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The effect of kappa carrageenan on physical and textural properties as well as sensory parameters on plant protein mixture-based nuggets

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Abstract

This study investigated the effect of kappa carrageenan on physical properties (cooking loss and color), texture profile analysis (TPA) and sensory evaluation of 2 recipes plant based nuggets namely soy and pea protein mixture-based nuggets (SPPN) and gluten-free plant protein mixture-based nuggets (GFPN) The ratio of soy protein to pea protein was carried out at three levels: 25:75, 50:50 and 75:25. TPA parameters including hardness, cohesiveness, and chewiness were increased as soy protein content increased ($P<0.05$), but cooking loss decreased ($P>0.05$). SPPN at all levels showed different color values from chicken nuggets. SPPN at a 50:50 level was chosen to produce GFPN based on the highest sensory evaluation score. Three levels of kappa carrageenan were added (1%, 3% and 5% by weight) to GFPN and compared with SPPN without kappa carrageenan. The increment of kappa carrageenan levels increased hardness and chewiness ($P<0.05$), but decreased springiness, cohesiveness and cooking loss ($P>0.05$). No significant color difference was found between GFPN with 1% kappa carrageenan and the control sample. GFPN with 5% kappa carrageenan was chosen because exhibited the highest ratings for appearance, aroma, texture and overall liking scores ($P<0.05$). Comparing GFPN with 5% kappa carrageenan to chicken nuggets, crude fiber, fat, protein, carbohydrates and energy were decreased 22%, 15.03%, 4.75%, 17.34% and 11.81%, respectively whilst increased 47.19% and 6.26, respectively. The combination of carrageenan and a plant-protein mixture (without gluten-free) shows the possibility of developing nuggets.

Keywords: Plant based-nuggets, Soy protein, Pea protein, Carrageenan, Gluten-free products

1. Introduction

The production of meat results in the excessive use of land and water resource, high risk of animal diseases, negative impact on terrestrial and emission of greenhouse gases (Singh *et al.,* 2021). Moreover, the consumption of meat-based products was found to be related to the risk of fatal diseases including colorectal cancer and cardiovascular diseases (Kamani *et al.,* 2019; Singh *et al.,* 2021). For all this awareness, consumption has shifted to plant-based products. Meat-based nuggets consists mainly of proteins and fat, a lesser content of seasoning, salt and wheat crumb, starched and fibers as binders (Kyriakopoulou *et al.,* 2021). To replace meat protein in the product, various plant proteins are incorporated in meat analog including soy, pea, wheat and fungi protein (Yuliarti *et al.,* 2021)

Soy ingredients (soy flour, soy protein concentrate and soy protein isolate) commonly have been used in meat analogs due to their characteristic functions including water-holding agent, gelling agents, fat-absorbing agent, and emulsifying agent (Kyriakopoulou *et al.,* 2021). Regarding to protein digestibility-corrected amino acid scores (PDCAAS), soy protein isolate obtains of PDCAAS 1.00. This is comparable to animal-based foods such as meat, eggs, and dairy (Bohrer, 2019). Pea protein is the most promising legume protein for meat-analog manufacturers by high-moisture extrusion in recent years (Bohrer, 2019; Kyriakopoulou *et al.,* 2021). This legume is considered as an alternative for soy protein (not labeled as allergenic, less relevant to the GMO issue, and growing in a temperate environment) (Schreuders *et al.,* 2019). The combination of different plant proteins has the potential to improve the textural attributes of meat analogs. Yuliarti *et al.* (2021) found that the incorporation of pea protein appeared to increase the hardness, chewiness and the viscoelastic properties of plant-based nugget.

The most concerningproblem related to the gluten-free plant-based product is imitating the functional and rheological attributes (Nanta *et al.,* 2021). Because wheat gluten is regarded as the primary binder holding the fiber together in the structure that mimics meat (Rizvi *et al*., 1980), it is used to improve the texture and functional properties. However, it is related to an immune reaction in gluten-intolerant people (Nanta *et al.,* 2021). To manipulate these attributes, a protein gel matrix can be formed in the presence of hydrocolloids, which have the potential to improve the nutritional and textural attributes of meat analogs (Yuliarti *et al.,* 2021). To date many various hydrocolloids have been used in meat analogsincluding pectin, guar gum and cellulose-derived products (Ziobro *et al.*, 2012) and iota carrageenan (Palanisamy *et al.,* 2018). From previous study of Nanta *et al*. (2021), they found that the addition of kappa carrageenan had a positive effect on the viscosity and textural properties of the gluten-free meat analog. Therefore, kappa carrageenan concentration was applied and the effect on plant-based nugget properties were investigated.

Kappa carrageenan is sulfated anionic polysaccharide derived from red algae (Hirota and Nagai, 2022). It has a linear structure with helical conformation and has one sulfate group for every two-galactose molecule (Tecante *et al.,* 2012). Due to its hydrophilic nature, kappa carrageenan can act as a stabilizing, thickening, and gelling agent (Shafie *et al.,* 2022). In the presence of cations including potassium ions and calcium ions, the kappa carrageenan is capable of forming a rigid gel with a helical structure (Tecante *et al.,* 2012). The gel matrix formation is made of aggregated chains in the presence of the cation (Pereira, 2016). Moreover, the viscosity, degree of plasticity and texture of the gluten-free meat analog was improved by the addition of kappa carrageenan (Nanta, *et al*., 2021).

Therefore, this study focused on the effect of kappa carrageenan addition to soy and pea protein mixtures-based nuggets (SPPN) (physical properties, texture profile analysis (TPA) and sensory evaluation) to produce a gluten-free plant-based nugget (GFPN) analog.

2. Materials and Methods

2.1 Materials

Soy protein isolate was purchased from Krungthepchemi (Bangkok, Thailand). Pea protein isolate was purchased from AW Nutrition (Nonthaburi, Thailand). Kappa carrageenan was purchased from T.C.S Pacific Ltd. (Bangkok, Thailand). The chicken meat was purchased from Siam Makro Co., Ltd. (Bangkok, Thailand). Wheat gluten was purchased from PTK Solution and Supplies Co., Ltd. (Thailand). Other ingredients were purchased from a local department store (Bangkok, Thailand).

2.2 Soy and pea protein mixture-based nuggets (SPPN) preparation

The SPPN was prepared following Kubola *et al.* (2021) with slight modification. A total of 3 different ratios of soy protein to pea protein were conducted including 25:75 (SPPN25), 50:50 (SPPN50) and 75:25 (SPPN75). Each composite ration contained 24.31% plant protein, 68.07% water (~25°C), 1.36% salt, 0.92% pepper powder, 0.61% sugar, 4.67% wheat gluten and 0.05% chicken flavor in order to make 100 g of each composite protein analog. The analog was prepared by thoroughly mixing plant protein mixture in NaCl solution in a food processor (Philips, Singapore) for 3 min at low speed. After the mixture was left at room temperature (~30°C) for 30 min, all ingredients were added and mixed in a food processor with low speed for a total time of 5 min. Then the 20 g of SPPN batter was molded. The molded samples were coated with batter and deep-fried at 180ºC for each piece. The batter for coating was prepared from all-purpose flour and water with a ratio of 1 : 2. The sample was then coated with egg and breadcrumbs, respectively. All SPPN samples were promptly cooled at room temperature (~30°C) after cooking until the core temperature of the sample dropped below 30°C (25-30°C) before analysis. As a control, a sample was prepared from chicken meat instead of plant protein.

2.3 Gluten-free plant protein mixtures-based nugget (GFPN) preparation

The GFPN was prepared from soy protein and pea protein with a ratio of 50:50 following Kubola *et al.* (2021) with slight modification as described above. The wheat gluten in the formulation was removed in order to investigate the effects of kappa carrageenan. The kappa carrageenan content was investigated at 0%, 1%, 3%, and 5% (w/w). The control sample was formulated from SPPN with a ratio of 50:50 with wheat gluten. This formulation was chosen because it showed the highest score in terms of appearance, color, aroma, texture and overall liking (P<0.05).

2.4 Physical properties determination

2.4.1 Color measurement

The color of the samples was determined using a colorimeter (HunterLab, ColorFlex CX2687, USA) with a D65 illuminant as described by Sakamut and Sajjabut (2021). The total color difference (ΔE) of the samples was calculated according to Yuliarti *et al.* (2021). L* (lightness), a* (redness) and b* (yellowness) was recorded.

2.4.2 Cooking loss

The samples were cut into 3.3 × 5.5 × 1 cm^3. The sample was then deep-fried at 180°C for each piece. The mass before (W_1) and after (W_2) frying was measured. Cooking loss is calculated according to AACC (1995).

2.5 Textural properties determination

The textural properties were measured using a texture analyzer (Model TA-XT2i, Stable Micro Systems, England) according to Yuliarti *et al.* (2021). The samples were cut into 2 × 2 × 2 cm^3 and a cylinder probe of 50 mm diameter (P50) was used to compress twice to 40% of its original thickness at a speed of 5.0 mm/s at ambient temperature (25°C) The hardness, springiness, cohesiveness, gumminess, chewiness was determined.

2.6 Sensory evaluation

The sensory attributes difference test of the samples was evaluated with 30 untrained panelists. The samples were cut into 2.5 × 2.5 × 2 cm^3 (L × W × H) before serving. The panelist was asked to rate the sample's sensory qualities, including appearance, color, aroma, taste, texture, and overall liking by using a 1-9 point descriptive scale: where one is denoted as extremely undesirable and nine as extremely desirable.

2.7 Proximate analysis

The proximate analysis including moisture content, protein, crude fiber, fat, ash, carbohydrates and energy of the analogs was measured according to the AOAC (2000).

2.8 Statistical analysis

A completely randomized design (CRD) was used in the experiment. ANOVA was used to perform analysis of variance in the data. At P≤0.05, Duncan's New Multiple Range Test was employed to assess differences between sample means using SPPS statistics Version 20.0. All of the trials were carried out twice.

3. Results and Discussion

3.1 Effect of plant protein ratio on physical properties of SPPN

3.1.1 Color (L*a*b*)

The color (L*a*b*) and total color difference (ΔE) of SPPN with various ratio of soy protein and pea protein is shown in Table 1. Control sample had the highest L* value of 42.83 but was not significantly different (P>0.05) to the SPPN25 and SPPN50, while SPPN75 had the lowest L* value of 40.29 (P≤0.05). It appeared that a significant difference a* value (8.44-9.06) was not observed in any sample (P>0.05). The control sample had the highest b* value of 28.38 and was significantly different from the other samples (26.20-27.59) (P≤0.05). In the current study, the b* value decreased as the pea protein isolate decreased. It might be due to pea protein isolate exhibited a higher b* value than soy protein isolate (Tunnarut *et al*., 2022) The ΔE value showed that the values in all SPPN samples were greater than one unit that is SPPN can be differentiated from the control sample by human eyes (Poynton, 1996). It might be related to the protein isolate's natural color, which was used in the formulation.

Table 1 Color (L*, a*and b*) and ΔE* values of samples with different protein ratios.

Sample	L*	a* NS	b*	ΔE*
Control	42.83±1.53[a]	8.98±1.69	28.38±1.05[a]	0.00
SPPN25	42.37±2.30[a]	9.06±1.61	27.59±1.33[b]	2.11
SPPN50	42.50±1.44[a]	8.44±1.03	27.53±0.74[b]	4.07
SPPN75	40.29±3.07[b]	8.74±1.83	26.20±1.41[c]	2.51

All values are presented as the mean and standard deviation.
Data within a column with different letters are significantly different (P≤0.05).
NS Non-significantly different (P≤0.05).

3.1.2 Cooking loss

The cooking loss of SPPN with various ratios of soy protein and pea protein is shown in Fig 1. Results showed that the substitution of pea protein with soy protein had no significant effect on the cooking loss of samples (P>0.05). It appeared that cooking loss decreased as the substitution of pea protein with soy protein in SPPN increased (P>0.05). The SPPN75 had the lowest cooking loss (P>0.05) which was 36.17% lower than the control sample. This result supported with those for hardness value, revealing that the strong network of SPPN75 can retain water within its structure. From the results of Fig 1, the cooking loss tend to decrease as the substitution of pea protein with soy protein increased. This implies that the water and fat in the SPPN75 structure were more tightly bound than in other SPPN samples, which reduced cooking loss (P>0.05). It might be due to soy protein exhibited a much higher gel-forming capacity than pea protein (Bildstein *et al*., 2008), revealing that the strong network of SPPN75 can retain water within its structure.

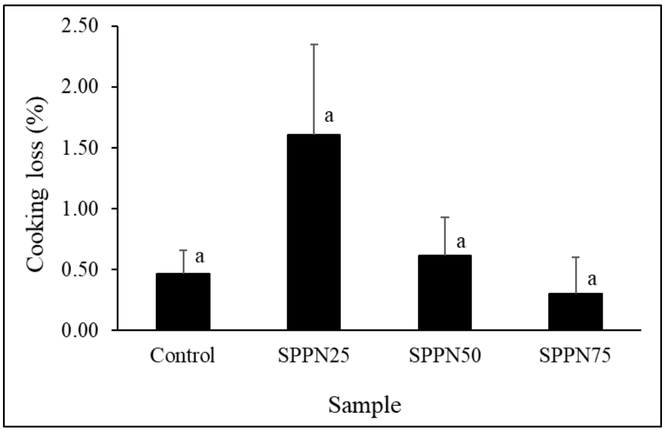

Fig 1 Cooking loss values of samples with different protein ratios. Bars represent the standard deviation. Different letters on each bar indicate significant differences (P≤0.05).

3.2 Effect of plant protein ratio on textural properties of SPPN

The textural properties of SPPN with various ratio of soy protein and pea protein by texture profile analysis (TPA) is shown in Table 2. In this investigation, the hardness and chewiness values of control samples were substantially greater (P≤0.05) than those of the SPPN samples. This might be a result of the myofibrillar proteins' stronger network, which is important for forming a strong structure (Kamani *et al.,* 2019). As a result, a control sample was resistant to compression, cohesiveness and springiness which showed the lower value than those of SPPN samples. It might be because the protein matrix of myofibrillar proteins could retain more water and fat content, filling the interstitial gaps and reducing springiness (Kamani *et al.,* 2019). The substitution of pea protein with soy protein in SPPN samples had a significant effect on hardness, chewiness and cohesiveness (P≤0.05). The SPPN75 sample had the highest hardness (P≤0.05) which was 51.46% higher than the SPPN25. Similar trends can be observed in the chewiness and cohesiveness values. The chewiness and cohesiveness values of the SPPN75 sample were higher than the SPPN25 by 3.49% and 56.97%, respectively. The amount of added soy protein increases the amount of force required to disintegrate the sample for swallowing (Kitcharoenthawornchai and Harnsilawat, 2015). In the case of springiness, the SPPN sample showed an increasing trend with a decreasing amount of pea protein (P>0.05). The result implied the greater ability of samples to regain their original shape after being deformed was required (Kitcharoenthawornchai and Harnsilawat, 2015). These TPA characteristics are dramatically increased in SPPN when soy protein substitutes pea protein, indicating a significantly harder texture. It might be due to structuring of pea protein exhibited a much lower gel-forming capacity than soy protein (Bildstein *et al.,* 2008). Furthermore, soy protein isolate gels are stronger than pea protein isolate gels when heated (O'Kane *et al.,* 2006; Shand *et al.,* 2007).

Table 2 TPA parameters of samples with different protein ratios.

Sample	Hardness (g)	Cohesiveness	Springiness (mm)	Chewiness (g/mm)
Control	3133.64±33.74[a]	0.76±0.02[c]	0.87±0.03[b]	2104.62±42.38[a]
SPPN25	553.39±83.09[d]	0.83±0.02[b]	0.95±0.02[a]	399.68±41.89[d]
SPPN50	890.72±87.01[c]	0.84±0.02[b]	0.96±0.03[a]	660.01±75.26[c]
SPPN75	1140.09±198.56[b]	0.86±0.03[a]	0.96±0.05[a]	928.88±146.39[b]

All values are presented as the mean and standard deviation.
Data within a column with different letters are significantly different (P≤0.05).

3.3 Effect of plant protein ratio on sensory evaluation of SPPN

The sensory evaluation result of SPPN with various ratio of soy protein and pea protein is shown in Table 3. The substitution of pea protein with soy protein in SPPN had a significant effect on those sensory parameters (P≤0.05). All sensory scores increased as the substitution of pea protein with soy protein increased to 50% (P≤0.05). There was no obvious difference in any of the parameters between the SPPN50 and SPPN75 samples (P>0.05). SPPN50 showed the highest score in terms of appearance, color, aroma, texture and overall liking (P>0.05). This could be related to the fact that the flavor of the sample may be impacted by the higher concentration of soy-based components, which panelists were able to taste. (Kamani *et al.*, 2019). Moreover, the lighter of SPPN50 sample could be accepted by panelists than the other sample.

Table 3 Sensory parameters of samples with different protein ratios.

Sample	Appearance	Color	Aroma	Taste	Texture	Overall liking
SPPN25	6.97±1.10[b]	6.70±1.42[b]	5.68±1.72[b]	5.52±1.48[b]	5.80±1.45[b]	5.90±1.45[b]
SPPN50	7.42±0.98[a]	7.22±1.03[a]	6.60±1.67[a]	6.30±1.54[a]	6.58±1.28[a]	6.87±1.14[a]
SPPN75	7.03±1.21[ab]	6.98±1.14[ab]	6.48±1.54[a]	6.32±1.59[a]	6.37±1.66[a]	6.55±1.48[a]

All values are presented as the mean and standard deviation.
Data within a column with different letters are significantly different (P≤0.05).

3.4 Effect of kappa carrageenan on physical properties of GFPN

3.4.1 Color (L*a*b*)

The color (L*a*b*) and total color difference (ΔE) of GFPN with various carrageenan contents are shown in Table 4. The control sample had an L* value of 39.43 and did not differ from the other GFPN samples significantly (P>0.05). The control sample had the lowest a* value of 9.38 and was significantly different from the other samples (9.48-11.89) (P≤0.05). The a* value and b* value seem to have increased as the carrageenan content increased. According to Palanisamy *et al.* (2018), the low concentration of iota carrageenan (0.75-3%) did not significantly impact the appearance of the color of the cooked extrudates. The result for the ΔE* indicated that the GFPN3 and GFPN5 samples had values above one unit. It implied that the samples could be identified by human eyes (Poynton, 1996).

Table 4 Color (L*, a* and b*) and ΔE* values of samples with various carrageenan contents.

Sample	Carrageenan content (%)	L*	a*	b*	ΔE*
Control	0	39.43±3.61[ab]	9.38±3.23[c]	24.66±1.56[c]	0.00
GFPN1	1	40.43±3.77[a]	9.48±3.03[c]	26.51±1.27[b]	0.92
GFPN3	3	37.96±2.54[b]	11.89±1.13[a]	27.51±1.75[a]	1.06
GFPN5	5	38.69±3.20[ab]	10.62±1.78[b]	26.71±1.11[ab]	3.36

All values are presented as the mean and standard deviation.
Data within a column with different letters are significantly different (P≤0.05).

3.4.2 Cooking loss

The weight loss of samples with varied carrageenan contents after frying is shown in Fig 2. Results showed that the addition of carrageenan had no significant effect on cooking loss in any sample (P>0.05). A decreasing trend with an increasing carrageenan content was found. The addition of 1%, 3% and 5% carrageenan had the cooking loss (P>0.05) which was 29.16%, 63.33% and 52.06% lower than the control sample. The capacity of carrageenan to form hydrogen bonds with water may account for the reduction in cooking loss (Hirota and Nagai, 2022). Rather than functioning through chemical interactions with proteins, kappa carrageenan reduced cooking loss by retaining the water in the gel network's interstitial spaces (Bernal *et al.*, 1987). The results in this study were supported by the textural properties result which showed an increasing value in hardness and chewiness in GFPN samples than in control. Pietrasik and Jarmoluk (2003) reported that increasing kappa carrageenan content in pork muscle gels to 0.8% content had lower cooking losses than the samples containing only 0.4% and without the hydrocolloid.

3.5 Effect of kappa carrageenan on textural properties of GFPN

TPA results of GFPN with various carrageenan content areshown in Table 5. The hardness and chewiness of the sample increased as the carrageenan content increased. The addition of 1%, 3% and 5% carrageenan had hardness of 40.03%, 55.76% and 69.70%, respectively, which was higher than that of control sample, respectively. The comparable increase in the chewiness was 31.70%, 48.84% and 63.02%, respectively. The force required to compress and disintegrate the sample (for swallowing) increased as a result of the compact protein network with carrageenan forming. The GFPN5 had the highest hardness and chewiness (P≤0.05). It appeared that the network structure becomes more compact when increasing carrageenan content up to 5%. Comparing the cohesiveness and springiness values of GFPN with 1%, 3% and 5% carrageenan to the control, the corresponding decreases were 9.05%, 7.36% and 12.07% and 3.31%, 6.19% and 6.92%, respectively. This was attributed to the gluten function, which forms a cohesive viscoelastic network in control sample (Chiang *et al.*, 2019). This is consistent with the results of Nanta *et al.* (2021), who found that the cohesiveness decreased (P≤0.05) when substitution of gluten with 1-3% carrageenan in meat analogue sample and the springiness exhibited a decreasing trend with an increasing amount of carrageenan (1-3%) in the formulation (P>0.05).

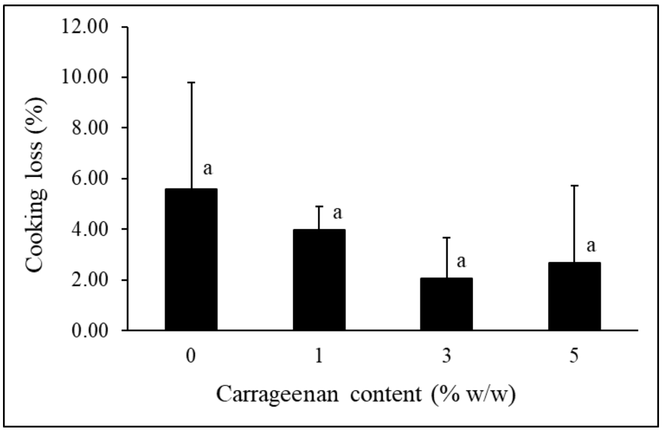

Fig 2 Cooking loss values of samples with various carrageenan contents. Bars represent the standard deviation. Different letters on each bar indicate significant differences (P≤0.05).

Table 5 TPA parameters of samples with various carrageenan contents.

Sample	Carrageenan content (%)	Hardness (g)	Cohesiveness	Springiness (mm)	Chewiness (g/mm)
Control	0	934.25±166.58^{d}	0.94±0.02^{a}	0.83±0.01^{a}	722.51±125.58^{d}
GFPN1	1	1,557.88±287.19^{c}	0.91±0.04^{b}	0.75±0.02^{b}	1057.89±187.09^{c}
GFPN3	3	2,111.77±426.80^{b}	0.88±0.03^{c}	0.77±0.04^{b}	1412.13±254.32^{b}
GFPN5	5	3,083.30±81.32^{a}	0.87±0.03^{c}	0.73±0.03^{c}	1953.62±110.58^{a}

All values are presented as the mean and standard deviation. Data within a column with different letters are significantly different (P≤0.05).

3.6 Effect of kappa carrageenan on sensory evaluation of GFPN

The sensory evaluation result of GFPN with various carrageenan content is shown in Fig 3. The addition of carrageenan in GFPN had a significant effect on those sensory parameters (P≤0.05), except that of taste. The sensory scores in terms of color, aroma, texture and overall liking of GFPN3 and GFPN5 were significantly higher than the control sample (P≤0.05). No significant change (P>0.05) in all parameters between GFPN3 and GFPN5 samples was observed. GFPN5 showed the highest score in terms of appearance, aroma, texture and overall liking (P>0.05). It is possible due to the compact structure and also the less cohesive of product with carrageenan created the feeling of more fibrousness in the mouth, which is preferred by panelists. Moreover, the addition of carrageenan in GFPN also improved the aroma of GFPN products. As a result, a higher parameter score can be obtained from the GFPN, compared to the control.

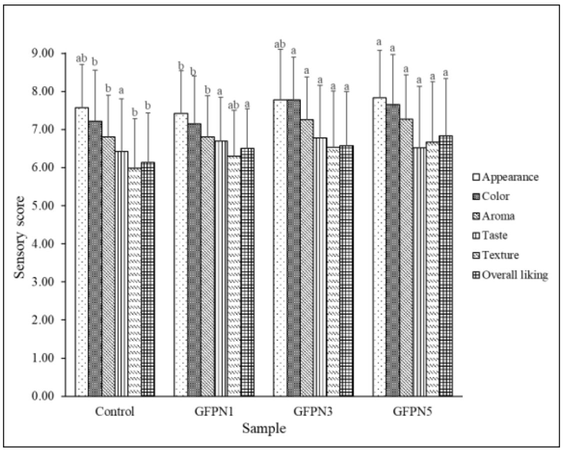

Fig 3 Sensory score of samples with various carrageenan contents. Bars represent the standard deviation. Different letters on each bar indicate significant differences (P≤0.05).

3.7 Proximate analysis

From the result of proximate analysis, the chemical composition of chicken nugget was 54.60% moisture, 20.01% protein, 9.05% fat, 2.67% ash, 1.00% crude fiber, 13.67% carbohydrate and 216.17 kcal (base on wet basis). In case of the GFPN5 sample, the chemical composition was 58.02% moisture, 19.06% protein, 7.69% fat, 3.93% ash, 0.78% crude fiber, 11.30% carbohydrate and 190.65 kcal The protein, fat, fiber and carbohydrate content in chicken nugget were higher than GFPN5 sample, while that of ash content were lower. Protein content showed is similar between the samples. The GFPN5 sample had the ash which were 47.19% higher than the control sample. Since chicken meat composes of iron and sodium 6.30 and 240 mg/100 g, respectively, while soy protein composes of iron and sodium 14.5 and 1,005 mg/100 g (Benamirouche *et al.,* 2020). Moreover, the GFPN5 sample had fiber and fat content which were 22.00% and 15.30% lower than chicken nugget, respectively. In the case of the moisture content, the GFPN5 had the higher value than that of the chicken nugget. It might be due to addition of water during GFPN batter preparation. The GFPN5 sample had the energy which was 11.81% lower than chicken nugget. This might be possible that chicken nugget contains higher protein, fat and carbohydrate content than GFPN5 sample.

4. Conclusion

The effect of plant protein ratio on SPPN and carrageenan content on GFPN properties was investigated. The substitution of pea protein with soy protein in SPPN had a positive impact on the cooking loss and textural properties of SPPN, with the exception of springiness and cohesiveness. To obtain the better physical and sensory characteristics, SPPN can be formulated at a 50:50 ratio. Additionally, the addition of carrageenan improved the GFPN properties, such as cooking loss, textural properties, and sensory attributes, excluding those related to taste. Carrageenan (GFPN5) with a 5% addition appears to provide the better physical, textural, and sensory characteristics. The GFPN5 sample had a higher crude fiber, fat, protein, carbohydrate content, and energy when compared to chicken nuggets. In order to comprehend the qualitative aspects of nuggets made from plant protein mixtures, future research should emphasize on the interaction between plant proteins and hydrocolloids.

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