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Valorization of under-utilized whey: its effect on rheological and functional properties in black wheat *idli*

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ABSTRACT

This study focuses on the dual objectives of utilizing dairy by-products for waste valorization and the creation of functional foods. In this study, novel functional black wheat *idli* was developed using paneer whey (BWP), cheese whey (BWC), and whey protein concentrate (BWWPC). Whey-enriched *Idli* exhibited the highest protein content (g/100 g) in WPC (24.76), paneer (20.34), and cheese whey (21.03) compared with the control (18.7). The antinutritional factors were reduced with WPC exhibiting the lowest levels of phytic acid (5.52 mg/100 g) and saponin (616 mg/100 g). Rheological evaluation demonstrated that paneer whey batter exhibited the highest elasticity ($G' = 9821$ Pa). Paneer whey also improved cohesiveness (0.692) and adhesiveness (−45.12 N s), while the WPC formulation provided the firmest texture. Thermal analysis indicated that cheese whey formulations had lower gelatinization temperatures ($T_p = 84.19$ °C), while paneer whey exhibited higher stability ($T_p = 102.84$ °C). Structurally, XRD analysis revealed sharper peaks in the paneer whey formulations, indicating enhanced starch crystallinity. High-resolution accurate mass spectrometry detected more bioactive metabolites in cheese whey formulations, showing higher levels of threonine and quercetin potential for enhanced nutrient bioavailability. This approach successfully demonstrates a sustainable strategy for valorizing dairy by-products by creating a novel, nutrient-dense functional food.

1. Introduction

The food industry is rapidly evolving, driven by innovation and changing consumer demand. In an era of increasing environmental awareness and resource scarcity, the transition to a circular economy is critical to the global food system. Innova Market Insights (2024) highlights recent food trends, such as personalized nutrition, gut health, and sustainability, with circular economy principles reviving regional crops and by-products to strengthen the agri-food system.

Simultaneously, the Indian dairy industry generates large volumes of whey during paneer and cheese production (180–190 million tons annually), which presents environmental challenges because of its high biochemical oxygen demand. This necessitates the development of sustainable valorization strategies to ensure effective waste management (Buchanan et al., 2023). Studies have shown that incorporating whey protein concentrate and pectin into foods can improve water retention

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and reduce waste (Gyawali & Ibrahim, 2018), whereas whey proteins have antioxidant, immunostimulatory, and anti-carcinogenic properties (Kumar et al., 2022).

Incorporating whey into foods is a promising valorization strategy that requires a nutritionally compatible and consumer-accepted base matrix that can also serve as a source for developing functional foods. Black wheat, a pigmented variety developed at the National Agri-Biotechnology Institute (Mohali, Punjab), has emerged as a promising ingredient because of its fiber-dense characteristics, high anthocyanin content, and other bioactive compounds associated with antioxidant, anti-inflammatory, and cardioprotective benefits (Aggarwal & Verma, 2025; Zhang et al., 2022) and appeals to health-conscious consumers, making it an ideal vehicle for functional food development. Black wheat is a novel base for enhancing functional foods, such as biscuits, cakes, flatbreads, and rusk (Ali et al., 2024; Devpal et al., 2025; Kumari, Sharma, et al., 2020). Because of the numerous health benefits of black wheat, it was selected for *idli* preparation of *idli*. *Idli* was selected for its high traditional consumption, natural fermentation process, suitability as a medium for functional ingredients, enhanced digestibility, bioavailability, and probiotic potential (Mandhania et al., 2019).

Fermentation enhances nutrient bioavailability and reduces anti-nutritional factors, such as phytic acid, tannins, and saponins (Malik et al., 2022). *Idli* is a traditional South Indian dish made from fermented batter. It is ideal for developing functional foods such as rawa *idli*, a popular variant made from fermented wheat semolina (rawa) (Aggarwal & Verma, 2025). By leveraging lactic acid bacteria, *idli* improves digestibility and allows the inclusion of probiotic and functional ingredients (Sharma et al., 2020). Recent investigations have explored the substitution of conventional ingredients in *idli* (rice or wheat) with nutrient-dense alternatives, such as chickpea (Chakraborty et al., 2022) and finger millet (Rani et al., 2019) which provide opportunities to diversify formulations and enhance nutritional profiles.

Repurposing whey into functional foods not only addresses some environmental issues, but also boosts the nutritional value of traditional foods. Although previous studies have examined whey-starch blends for various applications (Gyawali & Ibrahim, 2018; Kumar et al., 2022), no research has yet been conducted to study the combined effects of whey fortification and black wheat fermentation on the rheological, nutritional, and metabolic profiles of *idli*. The present study focused on the development and analysis of novel black wheat *idli* recipes by substituting water with various types of whey solutions during the preparation process. Specifically, four distinct formulations were devised: (i) Control black wheat *idli* with water (BWI), (ii) Black wheat *idli* with a paneer whey (BWP), (iii) Black wheat *idli* with cheese whey (BWC), and (iv) Black wheat *idli* with whey protein concentrate (BWWPC). These *idli* were subjected to a comprehensive evaluation of their physicochemical, nutritional, microbiological, sensory, metabolomic, rheological, and thermal properties to ascertain the effect of different whey types on their composition, structure, and consumer appeal. This study contributes to expanding the utilization of dairy by-products and promoting the diversification of functional cereal-based foods.

2. Materials and methods

2.1. Materials and reagents

The black wheat variety "NABIMG11″ was acquired from the National Agri-Biotechnology Institute in Mohali, Punjab, India. Black wheat grains were ground into semolina (rawa) using a disc mill and sieved through a 60-mesh sieve to obtain a uniform particle size of ~250 μm. Local stores in Varanasi, India, provided eno® and Amul® dahi (proximate composition (per 100 g) ~‘4.4g carbohydrates, 4g protein, and 3.1g fat). HiMedia, Pvt. Ltd., Mumbai, India furnished the following chemicals: Folin–Denis reagent, Sodium carbonate (Na_2CO_3), Ammonium thiocyanate (NH_4SCN), Ferric chloride ($FeCl_3.FeCl_2$), ethanol, Diethyl ether, Butanol, sodium chloride (NaCl), Hydrochloric acid (HCl) sodium acetate, sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), methanol, Folin-Ciocalteu (FC) phenol reagent, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonate) (ABTS), gallic acid, potassium persulfate ($K_2S_2O_8$). Borosil®, Pvt. Ltd., Varanasi, India, supplied laboratory essentials and glassware, including Whatman filter papers. Paneer whey (per 100 g: 5.9 g TS, 0.13 g fat, 0.38 g protein, 5.08 g lactose, 0.62 g ash, pH 5.2) and cheese whey (per 100 g: 6 g TS, 0.13 g fat, 0.7 g protein, 4.8 g lactose, 1.32 g ash, pH 6.0) were obtained from standardized cow milk (3.0 g fat, 8.5 g SNF per 100 g). Whey protein concentrate (80g/100g protein) was sourced from Bulk Supplements (Henderson, Nevada). *Leuconostoc mesenteroides* (NCDC 744) from NDRI was activated in 10 g/L MRS broth at 32 °C for 18 h before use in black wheat *idli* formulations.

2.2. Preparation of black wheat rawa idli

Four formulations of *idli* mix were prepared according to a previous study (Moura-Alves et al., 2025) and a pilot study to formulate three variants of black wheat *idli* fortified with whey *i.e.,* BWP, BWC, BWWPC, and control (BWI). For BWI, 100 g of broken BW was immersed in 100 mL water. In the treatment groups, water was fully substituted with an equal volume of paneer whey, cheese whey, or a WPC solution (1:1 w/v ratio). All the four treatment groups subjected to fermentation with 1.375 g/L *Leuconostoc mesenteroid* (NCDC 744) culture (National Dairy Research Institute, Karnal) at 32.5 °C for 4 h in a temperature-controlled incubator (Narang Scientific work Pvt, New Delhi, India). These fermentation conditions were optimized based on our previous study on black wheat *idli* (Aggarwal & Verma, 2025) in which demonstrated that the best combination of batter resulted in desirable batter characteristics such as improved volume, pH, titratable acidity, and density. Subsequently, the fermented batters were spread into a thin, uniform layer (approximately 5 mm thick) on stainless steel trays and dried in a tray dryer (Narang Scientific Works Pvt., New Delhi) at 55 °C for 6–8 h, until a final moisture content of < 5g/100 g was achieved for instant rawa *idli* mix with different types of whey formulations.

2.3. Characterization of idli/batter

2.3.1. Physico-chemical

The batter characteristics, including volume (the volume change was recorded after placing 10 g of batter in a measuring cylinder at 36 °C for 4 h), density, pH (Scientific Instrument, India), and titratable acidity (a mixture comprising 20 g of batter was combined with 40 mL of distilled water, utilizing 1/10 mol/L NaOH and phenolphthalein as the indicator) were measured (Aggarwal & Verma, 2025). The ash, protein, fat and dietary fiber contents were determined for proximate composition using the AOAC (2000) method.

2.3.2. Textural

Texture analysis of *idli* was performed using a Texture Analyzer (Model: CT3, Brookfield, Mumbai, India) equipped with a 36 mm diameter cylindrical aluminum probe. The procedure involved a two-compression cycle applied to 50 % of the sample initial height, with a pre-test speed of 1.0 mm/s, a test speed of 0.5 mm/s, and a post-test speed of 1.0 mm/s, maintaining 5 s intervals between compressions. Cylindrical samples (25 mm diameter, 20 mm height) were extracted from the center of freshly steamed *idli* and equilibrated at 25 °C.

2.3.3. Sensory evaluation

Sensory evaluation of *idli* samples was carried out using a nine-point hedonic scale with 20 trained panelists undertaking food-related studies (10 males and 10 females) from the Department of Dairy Science and Food Technology and Rasa-Shastra and Bhaishajya Kalpana at Banaras Hindu University, Varanasi, and maintaining an equal male-to-female

ratio. During the testing sessions, purified water and unsalted crackers were used as palatal cleansers. In each evaluation session, the panelists randomly assessed and scored samples from the four groups. The evaluators conducted sensory evaluations of *idli* based on three criteria: aroma, flavor, and overall acceptability (Durgadevi & Shetty, 2014). The advisory/ethical Committee (ref. No. DSFT/2024–2025/AEC/01) approval was obtained for sensory evaluation and complied with the ethical principles of the Declaration of Helsinki, with prior consent from all participants who participated voluntarily and maintained confidentiality.

2.3.4. Microbial quality

The microbial quality of the instant mix was evaluated using standard plating methods by serial dilutions on various media i.e., nutrient agar for total plate count, potato dextrose agar for yeast and mold counts, and Violet Red Bile Agar for coliform counts. The incubation conditions were as follows: 37 °C for 24 h for total plate and coliform counts (log CFU/g), and 25 °C for 48 h for yeast and mold counts (CFU/g) (Aggarwal & Verma, 2025).

2.3.5. Assessment of antioxidant potential

Antioxidant potential, specifically free radical scavenging capacities (DPPH inhibition, FRAP, and ABTS), Total Phenolic Content (TPC), and Total Flavonoid Content (TFC) were determined following the methodology described by Aggarwal and Verma (2025).

2.4. Determination of antinutritional factors of black wheat idli mix

2.4.1. Total phytates (phytic acid)

A 4 g specimen was immersed in 100 mL of 0.6 mol/L HCl solution for 3 h and then filtered using Whatman filter paper. In a 250 mL conical flask, 25 mL of the resulting filtrate was combined with 5 mL of 3 g/L NH_4SCN solution and 53.5 mL of distilled water. The mixture was titrated with $FeCl_3$ solution (0.00195 g/ml). The result was multiplied by 1.95 and 3.55 to obtain phytate phosphorus (P) and total phytate content, respectively (Saah et al., 2020).

2.4.2. Total tannin contents

The sample *idli* powder (0.5 g) was heated in 5 mL of distilled water and subsequently centrifuged, and the volume was adjusted to 10 mL. This mixture was added to 5 mL of Folin–Denis reagent and 10 mL of Na_2CO_3, with the total volume adjusted to 100 mL. The resulting solution was agitated and allowed to stand for 30 min, followed by measurement of the absorbance at 700 nm using a UV spectrophotometer (Bizuayehu et al., 2016). Tannin was expressed as mg per 100 g, calculated using a standard tannic acid curve (5–100 mg/L) and its associated regression using Eq. (1).

$$\text{Absorbance} = 0.0007 \text{ tannin (mg/100 g)} + 0.0224 \ (R^2 = 0.90) \quad \text{Eq. (1)}$$

2.4.3. Total saponin content

The double solvent extraction gravimetric technique was employed to determine the saponin content. The procedure involved combining 2 g of the sample with 50 mL of 200 mL/L aqueous ethanol, heating the mixture at 55 °C for 90 min, and repeating the extraction until the combined extracts reached 40 mL at 90 °C. The combined extracts were reduced, mixed with diethyl ether, and re-extracted until they were clear. Saponins were extracted with butanol, washed with 5 g/L NaCl, dried, weighed, and calculated using Eq. (2).

$$\textit{Saponins}\ (\text{g}/100\ \text{g}) = \frac{W2 - W1}{\textit{Weight of sample}} \times 100 \quad \text{Eq. (2)}$$

where:

W1 = Weight of the empty evaporating dish.

W2 = Weight of the evaporating dish with the dried sample.

2.5. Colour properties

A Hunter Lab Color reader (Hunter Associates Laboratory Inc., Reston, Virginia, U.S.A.) was used to assess the color of the sample. The device measured lightness (L*), which indicates color brightness from 0 (black) to 100 (white); redness (a*), which denotes redness (positive) or greenness (negative); and yellowness (b*), which represents yellowness (positive) or blueness (negative). These data were subsequently converted into various color indices, including the yellowness index (YI) (Eq. (3)), chroma (C) (Eq. (4)), Hue Angle (H) (Eq. (5)), Browning Index (Eq. (6)), and color index (Eq. (7)) (Dharshini & Meera, 2023).

$$YI = \frac{142.86 \times b^*}{L^*} \quad \text{Eq. (3)}$$

$$C = \sqrt{(a^*)^2 + (b^*)^2} \quad \text{Eq. (4)}$$

$$H = tan^{-1}\left\{\frac{b^*}{a^*}\right\} \times \frac{360}{2\pi} \quad \text{Eq. (5)}$$

$$BI = 100 \times \frac{x - 0.31}{0.17} \quad \text{Eq. (6)}$$

Where $x = \frac{a^* + 1.75L^*}{5.645L^* + a^* - 0.3012b^*}$

$$\textit{Colour Index} = \frac{1000 \times a^*}{L^*\ b^*} \quad \text{Eq. (7)}$$

2.6. Rheological measurements

Rheological characteristics of *idli* batters were evaluated at 25 °C using a rheometer (MCR 702, Anton Paar, Austria) equipped with a parallel plate geometry consisting of a lower plate (PP50) and an upper plate (PP25). This methodology was based on the procedure described by Kumari, Bhinder, et al. (2020), with a minor modification in the angular frequency range (1–100 rad/s). The samples were loaded and allowed to rest for 5 min for structural recovery and temperature equilibration. A strain sweep (0.01–100 % strain at 1 Hz) determined the linear viscoelastic region (LVER). Using a 0.5 % strain within the LVER, a frequency sweep was performed over 1–100 rad/s. The storage modulus (G′), loss modulus (G″), and complex viscosity (η*) were recorded.

2.7. Differential scanning calorimetry (DSC)

DSC (DSC-60 Plus, Shimadzu, Kyoto, Japan) calibrated with indium was utilized to determine the transition temperatures. Approximately 10 mg of samples enclosed in hermetically sealed aluminum containers were equilibrated at 20 °C for 1 h, followed by heating from 20 °C to 200 °C at 10 °C/min. This temperature range was selected to comprehensively analyze the thermal changes in the *idli* sample. This study examined parameters such as the peak transition temperature (T_p) and enthalpy change (ΔH).

2.8. X-ray diffraction (XRD)

XRD was performed using a Malvern Panalytical X-ray Diffractometer with Cu Kα radiation (λ = 0.15406 nm). The instrument was calibrated using silicon powder (Operate: 45 kV and 40 mA with a scan rate of 2°/min, step size of 0.013°, and 2θ scanning range of 10°–80°).

2.9. Scanning electron microscopy (SEM) with energy-dispersive X-ray spectroscopy (EDX)

SEM images of black wheat *idli* mix powder with different types of

whey were obtained using a Quanta 200F (Thermo-Fischer, USA) equipped with EDAX at 200 × magnification, 9.5 mm working distance, large field detector, and 20.00 kV.

2.10. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of black wheat *idli* mix samples with varying whey additions were obtained using an FTIR spectrophotometer (PerkinElmer Spectrum version 10.03.05) in transmission mode with KBr pellets. Spectra were acquired from 400 to 4000 cm^{-1} at 4 cm^{-1} resolution, averaging 20 scans at room temperature. Raw data were exported and analyzed using Origin-Pro 2024b (OriginLab Corporation, Northampton, MA, USA), as shown in Fig. 5.

2.11. High-resolution accurate mass spectrometry (HRAMS) based untargeted metabolomics profiling

The HRAMS-based metabolomics study complemented traditional analyses to reveal the molecular-level effects of whey fortification on *idli*, thereby offering novel insights into its functional potential. A 0.25 g sample was extracted with 2 mL of methanol, filtered (0.22 μm Allpure-GN nylon syringe filter), and analyzed using Dionex UltiMate 3000 RS UHPLC system coupled with an Orbitrap Eclipse Tribrid mass spectrometer (Thermo Fisher Scientific) with C_{18} column (1.9 μm, 2.1 × 100 mm) and specific mobile phases used, i.e., water–methanol with formic acid (0.3 mL/min) with a flow rate (0.3 mL/min), sheath gas (50 arb), auxiliary gas (10 arb), sweep gas (1 arb), vaporizer (350 °C), static spray voltage, and ion transfer tube (325 °C) (Jaspal et al., 2024). Data processing and peak annotation were processed using MS-DIAL and Compound Discoverer 3.3.2.31, and normalized using the quality-control-based robust locally estimated scatterplot smoothing signal correction method. Metabolites were identified using the Human Metabolome (HMDB) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases using the MetaboAnalyst 5.0. Statistical analyses including Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA), and heat map visualization, were performed using Origin-Pro 2024b (OriginLab Corporation, Northampton, MA).

2.12. Statistical analysis

All experiments were conducted in triplicate (n = 3) as independent biological replicates of the four formulations (BWI, BWP, BWC, and BWWPC) prepared on the same day to ensure process reproducibility. Data normality and homogeneity were confirmed before performing one-way ANOVA and Duncan's multiple range test ($p < 0.05$) to determine the significant differences among the four *idli* formulations. Statistical analyses of the physicochemical and sensory data were conducted using GraphPad Prism (version 5.0c, GraphPad Software, San Diego, CA, USA), and Principal Component Analysis (PCA), metabolomic heatmap generation, rheological parameters, and FTIR were performed using Origin-Pro 2024b (OriginLab Corporation, Northampton, MA, USA).

3. Results and discussion

3.1. Physicochemical properties

The batter volume was significantly ($p < 0.05$) highest in the BWWPC (41 ± 0.67 cm^3) because of the whey proteins' ability to incorporate and stabilize air bubbles. The addition of BWWPC increased the pH of the black wheat *idli* mix because proteins and minerals act as acid neutralizing buffers (Sharma et al., 2017). Titratable acidity was inversely correlated with BWWPC, displaying the lowest acidity ($p < 0.05$) which may be due to altering the fermentation dynamics of whey, potentially reducing acid production (Rani et al., 2019). Nutritional analysis revealed that the protein content was significantly ($p < 0.05$) higher, whereas dietary fiber, fat, and ash content exhibited no significant variations among the samples. WPC-enriched *idli* provided 24.76 g of protein per 100 g, meeting approximately 50g/100g of the daily protein requirement (56 g for men and 46 g for women). This makes it suitable for individuals in protein-deficient populations, which can be further confirmed by in vivo studies.

3.2. Sensory and textural evaluation

The sensory and textural properties are crucial for the acceptability of fermented foods. The BWI *idli* mix exhibited overall acceptability and texture (softness, adhesiveness, and springiness), making it an acceptable product. However, fortifying black wheat *idli* with whey, particularly paneer whey (BWP) and whey protein concentrate (BWWPC) significantly diminished the overall acceptability ($p < 0.05$). Leksrisompong et al. (2010) identified key aroma compounds in whey protein hydrolysates, such as dimethyl sulfide (DMS), 3-methyl butanal, 2-methyl butanal, and methional, which are products derived from Strecker degradation. These compounds can affect the aroma of wheat-fortified products.

BWC exhibited significantly ($p < 0.05$) higher hardness than BWI, primarily because of its elevated calcium content, which stabilizes β-lactoglobulin and promotes stronger protein aggregation and cross-linking with the starch matrix as well as its distinct HRAMS metabolite profile rich in specific amino acids (Table 3) and calcium content (Table 6). The WPC-fortified *idli* (BWWPC) exhibited significantly ($p < 0.05$) higher hardness (20.12 N) than the control (10.1 N), mainly because of its dense protein matrix (70–80g/100g WPC), resulting in a firmer, denser texture that may appeal to consumers preferring less spongy *idli*. BWP reduced adhesiveness via soluble components such as lactose, whereas BWC formed a drier crumb through mineral interaction and BWWPC by creating a firm protein network (Table 1). Cohesiveness improved with BWP due to whey protein binding, varied in BWC due to the mineral–acid balance, and was highest in BWWPC due to its strong protein matrix countering black wheat fiber crumbliness. Similar textural properties have been observed with whey protein addition to rye bread (Moura-Alves et al., 2025). Overall acceptability was highest for BWC, with BWP being more acceptable than BWWPC, reflecting their balanced textural and sensory characteristics. Nevertheless, the control sample remained the most preferred overall, whereas the BWWPC received a slightly lower score owing to excessive hardness. These findings demonstrate the potential of whey variants to modulate the functional and sensory properties of black wheat *idli* mix.

3.3. Microbial quality

Microbial analysis revealed no statistically significant differences in total plate count, coliforms, and yeast and mold counts among the BWI, BWP, BWC, and BWWPC samples, which may be due to the hygienic preparation processes, which involve thorough steaming during batter preparation and subsequent drying using a tray dryer. These thermal treatments effectively reduced the microbial load, particularly coliforms (undetected in any sample), and maintained low and comparable levels of total plate count and yeast and mold within microbial standards and limits (Food Safety and Standards Authority of India, 2021).

3.4. Antioxidant potential

The antioxidant potential of BWI *idli* with different types of whey samples, *i.e.,* BWP, BWC, and BWWPC (Table 2): free radical scavenging capacities (DPPH inhibition, FRAP, and ABTS), TPC, and TFC. In our previous study on the antioxidant potential of BWI (Aggarwal & Verma, 2025), it was found that BWI exhibited significantly ($p < 0.05$) higher DPPH, FRAP, and ABTS activities, but lower TPC values than the *idli* prepared with the addition of different types of whey (Table 2). The increase in TPC but reduction in antioxidant activities (DPPH, FRAP,

Table 1
Physicochemical, Sensory, Textural properties, and Microbial properties of black wheat rawa *idli* enriched with different type of whey.

Parameter	BWI	BWP	BWC	BWWPC
Batter Properties				
Batter volume (cm^3)	24.197 ± 0.38^{d}	31 ± 0.52^{b}	27 ± 0.45^{c}	41 ± 0.67^{a}
pH	4.07 ± 0.01^{c}	4.42 ± 0.02^{b}	4.5 ± 0.03^{b}	4.67 ± 0.04^{a}
Titratable acidity (g lactic acid/100 g)	0.53 ± 0.01^{a}	0.498 ± 0.01^{b}	0.478 ± 0.01^{b}	0.432 ± 0.01^{c}
Density (g/cm^3)	0.81 ± 0.02^{a}	0.667 ± 0.01^{c}	0.73 ± 0.01^{b}	0.589 ± 0.01^{d}
Physio-chemical properties of *idli*				
Protein content (g/100 g)	18.71 ± 0.28^{c}	20.34 ± 0.32^{b}	21.03 ± 0.29^{b}	24.76 ± 0.36^{a}
Fat (g/100 g)	1.85 ± 0.01^{a}	1.82 ± 0.03^{a}	1.95 ± 0.02^{a}	2.04 ± 0.02^{a}
Dietary fiber (g/100 g)	4.76 ± 0.12^{a}	4.75 ± 0.10^{a}	4.69 ± 0.08^{a}	4.79 ± 0.11^{a}
Ash content (g/100 g)	2.37 ± 0.04^{a}	2.64 ± 0.05^{a}	2.75 ± 0.04^{a}	2.61 ± 0.06^{a}
Sensory and textural properties of *idli*				
Flavour	6.7 ± 0.06^{a}	6.9 ± 0.11^{b}	7.1 ± 0.15^{b}	7.32 ± 0.12^{c}
Overall acceptability	7.7 ± 0.19^{a}	7.5 ± 0.21^{b}	7.62 ± 0.19^{b}	6.9 ± 0.22^{c}
Aroma	6.9 ± 0.26^{a}	5.6 ± 0.13^{c}	6.2 ± 0.27^{b}	5.3 ± 0.61^{d}
Hardness (N)	10.1 ± 0.23^{d}	12.0 ± 0.15^{c}	13.5 ± 0.17^{b}	20.12 ± 0.32^{a}
Adhesiveness (N.sec)	-41.22 ± 1.75^{c}	-45.12 ± 1.45^{d}	-35.21 ± 1.39^{b}	-22.32 ± 0.98^{a}
Springiness (mm)	0.804 ± 0.01^{a}	0.815 ± 0.01^{a}	0.786 ± 0.01^{b}	0.834 ± 0.01^{a}
Cohesiveness	0.643 ± 0.01^{c}	0.692 ± 0.01^{b}	0.683 ± 0.01^{b}	0.732 ± 0.01^{a}
Microbial quality of *idli*				
Total plate count (log CFU/g)	1.05 ± 0.03^{a}	0.97 ± 0.02^{a}	1.10 ± 0.04^{a}	1.15 ± 0.05^{a}
Coliform (CFU/g)	ND	ND	ND	ND
Yeast and mold (CFU/g)	18 ± 2.0^{a}	17 ± 1.0^{a}	19 ± 1.0^{a}	17 ± 1.0^{a}

Note: Values are presented as the mean ± standard error of three independent experimental replicates (n = 3). Means within the same row followed by different superscript letters (a, b, c, d) are significantly different, according to Duncan's multiple range test ($p < 0.05$).
* CFU/g – colony-forming units per gram.
**BWI: Black wheat rawa *idli*; BWP: Black wheat rawa *idli* with paneer; BWC: Black wheat rawa *idli* with cheese whey; BWWPC: Black wheat rawa *idli* with whey protein concentrate.

Table 2
Antioxidant properties and Antinutritional factors of black wheat rawa *idli* enriched with different type of whey.

Antioxidant properties	BWI	BWP	BWC	BWWPC
DPPH (% Inhibition)	72.20 ± 5.6^{a}	64.72 ± 2.6^{b}	64.75 ± 2.3^{b}	64.73 ± 2.8^{b}
FRAP (mmol/100 g)	55.84 ± 4.5^{a}	27.90 ± 1.1^{b}	27.84 ± 1.9^{b}	27.65 ± 1.7^{b}
ABTS (mg AAE/100g)	8.81 ± 1.07^{a}	5.37 ± 1.5^{b}	5.23 ± 1.3^{b}	5.07 ± 1.2^{b}
TPC (mg GAE/100g)	210.54 ± 7.85^{b}	224.77 ± 4.85^{a}	224.11 ± 3.92^{a}	225.90 ± 4.85^{a}
TFC (mg QE/100g)	205.6 ± 5.11^{a}	205.6 ± 3.11^{a}	196.0 ± 4.87^{b}	148.8 ± 4.32^{c}
Antinutritional factors				
Total phytate (mg/100g)	8.307 ± 0.499^{a}	6.72 ± 0.21^{b}	6.52 ± 0.14^{b}	5.52 ± 0.12^{c}
Saponin (mg/100g)	1087 ± 13.79^{a}	891 ± 12.21^{b}	887 ± 12.34^{b}	616 ± 10.23^{c}
Tannin (mg/100g)	350.857 ± 6.81^{b}	192.286 ± 3.85^{c}	186.32 ± 2.71^{d}	396.571 ± 3.57^{a}

Note: Values are presented as the mean ± standard error of three independent experimental replicates (n = 3). Means within the same row followed by different superscript letters (a, b, c, d) are significantly different, according to Duncan's multiple range test ($p < 0.05$).
DPPH- 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity; AAE- Ascorbic acid equivalent; FRAP-ferric ion reducing antioxidant capacity; ABTS-2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay; TPC-total phenolic content; GAE- Gallic acid equivalent; TFC- Total flavonoid content; QE-Quercetin equivalent.
**BWI: Black wheat rawa *idli*; BWP: Black wheat rawa *idli* with paneer; BWC: Black wheat rawa *idli* with cheese whey; BWWPC: Black wheat rawa *idli* with whey protein concentrate.

ABTS) in whey-fortified samples may be due to non-covalent binding of phenolics with β-lactoglobulin that mask active sites, assay interference from the Folin–Ciocalteu reagent reacting with whey-derived amino acids such as tryptophan and tyrosine (Johari & Khong, 2019; Thongzai et al., 2022). The DPPH and TPC of the samples (BWP, BWC, and BWWPC) showed no significant differences in this assay, highlighting their strong potential for scavenging free radicals. BWP and BWC exhibited similar FRAP values, whereas BWWPC exhibited a significantly ($p < 0.05$) lower value (507.6 mmol/100 g), indicating differences in ferric ion reduction. However, significant ($p < 0.05$) reduction of TFC in WPC enriched *idli* mix (148.8 mg QE/100g) compared to BWP (205.6 mg QE/100g) is likely due to non-covalent interactions such as hydrogen bonding and hydrophobic interactions between whey proteins (especially β-lactoglobulin) and flavonoids, which may decrease measurable flavonoid content (Sagar et al., 2020).

3.5. Determination of antinutritional factors

Phytic acid, tannins, and saponins reduce nutrient bioavailability and digestion by binding to minerals and by inhibiting enzymes. BWI exhibited significantly ($p < 0.05$) higher levels of both phytate and saponin than BWP, BWC, and BWWPC *idli* mix (Table 2). However, the significantly ($p < 0.05$) higher tannin content in BWWPC than in the control (BWI) was mainly due to the whey protein containing high tannins that can bind to proteins, forming strong complexes, and hindering the action of digestive enzymes (pepsin and trypsin), which could also affect protein digestibility (Kamal et al., 2024). The significant ($p < 0.05$) reduction in phytic acid in BWWPC (5.52 mg/100 g) as compared to the BWI (8.31 mg/100 g) is attributed to microbial phytase activity and whey mineral-chelating action during fermentation at pH 5.5 (Joudaki et al., 2023). Additionally, lactoferrin in whey may disrupt

Table 3
List of metabolites predominantly found in BWI with addition of different type of whey.

S.no	Name	Formula	Calc. MW	m/z	BWI		BWP		BWC		BWWPC		Category
					RT [min]	Area (Max)	RT	Area (max)	RT	Area (max)	RT	Area (max)	
Amine and Amide													
1	Betaine	$C_5H_{11}N0_2$	117.07	118.08	0.83	1.10×10^{10}	0.84	8.91×10^9	0.82	8×10^9	–	–	Amine
2	Choline	$C_5 H_{13}N O$	103.09	104.10	0.79	2.95×10^9	0.78	2.4×10^9	23.70	2×10^7	0.8	2.85×10^9	Amine
3	Hexadecanamide	$C_{16} H_{33}N 0$	255.25	256.26	25.21	8.04×10^8	25.18	1.15×10^9	25.47	3×10^9	24.09	1.2×10^9	Palmitamide
4	Hydroxypropionylcarnitine	$C_{10} H_{19} N O_5$	233.12	292.14	1.08	4.82×10^8	1.09	5.09×10^8	1.07	2×10^7	–	–	Acylcarnitine Amide
5	2′-Deoxyadenosine	$C_{10}H_{13}N_5 O_3$	251.1	252.1	0.92	3.90×10^8	0.91	2.08×10^8	–	–	–	–	Purine Derivative
Organic acid & derivative													
6	3-Hydroxy-3-methylglutaric acid	C_6 H10 O5	162.05	161.04	1.05	3.14×10^8	0.78	6×10^7	–	–	1.05	2×10^8	Organic Acid & Derivative
7	Ethyl levulinate	$C_7 H_{12} O_3$	144.07	145.08	3.41	3.11×10^8	5.26	1.27×10^9	3.38	2×10^9	5.37	7.67×10^8	Organic Acid & Derivative
8	4-Phenylbutyric acid	$C_{10} H_{12} O_2$	164.08	165.09	27.98	3.04×10^8	26.13	5×10^7	27.94	5×10^7	27.96	5×10^7	Organic Acid & Derivative
9	Citric acid	$C_6 H_8 O_7$	192.02	191.02	0.85	1.73×10^8	0.93	1.01×10^9	0.85	4×10^9	0.92	4×10^8	Organic acid
Fatty acid													
10	Oleic acid	$C_{18} H_{34} O_2$	282.25	281.24	–	–	25.42	1.09×10^9	–	–	–	–	Monounsaturated fatty acid
11	α-Eleostearic acid	$C_{18} H_{30} 0_2$	278.22	279.23	21.98	3.4×10^9	24.20	1.01×10^9	21.96	5×10^9	21.96	3.56×10^9	Polyunsaturated fatty acid
12	Linoleic acid	$C_{18} H_{32} 0_2 0_3$	280.24	279.23	24.58	3.36×10^9	24.56	4.84×10^9	24.55	1E+10	24.56	5.39×10^9	Polyunsaturated omega-6 fatty acid
13	α-Linolenic acid	$C_{23} H_{44} N 0_7 P$	477.28	476.27	22.87	1.54×10^9	23.89	2.57×10^9	23.88	4×10^9	23.90	2.17×10^9	Phospholipid
14	Oxooctadecadienoic acid	$C_{18} H_{30} O_3$	294.21	277.21	21.86	5.32×10^8	21.83	5.24×10^8	–	–	–	–	Fatty Acid
15	Linoleoyl ethanolamide	$C_{20} H_{37} N 0_2$	323.28	324.28	23.56	3.19×10^9	23.54	3.14×10^9	23.53	4×10^9	23.53	5.37×10^9	Fatty acid ethanolamide
16	Oleoyl ethanolamide	$C_{20} H_{39} N O_2$	325.29	326.3	24.29	4.10×10^8	22.49	7.2×10^8	24.60	2×10^8	24.26	1.05×10^9	Fatty acid
17	6-Hydroxycaproic acid	$C_6 H_{12} 0_3$	132.07	131.07	7.89	2.97×10^9	7.21	3.03×10^9	7.48	3×10^8	7.20	1.87×10^9	Hydroxy fatty acid
18	Monoolein	$C_{21} H_{40} 0_4$	356.29	379.28	24.7	2.38×10^9	24.68	1.69×10^9	24.68	4×10^9	24.68	1.07×10^9	Monoacylglycerol lipids
19	octadecadienoyl)-sn-glycerol	$C_{39} H_{68} O^5$	616.50	617.51	23.64	61845875	22.76	5.45×10^9	–	–	–	–	Glycerolipid
20	Hydroxyvaleric acid	$C_5 H_{10} O_3$	118.06	117.05	–	–	1.90	5.16×10^8	–	–	1.92	6.18×10^8	Hydroxy fatty acid
Amino acids													
21	Leucylproline	$C_{11} H_{20} N_2 O_3$	228.14	229.15	1.21	5.52×10^8	–	–	–	–	–	–	Amino Acid
22	DL-Arginine	$C_6 H_{14} N_4 0_2$	174.11	175.11	0.78	8.96×10^8	0.74	5.52×10^8	0.73	3×10^8	0.75	3.26×10^8	Amino Acid
23	Histidine	$C_6 H_9 N_3 O_2$	155.06	156.07	–	–	–	–	0.75	5×10^7	–	–	Essential amino acid
24	DL-Lysine	$C_6 H_{14} N_2 O_2$	146.10	147.11	0.744	1.2×10^8	0.736	1.01×10^8	0.73	4×10^7	0.745	3×10^7	Essential amino acid
25	Threonine	$C_4 H_9 N O_3$	119.05	120.06	–	–	–	–	0.88	3×10^7	–	–	Essential amino acid
26	Valine	$C_5 H_{11} N O_2$	117.07	118.09	–	–	–	–	–	–	22.133	7×10^6	Essential amino acid
27	Isoleucine	$C_6 H_{13} N 0_2$	131.09	132.10	1.09	1.79×10^{10}	1.07	1.63×10^{10}	1.06	7×10^9	1.12	1.2×10^{10}	Essential Amino Acid
28	L-Phenylalanine	$C_9 H_{11} N O_2$	165.07	164.07	1.29	6.07×10^8	1.34	2.2×10^{10}	1.30	3×10^8	1.30	2.18×10^8	Essential amino acid
29	DL-Tryptophan	$C_{11} H_{12} N_2 0_2$	204.08	188.07	1.20	3.08×10^9	1.18	2.52×10^9	1.02	2×10^9	1.13	1.71×10^9	Essential Amino Acid
30	L-(−)-Methionine	$C_5 H_{11} N 0_2 S$	149.05	150.05	0.94	1.01×10^9	0.93	8.07×10^8	0.95	3E+08	0.93	4.86×10^8	Essential amino acid
31	Leucine	$C_8 H_{16} N_2 O_3$	188.11	189.12	1.96	2.48×10^8	1.94	2.10×10^8	–	–	2.07	1.25×10^8	Essential amino acid
Polyphenols													
32	Caffeic acid	$C_9 H_8 O_4$	180.04	179.03	6.30	1.39×10^8	6.31	1.57×10^8	6.30	6×10^7	6.37	8×10^7	Phenolic Acid
33	Luteolin	$C_{15} H_{10} O_6$	286.04	285.04	14.65	1.41×10^8	14.61	1.35×10^8	15.67	4×10^7	14.61	1.3×10^8	Flavonoid
34	Gentisic acid	$C_7 H_6 O_4$	154.02	153.01	1.23	6×10^7	1.67	1.68×10^8	1.67	1×10^8	1.734	1.39×10^8	Phenolic Acid
35	Sinapinic acid	$C_{11} H_{12} O_5$	167.09	185.12	10.34	2×10^7	10.32	3.1×10^7	10.34	4×10^7	–	–	Polyphenols
36	Diosmetin	$C_{16} H_{12} O_6$	300.06	301.07	15.96	1.09×10^9	15.92	1.02×10^9	15.92	8×10^8	15.92	1.23×10^9	Flavonoids
37	Apigenin	$C_{15} H_{10} O_5$	270.05	271.05	15.85	1.13×10^8	15.79	1.09×10^8	15.79	9×10^7	15.80	1×10^8	Flavonoid
38	Kojic acid	$C_6 H_6 O_4$	142.02	143.03	2.18	1.14×10^8	–	–	–	–	–	–	Polyphenols
39	Isoferulic acid	$C_{10} H_{10} O_4$	194.05	195.06	9.16	1.23×10^8	15.85	2×10^7	22.59	5×10^8	28.72	1.67×10^8	Phenolic Acid
40	Gingerglycolipid A	$C_{33} H_{58} O_{14}$	678.38	701.37	22.83	6.29×10^8	22.52	1×10^7	22.14	4×10^7	–	–	Polyphenols
41	D-(−)-Quinic acid	$C_7 H_{12} 0_6$	192.06	251.07	0.85	1.38×10^9	–	–	0.83	6×10^7	0.85	4×10^7	Hydroxycinnamic Acids
42	Ferulic acid	$C_{10} H_{10} O_4$	194.05	177.05	9.99	4.42×10^8	9.98	3.25×10^8	9.10	3×10^8	9.95	1.02×10^8	Phenolic Acid
43	Quercetin	$C_{15} H_{10} O_7$	302.04	301	13.65	1×10^7	13.61	1×10^7	14.45	6×10^7	14.47	3×10^7	Flavonoid

(*continued on next page*)

Table 3 (*continued*)

S.no	Name	Formula	Calc. MW	m/z	BWI		BWP		BWC		BWWPC		Category
					RT [min]	Area (Max)	RT	Area (max)	RT	Area (max)	RT	Area (max)	
44	4-Methoxycinnamic acid	$C_{10}H_{10}O_3$	178.06	161.0	23.12	2×10^7	23.10	2×10^7	23.09	5×10^7	23.09	3×10^7	Phenolic Acid
Vitamins													
45	D-Pantothenic Acid	$C_9H_{17}NO_5$	219.11	220.1	1.26	2×10^8	–	–	–	–	1.29	1.3×10^8	Vitamin B5
46	Ascorbyl stearate	$C_{24}H_{42}O_7$	442.29	460.32	23.6	6×10^7	23.55	8×10^7	–	–	–	–	Vitamin C
47	Biotin sulfoxide	$C_{10}H_{16}N_2O4S$	260.08	259.07	1.03	3×10^7	–	–	–	–	–	–	Vitamin B7
48	alpha-tocotrienol	$C_{29}H_{44}O_2$	424.33	425.34	27.98	3.5×10^8	27.12	7×10^7	27.12	3×10^7	26.55	5.8×10^7	Vitamin E

*BWI: Black wheat rawa *idli*; BWP: Black wheat rawa *idli* with paneer; BWC: Black wheat rawa *idli* with cheese whey; BWWPC: Black wheat rawa *idli* with whey protein concentrate.
**Calc. MW: Calculated Molecular Weight; m/z: Mass-to-Charge Ratio; RT [min]: Retention Time in minute.
*** (–) indicates that the metabolite was not detected or was below the limit of quantification in the respective sample.

Table 4
Colour properties of black wheat rawa *idli* enriched with different type of whey.

Colour properties	BWI	BWP	BWC	BWWPC
L*	56.99 ± 0.30^c	59.54 ± 0.25^b	65.36 ± 0.40^a	56.33 ± 0.35^c
a*	6.99 ± 0.15^a	7.40 ± 0.12^a	5.20 ± 0.10^b	7.98 ± 0.18^a
b*	13.06 ± 0.25^a	13.48 ± 0.20^a	12.83 ± 0.22^a	13.83 ± 0.30^a
YI	32.49 ± 0.50^b	32.34 ± 0.45^b	28.04 ± 0.55^c	35.07 ± 0.60^a
C	14.48 ± 0.20^b	15.38 ± 0.18^a	13.84 ± 0.25^c	15.97 ± 0.30^a
H	62.7 ± 0.50^b	61.23 ± 0.45^b	67.94 ± 0.55^a	60.01 ± 0.60^b
BI	10.57 ± 0.20^b	11.03 ± 0.18^b	7.61 ± 0.15^c	12.43 ± 0.25^a
CI	9.06 ± 0.15^b	9.22 ± 0.12^b	6.20 ± 0.10^c	10.24 ± 0.18^a

Note: Values are presented as the mean ± standard error of three independent experimental replicates (n = 3). Means within the same row followed by different superscript letters (a, b, c, d) are significantly different, according to Duncan's multiple range test ($p < 0.05$).
L*, Lightness; a* - Red or Green; b*, Yellow or Blue; YI - Yellowness Index; H - Hue Angle (°); BI, browning index; CI, color index.
**BWI: Black wheat rawa *idli*; BWP: Black wheat rawa *idli* with paneer; BWC: Black wheat rawa *idli* with cheese whey; BWWPC: Black wheat rawa *idli* with whey protein concentrate.

phytic acid–mineral complexes by binding essential minerals (e.g., Fe^{2+}, Zn^{2+}, Ca^{2+}) (Mazorra-Manzano et al., 2020). Fermentation with lactic acid bacteria such as *Lactobacillus plantarum* and *Saccharomyces cerevisiae* reduces antinutritional factors through enzymatic and acid activity (Terefe et al., 2021). Previous studies (Malik et al., 2022) showed that paneer whey in millet significantly reduced tannins (69.17 % in kodo millet and 82.8 % in proso millet) and phytic acid (9.21 % in kodo millet) which supports its potential to improve nutrient bioavailability in black wheat rawa *idli* mix.

3.6. Untargeted metabolomics profile

Table 3 shows the primary metabolites of BWI with different whey types (BWP, BWC, and BWWPC) identified using the HMDB and KEGG databases. In total, 452 metabolites were identified in BWI by adding different types of whey (485 in BWP, 535 in BWC, and 460 in BWWPC). A detailed description of the 48 predominant metabolites and classification-based amines, amides, organic acids and derivatives, fatty acids, amino acids, polyphenols, and vitamins is provided in Table 3. The identification of essential amino acids (threonine in BWC and valine in BWWPC) (Table 3) which offer molecular-level evidence supporting the enhanced protein quality observed in the proximate analysis of these diets. Notably, threonine serves as a precursor for glycine and serine which are vital for the synthesis of connective tissue, including collagen. This finding suggests not only an improvement in nutritional value but also a potential contribution to gut health and integrity, thereby adding a functional aspect beyond protein quantification. Metabolites such as caffeic acid, luteolin, ferulic acid, and quercetin have exhibited antibacterial, antioxidant, anti-inflammatory, and antidiabetic properties in various preclinical trials (Barreca et al., 2021). Amines and amides have been well-studied for their anti-inflammatory properties (Qiao et al., 2024). Various essential amino acids were identified by the addition of whey (threonine (BWC) and valine (BWWPC)) and various essential fatty acids (linoleic acid and linolenic acid) and vitamins (B5, C, B7, and E) were identified (Table 3). The addition of whey (paneer whey, cheese whey, or whey protein concentrate) to BWI can alter the functional properties of oxidized phenolic compounds and antioxidant activity (Thongzai et al., 2022), *i.e.,* hydroxypropionylcarnitine, oxooctadecadienoic acid, 1,2-Di-(9Z,12Z-octadecadienoyl)-sn-glycerol, leucine, histidine, kojic acid, ascorbyl stearate, and biotin sulfoxide.

HRAMS offered molecular-level insights beyond conventional analysis e.g., BWP and BWC had similar protein levels, and BWC was richer in threonine and histidine (Table 3). Metabolic differentiation analysis using PCA and HCA revealed distinct group separation, with PC1 and PC2 accounting for 30.30 % and 25.12 % of variance, respectively

Table 5
Rheological and Thermal properties of black wheat rawa *idli* enriched with different type of whey.

Rheological properties	BWI	BWP	BWC	BWWPC
Storage modulus (Pa)	141.8 ± 5.7^{d}	9821 ± 96.2^{a}	3746.8 ± 65.3^{c}	4825.2 ± 70.1^{b}
Loss modulus (Pa)	96.09 ± 4.3^{c}	5423.2 ± 87.5^{a}	2976.4 ± 54.2^{b}	2772.5 ± 58.9^{b}
Loss factor (tan δ)	0.67 ± 0.02^{b}	0.55 ± 0.01^{c}	0.79 ± 0.01^{a}	0.57 ± 0.01^{c}
Complex viscosity (mPa·sec)	1712.9 ± 44.1^{d}	112190 ± 925^{a}	47854 ± 512^{c}	55649 ± 611^{b}
Thermal properties				
T_0 (°C)	59.17 ± 0.45^{d}	100.40 ± 0.83^{a}	79.59 ± 0.79^{c}	96.54 ± 0.62^{b}
T_p (°C)	63.84 ± 0.48^{d}	102.84 ± 0.89^{a}	84.19 ± 0.85^{c}	99.51 ± 0.66^{b}
T_c (°C)	68.30 ± 0.51^{c}	105.40 ± 0.92^{a}	86.25 ± 0.87^{b}	101.44 ± 0.71^{a}
ΔH (KJ/kg)	0.09 ± 0.001^{b}	0.42 ± 0.001^{a}	−0.09 ± 0.001^{d}	−0.02 ± 0.001^{c}

* Values are presented as means ± standard error of three independent experimental replicates (n = 3). Means within the same row followed by different superscript letters (a, b, c, d) are significantly different, according to Duncan's multiple range test ($p < 0.05$).
**BWI: Black wheat rawa *idli*; BWP: Black wheat rawa *idli* with paneer; BWC: Black wheat rawa *idli* with cheese whey; BWWPC: Black wheat rawa *idli* with whey protein concentrate.
***To = onset temperature of gelatinization endotherm; Tp = peak temperature; Tc = conclusion temperature; ΔT = Tc – To; ΔH = enthalpy.

Table 6
Energy dispersive x-ray spectroscopy of black wheat rawa *idli* enriched with different type of whey.

EDX (g/100 g Weight composition)	BWI	BWP	BWC	BWWPC
Magnesium (Mg)	13.1 ± 1.70^{a}	5.8 ± 0.96^{b}	0.1 ± 0.00^{d}	4.2 ± 0.84^{c}
Phosphorus (P)	23.5 ± 1.75^{a}	20.5 ± 1.15^{b}	15.8 ± 1.21^{d}	18.1 ± 1.57^{c}
Sulfur (S)	7.8 ± 2.60^{b}	6.9 ± 2.08^{c}	3.0 ± 1.91^{d}	11.0 ± 2.45^{a}
Chlorine (Cl)	Nil	7.2 ± 2.23^{b}	6.3 ± 3.80^{c}	8.0 ± 2.84^{a}
Potassium (K)	23.8 ± 3.29^{d}	32.1 ± 3.40^{c}	37.4 ± 4.90^{a}	33.4 ± 4.28^{b}
Calcium (Ca)	19.3 ± 4.15^{b}	18.8 ± 3.14^{c}	22.3 ± 5.60^{a}	17.1 ± 4.00^{d}
Manganese (Mn)	5.4 ± 3.23^{b}	3.6 ± 2.20^{d}	6.2 ± 3.72^{a}	4.3 ± 2.58^{c}
Zinc (Zn)	7.1 ± 4.39^{b}	5.0 ± 3.17^{c}	8.9 ± 5.56^{a}	3.9 ± 2.63^{d}

* Values are presented as means ± standard error of three independent experimental replicates (n = 3). Means within the same row followed by different superscript letters (a, b, c, d) are significantly different, according to Duncan's multiple range test ($p < 0.05$).
**BWI: Black wheat rawa *idli*; BWP: Black wheat rawa *idli* with paneer; BWC: Black wheat rawa *idli* with cheese whey; BWWPC: Black wheat rawa *idli* with whey protein concentrate.

(Fig. 1). This differentiation highlights the influence of whey fortification on metabolite composition. The control group (BWI) exhibited a characteristic profile that was rich in secondary metabolites (anthocyanins and carotenoids), sugars, and organic acids, reflecting the inherent antioxidant and nutritional properties of black wheat. In contrast, BWP, BWC, and BWWPC formed separate clusters from BWI, suggesting that the whey additives introduced unique metabolites. The hierarchical heatmap illustrates correlations with red/green indicating metabolite increases and blue indicating metabolite decreases (Fig. 1D). The metabolomic profiling of the black wheat *idli* group with different types of whey (BWI, BWP, BWC, and BWWPC) revealed distinct clustering patterns (Fig. 1A–C) in the PCA, with PC1 (30.30 %) and PC2 (25.12 %) between the control and whey-fortified samples, highlighting whey type as a key driver of metabolite diversity in the *idli* matrix. The eigenvalue plot (Fig. 1B) further validated the importance of PC1 and PC2, both exceeding the threshold of 1, affirming their contribution to the overall variation and suitability for interpretation according to the Kaiser criterion. The distinct clustering observed in the PCA score plot (Fig. 1C) demonstrates that whey fortification (BWP, BWC, and BWWPC) significantly alters the metabolomic profile of black wheat *idli*. This shift is not merely quantitative but qualitative, reflecting how fermentation and whey components drive distinct biochemical pathways that generate unique bioactive metabolites in each fortified formulation. The subsequent analysis focused on key metabolites that differentiated these groups and hypothesized their contribution to the observed functional and nutritional properties (Stanstrup et al., 2014).

The hierarchical heat map (Fig. 1D), based on Pearson's correlation of over 400 identified metabolites, provides an in-depth view of the metabolite distribution. Strong correlations were observed among various secondary metabolites, *i.e.,* anthocyanins, carotenoids, fatty acids, organic acids, and sugars. Positive correlations were found for eicosenoic acid, DL-tryptophan, DL-arginine, and 12-oxo phytodienoic acid, which are associated with lipid metabolism, stress responses, and signaling. In contrast, compounds such as sucrose and L-aspartic acid showed negative correlations with several nitrogenous and aromatic metabolites, highlighting the complex interplay between the biosynthetic and degradation pathways during fermentation (Schaan & Hughes, 2024). These findings suggest that the addition of different types of whey not only enhances protein content, but also contributes to functional bioactive profiles, including defense metabolites and hormone-like compounds. The presence and diversity of these secondary metabolites can influence the nutritional and health-promoting attributes of the final product (Schaan & Hughes, 2024).

Additionally, HRAMS revealed more bioactive compounds such as quercetin and caffeic acid, in the whey-fortified samples, highlighting the functional benefits of whey fermentation, and *their health implications require clinical confirmation.* The distinctive lipid profile of BWP, characterized by the presence of compounds like eicosenoic acid and oxylipin 12-oxophytodienoic acid may contribute to its rheological properties. Oxylipins, derived from fatty acids, may interact with starch–protein networks to enhance membrane dynamics and contribute to the robust, high batter elasticity (G' = 9821 Pa) and uniform pore structure (Fig. 4B). Furthermore, whey proteins combined with black wheat phenolics boosted antioxidant activity and reduced lipid oxidation (Thongzai et al., 2022), thereby enhancing black wheat *idli* functional, textural, and sensory attributes of black wheat. BWC altered amino acid (threonine and histidine) patterns, whereas BWWPC showed increased protein-lipid interactions (higher levels of 1-stearoylglycerol, which is a lipid-protein compound, and 1-tetradecylamine), improving texture and satiety.

The metabolite profiles of BWI with the whey variants (BWP, BWC, and BWWPC) showed distinct differences (Fig. 2). BWI contains 1-linoleoyl glycerol and ethyl levulinate, which are associated with antioxidant activity and health benefits (Bhonsle et al., 2022). BWP introduces bis(4-ethylbenzylidene)sorbitol and schaftoside to aid lipid metabolism and reduce oxidative stress (Mohd Sahardi et al., 2023). BWC contains elevated levels of α-eleostearic acid, which is known for its anti-inflammatory and cardioprotective effects (Yoshime et al., 2016). The BWWPC showed the highest levels of bis(4-ethylbenzylidene)

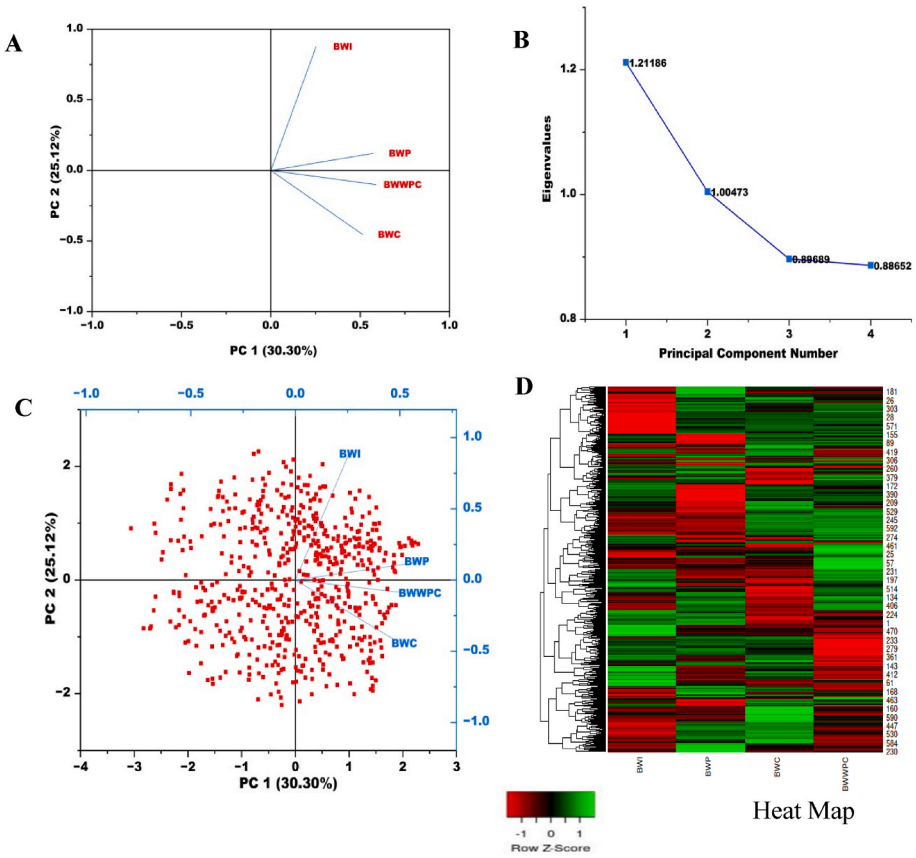

Fig. 1. Metabolomics analysis of four black wheat *idli* formulation. The panels compare the control (BWI) with whey-fortified samples (BWP, BWC, BWWPC): (A) PCA loading plot showing the contribution of individual metabolites to the principal components, (B) Eigenvalue plot displaying the percentage of variance explained by each principal component, (C) PCA score plot showing metabolic separation of the formulations with PC1 and PC2 accounted for 30.30 % and 25.12 % of the total variance, respectively and (D) Hierarchical clustering heat map of identified metabolites where green indicates higher relative abundance, red indicates lower relative abundance, and black indicates non-significant changes. Formulations: BWI (Control); BWP (Paneer Whey); BWC (Cheese Whey); BWWPC (Whey Protein Concentrate).

sorbitol, α-eleostearic acid, and 1-linoleoyl glycerol, indicating better nutrient bioavailability and metabolic benefits. Although the nutritional and bioactive profiles were improved in treatment samples, further in vivo or clinical studies are needed to confirm the health benefits. This approach supports both, functional food innovation and sustainability.

3.7. Colour properties

The color attributes of *idli* samples were measured using a Hunter Lab digital colorimeter (Table 4). The L* value, which represents lightness, ranged from 56.33 to 65.36, with the highest value observed in BWC. The a* value, indicating redness, was highest in BWWPC, followed by BWP, and lowest in BWC *idli*. The b* values showed no significant variations among the samples. These results align with those of previous studies (Kannan et al., 2015), that reported higher L*, b*, and positive a* values for *idli*. The hue angle (h°) is a qualitative color that distinguishes shades, *i.e.,* redness (0°), yellowness (90°), greenness (180°), and blueness (270°). The hue angles of all samples (60.01–67.94°) indicated a yellowish tone in *idli*, which is influenced by pigmented black wheat and is similar to the yellowish hue reported by Susmitha et al. (2022) in black rice *idli*.

Chroma quantifies the color intensity and degree to which a hue is distinguishable from a gray of equivalent luminance. In black wheat *idli* incorporated with different types of whey, chroma values ranged from 13.84 to 15.97, with BWWPC having significantly ($p < 0.05$) higher chroma values than BWC. However, no significant differences were observed between BWWPC and BWP samples.

The browning index primarily indicates non-enzymatic activity due to protein-starch interactions based on color changes during processing. The highest browning index was observed in BWWPC, whereas BWC exhibited the lowest index.

BWC had a significantly ($p < 0.05$) lower browning index than BWP *idli* owing to differences in protein and mineral content. Previous research has indicated that cheese whey has higher protein and mineral content than paneer whey, and this compositional difference may influence the browning index of whey-based products (Baba et al., 2016). The results also indicate that the browning index is inversely proportional to lightness (L*), with BWWPC having the highest browning index and lowest L* value (darkest appearance). In comparison, BWC showed the lowest browning index and highest L* value (lighter appearance).

3.8. Rheological properties

3.8.1. Storage and loss modulus

Key findings (Table 5) from oscillatory tests include the storage modulus (G′), representing the elastic behavior of the material, and the loss modulus (G″), which reflects its viscous or flow behavior. G′ quantifies how much energy is stored and recovered per unit volume during each oscillation, whereas G″ measures the amount of energy dissipated as the material flows. In a frequency sweep test, if G′ is higher than G″ the material behaves as a solid with viscoelastic properties (Fig. 3). Conversely, if G″ is greater than G′, the material behaves as a liquid with viscoelastic characteristics. In black wheat rawa *idli* batters, a higher G′ than G″ indicates a more elastic, solid-like texture mainly due to fiber-

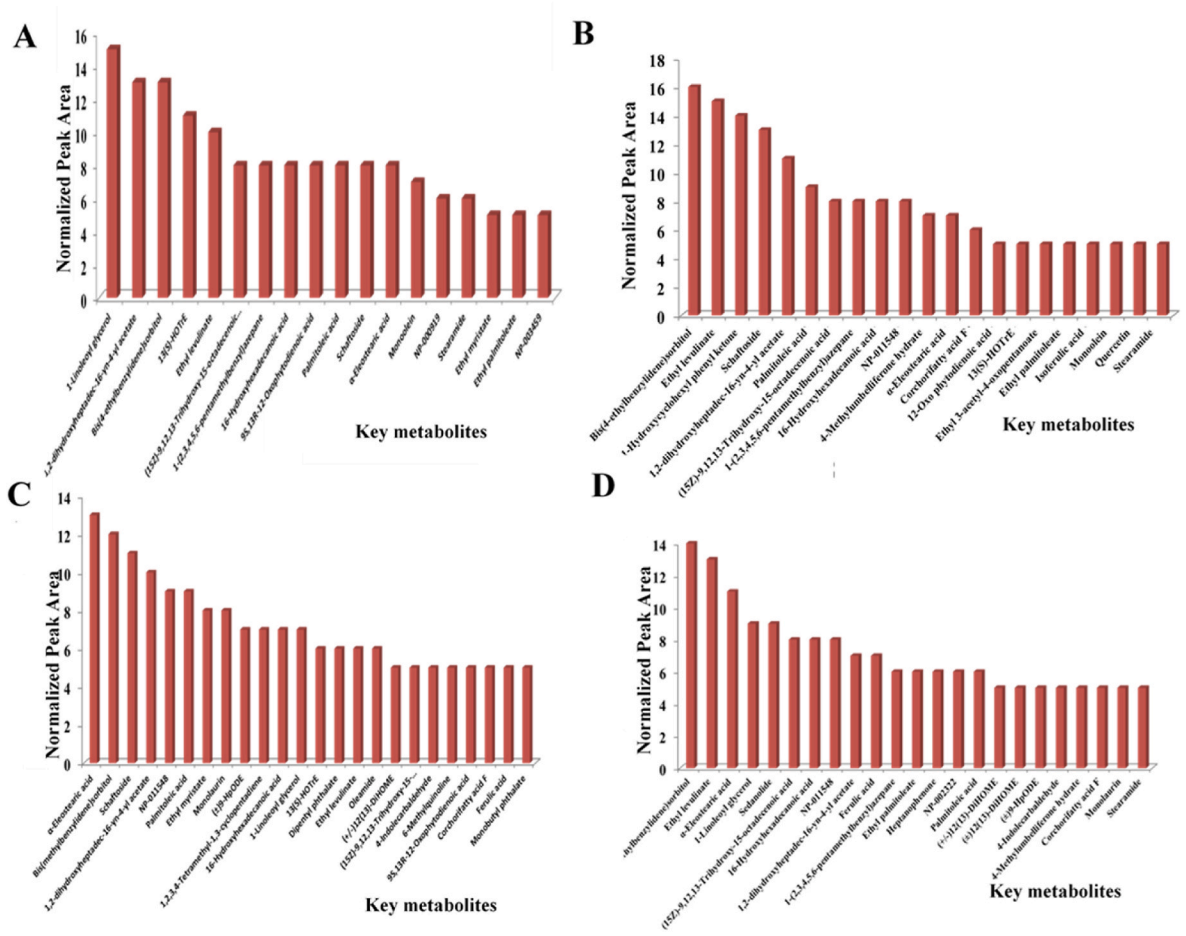

Fig. 2. Relative abundance of key differentiating metabolites across black wheat *idli* formulations. The bar chart displays the normalized peak areas from HRAMS analysis for the most significant metabolites: (A) Control/water (BWI), (B) Paneer Whey (BWP), (C) Cheese Whey (BWC), and (D) Whey Protein Concentrate (BWWPC).

enhanced water retention which improves batter viscosity and elasticity in similar grain-based batters such as chickpea flour (Herranz et al., 2016), whereas the whey proteins (β-lactoglobulin and α-lactalbumin) form a strong elastic network (Scott & Awika, 2023). Ma et al. (2021) also observed reduced G′ and G″ in wheat dough coated with anthocyanin-rich black wheat soybean extract. Batters with paneer whey, cheese whey, and WPC had significantly higher moduli than the control. The BWP (paneer whey) batter exhibited the highest G' (9821 Pa), which indicates the strongest elastic network, while the BWC and BWWPC also showed enhanced elasticity compared to the control (Table 5).

The influence of different whey types on the rheological properties of the batter is shown in Fig. 3. All batter formulations exhibited non-Newtonian shear-thinning (pseudoplastic) behavior, as shown by the decrease in complex viscosity (η*), storage modulus (G′), and loss modulus (G″) with increasing angular frequency (ω). This behavior is characteristic of complex food systems in which the internal structure of the material breaks down under shear, leading to reduced flow resistance. The BWP demonstrated a significantly higher complex viscosity and storage modulus across the frequency range than all other samples, indicating the formation of a stronger, more robust elastic network. From a processing perspective, the observed increase in elasticity (G′) in the BWP batter (Table 5) indicates enhanced shape retention during steaming and improved handling properties on a commercial scale.

3.8.2. Loss tangent

The loss tangent (tan δ) was determined from the ratio of the loss modulus (G″) to the storage modulus (G′). A value less than 1 indicates that elastic behavior is predominant, whereas values greater than 1 indicate viscous behavior. In the black wheat rawa *idli* batter, the loss tangent values for all samples (Table 5) containing paneer whey, cheese whey, and WPC were less than 1, indicating a gel-like structure in the batter. Similarly, Yıldız et al. (2018) observed that samples containing whey protein exhibited a higher loss tangent, indicating a gel-like structure. In contrast, those with guar gum exhibited a lower loss tangent, suggesting a more solid-like consistency.

3.8.3. Apparent viscosity

The influence of different types of whey on the apparent viscosity of the batter at various angular frequencies is illustrated in Fig. 3. All the batter samples (*complex viscosity (η)* increases and **angular frequency (ω)** decreases) exhibited non-Newtonian pseudoplastic behavior (Table 5). The superior elasticity of the BWP batter, indicated by its significantly ($p < 0.05$) higher storage modulus (G′) directly influences the processing and sensory outcomes by forming a robust network that traps fermentation gases more efficiently during steaming, resulting in a softer and more uniform porous structure (Fig. 4b) with higher sensory acceptability as compared to BWWPC (Table 1), which highlights a clear link between rheology and consumer perception through integrated analysis (Table 5). This increase in viscosity can be attributed to the ability of whey to absorb water and interact with proteins and starches from flour, forming a network that traps free water (Ahmadi et al., 2022). Yıldız et al. (2018) also observed a similar shear-thinning behavior (pseudoplasticity) in cake batter containing buckwheat flour,

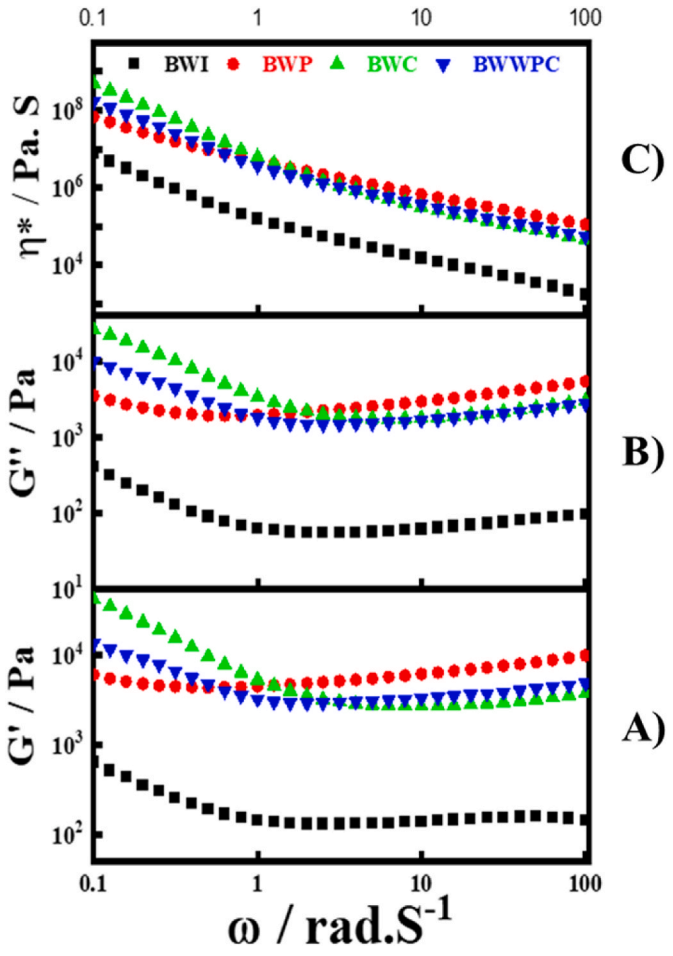

Fig. 3. Rheological properties of the black wheat *idli* batter formulations fortified with different whey as a function of angular frequency (ω) are: **BWI** (Control, ■), **BWP** (Paneer Whey, ●), **BWC** (Cheese Whey, ▲), and **BWWPC** (Whey Protein Concentrate, ▼) for (A) Storage modulus (G′), (B) Loss modulus (G″), and (C) Complex viscosity (η*).

gums (xanthan and guar), and proteins (soy and whey proteins).

3.9. Thermal properties by differential scanning calorimetry

Starch granules are characterized by their semicrystalline structure and undergo swelling as water infiltrates during gelatinization. DSC is instrumental in monitoring the heat flow during critical transitions for starch gelatinization and protein denaturation, which are essential in food processing (Kaur & Gill, 2021). Whey proteins (β-lactoglobulin) may influence the gelatinization properties of black wheat starch. The control sample (BWI) exhibited the lowest peak gelatinization temperature (Tp = 63.84 °C). Among the fortified samples, the Paneer whey formulation (BWP) exhibited a significant increase in T_p (102.84 °C) compared with the BWC (84.19 °C) and BWWPC (99.51 °C) formulations. The increase in gelatinization temperature is attributed to whey protein–starch interactions, where hydrophilic proteins promote a more ordered starch network and enhanced crystallinity, thereby increasing thermal stability (Kumar et al., 2021). Meanwhile, BWWPC (99.51 °C) significantly lower peak gelatinization temperature was observed as compared to BWP (102.84 °C) primarily because of heat-induced denaturation of whey proteins, exposing hydrophobic regions that bind to starch which hinders water penetration and slightly lowers the gelatinization temperature (Kan et al., 2021; Kumar et al., 2022) (Table 5; Supplementary Fig. 1). Additionally, whey proteins may improve the mechanical strength and water absorption of starch gels by forming a continuous protein matrix around swollen starch granules (AGUILERA & Baffico, 1997). The lower ΔH in BWWPC can be attributed to whey protein concentrate–starch interactions that disrupt the crystalline order, alter water distribution and weaken amylose–amylopectin associations, thereby reducing the energy required for gelatinization of starch granules (Kumar et al., 2022). These findings demonstrate that the pasting and thermal properties of whey protein-fortified starch systems can be elucidated using thermodynamic concepts such as free volume which describe the phase behavior in asymmetric biopolymer systems (Van der Sman, 2012).

3.10. X-ray diffraction

The BWI *idli* exhibited a typical A-type crystalline structure with firm single diffraction peaks observed at approximately 15° (2θ) and 23° (2θ), and double diffraction peaks near 17° and 18° (2θ). These peaks are characteristic of cereal starches, indicating retention of the native starch crystalline structure in black wheat. Similar to the findings of Qiu et al. (2015), any modifications in the starch structure likely occurred in the amorphous regions of the granules, because the crystallization pattern remained consistent across treatments. However, variations in whey type influenced the peak intensity and sharpness. The A-shaped curve of BWI exhibited distinct sharp peaks which indicate a well-organized crystalline structure. In contrast, the addition of whey (BWP, BWC and BWWPC) resulted in broader or less resolved peaks, suggesting a reduction in starch crystallinity or a disruption of the crystalline arrangement of starch. The BWP samples, which contained paneer whey, displayed sharper peaks at 15.0134° (2θ) and 17.3278° (2θ) than BWI. This could be attributed to the higher residual lactose and organic acids in paneer whey, which can form crosslinks with starch molecules and enhance starch crystallinity through ionic interactions, leading to sharper diffraction peaks in the XRD patterns (Sun et al., 2024). In contrast, BWC (cheese whey) exhibited slightly broader peaks at 15.0885° (2θ) and 17.1804° (2θ) which may be due to the higher calcium content that interacts with the starch granules, disrupting the starch lattice and resulting in broader diffraction peaks (Madureira et al., 2013). The BWWPC showed diffuse diffraction peaks, particularly at 15.1479° (2θ) and 17.2799° (2θ), indicating protein-starch interactions that might have altered crystallinity (Kumar et al., 2021). Furthermore, protein–starch interactions have been reported to have a delaying effect on amylopectin retrogradation which reduces crystallinity and enhances thermal stability. This aligns with recent research demonstrating a positive correlation between the proportion of resistant starch and the quantity of protein added (Yang et al., 2019). This effect of protein fortification may be attributed to the enhanced ordered structure of amylose and amylopectin in the compact system.

3.11. SEM with EDX

BWI exhibited a smooth surface with visible pores and indistinct starch granule boundaries, which suggests minimal component interactions (Fig. 4a). Conversely, paneer whey (BWP) (Fig. 4b) and cheese whey (BWC) (Fig. 4c) addition in *idli* enhanced starch-protein interactions, embedding starch granules in a finer protein matrix, resulting in a finer texture and more uniform pores compared to BWI. BWWPC (Fig. 4d) displayed weaker starch-protein interactions, a more porous structure, irregular pores, and nearly disappeared starch morphology, possibly due to the covalent binding of WPI to starch molecules and destruction of the protein secondary structure, allowing full molecule extension and globular structure loss. Gelatinization of WPI before the formation of starch granules may hinder starch swelling (Ren et al., 2017). Similar results have been observed with the addition of WPI to proso millet starch (Zheng et al., 2020).

Potassium (K) exhibited the highest concentration across all samples, particularly in BWC and BWWPC (Table 6; Supplementary Fig. 2). Calcium (Ca) was consistently present at significant levels, with BWC

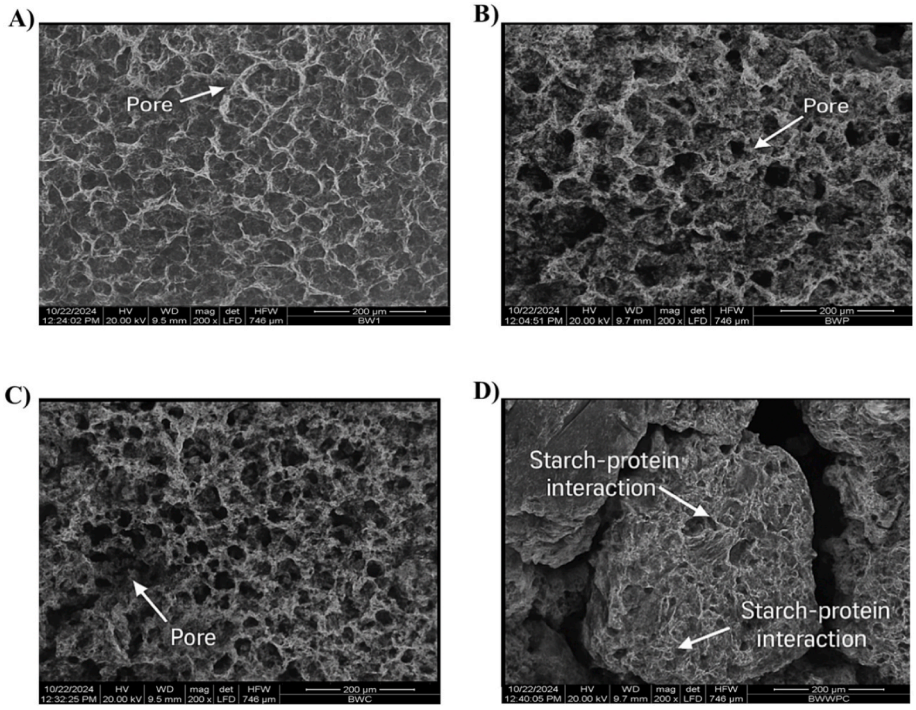

Fig. 4. Scanning electron microscopy (SEM) micrographs of the dried black wheat *idli* mix from the four formulations: (A) BWI (Control, prepared with water), (B) BWP (Paneer Whey) and (C) BWC (Cheese Whey), (D) BWWPC (Whey Protein Concentrate). All images taken at 200 × magnification; scale bar = 200 μm.

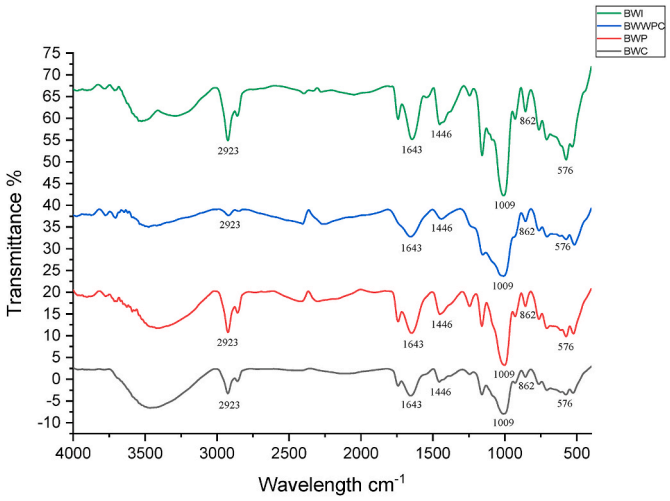

Fig. 5. Fourier Transform Infrared (FTIR) spectra of the four dried *idli* mix formulations. The spectra correspond to: BWI (Control), BWP (Paneer Whey), BWC (Cheese Whey), and BWWPC (Whey Protein Concentrate).

demonstrating the highest concentration. Magnesium (Mg) substantially reduced the BWC, indicating markedly lower levels in this sample. Sulfur (S) was notably elevated in BWWPC, potentially attributable to sulfate compounds in the whey protein concentrate. Chlorine (Cl) was detected in BWP, BWC, and BWWPC, but was absent in BWI.

3.12. FTIR spectroscopy

The FTIR spectra of the black wheat rawa *idli* instant mix with different types of whey are presented in Fig. 5. Starch granules are primarily composed of amylose and amylopectin, which possess a semi-crystalline structure that undergoes swelling, melting, and disruption when heated in the presence of water. Whey proteins contribute to the stabilization of amorphous regions, thereby elevating the glass transition temperature and potentially impeding gelatinization. A broad peak around 3400 cm^{-1}, attributed to O–H stretching and showed slight shifts from 3399 cm^{-1} in BWI to higher wavenumbers (3413 cm^{-1} in BWP, 3410 cm^{-1} in BWC, and 3405 cm^{-1} in BWWPC), indicating enhanced hydrogen bonding due to whey incorporation. The consistent C–H stretching peak at ~2923 cm^{-1} across all samples suggests the presence of aliphatic chains. Notably, the Amide I region (1600–1700 cm^{-1}) intensified in whey-fortified samples particularly around 1655 cm^{-1} which reflect β-sheet formation from protein denaturation and aggregation, thereby strengthening the gel matrix (Zhang et al., 2022). The Amide II peak (~1556 cm^{-1}) and CH_2 vibrations (~1415 cm^{-1}) correspond to the CH_2 groups of the amino acid side chains. Peaks near 1000 cm^{-1} (C-O stretching vibrations) were observed at 998 cm^{-1} for BWI, and ~990-992 cm^{-1} for BWP, BWC, and BWWPC. The band intensity ratio at 1022–950 cm^{1} indicates short-range order which reflects the double-helical structure and amylose content. Minimal changes in starch and protein absorption peaks suggest covalent bonding between the proteins and starch molecules.

4. Conclusions

This study successfully demonstrated the valorization of dairy by-products through the fortification *of* semolina (rawa) black wheat *idli*, comprehensively characterizing its functional, nutritional, rheological, and metabolomic properties. The integration of whey-based ingredients with traditional fermented foods presents interesting possibilities within the framework of food innovation and sustainability. Nutritionally, *idli* fortified with whey protein concentrate (WPC) exhibited the highest protein content (24.76 g/100 g) and the lowest concentrations of phytate and saponin. However, it received the lowest sensory scores because of its excessive hardness, necessitating texture improvement via hydrocolloids, fermentation optimization, or enzymatic treatments to alter protein structure and enhance palatability without compromising its nutritional advantages. Functionally, paneer whey (BWP) resulted in a highly elastic batter with a superior, uniformly porous structure, while

cheese whey (BWC) offered a unique profile of minerals and essential amino acids (threonine) which contribute to its high overall acceptability. Metabolomics identified metabolites in whey fortified formulations, including BWC (535), BWP (485), and BWWPC (460), and bioactive compounds including amino acids, fatty acids, polyphenols, and vitamins with higher threonine and quercetin levels in the BWC, offering molecular-level evidence for its enhanced nutrient bioavailability and functional potential. By integrating rheological, physicochemical, sensory, and metabolomic analyses, this study provides valuable insights into the interactions between milk proteins and black wheat components during fermentation, thereby supporting the development of nutrient-rich functional foods. Although promising, these findings are currently based solely on metabolomic data with limited sensory evaluation and require validation through in vivo studies, clinical trials, large-scale consumer acceptance testing, and pilot-scale trials to confirm the health benefits, market potential, and economic feasibility of fortified *idli* formulations. This study presents a promising and well-characterized strategy for sustainable food innovation by upcycling dairy by-products into nutrient-rich food which proposes a potentially scalable strategy to reduce dairy waste, utilize underutilized black wheat, and contribute to the principles of a circular economy. However, more research is required to assess scalability and environmental impact.

CRediT authorship contribution statement

Ankur Aggarwal: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Tarun Verma:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Kalyan Ghadei:** Writing – review & editing. **Pralay Maiti:** Writing – review & editing. **Rohit Sharma:** Writing – review & editing. **Satyender Singh Yadav:** Writing – review & editing. **Sunil Kumar:** Writing – review & editing. **Alok Kumar:** Writing – review & editing. **Kow-Tong Chen:** Writing – review & editing, Funding acquisition.

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethics declarations

Sensory evaluation procedures were approved by the advisory/ethical committee (Ref. No. DSFT/2024–2025/AEC/01 dated July 16, 2024).

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2025.118637.

Data availability

Data will be made available on request.

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