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Structural and physicochemical properties of plant-based meat analogues from transglutaminase-modified soybean protein

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Abstract

Plant-based meat analogues (PBMA) have been developed for decades. The resemblance in PBMA to meat, in terms of physical properties and sensory attributes, is important for consumers. This study aims to investigate the effects of transglutaminase (TG) concentration (1, 3, 5% (w/w)) on modified textured vegetable soybean protein (TVP). The changes in TVP protein molecular weight, microstructure, texture profile, sensory quality and nutritional value of PBMA were investigated. The first set of analyses examined the impact of TG concentration on TVP structure and the results showed that the TVP protein band from SDS-PAGE were changed to a larger band at 36-40 kDa as compared to the control indicating the formation of a cross-linked protein. The modified TVP was used as a raw ingredient for PBMA development. The appearance and microstructure of PBMA from the TG-modified TVP showed that the uncooked PBMA with TG-modified TVP were not different from those of the control. With regard to the texture profile of PBMA, an increase in TG concentration resulted in an increase in hardness. A sensory evaluation revealed that the PBMA with 1%TG provided the highest overall acceptance score. Hence, the PBMA with 1%TG was selected for a nutritional analysis comparing steak to meat. The carbohydrate and fat content of PBMA with 1%TG were higher than those of meat while the protein content of PBMA with 1%TG consisted of a maximum of 70% meat. This research could lead to alternative plant-based foods in the food industry.

1. Introduction

Nowadays, the PBMA market is expected to generate a rising revenue. The PBMA from different protein sources were created by several food industries in order to supply customers. The benefits of PBMA are lower or zero cholesterol, and lower total and saturated fat than real meat. The development of a plant-based meat nutritional profile, organoleptic properties and stability have been continuously developed according to consumer preferences. Plant-based meat analogues entail an advanced understanding of the interactions between ingredients such as carbohydrates, fats and proteins and other minor compositions such as oils, vitamins, minerals, as well as coloring agent and flavor. The core of developing PBMA is protein. Historically, soybean protein (comprising 60-70%) or isolated (composed of at least 90% protein) is used as the predominant protein source for plant-based meat (Bohrer, 2019). Several research studies have been used to form comprehensive reviews on the positive, health-improving effects of soy protein consumption with improvement in lipid metabolism (Anderson *et al*., 1995; Xiao, 2008) and cardiovascular health (Sacks *et al*., 2006). The challenge of this development is the restructuring of globular proteins in isolated soybean protein: globular proteins consist of polypeptide chains that fold into tightly packed structures, which resemble golf balls. It requires chemical, mechanical, or biological processing to mimic the fibrous structure of animal proteins. During restructuring, globular proteins are denatured, unfolded, aligned, and crosslinked through interactions like hydrophobic effects, van der Waals, hydrogen bonding,

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electrostatic effects, and disulfide bonding (McClements and Grossmann, 2021). Various functional ingredients are also required to create and mimic the type of texture, appearance, flavor, and mouthfeel of animal protein-based products. The formation of meat-like fibrous structures entails intensive processing, for example, thermos-extrusion, shear, spinning, and cross-linking (Sha and Xiong, 2020).

A common biological cross-linking protein is transglutaminase (TG), which catalyzes the reaction between glutamine and lysine residues within and between soybean protein structures. TG (EC 2.3.2.13) has been used as a gelling agent for animal, soy, wheat, rice, pea, lupin, sunflower, and other proteins (Dube *et al*., 2006). As mentioned, TG can promote protein gelation, which affects the texture of the end product and can be added before or after mechanical processes such as shear cell texturization (Manski *et al*., 2007). A protein-enriched restructured beef steak, containing plant -based ingredients, pea protein isolate, rice protein and lentil flour can improve the TG binding strength of a fibrous structure (Baugreet, *et al*., 2018). There has also been a study which applied microbial TG to isolated soy protein to improve the protein structure (Yang and Zhang, 2019). The textural properties such as hardness and chewiness of the restructured isolated soy protein were increased when using TG.

The objective of this research is to improve the structural properties of texturized soybean protein (TVP) using different dosages of TG. TG treated with TVP was used as the main ingredient of PBMA in steak recipes. Hence, cross-linked protein structure, the details of PBMA internal structure, texture properties, sensory quality and nutritional values of PBMA steak were investigated. The results of this research provide an alternative direction for improving the textural properties of PBMA.

2. Materials and methods

2.1 Materials

The materials used in this research were textured soy protein from the Institute of Food Research and Product Development (IFPD), Kasetsart University, and transglutaminase was purchased from Biobond (Kinry Food Ingredients co., Ltd.).

2.2 Preparation of transglutaminase-modified soybean protein

The textured soybean protein was modified by TG type A and type B. The activity of TG was analyzed by using an assay kit from Sigma-Aldrich (CS1070 Spruce Street, St. Loui, MO). Textured soybean protein (100 g) was added to 400 g of distilled water, then pre-heated in a shaking water bath at 45°C for 30 mins. 1%, 3% and 5% (w/w) of TG were added to the substrate and incubated in a shaking water bath (160 rpm) at 45°C for 3 hrs. The reaction was terminated by heating in the boiling water bath for 10 mins. The TG-modified protein was dried by tray dryer at 60°C for 24 hrs. All samples were kept in a ziplock for further analysis.

2.3 SDS-PAGE

Samples were weighed (100 mg) and suspended in 1 mL of deionized water. The protein concentration was determined by the Bradford method and approximately 20 µg of the sample was analyzed for its molecular weight and the formation of a cross-linked structure by SDS-PAGE analysis.

2.4 Preparation of the meat analogues

Preparation of the main ingredients used textured soybean protein (Control, 1%, 3% and 5%), all-purpose wheat flour, water, seasonings, and beetroot powder (the recipe compositions were not shown). All ingredients were mixed and left at room temperature. The emulsion was prepared by using water, coconut oil and hydroxypropyl methylcellulose (the recipe composition was not shown). The main ingredients (with a TVP diameter of approximately 1-3 mm) and emulsion were mixed at a ratio 35:100 and the mixture was shaped into a flat sphere. The meat analogues were steamed for 10 mins and frozen for 1 hr. The grilled meat analogue was prepared by using olive oil. The finished products were kept in a fridge for further analysis.

2.5 Confocal laser scanning microscopy

The internal structure of plant-based meat was studied by confocal laser scanning microscopy (CLSM) following the methods of Sorndech and Lekhavat (2021) with slight modifications. The dyes which were used in this study were Nile blue A, fluorescein isothiocyanate isomer (FITC) and rhodamine B-isothiocyanate isomer (RITC). Nile blue A was prepared in deionized water at 0.001% (w/v), while FITC was prepared in acetone at 0.001% (w/v) and RITC was prepared in methanol at 0.001% (w/v). The raw plant-based meat analogues (2 g) were weighed then 200 µL of nile blue A was added and incubated for 2 mins. The FITC and RITC were further added to the meat analogues using the same procedure as for nile blue A. The samples were kept in the refrigerator at 5°C for 2 days. The sample was gently spread on a slide and the internal structure was observed using CLSM and the magnification of the objective lens was 10× (Sorndech and Lekhavat, 2021).

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2.6 Texture profile analysis

The sample size 2.5 × 2.5 cm was prepared and the texture profile was measured using a texture analyzer (LLOYD Instruments, TA plus Ametek, UK) and cylinder probes sized 45 mm. The parameters consisted of hardness, cohesiveness, adhesiveness, and springiness.

2.7 Sensory evaluation

For the sensory test perception of consumers, an experiment was planned by assessing acceptability in terms of appearance, color, odor, bitterness, juiciness, texture, and overall preference using a 9-point hedonic scale method. Thirty untrained panellists, who declared themselves regular consumers of plant-based food were asked to rate the samples on the basis of a 9-point hedonic scale. The results were statistically analyzed with the SPSS statistics program (SPSS, Inc., Chicago, IL) and the differences in the mean scores were then compared at a confidence level of 95%.

2.8 Nutritional values

2.8.1 Fat content

The ground sample was weighed (3 g) into a thimble and dried at 105°C for 5 hrs. After drying, the samples were removed from the oven and placed in a desiccator. The fat extraction was carried out by using a Soxhlet extraction. The petroleum ether (250 mL) was placed into a bottle with a heating mantle and it was heated for 14 hrs. The solvent was evaporated by using a vacuum condenser. The samples were removed from the extractor after extraction and placed in an 80-90°C gravity convection oven and dried for 30 mins. The samples were then cooled in a desiccator and weighed. The fat content was calculated according to Association of Official Analytical Collaboration (AOAC) International (2000) as follows (AOAC International, 2000).

$$\text{Fat Content (\%)} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$$

2.8.2 Protein content

The ground sample was weighed (1 g) in a digestion flask, 5 g of Kjedahl catalyst and 200 mL of concentrated H_2SO_4 were added to the sample. The blank was prepared by using only the Kjedahl catalyst and concentrated H_2SO_4. All blank and samples were gently heated and boiled until the solution cleared. The samples were cooled and distilled water (60 mL) was added to them. The flasks were immediately connected to the digestion bulb on a condenser and 5-7 drops of mix indicator was added to the samples then heated until all the NH_3 was distilled. The distilled acid was titrated with a standard NaOH solution. The protein content was calculated according to AOAC International (2000) as follows:

$$\text{Protein content (\%)} = \frac{(A - B) \times N \times 14.007 \times 6.25}{W}$$

Where A = volume (mL) of 0.2 N HCl used for sample titration, B = volume (mL) of 0.2 N HCl used for blank titration, N = normality of HCl, W = weight (g) of sample, 14.007 = atomic weight of nitrogen and 6.25 = protein-nitrogen conversion factor

2.8.3 Carbohydrate content

The colorimetric method for carbohydrate determination described by AOAC International (2000) was used to determine the content of the carbohydrates. Two milliliters of sample were mixed with 1 mL of 5% aqueous solution of phenol in a test tube. Subsequently, 5 mL of concentrated H_2SO_4 were added rapidly to the mixture. The test tubes were allowed to stand for 10 mins and vortexed for 30 s and then placed for 20 mins in a water bath at room temperature for color development. The light absorption at 490 nm was recorded on a spectrophotometer. Reference glucose solutions were prepared in an identical manner as above. The UV was programmed to produce a calibration curve with the following concentrations: 10, 20, 30, 40, 50, 60 and 70 mg/L. The sample was measured in triplicate.

2.9 Statistical analysis

The experiment used a completely randomized design (CRD), and all data were statistically analyzed using Duncan's multiple range tests (DMRT) which were performed at $p \leq 0.05$ by SPSS (SPSS, Inc., USA).

3. Results and discussion

3.1 SDS-PAGE of transglutaminase-modified textured vegetable soybean protein

The molecular weights of the proteins from control and TG-modified TVP at 1%, 3% and 5%TG are shown in Figure 1. The protein concentrations of control, 1%, 3% and 5%TG were 22, 22, 22 and 18 µg, respectively, and the samples were run by using 15% polyacrylamide gel electrophoresis in a tricine buffer system. In the case of control, the results showed that the protein band displayed high intensity at 36 and 23 kDa while the 1% and 3% TG treated samples showed high intensities at 40, 36 and 23 kDa. These results suggest that the TG could form a lysine-glutamine cross-linked structure resulting in the formation of a 40 kDa protein band. However, for the 5% TG-treated samples, the protein band displayed a high intensity at 23 kDa while the other bands disappeared. This could imply that a high degree of cross-linking protein forms insoluble aggregates when using a high concentration of TG. For the TG-treated samples, an increase in the band intensity on the top of

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the separating gels in each lane (lanes 2-4) corresponded to the formation of insoluble cross-linked protein resulting in a decrease in band intensity especially in lane 4. These results reflect those of Marco *et al*. (2008) who studied the effects of TG on protein electrophoretic patterns of rice, soybean, and rice-soybean blends. The results found that soybean proteins interact with rice proteins, yielding protein aggregates of high molecular weight.

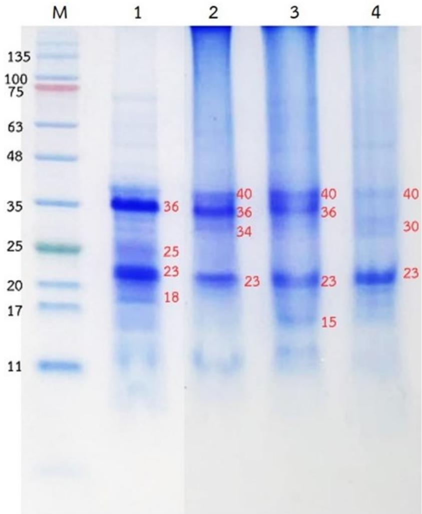

Figure 1. SDS-PAGE analysis of proteins from control sample and TG treated TVP. Lane M: protein molecular weight marker; lane 1: Control, lane 2-4: 1% TG, 3% TG, and 5% TG, respectively.

3.2 Visible appearance and internal structure of plant-based meat analogues steak

The appearance and internal structure of uncooked plant-based steak are shown in Figure 2. To observe the internal structure of the PBMA, it was cut vertically. All the samples (Figures 2-A1, B1, C1 and D1) displayed a light brown color. The aggregation of the ingredients revealed small to wide pores, especially in the aggregated proteins. The internal structure was determined by CLSM, and generally, the green area represented the color of the FITC-protein complex while the red area represented the color of the RITC-fat complex and the blue area represented the color of the nile blue A-carbohydrate complex. In the case of control (Figure 2-A2), the CLSM displayed a blue-green structure which represented the high intensity of the carbohydrate and protein structure (Figure 2-A3, A4). However, there were no differences among the 1% (Figure 2-B2), 3% (Figure 2-C2) and 5% (Figure 2-D2) of the TG-treated samples. A possible explanation for this might be that there was no effect of TG concentration on the internal structure of the plant-based steak. The RITC-fat complex of 3% (Figure 2-B5) TG-treated samples exhibited a high intensity of red indicating a high interaction among fats (emulsion), proteins and carbohydrates. This finding is consistent with that of Dreher *et al*. (2020) who studied the effects of plant-based emulsified and cross-linked fat crystal networks on the formation of analogues of animal fat tissue. The data from the CLSM indicated that solid fat concentrations resulted in some structural differences between samples.

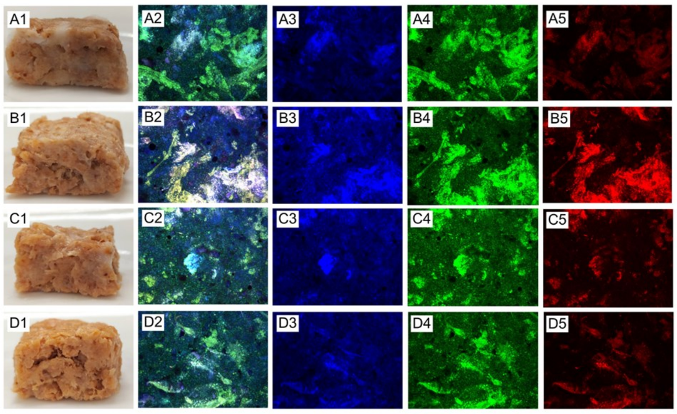

Figure 2. Appearance and internal structure of plant-based meat analogues (PBMA) steak by CLSM. A = Control, B = 1%TG, C = 3%TG and D = 5%TG. Cross section of uncooked PBMA steak (1) and TG treated textured soy protein observed by CLSM (2-5) with a scale bar of 100 µm.

3.3 Texture properties of plant-based meat analogues steak

The texture profile analysis of PBMA steak derived from different enzymes of 1%, 3% and 5%TG were determined. Adhesiveness, hardness, cohesiveness and springiness were measured. Figure 3 illustrates the texture profile and the results show that the hardness in PBMA steak increased with an increase in the percentage of TG concentration. However, adhesiveness, cohesiveness and springiness were not affected. These factors may explain the relatively good correlation between TG concentration and hardness. Normally, plant -based steak is mainly composed of soy protein. An increase in TG concentration could increase TG activity by the formation of a cross-linking reaction between molecules and within proteins resulting in an increase in hardness. In accordance with the present results, previous studies have demonstrated that PBMA prepared

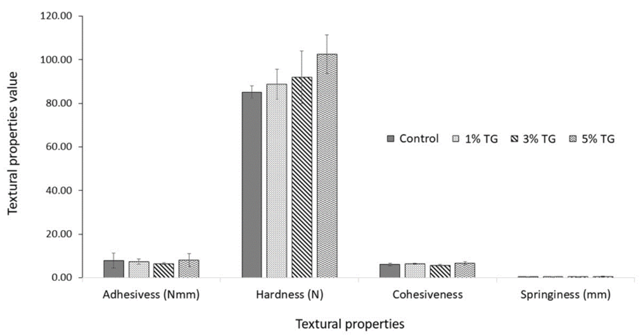

Figure 3. Texture profile of plant-based meat analogues (PBMA) steak.

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from soy protein showed a greater hardness than other proteins (Tunnarut *et al*., 2022). Moreover, this finding is consistent with that of Forghani *et al*. (2017) who studied the effects of microbial TG on physicochemical properties, electrophoretic patterns and sensory attributes of veggie burgers. The results showed that the addition of microbial TG and sodium-caseinate positively affected shrinkage and also increased the hardness of the veggie burgers. Hence, TG can improve the hardness of PBMA by forming a fibrous structure.

3.4 Sensory quality of plant-based meat analogues steak

After modification of the TVPs with different TG concentrations (1%, 3% and 5% w/w), the modified TVPs were used as an ingredient and then cooked to form the PBMA. The sensory evaluation scores on a 9-point hedonic scale of PBMA steak are shown in Table 1. It was found that the highest overall preference score was for the PBMA with 1%TG (5.90) and the acceptance per cent was 66.67%. Additionally, the texture, odor and juiciness scores for PBMA from TG-treated TVP were higher than that of the control. Thus, PBMA with 1%TG was selected for further nutritional analysis. These results corroborate the findings of a great deal of the previous work of Kim *et al*. (2022) who reported that the addition of TG could increase the sensory score of hardness, elasticity, compactness, and juiciness. Moreover, in accordance with the present results, previous review articles have demonstrated that the appearance and taste of PBMA were the main drivers of seduction to consume meat substitutes, regardless of the origin of the consumer's country and recipe (Weinrich, 2019). However, the previous findings suggest that sensory evaluation combined with instrumental measures, such as electronic nose, electronic tongue, texture profile and color value, can be advantageous and support to improvement the final PBMA product.

3.5 Nutritional values of plant-based meat analogues steak

The nutritional values of different types of steak composed of meat, PBMA control and PBMA with 1% TG are shown in Table 2. It can be seen from the data that the carbohydrates and the fat content of PBMA with 1%TG were higher than meat while the protein content of PBMA with 1%TG was 15.4 g/100 g which is equal to 70% of meat. This finding is consistent with that of Romão *et al*. (2023) who reviewed the nutritional compositions of meat substitutes commercialized worldwide and the results showed that in meat substitutes, the proportion of carbohydrates is higher than the protein concentration. According to the previous study of van Vliet *et al*. (2021) who reported a metabolomics comparison of plant-based meat and grass-fed meat, the results showed that nutrients such as amino acids, phenols, vitamins, unsaturated fatty acids, and dipeptides were absent in the plant-based meat alternative when compared to beef. Moreover, a cross-sectional survey of 207 PBM and 226 meat products available in the UK showed that as compared to meat, PBMA had significantly lower calorie, total fats content, saturated fats content, protein content, and significantly higher fiber and salt content (Alessandrini *et al*., 2021). These data led to the development of novel PBMA recipes which matched the protein content of meat. Hence, the fortified ingredients such as essential amino acids, vitamins and minerals which are found in red meat (for example, vitamins B12, zinc, and iron) are provided

Table 2. Nutritional values of difference types of steak.

Compositions	Types of steak		
	Meat	TVP control	1%TG treated TVP
Total carbohydrates (kcal/100 g)	0.01	27.52	25.25
Total proteins (g/100 g)	21.61	15.30	15.40
Total fats (kcal/100 g)	10.96	12.14	14.20

Table 1. Sensory quality of plant-based meat analogues (PBMA) steak.

Attributes	PBMA steak formulas			
	Control	1%TG	3%TG	5%TG
Appearance	6.47 ± 0.00^{B}	6.07 ± 1.14^{A}	5.97 ± 1.16^{A}	6.10 ± 1.18^{A}
Color	6.10 ± 1.04^{A}	6.13 ± 0.94^{A}	6.03 ± 1.13^{A}	$6.07\pm.1.20^{A}$
Texture	5.40 ± 1.35^{A}	5.50 ± 1.53^{A}	5.70 ± 1.17^{A}	5.47 ± 1.47^{A}
Odor	5.34 ± 1.19^{A}	5.97 ± 1.45^{B}	5.47 ± 1.13^{AB}	5.73 ± 1.44^{AB}
Juiciness	4.97 ± 1.47^{A}	5.23 ± 1.59^{A}	5.80 ± 1.22^{A}	5.30 ± 1.53^{A}
Bitterness	5.80 ± 1.24^{A}	5.83 ± 1.46^{A}	4.97 ± 1.20^{A}	5.83 ± 1.34^{A}
Overall preference	5.40 ± 1.13^{A}	5.90 ± 1.49^{B}	5.50 ± 1.11^{AB}	5.50 ± 1.38^{AB}
Acceptance	60.00%	66.67%	60.00%	66.67%

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p\leq0.05$).

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for a more direct nutritional adjustment in PBMA. The PBMA steak products in this study were designed to accurately mimic the desirable characteristics of the original animal-based versions.

4. Conclusion

The PBMA steak in the present study was designed to accurately mimic the desirable characteristics of the original animal-based steak. The commercial TG (1%, 3% and 5% w/w) was used to modify TVP structure and the SDS-PAGE showed that the TG-treated TVP increased protein band intensity at 40, 36 and 23 kDa indicating the formation of a cross-linked protein structure. The visible appearance displayed a light brown color and the internal structure of PBMA steak showed the aggregation of the ingredients and generated some pores between the aggregated ingredients. The internal structure which was determined by the CLSM images indicated that there were no differences in the internal structure among the 1%, 3% and 5% TG-treated samples. The texture profile analysis of the PBMA steak showed that the hardness in PBMA steak increased with an increase in the percentage of TG concentration and using 5% TG resulted in the greatest hardness. With regard to sensory quality, it was found that the highest overall preference score was the PBMA with 1% TG (5.90 out of 9) and the acceptance per cent was 66.67% and this sample was selected for the nutritional evaluation. Additionally, the texture, odor and juiciness scores of PBMA from TG-treated TVP were higher than those of the control. The protein content of PBMA with 1% TG was 15.4 g/100 g which is equal to 70% of the original animal-based steak. This study could lead to the development of PBMA recipe ideas and opportunities for the food industry in alternative plant-based foods.

Conflict of interest

The authors declare no conflict of interest.

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