Malaysian Applied Biology
https://doi.org/10.55230/mabjournal.v55i2.3653

Research Article

Effects of Duckweed and Pigments on the Chemical Composition, Physicochemical Properties, and Sensory Attributes of Plant-Based Patties

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ABSTRACT

The present study evaluated the influence of duckweed incorporation (0%, 1%, and 2%) and two pigment types (beet and soy leghaemoglobin, 2%) on the chemical composition, physicochemical properties, visible appearance, and sensory attributes of plant-based patties (PBPs). Increasing duckweed levels improved protein content and moisture retention while reducing cooking loss. Soy leghaemoglobin enhanced redness (a*), whereas beet pigment intensified yellowness (b*). This was also evident in the visible appearance, with leghaemoglobin formulations appearing noticeably redder. Higher duckweed concentrations decreased hardness but lowered sensory acceptance, whereas 1% duckweed, particularly with beet pigment, improved flavour, colour, and overall liking. These results indicate that strategic duckweed inclusion combined with natural pigments can enhance the nutritional value and physicochemical properties of PBPs, although excessive duckweed may compromise sensory quality. Thus, this work offers a new approach to developing PBPs by replacing common ingredients and pigments, supporting advances in future food product development.

Key words: Beet, leghaemoglobin, meat analogue, PCA, protein alternative, sensory evaluation

INTRODUCTION

The increasing global population and rising awareness of environmental and health issues have driven the demand for sustainable and nutritious food alternatives. Meat analogues, which can be meat-free or partially substituted with a small quantity of meat, are high-quality products that have characteristics similar to meat in terms of appearance, texture, flavour, and, to some extent, nutritional content (Ismail *et al.*, 2020; Ismail & Huda, 2022). However, in comparison to conventional animal-based meat, the appearance, texture, and flavour of plant-based meat analogues (PBMAs) remain unsatisfactory despite the advancement and optimization of plant-based meat production technology. For example, the majority of PBMA products lack the colour and visible appearance of conventional meat based food, which makes them less economically viable (Wu *et al.*, 2024). To produce PBMA with actual meat like properties, meat extender applications, in which high-protein non-meat ingredients partially substitute meat, serve as a basis for the development of numerous plant-based emulsion-type products (Kyriakopoulou *et al.*, 2021; Mohd Radzi *et al.*, 2025).

Duckweed often referred as *Wolffia globosa* belongs to the *Lemnaceae* family, is a type of free-floating aquatic plant. The significant amounts of protein, dietary fibre, fat, and phytochemicals found in duckweeds have gained interest recently (On-Nom *et al.*, 2023). They have been proposed as practical, economical, and innovative sources of macro and micronutrients that decrease environmental problems and improve food security (Xu *et al.*, 2023). Up to 30–45% of the total dry-weight protein, all nine necessary amino acids, potassium, iron, and carotenoids, particularly lutein and zeaxanthin, are found in duckweeds (Stewart *et al.*, 2020). The proteins found in duckweed have desirable techno-functional qualities that can be used in a variety of food compositions, such as fluid holding, emulsifying, foaming, and gelling (Ofoedu *et al.*, 2025). Additionally, duckweed has a starch level of up to 70%, which makes it a potential source of carbohydrates for food (Guo *et al.*, 2023).

Organic substances derived from plants are used as natural food colouring. For example, plant-based pigments such as betalain, curcuminoids, carotenoids, and anthocyanin have been utilized in the food sector. However, due to their extreme sensitivity to heat, light, and oxygen, natural colorants are difficult to incorporate into PBMAs (Bakhsh *et al.*, 2023a). Furthermore, these pigments may interact adversely with other plant-based constituents to produce unwanted flavours and colours. Beetroot contains a red pigment called betalain, which is gaining popularity due to its distinctive structure, appealing red colour, and significant funtional characteristics (Chaudhary & Singh, 2020). The enzyme polyphenol oxidase (PPO) in betalain oxidizes

Article History
Accepted: 11 June 2026
First version online: 30 June 2026
Cite This Article:
Weini, W., Mohd Radzi, N.R.S., Wan Mokhtar, W.M.F., Manab, A., Andriani, R.D., Huda, N. & Ismail, I. 2026. Effects of duckweed and pigments on the chemical composition, physicochemical properties, and sensory attributes of plant-based patties. Malaysian Applied Biology, 55(2): 64-71. https://doi.org/10.55230/mabjournal.v55i2.3653

phenolic hydroxyl groups, producing a brown hue when boiled, which gives PBMA a colour similar to cooked beef patties (Zeece, 2020).

Soy leghaemoglobin is a protein, which has an iron-containing compound called a heme group, is extracted from soybean root nodules. This leghaemoglobin is a monomeric hemoprotein comparable to mammalian myoglobin (Ahmad *et al.*, 2023). The process by which oxygen is delivered to peripheral tissues is extremely conserved and resembles that of animal haemoglobin. The addition of soy leghaemoglobin to meat analogue can provide a heme iron source equivalent to red meat, in contrast to traditional meat analogue product like soy burgers or vegetables, which typically only provide non-heme iron. Therefore, for the first time, PBPs were formulated with duckweed as a promising human food protein in combination with two distinct colouring systems (beet and soy leghaemoglobin) to elucidate their combined effects on composition, physicochemical characteristics, and sensory quality. Duckweed is an emerging, sustainable protein source (Song *et al.*, 2025), that has seen novel ingredient in structured PBMAs (Bakhsh *et al.*, 2023b). Thus, this study assessed the effects of duckweed (0%, 1%, and 2%) and colour pigments (2%) from beet and soy leghaemoglobin on the chemical composition, physicochemical properties, and sensory attributes of plant-based patties.

MATERIALS AND METHODS

Materials

Duckweeds and soy leg haemoglobin were bought from RT-Mart (Hefei, China). Textured vegetable protein (TVP), wheat gluten flour, tapioca starch liquid smoke flavour, garlic powder, onion powder, mushroom seasoning, black pepper, salts, sugar, cocoa butter, shortening, beet powder were obtained locally. Meanwhile, methylcellulose was bought from Modenist Pantry (USA).

Sample preparation

TVP was hydrated by soaking in warm water (60°C) at a 1:2 (w/v) ratio for 2 h. Meanwhile, for seitan, gluten flour and water were mixed and kneaded until smooth. Then, the dough was washed until it turned milky to clear and sieved. The duckweed incorporation level in our formulation was based on Bakhsh *et al.* (2023b), with modifications. All the ingredients in Table 1 were weighed, mixed, and ground together with TVP and seitan for 2 min using a food processor (MK-5078M, Osaka Japan). The mixture was shaped into 60 g portions using a patties moulder. The patties were steamed for 35 min and chilled overnight at 4°C prior to analysis (Mohd Radzi *et al.*, 2025).

Chemical composition

The chemical composition of plant-based patties (PBPs) was analysed based on AOAC (2002). Moisture content was determined by weighing 3 g of sample and drying it in an oven at 105°C until a constant weight was achieved. The percentage of moisture was calculated based on the weight before and after drying. Protein content was determined using the Kjeldahl method, where 1 g of sample was digested and the protein value was calculated using the conversion factor of 6.25. Ash content was measured by incinerating 3 g of sample at 550°C in a muffle furnace to constant white ash, expressed as ash weight/sample weight. Fat content was measured using the Soxhlet method (5 g sample, petroleum ether, 8 h extraction), with solvent removed by rotary evaporation. Fat content was expressed as oil weight/sample weight. Crude fibre was measured by Fibretherm digestion (1 g sample), oven-dried (105°C, overnight), and ashed (500°C, 4 h). Crude fibre content was measured as the difference between the weight of the digested sample and the weight of ash, relative to the sample weight.

Table 1. Formulation of plant-based patties containing different pigments and different concentration of duckweed

Ingredient (%)	Formulation (%)					
	0%DB	0%DL	1%DB	1%DL	2%DB	2%DL
Duckweed	0	0	1	1	2	2
Beet powder	2	-	2	-	2	-
Soy leghaemoglobin	-	2	-	2	-	2
TVP	24	24	23	23	22	22
Seitan	56	56	56	56	56	56
Methylcellulose	2	2	2	2	2	2
Tapioca starch	1.3	1.3	1.3	1.3	1.3	1.3
Cocoa butter	2	2	2	2	2	2
Shortening	2	2	2	2	2	2
Liquid smoke	0.1	0.1	0.1	0.1	0.1	0.1
Garlic powder	1.6	1.6	1.6	1.6	1.6	1.6
Onion powder	1.6	1.6	1.6	1.6	1.6	1.6
Mushroom seasoning	3.2	3.2	3.2	3.2	3.2	3.2
Black pepper	0.4	0.4	0.4	0.4	0.4	0.4
Salt	2.1	2.1	2.1	2.1	2.1	2.1
Sugar	1.7	1.7	1.7	1.7	1.7	1.7

0%DB: 0% duckweed incorporated with beet; 0%DL: 0% duckweed incorporated with leghaemoglobin; 1%DB: 1% duckweed incorporated with beet; 1%DL: 1% duckweed incorporated with leghaemoglobin; 2%DB: 2% duckweed incorporated with beet; 2%DL: 2% duckweed incorporated with leghaemoglobin.

Physicochemical properties

For the cooking loss, the PBPs were pan fried for 5 min at 150°C on each side and then kept at room temperature until reaching 26 ± 1°C prior to analysis. Cooking loss was determined by measuring the weight difference of patties before and after cooking. The exudate was collected, and its volume was recorded. Water-holding capacity (WHC) was measured by the weight difference of Whatman no. 1 after compressing 3 g of patty with 2 kg load (Ismail *et al.*, 2019). The surface colour of the cooked PBP was measured using a colorimeter (Konica Minolta Chromameter, CR-300, Japan). The values of L*, a*, and b* representing lightness, redness, and yellowness respectively, were determined using a colorimeter calibrated with a ceramic plate (Y = 93.5, X= 0.3132, y = 0.3198).

Visible appearance and textural properties

Visible appearance of PBPs were assessed using the method of Mohd Radzi *et al.* (2025) by documenting the visual aspect of patties using digital camera (EOS 700D, Canon, Tokyo, Japan). The images were captured in a portable photo studio mini light box with a white background (PYXEL 23 × 23 cm Ligh Box, China). Textural profile analysis (TPA) was carried out using a texture analyser equipped with a 10 kg load cell (Stable Micro System, London, UK) to measure the hardness, cohesiveness, chewiness, springiness, and gumminess of PBPs. The samples were prepared by cutting them into cubes of 1 × 1 × 1 cm. Each cube was then placed on the platform and subjected to compression using a cylindrical probe (P/75 compression platen). The pre-test, test, and post-test constant speeds were adjusted to 5.00 mm/sec in accordance with the default settings of patty. Samples underwent a two-cycle compression process at the 50% final strain. The surface area of the samples was measured using a Volscan profiler (VSP 600, Stable Microsystem Ltd., Surry, England). Calibration of the system was carried out following the manual procedure, which included tare weight adjustment and manual weight settings. The analysis was conducted with a vertical step of 10.0 mm, a standard rotation speed, and a round shape parameter (Mohd Radzi *et al.*, 2025). The surface area of the PBPs were recorded as volume (cm^3).

Sensory evaluation

The sensory evaluation protocol for PBPs was approved by the UniSZA Human Research Ethics Committee (UHREC) under reference number UniSZA/UHREC/2025/313. The sensory evaluation was carried out in the sensory laboratory of the School of Food Industry, Universiti Sultan Zainal Abidin (UniSZA). A total of 30 semi-trained panellists, all Food Science students, took part in the session, and informed consent was obtained from each participant prior to evaluation. The panellists were enrolled in a sensory evaluation course and had prior theoretical and practical experience in assessing sensory attributes (Nora'zizi *et al.*, 2025). The sensory assessment of PBPs was conducted using a hedonic test. Before the sensory evaluation begins, the panellists were instructed to abstain from food and drinks for half an hour. Six samples in the same shape 1 × 2 × 2 cm were cut, and three-digit random numbers were used to code the samples, and the presentation was random. During each session, panellists were served warm samples within 30 min of cooking (60°C ± 4.0°C – the common serving temperature for patties) (Monego *et al.*, 2018). Product liking was rated on a 9-point hedonic scale (1 = dislike extremely; 9 = like extremely). The measured sensory attributes were flavour, aroma, colour, texture, aftertaste, and overall acceptability.

Statistical analysis

Statistical analysis was conducted with three replications for each analysis. Analysis of variance was performed on PBPs formulated with different concentrations of duckweed and types of colour pigments to evaluate their chemical composition, physicochemical characteristics, textural attributes, and sensory properties (n = 3 replicates per treatment). Statistical analyses were fitted using SPSS version 29 (IBM Corp., Armonk, NY, USA). Data were expressed as mean ± standard deviation, and Duncan's test was applied to compare mean scores between formulations at a significance level of $p < 0.05$. Sensory data were subjected to principal component analysis (PCA) with XLSTAT software (Addinsoft, 2022, NY, USA).

RESULTS AND DISCUSSION

Chemical composition

The chemical composition of PBPs incorporated with various duckweed concentrations and pigments from beet and soy leghaemoglobin is showed in Table 2. The moisture content of PBPs ranged from 50.49% to 53.29%. An increasing trend in moisture content was observed with higher levels of duckweed in both beet- and leghaemoglobin-incorporated patties. This effect can be attributed to the enhanced water retention capacity at higher duckweed concentrations (Dhamaratana *et al.*, 2025). For protein content, patties incorporated with leghaemoglobin exhibited higher values than those with beet, regardless of duckweed concentration. Although leghaemoglobin is a heme-containing protein, it contributes only a minor portion to the overall protein content of the PBPs. The observed increase is largely due to duckweed, which is rich in protein. Previous studies have reported that duckweed protein contains a complete spectrum of essential amino acids, comparable to that of animal protein (Appenroth *et al.*, 2018). The ash content showed a marginal increased trend, with significant differences among samples as duckweed concentration increased ($p < 0.05$). Similar to the present study, Bakhsh *et al.* (2023b) reported higher ash content at increased duckweed levels. The fat content showed significant difference ($p < 0.05$) between formulations, ranging from 3.08% to 3.96%, which is considered relatively low in PBPs. The variation in fat content may be attributed to the non-homogenous emulsion fat gel within the patties, which can lead to fat loss during cooking (Bakhsh *et al.*, 2023b). Irrespective of the different concentrations of duckweed and pigments, no significant differences were observed in fibre content ($p > 0.05$). This may be due to the relatively low incorporation levels, suggesting that duckweed and pigments were not the main contributors to fibre content, which instead derived primarily from TVP and seitan. The presence of fibre in plant-based meat analogues is largely attributed to the plant-based ingredients and polysaccharides incorporated into the formulation (Bakhsh *et al.*, 2021).

Table 2. Chemical composition of PBPs with different concentrations of duckweed and pigment types

Sample	Moisture (%)	Protein (%)	Ash (%)	Fat (%)	Fibre (%)
0%DB	51.97±0.84[a]	23.97±0.61[c]	3.29±0.07[d]	3.08±0.06[e]	1.52±0.29[b]
1%DB	50.52±1.10[b]	24.98±0.66[b]	3.40±0.03[c]	3.96±0.13[a]	1.64±0.12[ab]
2%DB	53.22±0.51[a]	25.13±0.45[b]	3.81±0.09[a]	3.52±0.05[bc]	1.87±0.24[a]
0%DL	50.49±0.86[b]	26.90±0.30[a]	3.24±0.02[d]	3.66±0.10[b]	1.77±0.27[ab]
1%DL	52.61±0.14[a]	26.52±0.60[a]	3.40±0.06[c]	3.31±0.03[d]	1.72±0.02[ab]
2%DL	53.29±0.31[a]	26.95±0.38[a]	3.58±0.01[b]	3.43±0.06[cd]	1.77±0.18[ab]

Results are expressed as mean ± standard deviation. Values with different superscript letter (a-e) differ significantly ($p < 0.05$).

Physicochemical properties

The results of cooking loss, WHC, and colour properties of PBPs are presented in Table 3. Cooking loss was lowest in formulations with higher duckweed concentrations, indicating that duckweed enhances the moisture retention capacity of PBPs (Dhamaratana *et al.*, 2025). In contrast, pigment incorporation did not significantly affect cooking loss ($p > 0.05$), as no differences were observed between pigment types. Overall, cooking loss in PBPs was relatively low compared to conventional meat, which typically exhibits higher cooking loss (Furuhashi *et al.*, 2021; Mohd Radzi *et al.*, 2025). The reduced cooking loss in PBPs can be due to the seitan network and the water retention capacity of duckweed, in which disulfide protein linkages help prevent excessive water loss. This network forms a fibrous structure that functions as both a binder and a structuring agent within gluten (Kyriakopoulou *et al.*, 2021). The WHC of all PBPs showed a consistent trend with no significant differences among treatments ($p > 0.05$), which corresponds to the lower cooking loss observed. In addition, Samard and Ryu (2019) reported that meat analogue exhibit higher WHC due to the presence of soy-based water-soluble protein, good hydration capacity, and TVP porosity, all of which strongly influence WHC. Moreover, the inclusion of methylcellulose in the formulation also contributed to water retention. As noted by Bakhsh *et al.* (2021), methylcellulose at concentrations of 1.5% to 4.0% lowered water release and cooking loss due to its hydrocolloid properties, which enable it to bind moisture and form a gel upon heating.

Table 3. Physicochemical properties of PBPs with different concentrations of duckweed and pigment types

Sample	Cooking loss (%)	WHC (%)	L*	a*	b*
0%DB	11.94±0.19[a]	99.56±0.17[a]	46.29±0.08[a]	4.41±0.06[b]	9.77±0.03[a]
1%DB	11.71±0.10[a]	99.56±0.00[a]	42.93±0.08[b]	2.84±0.02[e]	8.26±0.03[b]
2%DB	10.34±0.82[b]	99.67±0.00[a]	42.09±0.05[c]	2.05±0.04[f]	7.72±0.08[c]
0%DL	11.83±0.33[a]	99.45±0.19[a]	41.58±0.12[d]	5.30±0.11[a]	5.09±0.10[d]
1%DL	11.21±0.09[ab]	99.67±0.00[a]	41.86±0.33[c]	3.51±0.12[c]	4.18±0.14[e]
2%DL	10.04±0.67[b]	99.67±0.00[a]	39.58±0.05[e]	3.19±0.04[d]	4.04±0.09[e]

Results are expressed as mean ± standard deviation. Values with different superscript letter (a-f) differ significantly ($p < 0.05$).

The L*, a*, and b* values of PBPs differed significantly depending on the duckweed concentration and pigment type used (Table 3). Although the pigments were incorporated at only 2%, the green colour of duckweed contributed to complex colour formation in combination with either beet or leghaemoglobin. The L* values are generally influenced by the scattering of light waves, with surface homogeneity determining lightness dispersion (Vu *et al.*, 2022; Mohd Radzi *et al.*, 2025). Notably, the L* values of PBPs were significantly lower with leghaemoglobin incorporation compared to beet ($p < 0.05$). The a* values of PBPs showed that at 0% duckweed, the redness contributed by beet and leghaemoglobin was more pronounced. However, increasing the duckweed concentration reduced redness due to the presence of chlorophyll, which imparts a green pigment. Between the two pigment types, leghaemoglobin produced a more intense red colour than beet. Leghaemoglobin, contains a heme group-an iron-containing molecule that imparts both colour and flavour to plant-based meat products (Ahmad *et al.*, 2023). It provides PBPs with a red colour prior to cooking, which then undergoes browning through the Maillard reaction during cooking (Kumari *et al.*, 2024). The colour properties imparted by leghaemoglobin are comparable to those of conventional meat, as leghaemoglobin undergoes chemical changes during cooking which gradually degrade myoglobin and alter the structure of the heme group, resulting in a colour shift from reddish to brownish (Vu *et al.*, 2022). As the concentration of duckweed increased, the b* values decreased for both pigment types. However, the b* values for beet were significantly higher than those for leghaemoglobin ($p < 0.05$). The pigments present in beet belong to the betalain group, which are water-soluble and divided into two classes: betacyanins (impart a red colour) and betaxanthins (impart a yellow colour). Exposure to heat triggers several degradation pathways in betalains, including oxidation through dehydrogenation, aldimine bond hydrolysis, and decarboxylation, which together cause the pigments to shift in colour toward yellow-orange (Miguel, 2018). In addition, beet contains the enzyme polyphenol oxidase, which oxidize the phenolic hydroxyl groups in betacyanins to quinones, resulting in a brown hue. Thermal treatment further facilitates this process (Zeece, 2020).

Visible appearance and textural properties

The visible appearance of uncooked and cooked PBPs with different concentrations of duckweed and pigment types is shown in Figure 1. Uncooked patties incorporated with beet displayed a lighter red colour at 0% duckweed, which developed into a red-brown colour with 2% duckweed. In contrast, uncooked patties incorporated with leghaemoglobin appeared slightly red-purple at 0% duckweed and developed into a deeper purple colour with 2% duckweed. The red colour in beet patties was attributed to the presence of betalain pigments (Miguel, 2018), while the red-purple colour in leghaemoglobin patties was due to myoglobin-like pigments (Vu *et al.*, 2022). After cooking, leghaemoglobin patties developed a dark brown colour, whereas beet-incorporated PBPs exhibited a distinct yellow colour, consistent with the colourimetic results discussed above. The dark brown colour of leghaemoglobin patties resulted from thermal denaturation of globular proteins, during which myoglobin denatures and produces a brown colour (Vu *et al.*, 2022).

The TPA results of PBPs, which measured hardness, cohesiveness, springiness, gumminess, and chewiness are shown in Table 4. Overall, cohesiveness and springiness results were almost consistent between treatments. The hardness, gumminess, and chewiness decreased with increasing duckweed concentrations for both pigment types. This trend can be due to the higher water content, consistent with the moisture values reported in Table 2, where duckweed inclusion increased moisture levels. According to Xia *et al.* (2023), hardness , chewability, and shear force in meat analogues gradually decrease with increasing moisture content. However, our results contrast those of Bakhsh *et al.* (2023b), who incorporated duckweed at 0.5, 0.7, and 1.0% and reported no significant differences (p > 0.05). The lack of significant differences in their study may be due to the small variations in duckweed concentration applied. Kyriakopoulou *et al.* (2021) further noted that the addition of certain proteins in plant-based meat analogues can influence gelation and overall structure, thereby affecting hardness. While some proteins form strong gels, others, when combined with plant flours or additional ingredients, reduce gel elasticity and produce a softer final product.

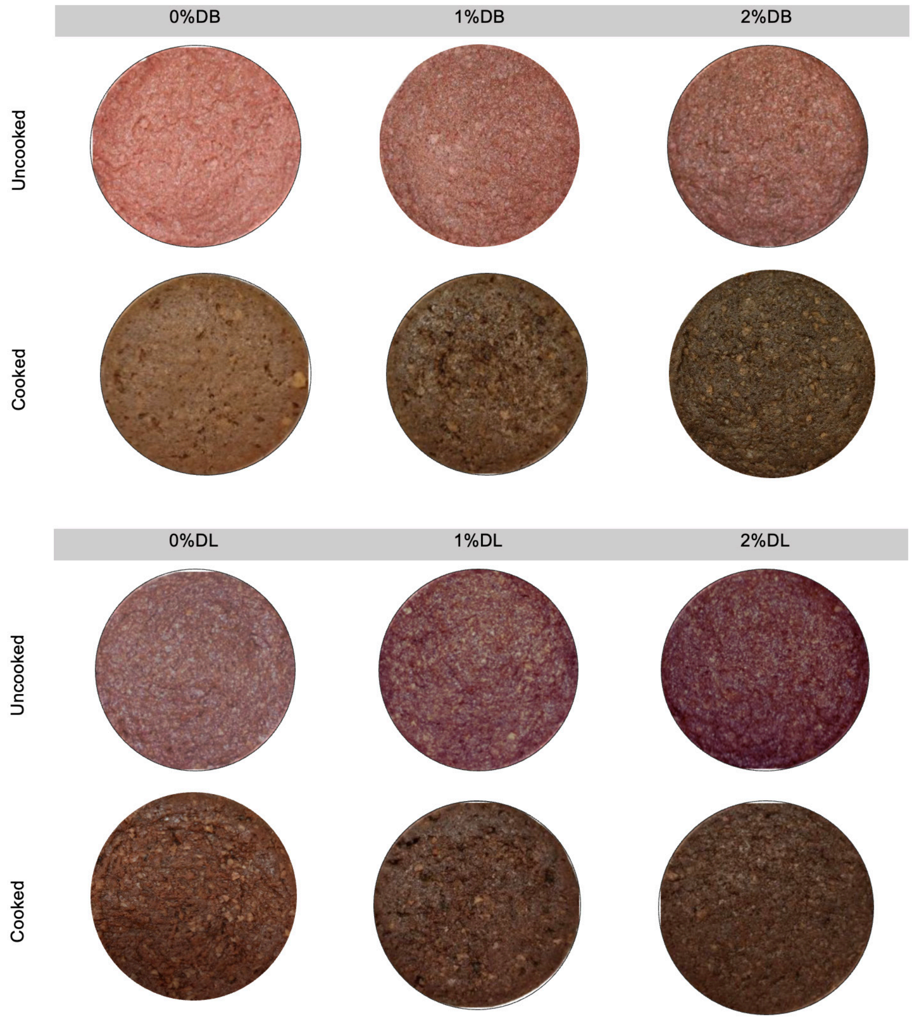

Fig. 1. Visible appearance of PBPs incorporated with different concentrations of duckweed and pigment types.

Table 4. Texture profile properties and volume of PBPs with different concentrations of duckweed and pigment types

Sample	Hardness (N)	Cohesiveness (%)	Springiness (mm)	Gumminess (N)	Chewiness (N)	Volume (cm^3)
0%DB	21.87±0.05^{bc}	0.57±0.00^{b}	0.66±0.01^{c}	12.75±0.05^{b}	7.85±0.02^{c}	41.50±0.71^{d}
1%DB	18.34±0.09^{d}	0.56±0.02^{b}	0.69±0.01^{b}	10.30±0.03^{d}	6.77±0.04^{d}	42.00±0.00^{d}
2%DB	18.44±0.01^{d}	0.56±0.01^{b}	0.61±0.01^{d}	10.10±0.00^{d}	6.18±0.00^{e}	45.00±0.00^{c}
0%DL	25.89±0.04^{a}	0.61±0.01^{a}	0.70±0.01^{b}	15.00±0.02^{a}	10.98±0.02^{a}	42.50±0.71^{d}
1%DL	22.85±0.06^{b}	0.57±0.00^{b}	0.70±0.01^{b}	12.55±0.06^{b}	8.53±0.03^{b}	47.00±0.00^{b}
2%DL	20.99±0.06^{c}	0.54±0.01^{c}	0.71±0.01^{a}	11.18±0.04^{c}	7.94±0.01^{c}	48.50±0.71^{a}

Results are expressed as mean ± standard deviation. Values with different superscript letter (a-e) differ significantly (p < 0.05).

Volume was measured to determine the surface area of PBPs. The decrease in volume was closely related to shrinkage, which in turn is related with cooking loss, lower WHC, and lower moisture content. Based on the result in Table 4, samples with higher levels of duckweed incorporation exhibited significantly greater retained volume ($p < 0.05$), indicating less shrinkage compared to the 0% duckweed. Vu *et al.* (2022) reported that heating induce shrinkage through water evaporation and structural reorganization of protein matrices, thereby altering their water-holding properties. The reduced shrinkage observed at higher duckweed concentrations may be due to the protein in duckweed, which possesses desirable techno-functional properties including water retention, emulsification, foaming, and gelling (Ofoedu *et al.*, 2025). Furthermore, duckweed contains up to 70% starch, which acts as an effective filler, reinforcing the structural matrix of PBPs (Takács *et al.*, 2025).

Sensory evaluation

Figure 2 presents the PCA of sensory attributes, where the first principal component (F1) explained 71.20% of the variance and the second principal component (F2) accounted for 10.10%, resulting in a cumulative variance of 81.30% in the sensory evaluation data. Sensory variables—colour, aroma, flavour, texture, aftertaste, and overall acceptability—are represented by the red vectors. All vectors were clustered on the right-hand side (positive F1), indicating that samples positioned in this region received higher ratings for these attributes. Based on the results, the 0%DB samples (green) and 0%DL samples (red) were positioned on the positive F1 axis. The 0%DB samples showed stronger associations with colour, aroma, flavour, and overall acceptability, indicating that panellists preferred these samples for their sensory qualities. In contrast, the 0%DL samples were more closely aligned with aftertaste and texture, suggesting good performance in these attributes. Overall, the most favourable sensory evaluations were observed in PBPs incorporated with beet pigment but without duckweed. In addition, the 1%DB samples (yellow) were positioned closer to the positive F1 axis and overlapped with vector such as flavour, colour, and overall acceptability, indicating that 1%DB improved sensory quality compared to 1%DL sample (blue), which were shifted toward the negative F1 axis. Meanwhile, the 2%DB (brown) and 2%DL (purple) samples shifted further toward the negative F1 side, away from the sensory vectors, suggesting that higher duckweed concentration (2%) were less favourable in sensory performance. Consistent with On-Nom *et al.* (2023), duckweed was reported to cause off-flavour and poor texture, reducing texture and overall liking scores. Duckweeds generally exhibit green, fresh, and fruity notes similar to spinach and dandelion, but some volatile compounds are also associated to less desirable waxy, fatty, bitter, and pungent descriptors (Smith *et al.*, 2024). These negative notes are likely to be intensified at 2% duckweed inclusion and thus contributed to the lower sensory acceptance observed in this study.

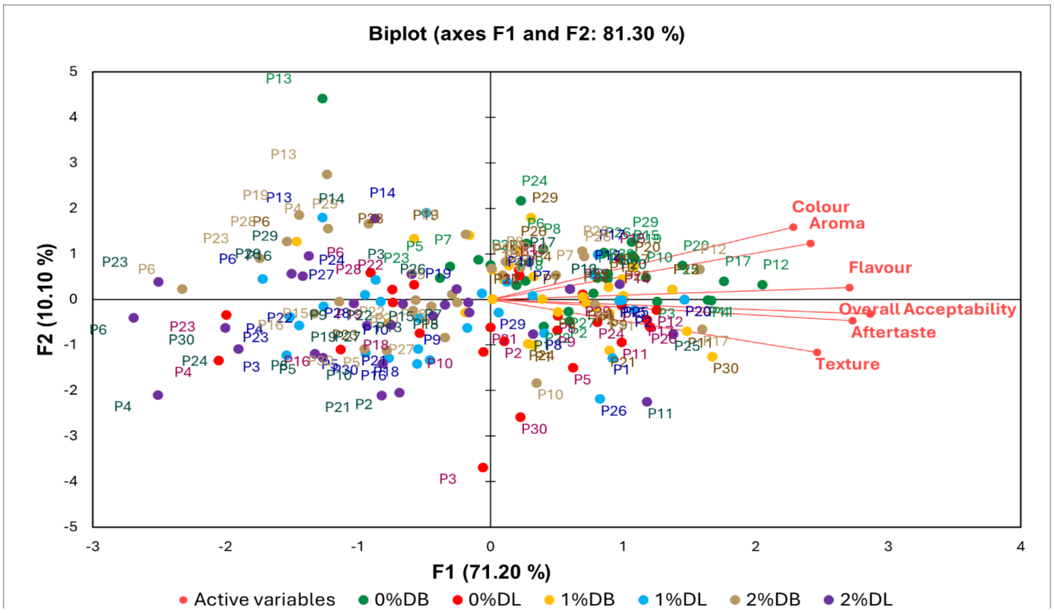

Fig. 2. PCA biplot of the sensory evaluation of PBPs incorporating different concentrations of duckweed and pigment types.

CONCLUSION

PBPs formulated with varying duckweed concentrations and pigment types showed significant differences in chemical composition, physicochemical properties, and sensory attributes. Higher duckweed concentrations increased moisture content, while the combination of duckweed with leghaemoglobin enhanced protein content. However, ash, fat, and fibre contents showed only marginal differences between formulations. Lower cooking loss and higher WHC were associated with formulations containing higher levels of duckweed. PBPs formulated with leghaemoglobin exhibited a redder and darker appearance due to the presence of the heme group, while beet pigments enhanced yellowness owing to their betalain content. Texture analysis revealed that hardness, gumminess, and chewiness decreased with higher duckweed incorporation. In sensory evaluation, PBPs with 2% duckweed were rated less desirable, while 1% duckweed, particularly when combined with beet pigment, was sufficient to improve flavour, colour, and overall acceptability compared to leghaemoglobin-based formulations. Thus, to enhance product development, future work should refine the texture of duckweed-based formulations, evaluate pigment stability, and conduct comprehensive consumer acceptance and shelf-life studies.

ACKNOWLEDGEMENTS
This research was supported by Ministry of Higher Education (MoHE) through Fundamental Research Grant Scheme (FRGS/1/2022/TK0/UNISZA/02/2) with the project number RR445 provided by Ministry of Higher Education of Malaysia (MoHE). The authors also acknowledge Universitas Brawijaya (UB), Indonesia, through the Visiting Lecturer Program 2025, for providing collaborative opportunities that contributed to joint academic activities and publications.

ETHICAL STATEMENT
The sensory evaluation protocol for plant-based patties was reviewed and approved by the UniSZA Human Research Ethics Committee (UHREC) under reference number UniSZA/UHREC/2025/313. All participants have read and signed the Research Informed Consent Sheet (UniSZA-PTPIP-42-GP 001-BR 008[01]) prior to participation.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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