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Textural evaluation of sausages as affected by partial meat substitution using okara tempe

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Abstract — Okara tempe has been traditionally consumed as low-cost meat substitute. This study aims to evaluate the textural properties of sausage as affected by partial meat substitution using okara tempe. Two types of sausages were tested, namely cellulose-cased and collagen-cased sausages with the level of meat substitution at 0, 10, and 20 %. The scope of assessment includes texture profile analysis, scanning electron microscopy, and sensory evaluation. The results showed that increasing okara tempe concentration led to a decreasing hardness and resilience of sausage samples. Microscopy images revealed fractal structure on the inner surface of okara tempe-substituted sausages that might be responsible for weakening of the structure. Consumers' appreciation on sausage texture tended to be decreased with the increasing concentration of okara tempe. Agglomerative hierarchical clustering analysis distributed the consumer panels into two clusters with slightly different hedonic scoring pattern. Lastly, although the okara tempe-substituted sausages had a weaker structure, the means of hedonic scores of all sausage samples were within positive response range indicating consumers' acceptance to the okara tempe-substituted sausages.

***Keywords* — sausage, meat, tempe, okara, soybean, texture.**

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INTRODUCTION

Consumers are getting more conscious on the environmental impact of what they eat. It has been reflected from a growing popularity of plant-based foods, particularly the one rich in protein. Food proteins from plants are considered more environmentally friendly due to lower emission of greenhouse gases (Day, 2013). Dietary shifting to plant-based foods and reducing consumption of animal-sourced foods have been campaigned wide and becoming a global trend (Wickramasinghe, et al., 2021). This circumstance has driven many researchers to explore alternative food proteins harvested from various plant sources, including valorisation of agricultural by products (Sozer, et al., 2017).

One of the widely generated by-products in Asian countries is known as okara, i.e. the soybean curd residue (SCR) from tofu production. Although considered as waste, nutritional quality of okara is suitable for human consumption. Dry okara are rich in fibre and protein, at around 40-60% and 15-30% respectively depending on the soybean cultivar and soymilk processing method. Valorisation of okara as food ingredients with satisfying sensorial properties have been the topic of numerous research for decades. In recent years, studies on okara fermentation have been sought as a promising means to improve its nutritional and sensorial quality for food ingredient application (Feng, et al., 2021, Vong and Liu, 2016).

Fermented okara has been part of traditional cuisines in several Asian cultures, including Indonesia. The okara can be converted into tempe through solid state fungal fermentation by using *Rhizopus oligosporus*. Okara tempe can be easily distinguished from conventional soybean tempe by its irregular particulate appearance and softer texture. The fermentation is reported to improve nutritional quality of okara by increasing the amount of soluble fibre and free amino acids (Vong, et al., 2018).

Okara tempe has been traditionally consumed as low-cost meat substitute in the form of skewers (Wardani, et al., 2018). Up to the authors' knowledge, there are extremely limited study reporting the utilisation of okara tempe as ingredient in meat product. Therefore, the current study aims to evaluate the effect of incorporating okara tempe in sausage formulation as meat substitute. The evaluation is focusing on textural properties, noting its importance in characteristics of meat product. This study is expected to provide insight on possibility to develop okara tempe as low-cost ingredient for both meat processors and meat-alternative industries.

MATERIALS AND METHODS

A. Materials

Chicken breast meat, okara tempe, sausage casings, and all other ingredients were obtained from local suppliers. Dry ingredients used in this study were soy protein isolate

(Marksoy 90, PT. Markaindo Selaras, Bogor, Indonesia), table salt (Dolpin, PT. Susanti Megah, Surabaya, Indonesia), garlic and onion powders (Jay's Kitchen, PT. Hoka Jaya Internasional, Sidoarjo, Indonesia), frankfurter premix seasoning powder (Wiberg®, FRUTAROM Savory Solutions Austria GmbH, Salzburg, Austria), and polyphosphate blend (Phosmix, Reephos Food Ingredients Co. Ltd., Jiangsu, China). The vegetable fat used was FILMA shortening (PT. Sinar Mas Agro Resources and Technology, Jakarta, Indonesia). The meat and okara tempe were purchased within two days prior to sausage production and kept frozen. Two different types of sausage casings with 26 mm diameter size were also used in this study, i.e. inedible cellulose (ViskoTeepak N.V., Lommel, Belgium) and edible collagen casings (Devro Pty. Ltd., New South Wales, Australia).

B. *Preparation of sausage*

Basic formulation of control sample consisted of 50% (w/w) meat, 10% (w/w) fat, 25% (w/w) ice cubes, 10% (w/w) soy protein isolate, and 5% (w/w) other dry ingredients. The okara tempe was used in the formulation of treated sample to substitute 10% and 20% of meat. Our preliminary study showed that meat substitution at higher than 20% were unable to exhibit an acceptable sausage texture. Description of control and treated samples are shown in Table 1.

Table 1. Sample codes and descriptions

Sample Code	Sample Description	
	Casing Type	Meat substitution by okara tempe (%)
CEL 0	Cellulose	0
CEL 10	Cellulose	10
CEL 20	Cellulose	20
COL 0	Collagen	0
COL 10	Collagen	10
COL 20	Collagen	20

Chicken meat and okara tempe were thawed in refrigerator for few hours prior to sausage production. Sausages were prepared by grinding the meat, okara tempe, ice cubes, and salt in a Mitochiba CH-200 food chopper (Mito Electronic, Indonesia). Subsequently, dry ingredients and vegetable shortening were blended into meat batter. The batter then stuffed into sausage casings by using manual sausage stuffer. The sausages were cooked in water at 60 ºC for 30 min then followed by further heating at 90 ºC for 15 min. The cooked sausages were immersed in cold water for 2 hours and immediately stored at refrigerator prior to analysis.

C. *Texture profile analysis*

Texture profile analysis was conducted by using the Brookfield CT3-100 Texture Analyzer with 4500 g load cell. Inedible casing was firstly removed from the samples prior to testing, whereas the edible casing was left intact. The samples were cut into 2 cm height cylinder shape and placed vertically on the middle of platform. The samples were subjected to two uniaxial compression cycles until reaching 75% of its original height at the speed of 1 mm/s using a TA4/1000 probe. Measurements of each sample type were repeated at least three times. The obtained texture profile analysis curves were then extracted into several parameters, namely hardness, springiness, and resilience. Hardness was obtained from the peak force resulting from the first compression. Both springiness and resilience are closely associated to the elastic textural properties but differ in approach. Springiness is a ratio of recovered sample's height that is measured when the second compression start, whereas resilience is a ratio of energy that is measured on the withdrawal of the first compression (Giongo et al., 2022, Gravelle et al., 2017, Nishinari, et al., 2019).

D. *Scanning electron microscopy*

Microstructure of sausage samples were evaluated using JEOL Scanning Electron Microscope (JSM-IT 200). Prior to scanning, the samples were sliced into 5 mm thick and subsequently dried via solvent exchange drying with ethanol. The dried samples were then coated with gold and scanned at accelerating voltage of 15 kV and around 7 mm working distance.

E. *Sensory evaluation*

Sensory evaluation was conducted as in a central location and involving 25 consumer panels. Each panel was served with a tray containing six pre-warmed sausage samples in cylindrical shape and 2 cm long. The panels were asked to record their hedonic response in a single paper ballot using 9-points hedonic scale (1 = strongly dislike and 9 = strongly like). The evaluated attribute was focus on texture of samples.

F. *Statistical analysis*

All data obtained from texture profile analysis and sensory evaluation were subjected to analysis of variance (ANOVA) test at 95% confidence interval. Comparison of means was carried out by Tukey's post hoc test. Agglomerative Hierarchical Clustering analysis was also conducted to evaluate the responses of consumer panels in sensory evaluation. The XLSTAT software (Addinsoft Inc., New York, USA) was used for all statistical analysis purpose.

RESULTS AND DISCUSSIONS

Texture Profile

The textural profile of sausages as affected by partial meat substitution using okara tempe are shown in Figure 1. Generally, increasing okara tempe concentration has led to a decreasing hardness of sausages. Sausages without meat substitution exhibited the highest hardness values among each sausage type, i.e. cellulose-cased and collagen-cased. However, the latter type showed a harder texture due to the contribution of unremoved collagen casing on the outer surface of the sausage samples during the test. The use of 10% okara tempe for meat substitution slightly reduced the hardness of sausages. At this level of substitution, the collagen-cased sausage texture remained harder compared to the cellulose-cased sample. Subsequently, further substitution containing 20% okara tempe has significantly ($p < 0.05$) reduced the hardness of both cellulose and collagen-cased sausages. Noting that all samples were measured at the same size, the reduction in hardness can be considered as a less rigid sausage's internal structure. In this study, the major component of sausage physical structure is composed of the meat's myofibrillar protein. Substituting the meat with okara tempe has reduced the quantity of myofibrillar protein network, hence weakening the internal structure. This textural softening effect was not surprising, since our preliminary research showed that okara tempe texture was not as hard as the meat and getting softer when it was cooked (data not shown). Similarly, the hardness reduction effects have also been reported when substituting meat with other plant-based protein-rich materials (Hidayat et al., 2017, Kamani et al., 2019).

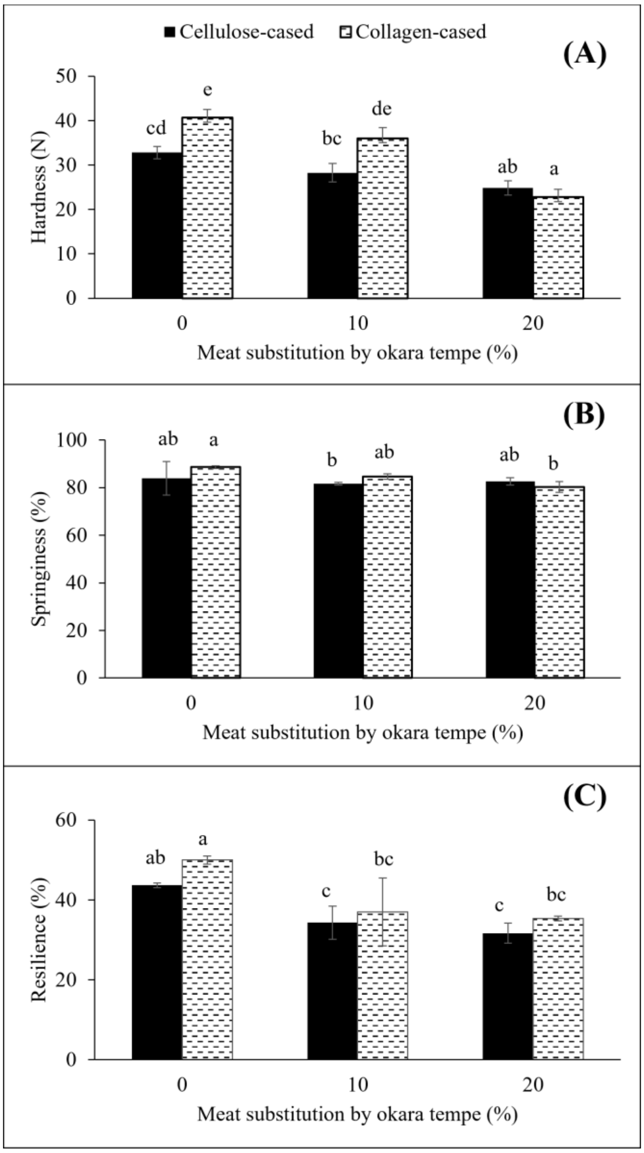

Figure 1. Hardness (A), springiness (B), and resilience (C) of sausage samples obtained from texture profiles analysis as affected by different okara tempe concentration for meat substitution. Different superscript letters indicate significant differences among samples ($p < 0.05$)

The instrumental evaluation of texture profiles has also provided the information on springiness and resilience of the sausage samples. Meat substitution by okara tempe did not show significant modification effects on springiness. In contrast, the resilience of sausage samples was evidently reduced with the increasing concentration of okara tempe. This finding led to an interpretation that the structural weakening has occurred. A lower hardness value indicated the softening of internal structure, whereas a lower resilience value implied that the internal structure was hardly recovered right after the first deformation. This study also suggested that when the textural profile analysis is conducted to evaluate sample's elasticity, it is important to look at both springiness and resilience parameters to avoid misleading interpretation. A previous report by Gravelle et al. (2017) showed that the use of hardness and resilience data was sufficient in providing insightful result from texture profile analysis of meat protein gels.

Microstructure

The obtained information on textural profiles can be further examined by the support of scanning electron microscopy images as shown in Figure 2. Comparison of sausage's inner surface are visualised at 100 times magnification. Noticeable differences on surface microstructure are clearly observed between zero and 20% substituted sausages in both cellulose and collagen-cased types. Sausages without okara tempe substitution exhibited a more even and solid surface of myofibrillar protein matrices, with the presence of negligible small pores. However, the magnified surface image of okara tempe-substituted sausages revealed fractal structures at several micrometres long surrounding and between the small pores. The observable fractures provided useful evidence that the substituted sausages have discontinuity and less rigid internal structure, hence it deforms easily at lower forces. This fracture may also indicate a weak interaction between okara tempe and myofibrillar protein matrices that might be contributed by large amount of okara tempe insoluble fibre. Gravelle et. al. (2017) suggested that the integration between meat protein matrix and insoluble fibre materials requires a comparable small size of protein capillary network and fibre particles. In this study, the fresh okara tempe was ground directly in the meat processor resulting in coarse particulates. Therefore, it might be the reason that okara tempe loosely attached to the meat protein network and creating fracture.

Sensory Evaluation

In addition to instrumental evaluation, sensory analysis of sausages was also conducted to obtain information on how consumers perceive the textural changes. Table 2 shows hedonic scores of all sausage samples. The calculated means of hedonic scores show that sausages without okara tempe substitution are significantly more favoured by consumers than the substituted sausages. However, consumers' appreciations on sausage samples are varied as can be seen from the minimum and maximum hedonic scores given.

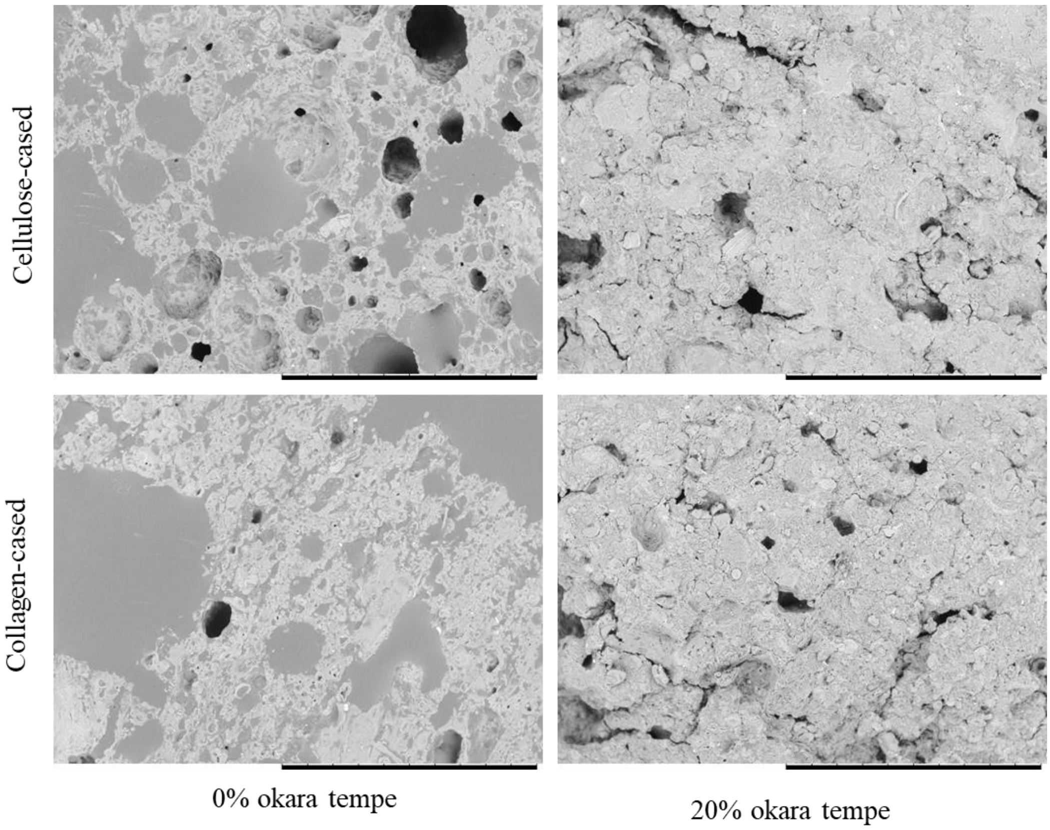

Figure 2. Scanning electron microscopy images of sausage's inner section with and without meat substitution by okara tempe. The captured image was at 100x magnification. The black-colored scale bar at the right bottom corner indicates 1 mm

Table 2. Hedonic scores on sausage textural attributes

Samples	Minimum Score	Maximum Score	Mean Score
CEL 0	4	9	7.24 ± 1.23^{ab}
CEL 10	5	8	6.56 ± 1.12^{bc}
CEL 20	2	9	6.16 ± 1.99^{bc}
COL 0	6	9	8.04 ± 0.94^{a}
COL 10	4	9	6.64 ± 1.22^{bc}
COL 20	4	9	6.04 ± 1.62^{c}

Different superscript letters indicate significant differences among samples ($p < 0.05$)

Table 3. Cluster characteristics of consumer panels derived from sensory evaluation data

Clusters of Consumer Panels	Centroids of hedonic score					
	CEL 0	CEL 10	CEL 20	COL 0	COL 10	COL 20
1 (n=13)	7.92	6.54	7.65	7.85	6.46	6.23
2 (n=12)	6.50	6.58	4.58	8.25	6.83	5.83

Sensory evaluation data were further analysed to provide more insights on hedonic response interpretation. Agglomerative hierarchical clustering has successfully distributed consumer panels into two clusters, as shown in Figure 3. Each cluster was nearly equal in number. Characteristics of each cluster can be observed in Table 3, showing the hedonic scoring pattern to all tested samples.

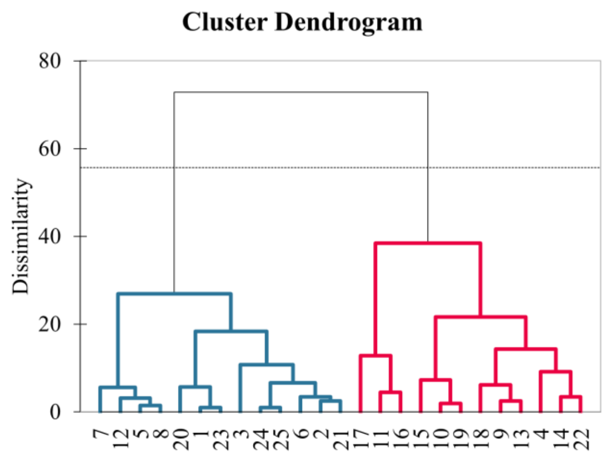

Figure 3. Cluster dendogram of consumer panels

The first cluster tended to provide a narrower range of hedonic scores. This group of consumer panels was highly appreciative to the tested samples. However, consumer panels of the second cluster seem to score in a wider range. These panels did not tolerate the undesirable textural change in sausage samples caused by okara tempe substitution. It is reflected from the score differences between samples without substitution and the samples with increasing okara tempe concentration.

CONCLUSIONS

Textural evaluation of okara tempe-substituted sausages have been presented. A reduced hardness due to structural weakening and fractures is acknowledged. A more appropriate treatment is required to improve compatibility and integrity of okara tempe with meat protein network structure. This study provides an initial baseline for development of okara tempe as low-cost ingredient for meat product.

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