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Physicochemical and Microstructural Properties of Buffalo Meat Batter Incorporated with Ultrasound-treated Coconut Flesh as a Meat Substitute

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ABSTRACT

Coconut flesh exhibits potential as a meat substitute due to its physicochemical properties (pH, cooking yield, and water-holding capacity (WHC)), which can be further enhanced through ultrasound treatment. This study aimed to evaluate the effects of ultrasound treatment and varying concentrations of coconut flesh on the physicochemical, rheological, and microstructural properties of buffalo meat batter. Five formulations were formulated, which are: a control (100% buffalo meat), 50% buffalo meat with 50% untreated coconut flesh (50%C), 100% untreated coconut flesh (100%C), 50% buffalo meat with 50% ultrasound-treated coconut flesh (50%USC), and 100% ultrasound-treated coconut flesh (100%USC). The results indicated that the 50%USC formulation effectively replaced buffalo meat without inducing significant ($p>0.05$) alterations in the physicochemical properties. This formulation exhibited a more stable emulsion and superior moisture retention compared to the control, as well as lower fat content and higher protein levels than the other treated samples ($p<0.05$). Furthermore, the texture profile of the 50%USC formulation was acceptable and comparable to the control formulation (100% buffalo meat). Notably, 50%USC also outperformed the control in terms of rheological and microstructural characteristics. Additionally, the 50%C formulation emerged as a viable alternative, displaying physicochemical, textural, and water-holding capacity (WHC) properties comparable to the control, with no statistically significant ($p>0.05$) deterioration in these quality attributes. These findings suggest that substituting buffalo meat with coconut flesh, particularly when subjected to ultrasound

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treatment, may enhance the quality and structural integrity of meat batter while maintaining desirable functional properties.

Keywords: Emulsion stability, healthier meat products, meat analogue, meat emulsion, meat replacement

INTRODUCTION

Meat substitutes are food materials that replace animal protein with plant-based sources like grains, beans, or cereals (Sze Wei et al., 2024). Cristina et al. (2021) classified non-animal meat products into three main types: meat extenders, which are non-meat ingredients with high protein content; meat substitutes, which replicate the texture, appearance and functionality of meat; and total protein products, which are entirely free from animal-derived proteins. Meat substitutes are formulated to act as direct analogues to conventional meat, enabling their incorporation into food products that traditionally rely on animal-derived meat components. Traditions used tempeh, wheat gluten, and tofu as protein sources to replace meat consumption (Jiang et al., 2020). Meat substitutes may be made from a variety of protein sources, including soy protein, cereal protein, and mycoprotein (Ismail et al., 2020). According to Riaz (2005), they were successful in providing the desired quality of the meat-replacement product compared to the meat-based product. The consumption of meat substitutes is associated with several health benefits, including improved quality of life, reduced risk of obesity, cardiovascular protection, lower blood cholesterol levels, decreased risk of cancer, and enhanced bone mass development. The ingredients commonly used in plant-based meat alternatives can improve the nutritional profile of the final product (Tuso et al., 2013). This recognition has led to growing consumer demand for meat substitutes.

Coconut, scientifically known as *Cocos nucifera* L., may be employed in its entirety, from the tree's trunk to its leaves. Coconut ranks as the fourth most important industrial crop in Malaysia, following palm, rubber, and paddy cultivation (Rozhan & Rohany, 2021). The flesh is the white, meaty component of a coconut seed. Coconut flesh is classified into two types: matured coconut kopyor (water with meat particles floating in it) and immature coconut. Wynn (2017) observed slight variations in the macronutrient composition between mature and immature coconut meat. In the matured coconut meat, a decrease in crude fat was observed alongside a reciprocal increase in total carbohydrates (calculated by difference). The protein found in coconut flesh is certainly derived from the oilseeds; nevertheless, it is lacking in methionine, lysine and threonine (Tenda et al., 2021). The proportion of essential amino acids in coconut flesh is naturally lower than that found in animal proteins. Nevertheless, proteins derived from mature coconut possess a generally favourable amino acid composition and exhibit comparatively good nutritional quality (Ching et al., 2015; Wynn, 2017). Fresh coconut flesh has been shown to have excellent protein-emulsifying properties, suggesting its potential applicability in the formulation of meat products (Onsaard et al., 2006). In addition, it has an aromatic flavour and odour (Jangchud et al., 2007), and the sweet taste of regular coconut is mainly due to sucrose (Wynn, 2017), which could beneficially affect the sensory acceptance of the final product.

An ultrasound homogeniser, also known as a sonicator, is a device equipped with a vibrating probe that generates shear forces, inducing cavitation within liquid samples. This instrument is essential for sample homogenisation, cell lysis, particle size reduction in suspensions, extraction of biological materials, and the enhancement of chemical processes (O'Sullivan et al., 2017). Aside from that, they may be used to produce an emulsion. The structure of proteins can be altered by utilising ultrasound techniques. Ultrasound treatment enhances the solubility and viscosity of soy protein isolate dispersions, thereby improving subsequent processing steps (Hu et al. 2013). This discovery was supported by Tian et al. (2020), who found that ultrasound exerted a substantial influence on the protein's structural and physicochemical characteristics, promoting the breakdown of peptide aggregates by disrupting non-covalent interactions. Various studies have reported that high-frequency ultrasound can improve protein solubility, reduce protein particle size, and enhance their functional properties (Arzeni et al. 2012; Jambrak et al. 2008). Jambrak et al. (2008) examined the impact of low-intensity, high-energy ultrasound (20-40 kHz) applied for 15 to 30 minutes on whey protein and found that the treatment improved both its solubility and hydrolysis. These improvements were attributed to conformational changes in the globular structure of proteins, which exposed hydrophilic regions to water. Similarly, Arzeni et al. (2012) examined the impact of high-frequency ultrasound on protein degradation, aggregation formation and solubility. Their findings suggested that increasing the temperature could facilitate the dissociation of small protein aggregates and reduce particle formation. Concurrently, ultrasound treatment promoted protein-water interactions, further enhancing protein solubility.

As coconut flesh has potential as a meat substitute, pre-treatment with ultrasound could enhance its physicochemical properties compared with untreated samples. Therefore, this study aimed to evaluate the use of ultrasound-treated and untreated coconut flesh as substitutes for meat in the production of buffalo meat batter. The output could benefit the food industry and future research in finding a suitable meat substitute.

MATERIALS AND METHODS

Meat Batter Preparation

Fresh tenderloin, buffalo meat, and animal fats were acquired from the Pasar Borong Selangor, Seri Kembangan, Selangor, Malaysia. The dry ingredients, including semi-matured grated coconut flesh, salt, cornstarch, sodium tripolyphosphate (STPP), sugar, and garlic powder, were sourced from the Sri Serdang local market in Seri Kembangan,

Table 1
Formulation of buffalo meat batter with different amounts of coconut flesh or ultrasound-treated coconut flesh as meat substitutes

Ingredients %	Control	50%C	100%C	50%USC	100%USC
Meat	70	35	-	35	-
Coconut	-	35	70	-	-
Ultrasound-treated coconut	-	-	-	35	70
Animal fats	15	15	15	15	15
Corn starch	5	5	5	5	5
Salt	1.2	1.2	1.2	1.2	1.2
STPP	0.3	0.3	0.3	0.3	0.3
Garlic powder	1.0	1.0	1.0	1.0	1.0
Sugar	1.0	1.0	1.0	1.0	1.0
Ice water	6.5	6.5	6.5	6.5	6.5
Total	**100**	**100**	**100**	**100**	**100**

Selangor, Malaysia. The sample preparation for the meat batter emulsion followed the steps described by Ismail et al. (2021a), with several modifications. First, the lean meat and fat were ground separately in a mincer (Model 4822, Hobart, Offenburg, Germany) through an 8 mm plate, then stored at -18°C.

The ultrasound treatment on the coconut flesh was adapted from Sui et al. (2017) and Sun et al. (2019) with a few modifications. The shredded coconut flesh was soaked in deionised water, then homogenised at 5,000 rpm for 5 minutes in a chilled microcentrifuge (Kubota 3740, Japan). The goal of pre-homogenising is to produce a coarse o/w emulsion. The shredded coconut was then treated for 10 minutes in an ice bath using an ultrasound homogeniser set to 25% power (the full power is 500 W) with a Φ6 mm ultrasound probe. The basic formulation for the meat batter consisted of 70% minced meat, 15% fat, 6.5% ice water, 5% corn flour, 1.2% salt, 1% garlic powder, 1% sugar, and 0.3% STPP. Five formulations were prepared with 100% meat (control), 50% meat + 50% coconut flesh (50%C), 100% coconut flesh (100%C), 50% meat + 50% ultrasound-treated coconut flesh (50%USC), and 100% ultrasound-treated coconut flesh (100%USC) (Table 1). All samples were set up for three replications.

Emulsion Stability Analysis

Emulsion stability was assessed as described by Ismail et al. (2021a). A 10 g sample of meat batter was weighed and placed in a centrifuge tube. The sample was then centrifuged at 3000 rpm for 15 minutes at 4°C, resulting in the separation of a transparent upper layer and an optically ‘creamy’ bottom layer. Subsequently, the tube was incubated in a water bath at 75°C for 30 minutes. To determine the total expressible fluid (TEF) and expressible fat (EFAT), the centrifuge tube lids were left open and inverted over marked crucibles for 1 hour to allow the expressible fluid to drip. The pelleted samples were then weighed, and the collected supernatants were oven-dried overnight at 105°C. The quantities of TEF and EFAT were calculated using the Equations 1, 2, and 3.

$$TEF = (Weight\ of\ centrifuge\ tube + Weight\ of\ the\ sample) - (Weight\ of\ centrifuge\ tube + Weight\ of\ the\ pellet) \quad [1]$$

$$\%TEF = \frac{TEF}{Weight\ of\ sample} \times 100 \quad [2]$$

$$\%EFAT = \frac{(Weight\ of\ crucible + Weight\ of\ dried\ supernatant) - (Weight\ of\ empty\ crucible)}{TEF} \times 100 \quad [3]$$

Proximate Analysis

Proximate analysis was conducted to determine the contents of protein, fat, moisture, ash, carbohydrates, and fibre in the samples. The samples were chopped into smaller pieces, minced through a 2 cm sieve three times to ensure homogeneity, and chemically analysed according to the AOAC (2012) method. AOAC procedures were used to calculate the total proportion of carbohydrates, protein, fat, fibre, moisture, and ash (AOAC, 2012).

pH Analysis

A cooked sample weighing 5 g was homogenised with 45 mL of distilled water, and the pH was measured using a handheld pH meter (Jenway, U.K.) and evaluated according to the manufacturer's instructions.

Cooking Yield Measurement

A 5 g portion of meat batter was weighed, placed in a centrifuge tube, and centrifuged at 1000 g for 15 seconds (Kubota 3740, Japan) to remove air bubbles. The samples were then submerged in a warmed water bath at 50°C, with the temperature steadily raised until the sample reached an internal temperature of 72°C, as detected by a thermocouple. The centrifuge tubes were cooled in a cold-water bath for 5 minutes before the exudates were expelled; the cooked samples were then removed and wiped dry with filter paper. The cooking yield was calculated based on Ismail et al. (2021b) using the Equations 4 and 5.

$$Cooking\ loss\ (\%) = \frac{Weight\ of\ the\ uncooked\ \ sample\ - Weight\ of\ the\ pellet}{Weight\ of\ the\ uncooked\ \ sample} \times 100 \quad [4]$$

$$Cooking\ yield\ (\%) = 100\% - Cooking\ loss \quad [5]$$

Water Holding Capacity Analysis

The water-holding capacity (WHC) was determined according to the method described by Ming-Min and Ismail-Fitry (2023). In a centrifuge tube, 5 g of raw material were manually mixed with 32 mL of distilled water and left for 10 minutes. The material was then centrifuged for 25 minutes at 2900 rpm in a chilled microcentrifuge (Kubota 3740, Japan). The supernatant was discarded, and the sample was dried for 20 minutes at 50°C with a 10 to 20° downward tilt. The following Equation 6 was used to determine the WHC.

$$WHC = \frac{(b-a)-(c-a)}{b-a} \times 100\% \quad [6]$$

Where,
a = weight of empty centrifuge
b = weight of centrifuge with supernatant
c = weight of the dried centrifuge

Colour Measurement

A hand-held colourimeter (CR-40, Minolta Camera Co., Japan) with an aperture of 8 mm was used to measure CIELAB L*, a*, and b* values, which were used as indicators of samples' lightness, redness, and yellowness, respectively. The colour measurements for uncooked and cooked samples were examined according to Ismail et al. (2021b). The uncooked sample was evenly spread in a 5 cm-diameter transparent plastic container, while the cooked sample was thinly sliced and arranged in a similar round container. For each sample, the colour was recorded at 3 different points, and the mean value was used for analysis

Warner-Bratzler Shear Force Test

The measurement of the shear force was conducted according to Faridah et al. (2023) with minor modifications. The cooked sample was sliced horizontally, measuring 25 mm in length and 15 mm in diameter, using a texture analyser (TA-XT2i, Stable Micro System, UK) fitted with a Warner-Bratzler (WB) shear blade that features a triangular slow cutting edge (1 mm thickness) at a cutting speed of 1.5 mm/s. The maximum shear force (N) and shear work (N.sec) were measured according to Faridah et al. (2023).

Rheological Test

The dynamic rheological properties of the meat emulsion were estimated using a rheometer (Anton Paar Rheometer, MCR 302 Series, Austria) fitted with a 35 mm diameter stainless steel plate and a 1 mm gap. To determine the limit of the linear viscoelastic range, an oscillatory strain sweep (0.1-100%, frequency = 1 Hz) was performed (LVR). In addition, a frequency sweep test (0.1-100 Hz) was conducted at LVR (= 1%). The samples were tested for 5 minutes at 25 °C. The storage modulus (G') and loss modulus (G") were determined, and the phase angle (tan = G″/G′) was calculated according to the method of Faridah et al. (2023).

Texture Profile Analysis

The texture profile analysis was performed using the TA-XT2 texture analyser (Stable Micro Systems, Surrey, UK). The cooked sample was compressed twice to 75% of its original height using a P/75 probe connected to a 30 kg weight. During the measurement, the texture analyser was set with a test speed of 1.5 mm/s and a post-test speed of 1.5 mm/s.

Microstructure Analysis

The microstructure of the meat emulsion sample was observed using a variable-pressure scanning electron microscope (Leo / Zeiss 1455 VPSEM, UK). The sample, measuring 5 mm in thickness, was fixed with 0.1 mol/L phosphate buffer (pH 7.0) containing 2.5% glutaraldehyde at 4°C for 24 h and dehydrated in incremental concentrations of ethanol solutions (50, 70, 90, 95, and three times with 100%) for 10 mins per solution. Subsequently, the sample was freeze-dried in acetone, cooled in liquid nitrogen, and mounted onto the stubs using double-sided carbon tape. It was then sputter-coated with gold for 3 minutes using an automated sputter coater. The specimen was observed at 250× magnification (Ismail et al., 2021b).

Statistical Analysis

Minitab 19 (MiniTab Inc., United States of America) was used to conduct a one-way analysis of variance (ANOVA) at the 95% confidence level (p<0.05) to evaluate the significant differences between the collected data.

RESULTS AND DISCUSSION

Proximate Composition of Buffalo Meat Batter

Table 2 presents the proximate composition of cooked meat batter incorporating coconut flesh as a meat substitute. No significant differences (p>0.05) in moisture content were observed between the control and other samples. These findings indicate that coconut flesh has a water content comparable to that of meat, which is a crucial factor in its potential as a meat substitute. Furthermore, moisture content may significantly influence the water-holding capacity of the product (Serdaroğlu et al., 2017).

No significant changes ($p>0.05$) in protein contents were observed in the treated samples compared to the control. However, when combined with buffalo meat, 50%USC exhibited a higher ($p<0.05$) protein content than the other treated samples, indicating the effectiveness of ultrasound treatment in releasing protein. This may be due to modifications in protein structure during ultrasound processing. High-energy ultrasound is a complementary technique for studying the structural and biochemical characteristics of proteins. Evidence suggests that ultrasonic exposure can enhance protein foaming capacity by stabilising the foam structure, increasing foam expansion, and promoting particle size reduction. Jambrak et al. (2008) tested the foaming characteristics of whey protein concentrates (WPC), whey protein isolate (WPI), and whey protein hydrolysate (WPH) at 10% (w/v) using ultrasound. They concluded that 15 min of ultrasonic pulses to protein systems at 10% (w/w) improves foaming. A reduction in particle size was observed, attributed to the likely disruption of protein aggregates during sonication, thereby enhancing protein adsorption at the system interface. Overall, sonication improved the particle size, volume, and hydrophobicity of the protein isolate, thereby enhancing foaming behaviour

Substituting the meat with coconut flesh significantly reduced the fat content ($p<0.05$) for 50%C and 50%USC. Although the fat content for 100%C and 100%USC was not significantly different from the control ($p>0.05$), both were still lower than the control. The observed reduction in fat content may be linked to the quantity of intermuscular and intramuscular fat present in the meat, which remained inseparable during processing. Additionally, the measured fat content could also be influenced by the coconut flesh itself, as it has been reported to contain approximately 60% fat (U.S. Department of Agriculture,

Table 2
Proximate composition of buffalo meat batter with different amounts of coconut flesh or ultrasound-treated coconut flesh as meat substitutes

Samples	**Moisture (%)**	**Protein (%)**	**Fat (%)**	**Ash (%)**	**Carbohydrate (%)**	**Crude fibre (%)**
Control	65.02±0.16^{a}	15.76±0.82^{ab}	14.06±1.02^{a}	2.38±0.11^{a}	3.74±0.71^{b}	0.03±0.21^{c}
50%C	66.57±0.37^{a}	14.06±1.32^{b}	7.68±1.48^{b}	1.66±0.08^{b}	9.34±0.56^{a}	0.68±0.06^{b}
100%C	65.56±0.40^{a}	13.77±0.86^{b}	10.29±1.00^{ab}	1.66±0.13^{b}	8.00±0.60^{a}	0.92±0.60^{a}
50%USC	64.71±0.10^{a}	18.82±0.75^{a}	5.56±0.33^{c}	2.40±0.08^{a}	7.78±0.57^{ab}	0.72±0.87^{ab}
100%USC	66.02±0.29^{a}	14.52±2.70^{b}	8.81±1.85^{ab}	1.49±0.11^{b}	8.20±0.48^{a}	0.86±0.48^{a}

All values are mean ± SD
$^{a-c}$Means in the same column with different uppercase superscripts are significantly different ($p<0.05$). 100% meat (control), 50% meat + 50% coconut flesh (50%C), 100% coconut flesh (100%C), 50% meat + 50% ultrasound-treated coconut flesh (50%USC), and 100% ultrasound-treated coconut flesh (100%USC)

2019). Cofrades et al. (2000) demonstrated that adding fibre to meat products could increase fat retention. Kim et al. (2013) and Ktari et al. (2014) reported that adding fibre to meat products enhanced fat binding. All samples substituted with coconut flesh showed significantly higher fibre content than the control ($p<0.05$), consistent with the presence of dietary fibre in coconut flesh (Trinidad et al., 2006).

The ash content of all treated samples was reduced ($p<0.05$) compared with the control, except for 50%USC. The naturally lower ash content of coconut compared to animal meat likely contributes to the lower ash levels observed in samples using coconut flesh as a substitute (Slavin, 2008). The carbohydrate content of all treated samples was higher, particularly for 100%C and 100%USC ($p<0.05$), although 50%C also showed a significant difference. The higher carbohydrate values are expected because coconut flesh contributes carbohydrates mainly in the form of dietary fibre (non-starch polysaccharides), with smaller amounts of soluble sugars (U.S. Department of Agriculture, 2019); moreover, total carbohydrate was calculated by difference, which includes fibre, thereby increasing the apparent carbohydrate fraction in the coconut-substituted meat batter.

Emulsion Stability, Water Holding Capacity (WHC), Cooking Yield, and pH of Buffalo Meat Batter

Table 3 presents the emulsion stability, water-holding capacity (WHC), cooking yield, and pH values of the buffalo meat batter incorporated with coconut flesh as a meat substitute. The emulsion stability of the raw batter and the overall emulsifying quality of the meat substitute emulsion were evaluated using the percentages of TEF and EFAT. Evaluating emulsion stability is essential for determining the ability of a meat emulsion to maintain its structural integrity when subjected to potential variations in physicochemical properties. According to Serdaroğlu et al. (2016), a higher percentage suggests a less stable emulsion. Except for the 100% substitution samples (100%C and 100%USC), there was no significant difference ($p>0.05$) between the treatments and the control. These findings are likely linked to the sample protein concentration, as neither 100%C nor 100%USC contained any meat and had low crude protein levels. Strong interactions between proteins can hold lipid droplets in place, strengthening the protein matrix and enhancing emulsion stability (Smith, 1988). Myosin is the main protein in meat that binds it together. It also acts as a natural emulsifier and plays a crucial role in maintaining a stable meat emulsion structure (Álvarez et al., 2007). This is supported by Zorba and Kurt (2006), who reported that increasing the amount of chicken meat in the meat mixture effectively enhanced emulsion stability. This finding aligns with Kamani et al. (2019), who found that partial replacement of meat with a soy-based ingredient with a high protein content improved the emulsion of chicken sausage. Although there was no significant difference among the other four samples, the 50%USC exhibited the highest emulsion stability, with the lowest %TEF and

%EFAT values. Ultrasound plays a significant role in promoting the gelation of polymer residues. Arzeni et al. (2012) reported a positive effect on the elasticity of the resulting gel network, attributing this enhancement to protein polymer aggregation facilitated by hydrophobic interactions.

Water holding capacity serves as an indicator of the meat batter's ability to bind water with external influences, a crucial parameter for determining emulsion stability (Serdaroğlu et al., 2016). A strong water-retention capacity is essential not only for maintaining product quality but also for minimising exudation during storage and distribution, and for preserving juiciness after cooking before consumption. Substituting the meat with coconut flesh reduced the WHC for 100%C samples ($p<0.05$) (Table 3). This may be attributed to the most abundant proteins in coconut flesh being globulin and albumin, which are soluble in NaCl and water (Patil & Benjakul, 2017). Coconut flesh treated with ultrasound exhibited no changes ($p>0.05$) in WHC compared to the control. Hagenmaier et al. (1975) reported that some proteins present in the aqueous phase of the coconut milk emulsion have the potential to act as emulsifiers and stabilise fat globules. Ultrasound application can disrupt small protein aggregates, reduce particle size, enhance protein-water interactions, and increase protein solubility (Arzeni et al., 2012). These factors contributed to a better-emulsified coconut flesh solution, in which the addition of ultrasound-treated coconut flesh to the batter stabilised the WHC. A more stable protein matrix results in reduced water and fat release, thereby improving the binding properties of meat products (Pietrasik & Shand, 2003).

One of the most relevant measures used to rate the quality of comminuted meat products is cooking yield, which reflects moisture and fat losses during heating (Aaslyng et al., 2003). Cooking yield is strongly influenced by the formation of a heat-induced gel matrix from myofibrillar proteins that immobilise water and stabilise the fat phase (Varga-Visi

Table 3

The emulsion stability (%TEF and %EFAT), water holding capacity (WHC), cooking yield, and pH values of buffalo meat batter with different amounts of coconut flesh or ultrasound-treated coconut flesh as meat substitutes

Samples	%TEF	%EFAT	WHC	Cooking yield (%)	pH Raw	pH Cooked
Control	5.36±1.02^{b}	15.76±0.82^{ab}	86.05±0.90^{a}	96.93±3.17^{a}	6.33±0.06^{a}	6.53±0.06^{a}
50%C	6.68±0.48^{b}	17.82±0.75^{a}	87.91±0.41^{a}	96.12±7.21^{a}	6.17±0.00^{a}	6.27±0.06^{a}
100%C	21.81±0.80^{a}	13.77±0.86^{b}	78.38±0.83^{b}	86.22±6.86^{b}	6.00±0.06^{a}	6.20±0.00^{a}
50%USC	4.38±1.48^{b}	14.06±1.02^{ab}	91.60±0.20^{a}	98.95±2.92^{a}	5.71±0.00^{b}	6.37±0.06^{a}
100%USC	22.29±1.00^{a}	15.52±0.70^{ab}	88.55±0.90^{a}	91.87±3.03^{b}	5.56±0.00^{b}	6.20±0.00^{a}

All values are mean ± SD

$^{a-c}$Means in the same column with different uppercase superscripts are significantly different ($p<0.05$). 100% meat (control), 50% meat + 50% coconut flesh (50%C), 100% coconut flesh (100%C), 50% meat + 50% ultrasound-treated coconut flesh (50%USC), and 100% ultrasound-treated coconut flesh (100%USC)

& Toxanbayeva, 2017). In the present study, 100%C and 100%USC showed significantly lower ($p<0.05$) cooking yield than the control and other treatments. This reduction is likely due to the complete replacement of buffalo meat, which decreases the availability of myofibrillar proteins required for gelation and emulsification, resulting in poorer entrapment of water and fat during cooking. In contrast, partial substitution preserved cooking yield because the remaining meat proteins could still form a cohesive gel network capable of retaining moisture and fat (Abdul Wahab et al., 2024).

Establishing the pH is essential for determining meat-eating quality, as water-holding capacity (WHC) is strongly pH-dependent and, in turn, influences tenderness, juiciness, and microstructure. In this study, pH decreased significantly ($p<0.05$) as the concentration of coconut flesh increased. This may be linked to the organic acids profile; malic acid, one of the principal organic acids reported in coconut, contributes to acidity and can lower batter pH, thereby affecting protein charge and water retention. During coconut maturation, the concentrations of organic acids—particularly malic acid—are reported to change, with pH generally increasing from early developmental stages toward full maturity as acids are metabolised (Jackson et al., 2004; Santoso et al., 1996). After cooking, pH increased slightly, and no significant differences ($p>0.05$) were observed among samples, likely because thermal processing reduces moisture and shifts the acid-base balance, making the cooked product less acidic (Li et al., 2022). This reduction in acidity after cooking is important because an excessively low pH can increase cooking losses by moving proteins closer to their isoelectric point, weakening water binding; in contrast, a less acidic (higher pH) cooked product generally supports better WHC, improved juiciness/tenderness, and a milder, less sour flavour perception.

Texture Profile and Shear Force of Buffalo Meat Batter

The texture profile analysis and shear force test of the cooked batter with coconut flesh as a meat substitute are presented in Table 4. Samples with a 100% substitute formulation (100%C and 100%USC) exhibited lower values ($p<0.05$) for all texture parameters, shear force, and work of shearing than the control. A trend towards reduced value was observed across all parameters as the quantity of coconut flesh increased. The hardness results may be linked to the moisture-retention properties of coconut protein. Bernasconi et al. (2020) also reported that the beef patty was softer when soybean protein was used as a meat substitute, possibly due to enhanced moisture retention induced by soybean protein. This may account for the reduced hardness observed in the substituted samples, which likely contributed to the decreased resistance of the cooked batter to shredding, thus lowering both the maximum shear force and the work needed for shearing. Furthermore, the significant reduction in hardness in samples containing plant-based ingredients could be due to their high fibre content, which may interfere with the protein gel matrix, consequently softening the meat

Table 4
Texture profiles and Warner Bratzler shear force of buffalo meat batter with different amounts of coconut flesh or ultrasound-treated coconut flesh as meat substitutes

Samples	Hardness (g)	Springiness	Cohesiveness	Gumminess	Chewiness (g/m^3)	Resilience	Maximum Shear Force (N/mm)	Work of shearing (N/mm)
Control	11124.93±835.17^a	0.87±0.06^a	0.48±0.06^a	7966.53±336.12^a	5510.94±319.98^a	0.18±0.06^a	15.36±1.02^a	15.76±0.82^{ab}
50%C	9525.12±547.21^{ab}	0.71±0.06^b	0.37±0.01^a	5326.27±572.66^b	4513.69±380.81^a	0.12±0.01^{ab}	10.68±0.48^b	17.82±0.75^a
100%C	5525.22±626.86^c	0.40±0.02^c	0.10±0.02^b	4816.20±457.46^c	2220.51±388.48^b	0.08±0.00^b	4.81±0.10^c	13.77±0.86^b
50%USC	9022.46±592.92^b	0.84±0.02^a	0.43±0.02^a	5766.30±384.14^b	4083.18±211.26^a	0.13±0.00^{ab}	13.38±1.48^{ab}	14.06±1.02^{ab}
100%USC	5920.95±732.92^c	0.47±0.06^c	0.11±0.02^b	4766.37±402.16^c	1731.45±267.45^b	0.08±0.00^b	2.29±1.00^c	15.52±0.70^{ab}

All values are mean ± SD

$^{a-c}$Means in the same column with different uppercase superscripts are significantly different (p<0.05). 100% meat (control), 50% meat + 50% coconut flesh (50%C), 100% coconut flesh (100%C), 50% meat + 50% ultrasound-treated coconut flesh (50%USC), and 100% ultrasound-treated coconut flesh (100%USC) texture (Han et al., 2018). Wan Rosli et al. (2015) reported that frankfurters substituted with oyster mushroom powder showed a significant reduction in hardness compared to the control samples, while Kamani et al. (2019) reported that meat-free sausages displayed a noticeably softer texture than their meat-based counterparts. These reductions in hardness, gumminess, and chewiness may be attributed to the lower levels of insoluble muscle fibres and connective tissue present in the emulsion system (Ismail et al., 2021b; Ming-Min & Ismail-Fitry, 2023). This could be due to the myofibrillar protein network in buffalo meat being more resilient than plant fibre, thereby providing the flesh with greater compressive resistance.

Cohesiveness is defined as the extent to which a product withstands subsequent deformation relative to its resistance during the initial deformation. Springiness is characterised by the ability of the cooked meat batter to recover its original dimensions after experiencing compressive deformation (Nishinari et al., 2013). Fat and protein exert a synergistic effect on the springiness of meat products, as the protein matrix can bind lipid and water droplets, which may fill the air gaps within the emulsion matrix and reduce springiness as fat content increases (Ismail et al., 2021a; Youssef & Barbut, 2010). This finding generally aligns with previous research connecting meat texture to the incorporation of plant fibre. Song et al. (2016) found that the springiness of the frankfurter decreased significantly ($p<0.05$) as the amount of citrus fibre added into the product increased. Similarly, the addition of sea spaghetti and sugarcane dietary fibre to meat emulsion systems significantly reduced the cohesiveness (Fernández-Martín et al., 2009; Zhuang et al., 2016).

Texture profile is critical in developing plant-based meat alternatives, as replicating the textural properties of conventional patties is one of the most significant challenges in this field. Consequently, a meat emulsion with a complete (100%) substitution of animal components with plant-based ingredients may not achieve satisfactory final product quality. However, when the meat fraction was reduced to 50%, most parameters (cohesiveness, chewiness, and resilience) did not change significantly ($p>0.05$) compared to the control, and the significant differences ($p<0.05$) observed in hardness and gumminess remained within the range value of commercial meat products. Thus, it can be expected that coconut can replace up to 50% of buffalo meat in meat products without sacrificing textural qualities.

Colour Properties of Buffalo Meat Batter

Colour is often the first criterion that consumers consider when selecting a cut of meat. L* refers to lightness or darkness, a* refers to red and green and b* refers to blue and yellow. Buffalo meat is generally darker and redder than beef due to its higher myoglobin content, and therefore typically shows lower L* and higher a* values (Yie et al., 2023). Table 5 displays the colour properties of buffalo meat batter with varying amounts of coconut flesh or ultrasound-treated coconut flesh as meat substitutes. No significant difference ($p>0.05$)

Table 5
The colour properties of buffalo meat batter with different amounts of coconut flesh or ultrasound-treated coconut flesh as meat substitutes

Samples	Raw			Cooked		
	L*	a*	b*	L*	a*	b*
Control	$56.10{\pm}1.41^{a}$	$4.63{\pm}0.10^{a}$	$13.65{\pm}1.35^{a}$	$53.10{\pm}1.41^{b}$	$3.63{\pm}0.10^{a}$	$10.05{\pm}0.35^{a}$
50%C	$56.34{\pm}1.00^{a}$	$3.52{\pm}0.70^{ab}$	$14.09{\pm}0.11^{a}$	$56.73{\pm}1.20^{ab}$	$2.74{\pm}0.14^{b}$	$9.85{\pm}0.50^{a}$
100%C	$56.57{\pm}0.67^{a}$	$3.06{\pm}0.32^{b}$	$13.96{\pm}0.08^{a}$	$58.39{\pm}0.53^{ab}$	$2.24{\pm}0.14^{b}$	$10.08{\pm}0.16^{a}$
50%USC	$55.71{\pm}0.10^{a}$	$3.62{\pm}0.75^{ab}$	$14.19{\pm}0.08^{a}$	$62.60{\pm}0.43^{a}$	$2.42{\pm}0.13^{b}$	$10.97{\pm}0.66^{a}$
100%USC	$55.68{\pm}0.54^{a}$	$2.90{\pm}2.30^{c}$	$13.91{\pm}0.20^{a}$	$62.85{\pm}0.80^{a}$	$2.07{\pm}0.16^{b}$	$10.12{\pm}0.14^{a}$

All values are mean ± SD
$^{a\text{-}c}$Means in the same column with different uppercase superscripts are significantly different (p<0.05). 100% meat (control), 50% meat + 50% coconut flesh (50%C), 100% coconut flesh (100%C), 50% meat + 50% ultrasound-treated coconut flesh (50%USC), and 100% ultrasound-treated coconut flesh (100%USC)

was observed in the raw L* values of the samples. Although non-meat additives, particularly flour, can cause a pale tint in beef products, it was demonstrated that using coconut flesh did not affect the lightness of the samples. Similarly, Selani et al. (2015) found no changes in L* values of beef burgers when these were flavoured with either pineapple, passion fruit, or mango by-products. However, for the cooked samples, significant differences in L* values were observed between the treated and control samples. Incorporating passion fruit albedo into pork patties also increased their L* values, suggesting that the L* behaviour of cooked burgers with fruit albedo was due to the white parts in the raw material (López-Vargas et al., 2014). Another potential explanation is that myoglobin denaturation, changes in the heme group structure, and redox reactions involving myoglobin species have been hypothesised to contribute to the observed loss of redness (Carlez et al., 1995).

The a* values of the raw samples were influenced by the addition of coconut flesh, particularly for the 100%C and 100%USC (p<0.05). This was likely due to the control sample containing only minced buffalo meat, which has a high myoglobin concentration, as well as to the colour of the coconut flesh itself. All the cooked samples showed lower a* values than the control. The a* values of the cooked batter were also lower compared to the raw samples. This may be attributed to myoglobin denaturation or heme displacement under heat. In fact, heat treatment reduced the redness of the meat emulsion compared to raw sausages (Carlez et al., 1995). There were no significant differences (p>0.05) observed in b* values for raw and cooked samples, irrespective of the amount of coconut flesh added. Nonetheless, the yellowness b* value significantly decreased when the samples were cooked. This may relate to the implication of the grey-brown colour alongside the low a* value.

Viscoelastic Properties of Buffalo Meat Batter

G' and G" are two indicators commonly used to characterise gel properties. G' measures the elastic energy stored in a material, indicating its elasticity, while G" represents the viscous component of the emulsion, evaluated from the energy dissipated during deformation. According to Campo-Deaño et al. (2009) and Hrynets et al. (2010), an increased G' value correlates with greater rigidity and a denser network structure, resulting in enhanced gel strength. Moreover, samples that exhibit greater frequency dependence tend to display more fluid-like behaviour than elastic behaviour (Campo-Deaño et al., 2009). The 50%USC sample, which achieved the highest texture profile analysis score, also exhibited the highest G′ and G″ values among all formulations (Figure 1a). Owing to its well-organised

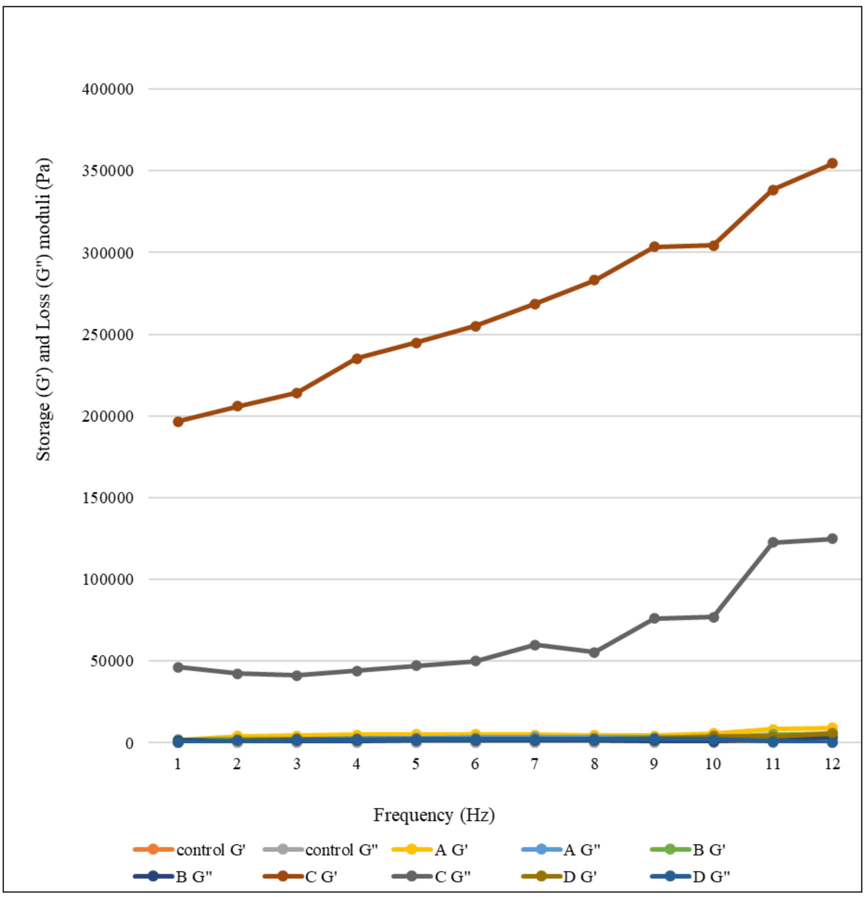

Figure 1a. The storage and loss moduli of buffalo meat batter with different amounts of coconut flesh or ultrasound-treated coconut flesh as meat substitutes

Note. 100% meat (control), 50% meat + 50% coconut flesh (sample A: 50%C), 100% coconut flesh (sample B: 100%C), 50% meat + 50% ultrasound-treated coconut flesh (sample C: 50%USC), and 100% ultrasound-treated coconut flesh (sample D: 100%USC) matrix structure (Kumar et al., 2017), this sample showed only minimal changes in G′ and G″ across the 1 to 10 Hz frequency range, suggesting a more stable emulsion network. Compared with other samples, the 50% USC showed higher G' at lower frequencies and a greater discrepancy between G' and G", suggesting the strongest network structure among all samples. Additionally, with increased moisture, texturisation occurs, leading to a degradation of the material's textural qualities.

The viscoelastic properties of the samples may also relate to their water-holding capacity (WHC) and moisture content. Kumar et al. (2017) reported that decreases in G' and G" with increased aloe gel incorporation were attributed to enhanced WHC and elevated moisture levels. A noticeable link exists between the texture profile analysis (TPA) value and the dynamic rheological characteristics of these samples. Increased moisture content promotes texturisation, resulting in a reduction of specific textural attributes. While it is established that ultrasonic treatments encourage protein unfolding, denaturation, aggregation, and gelation, protein gels induced by coconut shreds exhibit enhanced glossiness, smoothness, softness and elasticity compared to heat-induced gels. These properties are particularly significant in the context of meat product formulation (Chambale et al., 2014; Famelart et al., 1998; McClements et al., 2021; Messens et al., 1997; Supavititpatana & Apichartsrangkoon, 2007). A greater storage modulus implies a more potent gel and a more rigid structure (Campo & Tovar, 2008), resulting from the establishment of stable network structures or strong particle-particle interactions (Acevedo et al., 2014).

Figure 1b shows that, in the frequency analysis, all samples except the control exhibited a gel-like behaviour, as their loss factor values were less than 1. According to Agafonkina et al. (2019), larger tan δ values resulted in a more stable matrix. Although the control sample has the highest tan δ value ≈ 1.10, the 50%USC was still declared a stable matrix emulsion due to the small (p>0.05) variation in tan δ values relative to the control sample.

Microstructure of Buffalo Meat Batter

Figure 2 depicts the microstructure of a cross-section of buffalo meat emulsion incorporated with coconut flesh at different percentages. The control exhibits a homogenised protein matrix with few to no pores. Samples 100%C and 100%USC exhibit a less-dense matrix structure, with the most prominent pores and spaces. The larger pores and less dense matrix observed in the sample with 100% substitution were most likely caused by a lack of protein content, which destabilises the emulsion matrix as protein plays a significant role in the 3-D structure of emulsion (Alvarez et al., 2007; Devatkal et al., 2014). The proteins in coconuts are primarily water-soluble, enhancing the food's emulsifying potential. Moreover, the larger aggregate size of coconut protein is associated with a substantial hydrophobic surface, thereby indicating the most prominent matrix pores observed in samples 100%C

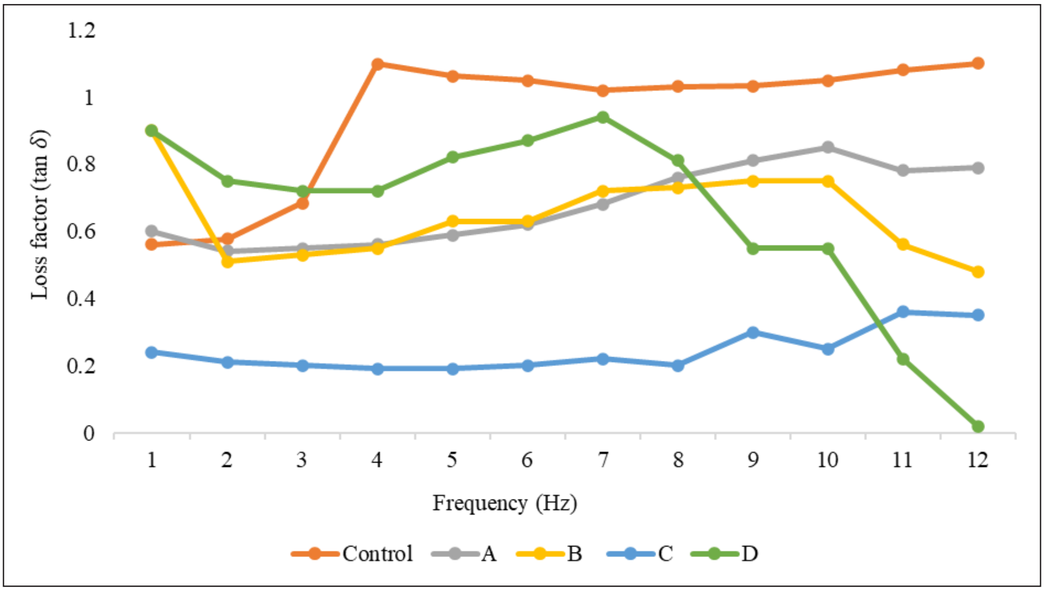

Figure 1b. Loss factor (tan δ) of buffalo meat batter with different amounts of coconut flesh or ultrasound-treated coconut flesh as meat substitutes
Note. 100% meat (control), 50% meat + 50% coconut flesh (sample A: 50%C), 100% coconut flesh (sample B: 100%C), 50% meat + 50% ultrasound-treated coconut flesh (sample C: 50%USC), and 100% ultrasound-treated coconut flesh (sample D: 100%USC)

and 100%USC. The 50%USC sample exhibits a spongy appearance due to dense protein aggregates. Ultrasound-treated coconut flesh samples display a protein network appearance similar to that of the control, but denser and more homogeneous. Overall, the gels appeared to have smaller pores than the controls, which supports the findings of increased elastic modulus.

This finding aligns with Liu et al. (2016), who investigated the effect of protein aggregates of varying sizes on the textural and microstructural features of fat-free acidified milk model systems. Nanoparticulated whey protein was found to produce acid gels exhibiting increased firmness, accelerated gelation, reduced whey separation, and a denser microstructure. Consequently, the intrinsic properties of protein aggregates emerge as a key factor influencing the extent to which the rheological characteristics of emulsion gels are modified. Similarly, our study showed that the addition of treated coconut yielded comparable outcomes, including improved texture, enhanced emulsion stability, and more organised protein networks. Moreover, it was observed that the textural modifications of protein aggregates were mainly due to interactions with other proteins within the continuous phase.

Microstructural analysis of the meat batter samples also revealed a slightly porous, coarse structure with minimal voids, consisting of finely suspended meat particles and fat

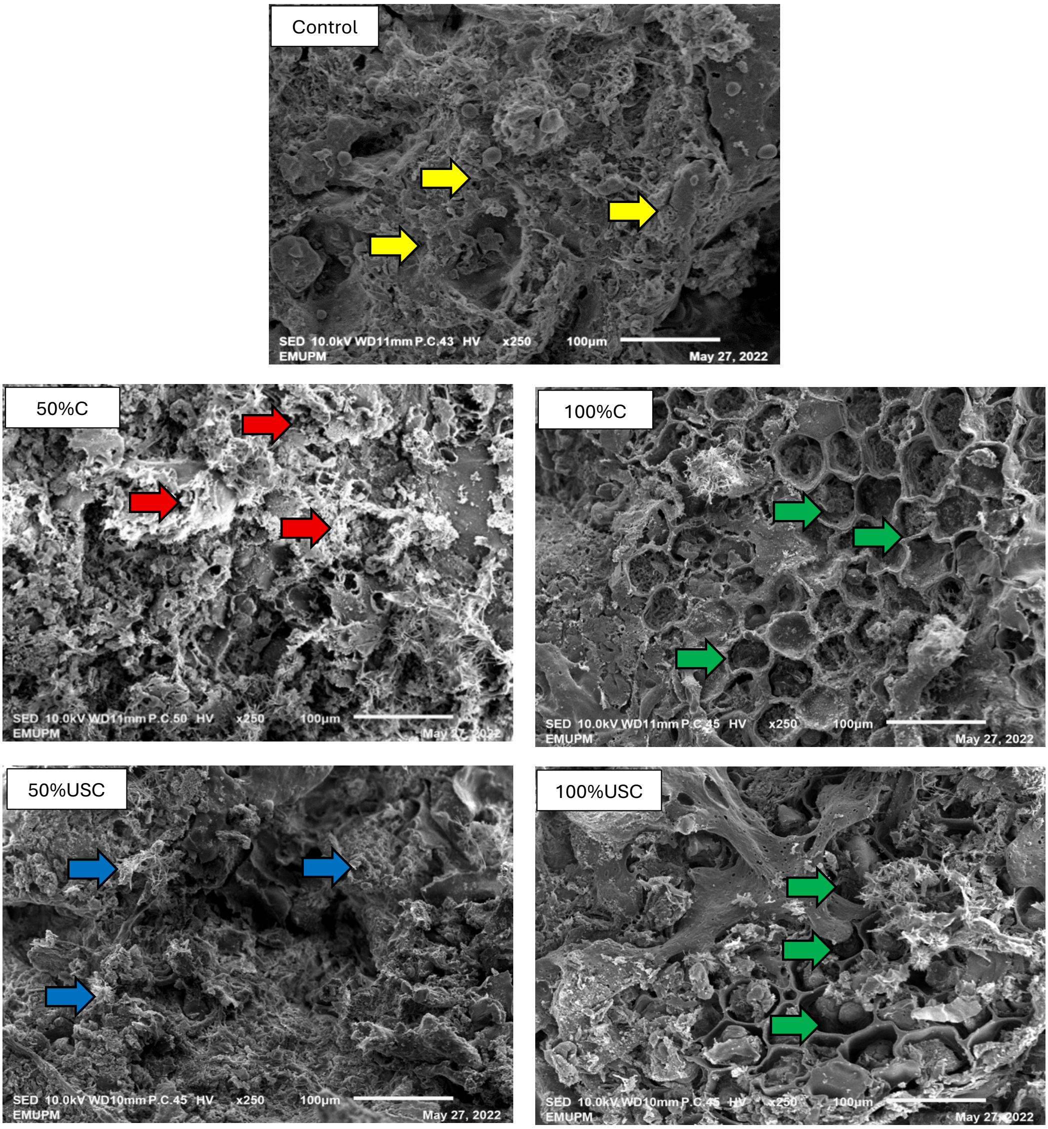

Figure 2. Scanning Electron Micrograph (250× magnification) of buffalo meat batter with different amounts of coconut flesh or ultrasound-treated coconut flesh as meat substitutes
Note. 100% meat (control), 50% meat + 50% coconut flesh (50%C), 30% meat, 100% coconut flesh (100%C), 50% meat + 50% ultrasound-treated coconut flesh (50%USC), 30% meat and 100% ultrasound-treated coconut flesh (100%USC)
Yellow arrows: homogenised meat matrices; Red arrows: coconut flesh with meat; Blue arrows: disrupted coconut flesh homogenised with meat; Green arrows: pores of coconut flesh batters

globules dispersed within a continuous protein matrix. This indicates that fat appeared to be interconnected with the protein network in the gel, as in the control. It has already been shown that as protein concentration increases, gel holes shrink and protein distribution becomes more uniform throughout the gel. In line with comparable structures from previous investigations, Jiménez et al. (2010) and Tahmasebi et al. (2016) identified that the size of fat globules decreases as the globulin-stabilised emulsion exhibits a lower coalescence index and flocculation factor. The fat globule shrinks as emulsion stability rises. This was corroborated by Kang et al. (2022), who partially replaced beef with soybean protein and found that increasing the soybean protein substitution ratio reduced pore size, thereby decreasing fat globule size. Thus, the addition of treated coconut flesh impacted the formation of the protein network and, consequently, the final texture of the batter emulsion.

CONCLUSION

This study shows that ultrasound-treated coconut flesh (50%USC) is a promising partial meat replacer in emulsified buffalo sausages. The 50%USC formulation produced the most stable emulsion, with improved moisture retention and higher fibre, while maintaining acceptable physicochemical and textural properties within the range of commercial products. Rheological and microstructural results further confirmed that ultrasound treatment improved the incorporation of coconut flesh without compromising product integrity. Untreated coconut flesh (50%C) also performed comparably to the control and may serve as a secondary option. Overall, using ultrasound-treated coconut flesh can support the development of healthier, lower-fat meat products and offers a sustainable approach for valorising coconut by-products.

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CONFLICTS OF INTEREST

The authors have declared no conflict of interest.

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