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RESEARCH ARTICLE

Effect of Substitution of Texturized Vegetable Protein Using Tempeh Towards Nutritional and Textural Quality of Plant-Based Nuggets

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A B S T R A C T

The development of Plant-based Meat Alternatives (PBMAs) has been focused on mimicking the textural, taste, appearance, aroma, and nutritional properties of real meat products. Texturized Vegetable Protein (TVP) has been widely used in food applications to develop plant-based meat products. However, the high energy cost to produce TVP leaves the price of TVP quite expensive. Therefore, this experiment aimed to see the effect of tempeh as an alternative to TVP on the nutritional and textural properties of plant-based nuggets (PBNs). In total, five PBN treatments were analyzed and compared to chicken nuggets for their nutritional and textural properties. The treatments were 1) C1: control (hung + ground TVP), 2) Hung TVP and tempeh (HT), 3) Ground TVP and tempeh (GT), 4) Tempeh only (TT), and 5) Hung TVP, ground TVP, and tempeh (HGT). From the results, all plant-based nugget treatments had significantly higher ($p<0.05$) protein and moisture content and lower fat content than commercial chicken nuggets. Whereas for textural properties, except C1 and HGT, all other PBN treatments had poorer hardness than commercial chicken nuggets. Overall, the addition or substitution of tempeh on hung and ground TVP treated PBN did not affect the protein, fat, and moisture content of PBNs, but significantly produced better nutritional properties than chicken nuggets. In terms of textural properties, the combination of Hung TVP, ground TVP, and tempeh provide the desirable textural properties as they can be comparable to chicken nuggets.

Keywords: ***Plant-based meat alternatives; Plant-based nuggets; Tempeh; TVP***

H I G H L I G H T S

- Changing TVP into tempeh increased protein and moisture content compared to actual chicken nuggets.
- Changing TVP into tempeh decreased fat and ash.
- Slight decrease of carbohydrate content in plant-based nuggets was observed although the difference was insignificant.
- Hardness value decreased if tempeh was substituted fully and/or partially with TVP.
- No difference in hardness value was observed if tempeh was processed with hung TVP and ground TVP.

INTRODUCTION

Currently, there is an increasing trend in adopting a flexitarian diet, as people are more aware of the health benefits and environmental impact of consuming plant-based products (Curtain & Grafenauer, 2019; Derbyshire, 2017). This event subsequently increases the consumption of plant-based meat products. Hence,

this resulted in a surge in market trends in plant-based meat alternatives (PBMAs), especially in the US, Canada, and Europe. As a result, it is predicted that the PBMA markets will reach 8.3 billion US dollars by 2025, including in Indonesia, as marked by the rising of foreign and local PBMA companies ("Green Butcher", 2021; "Plant-based Meat Market", 2020; Wan, 2018).

Several attempts have been made since the development of traditional PBMAs until the latest PBMA 2.0 generation to create PBMAs that mimic not only the texture but also the taste, appearance, nutritional value, and aroma of real meat products (He et al., 2020). Amid the vast development of PBMA 2.0 products, plant-based nugget is one of the most popular products among consumers. Plant-based chicken products and nuggets have been received well in the big restaurant chains such as KFC and A&W in some states in the USA, Canada, and the UK (Weston, 2019). It is also reported that the market for plant-based nuggets was the second-highest in Europe in 2019. However, introducing plant-based chicken alternatives into the market would be a challenge, as the cost of chicken is not as expensive as beef.

Matching the production cost of plant-based products as low as possible would be necessary to compete with chicken-based products in the market. Hence, soy-based textured vegetable protein (TVP) is commonly used for PBMAs development as it provides structural and textural properties that mimic the meat product, is relatively cheap, and is highly available in the global market (Riaz, 2011). However, despite its relatively low cost globally, the price of TVPs in Indonesia is considerably high, at Rp 71,000 per kg ("Texturized Soy Protein", n.d.). Therefore, considering the price of TVPs, an alternative ingredient may be essential to substitute or complement TVPs to reduce the production cost of plant-based nuggets.

Tempeh is an authentic Indonesian fermented food rich in protein similar to animal source proteins, such as chicken, beef, hamburger, and eggs (Shurtleff & Aoyagi, 1979). Many health benefits have been reported from tempeh consumption, such as healing digestion systems, reducing diarrhea incidence, increasing intestinal growth and proliferation, anti-oxidative properties, and reducing the risk of chronic degenerative diseases (Nout & Kiers, 2005). As of now, the utilization of tempeh towards plant-based meat products is still limited to certain products. For example, Vital *et al*. (2018) used tempeh to develop vegetarian burgers. However, the product was inferior in terms of texture and organoleptic properties when compared to meat burgers. Another experiment would be the addition of tempeh flour to sausage analogs, which is reported to successfully mimic the standard nutritional and textural properties of commercial meat sausages in Indonesia (Ambari *et al*., 2014). However, to the extent of the author's knowledge, there has not been much research covering the utilization of both TVP and tempeh in developing plant-based nuggets.

This study aimed to develop plant-based nuggets that closely mimic chicken nuggets in terms of nutritional and textural properties. The formula and methods for plant-based nugget processing were taken from Kumar et al. (2011), with some modifications in the formulation. The variables that are changed in this formulation are chunk TVP, ground TVP, and tempeh. The results of this research could provide insight into the utilization of tempeh and TVPs in improving both the nutritional and textural properties of plant-based nuggets and other PBMAs. At the same time, the utilization of tempeh may also reduce the production cost of plant-based nuggets.

MATERIAL AND METHODS

Materials

The materials used for the PBN dough were chunk and ground TVP (Hung Yang Food), soy protein isolate (PT Markaindo Selaras), tempeh (Soyagreen), extra-firm tofu (Morinaga), oyster mushroom, potato starch (Windmill), cornflour (Bob's Red Mill), mushroom broth (Sunbay), nutritional yeast (Brag), palm oil (Bimoli), salt, black pepper, garlic powder, and onion powder. Breadcrumb (Mamasuka) and all-purpose flour (PT Bogasari Flour Mills) were used to make the batter, whereas chicken nugget (Belfoods Royal Chicken Nugget Drummies) was used for the control.

Experimental design

Five plant-based nugget formulations were developed as follows: 1) Control positive (C1): Chunk and ground TVP, 2) Chunk TVP and tempeh (CT), 3) Ground TVP and tempeh (GT), 4) Tempeh only (TT), and 5) Chunk TVP, ground TVP, and tempeh (CGT). As mentioned previously, the formula and method for developing the PBNs were taken from Kumar et al. (2011) with a few modifications for each treatment. The plant-based nuggets were produced in 3 batches, where the samples in every batch were analyzed triplicate for both nutritional and textural analyses. Commercial chicken nugget was used as a negative control (C0) to compare the nutritional and textural properties of each PBN treatment. Finally, the ratios of the ingredients in each treatment (Table 1) were calculated by dividing the weight of each ingredient by the total formula weight.

Table 1. Plant-based nugget formulations (% w/w)

Ingredients	C1 %	HT %	GT %	TT %	HGT %
Hung TVP	7.81	9.62	0.00	0.00	4.46
Ground TVP	9.37	0.00	10.00	0.00	5.35
Tempeh	0.00	11.54	8.34	22.92	9.82
Soy Protein Isolate	6.25	7.70	6.67	8.34	7.15
Corn Flour	2.34	2.88	2.50	3.12	2.68
Potato Starch	3.90	4.80	4.16	5.20	4.46
Hot water	3.90	4.80	4.16	5.20	4.46
Nutritional Yeast	2.34	2.88	2.50	3.12	2.68
Seasonings	1.56	1.92	1.66	2.08	1.78
Palm Oil	6.25	7.70	6.67	8.34	7.15
Extra Firm Tofu	15.63	19.24	16.67	20.84	17.86
Vegetable Broth	25.79	8.66	20.84	1.04	15.19
Oyster Mushroom	10.16	12.50	10.83	13.54	11.61
Vital Wheat Gluten	4.68	5.76	4.99	6.24	5.35
Total	**100**	**100**	**100**	**100**	**100**

C1: control positive (hung + ground TVP); HT: hung TVP + tempeh; GT: ground TVP + tempeh; TT: tempeh only; HGT: hung TVP + ground TVP + tempeh

Plant-based nuggets processing

Firstly, the tempeh was cut into smaller pieces and steamed for 15 minutes. Then, the chunk TVP and mushrooms were boiled using vegetable broth (1:10 w/v) for 20 minutes. Next, the ground TVP was soaked in a hot vegetable broth with a ratio of 1:2 (w/v). Any excess water was drained, and the ingredients were ground using a food processor for 15 seconds until they showed a fibrous texture. The extra firm tofu was pressed using heavy objects and let sit for 30 minutes to let the water drain uniformly. Before mixing all of the ingredients according to the treatments, the gelatinized potato starch was prepared by mixing the potato starch with 70°C of 5 mL of hot water. Then, all of the ingredients left were mixed using a food processor until they formed a dry and moist dough.

The dough was then hand-shaped into a small rectangle shape with a thickness of 1 cm. The nuggets were steamed for 20 minutes, cooled in room temperature, and coated. The coating was made by preparing the wet batter mix and the breadcrumbs. The wet batter was made by mixing 70 grams of water and 20 grams of all-purpose flour. The breadcrumbs were homogenized using a food processor for 30 seconds and filtered using 20 mesh size separators to obtain uniform shapes. Before frying, the nuggets were frozen at -18°C for 30 minutes. The frozen nuggets were then deep-fried in pre-heated palm oil at 150°C for 3 minutes until the color of the outer layer was golden brown. The nuggets were then cooled down to room temperature and removed from their excess oil.

Proximate analysis

The Kjeldahl digestion method was used to determine the protein content (Nielsen, 2017). The Soxhlet method was used to determine the lipid content (Nielsen, 2017). The AOAC Official Method of Analysis (AOAC, 2019) was used to determine the ash content (936.03). The moisture analysis was done using a rapid moisture analyzer (MB45 Moisture Analyzer OHAUS). Finally, the total carbohydrate content was analyzed using by difference method.

Textural analysis

Textural analysis was conducted using Texture Analyzer (Perten TVT 6700) with TexCalc5 as the software to conduct the analysis. The batter of chicken and plant-based nugget samples were removed and cut into 10 mm height using a knife, then placed onto the Texture Analyzer plate. The setting parameters of the Texture Analyzer were as follows: single cycle, 35% compression, initial speed 1 mm/s, test speed 5 mm/s, and retract speed 1 mm/s. The textural parameter taken was hardness. The analysis was conducted three times per sample in each batch.

Statistical analysis

Data were statistically analyzed by IBM SPSS version 20 using one-way ANOVA, followed by the Tukey HSD test with a confidence level of 95% to test the significant difference of each treatment. Data were presented from the mean of 3 data ± standard deviations.

RESULTS AND DISCUSSION

Comparison of nutritional value

As shown in Table 2, all plant-based nugget treatments (CT, GT, TT, CGT, and C1) had significantly higher protein and moisture content and lower fat content ($p < 0.05$) than commercial chicken nuggets (C0). However, there were no significant differences ($p > 0.05$) for protein, fat, and moisture contents within all of the PBN treatments. In contrast, chicken nugget (C0) had a significantly higher ash content compared to all PBN treatments. Meanwhile, for the carbohydrate content, only TT and CGT treatments were found to be significantly different ($p < 0.05$) from each other and the other PBN and chicken nuggets. Despite the variability of the results achieved, the nutritional contents of all PBN treatments were found to be superior to the commercial chicken nugget analyzed and were in accordance with the standard nutritional parameters of chicken nugget from the Indonesian National Standard (SNI), except for carbohydrate content.

Table 2. Nutritional Composition of Standard and Commercial Chicken Nuggets and Plant-based Nuggets (%)

	Carbohydrate (%)	Protein (%)	Fat (%)	Moisture (%)	Ash (%)
Chicken Nuggets					
Standard	max. 20	min. 12	max. 20	max. 60	-
C0	32.32±6.35^{a}	11.07±2.11^{a}	18.00±1.68^{a}	34.96±3.24^{a}	3.53±0.26^{a}
Plant-based Nuggets					
C1	26.24±0.39^{a}	17.61±0.10^{b}	13.70±0.34^{b}	39.19±0.19^{b}	3.12±0.24^{b}
HT	24.66±0.94^{a}	18.45±0.73^{b}	12.04±0.11^{b}	41.93±1.15^{b}	2.74±0.49^{c}
GT	24.63±1.10^{a}	18.36±1.09^{b}	13.19±0.36^{b}	41.67±0.53^{b}	2.15±0.08^{def}
TT	24.31±2.32^{b}	19.55±2.05^{b}	13.46±0.27^{b}	40.83±0.53^{b}	1.70±0.26^{e}
HGT	23.62±0.59^{c}	19.03±1.71^{b}	12.41±0.49^{b}	43.04±1.27^{b}	2.24±0.11^{f}

C0: control negative (chicken nugget); C1: control positive (hung + ground TVP); HT: hung TVP + tempeh; GT: ground TVP + tempeh; TT: tempeh only; HGT: hung TVP + ground TVP + tempeh. The standard nutritional value for chicken

nuggets was derived from the Indonesian National Standard (SNI 01-6683-2002). Results in the same column sharing a different letter are significantly different ($p < 0.05$)

Moisture analysis

Following the results, the addition of tempeh caused significant differences ($p < 0.05$) in the nutritional composition of plant-based nuggets when compared to the commercial chicken nugget. However, it did not cause any significant differences ($p < 0.05$) within the plant-based nugget treatments. In addition, lower moisture content was observed in commercial chicken nuggets compared to all treatments. This could be caused by the production of commercial chicken nuggets that require high processing temperatures before freezing, such as pre-frying and pre-baking, therefore decreasing the moisture content of the chicken nuggets ("Model Rencana HACCP (Hazard Analysis Critical Control Point) Industri Chicken Nugget", 2006). Yoon et al. (1997) proved that the moisture content of chicken nuggets was found to be decreased by 1.1% and 1.4% after baking and frying, respectively.

In addition to that, commercial chicken nuggets may consist of not only chicken meats but also tapioca flours that act as fillers to reduce production costs (Ma'ruf et al., 2019). The addition of fillers in chicken nuggets may up to 15% (Heinz & Hautzinger, 2007). It was also reported by Dewi (2011) that the higher number of fillers would decrease the moisture content, which is caused by the low moisture content of the flours used when compared to the main ingredients (Dewi, 2011). Whereas in plant-based nuggets, the high moisture content is probably due to the inclusion of vegetable broth inside the formulations that can increase the water content, mainly from boiling and/or soaking the ingredients like mushroom, chunk TVP, and ground TVP. Moreover, according to Kotula & Berry (1986), the inclusion of SPI can increase moisture content by improving its water holding capacity (WHC). Therefore, SPI has the ability to absorb high amounts of water and retain it significantly during cooking (Kotula & Berry, 1986).

Ash analysis

Ash content is defined as all inorganic residues already removed from the food through heating at a very high temperature. The inorganic residues left in the combusted food are the total mineral content available in the food (McClements, n.d.). In this study, the commercial chicken nugget had a higher ash content than the PBNs. This result differed from Huda Faujan et al.'s (2018) study that reported significantly higher ash contents in chickpea flour-TVP-made nuggets instead of the commercial chicken nugget. Factors such as composition and ingredients might affect the ash content. For instance, naturally existing minerals in the water used in the production or the number of food additives used to preserve the quality of the chicken nugget (MacDonald & Reitmeier, 2017). Unfortunately, due to the lack of information about the composition and ingredients of the commercial chicken nugget, the discussion on ash content in this study is limited.

Among the PBN treatments, it can be observed that the PBN substituted with tempeh only had a significantly lower ash content. This might be caused by food processing, including steaming, boiling, and fermentation of tempeh, which can lower the mineral content by 20-40% (Bavia et al., 2012; Damanik et al., 2018; “Freezing, drying, cooking, and reheating”, n.d.). On the other hand, the extrusion process may also increase the iron content due to the iron contamination from the extrusion machine, causing the ash content of nuggets without tempeh in the formulations to be higher (Reddy & Love, 1999).

Protein analysis

Based on the results, the protein content in all PBN treatments was higher compared to commercial chicken nuggets. This could be caused by the protein content in the PBN ingredients. TVP can contain up to 50-70% crude protein, whereas other ingredients, such as tempeh (19.91%), soy protein isolate (85-90%), and vital wheat gluten (80%), are all high in protein (Jafari, 2019; Ortolan & Steel, 2017; Riaz, 2011; “Tempeh, Cooked”, 2019). Despite the usage of a very high amount of protein content, the total protein content

analyzed only lies between 17-18%. This is caused by food processing that reduces the protein content in the food. According to Tyagi et al. (2015), the protein loss caused by steaming and frying is 45% and 55%, respectively.

A theoretical calculation of the protein content of the PBNs was done based on the chunk TVP, ground TVP, and tempeh compositions without including the other ingredients. Since there is no specific information on the protein content of both chunk and ground TVP, the protein content used was based on the protein content of TVP in general, which is 51%. Based on the calculations, plant-based nuggets using control treatment (C1) had the highest protein content (51%), followed by GT (36.86%), CGT (35.45%), CT (34.04%), and lastly, TT (19.91%) treatment. However, the actual result was not expected as from the theoretical result. The protein content in the PBN treated with tempeh was the highest, whereas the control treatment was the lowest. Nevertheless, the protein content of all plant-based nugget treatments was similar to each other. This was also following similar results from Sharima-Abdullah et al. (2018), which found no significant differences in the protein content of plant-based nuggets treated with five different levels of chickpea flour and TVP (30:10, 25:15, 20:20, 15:25, 10:30).

Fat analysis

The fat content in the commercial chicken nuggets was found to be higher than the PBNs. This could be caused by the generally high-fat content in animal products compared to plant products (Makinson et al., 1987). Chicken contains about 18% fat, whereas TVP and tempeh contain about 1% and 11% fat, respectively (Bender, 2002; Riaz et al., 2005; "Tempeh, cooked", 2019). Despite that, the fat content of the plant-based nuggets lay between 12-13%. This might be caused by the addition of palm oil in production. Additionally, plant-based foods absorb a greater amount of fat after frying. This is caused by the different intercellular space capacities between animal and plant cells. In animal cells, the spaces are filled with fluids, unlike plant cells that are filled with air (Fillion & Henry, 1998). Therefore, plant-based foods uptake more fat after frying compared to animal-based foods.

Carbohydrate analysis

Based on the results, commercial chicken nuggets had the highest amount of carbohydrates compared to the PBNs. However, compared to previous studies by Bohrer (2019) and Polizer et al. (2015), the carbohydrate content of plant-based nuggets was found to be higher than chicken nuggets, which is not followed by the current study. Chicken meats do not contain any carbohydrates, whereas TVP and tempeh contain very low carbohydrate content (31% and 7.62%) ("Carbs in Chicken", n.d.; Johnson et al., 2008; "Tempeh, Cooked", 2019). Despite the low content of carbohydrates in both of the raw ingredients, the addition of fillers in the production of commercial chicken nuggets might cause the carbohydrate content of the nuggets to become higher. Fillers are commonly used to reduce the production cost by replacing the chicken meat with carbohydrate-rich ingredients, such as tapioca flours (Hafid et al., 2019). Heinz and Hautzinger (2007) reported that the total amount of fillers that can be used in chicken nugget production is up to 15%, whereas in plant-based nuggets is only 6%. Therefore, the higher ratio of fillers used in the production of the commercial chicken nugget might cause the carbohydrate content of the commercial chicken nugget to be higher than the current plant-based nugget formulations.

Moreover, according to USDA, the total amount of breading and batter used in the chicken nuggets can reach 30% of the total weight ("Chicken nuggets, fingers, strips, fritters, and patties, fully cooked, individually frozen", 2001). Compared to the current study, the amount of breading and batter used in the PBNs are only approximately 10-12%. Since the carbohydrate content in all of the nuggets was not directly analyzed, the carbohydrate content estimated may include other, not strictly speaking, carbohydrates like fibers and organic acids. Therefore, the carbohydrate content may be overestimated using this method ("Food energy – methods of analysis and conversion factors", 2002).

Comparison of hardness value

The average hardness value of both commercial chicken nuggets and all treatments of plant-based nuggets are shown in Table 3 below. It can be seen that the addition of tempeh decreased the hardness value significantly ($p<0.05$), except for the C1 and CGT, where there were no significant differences ($p>0.05$) compared to the commercial chicken nuggets (C0). However, these results differed from Kumar et al. (2015), which stated that chicken nuggets had a higher peak force than plant-based nuggets. In this experiment, some of the plant-based nugget treatments had similar hardness values compared to the chicken nuggets. This could be caused by the different samples used for the comparisons. In this experiment, the recipe's formulations used commercial chicken nuggets instead of handmade chicken nuggets.

Table 3. Hardness value (N/mm^2) of Chicken and Plant-based Nuggets

Parameter	Chicken Nugget (Control)	Plant-based Nugget				
	C0	C1	HT	GT	TT	HGT
Hardness (N/mm^2)	2.59±0.19^a	2.57±0.19^a	1.85±0.10^b	1.28±0.03^c	0.69±0.07^d	2.44±0.21^a

C0: chicken nugget; C1: control; HT: hung TVP + tempeh; GT: ground TVP + tempeh; TT: tempeh only; HGT: hung TVP + ground TVP + tempeh. Figures in the same row sharing a common letter are not significantly different ($p < 0.05$)

Based on Kumar et al. (2015), the hardness of chicken nuggets was 44.27 N/mm², whereas the hardness of plant-based nuggets was 33.54 N/mm². These results were far different compared to the current study. This could be caused by different compression areas of the nuggets. In Kumar et al.'s (2015) study, the area analyzed was the central cores of the nuggets. However, the crust layer could happen to be analyzed by the probe instead of only the cores of the nugget; hence it would affect the results ("Texture Analyzer", n.d.). Whereas in the current study, the crust layer of the nuggets was removed using a knife, leaving the flesh of the nuggets analyzed by the probe. Nevertheless, this study found a similar trend to Kumar et al. (2015), in which a higher hardness value was detected in the chicken nugget compared to the PBNs.

Per treatment-wise, plant-based nugget with tempeh treatment had the lowest peak force compared to the other treatment, meaning that tempeh treatment alone could neither mimic nor improve the textural attribute. However, the combination of tempeh with chunk TVP and ground TVP could improve the hardness of the plant-based nuggets, even though not higher than the control treatment. Based on an experiment done by Kassama et al. (2003), the inclusion of 5% TVP in meatless patties had a similar hardness value compared to beef patties (103.9 N and 105.9 N, respectively), which means that the hardness of TVP can be comparable to meat-based products (Kassama et al., 2003). The results showed that nuggets that had chunk TVP in the formulations had higher peak force than the ground TVP and tempeh formulations.

Ground TVP could not provide peak force as high as chunk TVP, which may be due to the shape and size of the ground TVP itself. Chunk TVP has a larger structure (15-25 mm thick and 50-70 mm long) compared to ground TVP (1.5-12 mm diameter). According to Riaz et al. (2005), TVP with larger sizes is harder to be hydrated, therefore, leaving the texture to be harder compared to the smaller sizes. Besides shape and size, densities also affect the hardness of TVP. TVP with lesser density, such as flakes, tends to have lower peak force value, as more water can hydrate inside the TVP, contributing to the softer texture. In contrast, denser TVP, such as granules and crumbles, will have higher peak force. This statement was proven by cooking the TVP granules and flakes in boiling water for 90 minutes and showed that the peak force of TVP granules was significantly higher than TVP flakes (72.99 g and 59.81 g, respectively) (Riaz et al., 2005).

In this experiment, ground TVP is classified as TVP flakes; therefore, the inclusion of ground TVP lowered the peak force of the plant-based nuggets compared to chunk TVP (Riaz et al., 2005). In comparison, the peak force value of plant-based nuggets treated with tempeh was found to be the lowest compared to the other treatments. This could be caused by the tempeh ingredients themselves that do not have properties like TVP, which can be hydrated and expandable to mimic the meat properties. However, when the tempeh was combined together with chunk TVP and ground TVP, the hardness value was comparable to the control and commercial chicken nugget treatment, which should not happen. This could be caused by the uneven size of the ingredients in the nugget, even after mixing. Therefore, when the nuggets were analyzed, the probes could have touched the parts of the ingredients that were not mixed evenly, leaving the hardness value was higher than it should be. A possible cause would be during grinding chunk TVP and mushroom for only a short time, therefore may leave the grinding process were not even.

CONCLUSION

The substitution of TVP with tempeh significantly increased the protein and moisture content and decreased the fat and ash content compared to commercial chicken nuggets. Although the carbohydrate content of some of the PBNs did not significantly differ from commercial chicken nuggets, the trend showed higher carbohydrate content in the commercial chicken nuggets. Partial or whole substitution of TVPs with tempeh in plant-based nuggets significantly decreased the hardness value of chunk TVP + tempeh, ground TVP + tempeh, and tempeh treated plant-based nuggets. Contrary, no significant differences were found in the hardness of the chunk + ground TVP and chunk TVP + ground TVP + tempeh treated plant-based nuggets compared to commercial chicken nuggets.

Many factors such as different food processing and ingredients may affect the nutritional composition and hardness of the PBNs. The short duration of grinding, for instance, caused the flesh of the PBNs to become uneven, hence limiting the interpretation of the different hardness values between treatments. For future study, increasing the grinding duration or substituting the tempeh with tempeh flour may address this limitation. Additionally, for a better understanding of the effects of composition and ingredients on the nutritional and textural properties of the nuggets, comparing the PBNs to a self-produced chicken nugget should be considered.

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REFERENCES

Ambari, D., Anwar, F., & Damayanthi, E. (2014). Formulasi sosis analog sumber protein berbasis tempe dan jamur tiram sebagai pangan fungsional kaya serat pangan. *Jurnal Gizi dan Pangan*, 9(1), 65-72.

Bavia, A., Silva, C., Ferreira, M., Leite, R., Mandarino, J., & Carrão-Panizzi, M. (2012). Chemical composition of tempeh from soybean cultivars specially developed for human consumption. *Food Science and Technology*, *32*(3), 613-620. https://doi.org/10.1590/s0101-20612012005000085.

Bender, A. (1992). *Meat and meat products in human nutrition in developing countries*. FAO. Retrieved 7 July 2021, from http://www.fao.org/3/T0562E/T0562E02.htm#Nutritional%20value%20of%20meat.

Bohrer, B. M. (2019). An investigation of the formulation and nutritional composition of modern meat analogue products. *Food Science and Human Wellness*, 8(4), 320-329.

Carbs in chicken. Carb-counter.org. Retrieved 2 July 2021, from http://www.carb-counter.org/poultry/search/Chicken/0500.

Chicken nuggets, fingers, strips, fritters, and patties, fully cooked, individually frozen. USDA. (2001). Retrieved 7 July 2021, from https://www.ams.usda.gov/sites/default/files/media/CID%20Chicken%20Nuggets%2C%20Fingers%2C%20Strips%2C%20Fritters%2C%20and%20Patties%2C%20Fully%20Cooked%2C%20Individually%20Frozen.pdf.

Curtain, F., & Grafenauer, S. (2019). Plant-based meat substitutes in the flexitarian age: an audit of products on supermarket shelves. *Nutrients*, 11(11), 2603.

Damanik, R., Pratiwi, D., Widyastuti, N., Rustanti, N., Anjani, G., & Afifah, D. (2018). Nutritional Composition Changes During Tempeh Gembus Processing. *IOP Conference Series: Earth and Environmental Science*, *116*, 012026. https://doi.org/10.1088/1755-1315/116/1/012026.

Dekkers, B. L., Boom, R. M., & van der Goot, A. J. (2018). Structuring processes for meat analogues. *Trends in Food Science & Technology*, 81, 25-36.

Derbyshire, E. J. (2017). Flexitarian diets and health: a review of the evidence-based literature. *Frontiers in Nutrition*, 3, 55.

Dewi, R. K. (2011). *Kajian komposisi kimia, kualitas fisik dan organoleptik duck nuggets dengan filler tepung maizena pada proporsi yang berbeda*. Universitas Sebelas Maret. Surakarta. Retrieved 5 July 2021, from http://perpustakaan.uns.ac.id.

Fillion, L., & Henry, C. J. K. (1998). Nutrient losses and gains during frying: a review. *International Journal of Food Sciences and Nutrition*, 49(2), 157–168.

Food energy – methods of analysis and conversions. (2002). FAO. Retrieved 22 February 2022, from https://www.fao.org/3/Y5022E/y5022e03.htm#bm3.3.

Freezing, drying, cooking, and reheating. Nutritiondata.self.com. Retrieved 25 June 2021, from https://nutritiondata.self.com/topics/processing.

Green butcher: Indonesia's first plant-based meat producer finalises seed round investment. vegconomist.com. (2021). Retrieved 15 April 2021, from https://vegconomist.com/investments/green-butcher-indonesias-first-plant-based-meat-producer-finalises-seed-round-investment/.

Hafid, H., Agustina, D., Ananda, S. H., Anggraini, D. U., & Nurhidayati, F. (2019). Chicken nugget nutrition composition with an additional variation of breadfruit flour. *In IOP Conference Series: Earth and Environmental Science*, 382(1), 1-7.

He, J., Evans, N. M., Liu, H., & Shao, S. (2020). A review of research on plant-based meat alternatives: driving forces, history, manufacturing, and consumer attitudes. *Comprehensive Reviews in Food Science and Food Safety*, 19(5), 2639-2656.

Heinz, G., & Hautzinger, P. (2007). Meat processing technology for small- to medium-scale producers (20th ed.). FAO.

Huda Faujan, N., Zaini Hassan, C., & Arifin, N. (2018). Physicochemical properties and consumer preference of imitation chicken nuggets produced from chickpea flour and textured vegetable protein. *International Food Research Journal*, *25*(3), 1016-1025. Retrieved 28 March 2022, from.

Hurrell, R. F., & Finot, P. A. (1983). Food processing and storage as a determinant of protein and amino acid availability. *Nutritional Adequacy, Nutrient Availability and Needs*, 44, 135–156.

Jafari, S. M. (2019). Biopolymer nanostructures for food encapsulation purposes. In biopolymer nanostructures for food encapsulation purposes (pp. 247-285). Academic Press.

Johnson, L. A., White, P. J., & Galloway, R. (2008). Soybeans. In soybeans (pp. 613-660). United States of America: Elsevier.

Kassama, L. S., Ngadi, M. O., & Raghavan, G. S. V. (2003). Structural and instrumental textural properties of meat patties containing soy protein. *International Journal of Food Properties*, 6(3), 519–529.

Kotula, A. W., & Berry, B. W. (1986). Addition of soy proteins to meat products. *ACS Symposium Series*, 7, 74–89.

Kumar, P., Chatli, M. K., Mehta, N., Singh, P., Malav, O. P., & Verma, A. K. (2015). Meat analogues: health promising sustainable meat substitutes. *Critical Reviews in Food Science and Nutrition*, 57(5), 923–932.

Kumar, P., Kumar, R., & Sharma, B. (2011). Product profile comparison of analogue meat nuggets versus chicken nuggets. *Fleischwirtschaft International*, 91, 72-75.

Kumar, P., Sharma, B., Kumar, R., & Kumar, A. (2012). Optimization of the level of wheat gluten in analogue meat nuggets. *Indian Journal of Veterinary Research*, 21(1), 54-59.

Kurniawan, R. (2014). *SNI 6683 2014 (Nugget Ayam)*. Academia.edu. Retrieved 8 July 2021, from https://www.academia.edu/36095611/Sni_6683_2014_nugget_ayam_.

Kyriakopoulou, K., Dekkers, B. L., & van der Goot, A. J. (2018). Plant-based meat analogues. In C. Galanakis (Ed.), Sustainable Meat Production and Processing (pp. 103-126). Academic Press.

Ma'ruf, W., Rosyidi, D., Radiati, L., & Purwadi, P. (2019). Physical and organoleptic properties of chicken nugget from domestic chicken (*Gallus domesticus*) meat with different corn flours as filler. *Research Journal of Life Science*, 6(3), 162-171.

MacDonald, R., & Reitmeier, C. (2017). Food processing. *Understanding Food Systems: Agriculture, Food Science, and Nutrition in the United States*, 1, 179-225.

Makinson, J. H., Greenfield, H., Wong, M. L., & Wills, R. B. H. (1987). Fat uptake during deep-fat frying of coated and uncoated foods. *Journal of Food Composition and Analysis*, 1(1), 93–101.

McClements, J. *Analysis of Ash and Minerals*. People.umass.edu. Retrieved 25 June 2021, from https://people.umass.edu/~mcclemen/581Ash&Minerals.html.

Model rencana HACCP (Hazard Analysis Critical Control Point) industri chicken nugget (2006). eBookPangan.com. Retrieved 15 April 2021, from http://tekpan.unimus.ac.id/wp-content/uploads/2013/07/MODEL-RENCANA-HACCP-INDUSTRI-CHICKEN-NUGGET.pdf.

Nielsen, S. (2017). Food Analysis Laboratory Manual (3rd ed.). Springer.

Nout, M. J. R., & Kiers, J. L. (2005). Tempe fermentation, innovation and functionality: update into the third millennium. *Journal of Applied Microbiology*, 98(4), 789–805.

Official Methods of Analysis of AOAC INTERNATIONAL (2019), AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 936.03.

Ortolan, F., & Steel, C. J. (2017). Protein characteristics that affect the quality of vital wheat gluten to be used in baking: a review. *Comprehensive Revision of Food Science and Food Safety*, 16, 369–381.

Plant-based Meat Market. (2020). Retrieved 22 January 2021, from https://www.marketsandmarkets.com/Market-Reports/plant-based-meat-market-44922705.html.

Polizer, Y. J., Pompeu, D., Hirano, M. H., Freire, M. T. de A., & Trindade, M. A. (2015). Development and evaluation of chicken nuggets with partial replacement of meat and fat by pea fibre. *Brazilian Journal of Food Technology*, 18(1), 62–69.

Reddy, M. B., & Love, M. (1999). The impact of food processing on the nutritional quality of vitamins and minerals. *Impact of Processing on Food Safety*, 459, 99–106.

Riaz, M. N. (2005). Textured soy protein utilization in meat and meat analog products. In Soy Applications in Food (pp. 148-197). Boca Raton: CRC Press.

Riaz, M. N. (2011). Handbook of food proteins (1st ed.). Cambridge: Woodhead Publishing.

Sharima-Abdullah, N., Faujan, N., Hassan, C., & Arifin, N. (2018). Physicochemical properties and consumer preference of imitation chicken nuggets produced from chickpea flour and textured vegetable protein. *International Food Research Journal*, 25(3), 1016-1025.

Shurtleff, W., & Aoyagi, A. (1979). The book of tempeh (1st ed.). New York: HarperCollins Publishers.

Tempeh, Cooked. USDA. Retrieved 15 April 2021, from https://fdc.nal.usda.gov/fdc-app.html#/food-details/172467/nutrients.

Texture Analyser. Arrow Scientific. Retrieved 28 June 2021, from http://www.arrowscientific.com.au/index.php?option=com_content&view=article&id=21:texture-analyser&catid=7&Itemid=37.

TVP® (Textured Vegetable Protein). Bob's Red Mill Natural Foods. Retrieved 19 April 2021, from https://www.bobsredmill.com/tvp-textured-veg-protein.html.

Tyagi, S., Kharkwal, M., & Saxena, T. (2015). Impact of cooking on nutritional content of food. *DU Journal of Undergraduate Research and Innovation*, 1(3), 180-186.

Vital, R., Bassinello, P., Cruz, Q., Carvalho, R., de Paiva, J., & Colombo, A. (2018). Production, quality, and acceptance of tempeh and white bean tempeh burgers. *Foods*, 7(9), 136.

Wan, L. (2018). *Asia dominates vegetarian markets but understanding local factors crucial for sales success*. foodnavigator-asia.com. Retrieved 15 April 2021, from https://www.foodnavigator-asia.com/Article/2018/07/02/Asia-dominates-vegetarian-markets-but-understanding-local-factors-crucial-for-sales-success#.

Widyastuti, R. (2021). *Harga kedelai impor naik, Kemendag: harga tempe bisa Rp. 16.000 per kg*. Tempo. Retrieved 15 April 2021, from https://bisnis.tempo.co/read/1428618/harga-kedelai-impor-naik-kemendag-harga-tempe-bisa-rp-16-000-per-kg.

Wulansari, R. (2013). *Mempelajari pengaruh penambahan hidrokoloid terhadap karakteristik nugget tempe*. 123dok.com. Retrieved 1 July 2021, from https://123dok.com/document/1y9mx5lq-mempelajari-pengaruh-penambahan-hidrokoloid-terhadap-karakteristik-nugget-tempe.html.

Yoon, H. R., Bednar, C., Czajka-Narins, D., & King, C. (1997). Effect of preparation methods on moisture content, fat content and sensory characteristics of breaded food items served in school lunches. *Journal of the American Dietetic Association*, 97(9), 97.

Weston, Z. (2019, 10 December). Why plant-based chicken is about to take off [Blog post]. Retrieved from: https://gfi.org/blog/2020-plant-based-chicken/.