

## Replacing animal fat with edible mushrooms: a strategy to produce high-quality and low-fat buffalo meatballs

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### Abstract

The present work investigated the effects of replacing the fat in meatballs with enoki, brown beech, shiitake, white oyster, brown, or king oyster mushrooms on the physicochemical and sensorial characteristics of the reduced-fat meatballs. The water holding capacity of the reduced-fat meatballs increased significantly (14.4 - 36.5%) in parallel to a significant increase in the moisture (3.3 - 5.6%) and protein (4.1 - 7.5%) contents, with a decrease in fat (16.3 - 86.3%) content as compared to the control. No differences were found for the cooking yield and texture properties of the samples, except for the king oyster mushroom which reduced the hardness of the reduced-fat meatballs. The redness of the reduced-fat meatballs was comparable with the control, but reduced in yellowness and lightness. Most of the reduced-fat meatballs had better sensory characteristics as compared to the control, in particular, the meatballs with shiitake mushroom. In conclusion, all the mushrooms tested have the potential to be used as a full fat replacer in meatballs, except for king oyster mushroom which reduced the texture quality of the reduced-fat meatballs.

### Keywords

low-fat meat products,  
fresh mushrooms,  
fat replacers,  
healthier meatballs,  
high-protein meatballs

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## Introduction

Healthier meat products are being formulated either by (1) promoting positive aspects such as enrichment with minerals, incorporation of prebiotics and probiotics, or improvement of amino acid quality, or (2) by reducing the health-issue factors such as reduction of fat, calories, cholesterol, sodium, phosphate, and/or nitrite in the meat products (Hathwar *et al.*, 2012; Olmedilla-Alonso *et al.*, 2013). As animal fat is associated with non-communicable diseases such as obesity and high cholesterol (Celada *et al.*, 2016; Drewnowski, 2018), the reduction of the fat content by replacing the animal fat fully or partially with other sources of protein (Aslinah *et al.*, 2018), carbohydrate, fibre-based ingredients (Carvalho *et al.*, 2019; Choe and Kim, 2019), or healthy lipid materials (Alejandre *et al.*, 2019; da Silva *et al.*, 2019) has become a topic of continuing research. Nevertheless, replacing the animal fat with healthier ingredients while retaining the functional properties generated by the fat, and maintaining the quality of the meat products produced is challenging (Brewer, 2012).

Olanwanit and Rojanakorn (2019) replaced the fat in Vienna sausages with hydrolysed collagen

and Man-sao powder which lowered the fat content and caloric value, while increasing the emulsion stability, moisture, and protein content. However, the structure of the sausages became more heterogeneous and compact. Furthermore, the panellists preferred the reduced-fat Vienna sausages with a maximum of 40% collagen and Man-sao powder. Ran *et al.* (2020) replaced the fat in meatballs with *Perilla* seed to reduce the fat content, increase the protein content, and improve the dietary fibre, while obtaining good emulsion stability and water retention. However, only 10% of *Perilla* seed could retain the texture and sensory attributes similar to the animal fat meatballs, as excessive *Perilla* seed deteriorated the hardness, elasticity, taste, and odour. Aslinah *et al.* (2018) used adzuki beans as a fat replacer to produce low-fat meatballs with a higher cooking yield and moisture content, but the hardness of the meatballs increased, and the panellists preferred 50% adzuki beans. One of the notable issues with the above-mentioned studies is that the ingredients used as the fat replacers could not be used to replace all the animal fat as they did not fully replicate the functional properties of the animal fat, thus affecting the final quality of the meat products, especially the sensory attributes. Such products are not considered low-fat meat products as

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they must contain 3% fat or less per 100 g (CAC, 1997).

Edible mushrooms have the potential to be used as a fat replacer due to their high protein (Moon and Lo, 2014) and fibre contents (Mehta *et al.*, 2015), contributing to the nutritional benefits (Chye *et al.*, 2008), and as a natural taste enhancer (Mattar *et al.*, 2018; Ang and Ismail-Fitry, 2019) of animal products. Mushrooms are rich in fungal proteins, chitin, essential amino acids, vitamins, minerals, and bioactive compounds identified as ergosterol (Chye *et al.*, 2008). Several studies have highlighted the application of mushrooms as meat analogues, such as the grey oyster mushroom in chicken patties (Wan Rosli *et al.*, 2011), and shiitake in pork sausages (Wang *et al.*, 2019a). Recently, there have also been attempts to use raw, boiled, fried, and deep-fried king oyster mushroom as replacements for pork back fat in sausages (Wang *et al.*, 2019b), and brown button mushroom in beef burgers (Patinho *et al.*, 2019). Different types of mushrooms and 100% fat replacement of fat may influence the physicochemical and sensorial properties of the meat products. Therefore, the present work evaluated the potential of enoki, brown beech, shiitake, white oyster, brown, and king oyster mushrooms to be used as a fat replacer in the production of reduced-fat meatballs.

## Materials and methods

### Sample preparation

Post-rigor topside buffalo meats were purchased from Giant Hypermarket, Seri Kembangan, Selangor. The buffalo meats and fat were prepared separately by cutting into small pieces, homogenised, and ground using a mincer with a 3.175 mm diameter disc (Hobart 4822, USA). Six types of fresh edible mushrooms [enoki (*Flammulina velutipes*), brown beech (*Hypsizygos tessellatus*), shiitake (*Lentinula edodes*), white oyster (*Pleurotus ostreatus*), brown (*Agaricus bisporus*), and king oyster (*Pleurotus eryngii*)] were also purchased from Giant Hypermarket. The mushrooms were rinsed with clean water, blanched, and ground using a blender (MX-900M Panasonic, Malaysia) to a uniform size. The mushrooms were blanched to inactivate proteolytic enzymes that can degrade myosin heavy chains and actin, thereby affecting the tenderisation of meats (Lee *et al.*, 2017).

The meatballs comprised of 74% meat, 10% fat, 4.2% corn flour, 1.2% sodium chloride (NaCl), 0.7% onion powder, 0.6% sugar, 0.5% sodium tripolyphosphate (STPP), 0.1% monosodium

glutamate (MSG), and 8.7% ice water. The fat (10%, w/w) in the control meatball (CM) was totally replaced with chopped mushrooms at a similar weight percentage. The six different types of the most commonly consumed species of mushroom were used for six different meatball formulations, and coded as EM (enoki), BBMM (brown beech), SM (shiitake), WOMM (white oyster), BMM (brown), and KOMM (king oyster). The experiments were performed in triplicate.

The meatballs were produced following the method of Aslinah *et al.* (2018) with slight modifications. All the ingredients were mixed using a bowl cutter (K3 Model-BenchType, Taiwan), except for NaCl, STTP, and ice water, for 15 min. Then, the NaCl and STPP were added, followed by ice water, and the mixing was continued for 5 min until the meat batter was homogenous. Each meatball weighing of  $10 \pm 0.5$  g was immersed in batches at 45°C water for 20 min, followed by boiling for 25 min at 80°C. The meatballs were drained and cooled before vacuum-packed and stored at -18°C. All samples were thawed to room temperature ( $27 \pm 1$ °C) before analyses.

### Water Holding Capacity (WHC)

The WHC was determined according to Dosh *et al.* (2016) with slight modifications. The sample (10 g) was thawed, ground, and mixed with 20 g of water using a homogeniser (Heidolph Diax 900, USA), then centrifuged (KUBOTA 5800, Japan) at 1,500 rpm for 5 min. The WHC was calculated using Eq. 1:

$$\text{WHC (\%)} = \frac{[(\text{water weight before centrifuge} - \text{water weight after centrifuge}) / (\text{sample weight})] \times 100}{\text{Eq. 1}}$$

### Cooking yield

The cooking yield was determined according to Serdaroglu *et al.* (2005) by calculating the weight difference for samples before and after cooking, using Eq. 2:

$$\text{Cooking yield (\%)} = \frac{(\text{final weight of cooked meatball} / \text{initial weight of uncooked meatball}) \times 100}{\text{Eq. 2}}$$

### Proximate analysis and calorific value

The AOAC method (AOAC, 2005) was used to determine the macronutrients' content of the meatballs. The moisture content was determined by drying the sample in an oven at 100°C overnight, and calculated based on the loss of weight upon drying.

The samples were ashed overnight at 550°C in a muffle furnace, and the percentage of ash after the sample was completely burned was determined (method no. 930.05). The crude protein content was determined by the Kjeldahl method, and the percentage of protein was obtained by multiplying the conversion factor (6.25) by the percentage nitrogen (method no. 978.04). The crude fat was determined by calculating the percentage of the oil in the sample with the Soxhlet extraction method (method no. 930.09). The carbohydrate was determined by difference. The experiments were performed in triplicate. The calorie content was determined based on the fat, protein, and carbohydrate contents, using Eq. 3:

$$\text{Calories (Kcal/100 g)} = (C \times 4) + (P \times 4) + (F \times 9) \quad (\text{Eq. 3})$$

where, C = carbohydrate content (g/100 g), P = protein content (g/100 g), and F = fat content (g/100 g).

#### Colour

The colour was determined according to Dzudie *et al.* (2002) with slight modifications. Approximately, 20 g of samples were thawed to room temperature ( $27 \pm 1^\circ\text{C}$ ), ground with a blender (MX-900M Panasonic, Malaysia), packed, and spread evenly in a 5 cm-diameter transparent packaging plastic. The samples were evaluated for lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ) using a chromameter CR-410 (Konica Minolta, Japan) with an aperture of 50 mm, and set up to illuminate D65. The analysis was performed in triplicate.

#### Texture profile analysis

The meatballs were reheated in a microwave for 2 min, cooled to room temperature ( $27 \pm 1^\circ\text{C}$ ), then trimmed to produce a similar diameter for the analysis. TPA was performed using a computer-assisted, Stable Micro Systems Texture analyser (TA.XT-Plus, London) to determine the hardness, cohesiveness, chewiness, and springiness. The samples were tested using a probe, 75 mm square compression platen type with double compression at 50% strain. The texture analyser was set with load cell = 25 kg, pre-test speed = 2.00 mm/s, test speed = 2.00 mm/s, post-test speed = 5.00 mm/s, distance = 10.00 mm, with an auto trigger type (5.0 g) (Huda *et al.*, 2010). The analysis was performed in triplicate.

#### Sensory evaluation

The sensory evaluation was scored using a

nine-point hedonic scale (1 = “dislike extremely”, 2 = “dislike very much”, 3 = “dislike moderately”, 4 = “dislike slightly”, 5 = “neither like nor dislike”, 6 = “like slightly”, 7 = “like moderately”, 8 = “like very much”, and 9 = “like extremely”). The thawed meatballs were reheated in the microwave, and served warm (30 - 40°C) to 30 untrained panellists in one session in a sensory laboratory, monadically. The samples were coded with three-digit random numbers, and the presentation was random. The sensory attributes assessed were the appearance, texture, juiciness, flavour, and overall acceptability of the meatball samples (Ikhlas *et al.*, 2011).

#### Statistical analysis

The statistical analysis was performed using Minitab Statistical Software version 17 (MiniTab Inc., USA). The data were analysed by one-way ANOVA, considering the treatments (different mushrooms) as the fixed effect, and the replicates as a random effect, with Tukey’s test at a significance level of 95% ( $p < 0.05$ ). The sensory data collected from 30 untrained panellists were analysed by one-way ANOVA, considering the treatments (different mushrooms) as the fixed effect, and the panellists (gender, age, and tasting order) as the random effect, with Tukey’s test at a significance level of 95% ( $p < 0.05$ ). Pearson correlation was conducted to determine the correlation between the selected data.

## Results and discussion

#### Water holding capacity and cooking yield

The WHC and cooking yields of the different meatball formulations are given in Figure 1. The replacement of fat in the meatballs with blanched and ground mushrooms significantly increased ( $p < 0.05$ ) the WHC by 15.5 to 36.5% as compared to the control (CM) without affecting their cooking yield ( $p > 0.05$ ). Enoki (77.24%) and shiitake (77.18%) mushrooms yielded the highest WHC, followed by brown mushroom (69.18%), white oyster mushroom (60.48%), brown beech mushroom (56.26%), and king oyster mushroom (59.16%). A high WHC is necessary to maintain the moisture content and juiciness of the product, thereby contributing to the necessary lubrication while chewing (Juárez *et al.*, 2012).

Cooking yield is a good indicator to explain the effect of WHC in meat products. In general, the cooking yield of a meat product is influenced by its ability to retain water and fat during cooking (Cheng and Sun, 2008). The present work demonstrated that

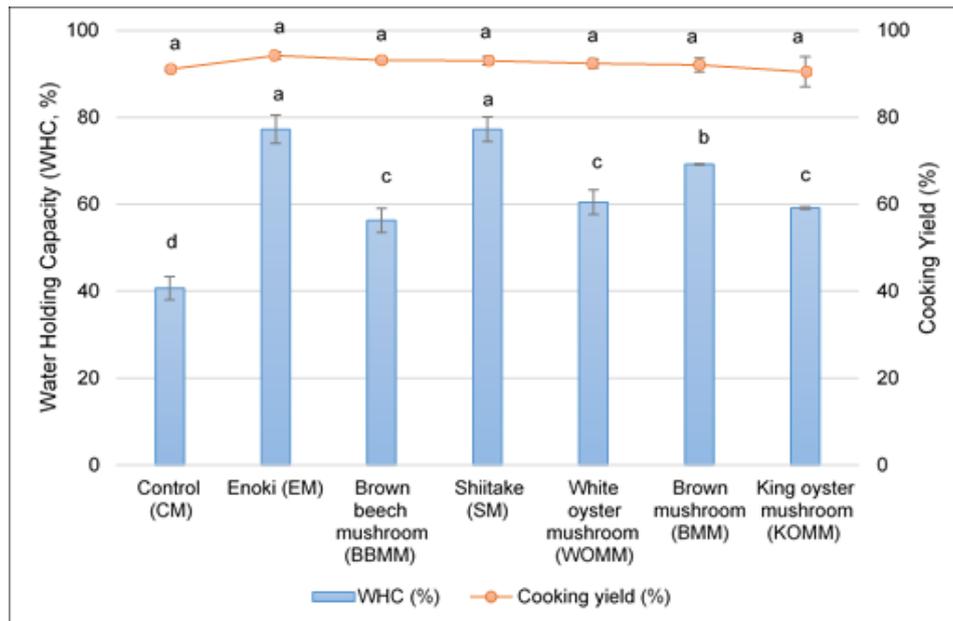


Figure 1. Water holding capacity (WHC) and cooking yield of meatballs incorporated with edible mushrooms as a fat replacer. Values are mean  $\pm$  standard deviation of three replicates ( $n = 3$ ). Means that do not share the same lowercase are significantly different ( $p < 0.05$ ).

the addition of mushrooms could significantly replace fat, and achieve the same cooking yield of the meatballs by increasing the WHC of the comminuted meat system. Mushrooms consist of largely polysaccharides and high-quality proteins that create a three-dimensional matrix to bind water in the meat system, thus contributing to comparable cooking performance, and mimicking the role of fat to create an emulsion.

Brewer (2012) described that the denaturation of protein by heat, pH, or enzymatic approaches can significantly affect the physicochemical properties of a product such as water holding ability, especially for plant-based proteins. Once the protein tertiary structure is denatured, the protein behaves more like fat. This is in line with Akoh (1998) who explained the utilisation of a protein-based fat replacer in a processed meat product to retain water, thus providing a mouth feel as well as texturing the final product similar to the role of fat.

#### Proximate composition

Table 1 provides the proximate compositions of the seven meatballs tested, while those of buffalo meat and mushrooms from published literatures are shown in Table 2 for comparison. All reduced-fat meatball samples exhibited a significantly higher ( $p < 0.05$ ) moisture content ranging from 71.40 to 73.20%, except for EM (70.89%), as compared to CM (67.57%). As shown in Table 2, mushrooms

contain about 87.9 to 91.6% moisture, which is slightly higher than that of the reduced-fat meatballs tested in the present work. Table 2 also shows that enoki and shiitake mushrooms contained the lowest moisture contents among the mushroom species, as similarly found in the reduced-fat meatballs tested in the present work.

The incorporation of mushroom into the meatball formulation significantly increased ( $p < 0.05$ ) the protein content of the meatballs by 4.1 to 7.5%, as compared to the control sample. The protein content of the reduced-fat meatballs ranged from 12.32% (KOMM) to 15.70% (BBMM) with no statistical difference between the samples ( $p > 0.05$ ). Reis *et al.* (2012) reported that brown mushroom and king oyster mushroom contain almost double the protein content as compared to the other commonly consumed mushroom species such as shiitake, oyster mushroom, and enoki mushroom as shown in Table 2. The most abundant amino acids in mushrooms are leucine, valine, glutamine, glutamic, and aspartic acids which are comparable to the amino acid composition of animal proteins (Longvah and Deosthale, 1998; Mattila *et al.*, 2002). The ash content of the reduced-fat meatballs ranged from 1.02 to 1.57%, which was comparable to CM (1.69%). In meatball products, ash is mainly sourced from the salt added during the meatball production (Hsu and Yu, 1999).

All reduced-fat meatballs significantly had a reduced ( $p < 0.05$ ) fat content as compared to CM

Table 1. Proximate analysis of meatballs incorporated with edible mushrooms as a fat replacer.

Sample	Moisture (% w/w)	Ash (% w/w)	Protein (% w/w)	Fat (% w/w)	Carbohydrate (% w/w)	Calorie (Kcal)
CM	67.57 ± 2.42 <sup>b</sup>	1.69 ± 0.50 <sup>a</sup>	8.19 ± 1.32 <sup>b</sup>	9.22 ± 0.22 <sup>a</sup>	12.98 ± 0.22 <sup>a</sup>	167.66 ± 5.22 <sup>a</sup>
EM	70.89 ± 1.70 <sup>ab</sup>	1.57 ± 0.12 <sup>a</sup>	13.93 ± 1.02 <sup>a</sup>	4.35 ± 2.68 <sup>bc</sup>	7.74 ± 3.21 <sup>bc</sup>	125.89 ± 10.05 <sup>c</sup>
BBMM	72.38 ± 1.12 <sup>a</sup>	1.32 ± 0.06 <sup>a</sup>	15.70 ± 1.84 <sup>a</sup>	7.72 ± 0.70 <sup>ab</sup>	2.53 ± 0.04 <sup>d</sup>	142.36 ± 3.83 <sup>b</sup>
SM	71.40 ± 0.95 <sup>a</sup>	1.02 ± 0.57 <sup>a</sup>	13.66 ± 0.44 <sup>a</sup>	1.53 ± 0.70 <sup>c</sup>	11.99 ± 0.34 <sup>ab</sup>	116.39 ± 1.74 <sup>cd</sup>
WOMM	72.93 ± 0.37 <sup>a</sup>	1.35 ± 0.01 <sup>a</sup>	14.69 ± 1.08 <sup>a</sup>	4.53 ± 0.35 <sup>bc</sup>	6.01 ± 0.52 <sup>cd</sup>	123.54 ± 2.47 <sup>cd</sup>
BMM	72.96 ± 0.47 <sup>a</sup>	1.25 ± 0.17 <sup>a</sup>	14.06 ± 0.40 <sup>a</sup>	1.27 ± 0.58 <sup>c</sup>	10.16 ± 0.52 <sup>abc</sup>	108.26 ± 0.70 <sup>d</sup>
KOMM	73.20 ± 0.40 <sup>a</sup>	1.38 ± 0.46 <sup>a</sup>	12.32 ± 1.96 <sup>a</sup>	2.92 ± 0.12 <sup>c</sup>	9.12 ± 0.47 <sup>abc</sup>	112.00 ± 7.77 <sup>cd</sup>

CM = control meatball; EM = enoki meatball; BBMM = brown beech mushroom meatball; SM = shiitake meatball; WOMM = white oyster mushroom meatball; BMM = brown mushroom meatball; and KOMM = king oyster mushroom meatball. Values are mean ± standard deviation of three replicates ( $n = 3$ ). Means in the same column that do not share the same lowercase superscript are significantly different ( $p < 0.05$ ).

Table 2. Proximate analysis of buffalo meat and mushrooms.

Sample	Moisture (% w/w)	Ash (% w/w)	Protein (% w/w)	Fat (% w/w)	Carbohydrate (% w/w)	References
Buffalo meat	76.3	0.98	20.39	1.37	0.96	
Enoki mushroom	87.87	0.88	0.47	0.21	10.57	
Brown beech mushroom	91.40	0.74	2.46	0.41	4.99	Tsai <i>et al.</i> (2019); Reis <i>et al.</i> (2012);
Shiitake mushroom	79.78	1.36	0.89	0.35	17.62	Naveena and Kiran (2014);
White oyster mushroom	89.17	0.62	0.76	0.15	9.3	
Brown mushroom	91.64	0.95	1.29	0.14	5.98	
King oyster mushroom	89.00	0.68	1.21	0.16	8.95	

(9.22% fat), except for BBMM (7.72% fat) ( $p > 0.05$ ), because mushrooms contain a very small amount of fat between 0.14 to 0.50%. Brown beech mushroom contained the highest fat amount (0.41%), almost double the amount of other mushrooms, resulting in meatballs with the highest fat content. According to Codex Alimentarius Commission (CAC, 1997), a product is 'low-fat' if its fat content is not more than 3 g per 100 g of sample. Therefore, KOMM (2.92% fat), SM (1.53% fat), and BMM (1.27% fat) could be categorised as low-fat meatballs, and serve as a healthier alternative to meat products.

Interestingly, the carbohydrate content of the reduced-fat meatballs was inversely correlated to their fat content, with a Pearson's correlation coefficient ( $r$ ) of -0.968. The low-fat meatballs, namely KOMM, SM, and BMM contained the highest carbohydrate at 9.12, 11.99, and 10.16%, respectively. However, they were lower than CM, thus providing advantages over conventional

meatballs. Regarding the caloric value of the reduced-fat meatballs, they ranged between 108.3 (BBM) to 142.4 Kcal/100 g (BBMM), which was 15 to 35.4% lower than CM (167.7 Kcal/100 g). The caloric value of the meatballs was strongly correlated with their fat content (Pearson's correlation coefficient,  $r = 0.952$ ) since fat is a dense energy source. The reduced-fat meatballs were BMM, KOMM, and SM with a calorie content around 110 Kcal/100 g, contributing to less than 5.0 and 5.8% of the recommended daily intake of energy for adult men and women, respectively, compared to CM at 7.7 and 8.8%, based on the consumption of 100 g meatballs according to the Recommended Nutrient Intakes for Malaysia (MOH, 2017).

#### Colour

The colour of meatball products is the primary factor that influences consumer acceptability, and may be affected by various factors such as chemical state and concentration of meat

Table 3. Colour parameters (lightness,  $L^*$ ; redness,  $a^*$ ; and yellowness,  $b^*$ ) of meatballs incorporated with edible mushrooms as a fat replacer.

Sample	$L^*$	$a^*$	$b^*$
CM	79.00 ± 0.10 <sup>a</sup>	5.36 ± 0.90 <sup>a</sup>	15.82 ± 0.72 <sup>a</sup>
EM	79.43 ± 0.67 <sup>a</sup>	8.98 ± 1.29 <sup>a</sup>	12.94 ± 0.13 <sup>bc</sup>
BBMM	76.82 ± 0.10 <sup>b</sup>	6.29 ± 0.28 <sup>a</sup>	12.84 ± 0.21 <sup>bc</sup>
SM	71.93 ± 1.07 <sup>d</sup>	6.83 ± 2.10 <sup>a</sup>	14.65 ± 0.78 <sup>a</sup>
WOMM	74.36 ± 0.04 <sup>c</sup>	6.55 ± 0.57 <sup>a</sup>	15.22 ± 0.03 <sup>a</sup>
BMM	73.10 ± 0.20 <sup>cd</sup>	5.81 ± 0.33 <sup>a</sup>	11.88 ± 0.09 <sup>c</sup>
KOMM	78.98 ± 0.28 <sup>a</sup>	6.90 ± 0.78 <sup>a</sup>	14.48 ± 0.07 <sup>ab</sup>

CM = control meatball; EM = enoki meatball; BBMM = brown beech mushroom meatball; SM = shiitake meatball; WOMM = white oyster mushroom meatball; BMM = brown mushroom meatball; and KOMM = king oyster mushroom meatball. Values are mean ± standard deviation of three replicates ( $n = 3$ ). Means in the same column that do not share the same lowercase superscript are significantly different ( $p < 0.05$ ).

pigments, physical state of the meat, and the presence of non-meat ingredients in the formulation (Sáyago-Ayerdi *et al.*, 2009). The effect of mushroom incorporation on the colour (lightness,  $L^*$ ; redness,  $a^*$ ; yellowness,  $b^*$ ) of the meatballs is shown in Table 3. There was no significant difference ( $p > 0.05$ ) in redness observed in the reduced-fat meatballs and CM. However, the lightness and yellowness were significantly affected. The lightness of EM and KOMM were comparable to CM ( $p > 0.05$ ), while other reduced-fat meatballs were significantly darker in colour ( $p < 0.05$ ) in the order of increasing intensity as follows: BBMM < WOMM ≤ BMM ≤ SM. This may be explained by the naturally black-brown appearance of fresh

shiitake as compared to the other mushrooms. Cerón-Guevara *et al.* (2020) also showed that the colour of frankfurter sausages was affected by the addition of white oyster mushroom and brown mushroom. The yellowness of SM, WOMM, and KOMM was comparable with CM ( $p > 0.05$ ), with only EM, BBMM, and BMM being significantly lower than CM ( $p < 0.05$ ). Moon and Lo (2014) stated that frozen mushrooms maintain their colour when soaked and blanched prior to storage and further processing. Overall, KOMM was the most comparable in colour to CM.

#### Texture

Table 4 presents the experimental TPA data

Table 4. Textural properties of meatballs incorporated with edible mushrooms as a fat replacer.

Sample	Hardness (kg)	Cohesiveness	Springiness (mm)	Chewiness (kg.mm)
CM	6.96 ± 1.32 <sup>ab</sup>	0.67 ± 0.03 <sup>a</sup>	0.84 ± 0.05 <sup>a</sup>	3.94 ± 0.90 <sup>a</sup>
EM	8.52 ± 2.08 <sup>ab</sup>	0.69 ± 0.07 <sup>a</sup>	0.87 ± 0.03 <sup>a</sup>	5.10 ± 1.46 <sup>a</sup>
BBMM	8.24 ± 0.91 <sup>ab</sup>	0.69 ± 0.04 <sup>a</sup>	0.90 ± 0.05 <sup>a</sup>	5.12 ± 0.74 <sup>a</sup>
SM	10.06 ± 0.43 <sup>a</sup>	0.69 ± 0.04 <sup>a</sup>	0.89 ± 0.01 <sup>a</sup>	6.22 ± 0.68 <sup>a</sup>
WOMM	8.44 ± 0.69 <sup>ab</sup>	0.67 ± 0.01 <sup>a</sup>	0.88 ± 0.01 <sup>a</sup>	5.03 ± 0.41 <sup>a</sup>
BMM	7.93 ± 3.13 <sup>ab</sup>	0.66 ± 0.06 <sup>a</sup>	0.89 ± 0.05 <sup>a</sup>	4.83 ± 2.43 <sup>a</sup>
KOMM	4.64 ± 0.58 <sup>b</sup>	0.70 ± 0.03 <sup>a</sup>	0.88 ± 0.02 <sup>a</sup>	2.88 ± 0.54 <sup>a</sup>

CM = control meatball; EM = enoki meatball; BBMM = brown beech mushroom meatball; SM = shiitake meatball; WOMM = white oyster mushroom meatball; BMM = brown mushroom meatball; and KOMM = king oyster mushroom meatball. Values are mean ± standard deviation of three replicates ( $n = 3$ ). Means in the same column that do not share the same lowercase superscript are significantly different ( $p < 0.05$ ).

that measured the intensity of hardness, cohesiveness, springiness, and chewiness of the reduced-fat meatballs. Overall, there was no significant difference observed between the reduced-fat meatballs and CM in all texture attributes studied ( $p > 0.05$ ), except for hardness. The reduced-fat meatballs had a comparable hardness (7.93 to 10.06 kg) to CM (6.9 kg), except for KOMM (4.4 kg) ( $p < 0.05$ ). BMM yielded the closest values in all attributes to CM, while KOMM had lower values, especially in hardness and chewiness. Overall, these values were better than the use of adzuki beans as a fat replacer in a previous study (Aslinah *et al.*, 2018).

The findings of the present work, however, disagree with Wan Rosli *et al.* (2011) who stated that the addition of different levels of grey oyster mushroom as meat replacer decreased the hardness of chicken patties. This shows that the incorporation of fresh mushroom into meat products is more successful for red meat-based products with a coarser myofibrillar protein texture as compared to the chicken-based product, because the texture of the mushroom fibres can replace the fibrous texture of the myofibrillar protein fibres. Also, substituting the fat with mushroom is a better strategy than replacing the meat as it did not jeopardise the texture. Therefore, the mushroom fibres at 10% (w/w) can integrate into the largely myofibrillar meatball matrix without affecting the textural integrity of the

meatballs. Serdaroğlu *et al.* (2005) described that the textural properties in meat products may be affected by the degree of myofibrillar proteins extracted, the content of stromal protein, as well as non-meat ingredient type and level. Nonetheless, the textural qualities of the meatballs must be evaluated along with a sensory evaluation to identify the most acceptable product for consumers.

### Sensory

The results of the sensory analysis of reduced-fat meatballs are summarised in Figure 2 in which panellists rated all samples using a nine-point hedonic scale. The scores obtained for all sensory attributes were in the range of 5.10 to 7.03, which fell under the category of 'Like'. The appearance was found to be the critical attribute that determined consumer acceptance. The appearance scores of all reduced-fat meatballs were comparable to CM ( $p > 0.05$ ) which were above 6.5, except BMM (5.1) which was lower ( $p < 0.05$ ). BMM was the darkest, and had the lowest yellowness (Table 3) as compared to other meatballs as discussed earlier.

There were no significant differences in the texture and juiciness attributes of the samples (noted with asterisk (\*) symbol in Figure 2). The texture was positively correlated to the TPA results, while the juiciness was positively correlated to the cooking yield values as earlier discussed, which showed no difference ( $p > 0.05$ ) between all the samples.

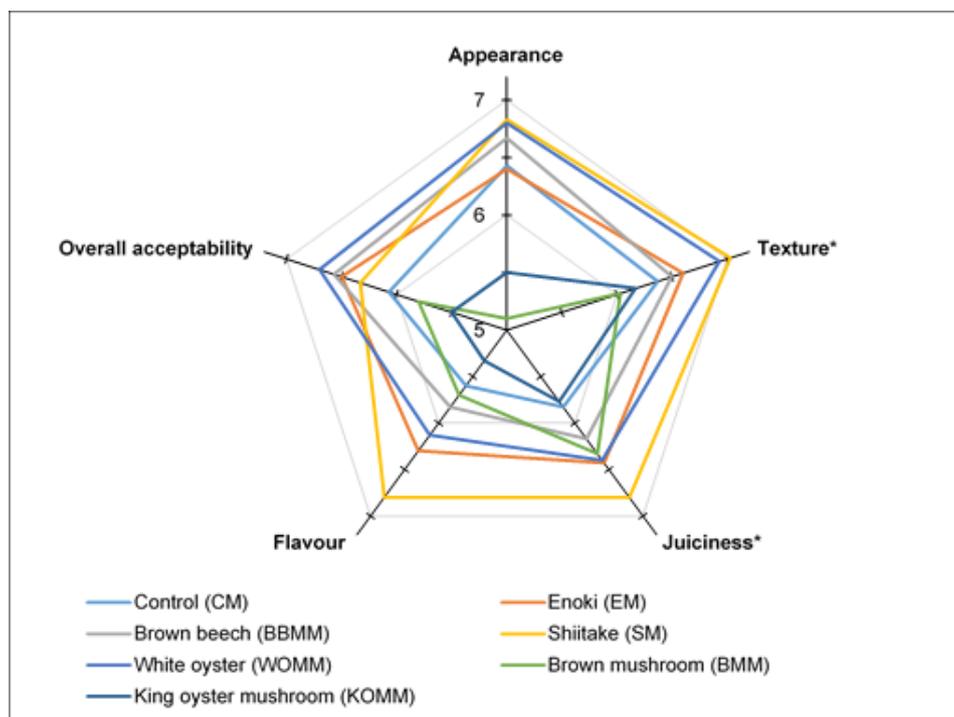


Figure 2. Sensory evaluation of meatballs incorporated with edible mushrooms as a fat replacer. Attributes with asterisk (\*) symbol are not significantly different ( $p > 0.05$ ) between samples.

Regarding the flavour attribute of the meatballs, SM (6.80) received the highest score, and KOMM (5.33) had the lowest, almost similar to CM (5.60). Patinho *et al.* (2021) reported that the overall score of beef burgers incorporated with cooked shiitake mushroom as a fat substitute was associated with the aroma score. As described by Pil-Nam *et al.* (2015), shiitake mushrooms promote the sensorial values of pork frankfurter by increasing the taste and flavour. Shiitake extract has been used in beef burgers as an alternative to sodium in the development of low-salt meat products (Mattar *et al.*, 2018).

Mushrooms contain valuable taste-active compounds including flavour 5'-nucleotides, which consist of the respective 5'-guanosine monophosphate, 5'-inosine monophosphate, and 5'-xanthosine monophosphate (Pil-Nam *et al.*, 2015). 5'-Guanosine monophosphate imparts a meaty and pleasant flavour that is much stronger than MSG (Beluhan and Ranogajec, 2011; Pil-Nam *et al.*, 2015); thus, is used as a natural flavour enhancer that contributes to umami or palatable taste when incorporated into the meat products (Dermiki *et al.*, 2013). This was proven when enoki, bunashimeji, and oyster mushrooms protein hydrolysates added to plain chicken soup showed better overall preference as compared to the control (Ang and Ismail-Fitry, 2019). Moreover, Qing *et al.* (2020) observed the enrichment of volatile compounds in meat products from the interaction effects with mushroom powder, thus contributing to the prominent aroma of 3-octanone and 1-octen-3-ol/3-octanol, which are the characteristic flavour of mushrooms.

For the overall acceptability of the reduced-fat meatballs, WOMM (6.70) was the most preferred, while KOMM (5.50) was the least preferred. This is similar to a study by Wang *et al.* (2019b) who reported that the panellists did not prefer the raw and boiled king oyster mushroom as a fat replacer in pork sausage. Overall, the present sensory analysis data showed that SM received the highest scores for all sensory attributes (Figure 2), specifically for appearance, texture, juiciness, and flavour, and had comparable overall acceptability to CM. Thus, the addition of shiitake to the meatball formulation could increase consumer acceptability of meatballs by improving the appearance, texture, juiciness, and flavour of the product.

## Conclusions

The present work demonstrated that mushrooms can be an excellent fat replacer in meatball formulations, providing a comparable

cooking yield with the control sample by increasing the water holding capacity of the meatballs. The replacement of 10% (w/w) fat with different types of blanched mushrooms significantly increased the protein and moisture contents, and reduced the fat content of the meatballs. The high-quality mushroom proteins integrate well with the red meat myofibrillar proteins to form a three-dimensional matrix with comparable texture properties (hardness, cohesiveness, springiness, and chewiness) to the control sample. The results of the texture attributes from the sensory evaluation showed an excellent agreement with the TPA data, while the juiciness attribute scores were in line with the cooking yield. The appearance and flavour attributes of the reduced-fat meatballs were comparable to the control sample, except for the king oyster mushroom meatball which was also the least preferred. Overall, the shiitake mushroom meatball had the highest score for all sensory attributes, especially flavour, and together with the brown beech mushroom meatball can be classified as 'low-fat' products according to Codex Alimentarius Commission. Therefore, the shiitake mushroom could be a potential fat replacer in meatball formulations. Future work should evaluate the shelf life, microbiological quality, water activity, and lipid oxidation of the reduced-fat meatballs.

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