Contents available at: Sri Lanka Journals Online

Tropical Agricultural Research

Journal Home Page: https://tar.sljol.info

RESEARCH

Formulation of Cookies Incorporated with Duckweed Powder and Evaluation of Its Physicochemical, Nutritional, Functional, Microbial, and Sensory Properties

W.M.S.U. Weerakoon[1], R. Hiththatiyage[2,4], M.A. Wickramasinghe[2], I. Rathnayaka[2], W.A.J.P. Wijesinghe[3], and R. Liyanage[2*]

[1]*Department of Export Agriculture, Faculty of Animal Science and Export Agriculture, Uva Wellassa University, Badulla 90000, Sri Lanka*
[2]*Nutritional Biochemistry Division, National Institute of Fundamental Studies, Hanthana Road, Kandy 20000, Sri Lanka*
[3]*Department of Food Science and Technology, Faculty of Animal Science and Export Agriculture, Uva Wellassa University, Badulla 90000, Sri Lanka*
[4]*Postgraduate Institute of Science, University of Peradeniya, Kandy, Sri Lanka*

ARTICLE INFO

Article history:
Received: 19 August 2025
Revised version received: 18 March 2026
Accepted: 19 March 2026
Available online: 01 April 2026

Keywords:
Cookies
Duckweed
Micronutrients
Protein

Citation:
Weerakoon, W.M.S.U. Hiththatiyage, R. Wickramasinghe, M.A. Rathnayaka, I. Wijesinghe, W.A.J.P. and Liyanage, R. (2026). Formulation of Cookies Incorporated with Duckweed Powder and Evaluation of Its Physicochemical, Nutritional, Functional, Microbial, and Sensory Properties. Tropical Agricultural Research, 37(2): 90-109.

DOI: https://doi.org/10.4038/tar.v37i2.8981

Weerakoon,W.M.S.U.
https://orcid.org/0009-0000-8701-9537

* Corresponding author- ruvini.li@nifs.ac.lk

ABSTRACT

Duckweed, a largely underexploited aquatic plant from the family Lemnaceae, is notable for its high protein content, encompassing all essential amino acids, and its abundance of micronutrients. This study explored the feasibility of incorporating duckweed powder into cookie formulations. Based on preliminary investigations, *Lemna perpusilla* and *Spirodela polyrhiza* were selected for the production of duckweed powder, which was subsequently assessed for its physicochemical, nutritional, and functional characteristics. Four cookie formulations were then prepared by substituting wheat flour with duckweed powder at levels of 10%, 20%, 30%, and 40%, alongside a control sample composed entirely of wheat flour. Sensory evaluation was performed using a 9-point hedonic test with 40 semi-trained panelists and identified the most preferred cookie, which was further assessed. According to proximate analysis, the selected cookie showed significant differences (p<0.05) from the control, with higher crude protein 12.25±0.02%, crude fat 21.45±0.03%, crude fiber 10.25±0.03%, crude ash 3.76±0.04%, and digestible carbohydrate 47.23±0.10%. The cookie provided 430.98±0.05 kcal/100g, with 22.43±0.04% sugar, 0.74±0.02% salt, and 1.64±0.03% total dietary fiber. Mineral content was significantly higher (p<0.05). Functional properties such as total phenolic content (1.62±0.03 mgGAE/g), flavonoid content (282.05±0.01 mgCAT/g), DPPH radical scavenging activity (212.36±1.20 mM TE/g), and α-amylase inhibition (IC_{50} 2.26±0.02 (μg/mL) were also high (p<0.05). Microbial levels remained within safe limits for one month, and all chemical and safety standards were met. The findings suggest that duckweed powder can serve as an effective partial replacement for wheat flour, enhancing both the nutritional profile and functional characteristics of cookie formulations.

INTRODUCTION

Cookies are among the most popular cereal-based baked products, characterized by their crispy texture and flat cake–like structure. They occupy an important position in the snack sector due to their wide variety of flavors, shapes, and types, as well as their ready-to-eat nature, affordability, and contribution of essential dietary and digestive components. (Giwa *et al*., 2012; Chaudhari *et al.*, 2018). In addition, cookies are valued for their good nutrient availability, palatability, compactness, and convenience. Their low moisture content (5–6%) restricts microbial growth, resulting in enhanced shelf stability and allowing them to be easily stored, distributed, and consumed almost anywhere and at any time. Consequently, considerable research has focused on modifying cookie formulations to improve their nutritional and functional attributes or to tailor them to the specific needs and preferences of different consumer groups (Vidyapeeth *et al.*, 2017).

At present, chronic non-communicable diseases (NCDs) constitute the leading cause of mortality, morbidity, and disability, exceeding the burden posed by communicable diseases. (Directorate of Non-Communicable Diseases Ministry of Health, 2022). One of the major contributors to this burden is protein–energy malnutrition, which results from inadequate intake of protein and energy and adversely affects growth, immune function, and overall health. Notably, malnutrition during early life not only compromises immediate health outcomes but also increases the risk of developing NCDs in later life. This challenge is especially severe in developing and least-developed countries, where substantial proportions of the population experience insufficient dietary protein and caloric intake.

To address this challenge, the use of plant-based protein–calorie sources has been proposed as a viable strategy, particularly when such proteins offer adequate nutritional quality and are already incorporated into traditional diets (Puranik, 2012). This approach is especially relevant in developing countries, where multiple forms of malnutrition, including widespread micronutrient deficiencies alongside protein–energy malnutrition are highly prevalent and often result in serious adverse health outcomes (Mejbel and Simons, 2018).

Within this framework, bakery products occupy a significant role in the food industry due to their consistent quality, wide acceptance, and affordability, making them suitable vehicles for improving population-level nutrition with minimal behavioral change. Fortification of these products with protein and essential micronutrients can substantially enhance their nutritional profile, thereby contributing to the mitigation of both protein–energy malnutrition and micronutrient deficiencies (Soni *et al.*, 2018).
Duckweed, one of the smallest free-floating aquatic plants, has gained increasing attention as a promising food ingredient owing to its exceptionally rapid growth up to 28 times faster than conventional crops and its high nutritional potential. (Pagliuso *et al.*, 2022). It belongs to the family Lemnaceae, which includes five genera (*Landoltia, Spirodela*, *Lemna, Wolffiella, and Wolffia*) comprising a total of 36 species (Baek *et al.*, 2021). These minute plants are widely distributed across the globe, except in polar regions, and thrive in still or slow-moving freshwater bodies such as ponds and lakes.

Duckweed exhibits considerable potential for human nutrition and offers multiple health benefits. Although its chemical and nutritional composition varies depending on species and cultivation conditions, it is consistently characterized by a high content of high-quality protein, typically ranging from 35 to 43% on a dry weight basis. Importantly, it provides all essential amino acids in proportions that meet or exceed FAO requirements, thereby supporting human growth and development (Xu *et al.*, 2011). When compared with conventional protein sources such as soybean (Glycine max), duckweed demonstrates several advantages, including faster growth rates, higher biomass yield, and greater annual protein productivity per unit area (Song e*t al*., 2025). Moreover, it contains valuable micronutrients and bioactive compounds, including vitamins, minerals, lutein, and β-carotene, which have

been linked to a reduced risk of chronic diseases (Xu *et al.*, 2011).

Based on these characteristics, this study aimed to formulate cookies by partially replacing wheat flour with duckweed powder and to comprehensively evaluate the physicochemical, nutritional, functional, microbial, and sensory qualities of the developed products, in order to determine the suitability of duckweed as a value-added, nutrient-rich functional ingredient in bakery applications.

METHODOLOGY

Materials

Two duckweed varieties, *Spirodela polyrhiza* and *Lemna perpusilla*, were obtained from the Nutritional Biochemistry Unit, National Institute of Fundamental Studies (NIFS), Kandy, Sri Lanka. The two duckweed varieties used in the present study were derived from the same batch of samples previously described and reported by Hiththatiyage *et al*. (2025). Wheat flour, white sugar, margarine, baking powder, vanilla essence, full cream milk powder and eggs were purchased from local supermarkets in Sri Lanka. All chemicals and reagents used in this study were of analytical grade.

Methods

Cultivation of duckweed plants

Two duckweed varieties were cultivated individually in wooden tanks lined with black polyethylene. Each tank maintained a water depth of 20 cm and had a surface area of 1.04 m^2 (Figure 1). Duckweed samples were cultivated under optimal growth conditions outlined by Bartošová *et al.* (2015), Baek *et al.* (2021), Hiththatiyage *et al*. (2025), and were harvested individually on a weekly basis.

Preparation of duckweed powder

Duckweed powder (DP) was prepared following the methods of Duangjarus *et al.* (2022) and Pagliuso *et al.* (2022) with slight modifications. The duckweed plants were harvested separately, and any debris was removed. The harvested plants were washed three times under running water and then air-dried until a constant weight was achieved. Once dried, the plants were ground individually using an electric mixer grinder and passed through a 250 μm standard sieve to obtain a fine powder. The powders from the two duckweed species were then combined in a 1:1 ratio. The resulting mixed duckweed powder was packed in double-layered aluminum foil pouches and stored at -20 °C until further analysis.

Duckweed powder properties

Moisture, crude protein, crude ash, crude fat, crude fiber, pH and water activity (a_w) were determined according to the *AOAC.* (2000). The yield percentage of duckweed powder were determined according to Xiao *et al.* (2013). The digestible carbohydrate content was determined by the difference (Nizam and Arampath, 2016). All the analyses were performed in triplicates.

(A)

(B)

(C)

Figure 1: Cultivation of duckweed plants (A and B - *Lemna perpusilla*, C - *Spirodela polyrhiza*)

Development of cookies

Cookies were prepared from different blend of wheat flour (WF) and duckweed powder using the AACC micro method (Quality and America, 1999) and the method by Soni *et al.* (2018) with slight modification. Five different cookie samples were formulated with varying proportions of duckweed powder as described in Table 1. Each cookie formulations comprised constant amounts of margarine, white sugar, egg, baking powder, full cream milk powder and vanilla extract. The ingredients were mixed, kneaded and made into dough. The dough was sheeted and shapes were cut using a cookie cutter (5 cm diameter and 5 mm thickness) (Kaushalya and Wijesinghe, 2024). Shaped cookies were baked at 160 ˚C for 20 min in a hot air oven. After baking, cookies were cooled to room temperature in a desiccator, packed in food-grade re-sealable black color triple layer zip lock standup pouch with a clear window.

Table 1: Different cookie formulations

Ingredients	T_0	T_1	T_2	T_3	T_4
Wheat flour	100%	90%	80%	70%	60%
Duckweed powder	0%	10%	20%	30%	40%

T: Treatments

Sensory evaluation

Sensory evaluation was carried out according to the Ikuomola *et al.* (2017) and Jayamali *et al.* (2022) with slight modifications. The evaluation aimed to identify the cookie sample with the most favorable sensory characteristics among four different wheat flour cookies containing varying proportions of duckweed powder. Sensory attributes assessed included appearance, color, aroma, flavor, texture, and overall acceptability. These were evaluated by 40 semi-trained panelists using a nine-point hedonic scale. All samples were presented simultaneously to each panelist at room temperature under standard lighting conditions and were coded with three-digit numbers. Drinking water was provided for rinsing the mouth, and crackers were supplied for palate cleansing between samples. Each panelist was given a ballot sheet and a pen to record their evaluations.

Microbial analysis of the control cookie sample and final product

The total plate count, as well as the yeast and mold counts, of both the final and control cookie samples were measured following SLS 516: Part 1 and SLS 516: Part 2 protocols, with minor modifications (Sri Lanka standards institution, 2021). Both analyses were performed in triplicate, initially 48 hours after preparation and subsequently at two-week intervals over the course of one month of storage.

Physicochemical properties of the control cookie sample and final product

The spread ratio and pH of the samples were measured following the AACC 10-50.05 and AOAC 943.02 methods, respectively, while baking loss was assessed according to Kuchtová *et al.* (2018). Water activity was determined using a water activity meter based on a modified AOAC 978.18 method. Color evaluation was performed with a chroma meter (CR-410, Japan) in accordance with Akhobakoh *et al.* (2022) and Oladunjoye *et al.* (2021). All measurements were carried out in triplicate.

Proximate composition of cookie samples

The moisture, crude ash, crude fat, and crude fiber content were determined according to the AOAC (2000) official methods (925.10, 923.03, 920.39, and 978.10, respectively).

The nitrogen content was determined using a CHNS(O) analyzer (PerkinElmer 2400 Series II) and protein content was calculated by multiplying nitrogen content (%) by a factor of 6.25 (Equation 1) (Nizam and Arampath, 2016). The total carbohydrate content was determined according to the method described by James, (1995) by using the Equation 2. All analysis was conducted in triplicate.

Crude protein (%) = Nitrogen (%) × 6.25 ..... Equation 1

%Digestible carbohydrates = 100 - (% moisture+ % protein + % fat +% fiber + % ash content) ... Equation 2

Energy (kcal per 100 g) = 4 (% Protein + % Carbohydrate) + 9 (% Fat) ..... Equation 3

Nutritional composition of the cookies

The energy content per 100 g of cookies was calculated using Equation 3 as described by Oladunjoye *et al.* (2021) by using the Equation 3. Acid-insoluble ash and the acidity of extracted fat were measured following SLS 251:2010. Total dietary fiber was determined according to the AOAC 985.29 method (McCleary, 2023), while total sugar and salt contents were assessed based on SLS 586:1982 and ISO 2006a (ISO, 2006), respectively, with minor modifications. All analyses were performed in triplicate.

Mineral composition of the duckweed powder, control cookie sample and final product

The mineral content of samples was determined by inductively coupled plasma optical emission spectroscopy (*ICP-OES*; Thermo scientific, *iCAP* 7000 series, Germany) according to AOAC 985.01 method with minor modifications. Triplicate measurements were done.

Anti-oxidant Potential and Anti-Diabetic Activity (α-amylase Inhibition Assay) of duckweed powder and control cookie sample and final product

Preparation of crude extracts

One gram of powdered sample was extracted into 20 mL of 60% ethanol by sonicating (CL-188, USA) for 30 min at 40 kHz. Then the content was centrifuged (5430R, Germany) for 15 min at 7500 rpm, and the supernatant was separated. Finally, concentrated sample (50 mg/mL) crude extract was stored at -20 ˚C until analysis.

Total Phenolic Content (TPC)

The total phenolic content was determined using Folin-ciocalteu reagent, according to the method described by Singleton *et al.* (1999) with minor modifications. A 50 μL aliquot of the sample solution was combined with 15 μL of distilled water and 105 μL of 10% Folin–Ciocalteu reagent in a 96-well microplate. The mixture was incubated at room temperature for 3 min, after which 80 μL of 7.5% Na_2CO_3 was added, followed by further incubation for 30 min at room temperature. Absorbance was measured at 760 *n*m using a UV–visible microplate spectrophotometer (Omega 415–3441, Germany). All measurements were performed in triplicate. Gallic acid, at concentrations ranging from 0 to 0.01 mg/mL, was used as the calibration standard, and the results were expressed as milligrams of gallic acid equivalents (mg GAE) per gram of dry weight.

Total Flavonoid Content (TFC)

TFC of the sample was determined according to the procedure of Agbo *et al*. (2015) with slight modifications. A 20 μL of the sample solution was mixed with 20 μL of 5% freshly prepared $NaNO_2$ and 30 μL distilled water in a 96 well micro-plate. It was allowed for a 6 min incubation at room temperature. After that, 20 μL of 10% $AlCl_3$ was added and the mixture was incubated for 6 min at room temperature. Then, 200 μL of 4% NaOH was added and the final mixture was incubated for 15 min at room temperature. The absorbance was measured at 510 nm using the UV–visible microplate spectrophotometer. Triplicate was conducted. Catechin was used as the calibration standard at concentrations ranging from 0 to 40 mg/mL.

Results were expressed in milligrams of catechin equivalents (CE) per gram dry weight (DW).

DPPH Radical Scavenging Activity

The procedure described by Blois, 1958 with slight modifications was used. A 50 μL aliquot of the sample solution was combined with 150 μL of DPPH dissolved in 70% methanol in a 96-well microplate. The mixture was incubated in the dark for 30 min and absorbance was subsequently measured at 517 *n*m. All measurements were performed in triplicate. Trolox, at concentrations ranging from 0 to 0.3 mM, was used as the calibration standard, and the results were expressed as milligrams of Trolox equivalents (TE) per gram of dry weight (DW).

Anti-Diabetic Activity (α-amylase Inhibition Assay)

α-amylase inhibitory activity was determined according to the method described by Wickramasinghe *et al.* (2023) and Visvanathan *et al.* (2016) with minor modifications. α-Amylase (2U/mL) and 0.4 g/mL of starch were dissolved in 0.02 M (pH 6.9) Phosphate Buffer Saline (PBS). Forty microliters of PBS, 40 μL of sample extract and 40 μL enzyme were added to a 96 well micro-plate and incubated at 37 ˚C for 10 min. After that 40 μL of starch was added and again kept for incubation at 37 ˚C for 15 min. Then after 100 μL of glucose oxidase (GOD) was added and allowed to incubation at 37 ˚C for 15 min. The absorbance was measured at 505 *n*m using the UV visible microplate spectrophotometer. Triplicate was done. The inhibition activity was expressed as IC_{50} values using Acarbose standard with a concentration ranging from 0.001 to 0.04 mg/mL.

Comparison with SLSI (Sri Lanka Standards Institute) standards (SLS 25: :2010)

The final product was evaluated in accordance with SLSI standards for biscuits and cookies by comparing its chemical and microbial requirements, as well as heavy metal limits (SLS 25:2010). The chemical requirements assessed included moisture content (percentage by mass), acid insoluble ash (on a dry basis, percentage by mass), and the acidity of extracted fat (expressed as oleic acid, percentage by mass). The aerobic plate count and yeast and mold counts per gram were used to determine the microbial requirements. Additionally, the limits for heavy metals were compared, focusing on arsenic (mg/kg) and lead (mg/kg).

Statistical analysis

All the properties of the duckweed powder, final product, and the control cookie sample were analyzed in triplicate, with mean values and standard deviations using Excel 2021. IC_{50} values of α-amylase Inhibition Assay was calculated by using CompuSyn software. The sensory data were analyzed by using the non – parametric, Friedman test with 95% confidence level using Minitab 17 statistical package. Control and final cookie were compared using one-way ANOVA with Tukey's test in Minitab 17 at a 0.05 significance level.

RESULTS AND DISCUSSION

Properties of duckweed powder

The duckweed powder exhibited an average pH of 6.13±0.02, indicating a slightly acidic nature. In comparison, (Nitiwuttithorn *et al*., 2024) reported a pH of approximately 6.6±0.1 for rehydrated duckweed powder, suggesting minor variations potentially due to differences in processing or moisture content.

The average water activity (a_w) of the duckweed powder was 0.541±0.008. Water activity is a critical factor in controlling microbial proliferation and influencing storage stability (Tapía *et al*., 2020). In flour-based products, a_w levels determine both microbial growth and shelf life, with higher water activity generally supporting greater microbial development. Specifically, bacterial growth typically requires a minimum a_w of 0.91, while fungi can grow at a_w levels of at least 0.6, with each microorganism having a distinct threshold below which growth is inhibited (Cronin *et al*., 2021). The relatively low water activity of duckweed powder (0.541±0.008) thus contributes to its enhanced quality and extended shelf life.

The yield of duckweed powder obtained from air-dried plants was 4.54±0.01%. Nitiwuttithorn *et al.* (2024) observed that fresh duckweed yielded protein recovery approximately 2.5 times higher than oven-dried material. Additionally, the starch content of duckweed has been shown to vary depending on the nitrogen and phosphorus concentrations in the growth medium (Xiao *et al*., 2013). Differences in powder yield can be attributed to multiple factors, including the drying method employed (Pagliuso *et al*., 2022), cultivation conditions such as nutrient availability and plant density, and harvesting frequency, with more frequent harvesting generally resulting in higher yields (Nieuwland *et al*., 2021; Said *et al*., 2022).

Proximate composition of the duckweed powder

The proximate composition of a 1:1 mixture of *Spirodela polyrhiza* and *Lemna perpusilla* duckweed powder is presented in Table 2. Moisture content in duckweeds varies depending on species and environmental conditions (Chen *et al*., 2018). For instance, Said *et al.* (2022) reported a moisture content of 11.43±0.29% for *Spirodela polyrhiza*, while *Lemna* species have been documented to range between 10.8% and 82.5%. In line with these findings, the average moisture content of the 1:1 mixture of *Spirodela polyrhiza* and *Lemna perpusilla* in the present study was 17.43±0.01%.

The protein content in duckweed is influenced by the species and the specific growth conditions. Additionally, the extraction methods employed can significantly impact the protein content of the extract (Xu *et al*., 2023). According to the results of this study the average crude protein was 30.10±0.08%. According to Yu *et al*. (2011) *Spirodela polyrhiza* has a relatively high protein content of 34.5%, while Xu *et al.* (2023) reported a crude protein content of 29.1% for this species. For *Lemna* spp., crude protein content has been reported to range from 11% to 32% (Chrismadha *et al*., 2016). Specifically, Nieuwland *et al*. (2021) found that *Lemna* spp. had a crude protein content of 33.6±0.9%, whereas Méndez-Martínez *et al.* (2021) reported a crude protein content of 27.59±0.25% for the same genus. Furthermore, Andriani *et al.* (2022) reported crude protein content of the *Lemna perpusilla* was 24.93%.

The protein content observed in this study is comparable to these findings, particularly aligning closely with the values reported by Xu *et al.* (2023) and Méndez-Martínez *et al.* (2021). This suggests that the protein content of duckweed can vary widely based on species and conditions but generally falls within the reported ranges.

As presented in Table 2, the average crude ash content of the duckweed mixture was 22.38±0.02%. This value aligns with previously reported crude ash contents for species within the Lemnaceae family, where *Spirodela* ranges from 1% to 16% and *Lemna perpusilla* from 7% to 36% (Pagliuso *et al*., 2022). Nieuwland *et al.* (2021) reported a crude ash content of 18.0±0.4% for *Lemna* spp., while Andriani *et al.* (2022) recorded 14.56% for *Lemna perpusilla*. The relatively higher ash content observed in this study is likely due to the combination of the two species, resulting in an average of 22.38±0.02%. This value falls within the reported range and may reflect either a synergistic effect or compositional variation arising from the mixture of species.

Previous studies have reported the crude fat content of *Spirodela polyrhiza* as 1.74±0.07% on a dry weight basis (Said *et al*., 2022), while *Lemna perpusilla* has been reported to contain 3.7% (Khandaker *et al*., 2007) and 2.08±0.10% (Méndez-Martínez *et al*., 2021) on a dry weight basis. In the present study, however, the crude fat content was notably higher, measured at 4.89±0.06%. As noted by Said *et al*. (2022) fat content in duckweed is strongly influenced by species and growth conditions. Additionally, Pagliuso *et al.* (2022) highlighted that factors such as temperature, light intensity, nutrient availability, and water quality can significantly affect the crude fat content of duckweed.

According to the study by Méndez-Martínez *et al.* (2021) the crude fiber content of *Lemna perpusilla* was 7.22±0.22%. Yu *et al.* (2011) found the crude fiber content of *Spirodela polyrhiza* to be 8.8%. Additionally, Andriani *et*

al. (2019) reported a crude fiber content of 5.26% for *Lemna perpusilla*. In the current study, the average crude fiber content of the mixed duckweed powder was determined to be 11.36±0.04%, which is slightly higher than the values reported in previous studies. The higher crude fiber content observed in this study may be attributed to the synergistic effects of combining the two species, leading to a composite nutritional profile that exceeds the individual contributions of each species.

The average digestible carbohydrate content observed in this study was 13.85±0.12%. According to Andriani *et al.* (2019), the carbohydrate content of *Spirodela polyrhiza* was reported to be 8%, whereas (Nieuwland *et al*., 2021) reported that the digestible carbohydrate content of *Lemna* spp. was 3.3±0.2%.

The digestible carbohydrate content found in the current study is significantly higher compared to the values reported for *Spirodela polyrhiza* and *Lemna* spp. This discrepancy may be due to the combination of the two species in the sample, which could result in a higher overall carbohydrate content. Blending different species of aquatic plants has been reported to 8%, whereas (Nieuwland *et al*., 2021) reported that the digestible carbohydrate content of *Lemna* spp. was 3.3±0.2%. The digestible carbohydrate content found in the current study is significantly higher compared to the values reported for *Spirodela polyrhiza* and *Lemna* spp. This discrepancy may be due to the combination of the two species in the sample, which could result in a higher overall carbohydrate content. Blending different species of aquatic plants can lead to an improved nutritional profile, potentially offering a more balanced and energy-dense food source.

Sensory evaluation

The results indicated that all evaluated sensory attributes, including appearance, colour, aroma, flavour, texture, and overall acceptability, differed among the four cookie formulations at a 95% confidence level (p<0.05). Among the samples, the cookie containing 10% duckweed powder (DP) and 90% wheat flour (WF) received the highest median scores and sum of ranks across all sensory parameters, suggesting that it was the most preferred formulation among the panelists. These findings indicate that the formulation containing 10% DP was the most acceptable among the duckweed-incorporated cookies. The corresponding radar diagram illustrating the sensory profile of the formulations is presented in Figure 2.

Table 2. Proximate composition of duckweed powder

Parameter	Average Value (%)
Moisture	17.43±0.01
Crude protein	30.10±0.08
Crude ash	22.38±0.02
Crude fat	4.89±0.06
Crude fiber	11.36±0.04
Digestible carbohydrate	13.85±0.12

Values are average of triplicate analysis ± standard deviation

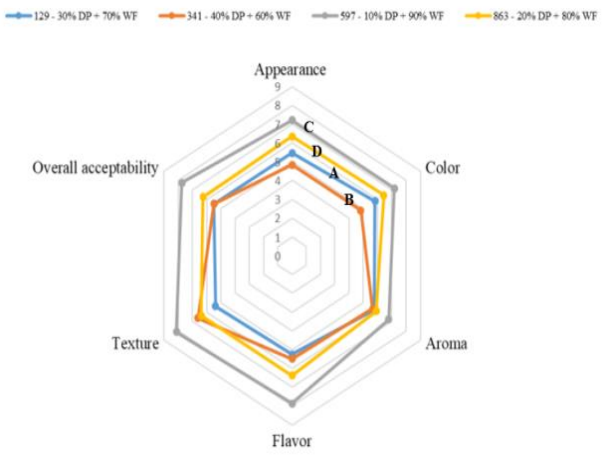

Figure 2: Radar diagram showing Friedman test–based sensory evaluation of samples (A). 129- 30% DP + 70% WF, (B). 341- 40% DP + 60%WF, (C). 597- 10% DP + 90% WF, (D). 863- 20% DP +80% WF

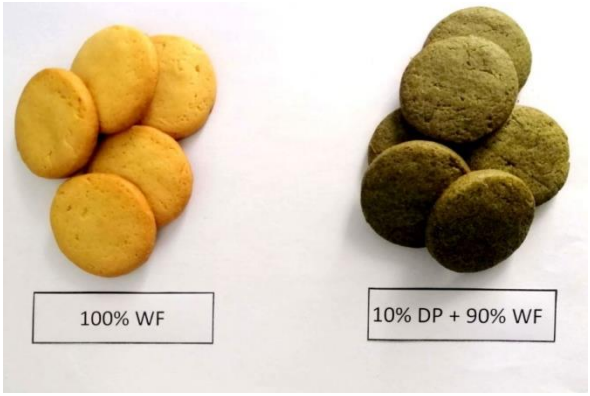

Figure 3: (A) Control cookie (100% Wheat flour) and (B) final cookie (10% Duckweed powder + 90% Wheat flour)

Microbial properties of the control cookie and final product

The mean total microbial load on nutrient agar was assessed for both the control and the final cookie samples. No microbial growth was detected at 48 hours post-preparation or at the two-week sampling point. After four weeks of storage, the control cookie exhibited a microbial load of (0.007±0.001) × 10^3 CFU g^{-1}, whereas the final cookie showed a lower load of (0.003±0.001)×10^3 CFU g^{-1}. Statistical analysis indicated a significant difference in total plate counts between the two samples (p<0.05). In the analysis of yeast and mold counts, neither the control nor the final cookie showed detectable levels at 48 hours, two weeks, or four weeks of storage.

Physicochemical properties of the control cookie and final product

The spread ratio is a key quality parameter for cookies, with higher values generally associated with more desirable products (Mudgil *et al*., 2017). Larger diameter and greater spread are considered important quality attributes (Handa *et al*., 2012), and the spread ratio tends to increase as the particle sizes of flour and sugar decrease (Boz, 2019). In this study, the average spread ratios of the control cookie and the final product were 5.48±0.08 and 5.53±0.08, respectively. While higher gluten content has been reported to reduce cookie spread (Pareyt *et al*., 2008), no significant difference was observed between the control (100% wheat flour) and the final cookie (10% DP + 90% wheat flour) (p>0.05). The minimal standard deviation observed indicates low variability, reflecting consistent cookie shape, which is advantageous for commercial production. Maintaining a uniform spread ratio is particularly important for packaging, as it ensures cookies have consistent size and shape.

According to this study, the average baking loss of the control cookie and the final product was 7.22±0.12% and 10.79±0.78%, respectively. A significant difference (p<0.05) in baking loss was observed between the final product and the control cookie. Typically, wheat flour contains 12% moisture, whereas duckweed powder has a moisture content of 17.43%. Furthermore, they noted that the percentage of weight loss during baking varied directly with the amount of salted butter used. Their findings indicated that weight loss in cookies tended to increase with the moisture content of the dough before baking.

The average pH of the control cookie and final product at 28 ˚C was 6.86±0.03 and 6.75±0.02, respectively. According to statistical analysis, the pH value of control cookie was significantly higher than the final cookie. Therefore, there was a significant difference between the two cookies (p<0.05). The pH of the cookies is slightly acidic according to the obtained results. Acidic products were more shelf-stable than non-acidic counterparts and an acidic pH was

associated with the development of a pleasant taste (Dias and Bandara, 2019). According to the research done by Chinwendu *et al.* (2017), pH of the wheat flour was 6.28±0.06%. According to the current study, pH of duckweed powder was 6.13±0.02%. Therefore, based on these findings, can conclude that the incorporation of duckweed powder, which has a slightly lower pH than wheat flour, contributes to the overall acidity of the cookies, potentially enhancing their shelf stability and taste.

The average water activity of control cookie was 0.54±0.00a_w and final product was 0.50±0.00 a_w at 24.91 ˚C. There was a significant difference (p<0.05) between the water activity of control and final cookie. The water activity of cookies is crucial for predicting their stability and safety concerning microbial growth and lipid oxidation rates. Bacteria, yeasts, and molds have specific water activity thresholds for spoilage, which are 0.90, 0.85-0.88, and 0.80, respectively (Dias and Bandara, 2019). The lower water activity of cookies in this study can be identified to be within safe limits of bacteria, yeast and mold.

The color was expressed in terms of L* (lightness, where 0 = black and 100 = white), a* (positive values indicate redness, negative values indicate greenness), and b* (positive values indicate yellowness, negative values indicate blueness), as defined by Charoenphun and Kwanhian (2019). The color of cookies was represented in L*, a*, and b* values in the Table 3. The control cookie showed a higher lightness (L* 67.31±1.00) than the final duckweed cookie (L* 38.44±0.07), indicating a darker look due to duckweed pigments. The a* value changed from positive in the control (4.58±0.67, red) to slightly negative in the duckweed cookie (-0.48±0.11, green), indicating the green color from chlorophyll in duckweed. The b* value decreased from 40.67±0.12 in the control to 24.84±0.07 in the final product, indicating lower yellowness with duckweed substitution. All differences were significant (p<0.05). The color of the control cookie (100% wheat flour) and the final selected cookie (10% DP + 90% WF) are shown in Figure 3.

Table 3: Color of cookies

Color parameter	T_0 – Control cookie	T_1 – Final product
L*	67.31±1.00^a	38.44±0.07^b
a*	4.58±0.67^a	-0.48±0.11^b
b*	40.67±0.12^a	24.84±0.07^b

Values are average of triplicate analysis ± standard deviation; values having the different superscript letters are significantly different (p<0.05)

Proximate composition of cookie samples

The proximate composition of the control cookie (100% WF) and final cookie (10% DP and 90% WF) selected from the sensory evaluation are presented in Table 4.

The moisture content significantly increased in the final cookie 5.05±0.03% compared to the control cookie 3.25±0.03%. Data indicated that, there was an incensement of the average moisture content with the incorporation of duckweed powder. This increase suggests that duckweed powder has a higher water retention capacity.

The final cookie shown a significant increase in crude protein content 12.25±0.02% compared to the control cookie 9.75±0.03%. According to the statistical analysis there was a significant difference between the two cookie samples at 95% significant level (p<0.05). Duckweed contains up to three times more protein than wheat (Xu *et al*., 2023). Wheat protein lacks some necessary amino acids and has a relatively low protein content (Khan *et al*., 2014).

The crude ash content, representing the mineral content, also increased significantly in the final cookie 3.76±0.04% compared to the control cookie 2.15±0.02%. This result indicates that duckweed powder is a rich source of minerals (Pagliuso *et al*., 2022) than wheat flour, contributing to the higher ash content in the final cookie.

The crude fat content showed a slight but significant increase in the final cookie 21.45±0.03% compared to the control 20.83±0.02%. Given that the crude fat content of wheat flour was reported to contain 1.81% (Waters *et al*., 2012), and the crude fat content of duckweed powder was 4.89±0.06%.

The final cookie had a considerably higher crude fiber content 10.25±0.03% than the control cookie 8.14±0.01%. This finding is predicted given that duckweed powder contains a higher amount of fiber.

The average digestible carbohydrate of the control cookie and final cookie was 55.87±0.1% and 47.23±0.10% respectively. There was a significant difference between the two cookie samples at 95% significant level (p<0.05). This decrease is a direct result of the higher protein, fiber, and ash contents in the final cookie, which reduces the relative proportion of digestible carbohydrates in the cookie.

Nutritional composition of the cookies

The control sample exhibited the highest (p<0.05) calorific value (449.96±0.24 kcal), whereas the final cookie formulation showed the lowest calorific value (430.98±0.05 kcal). The acid insoluble ash of the control cookie and the final cookie was 0.02±0.01% and 0.03±0.01% respectively.

Fat acidity is an indicator of the free fatty acid content in oils and fats and is commonly used to assess the extent of fat rancidity in bakery products such as cookies (Megeri *et al*., 2022). In the present study, the extracted fat from the control cookie exhibited a slightly higher (p<0.05) fat acidity (0.86±0.00%) compared to the final cookie (0.83±0.00%), indicating marginally lower rancidity in the final formulation.

Dietary fiber plays a crucial role in lowering the risk of severe health conditions, such as colorectal cancer, cardiovascular disease, and diabetes (Reyes-Pérez *et al*., 2013). Mechanically, fiber increases stool size and accelerates intestinal transit, limiting the contact time of possible carcinogens with the gut mucosa and encouraging the excretion of bile acids, which can lower circulating cholesterol levels (Slavin, 2013). Furthermore, soluble dietary fibers and their fermentation products, short-chain fatty acids (SCFAs), contribute to metabolic regulation by slowing glucose absorption, improving insulin sensitivity, enhancing glucagon-like peptide-1 (GLP-1) secretion, and reducing low-density lipoprotein (LDL) cholesterol levels, thereby lowering the risk of type 2 diabetes and cardiovascular diseases (Cronin *et al*., 2021; Slavin, 2013). In the present study, the total dietary fiber content was higher (p<0.05) in the final cookie (1.64±0.03%) compared to the control cookie (1.24±0.02%), indicating an improvement in dietary fiber content with the modified formulation

Table 4: Proximate composition of cookie samples

Parameter	T_0 - Control cookie (100% WF)	T_1 - Final cookie (10% DP and 90% WF)
Moisture (%)	3.25±0.03^{b}	5.05±0.03^{a}
Crude protein (%)	9.75±0.03^{b}	12.25±0.02^{a}
Crude ash (%)	2.15±0.02^{b}	3.76±0.04^{a}
Crude fat (%)	20.83±0.02^{b}	21.45±0.03^{a}
Crude fiber (%)	8.14±0.01^{b}	10.25±0.03^{a}
Digestible carbohydrates (%)	55.87±0.11^{a}	47.23±0.10^{b}
Energy (kcal/100g)	449.96±0.24^{a}	430.98±0.05^{b}

Values are average of triplicate analysis ± standard deviation; values having the different superscript letters are significantly different (p<0.05)

The mean total sugar content of the final cookie was 22.43±0.04%. According to the Food Regulations under the section 32 of the Food Act, 2019 – No 26/1980, solid or semi-solid foods having more than the 22 g sugar per 100 g of the food is considered as high sugar foods (Ministry of Health, 2020). According to final product (10% DP and 90% WF) cookie were identified as a high sugar product. Therefore, a red color code was given in the label.

The mean total salt content of the final cookie was 0.74±0.02%. The salt content may be attributed to the use of salted margarine as an ingredient, as no additional salt was added separately. According to the Food Regulations under the section 32 of the Food Act, 2019 – No 26/1980, solid or semi-solid foods having 0.25 g to 1.25 g salt per 100 g of the food is considered as medium salt foods (Ministry of Health, 2020). According to final product (10% DP and 90% WF) cookie were identified as a medium salt product. Therefore, amber color code was given in the label.

Mineral composition of the duckweed powder, control cookie sample and final product

The mineral composition of duckweed powder and cookie formulations is presented in Table 5. Among the minerals analyzed in duckweed powder, potassium was the most abundant, with a concentration of 41,135.17±2.01 mg kg^{-1}, followed by boron at 13,011.16±0.48 mg kg^{-1}. Magnesium was present at a considerable level (1,997.45 mg kg^{-1}), while sodium was detected at 452.41 mg kg^{-1}. Trace minerals included iron (16.47 mg kg^{-1}) and manganese (11.51 mg kg^{-1}), whereas chromium was detected at a much lower concentration (0.12 mg kg^{-1}).

As shown in Table 5, incorporation of 10% duckweed powder into the cookie formulation markedly enhanced (p<0.05) the mineral content compared to the control cookie prepared with 100% wheat flour. Boron was the predominant mineral in the final cookie (18,082.85±0.01 mg kg^{-1}) and was not detected in the control sample. Potassium content also increased substantially in the final cookie (4,435.49±0.08 mg kg^{-1}) compared to the control cookie (1,145.03±0.47 mg kg^{-1}). Similarly, the calcium concentration in the final cookie (1,400.73±0.08 mg kg^{-1}) was considerably higher (p<0.05) than that of the control (554.67 ± 0.09 mg kg^{-1}). Enhanced levels of sodium, magnesium, iron, and manganese were also observed in the final cookie, measuring 2,034.37±0.01, 263.51±0.05, 25.45±0.01, and 13.82±0.01 mg kg^{-1}, respectively, compared to 1,434.73±0.15, 91.27±0.08, 5.56±0.08, and 1.16±0.08 mg kg^{-1} in the control cookie. Chromium was detected at a low level in the final cookie (0.38±0.01 mg kg^{-1}) but was absent in the control sample.

Overall, the consumption of cookies enriched with 10% duckweed powder, owing to their improved mineral profile, may contribute to alleviating mineral deficiencies and enhancing dietary mineral intake.

Anti- oxidative potential and anti-diabetic activity (α-amylase Inhibition Assay) of duckweed powder, control cookie and final product

Total Phenolic Content (TPC)

The antioxidant effects of plant extracts are largely attributed to their polyphenol content (Dai and Mumper, 2010). Based on the results obtained, the average Total Phenolic Content (TPC) of the duckweed powder was 5.68±0.04mg GAE g^{-1}. In comparison, the study conducted by Gülçin *et al*. (2010) reported a TPC value of 16.70±0.00 μg mg^{-1} for duckweed.

The average TPC of the control cookie was 0.51±0.01 mg GAE g^{-1}, while the final cookie, incorporating duckweed powder, had a significantly higher (p<0.05) TPC of 1.62±0.03 mg GAE g^{-1} (Table 7). This suggests that the incorporation of duckweed powder significantly increased the phenolic content in the final cookie compared to the control.

Total Flavonoid Content (TFC)

Based on the results obtained, the average TFC of the duckweed powder was 805.75±16.67 mg CAT g^{-1}. In comparison, the study conducted by Gülçin *et al.* (2010) reported a TFC value of 17.4±0.1 μg mg^{-1} for duckweed. Current study shows a

considerably higher TFC in duckweed powder compared to the study by Gülçin *et al* (2010), likely due to methodology, species, and environmental factors.

The average TFC of the control cookie was 61.01±0.00 mg CAT g^{-1}, while the final cookie, incorporating duckweed powder, had a significantly higher (p<0.05) TFC of 282.05±0.01 mg CAT g^{-1}. This suggests that the incorporation of duckweed powder significantly increased the flavonoid content in the final cookie compared to the control.

DPPH radical scavenging activity

The average DPPH radical scavenging activity of the duckweed powder was measured at 160.23±1.54 mM TE g^{-1}. The control cookie exhibited a DPPH radical scavenging activity of 168.62±0.22 mM TE g^{-1}. However, the cookie incorporating duckweed powder demonstrated a significantly higher (p<0.05) DPPH radical scavenging activity, measured at 212.36±1.20 mM TE g^{-1}. (Table 6). These findings suggest that the addition of duckweed powder substantially increased the DPPH radical scavenging activity in the final cookie relative to the control.

***α*-amylase Inhibition Assay**

The *α*-amylase inhibition assay is a widely used *in vitro* technique to assess the anti-diabetic capacity of functional foods. Based on the findings, duckweed powder showed noticeable inhibitory activity when compared with both the control and duckweed-enriched cookies. The IC_{50} value of the final cookie was significantly lower than that of the control, indicating enhanced inhibitory strength (p<0.05) due to the inclusion of 10% duckweed powder (Table 6). These findings are consistent with previous work by (Hiththatiyage, 2025), which reported that *Spirodela polyrhiza* and *Lemna perpusilla*, the same varieties used in the duckweed cookies of the present study possess notable lipase inhibitory activity.

Although the inhibition levels of duckweed and cookies were lower than acarbose drug, the duckweed-enriched cookie shows promise as a dietary aid for managing blood sugar. Regular consumption may help regulate glucose with fewer side effects. Adding duckweed to baked goods could enhance their anti-diabetic potential effectively. However, further studies are needed to confirm these benefits before clinical application.

The IC_{50} value of the final cookie was significantly lower (p<0.05) than that of the control cookie, demonstrating that the 10% duckweed powder cookie was more effective in inhibiting *α*-amylase than the 100% wheat flour cookie.

Table 5: Mineral composition of the duckweed powder, control cookie, and final product

Parameter	Duckweed Powder	T_0 - Control cookie (100% WF)	T_1 - Final cookie (10% DP and 90% WF)
Magnesium (mg kg^{-1})	1997.45±0.81	91.27±0.08 ^{b}	263.51±0.05 ^{a}
Potassium (mg kg^{-1})	41135.17±2.01	1145.03±0.47 ^{b}	4435.49±0.08 ^{a}
Calcium (mg kg^{-1})	12511.84±2.36	554.67±0.09 ^{b}	1400.73±0.08 ^{a}
Sodium (mg kg^{-1})	452.41±0.05	1434.73±0.15 ^{b}	2034.37±0.01 ^{a}
Iron (mg kg^{-1})	16.47±0.82	5.56±0.08 ^{b}	25.45±0.01 ^{a}
Manganese (mg kg^{-1})	11.51±0.41	1.16±0.08 ^{b}	13.82±0.01 ^{a}
Chromium (mg kg^{-1})	0.12±0.01	N/D	0.38±0.01
Boron (mg kg^{-1})	13011.16±0.48	N/D	18082.85±0.01

Values are average of triplicate analysis ± standard deviation; values having the different superscript letters are significantly different (p < 0.05) (N/D = Not detected)

Table 6. Total Phenolic Content (TPC), Total Flavonoid Content (TFC), DPPH Radical Scavenging Activity, and α-Amylase Inhibition of Duckweed Powder

Total phenolic content (TPC)	Total flavonoid content (TFC)	DPPH radical scavenging activity	α-amylase inhibition assay
5.68±0.04 mg GAE g^{-1}	805.75±16.67 mg CAT g^{-1}	160.23±1.54 mM TE g^{-1}	IC_{50}1.39±0.01 (μg/mL) Acarbose IC_{50} 0.002±0.000 (μg/mL)

Values are average of triplicate analysis ± standard deviation

Table 7. Comparison of Total Phenolic Content (TPC), Total Flavonoid Content (TFC), DPPH Radical Scavenging Activity, and α-Amylase Inhibition of Control and final Cookie samples

Functional property	Control cookie	Final cookie
Total phenolic content (TPC)	0.51±0.01^{b} mg GAE g^{-1}	1.62±0.03^{a} mg GAE g^{-1}
Total flavonoid content (TFC)	61.01±0.00 ^{b} mg CAT g^{-1}	282.05±0.01 ^{a} mg CAT g^{-1}
α-amylase inhibition assay	IC_{50} 4.66±0.01 ^{a} (μg/mL)	IC_{50} 2.26±0.02^{b} (μg/mL)

Values are average of triplicate analysis ± standard deviation; values having the different superscript letters are significantly different (p<0.05)

Comparison with SLS standards

The chemical, microbial, and heavy metal parameters of the final cookie (10% DP + 90% WF) were evaluated against SLS standards, with the results summarized in Table 8. The moisture content of the cookie was 5.05 ± 0.03%, below the maximum limit of 6%, indicating enhanced shelf stability and reduced risk of microbial growth. Acid-insoluble ash was measured at 0.03 ± 0.01%, well within the SLSI limit of 0.05%, suggesting minimal contamination with extraneous materials such as sand or soil. The acidity of the extracted fat was 0.83 ± 0.00%, below the 1% threshold, reflecting good fat quality with negligible hydrolysis.

The aerobic plate count (APC) was very low at $(0.003 \pm 0.001) \times 10^3$ CFU/g, well within the permitted range of 10^3–10^5 CFU/g, demonstrating effective hygiene during processing. No yeast or mold was detected even after four weeks, indicating high microbiological stability and an extended shelf life. Heavy metal analysis showed no detectable arsenic, while lead was 0.45 ± 0.01 mg/kg, both safely below SLSI limits (1.0 mg/kg for As and 2.0 mg/kg for Pb), confirming the product's safety with respect to heavy metal contamination. Overall, all evaluated parameters met the SLS standards, confirming the final cookie's compliance with quality and safety requirements.

Table 8: Comparison of final product (10% DP + 90% WF) properties with SLSI Standards

	Parameter	SLSI requirement	Final product (10% DP + 90% WF)	Whether the parameters meet the SLSI standards or not
Chemical requirement	Moisture content (% mass)	Cookies – 6.0 (max.limit)	5.05±0.03	Yes
	Acid insoluble ash (on a dry basis, % mass)	0.05	0.03±0.01	Yes
	Acidity of extracted fat (expressed as oleic acid, % mass)	1.0	0.83±0.00	Yes
Microbial requirement	Aerobic Plate Count (CFU/g)	10^3 (min) - 10^5(max)	(0.003±0.001) × 10^3	Yes
	Yeast and Mold count	10^2 (min) – 10^3 (max)	Not detected (After 4 weeks)	Yes
Heavy metal limits	Arsenic, as As, mg/kg	1.0 (max)	Not detected (After 4 weeks)	Yes
	Lead, as Pb, mg/kg	2.0 (max)	0.45±0.01	Yes

CONCLUSIONS

The incorporation of duckweed powder, specifically a blend of *Spirodela polyrhiza* and *Lemna perpusilla*, enhanced the physicochemical, nutritional, sensory, functional, and microbial properties of cookies, with a 10% substitution meeting industry standards and consumer acceptability. These findings demonstrate the feasibility of integrating duckweed powder into bakery products, offering both nutritional enrichment and potential health-promoting benefits, thereby highlighting its value as a functional ingredient in the food industry.

ACKNOWLEDGEMENTS

Uva Wellassa University, Sri Lanka is acknowledged for academic knowledge. The National Institute of Fundamental Studies, Sri Lanka, should be acknowledged for providing research facilities and technical assistance.

AUTHORS' CONTRIBUTIONS

W.M.S.U. Weerakoon conceptualized the study, carried out the experiments, analyzed the data, and prepared the initial draft of the manuscript. R. Hiththatiyage provided guidance and support throughout the experimental work and contributed to the preparation of the manuscript. W.A.J.P. Wijesinghe and R. Liyanage were involved in study design, provided supervision, and critically reviewed and revised the manuscript. M.A. Wickramasinghe and I. Rathnayaka provided guidance and support throughout the study.

REFERENCES

Agbo, M. O., Uzor, P. F., Akazie-Nneji, U. N., Eze-Odurukwe, C. U., Ogbatue, U. B., & Mbaoji, E. C. (2015). Antioxidant, total phenolic and flavonoid content of selected Nigerian medicinal plants. *Dhaka University Journal of Pharmaceutical Sciences,* 14(1), 35–41. https://doi.org/10.3329/dujps.v14i1.23733

Akhobakoh, M., Zing, B. Z., Ngatchou, A., Mbassi, J. E. G., & Nchanji, E. B. (2022). Potato (*Solanum tuberosum* L.) flour enriched with date palm fruit (*Phoenix dactylifera* L.) powder and bean milk for cookies production. *Agricultural Sciences,* 13(9), 973–988. https://doi.org/10.4236/AS.2022.139060

Andriani, Y., Mulyani, Y., Iskandar, Megantara, S., & Levita, J. (2022). Molecular docking study, antioxidant activity, proximate content, and total phenol of *Lemna perpusilla* Torr. grown in West Java, Indonesia. *Rasayan Journal of Chemistry,* 15(2), 1182–1189. https://doi.org/10.31788/rjc.2022.1526925

AOAC. (2000). *Official methods of analysis* (17th ed.).

AOAC. (2010). *Official methods of analysis* (18th ed.).

Baek, G. Y., Saeed, M., & Choi, H. K. (2021). Duckweeds: Their utilization, metabolites and cultivation. *Applied Biological Chemistry,* 64(1), 1–15. https://doi.org/10.1186/s13765-021-00644-z

Baking Quality of Cookie Flour, AACC Micro Method (1999).

Bartošová, A., Sirotiak, M., & Fiala, J. (2015). Comprehensive study of duckweed cultivation and growth conditions under controlled eutrophication. *Research Papers Faculty of Materials Science and Technology Slovak University of Technology*, 23(36), 103–107. https://doi.org/10.1515/rput-2015-0012

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature,* 181(4617), 1199–1200. https://doi.org/10.1038/1811199a0

Boz, H. (2019). Effect of flour and sugar particle size on the properties of cookie dough and cookie. *Czech Journal of Food Sciences,* 37(2), 120–127. https://doi.org/10.17221/161/2017-CJFS

Charoenphun, N., & Kwanhian, W. (2019). Production of gluten-free cookies supplemented with durian rind flour. *Science and Technology RMUTT Journal,* 9(2), 23–38.

Chaudhari, P. D. N., Wandhekar, S. S., Shaikh, A. A., & Devkatte, A. N. (2018). Preparation and characterization of cookies prepared from wheat flour fortified with mushroom (*PleurotusSajorcaju*) and spiced with cardamom. *International Journal of Research and Analytical Review*, 5(4), 386–389.

Chen, G., Fang, Y., Huang, J., Zhao, Y., Li, Q., Lai, F., Xu, Y., Tian, X., He, K., Jin, Y., Tan, L., & Zhao, H. (2018). Duckweed systems for eutrophic water purification through converting wastewater nutrients to high-starch biomass. *RSC Advances,* 8(32), 17927–17937. https://doi.org/10.1039/C8RA01856A

Chrismadha, T., Sulawesty, F., Awalina, A., Mardiati, Y., Mulyana, E., & Widoretno, M. R. (2016). Growth performance of minute duckweed (*Lemna perpusilla*) In an integrated common carp (*Cyprinus carpio*) closed recirculation aquaculture. *Aquacultura Indonesiana*, 15(1), 16–26. https://doi.org/10.21534/ai.v15i1.21

Cronin, P., Joyce, S. A., O'Toole, P. W., & O'Connor, E. M. (2021). Dietary fibre modulates the gut microbiota. *Nutrients,* 13(5), 1655. https://doi.org/10.3390/nu13051655

Dai, J., & Mumper, R. J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules,* 15(10), 7313–7352. https://doi.org/10.3390/molecules15107313

Dias, N. A. I. S., & Bandara, G. M. W. R. (2019). Nutritive assessment of composite flour biscuit incorporated with herbal plant powder. *Journal of Food and Agriculture,* 12(1), 17–24. https://doi.org/10.4038/jfa.v12i1.5217

Directorate of non-communicable diseases ministry of health. (2022). *The national policy and strategic framework for prevention and control of chronic non-communicable diseases in Sri Lanka. Wb 2008, 1–14.*

Duangjarus, N., Chaiworapuek, W., Rachtanapun, C., Ritthiruangdej, P., & Charoensiddhi, S. (2022). Antimicrobial and functional properties of duckweed (*Wolffia globosa*) protein and peptide extracts. *Foods,* 11(15), 2348. https://doi.org/10.3390/foods11152348

Giwa, O. E., Babalola, R. O., & Atanlogun, S. K. (2012). Microbial, physical and sensory attribute of cookies produced from wheat flour fortified with *Termitomyces robustus* and spiced with curry leaves (*Murraya koenigii*). *International Journal of Applied Biology and Pharmaceutical Technology*, 3(2), 301–307. http://www.ijabpt.com/pdf/42044-Oluwamodupet[1].pdf

Ikuomola, D. S., Otutu, O. L., & Oluniran, D. D. (2017). Quality assessment of cookies produced from wheat flour and malted barley (*Hordeum vulgare*) bran blends. *Cogent Food and Agriculture*, 3(1). https://doi.org/10.1080/23311932.2017.1293471

Handa, C., Goomer, S., & Siddhu, A. (2012). Physicochemical properties and sensory evaluation of fructooligosaccharide enriched cookies. *Journal of Food Science and Technology,* 49(2), 192–199. https://doi.org/10.1007/s13197-011-0277-4

Hiththatiyage, R., Senavirathne, S., Wickramasinghe, M., Rathnayaka, I., Madhujith, T., Jayawardana, B., Wijesundara, S., & Liyanage, R. (2025). Nutritional composition and bioactive properties of four duckweed varieties in Sri Lanka. *BMC Agriculture*, 1–26. Preprint. https://doi.org/10.21203/rs.3.rs-6819490/v1

James, C. S. (1995). A*nalytical chemistry of foods*. Seale-Hayne Faculty of Agriculture, Food and Land Use, Department of Agriculture and Food Studies University of Plymouth.

Jayamali, N. A. I., Wijesinghe, J., & Silva, P. A. P. M. D. (2022). Green tea incorporated edible coating extends the postharvest life of strawberry fruits (*Fragaria ananassa*). *Advances in Technology*, 2(4), 382–393. https://doi.org/10.31357/ait.v2i4.6031

Kaushalya, S. D. N., & Wijesinghe, W. A. J. P. (2024). Gluten-free biscuit prepared using jackfruit seed flour. *Tropical Agricultural Research and Extension,* 27(4), 225–235. https://doi.org/10.4038/TARE.V27I4.5720

Khan, M.S., Ehsan, A., Ali S., & Khan, W.M. (2014). Assessment of Essential Amino Acids in Wheat Proteins : A Case Study. *Journal of Biodiversity and Environmental Sciences* 4(6), 185-189.

Khandaker, T., Khan Md.J., Shahjalal, Md., & Rahman, Md.M. (2007). Use of Duckweed (*Lemna Perpusilla*) as a Protein Source Feed Item in the Diet of Semi-Scavenging Jinding Layer Ducks. *Journal of Poultry Science*, 44(3), 314–21. https://doi.org/10.2141/JPSA.44.314

Kuchtová, V., Kohajdová, Z., Karovičová, J., & Lauková, M. (2018). Physical, textural and sensory properties of cookies incorporated with grape skin and seed preparations. *Journal of Food and Nutrition Sciences*, 68(4), 309–317. https://doi.org/10.2478/PJFNS-2018-0004

McCleary, B. V. (2023). Measurement of dietary fiber: Which AOAC official method to use. *Journal of AOAC International,* 106(4), 917–930. https://doi.org/10.1093/jaoacint/qsad051

Megeri, T. R., Meti, B. S., Hadimani, G., & Harke, S. (2022). Development of multigrain cookies. *International Journal of Research in Engineering and Science (IJRES) ISSN*, 10(10), 104–131. https://doi.org/10.22271/chemi.2021.v9.i1l.11336

Mejbel, H. S., & Simons, A. M. (2018). Aberrant clones: Birth order generates life history diversity in greater duckweed, *Spirodela*

polyrhiza. *Ecology and Evolution*, 8(4), 2021–2031. https://doi.org/10.1002/ECE3.3822

Méndez-Martínez, Y., Torres Navarrete, Y. G., Pérez Tamames, Y., Romás Viltres, M., & Cortés-Jacinto, E. (2021). Effect of duckweed meal dietary inclusion on growth performance and survival of African catfish fingerlings. *Rev. Fac. Agron. (LUZ)*, 38(1), 84–104. https://doi.org/10.47280/RevFacAgron(LUZ).v38.n1.05

Ministry of Health (2020). Review of Colour-coding-solids-English [on line] Available at https://eohfs.health.gov.lk/food/images/pdf/regulations/Colour-coding-solids-English.pdf [Accessed on 02.10.2024].

Mudgil, D., Barak, S., & Khatkar, B. S. (2017). Cookie texture, spread ratio and sensory acceptability as a function of soluble dietary fiber. *LWT - Food Science and Technology*, 80, 537–542. https://doi.org/10.1016/j.lwt.2017.03.009

Nieuwland, M., Geerdink, P., Engelen-Smit, N. P. E., van der Meer, I. M., America, A. H. P., Mes, J. J., Kootstra, A. M. J., Henket, J. T. M. M., & Mulder, W. J. (2021). Isolation and gelling properties of duckweed protein concentrate. *ACS Food Science and Technology*, 1(5), 908–916. https://doi.org/10.1021/acsfoodscitech.1c00009

Nitiwuttithorn, C., Wongsasulak, S., Vongsawasdi, P., & Yongsawatdigul, J. (2024). Effects of alkaline and ultrasonication on duckweed (*Wolffia arrhiza*) protein extracts, physicochemical and techno-functional properties. *Frontiers in Sustainable Food Systems*, 8, 1343615. https://doi.org/10.3389/FSUFS.2024.1343615/BIBTEX

Nizam, N., & Arampath, P. C. (2016). Development of Olu (*Nymphaea pubescens Will*) seed flour incorporated cookies and determination of organoleptic properties. *Tropical Agricultural Research*, 27(4), 420. https://doi.org/10.4038/tar.v27i4.8218

Oladunjoye, A. O., Eziama, S. C., & Aderibigbe, O. R. (2021). Proximate composition, physical, sensory and microbial properties of wheat-hog plum bagasse composite cookies. *LWT - Food Science and Technology*, 141, 111038 https://doi.org/10.1016/j.lwt.2021.111038

Pagliuso, D., Grandis, A., Fortirer, J. S., Camargo, P., Floh, E. I. S., & Buckeridge, M. S. (2022). Duckweeds as promising food feedstocks globally. *Agronomy*, 12(4), 796. https://doi.org/10.3390/agronomy12040796

Pareyt, B., Wilderjans, E., Goesaert, H., Brijs, K., & Delcour, J. A. (2008). The role of gluten in a sugar-snap cookie system: A model approach based on gluten-starch blends. *Journal of Cereal Science*, 48(3), 863–869. https://doi.org/10.1016/j.jcs.2008.06.011

Puranik, V. (2012). Development and compositional analysis of protein rich soybean-maize flour blended cookies. *Journal of Food Processing & Technology*, 03(09). https://doi.org/10.4172/2157-7110.1000182

Reyes-Pérez, F., Salazar-García, M. G., Romero-Baranzini, A. L., Islas-Rubio, A. R., & Ramírez-Wong, B. (2013). Estimated glycemic index and dietary fiber content of cookies elaborated with extruded wheat bran. *Plant Foods for Human Nutrition*, 68(1), 52–56. https://doi.org/10.1007/s11130-013-0338-0

Said, D. S., Chrismadha, T., Mayasari, N., Widiyanto, T., & Ramandita, A. (2022). Nutritional content and growth ability of duckweed *Spirodela polyrhiza* on various culture media. *IOP Conference Series: Earth and Environmental Science*, 1062(1). https://doi.org/10.1088/1755-1315/1062/1/012009

Shahane, A.A., & Shivay Y.S. (2024). Increase in above Ground Dry Matter Accumulation with Application Microbial Consortia and Zinc Fertilization in Different Cultivation Methods of Wheat. *International Journal of Research in Agronomy* 7(11), 197–203. https://doi.org/10.33545/2618060X.2024.v7.i11c.1961

Sielicka, M., Malecka, M., & Purlan, M. (2014). Comparison of the antioxidant capacity of lipid-soluble compounds in selected cold-pressed oils using photochemiluminescence assay (PCL) and DPPH method. *European Journal of Lipid Science and Technology*, 116(4), 388–394. https://doi.org/10.1002/ejlt.201300356

Singleton, V.L., Orthofer, R. & Rosa, M. Lamuela-Raventós. (1999). Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. *Methods in Enzymology*, 299, 152–78. https://doi.org/10.1016/S0076-6879(99)99017-1.

Song, Y., Hu, Z., Liu, S., Luo, S., He, R., Yang, X., Li, S., Yang, X., An, Y., & Lu, Y. (2025). Utilization of Microalgae and Duckweed as Sustainable Protein Sources for Food and Feed : Nutritional Potential and Functional Applications. *Journal of Agricultural and food chemistry*. 73, 4466−4482 https://doi.org/10.1021/acs.jafc.4c11610.

Soni, N., Kulkarni, A. S., & Patel, L. (2018). Studies on development of high protein cookies. *International Journal of Chemical Studies*, 6(6), 439–444.

Slavin, J. (2013). Fiber and prebiotics: Mechanisms and health benefits. *Nutrients,* 5(4), 1417–1435. https://doi.org/10.3390/nu5041417

Sri Lanka standards institution. (2021). Sri Lanka standards catalogue. 17.

Sri Lanka standard specification for biscuits (Second Revision), SLS 251(2010)

Tapía, M. S., Alzamora, S. M., & Chirife, J. (2020). Effects of water activity (aw) on microbial stability as a hurdle in food preservation. *Water Activity in Foods*. *Fundamentals and Applications*, 323–355. https://doi.org/10.1002/9781118765982.CH14

Vidyapeeth, M. K., Vs, I. P., Syed, I., Sharma, I. D., Ag, K., Vs, P., Hm, S., & Sharma, D. (2017). Development and evaluation of organoleptic and microbial quality of guar gum incorporated composite flour cookies. *Journal of Pharmacognosy and Phytochemistry*, 6(5), 716–720.

Visvanathan, R., Jayathilake, C., & Liyanage, R. (2016). A Simple Microplate-Based Method for the Determination of α-Amylase Activity Using the Glucose Assay Kit (GOD Method). *Food Chemistry,* 211, 853–59. https://doi.org/10.1016/J.FOODCHEM.2016.05.090.

Waters, D. M., Jacob, F., Titze, J., Arendt, E. K., & Zannini, E. (2012). Fibre, protein and mineral fortification of wheat bread through milled and fermented brewer's spent grain enrichment. *European Food Research and Technology*, 235(5), 767–778. https://doi.org/10.1007/s00217-012-1805-9

Wickramasinghe, M. A., Nadeeshani, H., Sewwandi, S. M., Rathnayake, I., Kananke, T. C., & Liyanage, R. (2023). Comparison of nutritional composition, bioactivities, and FTIR- ATR microstructural properties of commercially grown four mushroom species in Sri Lanka; Agaricus bisporus, Pleurotus ostreatus, Calocybe sp. (MK-white), Ganoderma lucidum. Food Production, Processing and Nutrition,5(1).https://doi.org/10.1186/s43014-023-00158-9

Xiao, Y., Fang, Y., Jin, Y., Zhang, G., & Zhao, H. (2013). Culturing duckweed in the field for starch accumulation. *Industrial Crops and Products*, 48, 183–190.

https://doi.org/10.1016/j.indcrop.2013.04.017

Xu, J., Cui, W., Cheng, J. J., & Stomp, A. M. (2011). Production of high-starch duckweed and its conversion to bioethanol. *Biosystems Engineering*, 110(2), 67–72. https://doi.org/10.1016/J.BIOSYSTEMSENG.2011.06.007

Xu, J., Shen, Y., Zheng, Y., Smith, G., Sun, X. S., Wang, D., Zhao, Y., Zhang, W., & Li, Y. (2023). Duckweed (*Lemnaceae*) for potentially nutritious human food: A review. *Food Reviews International,* 39(7),3620–3634. https://doi.org/10.1080/87559129.2021.2012800

Yu, G., Liu, H., Venkateshan, K., Yan, S., Cheng, J., Sun, X. S., & Wang, D. (2011). Functional, physiochemical, and rheological properties of duckweed (*Spirodela polyrhiza*) protein, *American Society of Agricultural and Biological Engineers*.54(2),555–561. https://doi.org/10.13031/2013.36459