Current Research in Food Science 10 (2025) 101057

Contents lists available at ScienceDirect

Current Research in Food Science

journal homepage: www.sciencedirect.com/journal/current-research-in-food-science

Techno-functional of peanut flour substituting on hybrid chicken meatball physico-chemical and nutritional qualities

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ARTICLE INFO

Handling Editor: Professor A.G. Marangoni

Keywords:
Chicken
Hybrid
Meat ball
Nutrition
Plant-based
Peanut

ABSTRACT

Consumer demand for healthy and sustainable food has driven the development of alternative meat products. This study investigated the effects of substituting peanut flour for chicken meat in meat balls. The hybrid chicken meat balls (HCM) were prepared by replacing chicken breast with 0, 25, 50, and 75 % peanut flour. Proximate composition, physico-chemical properties, textural quality, and protein patterns of HCM were determined. The protein (18.15–20.78 %), fat (0.13-18.71%), fiber (0.56–1.82 %), and ash (1.53–2.32 %) content increased with an increase in peanut flour. The highest cooking yield (99.16 %) was observed at 75 % peanut flour HCM, resulting in the highest juiciness (14.92 %). Texture profile analysis suggested that sample 25 % peanut flour HCM exhibited the most promising textural attributes and higher flour contents resulted in a decline in most textural attributes. HCM with 25 % peanut flour displayed a dense and continuous protein network, whereas 50 and 75 % peanut flour HCM had a looser, more porous structure. The amino acid profile of 25 % peanut flour HCM showed a significant presence of essential amino acids. The study concludes that substituting 25 % peanut flour improves protein and fiber content while maintaining desirable textural attributes, suggesting that peanut flour is a promising and sustainable option for meat ball production.

1. Introduction

Meat has been considered an essential part of the human diet since ancient times (Stanford and Bunn, 2001). Meat demand has increased by more than 58 % during the previous two decades, owing to significant population expansion and substantial economic growth (Whitnall and Pitts, 2019). However, there are an increasing number of consumers who are decreasing their meat consumption. There is an increasing number of consumers who can’t consume meat and meat products because of their health problems, thus they must limit their meat consumption as much as possible (Turnes et al., 2023). Almost half of persons aged 65 and above suffer a few chronic health problems, including high blood pressure, type-2 diabetes, hypertension, heart disease, digestive issues, and obesity. Controlling diet is necessary for them. By 2025, more than 15 % of Thai people claim to not consume meat and meat products (Statista, 2023). Worldwide, more than 1.5 billion individuals eat no meat at all (Phoenix, 2024). While awareness of the negative aspect of meat consumption is increasing day by day, the demand for vegetarian and vegan meat substitutes among meat consumers in Western people remains low (Neville et al., 2017; Tarrega et al., 2020; Profeta et al., 2021a, 2021b). Moreover, vegan meat substitutes frequently lack essential nutrients and vitamins (Bohrer, 2019;

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https://doi.org/10.1016/j.crfs.2025.101057
Received 9 February 2025; Received in revised form 15 April 2025; Accepted 22 April 2025
Available online 23 April 2025

Boukid, 2020; Gehring et al., 2020) and contain limiting concentrations of the essential amino acids, including methionine, lysine, tryptophan, and threonine (Schönfeldt and Gibson Hall, 2012), make them inherently less healthy than conscious meat consumption. This could be due to the conserved structure of plant storage proteins, which results in a lack of similar amino acids across plant families, such as sulfuric amino acids in legumes and lysine in oilseeds and cereals (Stone et al., 2015; Dabbour et al., 2019). However, the alternative meat market is growing steadily, with projections of reaching $85 billion by 2030, a significant increase from $4.6 billion in 2018 (UBS, 2019). This growth is fueled by a diverse consumer base, including vegetarians, vegans, health-conscious individuals, and those seeking environmentally friendly food options.

Food scientists and food industries are trying their best to develop alternative proteins from non-meat source. Many plant-based foods offer a compelling nutritional profile, often rich in vitamins, minerals, and dietary fibre, making them ideal functional foods for promoting public health. Peanuts (*Arachis hypogaea*), which are legumes and belong to the *Fabaceae* family, provide a protein-rich alternative to nuts such as almonds and walnuts, with a protein content of 22–30 %, comparable to that of chickpeas and soybean (Arya et al., 2016). The chemical composition in 100g portion of dry-roasted peanuts flour was reported as water 1.81g, protein 24.4g, fat 49.7g, ash 2.92g, carbohydrate 21.3g, total dietary fiber 8.4g (Hosseini Taheri et al., 2024). Their PDCAAS is relatively low (0.7), but when combined with other proteins, they can provide a valuable source of protein (Arya et al., 2016). Moreover, they contain mono- and polyunsaturated fatty acids and are able to fix nitrogen in the soil. With a wholesale price of around $500–600/t, they offer a cost-effective protein source compared to animal products like cheese, milk, and red meat (Katidi et al., 2023). Studies suggest that diets rich in plant-based foods may contribute to a decrease in metabolic dysfunction and cardiovascular disease (Hever and Cronise, 2017; Langyan et al., 2021; Wanezaki et al., 2016) while offering potential immunological (Moudgil and Venkatesha, 2022), anti-inflammatory (Dinarello, 2010), and anticancer benefits (Nakata et al., 2017). Nonetheless, the market for plant-based meat alternatives, also known as meat analogs, is still nascent compared to the red meat industry, currently only holding a 1–2 % share (Statista, 2023). Plant-meat mixtures, so-called meat hybrids, have been proven to address a greater population share (Boukid et al., 2024; Tarrega et al., 2020; Profeta et al., 2021a, 2021b). These products may have better sensory acceptance than plant-based meat substitutes (Neville et al., 2017). These products could overcome the shortcomings of plant-based products (such as flavor and texture) but could still offer environmental and potentially also health benefits. Hybrid meat products also differ in terms of fillers and extenders. The addition of high-quality, protein-rich plant ingredients in hybrid meat products provides economical and process benefits with healthier and environment friendly food products (Grasso and Jaworska, 2020). With a 50 % plant protein substitution, meat consumption can be reduced by twofold, potentially reducing the environmental effect of the food system by 11 % (van Dooren et al., 2014). The sensorial properties and acceptability of hybrid meat products might be increased by textured protein isolates and pulse proteins in a product-dependent manner (Neville et al., 2017; Grasso et al., 2019; Zamuz et al., 2019). Research revealed that substitution of 50 % beef by plant protein could reduce the textural properties and sensory acceptance than 30 % beef substitute (Grasso et al., 2019). This is supported by a study conducted by Grasso and Jaworska (2020), which found that the majority of hybrid meat products with a plant component higher than 30 % that were introduced to the UK market are no longer available. According to consumer analysis, a lot of meat substitutes are called "hybrid" products that mix plants with insects (Smetana et al., 2019, 2021), plants and plants (Smetana et al., 2015), fungi and plants (Smetana et al., 2015; Hashempour-Baltork et al., 2020; Mazumder et al., 2023a), and biomass derived from animals and plants (Guyomarc'h et al., 2021). However, there aren't many comprehensive studies that would take into account processing characteristics, nutritional changes, and sustainability of hybrid products. For these reasons, this study suggests using peanuts to create value-added chicken meat balls as part of a healthy diet. Consequently, the goal of this present study was to find a suitable concentration of peanut flour to substitute chicken breast meat during the processing of hybrid chicken meat balls (HCM) and to compare the physico-chemical properties of the optimized HCM with control (chicken meat ball) samples including nutritional value, textural profile, microstructure and protein patterns.

2. Materials and methods

2.1. Raw materials

Fresh peanut grains were purchased from a Makro supermarket, Chiang Rai, Thailand. King oyster mushrooms were supplied by Bannhedkrang farm, Songkhla, Thailand. Other food-grade ingredients (Table 1) were bought from Krungthep Chemipan Co. Ltd., Bangkok, Thailand. Chicken breast meat (CP brand, Thailand) was purchased from the Big C supermarket, Chiang Rai, Thailand.

2.2. Preparation of peanut flour

Debris as well as premature peanuts were removed manually, followed by washing with potable water to remove any leftover dirt. Peanuts were parched at 45 °C for 14 h followed by roasting the peanuts in an oven at 177 °C for 10 min. After 10 min the nuts were removed, mixed, and roasted for another 10 min or until light golden brown and fragrant state. The dried peanuts were ground into a fine powder with a pulverizer machine (RT-04A, Rong Tsong Precision Technology Co., Taichung, Taiwan). The ground peanuts were sieved through a 70-mesh (0.212 mm) sieve and stored at −20 °C in a re-sealable HDP zipper bag (Mazumder et al., 2024a).

2.3. Preparation of mushroom powder

Mushroom was added in the recipe to increase the nutrient content of hybrid chicken meat ball especially amino acids and fiber. Fresh king oyster mushroom (*Pleurotus eryngii*) (ash = 6.01 ± 0.55, crude fat = 2.70 ± 0.01, crude protein = 22.25 ± 0.5, wt%, d.b) was supplied by Bannhedkrang farm, Songkhla, Thailand. Mushrooms were sliced 3 mm thick and steamed at 130 °C for 5 s. Blached mushrooms were chopped by a meat grinder (SOKANY®, SK-312, Zhejiang, China). The coarsely minced mushrooms were dried in a cabinet try dryer (BP-80, KN Thai TwoOp, Bangkok, Thailand) at 60 °C for 10 h. The dried mushrooms were pulverized into a fine powder using a pulverizer machine (RT-04A, Rong Tsong Precision Technology Co., Taichung, Taiwan) and sieved through a 60-mesh (0.250 mm) sieve (Mazumder et al., 2024a).

Table 1
Base formulation for hybrid chicken meat ball (HCM).

Ingredients (%, by weight)	Control	T_1	T_2	T_3
Peanuts flour	0	22.68	45.37	68.06
Chicken breast meat	91.16	68.06	45.37	22.68
Tomato powder	0	0.45	0.45	0.45
Rice bran	0	0.91	0.91	0.91
King oyster mushroom powder	0	3.63	3.63	3.63
Cassava starch	4.56	0	0	0
Wheat flour	3.65	3.63	3.63	3.63
Baking flour	0.46	0.45	0.45	0.45
Salt	0.18	0.19	0.19	0.19

Control = 100 % chicken meat ball. T_1 = 25 % peanut flour and 75 % chicken breast meat based hybrid chicken meat ball (HCM), T_2 = 50 % peanut flour and 50 % chicken breast meat based hybrid chicken meat ball (HCM), T_3 = 75 % peanut flour and 25 % chicken breast meat based hybrid chicken meat ball (HCM).

2.4. Preparation of meat balls

Residual tissues and fats were removed from the chicken breast meat. Chicken breast meat was chopped into small pieces and ground using a meat mincer (SIR1-TC12E, SEVENFIVE DISTRIBUTOR Co., Ltd., Nonthaburi, Thailand). Three HCM were formulated using 25, 50, and 75 % peanut flour instead of chicken breast meat (Table 1). 100 % chicken (no peanut flour) meat balls were used as a control. Meat balls were prepared as described by Ikhlas et al. (2011) with slight modifications. All the ingredients were measured on a weight-by-weight basis and blended using a lab-scale mixing machine for 4 min. The resulting batter was shaped into meat balls (1-inch diameter and 28 g weight) using a meat ball mold and cooked in two stages (first at 40 °C for 20 min, followed by 90 °C for 20 min) (Yu, 1994). The meat balls were packaged in high-density polyethylene bags and stored at −18 °C until further analysis.

2.5. Proximate composition analysis

The proximate composition of HCM was determined using AOAC methods, including moisture method 950.46 (AOAC International, 2019a), ash method 920.153 (AOAC International, 2019b), crude protein method 981.10 (AOAC International, 2019c), fat method 922.06 (AOAC International, 2019d), and crude fiber (AOAC International, 2005) using a hot air oven, muffle furnace, Kjeldahl apparatus, Soxhlet apparatus, and gravimetrically, respectively. The total carbohydrate content was calculated by deducting the moisture, fat, protein, fibre and ash from 100 percent, as per FAO recommendations (FAO, 2003).

2.6. Determination of physical properties of HCM

2.6.1. Cooking yield

The cooking yield was determined by measuring the difference in the sample weight before and after cooking (Mazumder et al., 2024b). Raw meat balls were weighed, then cooked and surface-dried with a filter paper and measured once more using an analytical scale balance. The cooking yield was calculated using the formula:

$$\%\ \text{cooking yield} = \frac{\text{Weight of cooked meat balls}}{\text{Weight of raw meat balls}} \times 100 \qquad \text{Eq 1}$$

2.6.2. Juiciness

Juiciness was determined by cutting a cooked meat ball sample into 3-mm pieces and placing it between two pre-weighed Whatman (No. 4) filter paper, covered with aluminum foil, and pressed for 1 min by 10 kg of force. The sample was removed, and the filter paper was weighed. The extracted juice was determined as follows (Gujral et al., 2002) (Eq (2)).

$$\text{Juiciness (\%)} = \frac{\text{Weight of filter paper after pressing - weight of filter paper before pressing}}{\text{Weight of sample}} \times 100 \qquad \text{Eq. 2}$$

2.6.3. Color measurement

Color attributes of the hybrid meat balls were determined at three randomly chosen spots in the samples by Hunter colorimeter (Hunter Lab/color Quest XE, Reston, Color Global, Bangkok, Thailand) using 10° standard and illuminant D65. The instrument was initially calibrated using white and black tiles to determine the color parameters such as L* (lightness), a* (redness), and b* (yellowness). The whiteness index was determined using equation (3) (Ramatsetse et al., 2024).

$$W = 100 - \sqrt{\left[(100\text{-}L^*)^2 + (a^*)^2 + (b^*)^2\right]} \qquad \text{Eq. 3}$$

2.6.4. Texture profile analysis (TPA) of HCM

The TPA of HCM was evaluated using a TA.XT equipped with an SMSP/75 fixture for compression analysis (Stable Micro Systems, Surrey, UK) according to Mazumder et al. (2024b). The HCM was cut into 1.5 × 1 × 1.5 cm^3 (length × width × height). The meat balls were compressed twice to 30 % of their initial height between leveled plates. The texture analyzer with a 3.6 cm diameter probe, linked up with a PC, and software provided by the Texture Operating System Corporation (Stable Micro Systems, Surrey, United Kingdom) was used. Hardness (maximum force of the first compression phase, N), springiness (length of the detected height of the sample on the succeeding (2nd) pressing split by the initial pressing length, mm/mm), cohesiveness (ratio of work of the second compression and first compression, J/J), chewiness and resilience (upstroke energy of first compression divided by downstroke compression energy, J/J) were obtained.

2.7. Microstructure analysis

Each sample of HCM was cut (5 mm × 5 mm × 5 mm) and put into a microcentrifuge. The sample was frozen in dry ice and lyophilized in a freeze-dryer (Labconco, FreeZone8L, MO, USA). The freeze-dried samples were examined using a scanning electron microscope (JSM-6420, Jeol, Akishima, Japan), and microscopic pictures of each treatment were taken and documented (Zhuang et al., 2009).

2.8. Biochemical assay

2.8.1. Electrophoresis profile of HCM

Dis-continuous SDS-PAGE (under denaturing condition) was applied to determine the protein patterns of HCM (Sathe et al., 2012). The samples were mixed in a 1:1 ratio with sample buffer (0.5 M Tris-HCl, pH 6.8, containing 4 % SDS, 20 % glycerol, and 0.03 % bromophenol blue with 10 % DTT) and boiled for 3 min. Protein samples were transferred into Roti-PAGE gradient (4–20 %) precast gels and separated at a continuous current of 60 mA in an electrophoresis buffer tank filled with solution using a Biostep® GmbH power supply (Jahns Dorf, UK). The gels were stained overnight in a staining solution (Coomassie Blue R-250-methanol-acetic acid) while shaking gently at 50 rpm. The gel was de-stained with de-staining solutions I and II (methanol, acetic acid, and water) until the background was clear.

2.8.2. Amino acid profile

The amino acid composition of hybrid chicken meat balls was analyzed at the Central Laboratory, Ltd., Muang, Chiang Mai, Thailand, using the in-house method (TE-CH-372) based on Official Journal of the European Communities, Commission Directive 98/64/EC, L257 (1998), and the results were reported as g/100 g dry sample.

2.9. Statistical analysis

Data were expressed as means ± standard deviation. The data were subjected to analysis of variance followed by Duncan's multiple range post hoc test using SPSS 26.0 for Windows. The 95 % confidence level ($p < 0.05$) was considered to be statistically significant among different samples.

3. Results and discussion

3.1. Proximate composition of HCM

The proximate compositions of HCM using different percentages of peanut flour are shown in Table 2. The HCM and control had a moisture, ash, protein, crude fiber, and fat content ranging from 37.55 to 75.04%, 1.50 to 2.95%, 17.55 to 21.70%, 0.57 to 6.60%, and 2.63 to 17.15%, respectively. The control meat balls (0 % peanut flour) had the highest moisture content (75.04 ± 1.35 %), followed by T_1 (25 % peanut flour, 59.04+1.50%), T_2 (50 % Peanut flour, 46.05+1.25%) and T_3 (75 % peanut flour, 37.55+1.10%). The moisture content thus exhibited an inverse relationship with peanut flour addition. This is likely due to the

Table 2
Proximate composition of hybrid chicken meat ball (HCM) and the control (% wet basis).

Sample	Moisture	Ash	Protein	Fiber	Fat	Carbohydrate
Control	75.04 ± 1.35^a	1.50 ± 0.15^c	17.55 ± 1.25^b	0.57 ± 0.15^c	2.63 ± 0.9^d	2.71 ± 0.25^d
T_1	59.05 ± 1.50^b	2.12 ± 0.09^b	19.94 ± 1.05^a	3.80 ± 0.90^b	6.92 ± 1.40^c	6.17 ± 0.75^c
T_2	46.05 ± 1.25^c	2.72 ± 0.25^b	20.88 ± 0.75^a	5.75 ± 0.15^a	13.10 ± 1.12^b	11.50 ± 0.70^a
T_3	37.55 ± 1.10^c	2.95 ± 0.90^a	21.70 ± 0.55^a	6.60 ± 0.40^a	17.15 ± 1.71^a	15.05 ± 1.20^b

The data are expressed as mean ± standard deviation. Means with different superscripts along each column are significantly different at $p < 0.05$. Control = 100 % chicken meat ball. T_1 = 25 % peanut flour and 75 % chicken breast meat based hybrid chicken meat ball (HCM), T_2 = 50 % peanut flour and 50 % chicken breast meat based hybrid chicken meat ball (HCM), T_3 = 75 % peanut flour and 25 % chicken breast meat based hybrid chicken meat ball (HCM).

lower moisture content of peanut flour compared to chicken meat.

Fiber content was higher in peanut flour-based HCM than in the control without added peanut flour. However, HCM prepared with 50 % peanut flour exhibited no significant difference in fiber content compared to that made with 75 % peanut flour, likely due to minor variations in the measurement accuracy. A high-fiber diet is suggested to normalize bowel movement, reduce cholesterol levels, control blood sugar, help maintain bowel health, and reduce body weight (Veronese et al., 2018). Moreover, dietary protein is especially needed for functional needs including healthy growth and muscle development and the addition of peanut flour significantly increased the protein content of the formulations (Drummen et al., 2018). Therefore, consumption of HCM may contribute to the recommended daily allowance (RDA) for protein with a recommended intake of 0.8 g of protein per kg body weight, depending on gender and age (USDA. Composition of Foods Raw, 2015).

Moreover, ash and fat content were increased by the addition of peanut flour. Peanut flour is a well-documented source of protein, fat, and fiber (Zhao et al., 2023), explaining the observed rise in these components within the HCM. The ash content is also likely to increase due to the mineral profile of peanut flour. The proximate composition measurements suggest that adding peanut flour to the meat ball formulation will have a considerable effect on its properties due to changes in moisture, protein, and fiber content. To find a suitable concentration of peanut flour, the physical properties of HCM were analyzed.

3.2. *Physical properties of HCM*

Table 3 shows the results of the cooking property measurements of the meat balls. Cooking yields are one of the most essential factors for the meat sector in predicting product behavior when cooking (Pietrasik and Li-Chan, 2002). During the cooking process, meat loses weight and volume due to expulsion of fluid (Purslow et al., 2016). Cooking induces loss of water as cooking loss and fat melts, which causes drips out of the product (Bejerholm et al., 2014). Shrinkage of meat balls during the cooking process might be due to the denaturation of the meat proteins, resulting in less water to be held and entrapped with the protein structure by capillary forces (Shahiri Tabarestani and Mazaheri Tehrani, 2014). Previous research indicates that incorporation of plant-based flour in meat products increased the cooking yield (Dzudie et al., 2002; Pereira et al., 2016; Leonard et al., 2019). Similarly, Fang et al. (2018) and Mena et al. (2020) demonstrated that the addition of 3 % sugarcane fiber decreased the cooking yield of chicken sausages. The cooking yield was the highest for the hybrid with 75 % peanut flour (99.16 %) HCM, followed by 50 % peanut flour (97.81 %) HCM, 25 % peanut flour (95.46 %), and 0 % peanut flour (95.06 %). However, the control and T_1 (25 % peanut flour) did not significantly differ from each other. In addition, Table 3 reports a juiciness of 3.04–14.92 % of the control and the HCMs. Meat balls using 75 % peanut flour had the highest ($p < 0.05$) juiciness compared to the 25 and 50 % peanut flour formulated HCM. In general, the juiciness increased with increasing peanut flour content, indicating potential positive effects on the sensory attributes with peanut flour addition.

Cooking yield, a key parameter for meat processors, displayed a significant correlation with peanut flour content (Kyriakopoulou et al., 2021). While all formulations experienced shrinkage during cooking due to protein denaturation and consequent water loss, meat balls with higher peanut flour content showcased superior cooking yield. This trend likely stems from the water-binding properties of peanut flour and the overall higher dry matter content for samples formulated with peanut flour, minimizing shrinkage and maximizing cooked product weight. Furthermore, peanut flour content positively impacted moisture retention and juiciness, both crucial sensory aspects of meat balls. These findings suggest that peanut flour not only enhances water holding during cooking but may also contribute to a juicier final product, potentially improving consumer palatability. Though proximate composition and physical properties (cooking yield, and juiciness) were higher in 75 % peanut flour HCM, color parameters and textural properties needed to be investigated to find the suitable concentration of peanut flour.

3.3. *Color attributes of HCM*

Table 4 shows the L*, a*, and b* values of HCM and the control after being cooked. All the color coordinates of the HCMs were significantly different ($p < 0.05$) compared to the control. The analyses show that the 0 % peanut flour (control) had the highest value of lightness (77.74) and

Table 3
Cooking yield and juiciness of hybrid chicken meat ball (HCM) using different percentages of peanut flour.

Sample	Cooking yield (%)	Juiciness (%)
Control	95.06 ± 1.06^c	3.04 ± 0.29^c
T_1	95.46 ± 0.12^c	4.42 ± 0.57^c
T_2	97.81 ± 0.23^b	10.84 ± 1.15^b
T_3	99.16 ± 0.66^a	14.92 ± 0.71^a

The data are expressed as mean ± standard deviation. Means with different superscripts along each column are significantly different at $p < 0.05$. Control = 100 % chicken meat ball. T_1 = 25 % peanut flour and 75 % chicken breast meat based hybrid chicken meat ball (HCM), T_2 = 50 % peanut flour and 50 % chicken breast meat based hybrid chicken meat ball (HCM), T_3 = 75 % peanut flour and 25 % chicken breast meat based hybrid chicken meat ball (HCM).

Table 4
Color parameter values of hybrid chicken meat ball (HCM) and the control using different percentages of peanut flour.

Sample	L*	a*	b*	Whiteness index
Control	77.74 ± 1.35^a	0.37 ± 0.23^c	15.11 ± 0.86^c	73.07 ± 1.09^a
T_1	64.26 ± 2.13^b	6.28 ± 0.36^a	20.64 ± 1.36^a	58.23 ± 2.28^b
T_2	51.27 ± 2.45^c	9.27 ± 0.45^b	21.60 ± 1.14^a	45.79 ± 1.94^c
T_3	51.77 ± 1.97^c	9.32 ± 0.26^b	21.53 ± 1.00^a	46.34 ± 1.41^c

The data are expressed as mean ± standard deviation. Means with different superscripts along each column are significantly different at $P < 0.05$. Control = 100 % chicken meat ball. T_1 = 25 % peanut flour and 75 % chicken breast meat based hybrid chicken meat ball (HCM), T_2 = 50 % peanut flour and 50 % chicken breast meat based hybrid chicken meat ball (HCM), T_3 = 75 % peanut flour and 25 % chicken breast meat based hybrid chicken meat ball (HCM).

that the HCM formulations generally exhibited lower lightness values. However, there was no significant difference ($p > 0.05$) between 50 and 75 % peanut flour HCM in terms of lightness (L*) value. HCM formulated with 75% peanut flour exhibited the highest (a*) value (9.32), followed by 50% (9.27), 25 (6.28), and 0 % peanut flour (0.37) HCM. For yellowness (b*), groups of 25, 50, and 75 % peanut flour HCM showed no significant difference ($p > 0.05$), however, these values were higher than the control (0 % peanut flour). Few research demonstrated that the addition of plant-based flour increased the darkness of the hybrid meat products. Leonard et al. (2019) obtained higher darkness and yellowness of the beef ball due to the addition of sugarcane fibre. Mean et al. (2020) also reported that higher yellowness for lupin-enriched beef sausages, which makes sense as sugarcane flour is visually similar to lupin flour. Samutsri et al. (2023) suggest that increasing the unripe jackfruit content increased the darkness of chicken meat ball.

The color characteristics of cooked HCM, as shown in Table 4, reveal a considerable impact of peanut flour addition to the formulation. This translates to a visually distinct appearance for HCM with varying peanut flour concentrations. The control formulation (0 % peanut flour) displayed the highest lightness value, indicating a lighter color. The chosen chicken breast meat is white, which means there is a chance of decreasing L* values with the addition of peanut flour. This is likely due to the inherent dark color of peanut flour compared to meat. As the peanut flour concentration increased (25, 50, and 75 %), the lightness values progressively decreased. This trend suggests a darkening effect on the meat balls with increasing peanut flour substitution. Other studies suggested that this darkening might be attributed to the presence of phenolic compounds in peanut flour, which can induce browning reactions during cooking (Qiu and Chin, 2020). The redness (a^*) values exhibited some interesting variations. The 75 % peanut flour HCM had the highest redness, followed by the samples formulated with 50 and 25% peanut flour, respectively. This variation might be due to natural pigment differences in peanut flour batches or interactions between peanut and meat myoglobin during cooking (McClements et al., 2015). Interestingly, the 25 % peanut flour HCM displayed a lower redness value ($a^* = 6.28$) compared to other treatments. Yellowness (b*) values, on the other hand, showed no significant difference ($p > 0.05$) between the 25, 50, and 75 % peanut flour formulations. This suggests a similar degree of yellowness in these groups. However, all peanut flour-incorporated HCM displayed higher yellowness values compared to the control group. This can be attributed to the natural yellow pigments present in peanut flour, likely carotenoids. Given the above explanation, it is reasonable to expect that an HCM product with 25 % peanut flour will have the best visual acceptance (Table 4). However, also textural attributes are important, which will be described in the next section.

3.4. Textural properties of HCM

The textural qualities of HCM with increased peanut flour were investigated. TPA measurements revealed that the addition of peanut flour to HCM had a significant impact on its textural properties. Hardness values ranged from 3.07 N (75 % peanut flour) to 8.75 N (0 % peanut flour), springiness from 0.60 mm (75 % peanut flour) to 0.84 mm (0 % peanut flour), and cohesiveness from 0.20 J/J (75 % peanut flour) to 0.47 J/J (0% peanut flour). Hardness and chewiness followed similar trends across treatments, with 0 % peanut flour having the highest values for both hardness and chewiness. Interestingly, the treatments with the highest peanut flour content (50 and 75 %) showed low hardness values (4.88 and 3.07 N, respectively) (Table 5). The results showed that this treatment significantly reduced the force required to compress the sample, which can have an impact on the mouthfeel of a product. This is likely due to increased porosity from the addition of peanut flour and the presence of undissolved flour as well as fat particles (Alakali et al., 2010), which may reduce cross-linking with myofibrillar proteins and is expected to decrease hardness in samples with higher levels of peanut flour (Mazumder et al., 2023c). In addition, the findings demonstrated that HCM prepared with an increasing peanut flour concentration lowered the sample's springiness, whereas HCM made with 75 % peanut flour had the lowest capacity to restore its original dimension after compression. A low springiness value indicates that the material was plastically deformed (Toontom et al., 2022). Furthermore, compositions with 0 % peanut flour exhibited the highest chewiness. The result correlates to the hardness value. While chewiness is a calculated metric that is partly generated from hardness and springiness, hardness takes precedence due to its higher value when compared to other treatments. Table 5 indicates that chewiness was dramatically reduced by more than 10 times with the addition of 75 % peanut flour. Zeraatkar et al. (2019) discovered that substitution of chicken meat either partially or entirely by plant-based protein increased the hardness of chicken sausages. In addition, a mixer of mushroom, chickpea protein and pea protein isolates increased the hardness of chicken sausages. Mazumder et al. (2024a) found that beef sausages (Thai style) exhibited better textural properties than plant-based sausages. The plant-based sausages had higher hardness and chewiness value compared to beef sausages (Thai style). Similarly, Mazumder et al. (2023c) demonstrated that plant-based emulsified sausages are associated with increased textural properties than chicken-based emulsified sausages. Research shows that original chicken nuggets had better textural properties than plant-based chicken nuggets (Abdullah et al., 2018).

Texture analysis suggests that the formulation with 25 % peanut flour HCM had better textural properties than other HCM and was more comparable to the control (no peanut flour addition). However, due to the negative impact of reduced chewiness, springiness, and hardness, a 75 % peanut flour treatment might decrease consumer acceptance of HCM. Given the above explanation, it is reasonable to expect that an HCM product with 25 % peanut flour will have the best structural properties (Fig. 1). To determine the answer to this question, a microstructure analysis was conducted.

3.5. Microstructure of HCM

Microscopic analysis revealed distinct microstructures in the HCM formulated with varying peanut flour content (Fig. 1). As expected,

Table 5
Textural quality of hybrid chicken meat ball using different percentages of peanut flour.

Parameter	Control	T_1	T_2	T_3
Hardness (N)	8.75 ± 3.15^a	8.88 ± 2.15^a	4.88 ± 1.53^{ab}	3.07 ± 0.19^b
Adhesiveness	0.72 ± 0.14^a	0.71 ± 0.17^a	0.57 ± 0.32^b	0.45 ± 0.10^c
Springiness (mm/mm)	0.84 ± 0.01^a	0.84 ± 0.01^a	0.71 ± 0.04^b	0.60 ± 0.01^c
Cohesiveness (J/J)	0.47 ± 0.03^a	0.44 ± 0.05^a	0.33 ± 0.02^a	0.20 ± 0.05^a
Gumminess	4036.15 ± 122.22^a	3825.08 ± 195.66^a	2666.89 ± 257.99^b	2212.70 ± 140.19^c
Chewiness	3383.74 ± 86.60^a	3206.72 ± 71.76^a	2107.90 ± 45.24^b	1936.89 ± 28.29^c
Resilience (J/J)	0.18 ± 0.02^a	0.15 ± 0.02^{ab}	0.13 ± 0.01^b	0.10 ± 0.01^c

The data are expressed as mean ± standard deviation. Means with different superscripts across each row are significantly different at $p < 0.05$. Control = 100 % chicken meat ball. T_1 = 25 % peanut flour and 75 % chicken breast meat based hybrid chicken meat ball (HCM), T_2 = 50 % peanut flour and 50 % chicken breast meat based hybrid chicken meat ball (HCM), T_3 = 75 % peanut flour and 25 % chicken breast meat based hybrid chicken meat ball (HCM).

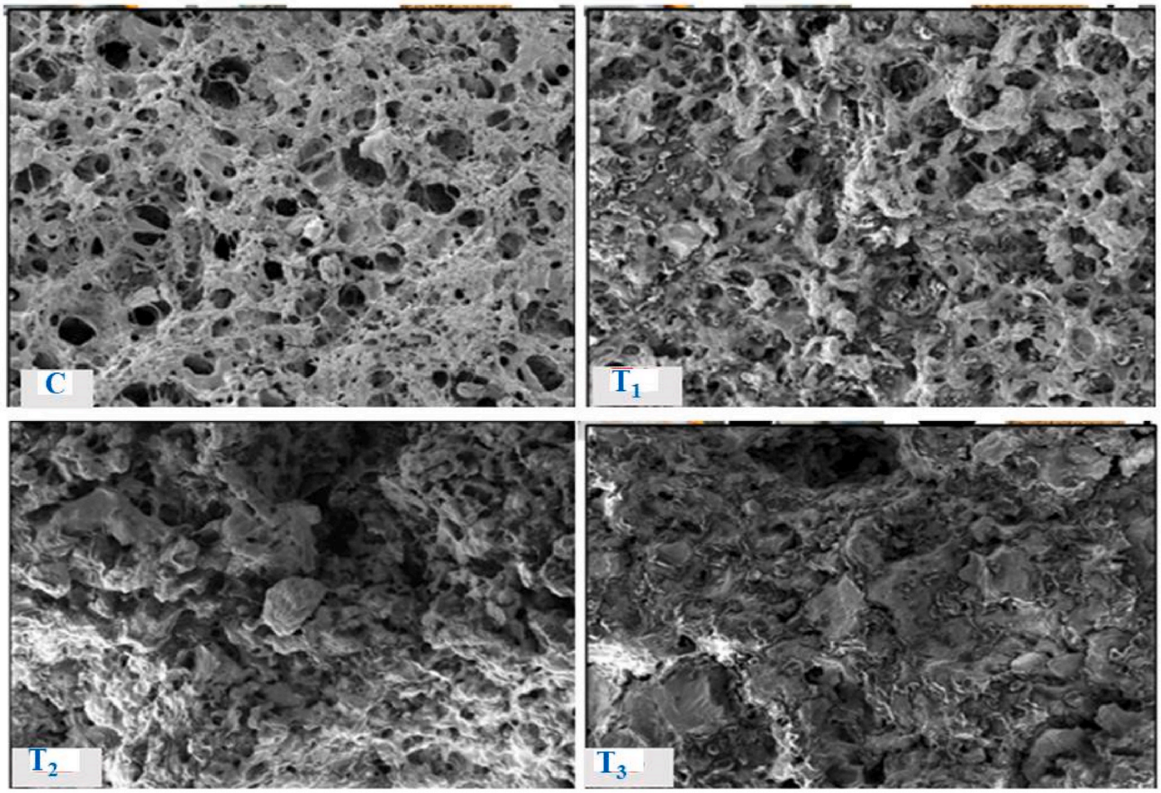

Fig. 1. Effects of substituting chicken breast meat with peanut flour on the microstructure of hybrid chicken meat ball (HCM). Control = 100 % chicken meat ball. T_1 = 25 % peanut flour and 75 % chicken breast meat based HCM, T_2 = 50 % peanut flour and 50 % chicken breast meat based HCM, T_3 = 75 % peanut flour and 25 % chicken breast meat based HCM.

control group and T_1 exhibited a dense and continuous protein network with small lipid pockets. This dense protein network likely contributes to a firmer and chewier texture in the meat balls (Huang et al., 2011; Sun et al., 2017). In contrast, samples T_2 and T_3 displayed a looser and more porous microstructure with larger visible pores and more fat particles dispersed throughout the sample (Fig. 1). This observation suggests that the incorporation of peanut flour may have disrupted the continuity of the chicken meat protein network during processing, potentially leading to a more discontinuous structure. This aligns with the theory that a denser protein network is associated with a firmer and chewier texture, while a looser structure is linked to a softer and less chewy bite (Shin and Choi, 2022). Research suggests that pea protein formed a rough-edged, unevenly shaped structure that trapped small air bubbles in the meat products. Higher concentrations of pea protein (9 and 12 %) resulted in larger voids in the myofibrillar protein gel matrix (Lin and Barbut, 2024). On the other hand, Zhu et al. (2022) demonstrated that adding pea protein enhanced the density of the pea-duck meat analogs by filling holes in the matrix. Lin and Barbut (2024) also demonstrated that brown rice substitution in meat products produced small aggregates which were dispersed throughout the myofibrillar protein gel matrix. This is due to the weak gelling capacity of brown rice, which explains why it had a lower influence on hardness than other plant proteins. In contrast, Santos et al. (2022) revealed that 50 % hydrated rice protein found a larger disruptions in the microstructure of hybrid meat products. Lin and Barbut (2024) indicates that the addition of faba bean produced a tiny and dispersed particles as well as some of the largest but poorly connected aggregates in the hybrid meat analogs. It might be due to the higher carbohydrate content in faba beans, which strongly bind with meat proteins.

3.6. Biochemical properties of HCM

3.6.1. Acid amino profile

The amino acid profile of the 25 % peanut flour HCM revealed a significant presence of various essential amino acids. These essential amino acids, including threonine (6.41g/100 g product), valine (8.16 g/100 g product), methionine (2.37 g/100 g product), isoleucine (6.57 g/100 g product), leucine (11.95 g/100 g product), phenylalanine (7.50 g/100 g product), histidine (6.17 g/100 g product), lysine (10.94 g/100 g product), and tryptophan (1.61 g/100 g product) (Table 6), are crucial for the body as they cannot be synthesized by the metabolism and must be obtained through diet (Aliu et al., 2018). Table 6 indicates that most of the essential amino acids were higher in T_1 (25 % peanut flour HCM) except methionine compared to control sample (chicken breast meat ball). This might be due to the presence of peanut, mushroom and chicken protein in the HCM. Research revealed that peanut flour contains threonine for only 2.6 g/100g protein; while FAO pattern is 4.0 g/100g protein, much lower than that of meat proteins (Singh and Singh, 1991). However, peanut flour contains valine for 5.3 g/100g protein; while FAO pattern is 5.0 g/100g protein. Other essential amino acids such as lysine (4.0 g/100 g protein), leucine (6.4 g/100 g protein) and isoleucine (3.2 g/100 g protein) were lower than FAO patter (5.5, 7.0 and 4.0 g/100 g protein, respectively). However, peanut flour contains tryptophan for 1.0 g/100g protein; while FAO pattern is 1.0 g/100g protein (Natarajan, 1980). Nonetheless, amino acid composition is required for the stability and contribution to the structure of meat substitutes (Ketnawa et al., 2023). For example, intermolecular disulfide bonds were the key component in stabilizing and contributing to the meatless meat structure (Hager, 1984). This profile suggests that the peanut flour and mushroom addition potentially contributes to the overall nutritional value of the meat ball by providing a source of essential amino acids. However, further research is required to examine

Table 6
Amino acid composition of hybrid meat ball (HCM) formulated with 25 % peanut flour and 75 % chicken breast meat (mg/100 g product).

Amino acid	Chicken meat balls (control)	Hybrid meat balls (25 % peanut flour and 75 % chicken breast meat)	FAO/WHO (2013) reference pattern for Children
Essential amino acids			
Threonine	5.57 ± 0.15[b]	6.41 ± 0.29[a]	3.10
Valine	6.25 ± 0.12[b]	8.16 ± 0.25[a]	4.30
Phenylalanine	4,87 ± 0.18[b]	7.50 ± 0.20[a]	4.50
Isoleucine	5.05 ± 0.10[b]	6.57 ± 0.35[a]	3.60
Leucine	8.15 ± 0.19[b]	11.95 ± 0.50[a]	6.60
Histidine	4.37 ± 0.20[b]	6.17 ± 0.21[a]	2.00
Lysine	7.99 ± 0.13[b]	10.94 ± 0.35[a]	5.70
Methionine	3.89 ± 0.17[a]	2.37 ± 0.20[b]	2.70
Tryptophan	0.95 ± 0.02[b]	1.61 ± 0.05[a]	–
Non-Essential amino acids			
Serine	5.22 ± 0.10[b]	7.18 ± 0.20 [a]	NA
Aspartic acid	9.93 ± 0.15[b]	16.31 ± 0.11[a]	NA
Glutamic acid	17.14 ± 0.19 [b]	27.88 ± 0.25[a]	NA
Glycine	5.07 ± 0.12[b]	7.45 ± 0.20[a]	NA
Alanine	6.36 ± 0.14[b]	8.28 ± 0.30[a]	NA
Tyrosine	3.12 ± 0.18[b]	4.99 ± 0.15[a]	NA
Cystine	ND	ND	NA
Proline	5.66 ± 0.11[a]	5.93 ± 0.14[a]	NA
Arginine	9.11 ± 0.16[b]	13.00 ± 0.30[a]	NA

ND = not detected; NA = not applicable; The data are expressed as mean ± standard deviation. Means with different superscripts across each row are significantly different at $p < 0.05$.

the digestibility of these products, as the availability of amino acids to the body can vary depending on the food matrix.

3.6.2. Electrophoresis profile of HCM

Electrophoresis analysis of the protein profiles under reducing condition (Fig. 2) revealed major protein bands in chicken (>245, 48 and 35 kDa) and peanut flour (about 40 kDa and 20 kDa). Overall protein profiles under these conditions showed that not big different among the samples. The control sample (con) showed the same protein patterns as presented in the chicken protein profile (chi). However, clearly observed the high density of the protein bands around 40 and 20 kDa when the amount of flour was increased in the recipe. In addition, the results

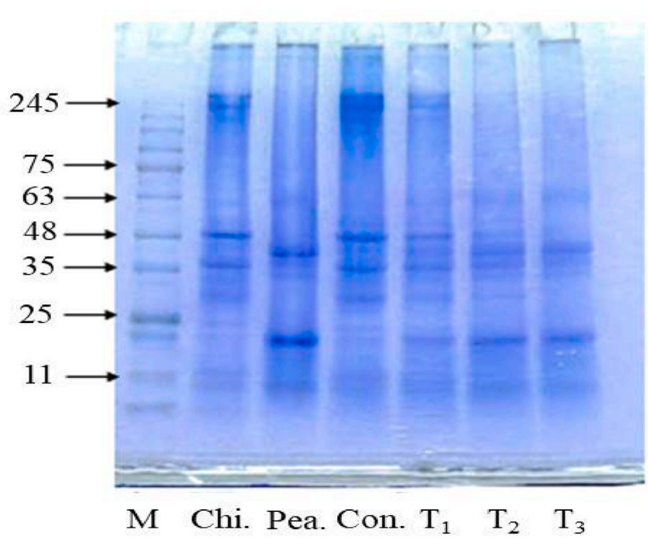

Fig. 2. Protein patterns of hybrid chicken meat balls (HCM) under reducing condition. M = Protein marker, pea. = peanut, chi. = chicken meat, con. = control, T_1 = 25 % peanut flour and 75 % chicken breast meat based HCM, T_2 = 50 % peanut flour and 50 % chicken breast meat based HCM, T_3 = 75 % peanut flour and 25 % chicken breast meat based HCM.

showed significantly different protein profiles between the control sample (con) and the hybrid meat ball (T_1-T_3). This might be due to the presence of different protein sources in the HCM. Steaming mushrooms may denature proteins and change their molecular weight profiles (Kyriakopoulou et al., 2019). Albumins, globulins, glutelin-like materials, glutelins, prolamins, and prolamin-like materials were the most abundant protein components in mushrooms. Riaz and Cheewapramong (2009) demonstrated that lysine, threonine, and methionine were the abundant protein components in peanut flour. High molecular weight protein components were cleary found in the control meat ball, while medium to low molecular weight proteins were found in hybrid meat ball. These results may resuting in different in textural and other physico-chemical properties. High molecular weight may responsible for high textural properties, especially hardness or springiness values. Under the denaturing conditions, the presence of abundant proteins and potential disulfide bonds was clearly observed in peanut flour. This technique separates proteins based on size, allowing estimation of their molecular weight (Laemmli, 1970). Control sample (chicken meat ball) is different from HCM protein patterns; the control meat ball has bigger protein fraction of >245 kDa. The protein pattern of chicken meat ball and HCM identified by electrophoresis gels demonstrated a clear different brand of protein extraction due to diferent protein structure. Further studies are warranted to explore the specific interactions between chicken and peanut flour proteins and identify the optimal peanut flour concentration for achieving the desired textural and functional properties in the final hybrid meat balls.

3.7. Implication of findings and alignment with current research

There are two types of alternative meat products rather than pure meat products throughout the world. The first one is plant-based (PB) meat alternatives, followed by hybrid meat alternatives. Vegetarians, vegans, and pescatarians accounted for one-third (or less) of all consumers. A strong emphasis on health and environmental issue may help to promote the use of PB meat substitutes (Mazumder et al., 2023b). The major challenges for PB meat alternatives are product formulations, product quality and allergens. Consumer acceptance of meat substitutes is influenced by a product's mouthfeel, color, and aroma. The sensory characteristics need to be modified to increase the palatability of the meat analogs (McClements, 2021). Previous research revealed that sensory characteristics and consumer acceptance of hybrid meat products might be achieved by using textured protein isolates along with pulse proteins in a product-dependent manner (Neville et al., 2017; Grasso et al., 2019; Zamuz et al., 2019). Admittedly, processing of protein isolates requires a significant amount of fresh water and energy (Berardy et al., 2015; Braun et al., 2016), and the protein texturization process also costs energy (Saerens et al., 2021). The addition of peanut flour as one of the main ingredients for HCM, might be a good option to reduce the consumption of huge amounts of fresh water and energy. Little research suggests that during the processing of hybrid meat products, less than 30 % plant protein should be used to find the optimum texture and mouth feel of the products (Baune et al., 2021). This study indicates that the inclusion of a maximum of 25 % peanut flour shows comparable color parameters, textural properties and microstructure of HCM to control meatballs. The protein quality of HCM was assessed *via* determination of the amino acid profile and electrophorsis profile. Compositional changes of HCM, based on the raw material composition, were calculated, the nutritional composition and finally, physical properties were analyzed to judge the probable acceptance of HCM.

4. Conclusion

This research reveals that peanut flour has the potential to be used as an alternative ingredient to substitute chicken meat to produce hybrid chicken meat balls with other appropriate ingredients. Nutritional analysis shows that increasing the peanut flour increased the ash, protein, fiber, and fat content. A lower fat content of T_1 (compared to T_2 and T_3) might indicate suitability for preparing HCM containing 25 % peanut flour and 75 % chicken breast meat. However, the fat content of T_1 was significantly higher than the control sample. Physical properties such as cooking yield and juiciness were comparable ($p > 0.05$) between control and sample T_1 (25 % peanut flour and 75 % chicken breast meat based HCM). Replacing chicken breast meat with 25 % peanut flour for the processing of HCM shows comparable textural properties, and microstructure with control samples. Nonetheless, control samples show better color parameters than sample T_1. The amino acid profile shows sample T1 shows a higher presence of all essential amino acids than the control sample except methionine. The amino acid profile shows higher presence of all essential amino acids such as leucine, isoleucine, and lysine in substantial amounts in 25 % peanut flour-based HCM when compared to the reference FAO pattern. Still, methionine content was lower than reference FAO pattern in 25 % peanut flour HCM. This study suggests that the addition of 25 % peanut flour in HCM reduces the methionine content compared to control (chicken meat ball). Peanut flour and chicken breast meat may be utilized as an alternative protein source that has the ability to replace up to 25:75 of HCM. Further research should focus on the bioavailability and allergenicity of HCM.

CRediT authorship contribution statement

Soklin Son: Data curation, Methodology, Software, Visualization, Writing – original draft. **Sreymom Hun:** Data curation, Formal analysis, Software, Visualization. **Md Anisur Rahman Mazumder:** Writing – original draft, Writing – review & editing. **Lutz Grossmann:** Writing – review & editing. **Passakorn Kingwascharapong:** Writing – review & editing. **Samart Sai-ut:** Writing – review & editing. **Pittaya Chaikham:** Writing – review & editing. **Young Hoon Jung:** Writing – review & editing. **Saroat Rawdkuen:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Ethical statement - studies in humans and animals

The authors did not use humans or animals in the present study.

Funding

The authors gratefully acknowledge the financial support from Mae Fah Luang University, Chiang Rai, Thailand *via* the Reinventing University Program Fund (F01-683R-04-045), The Office of the Permanent Secretary of the Ministry of Higher Education, Science, Research and Innovation. Received financial support for master tuition under contract number MFU-GS/M.67-11 from Mae Fah Luang University (Soklin Son).

Declaration of competing interest

The authors declare no conflict of interest. The funders had no role in the design of the study.

Acknowledgments

The authors thankfully to the Scientific and Technological Instruments Center, Mae Fah Luang University, Thailand for supporting both chemical and equipment during the project running.

Data availability

Data will be made available on request.

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