIALS AND METHODS

2.1. Raw Materials

All ingredients for the manufacture of plant-based burger patties were acquired from the supermarket in Thailand, consisting of mung bean (Raithip brand), soy protein isolate (MS brand), pea protein isolate (MS brand), button mushroom, instant oatmeal (QuakerTM), kidney beans (Tops brands), beetroot, garlic, refined sunflower oil (Naturel brand), coconut oil (Naturel brand), tapioca starch (Five Stars Fish brand), salt, pepper powder, and yeast extract. Chemicals consist of $MgSO_4$ for protein precipitation, chemicals for proximate and minerals analysis, Rhodamine B (Sigma-Aldrich), and Calcofluor White (Sigma).

2.2. Preparation of Fresh Mung Bean Protein

Using a modified approach of Ratnaningsih and Songsermpong (2021) methods, fresh mung bean proteins were precipitated by using magnesium sulfate salt under suitable conditions. The peeled mung bean was cleaned and soaked overnight in water. It was then rewashed to eliminate the beany flavor and green color before being ground with tap water (ratio of mung bean to water = 1:3). Basket centrifugation with a fine cloth filter was used to remove the crude fiber. The starch was then precipitated from the protein solution, and the protein solution was decanted.

Two liters of mung bean protein solution were heated to 80°C before 10 g/L of $MgSO_4$ was added and stirred until the protein precipitated. Fresh mung bean protein was suitable for use after three cycles of filtering using a cloth filter and washing with water to remove the $MgSO_4$ residue from the precipitated protein. By manually squeezing fresh protein through a cloth filter, the moisture content was reduced to around 80% wb. The fresh proteins were stored in the refrigerator overnight before use.

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2.3. Preparation of Plant-Based Burger Patties with Substitutions

Table 1 outlines the formula for producing plant-based burger patties. Prior to this, mung bean protein was conditioned at 80% wb. Similarly, SPI and PPI were conditioned to 80% wb by adding water until the moisture content reached 80% wb.

Each ingredient was weighed according to the formulation shown in Table 1. The coconut oil was frozen by storing it in the freezer. Garlic, mushrooms, and boiled, peeled kidney beans were chopped and stir-fried in sunflower oil until wilted and a scent emerged. Then, the ingredients were combined in a food processor with instant oatmeal, fresh mung bean protein, grated beetroot, salt, pepper powder, and yeast extract. Tapioca starch, hydrocolloid, and frozen coconut oil were added following the experimental protocol. After mixing thoroughly, around 50 g per patty was placed in a circular mold, steamed for five minutes, removed, and frozen at -18°C.

Table 1. Treatment and formulation of plant-based protein burger patties per 100 g ingredients.

Ingredient	Formula								
	MBP (4:0)	***MBP-SP (3:1)***	***MBP-SP (2:2)***	***MBP-SP (1:3)***	***SP (0-4)***	***MBP-PP (3:1)***	***MBP-PP (2:2)***	***MBP-PP (1:3)***	***PP (0-4)***
Mung bean protein (g)	50	37.5	25	12.5	-	37.5	25	12.5	-
Soy protein (g)	-	12.5	25	37.5	50	-	-	-	-
Pea protein (g)	-	-	-	-	-	12.5	25	37.5	50
Other[a]	50	50	50	50	50	50	50	50	50

Note: [a] With other ingredients for each formula: Buttom mushroom (14 g), instant oatmeal (5.5 g), boiled peeled kidney beans (14 g), grated beetroot (5.5 g), garlic (3 g), sunflower oil (2.5 cc), coconut oil (1.25 cc), tapioca starch (2 g), guar gum (0.5 g), salt (0.85 g), pepper powder (0.35 g), yeast extract (0.35 g).

2.4. Proximate Analysis

The moisture, ash, protein, fat, and crude fiber contents of protein isolates and plant-based burger patties were evaluated using AOAC methods (AOAC, 2016). Moisture content was determined using the oven drying procedure (AOAC method 925.10). The dry-ashing technique, according to AOAC methods 923.03, was used to evaluate the ash content. The total nitrogen content was measured using the Kjeldahl method, according to AOAC method 991.20, whereas the protein content was determined using a 6.25 conversion factor. The fat content was calculated using Soxhlet equipment in accordance with the protocol in AOAC method 922.06. The crude fiber was assessed using the residues remaining after acid and alkaline hydrolysis, in compliance with the procedure in AOAC method 978.10. Carbohydrates were measured using different methods. Total calories were calculated by multiplying the protein, carbohydrate, and fat contents by the factors 4, 4, and 9 kcal/g, respectively. The values were expressed in kilocalories per 100 g.

2.5. Mineral Analysis

Mineral compounds of the protein isolate and plant-based burger patties, including calcium, magnesium, iron, and zinc, were measured using an atomic absorption spectrophotometer according to AOAC method 984.27 (AOAC, 2016).

2.6. Color Analysis

The colors of the protein isolates and pre-cooked plant-based burger patties were determined instrumentally using a Hunter Colorimeter (Ultra Scan Pro, USA) employing the CIELAB system for L*, a*, and b*. L* values represented the lightness of samples (0 for darkness to 100 for whiteness). Positive a* values indicated redness, while negative a* values indicated greenness. Positive b* values represented yellowness, while negative b* values indicated blueness. Color differences (ΔE) were determined following Equation 1.

$$\Delta E = \sqrt{(L_i - L)^2 + (a_i - a)^2 + (b_i - b)^2} \quad (1)$$

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2.7. Texture Analysis

Texture profile analysis was conducted to analyze the pre-cooked plant-based burger patties using a texture analyzer (TexturePro CT V1.2, Stable Micro Systems, UK) following the modified Samard, Maung, Gu, Kim, and Ryu (2021) methods. A profile analysis of the material's texture was investigated using a 25 mm cylindrical probe in a two-cycle compression test. The samples were compressed twice to 50% of their original thickness at 1 mm/s, triggering a load of 5 kg. Hardness, adhesiveness, cohesiveness, springiness, and chewiness were recorded.

2.8. Cooking Properties

The burger patties were defrosted by placing them in the refrigerator overnight. Then, they were cooked in a pan fryer (non-stick frying pan) that was preheated to 120°C. Sunflower oil was used to lubricate the non-stick cooking surface before placing the samples in the pan. Two patties were cooked and flipped every 1 min. Burger patties were removed for testing after 4 min of cooking. Three replications were performed.

1. Cooking loss

The cooking loss of the burger patties was assessed using the modified Samard et al. (2021). The percentage of cooking loss was measured using Equation 2.

$$\text{Cooking loss (\%)} = \frac{\text{Raw patty weight (g)} - \text{Cooked patty weight (g)}}{\text{Raw patty weigt (g)}} \times 100 \quad (2)$$

2. Cooking yield

The cooking yield was estimated as described by Samard et al. (2021) using Equation 3.

$$\text{Cooking yield (\%)} = \frac{\text{Cooked patty weight (g)}}{\text{Raw patty weight (g)}} \times 100 \quad (3)$$

3. Moisture retention

Moisture retention was determined following the methods of Samard et al. (2021) and Equation 4. The moisture content of the cooked patty was evaluated by drying each sample at 105°C until constant weight, according to the standard AOAC protocol.

$$\text{Moisture retention (\%)} = \frac{\text{Cooking yield (\%)} \times \text{Moisture of cooked patty (\%)}}{100} \quad (4)$$

2.9. Microstructure Analysis

2.9.1. Scanning Electron Microscopy (SEM)

Using a scanning electron microscope (Zeiss Evo MA 10, Carl Zeiss Microscopy Ltd.), the morphology of the plant-based burger patties of MBP – SP and MBP – PP's dry samples was observed. The freeze-dried samples were placed on double-sided sticky conductive carbon tabs, and the samples were then gold-sputter coated. The samples were distributed uniformly over the surface of the carbon tabs using compressed air. 10 kV was the accelerating voltage. The SEM images were captured at a resolution of 500× magnification.

2.9.2. Confocal Laser Scanning Microscopy (CLSM)

Using a confocal laser scanning microscope (Digital Eclipse C2Si, Nikon), the structure of plant-based burger patties was observed on a microscopic scale. The MBP – SP and MBP – PP mixtures burger patties were frozen prior to cutting the samples into approximately 3 x 5 x 10 mm rectangular shapes. The samples were rapidly frozen using liquid nitrogen. With a surgical blade no. 24 (Techno Cut, ABC Medical, Thailand), the samples were sliced into specimens at 20°C. The specimens were then stained with a solution containing 0.002% Rhodamine B and 0.01% Calcofluor White at a ratio of 1:1, after which they were covered with a glass coverslip and kept in the dark for 1 hour prior to analysis. Two lasers supplied excitation light for CLSM: a 543 nm HeNe laser for Rhodamine B and a 405 nm blue/violet diode laser for Calcofluor White. To capture the images, a 10 EC Plan-Neofluar/0.5 objective lens was

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used. The images were analyzed using the red and blue channels of the ZEN program (Carl Zeiss Microscopy, Jena, Germany).

2.10. Statistical Analysis

The effect of soy and pea protein isolates on the characteristics of mung bean protein-based burger patties was analyzed by comparing the means of each treatment (formula). This study compared nine treatment groups (formulas): MBP (4:0), MBP-SP (3:1), MBP-SP (2:2), MBP-SP (1:3), SP (0:4), MBP-PP (3:1), MBP-PP (2:2), MBP-PP (1:3), and PP (0:4).

This study focused on determining whether at least one treatment mean was significantly different from the other treatments. It did not compare the effects of protein isolate type alone, nor the ratio alone. Therefore, data between treatments were analyzed directly using one-way ANOVA.

The effect of soy and pea protein isolates on the characteristics of mung bean protein-based burger patties was determined using one-way ANOVA with a 95% confidence interval. Duncan's multiple range tests were used after the ANOVA, with significant differences determined at the $p<0.05$ level. Three replications were used for all treatments in the experiment. The results were reported as mean ± SD.

3. RESULTS AND DISCUSSION

3.1. Characteristics of MBP, SPI, and PPI

3.1.1. Proximate Composition

Mung bean protein (MBP), soy protein (SP), and pea protein (PP) isolates are plant proteins widely utilized in the manufacturing of plant-based burger patties. The nutritional composition, mineral content, and color of dried MBP, SP, and PP are shown in Table 2. SP exhibited the highest protein content (87.68% dry basis), followed by PP and MBP, which had 86.61% and 78.61% dry basis, respectively. Regarding fat content, SP contained 7.95% dry basis, which was higher than MBP (5.60%) and PP (3.15%). This difference is related to the initial protein and fat content of soybean seeds, which are greater than those of mung beans or peas. The protein and fat contents of soybean seeds are reported to be 35-42% and 18-21%, respectively (Kumar & Pandey, 2020; Shrestha, van't Hag, Haritos, & Dhital, 2023) higher than in mung bean seeds which were 14.6-32.6% and 0.7-1.9%, respectively (Dahiya et al., 2015) and pea seeds contained 23.1-30.9% protein and 1.5-2% fat, respectively (Lam, Can Karaca, Tyler, & Nickerson, 2018).

3.1.2. Mineral Composition

Meanwhile, MBP had the greatest ash content at 6.89% db, followed by SP and PP at 5.62% db and 4.97% db, respectively. This was due to the mineral concentration of the protein isolate. MBP had more magnesium (1,064.4 ppm) than SP and PP (696.95 ppm and 621.26 ppm, respectively), as indicated in Table 2. This was relevant to the protein extraction technique from MBP that employed wet extraction with $MgSO_4$ salt, which resulted in increased magnesium levels in the precipitated protein (Ratnaningsih & Songsermpong, 2021).

3.1.3. Color of MBP, SPI, and PPI

Figure 1 illustrates the visual appearance of mung bean protein, soy protein isolate, and pea protein isolate used in this study. SP has the highest brightness level (82.94), followed by PP (81.01) and MBP (79.37). This variation is influenced by the initial color of the grains. Mung beans have a green pigment on their seed coat and contain phenolic compounds (Dahiya et al., 2015), which may affect the final color of the protein isolate, causing MBP to be darker than SP and PP. Additionally, browning during the drying process of fresh mung bean protein can contribute to the darker color, resulting from the browning reaction during temperature treatment.

The redness level (a*) of SP was the lowest, at 1.15, compared to MBP (3.83) and PP (3.72). The level of redness was probably influenced by reddish pigments such as anthocyanins, which were found in mung bean and pea.

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Meanwhile, the highest level of yellowness (b*) was observed in MBP (23.96), followed by PP (19.47) and SP (18.45). It is possible that the high level of yellowness was influenced by green or greenish-yellow pigments found in green beans (Dahiya et al., 2015), which caused the high level of yellowness in the obtained protein.

Table 2. Characteristics of mung bean protein, soy protein isolate, and pea protein isolate.

Parameters	Table Column Head		
	Dried MBP	***SPI***	***PPI***
Moisture, %db	8.56 ± 0.20	6.07 ± 0.25	6.58 ± 0.85
Ash, %db	6.89 ± 0.35	5.62 ± 0.06	4.97 ± 0.34
Protein, %db	78.61 ± 1.46	87.68	86.61
Fat, %db	5.60 ± 0.50	7.95	3.15
Crude fiber, %db	<0.1	0.19	0.38
Calcium, ppm	334.5	584.33	593.52
Magnesium, ppm	1,065.40	696.96	621.26
Iron, ppm	20.7	118.7	173.99
Zinc, ppm	-	29.18	96.69
L*	79.37 ± 1.57	82.94 ± 0.11	81.09 ± 0.06
a*	3.83 ± 0.55	1.15 ± 0.03	3.72 ± 0.03
b*	23.96 ± 0.19	18.45 ± 0.16	19.47 ± 0.08

Note: Values are expressed as mean ± SD. MBP = mung bean protein, SPI = soy protein isolate, PPI = pea protein isolate. (*) refers to the CIELAB color space.

[A] [B] [C]

Figure 1. Visual appearance of: [A] mung bean protein, [B] soy protein isolate, and [C] pea protein isolate used in this study.

3.2. Characteristics of Plant-Based Burger Patties with MBP-SPI and MBP-PPI

3.2.1. Proximate Composition

The results of the proximate analysis of plant-based burger patties are presented on a dry basis in Table 3. Increasing the application ratio of soy protein and pea protein in the formula had a significant effect on increasing the ash and protein content of the burger patties. However, increasing the application ratio of soy protein and pea protein in the formula significantly affected the decrease in the carbohydrate content of the burger patties.

Table 3. Proximate composition of plant-based burger patties.

Treatments	Proximate composition				
	Moisture (% db)	***Ash (% db)***	***Protein (% db)***	***Fat (% db)***	***Crude fiber (% db)***
MBP (4:0)	172.42±26.09^{c}	4.70±0.30^{d}	28.23±2.68^{d}	12.15±1.17^{d}	3.33±0.21^{c}
MBP-SP (3:1)	202.17±5.87^{abc}	5.37±0.19^{c}	36.04±0.64^{bc}	13.35±0.37^{abcd}	3.58±0.19^{bc}
MBP-SP (2:2)	185.24±16.01^{bc}	5.39±0.52^{c}	35.39±1.91^{bc}	13.71±0.40^{abc}	3.59±0.10^{bc}
MBP-SP (1:3)	187.81±11.07^{bc}	6.11±0.52^{ab}	38.57±1.53^{ab}	13.94±0.47^{ab}	3.60±0.13^{bc}
SP (0:4)	189.65±5.82^{abc}	6.40±0.27^{a}	41.27±0.37^{a}	14.24±0.65^{a}	3.50±0.21^{bc}
MBP-PP (3:1)	218.11±17.26^{a}	5.61±0.32^{bc}	33.35±2.16^{c}	12.61±0.56^{cd}	3.96±0.30^{a}
MBP-PP (2:2)	210.30±9.39^{ab}	6.02±0.73^{abc}	36.09±1.01^{bc}	12.90±0.16^{bcd}	3.77±0.23^{ab}
MBP-PP (1:3)	213.91±12.82^{ab}	6.15±0.24^{ab}	37.42±1.60^{b}	12.75±0.36^{bcd}	3.93±0.10^{a}
PP (0:4)	187.02±4.32^{bc}	5.74±0.17^{abc}	40.99±1.02^{a}	12.67±0.15^{cd}	3.32±0.14^{c}

Note: Values (mean±SD) in the same column with different lowercase superscripts (a, b, c, d, e) are significantly ($p<0.05$) different.

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Interestingly, increasing patties but soy protein had a significant effect on the fat content of the burger patties, but had no significant effect on the application of pea protein. Conversely, an increase in the ratio of soy protein had no significant effect on the water content, crude fiber, and calories of burger patties, but it had a significant effect on an increase in the water content, crude fiber, and calories of burger patties on an increase in the ratio of pea protein.

3.2.2. Mineral Composition

Mineral compounds of burger patties are shown in Table 4. It appeared that increasing the ratio of SP and PP in the burger patties formula significantly increased the mineral content of burger patties, including Ca, Mg, and Zn; however, it significantly lowered the Fe content of burger patties. The Ca content of burger patties increased significantly from 825.73 ppm for MBP-SP (4:0) to 3697.58 ppm for MBP-SP (0:4), and 2927.84 ppm for MBP-PP (0:4). The Mg content of burger patties significantly increased from 269.30 ppm to 1,112.95 ppm for MBP-SP (0:4), and 961.94 ppm for MBP-PP (0:4). The Zn content also significantly increased with the addition of the SP and PP ratios, reaching 13.84 ppm for MBP-SP (0:4) and 11.56 ppm for MBP-PP (0:4). Regarding Fe content, it significantly decreased from 21.17 ppm to 19.30 ppm for MBP-SP (0:4), and to 11.54 ppm for MBP-PP (0:4).

Table 4. Mineral content of plant-based burger patties.

Treatments	Mineral content			
	Ca (% db)	*Mg (% db)*	*Zn (% db)*	*Fe (% db)*
MBP (4:0)	425.73±13.43^{e}	269.30±6.41^{e}	8.14±0.17^{e}	21.17±014^{a}
MBP-SP (3:1)	1243.63±25.34^{d}	442.80±9.82^{d}	9.57±0.11^{d}	20.76±0.29^{b}
MBP-SP (2:2)	2061.64±15.71^{c}	615.62±2.73^{c}	10.99±0.35^{c}	20.24±0.35^{c}
MBP-SP (1:3)	2879.89±22.81^{b}	788.78±20.16^{b}	12.42±0.31^{b}	19.77±0.12^{d}
SP (0:4)	3697.58±71.76^{a}	1112.95±9.07^{a}	13.84±0.24^{a}	19.30±0.23^{e}
MBP-PP (3:1)	1051.68±14.37^{d}	334.89±2.09^{d}	9.00±0.27^{d}	18.82±0.23^{b}
MBP-PP (2:2)	1676.33±22.44^{c}	522.36±1.84^{c}	9.87±0.21^{c}	16.38±0.25^{c}
MBP-PP (1:3)	2302.35±23.08^{b}	674.05±4.42^{b}	10.71±0.24^{b}	13.96±0.35^{d}
PP (0:4)	2927.84±96.50^{a}	961.94±9.30^{a}	11.56±0.15^{a}	11.54±0.13^{e}

Note: Values (mean±SD) in the same column with different lowercase superscripts (a, b, c, d, e) are significantly ($p<0.05$) different.

3.2.3. Color Properties

Color is one of the important properties of burger patties, which can affect consumer acceptance. The color of the pre-cooked burger patties is shown in Figure 2 and Table 5. Increasing the ratio of SP addition to the formula significantly reduced the brightness level of burger patties, from 51.43 for MBP-SP (4:0) to 40.74 for MBP-SP (0:4). This reduction was possibly due to protein denaturation during the steaming process, which occurred at temperatures above 100°C. Additionally, the browning process also influenced the color of the burger patties formed. However, the addition of PP to burger patties tended to provide a more stable product brightness, except for the MBP-PP (2:2) and (1:3) ratios, which had brightness levels of 50.02 and 50.21 respectively, decreasing from the MBP-PP (4:0) color, which was 51.43.

For the level of redness, an increase in the SP ratio in the burger patties formula tended to be constant and did not significantly change the level of redness, except for the MBP-SP (0:4), which significantly decreased to 10.45. Meanwhile, the addition of PP to the burger patties formula significantly increased the redness of the burger patties. This is probably due to the reddish pigment found in the protein isolate used.

The addition of SP and PP to burger patties significantly increased the level of yellowness (b*), from 4.81 for MBP-SP (4:0) to 11.09 for MBP-SP (0:4). The increased level of yellowness was attributable to the yellow pigments from SP and PP, which were 18.45 and 19.47, respectively.

Burger patties received color differences (ΔE values) higher than 2.3, indicating a noticeable difference between MBP in 4 ratios. Burger patties with the addition of SP produced an even more striking color difference. However, the

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color changes for the MBP-PP burger patties (3:1), (2:2), and (1:3) were not significantly different, with values of 4.68, 4.19, and 4.40, respectively.

Table 5. Color parameters of plant-based burger patties.

Treatments	Color Parameter			
	L*	***a****	***b****	***ΔE***
MBP (4:0)	51.43±0.54^{a}	12.74±0.30^{d}	4.81±0.14^{e}	0^{g}
MBP-SP (3:1)	44.88±0.40^{d}	11.80±0.32^{d}	4.82±0.62^{e}	6.98±0.38^{c}
MBP-SP (2:2)	45.74±0.42^{c}	12.40±0.96^{d}	6.25±0.37^{d}	6.25±0.42^{d}
MBP-SP (1:3)	44.65±0.43^{d}	11.85±0.49^{d}	8.08±0.40^{bc}	7.90±0.28^{b}
SP (0:4)	40.74±0.43^{e}	10.45±1.10^{e}	7.27±1.60^{c}	11.65±0.25^{a}
MBP-PP (3:1)	51.50±0.31^{a}	16.53±0.03^{a}	7.75±0.29^{bc}	4.68±0.20^{e}
MBP-PP (2:2)	50.02±0.82^{b}	15.48±0.34^{b}	7.50±0.53^{bc}	4.19±0.26^{f}
MBP-PP (1:3)	50.21±0.13^{b}	14.13±0.30^{c}	8.41±0.38^{b}	4.40±0.39^{f}
PP (0:4)	51.17±0.66^{a}	15.27±0.55^{b}	11.19±0.25^{a}	6.86±0.41^{c}

Note: Values (mean±SD) in the same column with different lowercase superscripts (a, b, c, d, e, f, g) are significantly ($p<0.05$) different.
(*) refers to CIELAB color space.

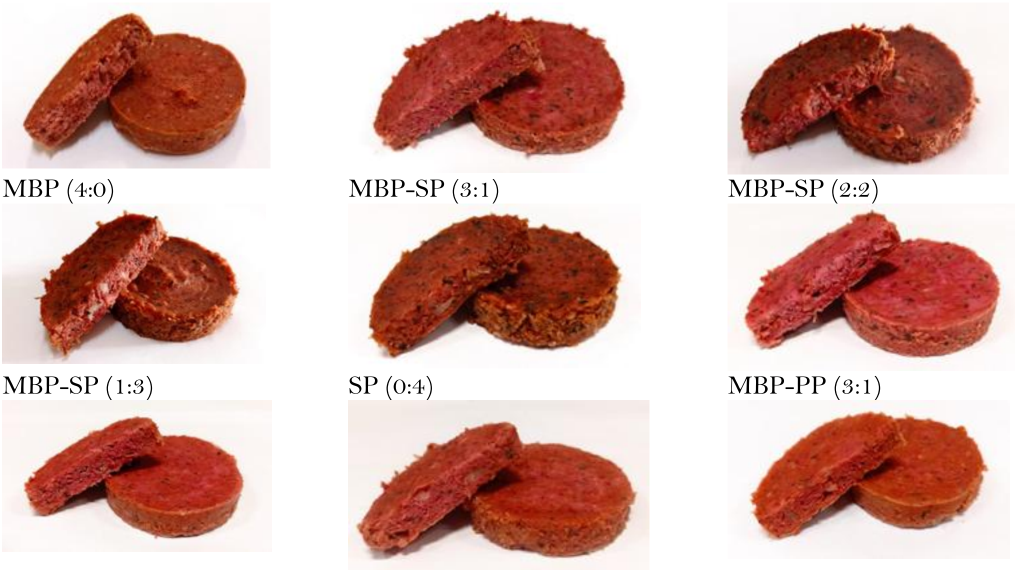

Figure 2. Visual appearance of precooked plant-based patties from several treatments.

3.2.4. Texture Properties

The texture profile analysis measures many texture characteristics, including hardness, cohesiveness, gumminess, springiness, and chewiness. The term hardness refers to the burger patties' maximal resistance to being bitten by teeth. Burger patties' cohesiveness impacts their ability to maintain structural integrity. Springiness refers to the ability of burger patties to rebound after deformation, whilst gumminess (the combination of hardness and cohesiveness) and chewiness refer to the effort needed to chew the patties' products (the combination of gumminess and springiness).

The increase in the SP ratio in the formula significantly softened burger patties compared to burger patties made from the MBP-SP formula (4:0), i.e., from 1.653 kgf to 0.91 kgf for burger patties with the MBP-SP formula (0:4). Likewise, with burger patties with an increase in the PP ratio, from 1.653 kgf to 1.101 kgf for MBP-PP burger patties (0:4).

Increasing the SP ratio in the burger patties formula significantly reduced the springiness level from 0.564 for MBP - SP (4:0) to 0.397 for MBP - SP (1:3), and to 0.350 for MBP - SP (0:4). While the increase in the PP ratio in the formula had no significant effect on springiness, except for MBP - PP (3:1), which significantly increased the springiness level of burger patties from 0.564 for MBP - PP (4:0) to 0.684.

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The increase in the SP ratio in the formula had no significant effect on the cohesiveness of burger patties, except for MBP - SP (1:3), which was 0.322. Meanwhile, the increase in the PP ratio in the formula had no significant effect on the cohesiveness level of the burger patties, except for the MBP-PP formula (0:4), which significantly increased the cohesiveness of burger patties to 0.299.

Increasing the SP ratio in the formula significantly reduced the gumminess level of burger patties from 0.413 kgf to 0.264 kgf and 0.235 kgf for MBP-SP (2:2) and MBP-SP (0:4), respectively. Meanwhile, the addition of the PP ratio to the burger patties formula had no significant effect on the burger patties, except for MBP-PP (1:3), which was 0.249 kgf.

Increasing the SP ratio in the formula significantly reduced the chewiness level of the burger patties from 0.231 kgf to 0.126 kgf, 0.131 kgf, and 0.084 kgf for MBP-SP (2:2), (1:3), and (0:4), respectively. Meanwhile, the addition of the PP ratio to the formula had no significant effect on the chewiness level of burger patties, except for MBP-PP (1 : 3), which reduced the chewiness level to 0.125 kgf.

Table 6. Texture profile of plant-based burger patties.

Treatments	Texture profile				
	Hardness(kgf)	*Springiness*	*Cohesiveness*	*Gumminess (kgf)*	*Chewiness (kgf)*
MBP (4:0)	1.653 ± 0.218^{a}	0.564 ± 0.038^{bcd}	0.258 ± 0.007^{c}	0.413 ± 0.058^{a}	0.231 ± 0.017^{ab}
MBP-SP (3:1)	1.199 ± 0.084^{bcd}	0.613 ± 0.054^{ab}	0.290 ± 0.021^{abc}	0.349 ± 0.042^{ab}	0.212 ± 0.009^{ab}
MBP-SP (2:2)	0.927 ± 0.085^{d}	0.472 ± 0.055^{de}	0.284 ± 0.022^{abc}	0.264 ± 0.043^{bc}	0.126 ± 0.034^{cd}
MBP-SP (1:3)	1.024 ± 0.097^{cd}	0.397 ± 0.030^{ef}	0.322 ± 0.035^{a}	0.329 ± 0.038^{abc}	0.131 ± 0.023^{cd}
SP (0:4)	0.910 ± 0.199^{d}	0.350 ± 0.071^{f}	0.256 ± 0.036^{bc}	0.235 ± 0.072^{c}	0.084 ± 0.033^{d}
MBP-PP (3:1)	1.385 ± 0.260^{ab}	0.682 ± 0.093^{a}	0.281 ± 0.011^{abc}	0.391 ± 0.089^{a}	0.270 ± 0.086^{a}
MBP-PP (2:2)	1.299 ± 0.086^{bc}	0.602 ± 0.095^{abc}	0.276 ± 0.033^{abc}	0.358 ± 0.030^{ab}	0.215 ± 0.032^{ab}
MBP-PP (1:3)	0.941 ± 0.127^{d}	0.503 ± 0.035^{cd}	0.268 ± 0.040^{bc}	0.249 ± 0.014^{c}	0.125 ± 0.011^{cd}
PP (0:4)	1.101 ± 0.164^{bcd}	0.524 ± 0.052^{bcd}	0.299 ± 0.012^{ab}	0.329 ± 0.040^{abc}	0.171 ± 0.018^{bc}

Note: Values (mean±SD) in the same column with different lowercase superscripts (a, b, c, d, e, f) are significantly ($p<0.05$) different.

3.2.5. Cooking Properties

For cooking properties, burger patties were analyzed for cooking loss, cooking yield, and moisture retention. The lowest cooking loss was achieved by burger patties with an MBP ratio of 4, namely 14.22%. MBP-SP (4:0) was not significantly different from MBP-SP (3:1), and significantly different from MBP-SP (2:2), MBP-SP (1:3), and MBP-SP (0:4), which were 19.61%, 21.66%, and 27.59%, respectively. Meanwhile, MBP-PP (0:4) was not significantly different from MBP-PP (3:1) and MBP-PP (2:2), and significantly different from MBP-PP (1:3) and MBP-PP (0:4), at 23.71% and 26.09%, respectively.

The highest cooking yield was achieved by burger patties with an MBP ratio of 4, at 85.78%. MBP-SP (4:0) was not significantly different from MBP-SP (3:1), but was significantly different from MBP-SP (2:2), MBP-SP (1:3), and MBP-SP (0:4). MBP-PP (4:0) was not significantly different from MBP-PP (3:1) and MBP-PP (2:2), but was significantly different from MBP-PP (1:3) and MBP-PP (0:4).

The highest moisture retention was achieved by burger patties with an MBP ratio of 4, at 46.98%. Moisture retention decreased significantly with the addition of the SP ratio, as well as with the addition of PP. MBP-SP (3:1), MBP-SP (2:2), and MBP-SP (1:3) were not significantly different in moisture retention of burger patties. MBP-PP (2:2) was not significantly different from MBP-PP (1:3), and MBP-PP (1:3) was not significantly different from MBP-PP (0:4).

Based on the cooking qualities, it was evident that a considerably higher MBP ratio resulted in fewer cooking losses, greater cooking yields, and more moisture retention. This was feasible because the SPI and PPI employed in the production of protein isolate had been denatured, hence diminishing their functional characteristics (Shrestha et al., 2023). The inference was that it was preferable to make burger patties or meat substitutes using fresh plant protein. In addition, the usage of larger quantities of hydrocolloids must be investigated further to improve their cooking qualities.

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Figure 3 illustrates the cooking properties of mung bean protein-based burger patties, including the cooking loss, cooking yield, and moisture retention of burger patties.

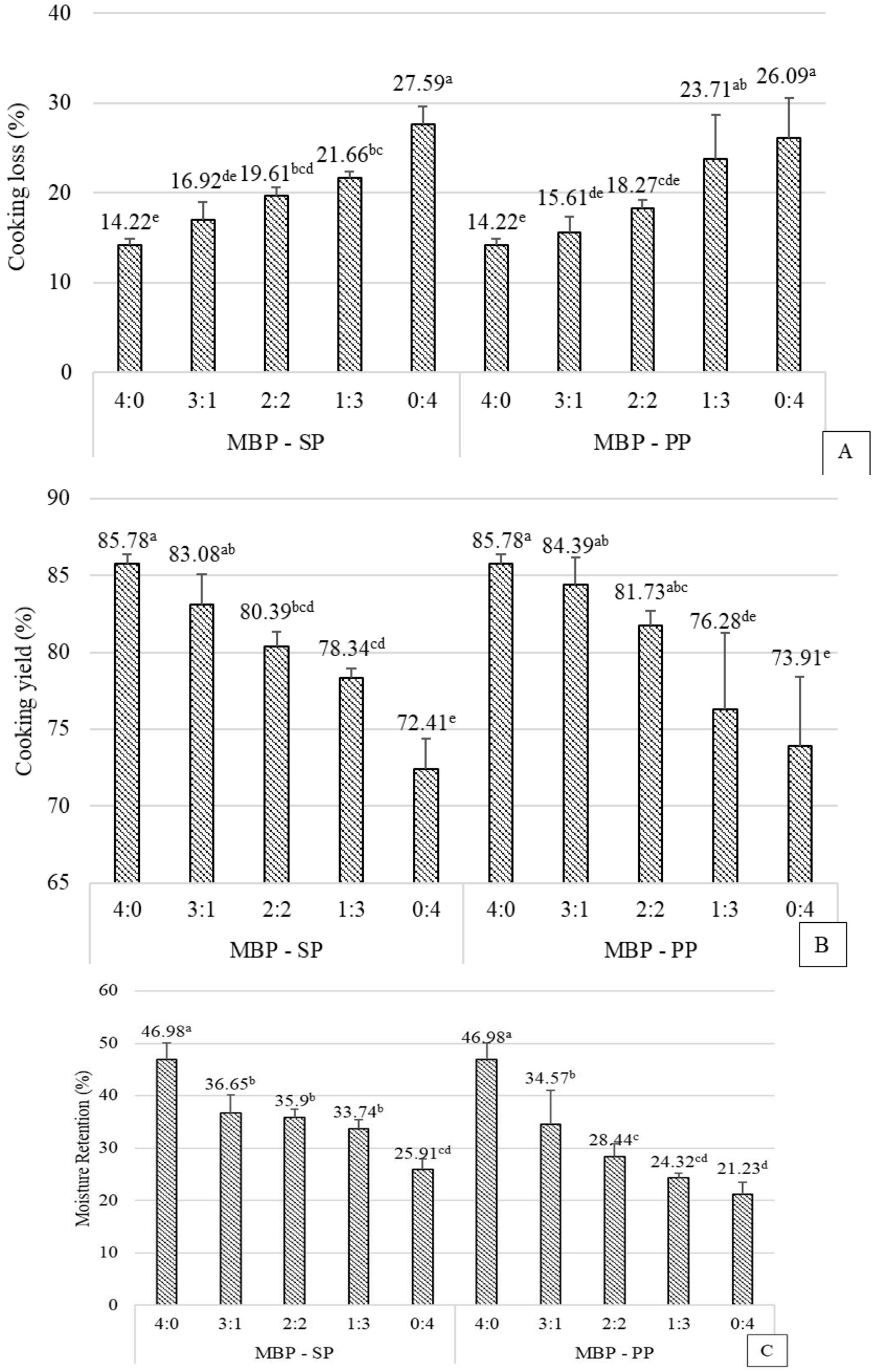

Figure 3. Cooking properties: [A] Cooking loss, [B] Cooking yield, and [C] Moisture retention of plant-based burger patties.

Note: Values (mean) in the same graph [A], [B], or [C] with different lowercase superscripts (a, b, c, d, e) are significantly ($p<0.05$) different.

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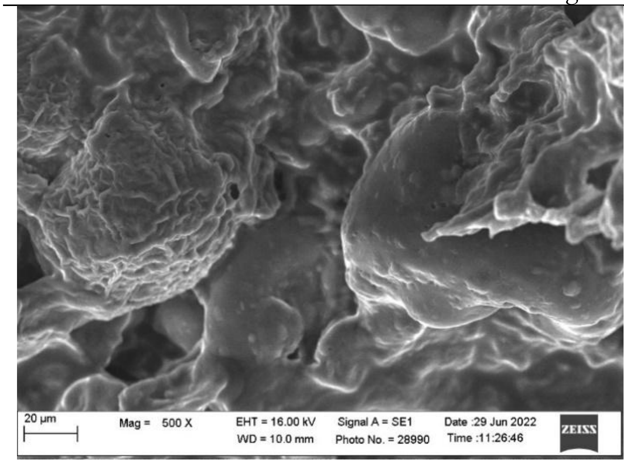
3.2.6. Microstructure

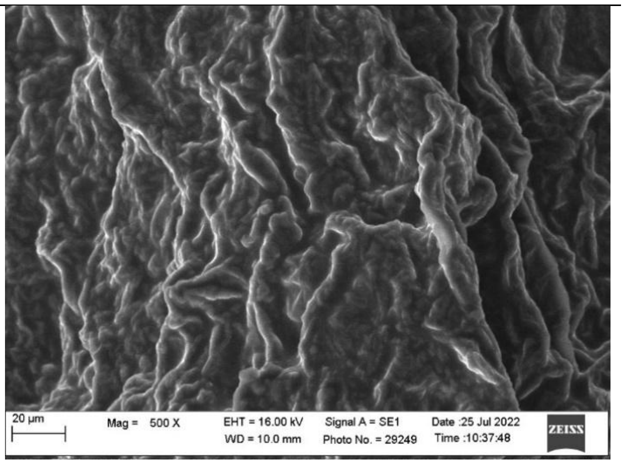
3.2.6.1. Scanning Electron Microscopy (SEM)

The visual characteristics of burger patties composed of MBP (ratio 4), MBP–SP (3:1), MBP–SP (2:2), MBP–SP (1:3), SP (ratio 4), MBP–PP (3:1), MBP–PP (2:2), MBP–PP (1:3), and PP (ratio 4) are illustrated in Figure 4, captured at magnifications of 500x. The surface appearance of the burger patties with MBP (ratio 4) is not significantly different from that of SP (ratio 4) and PP (ratio 4). The surface condition of the burger patties is likely influenced by the pressure exerted during the formation process in the circular mold. It is additionally affected by the steaming and cooling operations during manufacture. The surfaces of most patties remain uneven, lacking any discernible fibrous texture, except for the MBP-SP (2:2) and MBP-SP (1:3) formulations. The microstructure of the burger patties indicates that the binder effectively unites all the constituents; nonetheless, the protein and starch molecules remain in a globular configuration instead of a fibrous one.

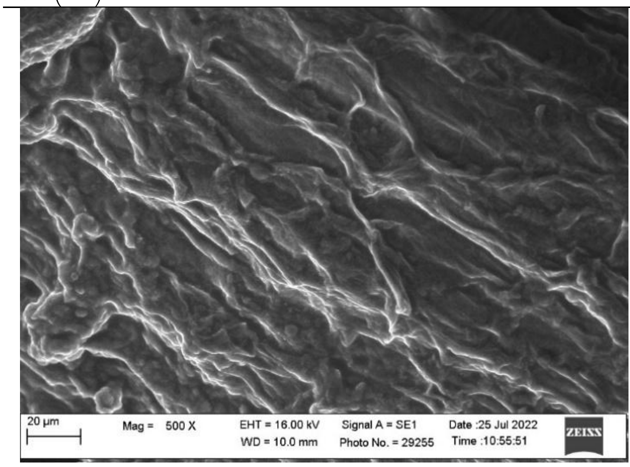
Magnification 500x



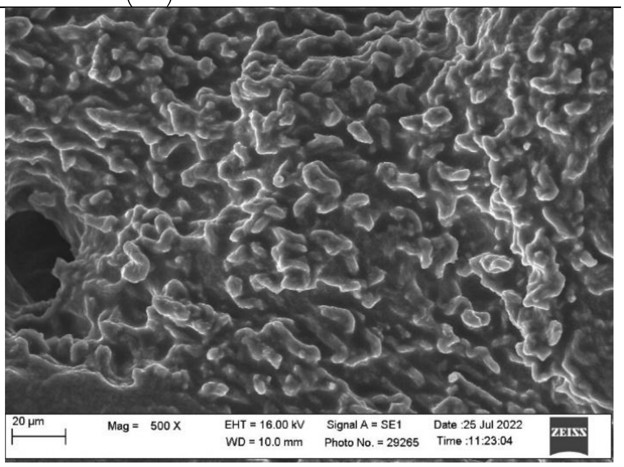
SP (0:4)



MBP – SP (1:3)



MBP – SP (2:2)



MBP – SP (3:1)

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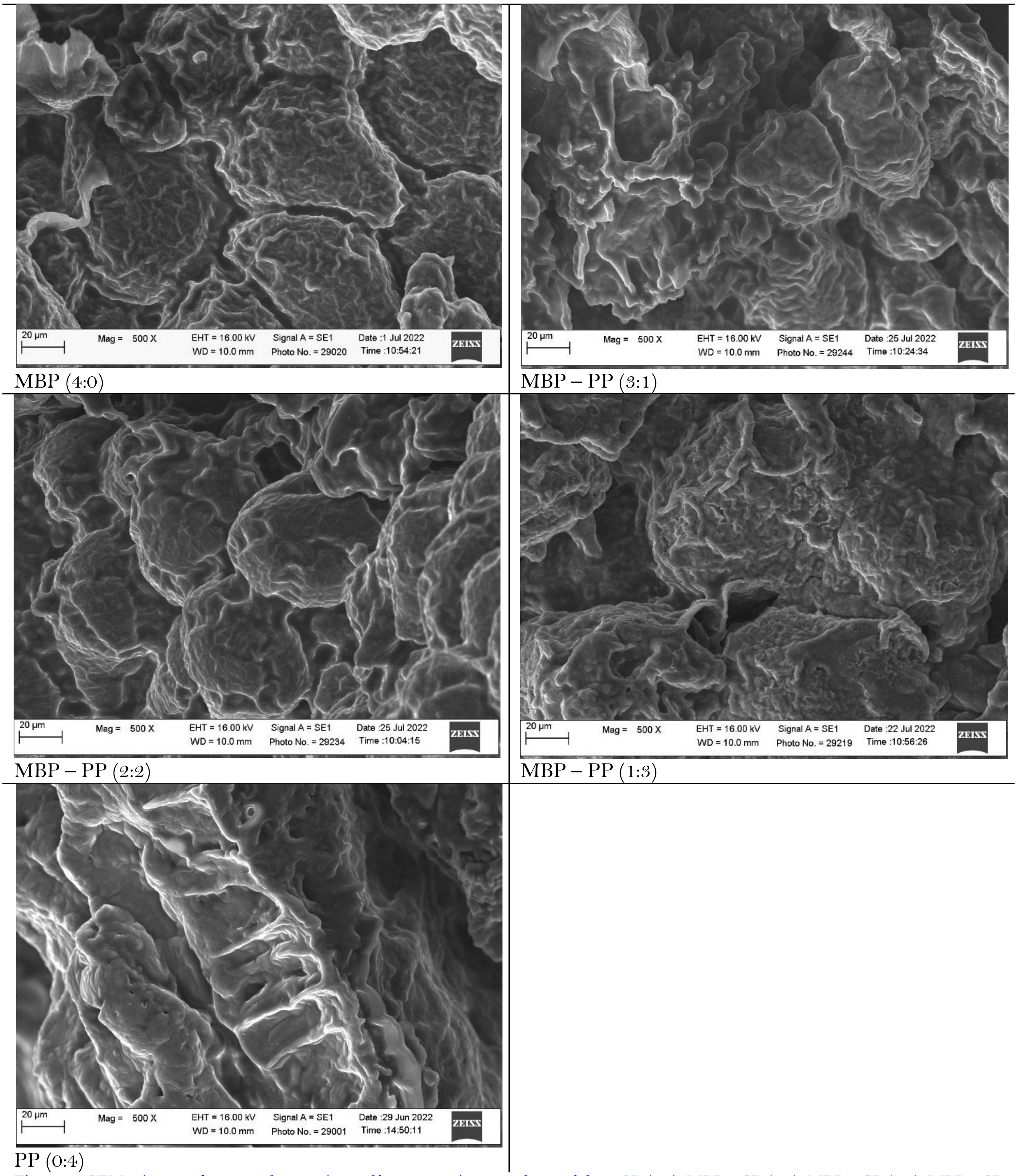

Figure 4. SEM micrographs on surface sections of burger patties manufactured from SP (0:4), MBP – SP (1:3), MBP – SP (2:2), MBP – SP (3:1), MBP (4:0), MBP – PP (3:1), MBP – PP (2:2), MBP – PP (1:3), and PP (0:4). (Scale bars: 500× magnification: 20 µm).

3.2.6.2. Confocal Laser Scanning Microscopy (CLSM)

CLSM was used to examine the microstructure and the distribution of proteins and polysaccharides in the plant-based burger patties. The results obtained with Rhodamine B and Calcofluor White fluorescence are shown in Figure 5. Burger patties with SP particles consist of proteins (orange-red stain) surrounded by polysaccharides (blue). Additionally, pink was also visible, which was a combination of orange-red and blue, representing the distribution of fat in the product. Burger patties with SP content showed a pink color, indicating that, apart from proteins and polysaccharides such as starch and hydrocolloids there was also oil present. For SP with a ratio of 1 to 4 levels, all exhibited a pink color, which was more intense than in burger patties with the MBP ratio scale of 4. The higher the

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level of the SP ratio, the greater the pink color intensity, which was associated with the presence of fat particles. This was possible because the burger patties contained fat from sunflower oil, coconut oil, and the oil contained within the SP itself. The oil content in soy was relatively high, ranging between 18-21% (Shrestha et al., 2023).

Burger patties with MBP level ratio of 4 consist of protein (represented in orange red), mixed with polysaccharides (blue), and fat (pink). Polysaccharides were made from tapioca starch, hydrocolloids, and fiber from the ingredients. Fat was from fat and oil ingredients. Burger patties with an MBP ratio of 4 showed a much darker color than burger patties with SP content. This showed that the distribution of fat on burger patties with MBP level 4 is much less.

While burger patties with PP content, the distribution of protein (orange red), polysaccharides (blue), and fat (pink) could be seen. The intensity of pink color was much higher in burger patties with a ratio of PP level of 4. The intensity of pink in burger patties with a ratio of PP 1 to 3 was much lower than in burger patties with SP content. It was related to the fat distribution in burger patties products.

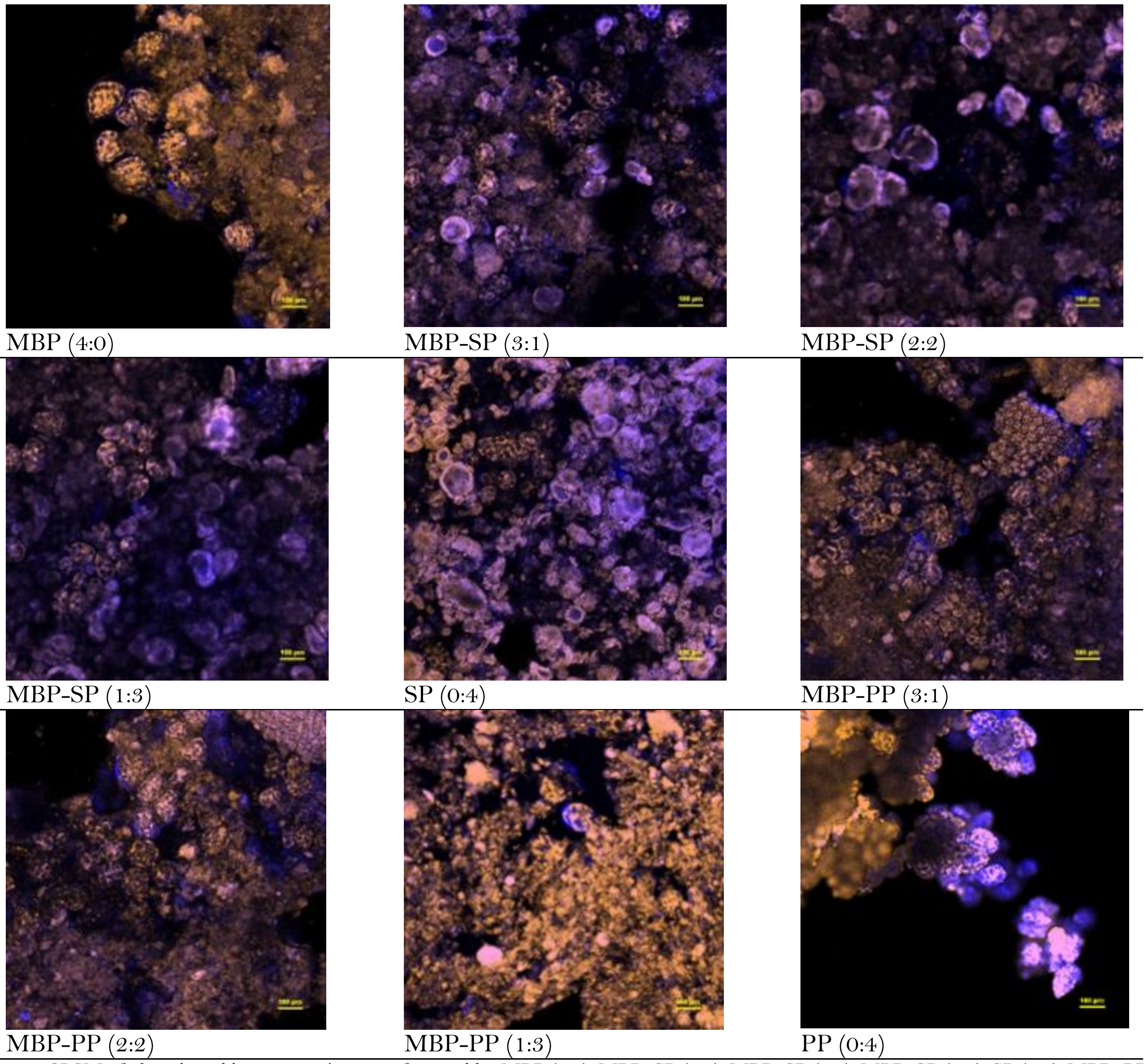

Figure 5. CLSM of plant-based burger patties manufactured by MBP (4:0), MBP–SP (3:1), MBP–SP (2:2), MBP–SP (1:3), SP (0:4), MBP–PP (3:1), MBP–PP (2:2), MBP–PP (1:3), and PP (0:4).

4. CONCLUSIONS

Both soy protein isolate and pea protein isolate can enhance the protein content of plant-based burger patties derived from mung bean protein. Nonetheless, both yielded a softer burger patty texture. The application of pea protein isolates exclusively on burger patties derived from mung bean protein resulted in superior coloration compared to the use of soy protein isolates. Both soy and pea protein isolates could enhance the Ca, Mg, and Zn content but decrease

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the Fe content in the patties. The increased addition of protein isolates resulted in greater cooking losses of the burger patties. The microstructure profile indicates that patties with soy protein isolate have a more consistent lipid distribution than those made with pea protein isolate.

The protein and mineral composition, texture, color, and cooking properties indicate that the MBP–PP (3:1) and MBP–PP (2:2) formulations possess the potential for further enhancement into a formula with superior attributes. Additional research is required to improve the properties of plant-based burger patties concerning textures and to assess their bioavailability and effects on allergenicity.

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