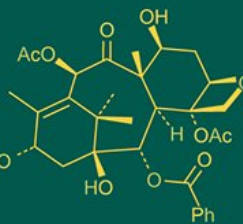
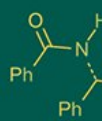


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(6): 482-488
www.biochemjournal.com
Received: 04-04-2026
Accepted: 08-05-2026

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Influence of fresh *Wolffia globosa* on the technological parameters of french macarons

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i6f.8669>

Abstract

French macarons are delicate bakery products in which nutritional fortification is challenging due to their dependence on precise formulation and structure. *Wolffia globosa*, a nutrient-dense aquatic plant rich in protein and bioactive compounds, was evaluated as a functional ingredient for macaron enrichment. French macarons were enriched with *Wolffia globosa* to evaluate its potential as a functional ingredient. Macarons were prepared using the French meringue method, replacing 10% of stiffened meringue with fresh *Wolffia globosa*. The control and enriched macarons were analyzed for proximate composition, antioxidant activity, colour, texture, and sensory acceptability. *Wolffia* incorporation increased protein content from $9.75 \pm 0.14\%$ to $10.30 \pm 0.65\%$ (dwb), while moisture showed a slight rise ($7.91 \pm 0.36\%$ to $8.12 \pm 0.32\%$). Antioxidant activity was markedly enhanced, with ABTS values increasing from $40.3 \pm 0.4\%$ to $60.77 \pm 0.12\%$ and DPPH from $24.9 \pm 0.7\%$ to $38.6 \pm 0.8\%$. Colour analysis indicated reduced lightness (L^* : 82.58 to 72.99) with increased redness and yellowness. Texture profile analysis showed increased hardness (338.51 to 9230.82 g) and chewiness, indicating structural modification. Sensory evaluation using a 9-point hedonic scale indicated improved aroma, texture, flavor, and acceptability in *Wolffia*-enriched macarons compared to the control, although colour preference was slightly reduced. The findings suggest that incorporation of 10% fresh *Wolffia globosa* can effectively improve the nutritional and antioxidant properties of French macarons while maintaining desirable sensory quality. Therefore, *Wolffia globosa* shows promising potential as a functional ingredient for the development of value-added bakery products.

Keywords: French macarons; *Wolffia globosa*; Functional food; Antioxidant activity; Sensory acceptability

Introduction

French macarons are popular Western desserts characterized by their small circular shape, crisp exterior, and soft, moist interior (Campbell & Mougeot, 1999) [8]. Although they originated in Italy, macarons were later refined in France, where they gained widespread recognition and eventually became globally popular (McGee, 2007; Wybauw, 2007) [19, 34]. These confections are primarily composed of meringue and almond flour, which together contribute to their distinctive structure and texture (McGee, 2007; Wybauw, 2007) [19, 34]. The standard macaron formulation includes egg whites, granulated sugar, powdered sugar, and almond flour (Bissar & Özcan, 2022; Oncică et al., 2025) [5, 25], with almond powder, egg whites, and sugar identified as the core ingredients critical for successful macaron production (Oncică et al., 2025) [25]. However, macaron production requires highly precise ingredient ratios to maintain characteristic texture and appearance, making ingredient substitution particularly challenging (Bissar & Özcan, 2022) [6].

Wolffia globosa is a nutrient-dense aquatic plant recognized for its high protein content and richness in essential micronutrients (On-Nom et al., 2023) [26]. The incorporation of fresh *Wolffia globosa* into French macarons offers a promising approach to nutritional enhancement while preserving sensory quality. However, the inclusion of novel ingredients such as *Wolffia globosa* necessitates careful evaluation of batter rheological behavior and matrix stability to ensure product quality, as alterations in the macaron matrix can significantly influence final product characteristics (Oncică et al., 2025) [25]. Therefore, the objective of the present study was to evaluate the effect of incorporating fresh *Wolffia globosa* into French macarons on their nutritional composition, antioxidant activity, colour attributes, texture profile, and sensory acceptability, with the aim of developing a

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Nutritionally enriched, value-added functional macaron without compromising consumer appeal (Campbell & Mougeot, 1999; Appenroth *et al.*, 2018) [4, 8].

2. Materials and methods

2.1. Fresh *Wolffia globosa* and ingredients

Fresh *Wolffia globosa* was collected from *Wolffia* tanks of College of Fisheries, Lembucherra and West Tripura. The ingredients used for the preparation of French macarons included almond flour, powdered sugar, granulated sugar, and egg whites for the macaron shells. For the experimental formulation, fresh *Wolffia globosa* was incorporated by

partially replacing the stiffened meringue. The cream filling was prepared using salted butter, fresh cream, lemon zest, vanilla essence, and vinegar.

2.2. Preparation of French Macarons

French macarons were prepared using the French meringue method. Egg whites were whipped until foamy and gradually beaten with granulated sugar to obtain a stiff and glossy meringue. Almond flour and powdered sugar were finely ground, mixed, and sieved to ensure uniform particle size and smooth batter consistency (Table 1).

Table 1: Quantity of ingredients required for the French Macaron preparation

Ingredient	Weight (g)	Percentage (%)
Almond flour	100	18.35%
Sugar	220	40.37%
Egg white	100	18.35%
Butter	125	22.94%
Total	545	100%

2.3. Optimization of *Wolffia globosa* to French Macaron

Optimisation was performed for 5, 10 and 15% of incorporation into meringue. However, the 10% was

optimised based on the “foot” formation and further to have nutritional enhancement (Fig.1).

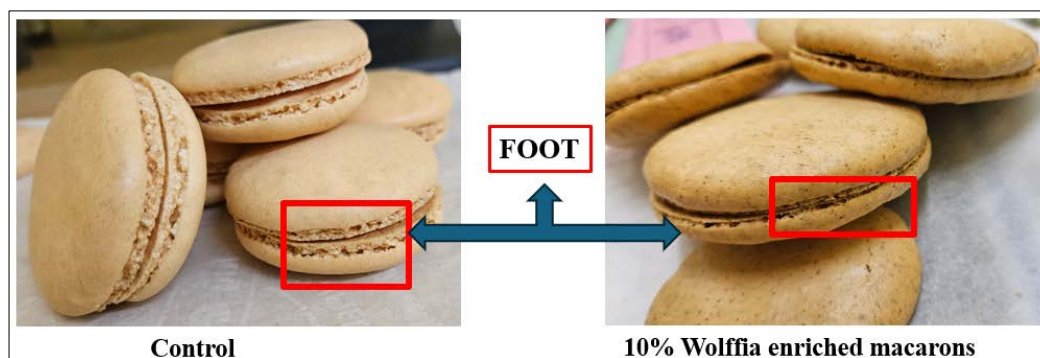


Fig 1: Observation of foot in the control macaron and 10% wolffia enriched macaron

2.4. Preparation and Incorporation of *Wolffia globosa*

Fresh *Wolffia globosa* was thoroughly washed with potable water to remove adhering impurities and gently pat-dried to eliminate excess surface moisture. For the experimental formulation, 10% of the stiffened meringue was replaced

with fresh *Wolffia globosa* (19 g replacement in 190 g of meringue). The prepared *Wolffia globosa* was gently folded into the meringue to avoid collapse of the aerated structure. The schematic diagramme is depicted in Fig.2.



Fig 2: Schematic process of preparing the French macarons with and without fresh *Wolffia*

2.5. Macaronage, Piping, and Resting

The almond flour-powdered sugar mixture was incorporated into the meringue (control) and *Wolffia*-meringue mixture (treatment) using the macaronage technique until a smooth, flowing batter was obtained. The batter was transferred to a piping bag and piped into uniform circular shells (approximately 4 cm diameter) on parchment-lined baking trays. The piped shells were rested at room temperature for 30-40 minutes to allow surface drying and skin formation.

2.6 Baking and Filling

Macaron shells were baked in a preheated oven at 150 °C for 14-16 minutes. The *Wolffia*-incorporated macarons required a slightly longer baking time (~17 minutes) due to higher moisture content. After baking, the shells were cooled to room temperature and filled with cream filling prepared using butter, fresh cream, lemon zest, and vanilla essence.

3. Influence of fresh *Wolffia globosa* on the technological quality of French macaron

3.1. Proximate composition analysis

The proximate composition analysis (moisture, crude protein, fat, and ash content) of control and *Wolffia*-added French Macarons were carried out using the following standard methods of AOAC (2016) [1].

3.2. Assay for antioxidant activity

3.3. 2, 2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) ABTS radical scavenging activity

Arnao *et al.* (2001) [5] measured ABTS of control and *Wolffia*-added French Macarons. The ABTS working solution was prepared by mixing equal volumes of 7.4 mM ABTS and 2.6 mM potassium persulfate, then kept in the dark at room temperature for 12 hours. It was diluted (1 mL ABTS + 60 mL methanol) to obtain an absorbance of 1.1 ± 0.02 at 734 nm. For the assay, 150 µl of sample was mixed with 2,850 µl of ABTS solution and incubated in the dark for 2 hours. Methanol was used as the control. Absorbance was measured at 734 nm, and ABTS radical inhibition (%) was calculated using the formula.

$$\text{ABTS scavenging activity (\%)} = (A_0 - A_1) / A_0 \times 100$$

Where,

A_0 = Absorbance of the control

A_1 = Absorbance of the sample

3.4. 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity of control and *Wolffia*-added French Macarons was determined according to Dhanabalan *et al.* (2017) [11]. The stock solution was prepared by dissolving 24 mg DPPH in 100 mL methanol and stored at -20 °C. The working solution was made by mixing 10 mL of stock with 45 mL methanol. For the assay, 150 µl of diluted sample was mixed with 2850 µl of DPPH solution and kept in the dark for 24 hours. Methanol was used as the control. Absorbance was measured at 515 nm, and inhibition (%) was calculated using the formula.

$$\text{DPPH scavenging activity (\%)} = (A_0 - A_1) / A_0 \times 100$$

Where,

A_0 = Absorbance of the control

A_1 = Absorbance of the sample

3.5. Colour analysis

The colour of the samples was determined in triplicate using a spectrophotometer (Colourflex EZ, Hunter Associates Laboratory, Inc., Reston, VA) with illuminant D65/10°. The instrument was calibrated with black and white reference plates before analysis. A slice of each sample was placed over the light source, and L^* , a^* , and b^* values were recorded. L^* represents lightness (0 = black, 100 = white), a^* indicates red/green (+ a^* red, - a^* green), and b^* indicates yellow/blue (+ b^* yellow, - b^* blue). The CIELAB (L^* , a^* , b^*) colour scale was used (Young and Whittle, 1985) [38].

3.6. Texture Profile Analysis (TPA)

Textural properties of all samples were determined using a texture analyzer (TA-XT PLUS, Stable Micro Systems, Surrey, England, and UK) with a 75 mm compression plate and 50 kg load cell. Samples were cut into 2.5 × 3.0 cm cubes, and three cubes per sample were analyzed. The test conditions were 60% compression, pre-test speed 1 mm s⁻¹, test speed 1 mm s⁻¹, post-test speed -5 mm s⁻¹, distance 8 mm, and trigger force 5 g. Hardness, adhesiveness, cohesiveness, and chewiness were recorded.

3.7. Sensory scores

French Macaron samples were conditioned to room temperature and served to students, faculty, and staff of the College of Fisheries, Lembucherra for sensory evaluation. Panelists were briefed on the 9-point Hedonic Scale (Meilgaard *et al.*, 2006) [20] before assessment. They evaluated general appearance, colour, texture, odor, flavour, and overall acceptability on a scale of 1 to 9 (9 = like extremely, 1 = dislike extremely).

4. Results and discussion

4.1. Proximate composition

The incorporation of *Wolffia* into French macarons resulted in minor but notable changes in their proximate composition and nutritional profile (Table 2). Moisture content showed a slight increase from 7.91% in the control to 8.12% in the *Wolffia*-added macarons, which may influence the texture, mouthfeel, and shelf stability of the product. Similarly, ash content increased marginally from 0.98% to 1.00%, indicating a possible enrichment of mineral components. This observation is consistent with previous reports that *Wolffia* species are naturally rich in minerals such as potassium and iron (Appenroth *et al.*, 2018) [4].

A more pronounced change was observed in the protein content, which increased from 9.75% in the control to 10.30% in the *Wolffia*-fortified macarons (dry weight basis). The enhancement in protein content suggests that *Wolffia* can serve as an effective functional ingredient for improving the nutritional quality of bakery products, aligning with current trends in the development of protein-enriched functional foods (Nitiwuttithorn *et al.*, 2024; On-Nom *et al.*, 2023; Zięć *et al.*, 2025) [23, 39]. The increase can be attributed to the naturally high protein content of *Wolffia* species, which reportedly ranges from 20-30% of freeze-dried weight (Appenroth *et al.*, 2018; Patcharabudsarakumkul *et al.*, 2025) [4, 28]. The lipid content remained relatively unchanged, with values of $14.23 \pm 0.08\%$ in the control and $14.12 \pm 0.74\%$ in the *Wolffia*-added macarons, indicating that *Wolffia* incorporation did not significantly alter the fat composition of the product. This finding is in agreement with the relatively low-fat content of

Wolffia, reported to range between 1-5% (Appenroth *et al.*, 2018) ^[4]. Interestingly, the fiber content decreased from 0.60% in the control to 0.41% in the Wolffia-added macarons. This observation contrasts with the general perception of Wolffia as a dietary fiber source, with reported fiber contents of approximately 25% of freeze-dried weight

(Appenroth *et al.*, 2018; On-Nom *et al.*, 2023) ^[4]. The reduction in measured fiber content may be attributed to factors such as the level of Wolffia incorporation, the specific form of Wolffia used, or interactions between ingredients during processing that could influence fiber determination.

Table 2: Proximate composition of the control and a *Wolffia globosa* incorporated French Macarons

Treatments	Moisture content (%)	Protein (dwb) (%)	Lipid (dwb) (%)	Ash (dwb) (%)	Fiber (dwb) (%)
Control	7.913±0.36	9.75±0.14	14.23±0.08	0.98±0.01	0.60±0.04
Wolffia added	8.12±0.32	10.30±0.65	14.12±0.74	1±0.04	0.41±0.06

4.2. Antioxidant activity

The incorporation of Wolffia significantly enhanced the antioxidant activity of French macarons, as presented in Table 3. The ABTS radical scavenging activity increased markedly from 40.3% in the control to 60.77% in the Wolffia-added macarons. Similarly, the DPPH radical scavenging activity showed a considerable rise from 24.9% in the control to 38.6% in the fortified product. These findings clearly indicate that the addition of Wolffia improves the free radical scavenging capacity of the macarons.

The substantial increase in both ABTS and DPPH antioxidant activities suggests that Wolffia contributes bioactive compounds with strong antioxidant potential, thereby enhancing the functional value of the product. The

improved antioxidant activity may provide additional health benefits by contributing to the dietary intake of natural antioxidants and helping to reduce oxidative stress. These results are in agreement with previous studies reporting the antioxidant and antiradicals properties of Wolffia species (On-Nom *et al.*, 2023; Pagliuso *et al.*, 2020) ^[27].

The enhanced antioxidant capacity observed in the Wolffia-fortified macarons can be attributed to the presence of various phytochemicals and bioactive compounds naturally occurring in Wolffia, including phenolics, flavonoids, vitamins, and pigments with antioxidant properties (Yadav *et al.*, 2024; Yadav *et al.*, 2025) ^[36]. Earlier in-vitro studies have also demonstrated significant antioxidant activities in Wolffia extracts, supporting the present findings (On-Nom *et al.*, 2023; Gülçin *et al.*, 2010).

Table 3: Antioxidant Activity of the control and a *Wolffia globosa* incorporated French Macarons

Treatments	ABTS%	DPPH%
Control	40.3±0.4	24.9±0.7
Wolffia added	60.77±0.12	38.6±0.8

4.3. Color attributes

The incorporation of Wolffia significantly influenced the color characteristics of French macarons, as shown in Table 4. The L* value, which represents lightness, decreased from 82.58±0.22 in the control to 72.99±0.34 in the Wolffia-added macarons, indicating the development of a darker product. In contrast, the a* value increased from 6.05±0.70 to 10.54±0.67, reflecting an increase in redness, while the b* value rose from 27.86±0.12 to 31.95±0.17, indicating enhanced yellowness in the fortified macarons. Overall, the incorporation of Wolffia resulted in a darker, more reddish-yellow appearance compared to the control.

These color modifications can primarily be attributed to the natural pigments present in Wolffia, including chlorophyll and other bioactive compounds that contribute greenish and yellowish tones (Gharehbeglou *et al.*, 2023; Yadav *et al.*, 2024) ^[17, 35]. The presence of such pigments is known to influence the visual characteristics of food products and can significantly alter their appearance during processing and baking (Molina *et al.*, 2023; Wijesekara & Xu, 2024) ^[21, 32]. Similar observations have been reported in studies involving

the incorporation of plant- and algae-based ingredients into bakery and snack products, where reductions in lightness and increases in yellowness or redness were commonly observed depending on the concentration and type of ingredient used (Gharehbeglou *et al.*, 2023; Yilmaz & Yüce, 2022) ^[17, 37].

The observed changes in color are important from a product development perspective, as visual appearance strongly influences consumer perception, acceptance, and expectations regarding quality, freshness, and nutritional value (Famakinwa *et al.*, 2023; Wadhera & Capaldi-Phillips, 2014) ^[14, 31]. Although some studies on Wolffia-fortified snack products have reported minimal changes in color (On-Nom *et al.*, 2023), the present findings suggest that Wolffia incorporation at the selected level had a pronounced effect on the appearance of French macarons. Therefore, optimizing the incorporation level of Wolffia may be important to balance improved nutritional functionality with desirable sensory characteristics and consumer acceptability (Daszkiewicz *et al.*, 2023; Wu *et al.*, 2024) ^[10, 33].

Table 4: Colour Attributes of the control and Wolffia *globosa* incorporated French Macarons

Treatments	L* (lightness)	a* (redness)	b* (yellowness)
Control	82.58±0.22	6.05±0.7	27.86±0.12
Wolffia	72.99±0.34	10.54±0.67	31.95±0.17

4.4. Texture profile analysis

The incorporation of *Wolffia* significantly affected the texture profile of French macarons, as presented in Table 4. Among the texture parameters, hardness showed the most pronounced increase, rising from 338.51 ± 101.71 g in the control to 9230.82 ± 1677.63 g in the *Wolffia*-added macarons. Similarly, gumminess and chewiness increased substantially from 81.13 ± 33.48 g to 2739.11 ± 628.28 g and from 42.62 ± 22.43 g to 1230.65 ± 346.28 g, respectively. Cohesiveness and resilience also increased, with cohesiveness rising from 0.24 ± 0.07 to 0.30 ± 0.04 and resilience nearly doubling from 0.08 ± 0.03 to 0.15 ± 0.02 . In contrast, springiness showed a slight decrease from 0.51 ± 0.09 mm to 0.45 ± 0.06 mm.

The marked increase in hardness, gumminess, and chewiness indicates the formation of a firmer and denser macaron structure following *Wolffia* incorporation. These Texture Profile Analysis (TPA) parameters are closely related to the force required to compress, disintegrate, and chew a product before swallowing (Alemu, 2023; Altan *et al.*, 2023; Dou *et al.*, 2023; Nwosisi *et al.*, 2019) [2, 3, 12, 24]. The observed changes suggest that the addition of *Wolffia* significantly modified the structural integrity of the macaron matrix. The increase in texture firmness may be attributed to the protein and fiber components present in *Wolffia*. Plant-based ingredients rich in proteins and dietary fibers are known to interact with starches and proteins within food systems, resulting in the formation of denser and more rigid networks that reduce water mobility and increase structural

compactness (Flory *et al.*, 2023; Fratelli *et al.*, 2023) [15, 16]. Similar findings have been reported in bakery products fortified with microalgae such as *Chlorella vulgaris*, where increased hardness and reduced softness were associated with the formation of a stiffer dough matrix (Dündar *et al.*, 2023) [13]. The relatively high protein content of *Wolffia* (Appenroth *et al.*, 2018; On-Nom *et al.*, 2023) [4] may have contributed to the development of stronger internal interactions within the macaron structure, thereby increasing hardness and cohesiveness. The increase in cohesiveness suggests improved internal bonding within the product matrix, while the higher resilience indicates a greater capacity of the macarons to recover after deformation (Alemu, 2023; Dou *et al.*, 2023) [2, 12]. However, the slight reduction in springiness reflects a decrease in elasticity, indicating that the *Wolffia*-added macarons became less flexible despite being structurally stronger.

These textural modifications are particularly important because texture is a major sensory attribute influencing consumer acceptance and eating quality (Giron *et al.*, 2025; Nasrollahzadeh *et al.*, 2024) [18, 22]. The substantial increase in hardness, gumminess, and chewiness may alter the characteristic delicate, soft, and airy texture traditionally associated with French macarons, which could influence consumer preference and overall acceptability. Therefore, optimization of the incorporation level of *Wolffia* may be necessary to achieve a balance between enhanced nutritional functionality and desirable sensory properties.

Table 5: Texture Profile Analysis of the control and *Wolffia globosa* incorporated French Macarons

Treatments	Hardness (g)	Springiness (mm)	Cohesiveness	Gumminess (g)	Chewiness (g)	Resilience
Control	338.51 ± 101.71	0.51 ± 0.09	0.24 ± 0.07	81.13 ± 33.48	42.62 ± 22.43	0.08 ± 0.03
<i>Wolffia</i>	9230.82 ± 1677.63	0.45 ± 0.06	0.30 ± 0.04	2739.11 ± 628.28	1230.65 ± 346.28	0.15 ± 0.02

4.5. Sensory evaluation

Sensory evaluation of the control and *Wolffia*-added French macarons was conducted to assess consumer perception across five attributes, namely color, aroma, texture, flavor, and appearance (Figure 1). The control macarons exhibited generally high acceptability scores, with color scoring approximately 8.2, aroma 7.6, texture 7.2, flavor 7.0, and appearance 7.8 on a nine-point hedonic scale. The *Wolffia*-added macarons demonstrated a distinct sensory profile, with slightly lower scores for color (approximately 8.0) but higher scores for aroma, texture, flavor, and appearance compared to the control.

The slightly lower color score of the *Wolffia*-added macarons may be attributed to the natural pigments present in *Wolffia*, particularly chlorophyll and other bioactive compounds, which contributed to darker and more yellowish-red color characteristics, as reflected in the L*, a*, and b* values (Gharehbeglou *et al.*, 2023; Yadav *et al.*, 2024) [17, 35]. Although these pigment-related changes slightly affected color preference, the appearance score of the fortified macarons was higher (approximately 8.4) than that of the control (7.8), suggesting that consumers found the product visually appealing despite the color modification (Bordim *et al.*, 2021) [7]. Aroma and flavor scores also

improved following *Wolffia* incorporation, with aroma increasing from approximately 7.6 in the control to 8.2 in the fortified macarons, and flavor increasing from around 7.0 to 8.4. These findings indicate that the addition of *Wolffia* enhanced the sensory quality of the macarons without imparting undesirable off-flavors, thereby contributing positively to consumer acceptability (Rabitti *et al.*, 2024) [29].

Texture received the highest improvement among all sensory attributes, increasing from approximately 7.2 in the control to 8.6 in the *Wolffia*-added macarons. This enhanced texture perception may be associated with the increased firmness, cohesiveness, and chewiness observed during texture profile analysis, which likely contributed to a more desirable mouthfeel and eating experience (Giron *et al.*, 2025; Nasrollahzadeh *et al.*, 2024; Flory *et al.*, 2023; Fratelli *et al.*, 2023) [15, 16, 18, 22]. The sensory evaluation results demonstrate strong consumer acceptance of *Wolffia*-fortified French macarons. Despite slight changes in color, the improvements observed in aroma, flavor, texture, and appearance suggest that *Wolffia* has considerable potential as a functional ingredient for developing nutritionally enriched confectionery products with desirable sensory

characteristics (Chala & Emire, 2022; Siegrist & Hartmann, 2020) [9, 30].

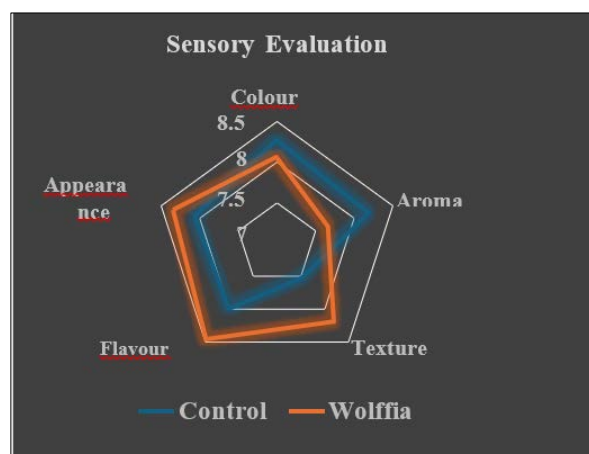


Fig 3: Radar chart illustrates sensory evaluation

Conclusion

The present study demonstrated that incorporation of 10% fresh *Wolffia globosa* is optimal for the development of nutritionally enriched macarons without compromising product quality. Macarons formulated at this level exhibited well-defined feet, uniform shape, and good rise, indicating effective protein network formation and proper steam expansion during baking. Texture profile analysis confirmed a desirable crisp outer shell with a soft interior, comparable to control macarons. Although a slight green hue was observed due to *Wolffia* addition, the colour remained visually acceptable and did not adversely influence consumer perception. Proximate composition analysis revealed enhanced protein content along with improved antioxidant activity, highlighting the nutritional advantage of *Wolffia* incorporation. Sensory evaluation using a 9-point hedonic scale indicated good acceptability across taste, texture, appearance, and overall quality. Overall, the study supports the potential of *Wolffia globosa* as a functional ingredient for developing nutrient-enriched macarons with satisfactory technological and sensory attributes.

Acknowledgments

The authors express their sincere gratitude to the Dean, College of Fisheries, Central Agricultural University (Imphal), for providing the necessary facilities, encouragement, and institutional support to carry out this student research project successfully. The authors also acknowledge the support extended by the Department of Aquaculture for providing fresh *Wolffia globosa* used in the study under the project (BT/PR41748/NER/95/1862/2021; Scheme: I&ED; 0155).

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