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Fat-Reduced Sausage Supplemented with Germinated Mung Beans: Physicochemical Properties and Sensory Acceptance

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The aim of this study was to evaluate the effects of germinated mung beans addition as a fat replacer in sausages on their physico-chemical quality and sensorial acceptance. Germinated mung bean powder was used to replace fat at different levels: 0 %, 25 %, 50 %, 75 %, and 100 %. The sausages were analyzed for their physicochemical properties (proximate composition, cooking quality, instrumental colour and texture), microstructure (using scanning electron microscopy), and overall sensory acceptability. The results show that increasing the fat replacement ratio from 0 to 100 % with germinated mung beans increased the carbohydrate, protein, and ash content by 1.4, 1.3 and 1.2 times and decreased the fat content and caloric value by 3.0 and 1.33 times. Simultaneously, the total phenolic content (TPC) and antioxidant activity increased by 58.5 % and 14.8 %. Adding germinated mung beans also improved the emulsion stability and the cooking yield of fat-reduced sausages. The sample with 50 % fat replacement had the colour and textural attributes closest to the control and was most preferred by the consumers. Thus, the germinated mung bean powder is a potential ingredient that can be used to replace fat in the sausages.

1. Introduction

Sausages are convenient and highly nutritive emulsified meat products with a significant fat content (20 - 30%). Overconsumption of fat in the diet can cause various chronic diseases, leading to the efforts in the development and consumption of reduced-fat sausages. Removing fat from sausages can adversely affect their texture, flavor, appearance, and cooking losses, reducing yield and profitability (Yashini M. et al., 2019). To solve this problem, several fat substitutes have been used including carbohydrate-based fat replacers like resistant starch (Garcia-Santos et al., 2019), konjac gel (Kim et al., 2019), oat fiber and inulin (Souza et al., 2019), citrus fiber (Song et al., 2016), protein-based fat replacers like hydrolysed collagen (Olanwanit et al., 2019). Protein from beans is considered a healthier fat-replacer, low in calories, high in essential amino acids, and potential as a protein supplement for diets (Rosa et al., 2019). Some authors have used soybean, chickpea, lentil (Zeb et al., 2021), and pigeon pea flour (Tahmasebi et al., 2021) as fat substitutes in sausage products. Currently, limited research has utilized germinated mung beans for meat processing. Germinated mung beans are low in lipid (1.3 % dry basis - DB), rich in carbohydrates (mainly starch, 66.7 % DB), protein (28.8 % DB), and minerals (3.2 % DB); the germination also increases polyphenol content and antioxidant capacity of the mung bean seeds (Hung et al., 2020). Carbohydrates and proteins are both macromolecules that produce specific organoleptic and physicochemical characteristics (e.g., thickness and viscosity), which may provide fat-reduced sausage with attributes comparable to those of a full-fat sausage. They also can naturally form microparticles similar in shape and size to those of emulsion droplets and fat globules, allowing them to mimic fats (Yashini et al., 2019). Liu et al. (2018) found an improvement in the functional characteristics of carbohydrate and protein (e.g. water binding, gelling and emulsifying capacity) during germination of mung beans, which are needed for their ability to mimic fat properties and reduce the negative effects of fat removal from sausages. A combination of carbohydrates and protein from germinated mung beans can be used to replace fat in sausages and provide nutritional benefits to the product, but excessive substitution ratio may lead to inadequate physicochemical quality and consumer acceptance. The aim of this study was to evaluate the influence of the fat replacement ratio by germinated mung beans on the chemical composition, physicochemical properties, and sensorial quality of fat-reduced sausages.

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2. Materials and Methods

2.1 Materials

Lean pork and pork backfat were purchased from Vissan stores in Ho Chi Minh City. Lean pork was selected with a ratio of lean over 80 %, light pink color, dried surface, and no drip loss. Pork backfat was trimmed to remove skin and tendons, white in color, meeting quality standards according to TCVN 6044-2013. Modified starches, salts, sugar, STPP (sodium tripolyphosphate), and synthetic flavors were provided by Ba Huynh Co., Ltd. The commercial Viscofan 24 mm collagen casings were used to shape the sausage samples.

Germinated mung beans: Mung beans were soaked in water with a bean-to-water ratio of 1:1 (w/v), germinated at room temperature (26-28 °C), 85 % of relative humidity in 48 h to obtain the optimal values of chemical composition, TPC and antioxidant activity, dry matter loss, and protein and carbohydrate functional properties (Hung et al., 2020). The germinated mung beans were then dried in a convective hot air dryer at 50 °C for 72 h until the moisture content reached 10-12 %. The seed coats were removed, and the beans were powdered; the powder was sieved through an 80-mesh screen and stored in vacuum bags in the fridge.

Chemicals used for quantitative analysis were supplied by Sigma-Aldrich company (USA). All chemicals and solvents were analytical grade, unless otherwise specified.

2.2 Sausage preparation

The sausage formulations were prepared with varying compositions. The control sample (C100) consisted of 55% lean pork, 25% pork backfat and 20% ice. In the samples T25, T50, T75 and T100, 25 %, 50 %, 75 % and 100 % fat were replaced with germinated mung bean powder, previously hydrated with water (1:1.5 w/v ratio). All formulations were supplemented with modified starches, NaCl, and other spices and additives based on a San Miguel Pure Foods company recipe. The raw materials were trimmed and minced to ensure uniformity. They were then fine ground to create a consistent emulsion, with additives, spices, and crushed ice added at 10 °C. The emulsion was shaped using collagen casings, pasteurized at 80-85 °C for 60 min, and rapidly cooled. Finally, the low-fat sausages were vacuum-packed and then stored at 4 °C for further experiments.

2.3 Analytical methods

2.3.1 Proximate analysis

Protein content of sausages was analyzed by the Kjeldahl method (AOAC 992.15). Fat was determined in a Soxhlet apparatus using petroleum ether as an extraction solvent (AOAC 985.15). The gravimetric method was applied to determine moisture content (AOAC 950.46). Ash content was analyzed using a furnace at 550 °C for 4 h (AOAC 920.153). Carbohydrate was estimated by deducting the percentage of protein, lipid, moisture, and ash content. Calorie value was calculated based on proximate composition (carbohydrate or protein provides 4 Kcal/g, lipid provides 9 Kcal/g, according to Garcia-Santos et al. (2019)).

2.3.2 Total phenolic content and antioxidant capacity

Sausage samples were mixed and ground with methanol at a 1:4 w/v ratio. The resulting mixture was filtered to obtain a clear filtrate. The total phenolic content was determined by using spectrophotometric method with Folin Ciocalteu reagent (Hung et al., 2020). The antioxidant capacity was spectrophotometrically determined using a free radical scavenging method with 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical solution (Hung et al., 2020).

2.3.3 Cooking quality

The cooking quality was evaluated based on the method described by Tahmasebi et al. (2016). The cooking yield was assessed by weighing sausage samples before and after cooking at 80 °C for 60 min; this value was expressed as a percentage. To analyze the stability of emulsion, the meat batter (10 g) was weighed into tubes, then centrifuged at 3000 g for 5 min at 4 °C. The tubes were heated in a Water bath Memmert WNB22 at 80 °C for 60 min, then cooled to about 4 °C. The tubes were then placed upside down for 45 min to assist the separation of fat and water layers. The amount of fat released was calculated based on the weight difference of total liquid released before and after drying at 100 °C for 8 h and expressed as a percentage of the sample weight. Evaporated water was determined and also expressed as a percentage of the sample weight.

2.3.4 Instrumental colour and Texture profile analysis (TPA)

Instrumental color was estimated by colorimetric method using a CR-300 chronometer (Konica Minolta, Japan) and CIE Lab system. The textural properties were determined according to Garcia-Santos et al. (2019) with some modification, using a cylindrical probe (ϕ 20 mm diameter), attached to a Texture Analyzer (CT3, AMETEK Co., Ltd., Surrey, UK). The following parameters were derived: hardness (N), cohesiveness (dimensionless), springiness (mm), chewiness (N x mm), and gumminess (N). Ten replicates were measured per sample.

2.3.5 Scanning electron microscopy (SEM)

The samples were cut into 1-cm-thick slices, freeze-dried for 48 h in VirTis Benchtop Pro cubes of approximately 1 cm in length, and examined by scanning electron microscopy (SEM, Hitachi TM3000 instrument, Tokyo, Japan).

2.3.6 Overall acceptability of fat-reduced sausages

Sensory evaluation of sausage was evaluated by acceptance test (Garcia-Santos et al., 2019). The number of panelists was 110 people recruited from Saigon Technology University students. They were required to rate their preference for the sausages on a 9-point hedonic scale, corresponding to the range from “extremely dislike” (1 point) to “extremely like” (9 points).

2.3.7 Data analysis

Experiments were done in triplicate and results were presented as mean ± standard deviation. Analysis of variance was conducted using XLSTAT 2022 software, followed by Tukey's test at a 5 % significance level to compare the differences between experimental results.

3. Result and Discussion

3.1 Proximate composition, TPC and antioxidant capacity

The proximate composition, caloric value, TPC, and antioxidant capacity of the fat-reduced sausages are presented in Table 1. The results show that, the content of all chemical components of the fat-reduced sausages were significantly different compared to those of the control ($p<0.05$).

Table 1: Proximate composition and cooking quality of sausages

Sample	C100	T25	T50	T75	T100
Carbohydrate (%)	6.86 ± 0.16^{a}	7.52 ± 0.03^{b}	7.86 ± 0.13^{c}	8.48 ± 0.04^{d}	9.34 ± 0.14^{e}
Protein (%)	16.08 ± 0.76^{a}	17.59 ± 0.67^{b}	18.34 ± 0.38^{c}	19.09 ± 0.03^{d}	20.26 ± 1.41^{e}
Lipid (%)	20.29 ± 0.93^{e}	15.51 ± 0.15^{d}	12.76 ± 1.24^{c}	10.77 ± 0.15^{b}	7.71 ± 1.02^{a}
Ash (%)	2.077 ± 0.08^{a}	2.084 ± 0.20^{b}	2.259 ± 0.09^{c}	2.164 ± 0.01^{bc}	2.487 ± 0.04^{d}
Moisture (%)	54.69 ± 1.05^{a}	57.29 ± 0.62^{b}	58.77 ± 0.85^{c}	59.64 ± 0.08^{cd}	60.20 ± 0.90^{d}
Calories (Kcal/100 g)	274.37^{a}	240.03^{b}	219.68^{c}	205.89^{d}	187.80^{e}
TPC (mgGAE/g DB)	1.47 ± 0.05^{a}	1.53 ± 0.05^{a}	1.81 ± 0.06^{b}	1.96 ± 0.08^{c}	2.33 ± 0.01^{d}
DPPH scavenging activity (mgTE/g DB)	2.63 ± 0.02^{a}	2.66 ± 0.01^{a}	2.68 ± 0.05^{a}	2.81 ± 0.05^{b}	3.02 ± 0.02^{c}
Cooking yield (%)	93.27 ± 0.26^{a}	96.64 ± 0.01^{b}	96.77 ± 0.23^{b}	96.55 ± 0.10^{b}	97.53 ± 0.06^{c}
Emulsion stability					
Water loss (%)	3.50 ± 0.92^{b}	0.53 ± 0.01^{a}	0.19 ± 0.05^{a}	0.12 ± 0.03^{a}	0.14 ± 0.06^{a}
Fat loss (%)	4.51 ± 0.43^{d}	2.82 ± 0.19^{c}	2.24 ± 0.01^{bc}	1.83 ± 0.02^{b}	1.15 ± 0.25^{a}

The results are the mean value of three replicates. The value with different characters (in row) indicates a significant difference ($p < 0.05$); GAE: gallic acid equivalent; TE: trolox equivalent; DB: dry basis

Table 1 shows that increasing the percentage of fat replaced by germinated mung beans powder significantly increased carbohydrate, protein and ash content ($p<0.05$); at 100 % fat replacement, these values increased by 1.4, 1.3 and 1.2 times, compared to the control. The high starch, protein and ash content in germinated mung beans (Hung et al., 2020) was responsible for this increase. The sausage’s fat content was reduced approximately three times, from 20.29 % in the control sample C100 to 7.71 % in the sample T100, as the fat was removed and replaced with low-fat germinated mung beans. For low-fat meat products, water-binding ability is an important attribute affecting the quality of the product (Song et al., 2016). Germinated mung bean powder was hydrated before being formulated in the sausages, which enhanced the water-binding ability of the beans' carbohydrates and protein. This also increased the moisture content of the fat-reduced sausages. Studies of Mokni et al. (2018) that added chickpeas to sausages also reported similar results of increased protein and carbohydrate content. In a study of Zeb et al. (2021), sausages with added beans, bean starch, and chickpea flour showed reduced fat and increased moisture. Despite high protein and carbohydrate content, low content of lipid in fat-reduced sausages resulted in significantly lower energy values compared to the control ($p<0.05$); the reduction was 1.33 times when the fat replacement ratio increased from 0-100 %. A similar trend was observed when oat fiber and inulin were used to replace fat in Brazilian cooked sausage (Souza et al., 2019). Supplementing fat-reduced sausages with germinated mung beans at different ratios significantly increased the

TPC ($p<0.05$). The T100 sample, when replacing 100 % fat with germinated mung bean, showed the highest TPC and antioxidant capacity, increased 58.5 % and 14.8 %, compared to those of the control sample.

3.2 Cooking quality

In the emulsion-filled gels of sausages, fat can be filled into gel matrix of meat protein in the form of emulsion droplets stabilized with emulsifiers. According to Zhao et al. (2018), when fat is removed, the gel network of protein can be weakened, leading to increased water and fat release; fat reduction significantly decreased water-holding capacity and emulsion stability and using fat replacers could enhance these properties of reduced-fat sausages. The Table 1 show that, the emulsion stability of fat-reduced sausages was improved by supplementing germinated mung bean powder. Compared with the control, the percentages of water and fat loss decreased significantly by 6.6 and 1.6 times ($p<0.05$) when replacing 25 % of pork backfat with germinated mung bean powder. Further reductions in fat and increases in germinated mung beans addition did not show a significant effect on water separation ($p>0.05$) but continued to significantly reduce fat separation ($p<0.05$). Plant-based proteins such as soybean, chickpea, lentil, and mung beans are hydrophilic, aiding water retention. During cooking, they also form a gel network with starch, acting as fat-encapsulating agents, stabilizing the emulsion and preventing fat separation (Kamani et al. 2019). Like the emulsion stability, the cooking yield of fat-reduced sausages also increased. The cooking yield of sample with 100 % fat replacement by germinated mung bean was the highest and 4.26 % higher than that of the control. This is possibly because during heating, the water effectively bound with the gel matrix formed by the mung bean protein; the high starch content in germinated mung beans also improved water binding ability, further enhancing cooking yield and reducing cooking loss, as also observed when adding Adzuki bean to sausages in the study of Aslinah et al. (2018).

3.3 Instrumental colour

The instrumental colour and texture, and sensory acceptance of fat-reduced sausages are shown in table 2. It can be obsserved that, when germinated mung bean powder was used to replace 25 % and 50 % of fat, the lightness (L*) increased, and the redness (a*) decreased ($p<0.05$). The yellow colour (b*) showed no difference in comparison with the control ($p>0.05$). The red colour in heat-processed meat products is attributed to myoglobin and hemoglobin denaturation (Zao et al., 2018). Colour differences across sausage samples may be caused by the dilution of meat myoglobin and the addition of germinated mung beans in different proportions. Germinated mung bean powder (without hull) was bright in colour, which could contribute to the lighter and less red appearance of fat-reduced sausages. The lightness (L*) decreased when germinated mung bean powder was used to replace 50 % and 100 % of the fat. Simultaneously, fat-reduced sausage's redness (a*) and yellowness (b*) rose compared to samples with lower amounts of germinated mung bean supplementation. High ratio of added germinated mung beans increased protein and carbohydrate content of the sausages. This resulted in interactions between amino groups (NH_2) and carbonyl groups (Maillard reaction) that produce melanoidin compounds with yellow to brown hues, which darkens the product, particularly after cooking. In the research of Mokni et al. (2018), the authors reported that the darkening of the product was caused by a decrease in fat content when fat was replaced with chickpea protein, which is probably related to the light-scattering attributes of fat.

Table 2: Instrumental colour and texture, and sensory acceptance of fat-reduced sausages

Sample	C100	T25	T50	T75	T100
L*	46.93 ± 1.38^{bc}	47.43 ± 0.61^{cd}	49.30 ± 0.608^{d}	45.23 ± 1.50^{ab}	44.73 ± 0.90^{a}
a*	25.37 ± 2.10^{c}	19.67 ± 0.11^{a}	21.70 ± 0.55^{ab}	23.83 ± 1.93^{bc}	22.33 ± 1.06^{b}
b*	19.63 ± 1.51^{ab}	16.93 ± 0.32^{a}	18.87 ± 0.25^{a}	21.00 ± 1.27^{b}	19.20 ± 0.95^{ab}
Hardness (N)	31.74 ± 3.39^{a}	29.64 ± 1.54^{a}	31.01 ± 0.28^{a}	35.57 ± 1.22^{b}	40.91 ± 2.57^{c}
Cohesiveness	0.65 ± 0.03^{b}	0.65 ± 0.01^{b}	0.65 ± 0.01^{b}	0.66 ± 0.01^{b}	0.56 ± 0.01^{a}
Springiness (mm)	0.88 ± 0.07^{ab}	0.88 ± 0.02^{a}	0.88 ± 0.01^{a}	0.89 ± 0.07^{a}	0.87 ± 0.08^{a}
Gumminess (N)	20.54 ± 1.57^{a}	19.59 ± 0.83^{a}	20.24 ± 0.40^{a}	23.27 ± 0.80^{b}	22.81 ± 1.08^{b}
Chewiness (N*mm)	18.12 ± 1.31^{a}	17.41 ± 0.47^{a}	17.77 ± 0.57^{a}	20.80 ± 0.82^{b}	19.83 ± 0.75^{b}
Sensory acceptance	6.86 ± 1.21^{a}	5.90 ± 1.41^{b}	6.50 ± 1.19^{a}	5.53 ± 1.64^{b}	4.72 ± 1.21^{c}

The results are the mean value of three replicates. The value with different characters (in row) indicates a significant difference ($p < 0.05$)

3.4 Textural properties

In semi-solid foods like sausages, fats often have a melting, lubricating effect that leads to properties such as adhesiveness, cohesiveness of the product; the small fat droplets can also be filled into the gel matrix of the meat protein, which enhances hardness (Yashini et al., 2019). According to Zhao et al. (2018), reducing fat decreased the hardness of sausages, the increase of water content in fat-reduced sausages could result in a soft texture. As can be seen in Table 2, reducing 25 % and 50 % of fat did not significantly affect the textural characteristics of the sausages ($p>0.05$); in this case, using germinated mung beans as a partial fat-replacer contributed to stronger textural property and helped to overcome the adverse effects caused by removing fat from the sausages. However, the hardness of T75 and T100 (75 and 100 % fat reduction) increased significantly and reached 35.57 N and 40.91 N, 12 % and 29 % higher than that of the control. The increase in the hardness also led to an increase in the chewiness and gumminess of the fat-reduced sausages compared to the control ($p<0.05$). The sausage samples with 75-100 % fat substitution with germinated mung beans had significantly higher protein content than that of the control sample, leading to better protein gel formation. The SH groups in the legume protein molecules can form disulfide bonds and create a more stable protein network during the heating process, which could be the reason for the increase in texture profile parameters (Kamani et al., 2019). Alongside the role of protein, substituting fat with an excessive amount of germinated mung beans also drastically increased carbohydrate content, which could influence the myofibril gel network of meat protein. The competition for water between meat protein and mung bean starch can lead to a dry and hard myofibril gel, resulting in excessive hardness and chewiness. Similar trends were found, when using citrus fiber (Song et al., 2016), konjac gel and vegetable powder (Kim et al., 2019) to replace fat in sausages.

3.5 Microstructure of the fat-reduced sausages

The SEM images in Figure 1 show the influence of germinated mung beans addition on the microstructure of low-fat sausages. The samples displayed a cross-linked, fibrous, porous, three-dimensional network structure. Cross-sectional image of the control sample (C100) showed the formation of various-sized pores, creating a sponge-like or honeycomb-like structure. Tahmasebi et al. (2016) also described a sponge-like three-dimensional gel structure of the sausages. The formation of these pores may be due to the expansion of fat, water, and air during heating processes such as steaming, frying, and grilling of the sausages (Olanwanit et al., 2019). As observed in the SEM images, the samples with 25–50 % fat substitution by germinated mung bean (T25 and T50) exhibited smoother cross-sectional surfaces and fewer porosity holes than the control sample. Similar result was reported by Song et al. (2016), the authors found that adding citrus fiber turned the gel network into a compact, continuous structure of high fiber density, resulting in higher water binding capacity. For the T75 and T100 samples, extreme fat reduction led to the development of a coarse network with large pores allowing water to escape the structures. The excessive hardness of these samples was probably responsible for the brittle and fragile texture.

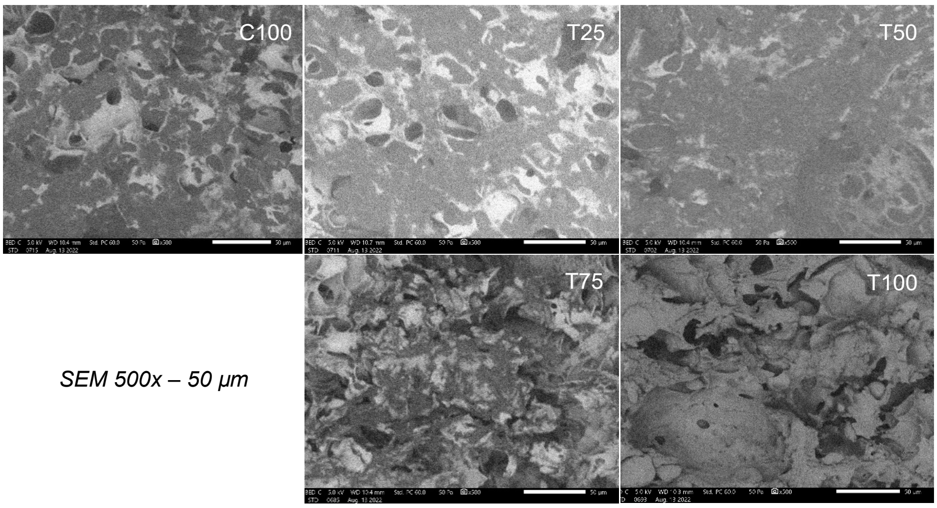

Figure 1: SEM images of fat-reduced sausages supplemented with germinated mung bean

3.6 Sensory acceptance

The results in Table 2 show a significant difference in acceptance scores assessed by students from Saigon Technology University among the samples ($p<0.05$). The control and T50 samples obtained the highest scores, 6.86 and 6.36, with no significant difference. Fat is a crucial component in sausages, and removing it affects sensory properties such as structure, appearance, and flavor. The consumers can perceive sensory differences when fat is replaced with alternative fat substitutes, which can influence their preference for the product (Yashini et al., 2019). In this research, replacing 50 % of the fat in sausages with germinated mung beans was feasible and did not adversely affect consumer preference. Similarly, in the study of Zhao et al. (2018), regenerated cellulose fiber was successfully applied to enhance the organoleptic properties of the emulsified sausages with 30 % fat reduction and maintain the same sensorial quality as the control sample.

4. Conclusions

Germinated mung bean is a potential ingredient that can be used to replace fat in sausages. Increasing the fat replacement ratio from 0-100 % with germinated mung beans increased carbohydrate, protein and ash content, reduced fat content and calorie value, enhanced the TPC and antioxidant capacity, and improved the cooking quality of the sausages. Replacing 25-50 % of the fat with germinated mung bean powder maintained the sausage's structure and color, while higher substitutions (75-100 %) resulted in darker color, excessive hardness, gumminess, and chewiness. Among the samples tested, the T50 sample, with 50 % fat replacement by germinated mung bean powder, exhibited the properties closest to the control sample and was the most preferred by the consumers.

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