Food and Humanity 6 (2026) 101235

Contents lists available at ScienceDirect

Food and Humanity

journal homepage: www.editorialmanager.com/foohum/journal_overview.html

Physicochemical, nutritional, antioxidant, and glycaemic properties of gluten-free cassava bread enriched with nettle leaf and watermelon peel powders

Srivathsa Kumbaji[a], Vahid Baeghbali[a,b,c], Tafadzwa Mkungunugwa[a,b], Ganiyat Olayinka Olatunde[d], Rania Harastani[a,b], Luca Serventi[e], Mary Nkongho Tanyitiku[a,b,*]

[a] *Food and Markets Department, Natural Resources Institute, University of Greenwich, Chatham Maritime, Kent ME4 4TB, United Kingdom*
[b] *Medway Food Innovation Centre, Natural Resources Institute, University of Greenwich, Chatham Maritime, Kent ME4 4TB, United Kingdom*
[c] *Bezos Centre for Sustainable Protein, University of Greenwich, Medway Campus, Central Avenue, Chatham Maritime, Kent ME4 4TB, United Kingdom*
[d] *Department of Food Science & Technology, Federal University of Agriculture, Abeokuta, Nigeria*
[e] *The New Zealand Institute for Plant and Food Research Limited, Private Bag 4704, Christchurch 8140, New Zealand*

ARTICLE INFO

Keywords:
Gluten-free bread
Cassava
Nettle leaf
Watermelon peel
Predicted glycaemic index
Celiac disease

ABSTRACT

Cassava-based bread is naturally gluten-free but nutritionally limited due to low protein, fibre, and bioactive content. This study investigated the incorporation of nettle leaf powder (NLP) and watermelon pecel powder (WPP) as sustainable functional ingredients to enhance its nutritional, physicochemical, and health-promoting properties. Composite breads were formulated by partially substituting cassava flour (CF) with increasing levels of NLP and WPP at CWN1 (85% CF: 10% WPP: 5% NLP), CWN2 (70% CF: 20% WPP: 10% NLP), CWN3 (60% CF: 25% WPP: 15% NLP), and CWN4 (50% CF: 30% WPP: 20% NLP). The inclusion of NLP and WPP resulted in expected increases in protein, dietary fibre, and total phenolic content, reflecting the intrinsic composition of the added materials. More importantly, significant ($p < 0.05$) increase in antioxidant activity were observed, with CWN4 being highest (DPPH·: 573.49 ± 0.60 μmolTE/g, ABTS·+: 547.69 ± 0.88 μmolTE/g). In vitro starch hydrolysis revealed a progressive reduction in digestibility, with hydrolysis index decreasing from 73.21 to 66.39 and predicted glycaemic index (pGI, dimensionless) from 71.54 to 62.12, indicating moderated glycaemic potential. Colour analysis revealed darker crumb appearance ($L^* = 15.75$), increased greenness ($a^* = -8.47$), and yellowness ($b^* = 27.84$), attributable to chlorophyll and phenolic pigments. Electronic taste further demonstrated distinct taste profiles, with increased bitterness and umami responses that reflected increasing phenolic and minerals. Overall, while physical bread quality was not improved, the incorporation of NLP and WPP significantly enhanced antioxidant capacity and reduced starch digestibility, highlighting their potential in the development of functional gluten-free products with improved metabolic relevance.

1. Introduction

Celiac disease (CD) is a chronic autoimmune enteropathy that occurs in genetically predisposed individuals following ingestion of gluten proteins found in wheat, barley, and rye (Aljada et al., 2021; NIH, 2020). In affected individuals, gluten exposure triggers immune-mediated damage to the small intestinal mucosa, resulting in villous atrophy, malabsorption, and systemic inflammation, which can lead to nutrient deficiencies and gastrointestinal symptoms distress (Rai et al., 2018). With increasing global awareness and diagnosis of CD and non-celiac gluten sensitivity, strict lifelong adherence to a gluten-free diet remains the only effective therapeutic strategy (NIH, 2020; Tanyitiku et al., 2024). Consequently, demand for nutritionally adequate and sensory-acceptable gluten-free staple foods, particularly bread, has risen markedly in recent years (Olawoye et al., 2020; Rai et al., 2018). However, many commercially available gluten-free breads rely heavily on refined starches and non-whole-grain ingredients, resulting in products that are often deficient in protein, dietary fibre, iron, folate, B

---

* Correspondence to: Natural Resources Institute, University of Greenwich, United Kingdom.
E-mail address: Mary.Tanyitiku@greenwich.ac.uk (M.N. Tanyitiku).

https://doi.org/10.1016/j.foohum.2026.101235
Received 1 January 2026; Received in revised form 1 May 2026; Accepted 11 May 2026
Available online 13 May 2026

vitamins, calcium, magnesium, and zinc (Eduardo et al., 2013; Olawoye et al., 2020). These nutritional shortcomings highlight the need for ingredient innovations that can enhance both the physicochemical and nutritional quality of gluten-free baked products.

Cassava (*Manihot esculenta* Crantz) is an essential staple crop for more than 700 million people worldwide and a primary caloric source across tropical regions (Tanyitiku, 2024; Ze et al., 2021). Cassava flour is valued for its high carbohydrate content, neutral flavour, fine particle size, and functional starch properties, making it a promising gluten-free ingredient for bread production (Sigüenza-Andrés et al., 2021; Ze et al., 2021). However, breads formulated solely with cassava flour often exhibit limited viscoelasticity due to the absence of gluten, resulting in technological and sensory shortcomings such as reduced loaf volume, weak crumb structure, rapid staling, and pale crust coloration compared with wheat-based breads (Eduardo et al., 2013; Halake & Chinthapalli, 2020; Sigüenza-Andrés et al., 2021). To overcome these drawbacks, the fortification of cassava flour with functional ingredients from legumes, leafy greens, fruit or vegetable by-products has gained interest, as these can supply proteins, minerals, and bioactive compounds, modify dough rheology through fibre and polysaccharides crosslinking networks, and impart natural pigments that enhance crumb and crust colour.

Stinging nettle (*Urtica dioica* L.) is widely distributed in Europe, Asia, Africa, and South America, and have a long ethnobotanical history of use as food and medicine (Tanyitiku et al., 2024). Its powder is a promising functional ingredient with the potential to improve the nutritional quality, antioxidant activity and potential glycaemic regulation in foods (Adhikari et al., 2016; Mohammadian et al., 2024; Wójcik et al., 2021). Studies incorporating nettle leaf powder into bakery products such as breads, cookies, and waffles, have consistently reported increased in protein, dietary fibre, essential minerals, carotenoids, and antioxidant compounds (Maietti et al., 2021; Tanyitiku & Njombissie Petcheu, 2025; Đurović et al., 2020).

Similarly, watermelon (*Citrullus lanatus*) peel, an underutilized by-product representing nearly one-third of the fruit's mass, has recently gained attention as a sustainable source of functional ingredient (Asoka et al., 2022; Gu et al., 2023). The peel and rind are rich in dietary fibre, L-citrulline, vitamin C and phenolic compounds, and demonstrate strong antioxidant activity in in vitro assays (Gu et al., 2023; Kataria & Kaur, 2023). Prior studies have shown that incorporating watermelon peel or rind powders into baked goods such as cookies and cakes could enhance dietary fibre and antioxidant content while lowering the predicted glycaemic response (Acun et al., 2025; Al-Sayed & Ahmed, 2013; Naknaen et al., 2016). Despite these promising attributes, the application of nettle leaf and watermelon peel flours has been predominantly limited to gluten-containing wheat bakery products (Mohammadian et al., 2024; Naknaen et al., 2016). Their functionality, nutritional potential, and sensory influence in gluten-free food matrices remain under-explored.

Therefore, this study aimed to evaluate the effects of nettle leaf and watermelon peel powders on physicochemical, nutritional, antioxidant, and glycaemic properties of gluten-free cassava bread.

2. Materials and method

2.1. Raw materials

Three principal raw materials were used in this study: cassava flour (CF), watermelon peel powder (WPP), and nettle leaf powder (NLP). Cassava flour was obtained from a local supplier (African taste, Jumbo, United Kingdom). Fresh stinging nettle (*Urtica dioica* L.) leaves were collected from a vegetable farm in Dockside, Chatham, United Kingdom, and identified by morphological characteristics (Tanyitiku et al., 2024). The leaves were washed thoroughly with deionised water and shade-dried at 25 ± 2℃ for three days. Watermelon (*Citrullus lanatus*) peels were obtained from fresh fruits purchased at a local supermarket (ASDA, Gillingham Pier, Chatham, United Kingdom). The outer green rind and white mesocarp were sliced, then oven-dried in an incubator (GenLab, United Kingdom) at 55℃ to a moisture content of below 10%. Dried nettle leaves and watermelon peels were separately milled to fine powders using a Thermomix TM6 (Vorwerk, Wuppertal, Germany) to obtain nettle leaf powder (NLP) and watermelon peel powder (WPP). Other ingredients: wheat flour, corn starch, dry yeast, salt, sugar, and rapeseed oil, were procured from the same supermarket. Before use, all flours and powders were sieved through a < 250 μm UWL 400 T sieve shaker (Haver & Boecker, VWR International, United Kingdom) and stored in airtight polyethylene bags at 25 ± 2℃ until bread preparation and analysis. All chemicals and reagents were of analytical grade.

2.2. Bread preparation

Six bread formulations were prepared by partially substituting cassava flour (CF) with increasing levels of NLP and WPP at CWN1 (85% CF: 10% WPP: 5% NLP), CWN2 (70% CF: 20% WPP: 10% NLP), CWN3 (60% CF: 25% WPP: 15% NLP), and CWN4 (50% CF: 30% WPP: 20% NLP). WWB: white wheat bread served as the reference bread for glycaemic index (HI = 100), CFB: cassava flour bread of control for the gluten-free series, CWN1: low substitution level, CWN2: moderate substitution level, CWN3: high substitution level, CWN4: very high substitution level. WF: wheat flour, CF: cassava flour. WPF: watermelon peel powder, NLF: nettle leaf powder. The substitution levels for watermelon peel powder (WPP) and nettle leaf powder (NLP) were selected based on preliminary formulation trials and literature reports (Al-Sayed & Ahmed, 2013; Naknaen et al., 2016; Rai et al., 2018) on fibre-rich ingredient incorporation in gluten-free bakery systems. The chosen ratios (CWN1-CWN4) were designed using a Breville bread maker (Model BBM800, Breville Group Ltd., Australia) to provide a progressive increase in functional ingredient content while maintaining dough workability and structural integrity. Higher substitution levels were avoided due to observed detrimental effects on dough handling and bread structure during preliminary testing. For each formulation, all ingredients were accurately weighed using a digital precision balance (±0.01 g) and sequentially added into the bread maker pan in the following order: 460 mL water, 6 g oil, 2.5 g sugar, 2.5 g salt, 300 g composite flour (cassava + watermelon peel + nettle leaf), 5 g corn starch, and 3 g of dry yeast. The white wheat bread (formulation A) was baked using the standard 'White Bread' program, while the gluten-free formulations (CFB, CWN1-CWN4) were prepared using the 'Gluten-Free' cycle (2 h 10 min total: 20 min mixing/kneading, 40 min proofing, and 70 min baking with intermittent kneading phases). To ensure uniformity across batches, the bread maker was pre-warmed before each run, and baking was performed in single-loaf mode (pan capacity: 1.2 L). Upon completion of the cycle, loaves were removed from the pan, cooled at ambient laboratory conditions (25 ± 2℃) for 2 h, and subsequently analysed for loaf volume, weight, moisture, and fat content. Each loaf was packaged in polyethylene bags to minimize moisture loss and stored at 4 ± 2℃ for subsequent analyses. To prevent cross-contamination, all equipment and utensils were thoroughly cleaned between batches, and gluten-containing breads were processed last.

2.3. Chemical analysis

Each flour, powder and bread sample was analysed for moisture, fat, protein, starch, and dietary fibre content. Moisture and fat were determined using a CEM Smart 6 moisture analyser (CEM Corp., Matthews, NC, USA) and Oracle fat analyser (CEM Corp., Matthews, NC, USA). Both moisture and fat determination was carried out according to the AOAC 2008.06 official method (Leffler et al., 2008). Protein content was quantified using the Bradford dye-binding assay kit (Sigma-Aldrich, St. Louis, MO, USA). Briefly, homogenized bread samples were extracted in phosphate-buffered saline (pH 7.9) and centrifuged at 4000 × g for 15 min. The resulting supernatant was reacted with Coomassie Brilliant Blue G-250 reagent, and absorbance was measured at 595 nm using a UV-Vis spectrophotometer (Jenway, Cole-Parmer, United Kingdom).

Protein concentration was calculated from a standard curve prepared with bovine serum albumin (BSA). Dietary fibre content was analysed using the Total Dietary Fibre Kit (Sigma-Aldrich, United Kingdom) following the enzymatic-gravimetric AOAC Method 991.43. Both soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) fractions were determined, and total dietary fibre (TDF) was expressed as the sum of SDF and IDF. Starch content was measured using the Total Starch Assay Kit (Sigma-Aldrich, United Kingdom) based on enzymatic hydrolysis of starch to glucose by thermostable α-amylase and amyloglucosidase. Released glucose was quantified spectrophotometrically at 510 nm using the glucose oxidase/peroxidase (GOPOD) reagent, and total starch was expressed as a percentage of dry matter.

2.4. *Specific volume*

Specific loaf volume was determined using the rapeseed displacement method according to AACC International Approved Method 10–05.01 (AACC Method 10–05.01, 2010). After cooling (25 ± 2℃, 2 h), loaves were weighed, and volume was measured. Specific volume (cm^3/g) was calculated as the ratio of loaf volume to loaf weight. Measurements were performed in triplicate.

2.5. *Colour measurement*

Bread colour was measured using a Minolta Chroma Meter (CR-400, Konica Minolta Sensing Inc., Osaka, Japan) calibrated with a standard white tile. Measurements were performed using illuminant D65 and a 10° standard observer with d/8° geometry. Slices (2 cm thickness) were analysed at five random points on crumb and crust surfaces using an 8 mm aperture. Colour parameters were recorded in the CIE Lab* system. Chroma (C*), hue angle (h°), and total colour difference (ΔE*) were calculated from L*, a*, and b* values relative to the control sample.

2.6. *Texture Profile Analysis (TPA)*

Texture Profile Analysis (TPA) of the bread samples was performed using a Texture Analyzer (TA-XT Plus, Stable Micro Systems, Godalming, Surrey, United Kingdom) equipped with a 50 mm diameter cylindrical probe. After 12 h of storage at ambient temperature (25 ± 2℃), loaves were sliced at the centre into 50 mm thick sections, and the crumb portion was used for analysis to avoid crust interference. Each sample was subjected to a double compression cycle to 50% of its original height at a test speed of 1.0 mm/s, with a trigger force of 5 g and a 5 s interval between compressions. Each measurement was performed on at least five slices per loaf, and mean values were calculated. The texture parameters, hardness, adhesiveness, resilience, cohesiveness, springiness, and chewiness, were derived automatically from the force-time curve using the Exponent Connect 32 software (Ver. 8.1, Stable Micro Systems, UK).

2.7. *Total phenolic content and antioxidant activity*

2.7.1. *Total phenolic content (TPC)*

The TPC of bread samples was determined using the Folin-Ciocalteu colorimetric method according to Lohvina et al. (2022) with slight modifications. Briefly, 1 g of bread crumb was extracted with 10 mL of 70% ethanol under agitation (350 rpm, 25℃ for 1 h) and centrifuged at 4000 g for 15 min. The supernatant (0.2 mL) was mixed with 1 mL of 10% (v/v) Folin-Ciocalteu reagent, followed by the addition of 0.8 mL of 7.5% sodium carbonate. After incubation in the dark at 25℃ for 30 min, absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Jenway, Cole Palmer, United Kingdom). TPC was expressed as mg gallic acid equivalents (GAE) per g dry weight using a gallic acid calibration curve (0–200 mg/L)

2.7.2. *DPPH· radical scavenging activity*

The antioxidant capacity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay (Yu et al., 2013). A 0.1 mM DPPH solution in methanol was freshly prepared. An aliquot of 0.2 mL of bread extract was added to 2.8 mL of DPPH solution, vortexed, and incubated in the dark at room temperature for 30 min. Absorbance was recorded at 517 nm, and results were expressed as Trolox equivalent antioxidant capacity (TE, μmol Trolox equivalents/g dry weight) using a Trolox calibration curve (0–500 μM) (Shen et al., 2019).

2.7.3. *ABTS$\cdot^{+}$ radical cation scavenging activity*

The ABTS$\cdot^{+}$ value of bread samples were determined according to Rachman et al. (2023). The ABTS$\cdot^{+}$ radical was generated by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and incubating in the dark for 16 h. The solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. To 3.0 mL of diluted ABTS$\cdot^{+}$ solution, 0.2 mL of bread extract was added, mixed, and incubated at room temperature (25 ± 2℃) for 6 min. Absorbance was measured at 734 nm, and antioxidant activity was expressed as μmol Trolox equivalents/g dry weight using a Trolox calibration curve (0–500 μM).

2.8. *Bread hydrolysis and predicted glycaemic index*

The hydrolysis index was determined as described by Pasqualoni et al. (2024). In brief, 100 mg of bread was incubated in glass vials at 37℃. During incubation, 4 mL of maleic buffer (pH 6) containing 40 mg of pancreatic amylase (3000 U/mg) and 4 mL of amyloglucosidase solution (300 U/mL) (Sigma-Aldrich, United Kingdom) were added. Aliquots (1 mL) were withdrawn at 0, 20, 60, 90, 120, and 180 min. Enzyme activity in the aliquots was immediately stopped by heating at 100 °C for 5 min, and samples were centrifuged at 4000 g for 10 min. Reducing sugar release was determined spectrophotometrically at 510 nm using a Sigma Glucose (GO) Assay Kit (Sigma-Aldrich, United Kingdom) according to the manufacturer's instructions. The hydrolysis index (HI) was determined as the percentage ratio of the area under the curve representing bread samples hydrolysis (0–180 min) to the area under the curve of a reference white bread (WWB). The pGI was estimated using Eq.1 according to (Goñi et al., 1997).

$$\mathrm{pGI} = \quad 39.71 + (0.549 \times \mathrm{HI}) \tag{1}$$

2.9. *Microstructural analysis by Scanning Electron Microscopy (SEM)*

The microstructure of the optimized gluten-free bread crumb was examined using a ZEISS Sigma Field Emission Scanning Electron Microscope (FE-SEM) (Carl Zeiss Microscopy GmbH, Germany). Bread samples were cut into approximately 5 × 5 × 5 mm from the crumb region and immediately frozen at −20℃ to minimize structural collapse. The frozen samples were subsequently freeze-dried (-50℃, 0.1 mbar, 48 h) using a FT33 MK11 vacuum freeze dryer (Armfield, United Kingdom), to remove moisture while preserving pore structure. The dried samples were then fractured to expose interior surfaces and mounted on aluminium stubs using double-sided carbon adhesive tape. Imaging was performed under high vacuum conditions using an accelerating voltage of 5–10 kV and a working distance of 8–10 mm. Micrographs were captured at magnifications ranging from 250 × to 1000 × to visualize the overall crumb porosity and cell wall integrity. The images were evaluated to assess pore size distribution, gas cell wall thickness, and starch-protein network formation.

2.10. *Instrumental taste evaluation*

The taste profile of bread samples was analysed using an electronic tongue system (TS-5000Z, Intelligent Sensor Technology Inc., Kanagawa, Japan). Prior to analysis, the system was calibrated with standard taste solutions to ensure accurate discrimination of each taste modality.

Bread samples were cut from the central portion of each loaf, including crust. Approximately 20 g of the crumb was homogenised with 40 mL of deionised water using a Thermomix TM6 (Vorwerk, Wuppertal, Germany) for 2 min at 25℃ to obtain a uniform slurry. The homogenate was centrifuged at $4000 \times g$ for 15 min, and the clear supernatant was collected for analysis. The supernatants were placed in sample trays and loaded into the e-tongue equipped with cross-selective lipid membrane sensors designed to mimic human taste perception. The system objectively quantified taste attributes including sweetness, saltiness, sourness, bitterness, umami, and overall taste intensity. All measurements were carried out in triplicates. Between measurements, the sensors were rinsed with especial washing solution and standard solutions and reconditioned in reference solution to prevent carryover effects.

2.11. *Statistical analysis*

Data were analysed using IBM SPSS Statistics using IBM SPSS v.31.0.0.0 (117) (IBM Corp., Armonk, NY, USA). The results are reported as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed to evaluate the effect of flour substitution levels on all measured parameters. When ANOVA indicated significant differences ($p < 0.05$), Tukey's Honestly Significant Difference (HSD) test was applied for pairwise comparisons among means. In addition to analysis of variance (ANOVA), Pearson's correlation analysis was performed to evaluate the relationships among physicochemical, antioxidant, colour, and texture parameters of the bread samples. Correlation coefficients (r) were calculated to determine the strength and direction of associations between variables.

3. Results and discussion

3.1. *Chemical composition of breads*

The macronutrient composition and antioxidant potential of cassava flour (CF), watermelon peel powder (WPP), and nettle leaf powder (NLP) are presented in Table 1. NLP exhibited notably higher protein, dietary fibre, and antioxidant-related parameters (TPC, DPPH·, ABTS$^{\cdot+}$), whereas CF was characterised by high starch content and minimal fat. These differences reflect the intrinsic composition of the raw materials and are consistent with previous reports (Adhikari et al., 2016; Asoka et al., 2022; Kumbaji et al., 2026). As the study focused on gluten-free formulations, comparisons were restricted to gluten-free raw materials rather than gluten-containing wheat flour.

The proximate composition of the formulated breads is shown in Table 2. Moisture and fat contents did not vary significantly among formulations, indicating that substitution with WPP and NLP had limited influence on water retention and lipid composition under the conditions applied. Significant differences ($p < 0.05$) were observed in protein, fibre, and starch contents. The white wheat bread (WWB) showed a high protein content (7.96%), which was statistically comparable ($p > 0.05$) to CWN3 and CWN4 but significantly higher ($p < 0.05$) than the other cassava-based formulations. The increase in protein and dietary fibre in composite breads (CWN1-CWN4) reflects the intrinsic composition of WPP and NLP rather than a processing-induced effect. Similar trends have been reported in fibre-enriched bakery products (Al-Sayed & Ahmed, 2013; Naknaen et al., 2016). Conversely, starch content decreased with increasing substitution levels, primarily due to dilution by non-starch components. While this reduction is expected, it may have important nutritional implications, particularly in relation to starch digestibility and glycaemic response, which are discussed in later sections. Overall, while compositional changes were largely predictable based on ingredient composition, they provide the basis for understanding the functional and health-related properties of the enriched breads.

Table 1
Macro-nutrients and antioxidant capacity of casava, watermelon peel and nettle leaf powders.

Composition	CF	WPP	NLP
Moisture (%)	10.21 ± 0.08^{a}	9.25 ± 0.00^{b}	8.35 ± 0.04^{c}
Protein (%)	1.26 ± 0.66^{c}	5.60 ± 0.0^{b}	28.53 ± 0.11^{a}
Fats (%)	0.07 ± 0.45^{c}	0.40 ± 0.02^{a}	0.23 ± 0.08^{b}
Starch (%)	87.59 ± 1.30^{a}	9.53 ± 0.34^{b}	6.29 ± 0.02^{c}
Soluble fibre (%)	1.27 ± 0.55^{b}	0.43 ± 0.71^{c}	2.40 ± 0.53^{a}
Insoluble fibre (%)	1.06 ± 0.04^{b}	0.58 ± 0.04^{c}	22.30 ± 0.08^{a}
Total dietary fibre (%)	2.43 ± 1.23^{b}	0.40 ± 0.88^{c}	23.43 ± 2.41^{a}
TPC (mg GAE/g)	0.17 ± 0.08^{c}	8.88 ± 0.46^{b}	53.09 ± 0.35^{a}
DPPH· (µmol TE/g)	140.06 ± 0.02^{c}	302.75 ± 0.01^{b}	463.90 ± 0.41^{a}
ABTS$^{\cdot+}$ (µmol TE/g)	194.56 ± 0.02^{c}	265.65 ± 0.08^{b}	386.71 ± 0.55^{a}

CF: cassava flour. WPP: watermelon peel powder, NLP: nettle leaf powder. Results are expressed as mean ± standard deviation ($n = 3$) and means with different superscripts in the same row are significantly ($p < 0.05$) different

3.2. *Total phenolic content and antioxidant activity*

The incorporation of WPP and NLP resulted in significant increases ($p < 0.05$) in total phenolic content (TPC) and antioxidant activity across all composite formulations (Table 1). Unlike proximate composition, these changes reflect functional enhancement, as they indicate the retention and activity of bioactive compounds following thermal processing. The highest TPC was recorded in CWN4 (7.68 mg GAE/g), followed by CWN3 (4.92 mg GAE/g), reflecting the progressive inclusion of nettle leaf powder (NLP) and watermelon peel powder (WPP), both rich in polyphenolic compounds (Table 1). This trend agrees with findings by Gu et al. (2023) and Maietti et al. (2021), who reported significant phenolic enrichment in bakery products enriched with watermelon rind or nettle leaf powders respectively. Similarly, Đurović et al. (2020) observed an increase from 2.90 ± 0.05 mg GAE/g (control wheat bread) to 11.34 ± 0.11 mg GAE/g in nettle-enriched breads. Maietti et al. (2021) reported TPC increases from 372 ± 14 µg GAE/g in white wheat bread to 597 ± 17 µg GAE/g in nettle-enriched bread, while Naknaen et al. (2016) observed elevated phenolics in cookies incorporated with watermelon rind powder, from 2.22 ± 0.36 mg GAE/g (control) to 6.72 ± 0.31 mg GAE/g (30% substitution).

A similar trend was observed for both DPPH· and ABTS$^{\cdot+}$ radical scavenging activities, confirming a dose-dependent enhancement in antioxidant capacity. Between the control samples, WWB exhibited higher DPPH· and ABTS$^{\cdot+}$ values than CFB (Table 3), consistent with previous reports (Shen et al., 2019). In that study, antioxidant activities ranged from 132 to 281 µmol TE/mg (crust) and 2–49 µmol TE/mg (crumb) for DPPH·, and from 420 to 1079 µmol TE/mg (crust) and 66–167 µmol TE/mg (crumb) for ABTS$^{\cdot+}$. In the present study, the composite breads significantly ($p < 0.05$) enhanced scavenging capacities compared to CFB, with CWN4 showing the greatest DPPH· inhibition (573.49 µmol TE/g) and ABTS$^{\cdot+}$ activity (547.69 µmol TE/g). These results suggest that bioactive compounds present in NLP and WPP retained their functional integrity during the baking process. The enrichment effect can be attributed to the abundance of polyphenols, flavonoids, and chlorophyll pigments in nettle leaves (Adhikari et al., 2016; Maietti et al., 2021; Man et al., 2019; Đurović et al., 2020), along with phenolic acids and antioxidant metabolites in watermelon peel (Al-Sayed and Ahmed, 2013; Gu et al., 2023). Similar enhancements in phenolic and antioxidant activity have been documented in nettle-enriched or watermelon rind-enriched bakery systems (Dubey et al., 2021; Maietti et al., 2021; Mohammadian et al., 2024). These findings suggest that phenolic compounds present in WPP and NLP remained stable during baking and contributed effectively to the antioxidant potential of the final product.

The enhanced antioxidant activity can be attributed to the presence of polyphenols, flavonoids, and pigments in nettle leaves, as well as phenolic acids and associated compounds in watermelon peel (Adhikari et al., 2016; Al-Sayed & Ahmed, 2013; Gu et al., 2023; Maietti et al.,

Table 2
Physicochemical characteristics of bread formulations.

Parameters	WWB	CFB	CWN1	CWN2	CWN3	CWN4
Chemical						
Moisture (%)	36.55 ± 2.93^{a}	38.85 ± 1.75^{a}	40.53 ± 2.00^{a}	41.12 ± 2.24^{a}	43.83 ± 5.57^{a}	41.39 ± 0.88^{a}
Protein (%)	7.96 ± 1.02^{a}	1.62 ± 0.08^{c}	1.98 ± 0.12^{c}	3.04 ± 0.04^{b}	5.38 ± 0.46^{a}	6.43 ± 0.88^{a}
Fats (%)	3.34 ± 0.16^{a}	1.30 ± 0.17^{b}	1.14 ± 0.78^{b}	1.24 ± 0.11^{b}	1.23 ± 0.33^{b}	1.14 ± 0.65^{b}
Starch (%)	63.56 ± 0.42^{a}	64.41 ± 0.51^{a}	63.21 ± 1.12^{a}	53.70 ± 0.04^{b}	52.41 ± 0.60^{b}	47.72 ± 0.34^{c}
SDF (%)	0.79 ± 1.14^{a}	0.90 ± 0.22^{a}	1.35 ± 0.08^{a}	1.71 ± 0.34^{a}	2.14 ± 1.42^{a}	2.43 ± 0.38^{a}
IDF (%)	2.43 ± 0.08^{d}	2.54 ± 0.60^{d}	3.67 ± 1.53^{d}	7.34 ± 0.98^{c}	10.25 ± 1.46^{b}	16.74 ± 1.76^{a}
TDF (%)	2.65 ± 0.22^{e}	3.80 ± 1.22^{e}	4.90 ± 1.42^{d}	6.02 ± 1.40^{c}	12.63 ± 1.32^{b}	15.49 ± 0.87^{a}
TPC and antioxidant capacity						
TPC (mg GAE/g)	0.41 ± 0.62^{e}	0.12 ± 0.02^{f}	1.19 ± 0.69^{d}	3.42 ± 0.11^{c}	4.92 ± 0.08^{b}	7.68 ± 1.31^{a}
DPPH· (μmol TE/g)	163.66 ± 0.45^{e}	158.65 ± 0.00^{f}	208.90 ± 0.45^{d}	352.75 ± 0.54^{c}	522.65 ± 0.08^{b}	573.49 ± 0.60^{a}
ABTS·+ (μmol TE/g)	243.66 ± 0.32^{e}	186.41 ± 0.08^{f}	256.45 ± 0.31^{d}	385.90 ± 0.04^{c}	396.05 ± 0.52^{b}	547.69 ± 0.88^{a}
Specific volume (mL/g)	4.41 ± 0.14^{a}	2.37 ± 0.75^{b}	2.24 ± 0.51^{b}	2.23 ± 0.28^{b}	2.19 ± 0.32^{b}	2.41 ± 0.58^{b}
Colour						
L*	83.56 ± 1.15^{a}	68.41 ± 1.98^{b}	37.91 ± 0.62^{c}	22.93 ± 0.46^{d}	21.42 ± 0.50^{d}	15.75 ± 0.88^{e}
a*	2.65 ± 0.48^{a}	1.78 ± 0.80^{b}	-1.69 ± 0.34^{c}	-4.53 ± 0.28^{d}	-4.66 ± 0.65^{d}	-8.47 ± 0.72^{e}
b*	16.18 ± 0.60^{d}	19.62 ± 0.78^{c}	20.21 ± 0.22^{c}	23.43 ± 0.83^{b}	26.01 ± 0.40^{a}	27.84 ± 0.66^{a}
Chroma (C*)	15.55 ± 2.45^{d}	20.50 ± 0.88^{c}	20.52 ± 0.17^{c}	22.63 ± 0.44^{b}	24.22 ± 0.36^{b}	26.66 ± 0.64^{a}
Hue angle (h°)	83.2	82.25	70.30	72.34	71.64	68.43
ΔE	8.64 ± 1.02^{d}	5.71 ± 0.43^{e}	10.82 ± 0.11^{c}	13.28 ± 0.73^{ab}	13.97 ± 0.02^{b}	15.34 ± 0.42^{a}
Texture profile analysis						
Hardness (N)	8.28 ± 2.50^{e}	10.53 ± 3.26^{e}	15.66 ± 2.05^{d}	18.03 ± 0.64^{c}	21.02 ± 1.64^{b}	32.78 ± 0.94^{a}
Adhesiveness (N.s)	0.00187 ± 0.00171^{a}	0.00011 ± 0.00353^{b}	-0.00111 ± 0.00479^{c}	-0.00241 ± 0.00736^{d}	-0.00383 ± 0.00350^{e}	-0.00414 ± 0.00946^{f}
Resilience	29.956 ± 3.49^{a}	13.495 ± 1.68^{c}	25.845 ± 3.34^{a}	19.459 ± 3.49^{b}	13.034 ± 2.29^{c}	13.439 ± 4.20^{c}
Cohesiveness	0.607 ± 0.034^{a}	0.522 ± 0.014^{b}	0.501 ± 0.063^{b}	0.448 ± 0.087^{b}	0.350 ± 0.050^{c}	0.347 ± 0.079^{c}
Springiness	93.403 ± 0.211^{a}	81.955 ± 2.106^{b}	68.778 ± 3.900^{c}	58.281 ± 5.417^{d}	35.922 ± 1.594^{f}	39.193 ± 7.589^{e}
Chewiness (N)	5.80 ± 6.74^{d}	8.05 ± 4.25^{c}	9.89 ± 3.39^{c}	11.41 ± 0.61^{b}	13.41 ± 0.72^{a}	14.62 ± 0.83^{a}

WWB (100% WF: 0% WPP: 0% NLP; gluten control), CFB (100% CF: 0% WPP: 0% NLP; gluten-free control), CWN1 (85% CF: 10% WPP: 5% NLP), CWN2 (70% CF: 20% WPP: 10% NLP), CWN3 (60% CF: 25% WPP: 15% NLP), and CWN4 (50% CF: 30% WPP: 20% NLP), WF: wheat flour, CF: cassava flour. WPP: watermelon peel powder, NLP: nettle leaf powder, means with different superscripts in the same row are significantly ($p < 0.05$) different.

Table 3
AUC, HI and pGI of formulated bread samples.

Formulation	AUC (mg.min/g)	HI (%)	pGI
WWB	$17,456.32 \pm 2.34^{a}$	100.00^{a}	94.45 ± 0.56^{a}
CFB	$13,687.54 \pm 0.88^{b}$	73.21 ± 1.28^{b}	71.54 ± 0.65^{b}
CWN1	$12,986.70 \pm 3.76^{c}$	70.47 ± 0.54^{c}	69.43 ± 0.72^{c}
CWN2	$11,496.56 \pm 4.56^{e}$	68.65 ± 0.81^{d}	64.21 ± 0.84^{d}
CWN3	$11,653.42 \pm 3.21^{d}$	67.90 ± 0.34^{d}	65.39 ± 0.44^{d}
CWN4	$11,107.65 \pm 2.11^{f}$	66.39 ± 1.04^{d}	62.12 ± 0.56^{e}

WWB (100% WF: 0% WPP: 0% NLP; gluten control), CFB (100% CF: 0% WPP: 0% NLP; gluten-free control), CWN1 (85% CF: 10% WPP: 5% NLP), CWN2 (70% CF: 20% WPP: 10% NLP), CWN3 (60% CF: 25% WPP: 15% NLP), and CWN4 (50% CF: 30% WPP: 20% NLP), WF: wheat flour, CF: cassava flour. WPP: watermelon peel powder, NLP: nettle leaf powder, means with different superscripts in the same column are significantly ($p < 0.05$) different.

2021). While similar enrichment trends have been reported in previous studies, the magnitude of improvement observed here highlights the potential of these underutilised materials in developing functional gluten-free bakery products. Importantly, these results demonstrate that the incorporation of WPP and NLP does not merely alter composition but significantly enhances the functional properties of cassava-based bread, particularly in terms of antioxidant capacity. This has direct implications for the development of value-added gluten-free products with potential health benefits.

3.3. *Specific volume and loaf characteristics*

The specific volume of the bread samples is presented in Table 2. The gluten control (WWB) exhibited the highest specific volume (4.41 mL/g), consistent with the viscoelastic gluten network and starch-rich nature of wheat flour that promote optimal gas retention and expansion during proofing and baking (Halake & Chinthapalli, 2020; Rachman et al., 2023). In contrast, the specific volume of the cassava-watermelon-nettle breads (CWN1-CWN4) showed no significant differences ($p > 0.05$) compared with the gluten-free cassava control (CFB), indicating that partial substitution with watermelon peel powder (WPP) and nettle leaf powder (NLP) did not markedly affect gas cell expansion or loaf aeration. Similar observations were reported by Eduardo et al. (2013) and Zhao et al. (2022) who found that moderate incorporation of maize or rice protein flours into gluten-free formulations did not significantly alter loaf volume when hydrocolloids or stabilizers were present. Likewise, Rachman et al. (2023) reported lower specific volume values (1.61–1.86 cm^3/g) in banana-cassava composite breads relative to wheat bread (3.54 cm^3/g).

In this study, cassava was selected as the gluten-free base due to its wide availability, affordability, and neutral taste, making it a suitable staple for gluten-intolerant populations. However, to address the shortcomings of cassava flour including nutritional (low protein, fibre, and bioactive compounds) and functional (crumbling texture, loaf volume) properties, nettle leaf and watermelon peel powders were incorporated as functional ingredients. The specific volume values for gluten-free samples ranged from 2.19 to 2.41 mL/g, suggesting that the structural integrity of cassava-based doughs was maintained across substitution levels. This relative stability may be attributed to the inherent starch content of cassava flour, which contributes to adequate batter viscosity and gas cell stabilization. Although WPP and NLP are rich in fibre and phenolic compounds (see Table 1), components that can impede gas retention, the inclusion levels used were likely below the threshold required to significantly disrupt dough rheology or expansion. Moreover, the combination of these ingredients not only enhances the nutritional and functional quality of cassava-based bread but also supports sustainability through the valorisation of underutilized and agro-industrial by-products.

Representative cross-sectional images of the gluten-free cassava breads are presented in Fig. 1. WWB exhibited a more open and aerated crumb structure, characterised by larger and more uniformly distributed gas cells. In contrast, CFB and CWN1-CWN4 displayed a comparatively denser and more compact crumb matrix with smaller and less uniformly distributed pores. This structural difference is consistent with the

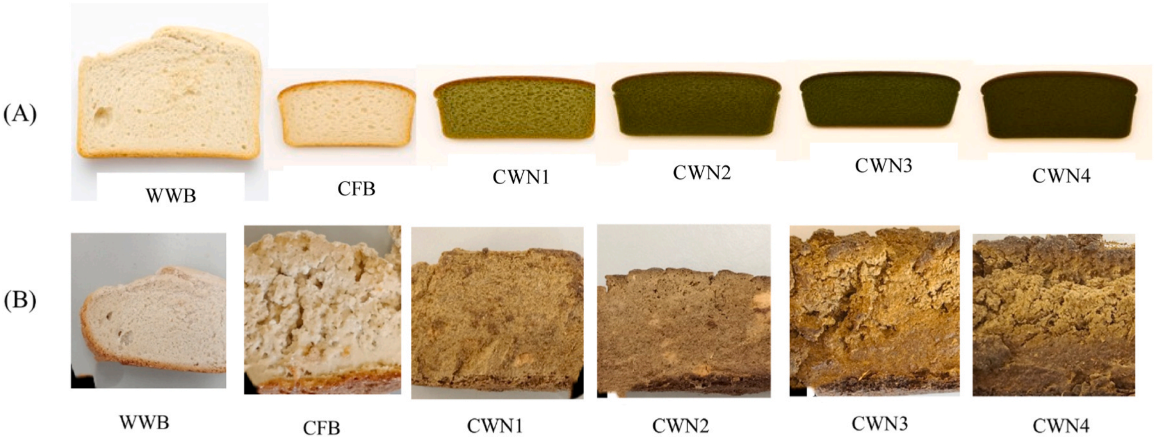

Fig. 1. Representative cross-sectional crumb structure of gluten-free cassava breads: (A) fresh bread crumb; (B) freeze-dried crumb prior to microstructural analysis. Freeze-drying was conducted to preserve structural features for subsequent SEM evaluation. WWB (100% WF: 0% WPP: 0% NLP; gluten control), CFB (100% CF: 0% WPP: 0% NLP; gluten-free control), CWN1 (85% CF: 10% WPP: 5% NLP), CWN2 (70% CF: 20% WPP: 10% NLP), CWN3 (60% CF: 25% WPP: 15% NLP), and CWN4 (50% CF: 30% WPP: 20% NLP), WF: wheat flour, CF: cassava flour. WPP: watermelon peel powder, NLP: nettle leaf powder.

significantly ($p < 0.05$) higher specific volume recorded for CWWB (4.41 mL/g) compared to the composite cassava breads (2.19–2.41 mL/g), which did not differ ($p > 0.05$) significantly among themselves. Although minor visual variations in pore distribution were observed among the composite formulations, the overall crumb compactness remained comparable (Fig. 2), reflecting the statistically similar specific volume values. The denser internal structure of the cassava-based breads corresponds with the higher hardness values obtained in texture profile analysis (Table 2), suggesting reduced gas retention and matrix expansion relative to the control formulation. Also, its darker crumb colour is likely attributable to the intrinsic pigmentation of nettle leaf and watermelon peel powders, as well as enhanced browning reactions during baking.

SEM micrographs (Fig. 2) revealed comparable microstructural features in WWB, CFB, CWN1 to CWN4. All samples exhibited a relatively continuous matrix with well-dispersed gas cells and partially gelatinized starch domains; no marked differences in pore size distribution, cell wall thickness, or granule fragmentation were evident at the magnifications examined. Visible porosity and apparent cell-wall integrity appeared similar across these samples, supporting the outcome of the specific volume analysis where no significant differences in loaf expansion were observed. The absence of notable microstructural divergence suggests that the substitution levels ($\leq$30% combined WPP + NLP in CWN1-CWN4) did not disrupt starch gelatinization or the formation of a cohesive crumb matrix under the applied baking conditions. Possible explanations include 1) the relatively low substitution levels being insufficient to exceed the threshold at which fibres and phenolics perturb matrix continuity, 2) effective action of the gluten-free processing aids, such as hydrocolloid/stabilizer, and cassava starch properties that maintained batter viscosity and gas-cell stabilization, and 3) thermal processing promoting comparable gelatinization across formulations, so starch-polyphenol interactions remained limited in their impact on gross microstructure. Comparable microstructural characteristics were reported by Zhao et al. (2022) in gluten-free breads fortified with rice protein flour, were moderate fibre inclusion enhanced matrix compactness without severely compromising loaf expansion. Functionally, these SEM findings corroborate the texture and specific volume data: samples with similar microstructure displayed comparable loaf volumes and TPA parameters (especially for CWN1-CWN2 vs CFB and WWB, see Table 3). From a product-development perspective, this indicates that moderate incorporation of WPP and NLP into gluten-free formulations can improve nutritional and antioxidant attributes while preserving microstructure and macro-textural quality.

3.4. Colour

The addition of nettle leaf powder (NLP) and watermelon peel powder (WPP) significantly ($p < 0.05$) altered both crumb and crust colour of the cassava-based gluten-free breads (Table 2). The control breads (WWB and CFB) exhibited pale, nearly white crumbs and light golden crusts, characteristic of refined wheat and cassava flours. With increasing substitution levels of NLP and WPP (CWN1-CWN4), a progressive reduction in lightness (L*) was observed, from 37.91 to 15.75, indicating a darker overall appearance. The a* and b* coordinates also reflected compositional effects, showing lower a* values (shift toward green tones) and slightly reduced b* values (less yellow intensity) at higher substitution levels. These trends correspond to the elevated polyphenol and antioxidant contents observed in the enriched breads (Table 2).

Also, the colour modifications can be attributed to the intrinsic pigments of the added flours. WPP contributed subtle yellow-orange hues due to carotenoids and natural flavonoid pigments, while NLP imparted a dark green tone from chlorophyll and phenolic constituents. The combined effect of these bioactive compounds likely produced the characteristic greenish-brown hue of enriched samples. Additionally, polyphenols and carotenoids from both sources may have participated in non-enzymatic browning reactions (Maillard and caramelization) during baking, further intensifying crust pigmentation. Comparable findings have been reported in breads fortified with nettle powder (Maietti et al., 2021; Đurović et al., 2020) and watermelon rind powder (Al-Sayed & Ahmed, 2013; Naknaen et al., 2016). To further interpret colour variation among samples, chroma (C*), hue angle (h°), and total colour difference (ΔE) were calculated from L*, a*, and b* values. Chroma, representing colour saturation or vividness, decreased with higher levels of NLP and WPP, indicating less intense but more natural pigmentation due to the blending of chlorophyll (green) and phenolic-derived brown pigments. Correspondingly, hue angle values shifted from 83.0° (WWB) and 82.3° (CFB) toward lower values (71.6–68.4°) in CWN3 and CWN4, reflecting a perceptible shift toward greenish-brown tones.

The total colour difference (ΔE) between the control and composite breads exceeded 3.0 in all cases, indicating visually perceptible colour differences to the human eye. These findings confirm that substitution with nettle and watermelon peel flours significantly modified bread pigmentation, consistent with the increased total phenolic and antioxidant contents. Similar trends in hue and chroma have been observed in other chlorophyll- or fruit by-product-enriched bakery products (Maietti et al., 2021; Naknaen et al., 2016; Wójcik et al., 2021), supporting the

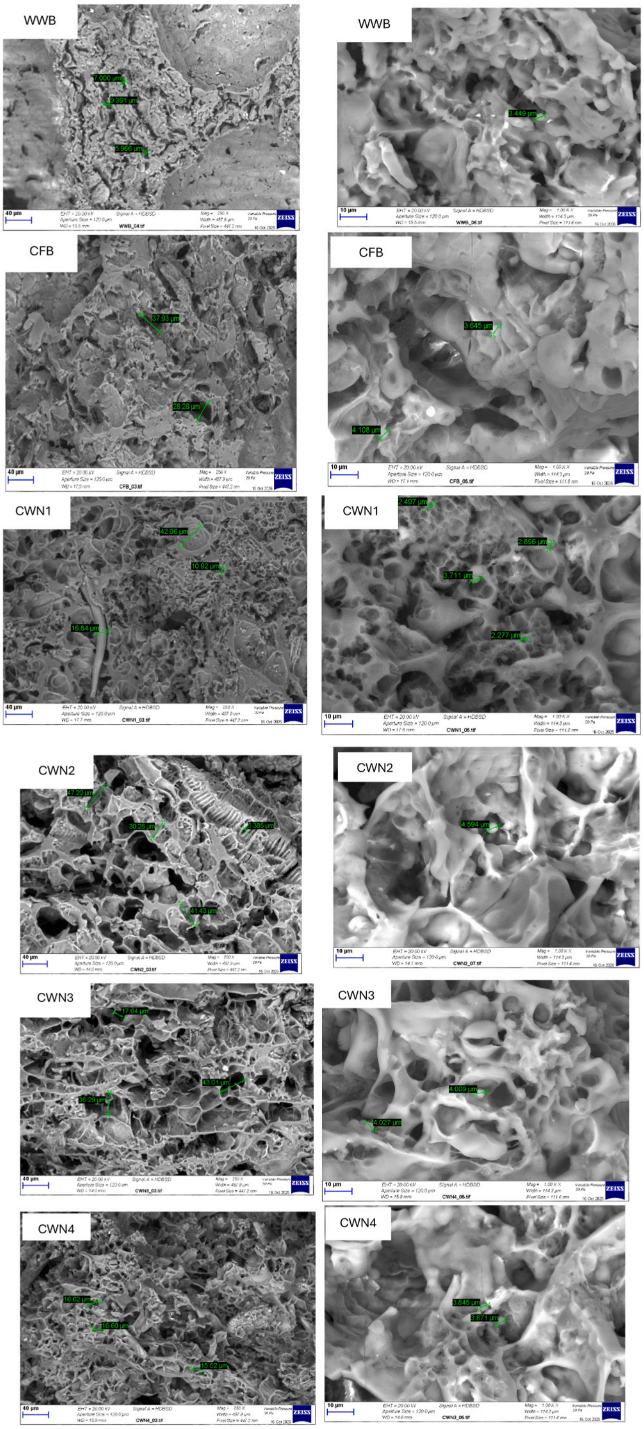

Fig. 2. SEM micrographs of bread crumb at 250 × and 1000 × magnification. WWB (100% WF: 0% WPP: 0% NLP; gluten control), CFB (100% CF: 0% WPP: 0% NLP; gluten-free control), CWN1 (85% CF: 10% WPP: 5% NLP), CWN2 (70% CF: 20% WPP: 10% NLP), CWN3 (60% CF: 25% WPP: 15% NLP), and CWN4 (50% CF: 30% WPP: 20% NLP), WF: wheat flour, CF: cassava flour. WPP: watermelon peel powder, NLP: nettle leaf powder.

role of plant-based functional food ingredients in enhancing both nutritional and aesthetic properties of gluten-free breads.

3.5. *Texture of formulated breads*

The texture profile parameters of the gluten-free cassava breads were significantly affected ($p < 0.05$) by the incorporation of watermelon peel powder (WPP) and nettle leaf powder (NLP) (Table 2). Hardness values increased significantly ($p < 0.05$) with increasing substitution of NLP and WPP. WWB exhibited the lowest hardness (8.28 N), followed by the cassava control bread (CFB) (10.53 N). The progressive incorporation of plant powders resulted in a marked increase in crumb firmness, with CWN4 showing the highest hardness value (32.78 N). The increase in hardness may be attributed to the high dietary fibre content of NLP and WPP, which can interfere with gas cell expansion and weaken the continuous starch matrix in gluten-free systems. Fibre particles likely disrupted the crumb structure and reduced moisture mobility, resulting in a denser and more compact internal matrix. Additionally, the absence of gluten in cassava-based formulations limits network elasticity, further contributing to increased firmness at higher substitution levels. Conversely, the wheat control bread (WWB) exhibited the lowest hardness (8.28 ± 2.50 N), consistent with its gluten network and superior gas retention capacity (Eduardo et al., 2013; Rai et al., 2018).

Chewiness followed similar trends to hardness, although no significant ($p > 0.05$) differences were observed between CWN3 and CWN4, reflecting the denser matrix and reduced aeration of the composite loaves. In contrast, adhesiveness increased slightly in the enriched breads (Table 2), possibly due to the higher soluble fibre fractions from WPP and NLP, which enhance crumb stickiness and moisture retention. Cohesiveness and springiness showed slight but consistent reductions with increasing WPP and NLP substitution, suggesting that the absence of gluten reduced internal elasticity and recovery after compression. Nonetheless, the moderate substitution levels (CWN1-CWN2) retained acceptable resilience and chewiness, indicating that limited incorporation of these flours can enhance nutritional value while maintaining desirable textural integrity. Comparable patterns have been observed in gluten-free breads fortified with fruit and vegetable by-products, where fibre- and phenolic-rich components modified dough rheology and crumb structure without severely impairing palatability (Acun et al., 2025; Eduardo et al., 2013; Naknaen et al., 2016; Rai et al., 2018). Overall, these results suggest that the incorporation of watermelon peel and nettle leaf powders enhances the nutritional and functional profile of cassava-based breads, though higher substitution levels may slightly compromise softness and elasticity. Optimisation of fibre ratios or hydrocolloid supplementation may further improve the texture of such composite gluten-free systems.

3.6. *In vitro starch hydrolysis and predicted glycaemic index*

Throughout the digestion period, white wheat bread (WWB) exhibited the highest rate of starch hydrolysis and reducing sugar release (Fig. 3). Cassava bread (CFB) demonstrated a moderate reduction in glycaemic response, releasing 179.5 mg/g of reducing sugars and yielding an area under the curve (AUC) of 13,687.54 mg·min/g. The

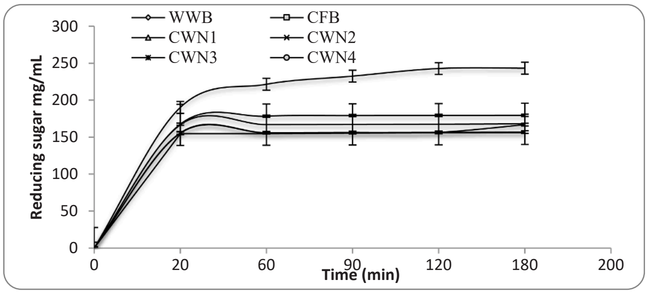

Fig. 3. Reducing sugar release of formulated bread samples. WWB (100% WF: 0% WPP: 0% NLP; gluten control), CFB (100% CF: 0% WPP: 0% NLP; gluten-free control), CWN1 (85% CF: 10% WPP: 5% NLP), CWN2 (70% CF: 20% WPP: 10% NLP), CWN3 (60% CF: 25% WPP: 15% NLP), and CWN4 (50% CF: 30% WPP: 20% NLP), WF: wheat flour, CF: cassava flour. WPP: watermelon peel powder, NLP: nettle leaf powder.

hydrolysis index (HI) was 73.21, corresponding to a predicted glycaemic index (pGI), a dimensionless parameter, of 71.54 (Table 3). In contrast, the incorporation of watermelon peel powder (WPP) and nettle leaf powder (NLP) (CWN1-CWN4) resulted in a progressive reduction in glycaemic potential. Reducing sugar release decreased from 168 mg/g in CWN1–156 mg/g in CWN4, while AUC values declined from 12,986.70 to 11,107.65 mg·min/g. The corresponding HI and pGI values were lowest in CWN4 (66.39 and 62.12, respectively).

According to the glycaemic index classification (Atkinson et al., 2021), WWB falls within the high-GI range (pGI $\geq$ 70), while CFB and CWN4 are classified as medium-GI foods (pGI = 56–69). The observed decline in HI and pGI with increasing WPP and NLP substitution suggests that these flours can effectively lower the glycaemic potential of cassava-based breads. This effect may be attributed to their high dietary fibre and polyphenol contents, which are known to impede enzymatic hydrolysis of starch and slow glucose diffusion during digestion (Englyst et al., 1992; Goñi et al., 1997; Gu et al., 2023). The reduction also aligns with the lower starch content and higher fibre levels observed in the composite flours, supporting a matrix-driven attenuation of starch bioavailability.

Similar trends have been reported for bakery products enriched with polyphenol-rich or fibre-dense plant materials, which exhibited reduced starch digestibility and glycaemic response (Acun et al., 2025; Goñi et al., 1997; Pasqualoni et al., 2024; Tanyitiku et al., 2024). For example, Tanyitiku et al. (2024) reported a decreased ($p < 0$ 05) of predicted glycaemic index (GI) (pGI) from 48.60% (100% corn control cookies) to 33.18% (20% stinging nettle incorporated cookies). Additionally, several in vitro and in vivo studies have reported the hypoglycemic activity of nettles suggesting it decreases the intestinal absorption of glucose as well as inhibits key carbohydrate digestive enzymes, including α-amylase and α-glucosidase (Ziaei et al., 2020). These findings suggest that WPP and NLP incorporation not only improves the nutritional and functional attributes of cassava bread but may also promote better glycaemic control, an important consideration for individuals with celiac disease or those managing blood glucose levels (NIH, 2020; Rai et al., 2018). The composite breads therefore hold promise as functional gluten-free foods that combine enhanced nutritional quality with moderated glycaemic response.

White wheat bread (WWB) exhibited the highest rate of starch hydrolysis and reducing sugar release throughout the digestion period (Fig. 3). The cassava control bread (CFB) showed a moderate reduction in glycaemic response, with a reducing sugar release of 179.5 mg/g and an area under the curve (AUC) of 13,687.54 mg·min/g. This corresponded to a hydrolysis index (HI) of 73.21 and a predicted glycaemic index (pGI, dimensionless) of 71.54 (Table 3). Incorporation of watermelon peel powder (WPP) and nettle leaf powder (NLP) (CWN1–CWN4) resulted in a progressive reduction in starch hydrolysis. Reducing sugar release decreased from 168 mg/g (CWN1) to 156 mg/g (CWN4), while AUC values declined from 12,986.70 to 11,107.65 mg·min/g. The lowest HI and pGI values were observed in CWN4 (66.39 and 62.12, respectively), indicating a clear attenuation of glycaemic potential with increasing substitution level.

According to glycaemic index classification (Atkinson et al., 2021), WWB falls within the high-GI range (pGI $\geq$ 70), whereas both CFB and the composite formulations are classified as medium-GI foods (pGI = 56–69). The reduction in HI and pGI observed in the composite breads represents a key functional outcome of this study, indicating improved starch digestibility characteristics relative to the control formulations. This effect can be attributed to the presence of dietary fibre and polyphenolic compounds in WPP and NLP, which are known to limit enzymatic access to starch, reduce diffusion of hydrolysis products, and potentially inhibit digestive enzymes (Englyst et al., 1992; Goñi et al., 1997; Gu et al., 2023). Additionally, the partial replacement of starch-rich cassava flour with fibre-rich materials contributes to a dilution of readily digestible carbohydrates, further reducing starch hydrolysis. Similar reductions in starch digestibility have been reported in bakery products enriched with fibre- and polyphenol-rich ingredients (Acun et al., 2025; Pasqualoni et al., 2024; Tanyitiku et al., 2024). For instance, Tanyitiku et al. (2024) reported a significant decrease in predicted glycaemic index from 48.60 to 33.18 with the incorporation of stinging nettle in cookies. Moreover, bioactive compounds present in nettle have been associated with inhibition of key carbohydrate-digesting enzymes, including α-amylase and α-glucosidase (Ziaei et al., 2020). Overall, these findings demonstrate that incorporation of WPP and NLP can effectively reduce the glycaemic potential of cassava-based gluten-free bread. This highlights their potential application in the development of functional gluten-free products with improved metabolic relevance, particularly for individuals requiring moderated postprandial glucose responses.

3.7. *Taste evaluation using electronic tongue*

The radar plot (Fig. 4) illustrates the electronic tongue responses across five primary taste attributes, bitterness, umami, sourness, saltiness, and aftertaste, for the various bread samples. Significant differences ($p < 0.05$) were detected among the formulations, indicating that incorporation of nettle leaf and watermelon peel powders (WPP and NLP) notably influenced the taste profile of the cassava-based breads. Cassava formulations exhibited higher sourness (CAO values ranging from −10.2 in CFB to −25.5 in CWN4) compared to the wheat control (3.4). CWN4 also recorded the highest intensities of bitterness (17.1), astringency/aftertaste (5.8), and umami (14.5). These increases are consistent with previous reports on functional breads fortified with nettle or other green plant powders (Maietti et al., 2021; Tanyitiku & Njombissie Petcheu, 2025; Wójcik et al., 2021). Tanyitiku and Njombissie Petcheu (2025) similarly observed enhanced bitterness (7.43) and aftertaste (7.63) in nettle-incorporated rice waffles, attributing these to organic acids and phenolic compounds inherent in nettle leaves. Such bioactive constituents are known to impart mild acidity, astringency, and complex mouthfeel in plant-based bakery matrices (Kohajdova et al., 2018).

Moderate substitution levels (CWN1-CWN2) yielded the most harmonious sensory balance, combining subtle umami enhancement with minimal bitterness, while higher substitutions introduced more pronounced plant-derived flavour notes. Despite the elevated sourness and bitterness observed at higher substitution levels, moderate incorporation (CWN1 and CWN2) produced relatively symmetrical radar plots, indicative of balanced sensory profiles. In contrast, CWN3 and CWN4 displayed more irregular shapes, reflecting intensified bitterness and aftertaste due to greater concentrations of polyphenols and chlorophyll pigments. These results suggest that the inclusion of WPP and NLP modulates the flavour complexity of cassava breads in a concentration-dependent manner. Overall, the radar plot profiles confirm that watermelon peel and nettle leaf powders can enrich the multidimensional flavour characteristics of gluten-free cassava breads, potentially appealing to niche consumer groups seeking health-oriented, functional bakery products with distinctive sensory identities.

3.8. *Correlation analysis*

The relationships among key chemical, and physical parameters of the formulated breads were examined using Pearson's correlation coefficients (Table 4). Strong positive correlations were observed between total phenolic content (TPC), antioxidant activities (DPPH· and ABTS·$^{+}$), and dietary fibre (r = 0.812–0.950, $p < 0.05$), confirming that the incorporation of nettle leaf and watermelon peel powders enhanced both nutritional and functional quality. Protein content also showed a moderate positive correlation with TPC (r = 0.642), reflecting the nutrient-dense nature of nettle powder.

Conversely, specific volume correlated negatively with fibre (r = -0.653 for IDF and r = -0.742 for SDF) and TPC (r = -0.727), indicating that higher levels of fibrous and phenolic compounds may have

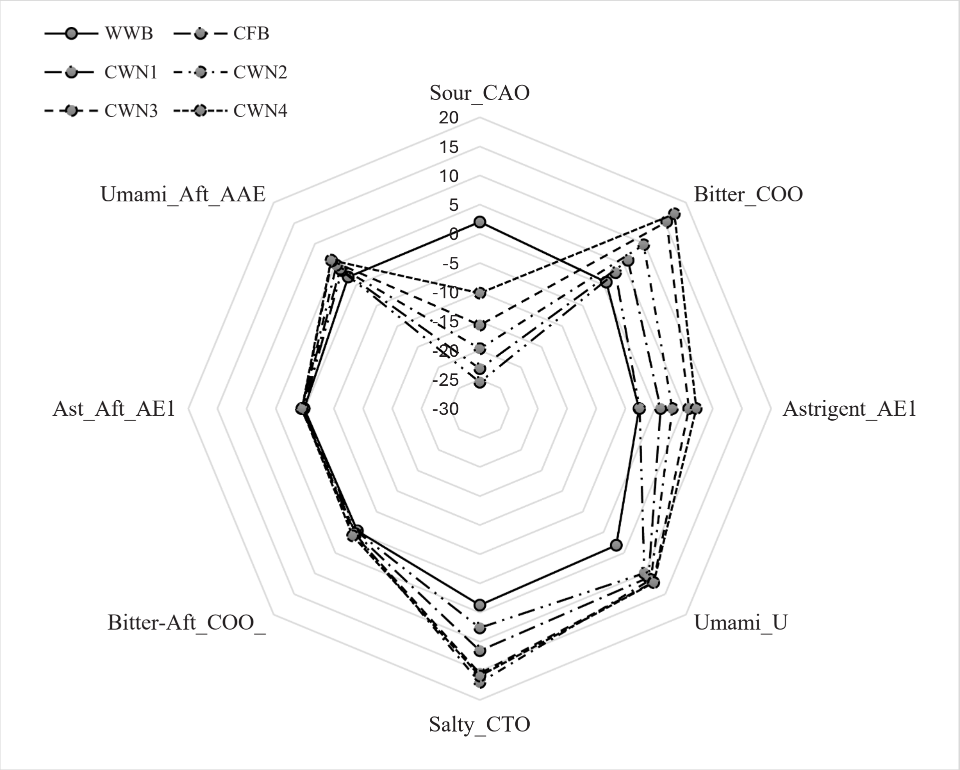

Fig. 4. Electronic tongue analysis of the formulated bread samples. WWB (100% WF: 0% WPP: 0% NLP; gluten control), CFB (100% CF: 0% WPP: 0% NLP; gluten-free control), CWN1 (85% CF: 10% WPP: 5% NLP), CWN2 (70% CF: 20% WPP: 10% NLP), CWN3 (60% CF: 25% WPP: 15% NLP), and CWN4 (50% CF: 30% WPP: 20% NLP), WF: wheat flour, CF: cassava flour. WPP: watermelon peel powder, NLP: nettle leaf powder.

interfered with gas retention and loaf expansion. Similarly, bread hardness exhibited a positive association with fibre ($r = 0.854$ for SDF and $r = 0.632$ for IDF), whereas springiness and cohesiveness showed inverse relationships with phenolic content ($r =$ -0.687 and -0.653, respectively). These trends are consistent with observations by Acun et al. (2025), who reported that fibre- and antioxidant-rich fortification tends to improve nutritional properties but can adversely affect crumb softness and elasticity. A significant negative correlation ($p < 0.05$) was also observed between TPC and both hydrolysis index (HI) and predicted glycaemic index (pGI), suggesting that phenolic compounds may interact with starch or digestive enzymes to retard hydrolysis and glucose release. Likewise, total dietary fibre correlated inversely with reducing sugar release, supporting the hypothesis that fibre creates a physical barrier limiting enzymatic access to starch. Similar interactions between fibre, phenolics, and starch digestibility have been reported by Gu et al. (2023) and Pasqualoni et al. (2024).

Overall, the correlation matrix underscores a distinct trade-off between nutritional enrichment and textural performance, highlighting the need to optimize the substitution levels of watermelon peel and nettle leaf powders to achieve a desirable balance between functionality, sensory acceptability, and health-promoting properties in gluten-free cassava bread formulations. Nonetheless, the use of nettle leaf and watermelon peel powders, particularly the valorisation of watermelon peel as an underutilised agri-food by-product, highlights the sustainability potential of the formulations by reducing food waste and contributing to more circular and resource-efficient food systems.

4. Conclusion

This study demonstrated that partial substitution of cassava flour with nettle leaf powder (NLP) and watermelon peel powder (WPP) modifies the compositional and functional properties of gluten-free bread. The incorporation of these plant-based ingredients led to expected increases in protein, dietary fibre, and phenolic content, resulting in significantly enhanced antioxidant activity (DPPH· and ABTS$\cdot_{+}$). Importantly, the composite formulations exhibited reduced in vitro starch hydrolysis and lower predicted glycaemic index values, indicating improved starch digestibility characteristics and potential for glycaemic moderation. These findings highlight the functional relevance of NLP and WPP in developing gluten-free products with enhanced health-related properties. However, the inclusion of these materials did not improve key physical attributes, as no significant changes in loaf specific volume were observed and crumb hardness increased with higher substitution levels. Colour and electronic tongue analyses further indicated notable changes in visual and taste profiles, reflecting the presence of plant-derived pigments and bioactive compounds. Overall, while physical bread quality was not enhanced, the incorporation of NLP and WPP offers a promising strategy for producing gluten-free bread with improved antioxidant capacity and moderated glycaemic response. Future work should focus on formulation optimisation, particularly through hydrocolloid systems, as well as sensory validation and shelf-life assessment to support industrial application.

CRediT authorship contribution statement

Mary Nkongho Tanyitiku: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Luca Serventi:** Writing – review & editing, Writing – original draft, Validation, Methodology, Data curation, Conceptualization. **Rania Harastani:** Writing – review & editing, Validation, Methodology, Conceptualization. **Ganiyat Olayinka Olatunde:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Tafadzwa Mkungunugwa:** Writing – review & editing, Writing – original draft, Validation, Investigation, Data curation, Conceptualization. **Vahid Baeghbali:** Writing – review & editing,

Table 4
Pearson's correlation between observed values.

	AUC	HI	SV	Moist	Pro	Fats	Starch	IDF	SDF	TPC	DPPH	ABTS	Hard	Adh	Coh	Res	Spri	Chew
pGI	0.436	-0.843**	-0.319	0.876	-0.665**	-0.720**	0.653*	0.321	-0.453	-0.927*	0.760	0.212	0.654	0.576	0.786	-0.765	0.653	0.876
HI	0.567		0.87**	-0.778**	-0.567	0.321	-0.654	0.765	0.210	-0.854*	0.532	0.542	0.542	0.186	-0.645	0.596	-0.734	0.764
SV	0.321			-0.754**	-0.890*	0.653	0.986	-0.653	-0.742	-0.727	0.139	0.286	-0.765*	0.156	0.231	0.584	0.435	-0.755
Mois	0.342				-0.713*	-0.552*	0.87**	-0.465	0.572	0.675	-0.532	0.873*	0.879**	0.287**	0.756*	0.563	0.213	0.845
Prot	-0.848*					-0.685*	0.129	0.463	0.684	0.642	0.674	0.778	-0.685	0.564	0.321	-0.543	0.574	0.673
Fats	-0.324						0.432	0.954	0.543	0.674	0.674	-0.532	0.423	-0.321	0.586	0.646	-0.190	0.643
Star	-0.321							0.211	-0.612	0.634	0.723*	0.432	0.843**	0.543	-0.572	0.127	-0.432	0.548**
IDF	-0.633								0.890	0.741	0.874	0.912	0.735*	0.576	0.732	0.856	0.463	0.5740
SDF	0.342									0.876	0.743	0.776	0.854	0.471	0.213	0.543	0.214	-0.454
TPC	-0.932										0.950	0.931	0.632	0.719	-0.653	0.456	-0.687	-0.793
DPPH	-0.845											0.812	-0.696	0.837	-0.756	-0.730	-0.654	0.232
ABTS	-0.454												0.876	0.734	0.645*	-0.875	0.154	0.645
Hard	0.432													0.438	0.563	0.736	-0.342	0.721
Adh	0.367														0.547	0.856	0.324	0.456
Coh	0.218															0.813	0.435	-0.845*
Res	0.871**																-0.754	0.867
Spri	0.472																	-0.661*
Chew	-0.818	-0.563*	-0.196*	0.275	-0.414*	-0.208	0.522	-0.791*	0.266*	-0.407	-0.474	-0.652	-0.341*	-0.252*	-0.462	0.194*	0.571*	1

SV: Specific volume, Moist: Moisture, Prot: Protein, Star: Starch, IDF: Insoluble dietary fibre, SDF: Soluble dietary fibre, Hard: Hardness, Adh: Adhesiveness, Coh: Cohesiveness, Res: Resilience, Spri: Springiness, Spri: Springiness, Chew: Chewiness. *Significant at $p < 0.05$. **, significant at $p < 0.01$.

Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Srivathsa Kumbaji:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

All authors confirm that this article's contents have no conflict of interest

Acknowledgements

This work was supported by the Postharvest Innovations Research Group, Centre for Food Systems Research (CFSR), Natural Resources Institute (NRI), University of Greenwich, through internal funding awarded to the corresponding author.

References

AACC Method 10-05.01 (2010). Guidelines for measurement of volume by rapeseed displacement.

Acun, S., Gül, H., & Seyrekoğlu, F. (2025). Comprehensive analysis of physical, chemical, and phenolic acid properties of powders derived from watermelon (Crimson Sweet) by-products. *Journal of Food Science, 90*(3), Article e70092. https://doi.org/10.1111/1750-3841.70092

Adhikari, B. M., Bajracharya, A., & Shrestha, A. K. (2016). Comparison of nutritional properties of stinging nettle (*Urtica dioica*) flour with wheat and barley flours. *Food Science & Nutrition, 4*(1), 119–124. https://doi.org/10.1002/fsn3.259

Al-Sayed, H. M. A., & Ahmed, A. R. (2013). Utilization of watermelon rinds and sharlyn melon peels as a natural source of dietary fiber and antioxidants in cake. *Annals of Agricultural Sciences, 58*(1), 83–95. https://doi.org/10.1016/j.aoas.2013.01.012

Aljada, B., Zohni, A., & El-Matary, W. (2021). The gluten-free diet for celiac disease and beyond. *Nutrients, 13*(11). https://doi.org/10.3390/nu13113993

Asoka, S., Shamshad Begum, S., & Vijayalaxmi, K. G. (2022). Physico-chemical properties and nutritional composition of watermelon (*Citrullus lanatus*) and its rind flour. *Biological Forum - An International Journal, 14*, 505–510.

Atkinson, F. S., Brand-Miller, J. C., Foster-Powell, K., Buyken, A. E., & Goletzke, J. (2021). International tables of glycemic index and glycemic load values 2021: a systematic review. *The American Journal of Clinical Nutrition, 114*(5), 1625–1632. https://doi.org/10.1093/ajcn/nqab233

Dubey, S., Rajput, H., & Batta, K. (2021). Utilization of watermelon rind (*Citrullus lanatus*) in various food preparations: A review. *Journal of Agriculture, Science Food Research, 14*(1). https://doi.org/10.35248/2593-9173.23.14.141

Đurović, S., Vujanović, M., Radojković, M., Filipović, J., Filipović, V., Gašić, U., & Zeković, Z. (2020). The functional food production: Application of stinging nettle leaves and its extracts in the baking of a bread. *Food Chemistry, 312*, Article 126091. https://doi.org/10.1016/j.foodchem.2019.126091

Eduardo, M., Svanberg, U., Oliveira, J., & Ahrné, L. (2013). Effect of cassava flour characteristics on properties of cassava-wheat-maize composite bread types. *International Journal of Food Science, 2013*, Article 305407. https://doi.org/10.1155/2013/305407

Englyst, H. N., Kingman, S. M., & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition, 46*, S33–50.

Goñi, I., Garcia-Alonso, A., & Saura-Calixto, F. (1997). A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research, 17*(3), 427–437. https://doi.org/10.1016/S0271-5317(97)00010-9

Gu, I., Balogun, O., Brownmiller, C., Kang, H. W., & Lee, S.-O. (2023). Bioavailability of citrulline in watermelon flesh, rind, and skin using a human intestinal epithelial caco-2 cell model. *Applied Sciences, 13*(8). https://doi.org/10.3390/app13084882

Halake, N., & Chinthapalli, B. (2020). Fermentation of traditional african cassava based foods: microorganisms role in nutritional and safety value. *Journal of Experimental Agriculture International*, 56–65. https://doi.org/10.9734/jeai/2020/v42i930587

Kataria, D., & Kaur, J. (2023). From waste to wellness: exploring the nutritional composition, health benefits and utilization of watermelon rind. *Journal of Food Chemistry and. Nanotechnology, 9*(S1), S478–S482. https://doi.org/10.17756/jfcn.2023-s1-060

Kohajdova, V. J., Karovicova, M., Laukova, & Laukova, M. (2018). Physical, textural and sensory properties of cookies in-corporated with grape skin and seed preparations. *Polish Journal of Food and Nutrition Sciences, 68*(4), 309–317. https://doi.org/10.2478/pjfns-2018-0004

Kumbaji, S., Baeghbali, V., Mkungunugwa, T., Olatunde, G., Harastani, R., Serventi, L., & Tanyitiku, M. N. (2026). Physicochemical and glycaemic characteristics of gluten-free cassava bread enriched with nettle leaf and watermelon peel flours. *Preprint)*. https://doi.org/10.2139/ssrn.6018256

Leffler, T. P., Moser, C. R., McManus, B. J., Urh, J. J., Keeton, J. T., Claflin, A., Adkins, K., Claflin, A., Davis, C., Elliot, J., Goin, P., Horn, C., Humphries, J., Ketteler, K., Perez, P., & Steiner, G. (2008). Determination of moisture and fat in meats by microwave and nuclear magnetic resonance analysis: Collaborative Study. *Journal of AOAC International, 91*(4), 802–810. https://doi.org/10.1093/jaoac/91.4.802

Lohvina, H., Sándor, M., & Wink, M. (2022). Effect of ethanol solvents on total phenolic content and antioxidant properties of seed extracts of fenugreek (*Trigonella foenum-graecum* L.) Varieties and determination of phenolic composition by HPLC-ESI-MS. *Diversity, 14*(1). https://doi.org/10.3390/d14010007

Maietti, A., Tedeschi, P., Catani, M., Stevanin, C., Pasti, L., Cavazzini, A., & Marchetti, N. (2021). Nutrient composition and antioxidant performances of bread-making products enriched with stinging nettle (*Urtica dioica*) leaves. *Foods, 10*(5). https://doi.org/10.3390/foods10050938

Man, S. M., Păucean, A., Chiș, M. S., Muste, S., Pop, A., Mureșan, A., & Marțiș, G. (2019). Effect of nettle leaves powder (*Urtica dioica* L.) addition on the quality of bread. *Hopita- and Medicinal Plants, 27*.

Mohammadian, M., Biregani, Z., Hassanloofard, Z., & Salami, M. (2024). Nettle (*Urtica dioica* L.) as a functional bioactive food ingredient: Applications in food products and edible films, characterization, and encapsulation systems. *Trends in Food Science & Technology, 147*. https://doi.org/10.1016/j.tifs.2024.104421

Naknaen, P., Itthisoponkul, T., Sondee, A., & Angsombat, N. (2016). Utilization of watermelon rind waste as a potential source of dietary fiber to improve health promoting properties and reduce glycemic index for cookie making. *Food Science & Biotechnology, 25*(2), 415–424. https://doi.org/10.1007/s10068-016-0057-z

NIH. (2020). *National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Disease (NIDDK)*. U.S. Department of Health and Human Services. 〈https://www.niddk.nih.gov/health-information/digestive-diseases/celiac-disease〉 (Available at).

Olawoye, B., Gbadamosi, S. O., Otemuyiwa, I. O., & Akanbi, C. T. (2020). Gluten-free cookies with low glycemic index and glycemic load: optimization of the process variables via response surface methodology and artificial neural network. *Heliyon, 6*(10), Article e05117. https://doi.org/10.1016/j.heliyon.2020.e05117

Pasqualoni, I., Tolve, R., Simonato, B., & Bianchi, F. (2024). The impact of selected ingredients on the predicted glycemic index and technological properties of bread. *Foods, 13*(16). https://doi.org/10.3390/foods13162488

Rachman, A., kie, S., Brennan, M., & Brennan, C. (2023). Effect of cassava-banana flour composition and soy protein isolate addition on bread quality and antioxidant properties of gluten-free bread. *IOP Conference Series: Earth and Environmental Science, 1246*, Article 012051. https://doi.org/10.1088/1755-1315/1246/1/012051

Rai, S., Kaur, A., & Chopra, C. S. (2018). Gluten-free products for celiac susceptible people. *Frontiers in Nutrition, 5*.

Shen, Y., Tebben, L., Chen, G., & Li, Y. (2019). Effect of amino acids on maillard reaction product formation and total antioxidant capacity in white pan bread. *International Journal of Food Science and Technology, 54*(4), 1372–1380. https://doi.org/10.1111/ijfs.14027

Sigüenza-Andrés, T., Gallego, C., & Gómez, M. (2021). Can cassava improve the quality of gluten free breads? *LWT - Food Science and Technology, 149*, Article 111923. https://doi.org/10.1016/j.lwt.2021.111923

Tanyitiku, M. N. (2024). Microbiological contamination of fermented cassava products sold in local markets, Yaounde (Cameroon). *African Journal of Agriculture and Food Science, 7*, 1–14. https://doi.org/10.52589/AJAFS-DPAMM68W

Tanyitiku, M. N., & Njombissie Petcheu, I. C. (2025). Technofunctional properties of stinging nettle (*Urtica dioica* L.) leaf flour and its enhancing pasting, physical and sensory characteristics in gluten-free rice waffles. *Journal of Food Quality, 2025*. https://doi.org/10.1155/jfq/9418554

Tanyitiku, M. N., Bessem, P., & Njombissie Petcheu, I. C. (2024). Gluten-free corn cookies incorporated with stinging nettle leaf flour: Effect on physical properties, storage stability, and health benefits. *International Journal of Food Science, 2024*, 1–13. https://doi.org/10.1155/2024/8864560

Wójcik, M., Różyło, R., Łysiak, G., Kulig, R., & Cacak-Pietrzak, G. (2021). Textural and sensory properties of wheat bread fortified with nettle (*Urtica dioica* L.) produced by the scalded flour method. *Journal of Food Processing and Preservation, 45*. https://doi.org/10.1111/jfpp.15851

Yu, L., Nanguet, A.-L., & Beta, T. (2013). Comparison of antioxidant properties of refined and whole wheat flour and bread. *Antioxidants, 2*(4), 370–383. https://doi.org/10.3390/antiox2040370

Ze, N. N., Engama, M.-J. M., & Ngang, J. J. E. (2021). New retting method of cassava roots improve sensory attributes of Bobolo and Chikwangue in Central Africa: An approach through Just About Right (JAR) test. *Emirates Journal of Food and Agriculture, 33*(6), 475–482. https://doi.org/10.9755/ejfa.2021.v33.i6.2716

Zhao, F., Li, Y., Li, C., Ban, X., Cheng, L., Hong, Y., & Li, Z. (2022). Insight into the regulations of rice protein on the gluten-free bread matrix properties. *Food Hydrocolloids, 131*, Article 107796. https://doi.org/10.1016/j.foodhyd.2022.107796

Ziaei, R., Foshati, S., Hadi, A., Kermani, M. A. H., Ghavami, A., Clark, C. C. T., & Tarrahi, M. J. (2020). The effect of nettle (*Urtica dioica*) supplementation on the glycemic control of patients with type 2 diabetes mellitus: A systematic review and meta-analysis. *Phytotherapy Research, 34*(2), 282–294. https://doi.org/10.1002/ptr.6535. Epub 2019. PMID: 31802554.