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Incorporation of shiitake (*Lentinula edodes*) and brown button mushroom (*Agaricus bisporus*) as fat replacers in meatballs processed with different mixing times

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Abstract

Shiitake and brown button mushrooms have high protein and fibre content and can potentially be utilised as a fat replacer in meat products like meatballs. Meanwhile, different mixing times during meatball manufacturing may also influence product quality. The present work investigated the physicochemical, microstructure and sensory properties of fat-replaced meatballs with shiitake (*Lentinula edodes*) and brown button mushroom (*Agaricus bisporus*) processed at different mixing times (5, 10 or 20 min) using bowl-cutter. No differences ($p<0.05$) were found for the linear expansion, water holding capacity, texture profile analysis and Warner Bratzler for both types of mushrooms and mixing times. Shiitake meatballs mixed at 20 min have a higher ($p<0.05$) cooking yield while the total soluble protein is higher ($p<0.05$) regardless of any mixing time. The redness (a*) value of shiitake meatballs increases ($p<0.05$) as the mixing time increases. Microstructure images showed improved surface compositions of meatballs as the mixing time increased. Nevertheless, brown button meatballs with a mixing time of 20 min have the highest ($p<0.05$) scores in terms of texture and juiciness. In conclusion, both shiitake and brown button mushrooms are suitable to be used as fat replacers but a longer mixing time produced better quality meatballs.

1. Introduction

Meat emulsion-based products such as meatballs are usually made up of meat protein, animal fat and water, along with non-meat additives including salt, phosphates, flour and spices (Purnomo and Rahardiyan, 2008; Khalid *et al*., 2021). Meatballs are referred to as 'bebola daging' in Malaysia (Aslinah *et al*., 2018). They are among the most popular snack items in Malaysian markets and are easily accessible (Huda *et al*., 2009). Meatballs are well known for their juiciness and flavourful taste due to the amount of animal fat that are being incorporated during the process of making meatballs (Huang *et al*., 2005). The organoleptic qualities of the meatball, such as flavour, texture, juiciness and binding capabilities that can greatly affect the eating quality of the meat product are greatly influenced by the animal fat (Yılmaz and Dağlıoğlu, 2003; Huang *et al*., 2005). Nevertheless, despite these benefits, meatballs still need a significant amount of added fat, and excessive consumption of these meat products are not recommended, especially for specific demographic groups (Asgar *et al*., 2010) as fat is directly related to health problems such as obesity and coronary heart disease (Yang *et al*., 2017). Therefore, in recent years, to avoid the chance of getting various diseases, the demand towards reduced-fat meat products is growing as consumers have shifted towards a healthier meat derivative (Asioli *et al*., 2017; Khalid *et al*., 2021). The incorporation of fat replacers to replace animal fat in meat products is one of the most vital approaches to enhance the health quality of meat products (Asyrul-Izhar *et al*., 2022).

Different types of edible mushrooms have the potential to be used as a fat replacer due to their high fibre and protein content (Moon and Loo, 2014; Mehta *et al*., 2015; Cardoso *et al*., 2019) and as a natural flavour enhancer for animal products (Mattar *et al*., 2018; Ang and Ismail-Fitry, 2019). Scientific studies have evaluated

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the nutritional value and functional potential of various edible mushroom species because of the nutritional properties and the significant growth in mushroom production and consumption (Patinho *et al*., 2019). Among mushrooms, shiitake mushroom (*Lentinula edodes*) and brown button mushroom (*Agaricus bisporus*) have a high potential to be utilised as a fat replacer in meat-based products due to their natural characteristics. Several studies have highlighted that the shiitake mushroom is characterised by its lower fat content, high nutritional value and contains several bioactive compounds, including polysaccharides and dietary fibre (Jiang *et al*., 2015; Ramle *et al*., 2021), and brown button mushrooms which have a high considerable amount of fibre content and antioxidant activity which displayed their potential for application in different foods (Manzi *et al*., 2001; Barros *et al*., 2008; Patinho *et al*., 2019).

A good mixing process could produce very high quality meat products. Generally, mixing is conducted to homogenise the ingredients, extract the protein, reduce the size of the meat particle, and enhance the interaction of the protein network to achieve a stable emulsion (Abdolghafour and Saghir, 2014; Murad *et al*., 2017). Various mixing techniques have been investigated to compare different mixing equipment, continuous or intermittent mixing procedures as well as mixing time (Murad *et al*., 2017; Asyrul-Izhar et *al*., 2021; Ismail *et al*., 2021) as a proper mixing process especially the mixing time could affect the properties of the final products. A shorter mixing time can result in insufficient binding, while a longer mixing time might overwork the mixture and produce undesirable product properties (Álvarez *et al*., 2007; Devine and Dikeman, 2014). Therefore, the present study evaluated the potential of shiitake and brown button mushrooms to be used as a fat replacer in reduced-fat meatballs produced with different mixing times. The findings are expected to be beneficial as a reference for meat product manufacturers and future research.

2. Materials and methods

2.1 Materials

Post-rigor topside buffalo meats were purchased from Shahrul Fresh and Frozen, Kajang, Selangor. Two types of fresh edible mushrooms [shiitake (*Lentinula edodes*) and brown button (*Agaricus bisporus*)] and other ingredients were bought at ECON SAVE, Cheras and Lotus's IOI CITY MALL, Putrajaya, Selangor. The buffalo meat was prepared by cutting them into small pieces, homogenized, and ground using a mincer with a 3.175 mm diameter disc (Hobart 4822, USA). The mushrooms were rinsed with clean water, blanched in boiling water for 40 seconds, and ground using a blender (MX-900M Panasonic, Malaysia) to a uniform size. The meatballs comprised 74% meat, 10% shiitake or brown button mushroom (to replace the 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.

2.1.1 Processing of meatballs

The meatballs were produced following the method of Ramle *et al*. (2021) with slight modifications. All the ingredients were weighed according to the designated formulation and were mixed using a silent bowl cutter (TQ-5, Fresh, Taiwan). All of the ingredients were placed in the silent bowl cutter and mixed for different mixing times (5 minutes, 10 minutes or 20 minutes) except for ice water that was added at the last stage of the mixing procedure to control the mixture temperature. The round meatballs were formed manually with a weight of 10±0.5g each and were immersed in batches at 45°C water for 20 min to set. The set meatballs were further placed in hot water at approximately 80°C for 25 minutes. Then, the excessive water was drained out and the meatballs were left to cool at room temperature before being vacuum-packed followed by storage in a freezer (-18°C) until further analysis. Three replications of each formulation were prepared.

2.2 Cooking yield

The cooking yield of buffalo meatballs was expressed in percentage using the method described by Serdaroğlu *et al*. (2005). The initial weight of the raw sample (Wi) is recorded before cooking and the final weight (Wf) was recorded after being cooked in hot water at 80°C for 25 min and cooled down for 10 minutes at room temperature. The equation for calculating cooking yield is:

$$\text{Cooking Yield (\%)} = Wf/Wi \times 100$$

2.3 Linear expansion

The initial diameter of the raw sample before cooking and the final diameter were measured after being cooked and cooling for 10 minutes at room temperature. The diameter was measured manually using a Vernier calliper (Mitutoyo, Japan). Then the measurement was calculated as described by Julianty *et al*. (1994):

$$\text{Linear expansion \%} = \text{(diameter final-diameter initial))/(diameter initial)} \times 100\%$$

2.4 Water holding capacity

The water holding capacity (WHC) of meatballs was

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measured using the expressible moisture method (Ramírez *et al*., 2002). The sample was thawed for 30 min at room temperature before being cut in half. The sample was trimmed carefully until the final weight of 8 g before being wrapped inside filter paper Whatman no.1 (601) size 125 mm and loaded in a 50 ml centrifuge tube. Then, the sample was centrifuged at 1500 rpm, 25ºC for 15 minutes using a high-speed centrifuge (Centrifuge Kubota 3740, Kubota Corp., Japan), using a modified parameter (Ramle *et al*., 2021). The weight of buffalo meatballs before and after centrifuge was recorded and calculated using the formula:

WHC (%) = (wt before (g)-wt after(g))/(wt before (g)) × 100%

2.5 Colour evaluation

Colour measurements were taken using a colourimeter (Chroma meter CR-210, Minolta, Japan). Colours (lightness = L*, redness = a*, and yellowness = b*) were measured on the surface of thawed buffalo meatball sample at random positions. Before each measurement, the apparatus was standardized against a white paper (Serdaroğlu *et al*., 2005).

2.6 Texture profile analysis

Thawed meatballs were steamed at 100°C for 10 min as a sample preparation step. The textural properties for each sample were measured using a cylinder probe (φ 50 mm diameter), set attached to a Texture Analyzer (TA-XT2i, Stable Micro System Ltd., UK). The test parameter was as follows: two cycles of compression; pre-test speed, 1.0 mm/s; test speed, 2.0 mm/sec; post-test speed, 5.0 mm/s; distance, 8.0 mm; strain 75%, trigger force 5.0 g (Huda *et al*., 2009). The texture profile analysis attributes were hardness [peak force on first compression (kg)], springiness [ratio of the sample recovered after the first compression], cohesiveness [ratio of active work done under the second force-displacement curve to that done under the first compression curve], gumminess [hardness (kg) × cohesiveness], and chewiness [hardness × cohesiveness × springiness (kg)] and resilient were automatically measured by machine and were recorded.

2.7 Shear test

The sample was randomly selected, thawed and steamed at 100°C for 10 min and cooled for 30 min before being analysed. The analysis was done using a Texture Analyzer (TA-XT2i, Stable Micro System Ltd., UK). Specimen loading, test conditions and specimen preparation followed the procedure described by Su *et al*. (2000). The samples were compressed once at a crosshead speed of 3.00 mm/sec to cut the whole meatball by using the Warner-Bratzler blade set with a 25 kg load cell. Shear force and the work of shearing samples were estimated with a Warner–Bratzler blade attached to the same texture analyser. The maximum force to cut the sample (shear force) and the work needed to move the blade through the sample (work of shearing) were recorded.

2.8 Total soluble protein analysis

For the extraction of protein, four grams of the minced meatball was homogenized with 80 ml of ice-cooled buffered solution in a homogenizing tube placed in ice for 4 mins. The homogenates solutions were centrifuged using a high-speed centrifuge (Centrifuge Kubota 3740, Kubota Corp., Japan) machine at 10,000 g for 1 hr at 4°C. After centrifugation, the protein isolate obtained from the supernatants was used for the determination of protein concentration by the Lowry method (Lowry *et al*., 1951), measuring absorbance at 660 nm using a spectrophotometer (Shimadzu UV Mini 1240, Japan). Bovine serum albumin was used as the standard.

2.9 Sensory analysis

Sensory evaluation was carried out using the acceptance test of a 9-point hedonic scale by 30 untrained panellists from faculty members. The hedonic scale used was by ranking each different buffalo meatball from 1 (dislike extremely) to 9 (like extremely). All six types of meatballs with different mixing times and mushroom types were evaluated on their appearance, aroma, flavour, texture, juiciness and overall acceptability (Ramle *et al*., 2021).

2.10 Scanning electron microscopy

Two types of meatballs varying in mixing time and mushroom type were selected randomly. They were thawed and prepared by cutting into 1 cm x 1 cm squares with a thickness of 1 mm approximately using a razor blade. The meat slices were then attached to the stub using carbon conductive adhesive 502 to fix the meat piece. Then they were put into a vacuum chamber of an SEM machine, (Variable Pressure Scanning Electron Microscope LEO 1455 VPSEM, Hi-Tech Instrument, Malaysia) to observe the micrograph of the meatball cut surface. The images were taken at 30× magnification.

2.11 Statistical analysis

The data obtained from all analyses except SEM was evaluated using Minitab Software (Minitab 16.0 for Windows, Minitab, USA). Data were analysed by two-way analysis of variance (ANOVA). Tukey's multiple comparison test was used as a post-ANOVA technique to

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Table 1. Cooking yield, linear expansion and water holding capacity of shiitake and brown button meatballs with different mixing times.

Analyses	Type of mushroom	Mixing time		
		5 min	10 min	20 min
Cooking yield (%)	Shi	88.95±1.63^{Aa}	89.74±2.94^{Aa}	92.03±0.93^{Aa}
	BB	89.16±2.30^{Aa}	90.81±2.94^{Aa}	90.06±0.62^{Ab}
Linear expansion (%)	Shi	-3.08±1.50^{Aa}	-2.68±0.95^{Aa}	-2.46±1.30^{Aa}
	BB	-3.73±0.42^{Aa}	-3.77±0.92^{Aa}	-3.61±1.09^{Aa}
WHC (%)	Shi	86.99±2.58^{Aa}	90.48±3.12^{Aa}	89.00±3.62^{Aa}
	BB	87.19±1.88^{Aa}	91.25±2.70^{Aa}	91.25±2.50^{Aa}

Values are presented as mean±SD. Values with different superscripts within the same column and same analysist are statistically significantly different (p<0.05)

determine significant differences among the means of the test at 95% (p<0.05).

3. Results and discussion

3.1 Cooking yield, linear expansion and water holding capacity

The cooking yield, linear expansion and water holding capacity of meatballs with different types of mushrooms as fat replacers and different mixing times are given in Table 1. Cooking yield is a good indicator that is used by the meat industry to explain the behaviour of their products during processing (Ulu, 2006). In general, the ability of a meat product to retain water and fat while cooking influences its cooking yield (Cheng and Sun, 2008). The present work demonstrated that at a longer mixing time, shiitake mushrooms had a significantly (p<0.05) higher cooking yield than brown button mushrooms. This is related to the polysaccharides and high-quality proteins, which form a three-dimensional matrix to bind water in the meat system, contributing to equivalent cooking performance and duplicating the role of fat in the formation of an emulsion (Ramle *et al*., 2021). Correspondingly, the linear expansion and water holding capacity for meatballs with different fat replacers also increase with mixing time. This indicates that an increase in the mixing time could create a three-dimensional protein structure which binds the fat and water in the meatballs. The mixing time may also influence the quality of the meat products produced; for example, a longer mixing time affected linear expansion, whereas a sufficient mixing time contributed to better expansion (Murad *et al*., 2017). This indicates that an increase in mixing time will increase the emulsification between mushroom, starch, meat and other ingredients into a stable meat batter to set the protein structure upon heating.

3.2 Colour analysis

The colour attributes of cooked meat products are mostly determined by the pigmentation of the meat from which they were created and the ingredients employed in their formulation (Serdaroğlu, 2006). The effects of mushroom incorporation on the colour (lightness, L*; redness, a*; yellowness, b*) of the meatballs are shown in Table 2. There was no significant difference (p<0.05) in lightness (L*) and yellowness (b*) for all the samples. However, the redness (a*) value of shiitake meatballs showed that the redness (a*) increases with mixing time. Shiitake mushrooms are rich in iron compounds that function to increase the binding of oxygen to haemoglobin (Reguła and Siwulski, 2007), thus, the redness colour increases over a longer mixing time, where the shiitake natural component is well emulsified and reacted with meat haemoglobin. In addition, a longer mixing time might also cause a more homogeneous dispersion of all the ingredients in the meat batter. Lightness (L*) value of meatballs with animal fats is higher if compared to fat-replaced meatballs as the animal fat itself is white or cream-white. This could be

Table 2. Colour of shiitake and brown button meatballs with different mixing times.

Colour analysis	Type of mushroom	Mixing time		
		5 min	10 min	20 min
L*	Shi	62.48±1.70^{Aa}	59.67±0.42^{Aa}	59.69±2.83^{Aa}
	BB	60.13±0.34^{Aa}	59.70±2.00^{Aa}	61.81±1.88^{Aa}
a*	Shi	3.39±0.34^{Ba}	3.87±0.40^{ABa}	4.33±0.19^{Aa}
	BB	3.62±0.45^{Aa}	3.96±0.45^{Aa}	3.80±0.40^{Aa}
b*	Shi	5.01±1.35^{Aa}	5.83±1.63^{Aa}	6.79±0.75^{Aa}
	BB	5.51±1.53^{Aa}	5.91±0.86^{Aa}	5.57±0.93^{Aa}

Values are presented as mean±SD. Values with different superscripts within the same column and same analysist are statistically significantly different (p<0.05). L* lightness, a* redness and b* yellowness

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due to the carotenoid pigment in feed and heme pigments from blood or drip (Hui *et al*., 2001). Matured shiitake and brown button mushrooms have dark brown colours on their cap, and this will directly turn meatballs into darker colours (Cheung, 2008). Also, compared to beef meat, buffalo meat is darker and the colour attribute is caused by higher myoglobin content which is (5.0 mg/g) (Maheswarappa *et al*., 2016). The results also showed that shiitake mushrooms had the highest value of yellowness (b*) regardless of mixing time. Changes in yellow might be attributed to the brownish colour of the shiitake aqueous extracts (Mattar *et al*., 2018).

3.3 Texture profile analysis and Warner Bratzler

Table 3 presents the experimental Texture Profile Analysis (TPA) data that measured the intensity of hardness, springiness, cohesiveness, gumminess, chewiness, resilience and Warner Bratzler of fat-replaced meatballs. Overall, there was no significant difference observed between the fat-replaced meatballs in all texture attributes studied ($p>0.05$). Nevertheless, the hardness value showed that brown button meatballs have a higher hardness value than shiitake meatballs. The high dietary fibre content of brown button mushrooms was found to improve the textural qualities of cooked beef emulsion (Kurt and Gençcelep, 2018). The gelation of myofibrillar proteins was shown to be closely related to the structural properties of cooked beef products (Kang *et al*., 2021). High ionic strength aids in the extraction of myofibrillar protein from meat during the manufacture of meat batters. Brown button mushroom powder is high in Ca, K, Mg, Na, and other elements, which raises the ionic strength of meat batters and facilitates the extraction of salt-soluble proteins from chicken, making it useful for the production of the three-dimensional structure of heat-induced gel (Mleczek *et al*., 2020).

Although all the TPA results did show any significant difference, the unique pattern of all the TPA attributes except resilience indicated that mixing time did have an effect towards the texture of meat products. The formation of a gel by myofibrillar protein, majorly actomyosin is important to determine the texture profile and mastication properties as they affect the emulsion formed after the meat batter was mixed at different mixing times. Sufficient mixing is needed to form a stable protein matrix with desirable texture characteristics and this network formation was comprehensively induced by protein components, actin and myosin (Hui *et al*., 2001). Besides the mixing process, the ingredient in meat batter also plays an important role in the emulsion and texture of meatballs. Mushrooms have fibrous tissue and can absorb and hold moisture, thus improving the texture of meatballs (Chun *et al*., 2005). The incorporation of fresh mushrooms into meat products is also more successful for red meat-based products with a coarser myofibrillar protein texture as the mushroom fibres' texture can replace the fibrous texture of the myofibrillar protein fibres (Ramle *et al*., 2021). The addition of salt and STPP also helped increase the hardness, springiness and chewiness properties of manufactured meat products caused by salt binding the meat protein (Somboonpanyakul *et al*.,

Table 3. Texture profile analysis and Warner Bratzler of shiitake and brown button meatballs with different mixing times.

Analyses	Types of mushroom	Mixing time		
		5 min	10 min	20 min
Hardness	Shi	790.3±188.2^{Aa}	1014.2±82.0^{Aa}	917.5±52.5^{Aa}
	BB	918.8±162.0^{Aa}	1141.8±147.5^{Aa}	905.6±85.7^{Aa}
Springiness	Shi	0.94±0.01^{Aa}	0.92±0.01^{Aa}	0.94±0.02^{Aa}
	BB	0.93±0.00^{Aa}	0.94±0.00^{Aa}	0.94±0.00^{Aa}
Cohesiveness	Shi	0.86±0.00^{Aa}	0.84±0.01^{Aa}	0.89±0.07^{Aa}
	BB	0.85±0.01^{Aa}	0.86±0.00^{Aa}	0.86±0.01^{Aa}
Gumminess	Shi	681.6±158.6^{Aa}	860.0±60.1^{Aa}	817.2±40.0^{Aa}
	BB	786.7±125.9^{Aa}	983.6±122.3^{Aa}	783.0±64.4^{Aa}
Chewiness	Shi	648.5±165.0^{Aa}	787.4±52.1^{Aa}	770.1±53.4^{Aa}
	BB	733.9±116.6^{Aa}	929.1±110.5^{Aa}	739.4±61.3^{Aa}
Resilience	Shi	0.52±0.01^{Aa}	0.49±0.02^{Aa}	0.51±0.01^{Aa}
	BB	0.51±0.00^{Aa}	0.51±0.00^{Aa}	0.51±0.00^{Aa}
Max shear force	Shi	2.61±0.38^{Aa}	3.18±0.67^{Aa}	2.33±0.99^{Aa}
	BB	2.72±0.40^{Aa}	2.69±0.25^{Aa}	2.49±0.32^{Aa}
Work of shear	Shi	21.05±3.09^{Aa}	23.09±3.14^{Aa}	18.56±5.82^{Aa}
	BB	23.80±4.80^{Aa}	23.18±2.28^{Aa}	18.57±3.62^{Aa}

Values are presented as mean±SD. Values with different superscripts within the same column and same analysist are statistically significantly different ($p<0.05$).

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2007).

3.4 Total soluble protein

Protein solubility is important not only for determining the best conditions for protein extraction but also for great importance applications in the food industry (Hrynets *et al*., 2011). Protein solubility is a good indicator of protein denaturation and is fundamentally related to the hydrophobicity/ hydrophilicity balance (Omana *et al*., 2010). The total soluble protein result in this study showed that there was no significant difference ($p<0.05$) in the mixing time between all of the samples (Table 4). This is similar to a study by Asyrul-Izhar *et al*. (2021), which indicated that the protein content of sausages was not influenced by mixing time. This result can also be correlated with linear expansion, water holding capacity and texture analysis which showed that they were not significantly affected by the difference in mixing time. Nevertheless, there was a significant difference ($p<0.05$) between different types of mushrooms used as fat replacers. Results indicated that shiitake meatballs had a higher total protein solubility compared to brown button meatballs regardless of mixing time. According to Cui (2010), tissue senescence is the key indicator for the decrease in the total soluble protein content of brown button mushrooms. The free amino acids formed by protein breakdown combined with quinones to generate melanins, which accelerated mushroom browning (Meng *et al*., 2010), thus leading to a lower total soluble protein content. The textural properties of meat products are also closely related to protein characteristics. For instance, the hardness results for both types of fat-replaced meatballs showed that brown button meatballs have a higher hardness value than shiitake meatballs. On the other hand, the higher total soluble protein content for shiitake meatballs could influence the texture of the meatballs as myofibrillar proteins and mushrooms appear to compete for water adsorption and influence product texture, making the food softer (Soltanizadeh and Ghiasi-Esfahani, 2015), thus decreasing the hardness for shiitake meatballs.

3.5 Sensory analysis

The results of the sensory analysis of reduced-fat meatballs are summarized in Table 5 in which the panellists rated all the samples using a nine-point hedonic scale. The scores obtained for all sensory attributes were in the range of 5.30 to 6.90, which fell under the category of 'Like'. As shown in the table, there were no significant differences ($p>0.05$) in terms of appearance, aroma, flavour and overall acceptability of all the samples. Nevertheless, the scores indicated that the panellist preferred the brown button meatballs if compared to shiitake meatballs. This might be attributed to the original umami taste of the button mushrooms

Table 4. The total soluble protein content of shiitake and brown button mushrooms with different mixing times.

Analyses	Types of mushroom	Mixing time		
		5 min	10 min	20 min
Total soluble protein (g/100 g)	Shi	$0.53±0.03^{Aa}$	$0.59±0.03^{Aa}$	$0.59±0.03^{Aa}$
	BB	$0.44±0.02^{Ab}$	$0.46±0.02^{Ab}$	$0.47±0.02^{Ab}$

Values are presented as mean±SD. Values with different superscripts within the same column and same analysist are statistically significantly different ($p<0.05$).

Table 5. Sensory evaluation of shiitake and brown button meatballs with different mixing times.

Analyses	Types of mushroom	Mixing time		
		5 min	10 min	20 min
Appearance	Shi	$6.26±1.61^{Aa}$	$6.23±1.59^{Aa}$	$5.96±1.29^{Aa}$
	BB	$6.06±1.68^{Aa}$	$6.30±1.48^{Aa}$	$6.43±1.56^{Aa}$
Aroma	Shi	$6.36±1.56^{Aa}$	$6.20±1.58^{Aa}$	$6.23±1.50^{Aa}$
	BB	$6.20±1.34^{Aa}$	$6.43±1.33^{Aa}$	$6.43±1.27^{Aa}$
Flavour	Shi	$5.83±1.89^{Aa}$	$5.70±1.89^{Aa}$	$5.30±1.87^{Aa}$
	BB	$6.33±1.42^{Aa}$	$6.50±1.61^{Aa}$	$6.23±1.79^{Aa}$
Texture	Shi	$6.43±1.50^{Aa}$	$6.26±1.68^{Aa}$	$5.83±1.66^{Ab}$
	BB	$6.90±1.29^{Aa}$	$6.26±1.33^{Aa}$	$6.86±1.47^{Aa}$
Juiciness	Shi	$6.46±1.59^{Aa}$	$5.96±2.02^{Aa}$	$5.96±1.67^{Ab}$
	BB	$6.86±1.59^{Aa}$	$6.10±1.66^{Aa}$	$6.46±1.67^{Aa}$
Overall acceptability	Shi	$6.36±1.47^{Aa}$	$6.10±1.72^{Aa}$	$5.80±1.24^{Aa}$
	BB	$6.76±1.35^{Aa}$	$6.46±1.27^{Aa}$	$6.53±1.59^{Aa}$

Values are presented as mean±SD. Values with different superscripts within the same column and same analysist are statistically significantly different ($p<0.05$).

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itself as the umami compounds enhance the overall taste of the food (Yamaguchi and Ninomiya, 2000; Dermiki *et al*., 2013). Mushrooms, particularly brown button mushrooms, are thought to be a rich source of umami flavour, including a wide range of umami-inducing chemicals such as asp, glu, and 5'-mononucleotides in significant quantities (Kalač, 2016). The texture and juiciness of the meatballs showed a significant ($p<0.05$) difference between shiitake and brown button meatballs at a mixing time of 20 minutes. This was positively correlated with the other results as discussed earlier which indicated that a longer mixing time gave a better result for the characteristics of the meatballs. A previous report by Ramle *et al*. (2021) reported that brown button meatballs had a higher moisture content than shiitake meatballs, thus, this might contribute towards the sensory evaluation of the meatballs in terms of texture and juiciness as moisture could contribute towards a better texture and juiciness of meat products. In addition, the higher total protein solubility content of shiitake meatballs leads to a softer meatball. Hence, the panellist preferred the meatballs with a higher hardness as indicated by the texture profile analysis result in this study.

3.6 Scanning electron microscopy

The scanning electron microscopic (SEM) images of shiitake and brown button meatballs with different mixing times are shown in Figure 1. The protein matrix of both the shiitake and brown button meatballs became more regular, compact and dense continuous phase as the mixing time increased. Different mixing times could enhance the internal structure of the meat products making them more homogeneous and promoting the ability to absorb water (Meng *et al*., 2022). The cooking yield, linear expansion and water holding capacity of the meatballs in this study also increase as there is an increase in mixing time and it is shown in the microscopic images of the meatballs that the protein structures were more uniform and able to hold water as mixing time increases. Nevertheless, the size of the cavities might also increase for both meatballs as the mixing time increases. This is similar to a study by Asyrul-Izhar *et al*. (2021) which indicated that bigger cavities were formed when the meat batter was mixed for 20 minutes. Larger cavities can also be seen in brown button meatballs if compared to shiitake meatballs and this can be correlated to the total soluble protein result in this study which reported that brown button meatballs had a lower amount of total soluble protein content. The smaller the amount of protein being extracted, it results in large the void/cavity formation (Somboonpanyakul *et al*., 2007).

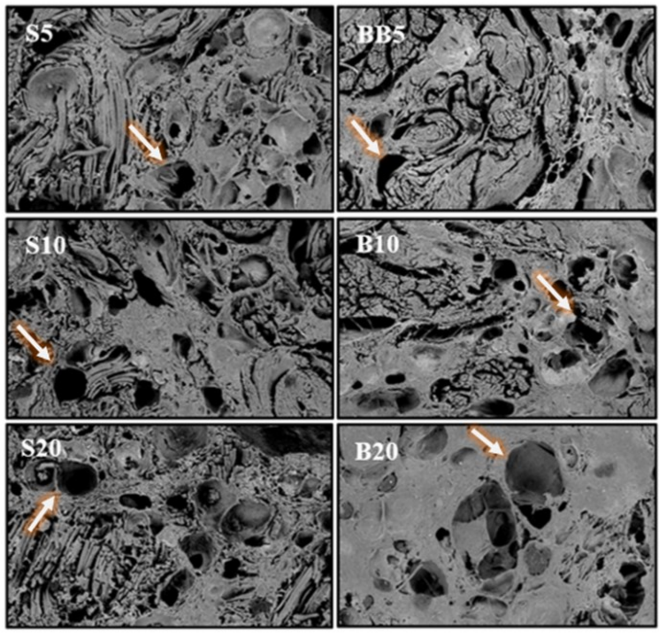

Figure 1. Scanning electron microscopy of shiitake and brown button meatballs with different mixing times at 30× magnification. Shiitake meatball with a mixing time of 5 mins (S5); Shiitake meatball with a mixing time of 10 mins (S10); Shiitake meatball with a mixing time of 20 mins (S20); Brown button meatball with a mixing time of 5 mins (BB5); Brown button meatball with a mixing time of 10 mins (BB10); Brown button meatball with a mixing time of 20 mins (BB20). The size of voids/cavities is shown using the arrows.

4. Conclusion

The use of shiitake (*Lentinula Edodes*) and brown button (*Agaricus Bisporus*) mushrooms to replace animal fat in meatballs can be considered a technological and sensory promising strategy. The results showed that there were no differences in terms of linear expansion, water holding capacity, texture profile analysis and Warner Bratzler for both types of mushrooms and mixing times. Shiitake meatballs mixed at a longer duration (20 minutes) showed a significantly higher value of cooking yield than brown button meatballs. The total soluble protein also showed that shiitake meatball has a significantly ($p<0.05$) higher total soluble protein content regardless of mixing time. The lightness (L*) and yellowness (b*) values of both types of mushrooms and mixing time do not show any significant differences but the redness (a*) value indicated that the redness value of shiitake meatballs increases as the mixing time increases. Scanning electron microscopy images showed differences in the surface composition between different types of meatballs as the mixing time increased. In addition, brown button mushrooms with a mixing time of 20 minutes presented a sensory profile with attributes that positively contribute to the texture and juiciness of the meatball. In conclusion, both shiitake and brown button mushrooms are suitable to be used as fat replacers but a longer mixing time resulted in better quality

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meatballs.

Conflict of interest

The authors declare no conflict of interest.

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