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Compositional variability in roller-mill processed streams: distribution of macro- and micronutrients, phytochemicals, and contaminants (heavy metals and anti-nutrients)

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This study investigated the micro- and macronutrient compositions, phytochemicals, heavy metals, and antinutritional factors of industrial wheat milling streams, including straight run flour (SRF), breaks (B1–B4), reductions (C1–C5), and by-products (fine bran, coarse bran, and germ). Specific streams such as B2, C1, C2F1, and C2F2 exhibited higher brightness, while the proximate composition varied significantly (p <0.05) across streams. Endosperm-rich flours were dominated by proline (6.07–14.19%) and glutamic acid (22.97–41.40%), whereas germ was richer in lysine (6.28%), arginine (7.13%), and threonine (4.27%). Break flours contained significantly (p <0.05) lower mineral levels but higher gluten strength (10.07–15.47%), while by-products were abundant in phenolics (127.16–138.80 mg GAE/100 g), carotenoids (0.115–0.141 mg/100 g), and antioxidant capacity (FRAP: 36.08–69.19 μmol Fe^{2+} per g). The particle size distribution (PSD) (<50 μm: 0.86–38.38%) influenced the functionality, with finer fractions showing significantly reduced phytochemical content. Heavy metals and anti-nutrients (tannins: 0.59–1.397 mg g^{-1}; phytic acid: 0.157–0.167%) were concentrated in bran fractions. Principal component analysis (PCA) revealed substantial variability, with PC1 and PC2 explaining 53.5% and 12.52% of the variation, respectively. Overall, these findings emphasize the potential of precision milling and strategic stream recombination to develop tailored ingredients, refined flours optimized for functionality, and by-products enriched for nutritional fortification. Additionally, understanding the distribution of acrylamide formation precursors (amino acids and reducing sugars) provides opportunities to minimize acrylamide formation in end products.

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Sustainability spotlight

This study highlights the untapped sustainability potential of industrial wheat milling by showing how different flour streams and by-products can be strategically valorized. Endosperm-rich fractions, such as B2, C1, C2F1, and C2F2, provided refined flours with desirable brightness, gluten strength, and functional suitability for baked goods. In contrast, bran and germ fractions, often relegated to by-products, were shown to be nutrient-dense reservoirs rich in essential amino acids, phenolics, carotenoids, and antioxidant capacity. By mapping micro- and macronutrients, bioactive compounds, heavy metals, and antinutritional factors across streams, the study underscores the value of precision milling as a tool for circular food systems. Rather than viewing bran, germ, and coarse fractions as waste, they can be repositioned as functional fortifiers in nutrition-focused formulations. Importantly, insights into particle size distribution and acrylamide precursors (amino acids and reducing sugars) offer pathways for reducing food-processing contaminants, aligning with sustainable production and safer consumption goals (SDG 12). Together, these findings show that targeted recombination of streams can reduce post-harvest losses, promote whole-grain utilization, and expand the nutritional portfolio of wheat-based foods. Its emphasis on by-product valorization and flour blending optimization for better nutrition and safety highlights its importance for sustainable milling practices and food system innovation.

1. Introduction

Wheat milling is one of the world's oldest and most widespread food industries, evolving significantly in technology, equipment, automation, and management. Key drivers of this change include globalization and food safety demands.[1] While globalization has expanded the market for milling products, a significant challenge remains to adhere to regional standards when

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exporting locally processed raw materials using domestic machinery. The valorization of roller-mill flour streams presents a significant opportunity to enhance flour-based products' nutritional quality and functional properties. These streams, consisting of various fractions obtained during wheat milling, exhibit diverse distributions of essential nutrients, including proteins, vitamins, amino acids, minerals, sugars, bioactive compounds, heavy metals, and anti-nutrients. Understanding their nutritional profile is crucial for optimizing their utilization in creating specialty flour/choosing the right flour streams for food fortification and functional food development.[2]

The endosperm is first separated from the bran layers and then crushed into many milling stream flours during the mechanical progressive reduction of milling wheat.[3] The quality of grain and milling process flow determines the number and quality of the milling fractions produced.[4] Roller mills are the most popular mills for making wheat flour used in the bakery and cereal-based industries. This procedure involves successively grinding cleaned and conditioned wheat with corrugated and smooth rolls, sifting, and purification. A certain amount of flour is created in each phase, removed, and blended in appropriate ratios to make the required flour.[5]

Proteins (10–15%), lipids (1.5–2%), and accessible and unavailable carbohydrates (65–70%) constitute the majority of the chemical components of wheat. Because of the uneven distribution of these elements throughout the wheat kernel, separate streams have varied compositions and functional features.[6] The composition of various anatomical sections and the endosperm zone from which they originate determines the functional qualities of the flour fractions acquired at different technological stages. The type of millstream affects the granularity, presence of damaged starch, protein content, ash level, fat content, and the degree of enzymatic activity and amount of fibers,[4] as well as the presence of amino acids, minerals, phytic acid, and bioactive compounds. With more breaks and reductions, protein and ash content increased.[7] The streams created during the later reduction passes include more lipids. Many experimental studies[8–11] have reported the distribution of hydrolytic (amylases, proteases, *etc.*) and oxidation–reduction (oxygenase, polyphenol oxidase, peroxidase, *etc.*) enzymes in different mill streams, typically about the dough and breadmaking. Every *et al.*[10,11] also investigated the breadmaking characteristics of wheat milling fractions with glutathione and oxidized glutathione concentrations.

Knowing different flour streams' composition, function, and quality characteristics is crucial for choosing the right ones for different end products.[4] Breaking, size splitting, and reduction are three milling operations that produce flour mill streams that vary in composition, rheological characteristics, and overall technical usefulness. As a result, flour mill streams may be used to determine how effectively milling processes work and how to blend flour mill streams in the right proportions to produce tail or end-milled flour. Such flours have specific end-use characteristics that satisfy consumer preferences.[12] The quality of the wheat grain dictates the type and amount of flour produced at each break, and the reduction rollers are located next to the sizing (sifter and purifier). A miller carefully combined the resultant mill streams to create a variety of flours with various qualities and levels of fineness for different end purposes. One may regulate the flour's baking qualities by carefully selecting the flour streams to connect.[13,14]

Numerous studies have shown that understanding the variety of physicochemical and rheological characteristics of mill streams is essential to maximize the quality of the end product.[15,16] It is important to note that straight-grade flour was created during milling by combining all flour streams from each roll. Specialty flour, on the other hand, is created by combining flour mill streams to obtain the appropriate quality for final goods such as bread, cookies, biscuits, and cakes.[14,17]

Many prior investigations on wheat milling streams were narrow in scope, focusing on a limited set of components such as basic macronutrients or isolated compound classes. However, few studies have comprehensively examined the co-distribution of essential nutrients, phytochemicals, and potential contaminants across roller-milled wheat streams. To bridge this gap, our study provides a multi-parameter analysis by exploring the compositional differences across industrial roller-milled streams (break, reduction, and by-products), simultaneously assessing macro- and micronutrients, amino acids, sugars, heavy metals, anti-nutrients, and bioactive compounds.

This integrated approach aims to identify high-value streams that can be leveraged to improve dietary intake, promote health benefits, and maximize the potential of wheat milling by-products, thereby reducing waste.

2. Materials and methods

2.1. Raw materials

Medium hard wheat (*Triticum aestivum*) was procured from a local market in Mysuru, Karnataka, India. They were cleaned using a Labofix laboratory cleaner (Brabender, Germany).

2.2. Wheat milling process

The wheat milling process began with controlled conditioning, where cleaned wheat kernels were hydrated to 15% moisture content using an automated moisture control system (MYFC-MOZF, Buhler AG, Switzerland) and tempered at 24 ± 2 °C for 24 hours. Milling was conducted using an industrial-scale Buhler roller mill with programmable logic control at CFTRI's facility in Mysuru, India. The mill configuration consisted of four break rolls (B1–B4), five reduction rolls (C1–C5), two purifiers (S1–S2), and bran finishers that separated three by-product streams: fine bran (FB), coarse bran (CB), and germ (GE). Operating at 20 metric tons per day capacity, the mill achieved a 72% flour extraction rate with 25–26% bran yield. Critical to the process was the break stream configuration, where adjustable roller gaps (0.1–2.5 mm) were precisely set according to kernel size distribution to optimize endosperm release, with the first two breaks (B1–B2) showing the most significant operational variability due to their initial kernel disintegration role. Two flour types were collected for analytical purposes: progressive-milled fractions from individual streams and

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straight-run flour as a composite blend. All milling parameters followed the AACC Method 26-21.02 standards, maintaining roller speed differentials of 2.5 : 1 for break rolls and 1.25 : 1 for reduction rolls to ensure consistent particle size reduction and separation efficiency.

2.3. Particle size analysis of wheat flour mill streams

The PSD of flour streams obtained from the roller mill was measured using a Microtrac S3500 particle size analyzer (Montgomeryville, PA). This instrument employs three red laser diodes for optimal alignment, ensuring accurate particle size characterization. Data analysis was performed using the integrated Flex software within the Microtrac system. To ensure reliability, triplicate measurements were taken for each sample, and the mean value was reported as the final result.

2.4. Color analysis of wheat flour mill streams

The stream color was assessed using a Hunter Lab Color Measuring System (LabScan XE, Reston, VA). Before measurement, the instrument was calibrated with a barium sulfate standard whiteboard, ensuring 100% reflectance. Samples were loaded into the holder, and reflectance values were recorded across a 360–800 nm wavelength range. The L^* value indicated lightness (0 = pure black; 100 = pure white), while a^* values represented red (positive) or green (negative) tones, and b^* values denoted yellow (positive) or blue (negative) hues. Total color difference (ΔE) was calculated using the standard formula.

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

$$\Delta L = L_2 - L_1,\ \Delta a = a_2 - a_1,\ \Delta b = b_2 - b_1$$

where L_2, a_2, and b_2 = standard values; L_1, a_1, and b_1 = sample values.

2.5. Nutritional analysis of milled streams

All streams were comprehensively analyzed for key quality parameters using approved AACC International methods.[18] Oven drying determined moisture content (Method 44-15.02), while protein content was measured *via* the Kjeldahl nitrogen procedure (Method 46-10.01) with a conversion factor of 5.7. Total fat content was quantified by ether extraction (Method 30-10.01), and ash content was assessed by incineration at 550 °C (Method 08-01.01). By hand washing, gluten quality was evaluated through dry gluten determination (Method 38-10.02). The wheat milled streams' reducing and total sugar levels were evaluated following the AOAC method 939.03.[19] Reducing sugars were reported as glucose equivalents in grams per 100 g of flour. Each analysis was conducted in triplicate, and the results were expressed as mean ± standard deviation (SD).

2.6. Amino acid analysis of milled streams

The amino acid composition of roller-milled stream samples was determined using cation-exchange chromatography following acid hydrolysis. Samples (100 mg) underwent hydrolysis with 6 M hydrochloric acid (1 mL containing 0.01% phenol) at 110 °C for 24 h in sealed tubes under a nitrogen atmosphere to prevent oxidative degradation. After evaporation of the hydrolysate, the residue was dissolved in 0.01 M HCl (1 mL) and neutralized with 0.01 M NaOH.

The analysis was performed on an S433 amino acid analyzer (Sykam GmbH, Germany) equipped with a sodium-based cation-exchange column (NACA K07/Li; 150 × 4.6 mm; 7 μm particle size). Before injection, samples were filtered through 0.2 μm membrane filters to remove particulate matter. The system employed post-column ninhydrin derivatization with dual-wavelength detection at 440 nm (proline) and 570 nm (primary amino acids). The method was adapted from an established protocol[20] with modification[21] to optimize separation conditions for wheat protein hydrolysates.

2.7. Heavy metal analysis

Approximately 0.5 g of each stream sample was weighed into Teflon tubes. Then, 6–7 mL of concentrated HNO_3 and 500 μL of H_2O_2 were added for digestion. Additionally, 500 μL of 10 ppm yttrium (Y) was added as an internal standard. Before sealing the tubes, the samples were allowed to stand for 10–15 minutes for pre-digestion. After complete digestion, the solutions were diluted to a final volume of 50 mL with deionized water. A PerkinElmer ICP-MS (operated in KED mode) was used for analysis. The instrument was calibrated using a minimum of five linearity points, followed by a blank run and subsequent sample measurements. The concentrations of heavy metals (Pb, As, Cd, Hg, Cu, and Sn) were determined and reported in ppm (mg kg^{-1}) on a sample weight basis.[18]

2.8. Phytochemical assays

2.8.1. Carotenoids. Carotenoids were extracted from 1 g of each milling stream using 7 mL acetone : petroleum ether (1 : 1 v/v) mixture, which were taken in 15 mL tubes and mixed by rotating (30 rpm, 1 h, and 25 ± 2 °C) the tubes. After centrifugation (3000×*g*, 7 min, and 20 °C), the supernatant was mixed with 5 mL of water, vortexed (15 s), and the petroleum ether layer was extracted. Absorbance (450 nm) was recorded (Shimadzu UV-1800) against a petroleum ether blank. Content was calculated *via* a β-carotene standard curve (0–10 μg mL^{-1}; R^2 >0.99) as μg β-carotene equivalents per g. The method, adapted from Giordano *et al.*,[22] included bran-specific optimizations for matrix effects.

2.8.2. Total flavonoid content (TFC). For TFC analysis, crude extracts from each stream (0.1 mL) were diluted to an optimal concentration using absolute methanol. The diluted samples were then reacted with 1 mL of freshly prepared aluminum chloride solution (2% w/v in methanol) and allowed to stand at ambient temperature (25 ± 1 °C) for 15 minutes to ensure complete complex formation. The reaction mixtures were subsequently analyzed using a UV-visible double-beam spectrophotometer (model UV-1800, Shimadzu Corporation, Kyoto, Japan) with absorbance measurements taken at 430 nm against a methanol blank. Quantification was performed using

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a quercetin standard calibration curve (0–100 μg mL^{-1}; R^2 = 0.998), with results expressed as milligrams of quercetin equivalents per 100 grams of dry sample (mg QE/100 g). This spectrophotometric method follows the modified aluminum chloride complexation procedure described initially by Okarter *et al.*,[23] with adaptations for wheat matrix analysis.

2.8.3. Total phenolic content (TPC). The TPC of flour stream extracts was quantified using the Folin–Ciocalteu spectrophotometric method with modifications for wheat matrices. In brief, 0.1 mL of methanolic extract was combined with 2.9 mL of deionized water in a 10 mL test tube. After adding 0.5 mL of Folin–Ciocalteu reagent (1 N), the mixture was vortex-mixed (30 s) and allowed to react for exactly 3 min at 25 ± 1 °C. Subsequently, 2 mL of sodium carbonate solution (20% w/v, prepared fresh daily) was added, and the reaction mixture was immediately vortexed again (60 s) before immersion in a 40 °C water bath for precisely 1 min to initiate color development. After cooling to room temperature in darkness (25 min), the absorbance of the blue chromophore was measured at 650 nm against a reagent blank using a UV-1800 spectrophotometer (Shimadzu). Quantification was achieved using a six-point gallic acid standard curve (0–800 μg mL^{-1}, R^2 >0.9979), with results expressed as milligrams of gallic acid equivalents per 100 grams of dry sample (mg GAE/100 g DW). This protocol was adapted from the classical method of Okarter *et al.*[23] with optimization for cereal samples.

2.8.4. Total antioxidant assay. The phosphomolybdenum method[23] was adapted to quantify the total antioxidant capacity across all milling fractions. For analysis, 100 μL of methanolic extract from each fraction was combined with 200 μL of ultrapure water and 1.8 mL of freshly prepared reagent solution (4 mM ammonium molybdate, 28 mM sodium phosphate, and 600 mM sulfuric acid) in screw-cap tubes. The reaction mixtures were incubated at 95 ± 1 °C for 90 minutes in a precision water bath to ensure complete complex formation. After cooling to room temperature (25 ± 2 °C), the absorbance of the green phosphomolybdenum complex was measured at 695 nm against a reagent blank using a UV-2600 spectrophotometer (Shimadzu) with 10 mm pathlength quartz cells. A five-point ascorbic acid standard curve (20–200 μg mL^{-1}; R^2 >0.9946) was prepared daily for quantification. Results were expressed as milligrams of ascorbic acid equivalents per 100 grams of dry sample (mg AAE/100 g DW).

2.8.5. Ferric-reducing antioxidant power (FRAP) assay. FRAP was measured by mixing 100 μL methanolic extract with 250 μL phosphate buffer (0.2 M, pH 6.6) and 250 μL potassium ferricyanide (1% w/v), incubating them (50 ± 0.5 °C and 30 min). Reactions were stopped by adding 250 μL trichloroacetic acid (10% w/v), centrifuged (8000×*g*, 10 min, and 4 °C), and the supernatants were reacted with ferric chloride (0.1% w/v). Absorbance (700 nm) was measured (Shimadzu UV-1800) against a blank. Quantification used an ascorbic acid standard curve (20–150 μg mL^{-1}; R^2 >0.9858), with results expressed as μmol AAE per g DW. Adapted from Okarter *et al.*[23] for wheat matrices.

2.8.6. Anti-nutrients. The distribution of tannins and phytic acids in milled fractions was analyzed using the Megazyme kit method. The procedure was carried out according to the instructions in the kit.

2.9. Statistical analysis

Statistical calculations were performed using Microsoft Excel 2016 (Microsoft Corporation, USA), with results expressed as mean ± standard deviation (SD). Data analysis was conducted using SPSS 16.0 (IBM, USA), where analysis of variance (ANOVA) was applied to determine the significance. Mean differences were adjusted using the Tukey multiple range test, with statistical significance set at p <0.05.

Multivariate analysis: principal component analysis (PCA) was applied to evaluate the chemical compositions of roller-milled fractions. The multivariate analysis of flour fractions was carried out using OriginPro 2025 (Learning edition) statistical software (version 10.2, OriginLab).

3. Results and discussion

The roller milling process produced SRF with 72% extraction rate, demonstrating efficient separation of wheat kernel components. Two flour types were collected for analytical purposes: progressive-milled streams and SRF as a composite blend. Our analysis revealed significant variations ($p \leq 0.05$) in physicochemical and nutritional characteristics across milling streams, including PSD, color parameters, phytochemicals/bioactive compounds, amino acid profiles, and heavy metal content. These components showed distinct partitioning patterns among the three main mill stream categories, break flours (B1–B4), reduction flours (C1–C5), and by-products (FB, CB, and GE). The GE contained 20–30% CB contamination, reflecting the practical challenges of achieving complete germ purity in our-scale milling operations. The comprehensive distribution patterns of these chemical compositions and phytochemical components across milling fractions are presented and discussed in detail in subsequent sections, highlighting opportunities for targeted stream utilization based on their unique compositional profiles.

3.1. Particle size distribution

Wheat flour fractions from the break (B1–B4) and reduction (C1–C5) streams showed significantly different ($p \leq 0.05$) patterns in their PSD (Table 1), indicative of their different compositions and milling sources. B1 and B2 displayed comparable distributions in the break streams, with B1 having a relatively uniform, medium-fine texture typical of early break flours, with 51.38% of its particles falling between 50 and 100 μm and 19.97% finer than 50 μm.[3] B2 contained a significantly greater percentage of particles between 100 and 150 μm (35.69%), indicating coarser fragmentation, whereas B3 maintained a balance between intermediate (28.67% at 100–150 μm) and finer (49.55% at 50–100 μm) particles. Among break streams, B4 was notable for having the highest percentage of particles <50 μm (38.38%) and the lowest fraction of particles >150 μm (3.52%), which reflected its finer, more refined nature as a result of progressive endosperm purification.[24]

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Table 1 Particle size distributions and color of streams[a,b]

	Particle size distribution				Color values			
Fractions	<50 μm	50–100 μm	100–150 μm	>150 μm	L^*	a^*	b^*	DE
B1	19.97	51.38	20.84	7.81	87.37 ± 0.01^{c}	0.62 ± 00^{g}	$8.98 \pm 0.02^{f,g}$	8.28 ± 0.05^{f}
B2	12.48	44.52	35.69	7.31	87.58 ± 0.06^{c}	0.56 ± 0.01^{h}	9.12 ± 0.01^{f}	8.23 ± 0.02^{f}
B3	15.67	49.55	28.67	6.11	87.22 ± 0.01^{c}	0.64 ± 00^{g}	9.65 ± 0.03^{f}	8.87 ± 0.06^{f}
B4	38.38	45.89	12.12	3.52	85.83 ± 0.07^{d}	0.79 ± 00^{f}	9.77 ± 0.00^{f}	9.88 ± 0.05^{e}
C1F1/C1F2	24.59	41.24	26.64	7.53	89.55 ± 0.02^{a}	0.25 ± 0.01^{j}	8.21 ± 0.01^{h}	6.35 ± 00^{h}
C2A	21.66	40.84	33.68	3.82	90.11 ± 0.01^{a}	0.23 ± 00^{j}	8.3 ± 0.01^{h}	6.84 ± 1.13^{h}
C2B	6.73	26.74	51.61	14.92	88.86 ± 0.02^{b}	0.44 ± 0.01^{i}	9.14 ± 0.02^{f}	7.52 ± 0.04^{g}
C3	0.86	8.79	57.27	33.15	84.36 ± 0.02^{e}	1.15 ± 0.02^{d}	11.17 ± 0.01^{d}	11.94 ± 0.06^{d}
C4	24.30	39.94	25.1	10.66	84.68 ± 0.02^{e}	1.06 ± 0.01^{e}	10.73 ± 0.06^{e}	11.4 ± 0.15^{d}
C5	3.93	9.37	34.84	51.85	81.06 ± 0.01^{f}	1.8 ± 0.01^{c}	11.61 ± 0.02^{d}	14.77 ± 0.05^{c}
FB	—	—	—	—	60.86 ± 0.03^{g}	5.72 ± 0.01^{b}	15.91 ± 0.01^{c}	34.98 ± 0.08^{b}
CB	—	—	—	—	56.79 ± 0.03^{i}	6.59 ± 0.01^{a}	16.33 ± 0.02^{b}	39.03 ± 0.01^{a}
GE	—	—	—	—	58.25 ± 0.01^{h}	6.21 ± 0.01^{a}	17.05 ± 0.06^{a}	37.86 ± 0.09^{a}
SRF	21.52	38.85	32.97	6.66	87.75 ± 0.15^{c}	0.42 ± 0.005^{i}	8.45 ± 0.01^{g}	7.6 ± 0.09^{g}

[a] Measurements with different letters (a, b, and c) are significantly different from each other ($p < 0.05$). Measurements that share the same letter are not significantly different. [b] Breaks – B1, B2, B3, & B4; reductions – C1F1/C1F2, C2F1, C2F2, C3, C4, C5; FB – fine bran; CB – coarse bran; GE – germ; SRF – straight run flour.

However, the reduction streams C1 and C2A showed no significant changes when compared to those of early break flours; according to Doblado-Maldonado *et al.*,[25] C1 had 41.24% particles in the 50–100 μm range and 24.59% particles of <50 μm size. The greater percentage of 100–150 μm particles (33.68%) in C2A was probably produced by leftover bran fragments. However, there were significant ($p \leq 0.05$) changes in C2B and C3, with C2B having 51.61% particles in the 100–150 μm range and C3 having 57.27% particles in this fraction and 33.15% of >150 μm size. This suggests that the material was coarser and less refined from later reduction stages. C5 was the coarsest stream, with 51.85% of particles of >150 μm size, indicating considerable bran contamination, whereas C4 returned to a finer profile (39.94% of 50–100 μm size).

With 38.85% particles in the 50–100 μm range and 32.97% particles in the 100–150 μm range, SRF flour showed a balanced distribution that closely resembled C1 and B1, suggesting that it was composite from early break and reduction streams. According to Posner and Hibbs,[26] the <50 μm fraction (21.52%) satisfies the requirements for premium flour in baking applications. For superior baking, break flours (B1–B3), which are finer and more consistent, were used. the later reduction streams (C3 and C5) are coarser, they might need to be blended or reprocessed. Similar trends were observed in the study by Mahajan *et al.*[27] and Hitlamani and Inamdar.[21] The intermediate particle size of straight-run flour contributes to its adaptability in industrial settings. As detailed in the following discussion, the PSD among the milled fractions directly affects their color, chemical composition, and bioactive content.

3.2. Color characteristics of fractions

Color parameters (L^*, a^*, b^*, and ΔE) revealed significant differences ($p \leq 0.05$) among milling streams, reflecting varying bran contamination and compositional features (Table 1). Break flours (B1–B3) maintained high brightness (L^*: 85.83–87.58), indicating pure endosperm material, while B4 showed slightly lower L^* (85.83) due to increased number of bran particles.[4] Yellowness (b^*) progressively increased from B1 (8.98) to B4 (9.77), suggesting carotenoid accumulation in peripheral layers.[28]

Reduction streams exhibited more pronounced variations, with early fractions (C1–C2A) displaying the highest purity (L^*: 89.55–90.11; a^*: 0.23–0.25; b^*: 8.21–8.30). Later reduction streams (C3–C5) showed significantly increased bran content (L^*: 81.06–84.68; a^*: 1.06–1.80; b^*: 10.73–11.61). The total color difference (ΔE) ranged from 6.35 (C1) to 14.77 (C5), demonstrating progressive color change from pure white flour.

By-products displayed distinct color profiles, with GE showing maximum yellowness (b^*: 17.05) and CB exhibiting the darkest color (L^*: 56.79) and highest redness (a^*: 6.59). These color variations have important quality implications, bright white flours (C1–C2A) suit refined products, while more colored fractions (B3–B4 and C3–C5) may serve whole grain applications. SRF showed premium quality characteristics (L^*: 89.10; ΔE: 6.95), confirming effective bran separation.[21] The results demonstrate how milling progressively segregates kernel components by color attributes, enabling targeted stream utilization based on end-use requirements.[26]

3.3. Nutritional composition

The nutritional composition of milled fractions varied significantly ($p < 0.05$) across different streams, as presented in Table 2 (on a dry weight basis). This distribution reflects the heterogeneous nature of wheat kernel components, with distinct nutritional profiles emerging from the break, reduction, and by-product fractions. The comprehensive quantitative data in Table 2 demonstrate how modern roller milling effectively segregates these nutritional components into specific flour streams, due to the PSD in flour streams.[21] Similar trends were

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Table 2 Nutritional profile and mineral distribution in streams[a]

Proximate (%)	SRF	Break				Reduction						By-products		
		B1	B2	B3	B4	C1F1/ C1F2	C2F1	C2F2	C3	C4	C5	FB	CB	Germ
Moisture	11.26 ± 0.02^{c}	12.12 ± 0.01^{a}	12.05 ± 0.04^{a}	12.01 ± 0.00^{a}	10.03 ± 0.01	12.84 ± 0.02^{a}	12.50 ± 0.01^{a}	12.57 ± 0.03^{a}	12.43 ± 0.05^{a}	11.92 ± 0.02^{b}	9.02 ± 0.05^{e}	10.56 ± 0.04^{d}	8.00 ± 0.08^{f}	11.83 ± 0.06^{b}
Ash	0.60 ± 0.00^{g}	0.61 ± 0.01^{g}	0.66 ± 0.01^{g}	0.78 ± 0.02^{f}	1.29 ± 0.01^{d}	0.71 ± 0.03^{f}	0.54 ± 0.01^{h}	0.72 ± 0.03^{f}	0.93 ± 0.01^{e}	1.37 ± 0.00^{c}	1.23 ± 0.02^{d}	4.57 ± 0.01^{b}	4.93 ± 0.03^{a}	4.60 ± 0.01^{b}
AIA[b]	0.01	0.09	0.07	0.08	0.10	0.05	0.03	0.01	0.02	0.03	0.21	0.22	0.19	0.23
Fat	1.67 ± 0.01^{g}	1.14 ± 0.05^{i}	1.15 ± 0.05^{i}	1.81 ± 0.07^{f}	2.61 ± 0.03^{d}	1.48 ± 0.01^{h}	1.53 ± 0.05^{h}	1.92 ± 0.05^{e}	4.13 ± 0.07^{c}	4.18 ± 0.16^{c}	4.21 ± 0.09^{b}	4.12 ± 0.02^{c}	4.78 ± 0.07^{a}	4.31 ± 0.17^{b}
Protein	9.63 ± 0.02^{f}	9.70 ± 0.05^{f}	11.11 ± 0.03^{e}	12.22 ± 0.01^{d}	10.95 ± 0.05^{e}	8.31 ± 0.01^{g}	8.60 ± 0.05^{g}	8.31 ± 0.05^{g}	9.42 ± 0.00^{f}	11.18 ± 0.03^{e}	9.37 ± 0.02^{f}	17.34 ± 0.14^{b}	16.79 ± 0.11^{c}	24.32 ± 0.19^{a}
Wet gluten	33.44 ± 1.5^{c}	28.69 ± 1.21^{g}	36.85 ± 1.9^{b}	43.79 ± 2.1^{a}	31.82 ± 2.34^{e}	33.30 ± 1.42^{c}	32.24 ± 0.5^{d}	30.74 ± 0.87^{f}	29.16 ± 0.19^{f,g}	27.17 ± 1.31^{h}	26.05 ± 1.81^{i}	—	—	—
Dry gluten	11.46 ± 0.21^{d}	10.07 ± 0.17^{f}	13.03 ± 0.21^{b}	15.47 ± 0.20^{a}	12.05 ± 0.19^{c}	11.32 ± 0.11^{d}	10.92 ± 0.09^{e}	10.49 ± 0.14^{e}	10.10 ± 0.21^{e}	10.25 ± 0.089^{e}	9.12 ± 0.24^{g}	—	—	—
Minerals (mg/100g)														
Sodium	4.81 ± 0.00^{g}	2.08 ± 0.01^{i}	9.07 ± 0.02^{e}	1.50 ± 0.00^{j}	4.95 ± 0.01^{g}	22.63 ± 0.02^{b}	21.91 ± 0.01^{c}	27.84 ± 0.01^{a}	3.23 ± 0.07^{h}	6.16 ± 0.03^{f}	12.63 ± 0.09^{d}	22.56 ± 0.05^{b}	28.02 ± 0.05^{a}	9.42 ± 0.09^{e}
Magnesium	3.64 ± 0.01^{k}	2.10 ± 0.01^{l}	5.99 ± 0.01^{i}	2.11 ± 0.01^{l}	4.62 ± 0.02^{j}	16.13 ± 0.06^{d}	15.17 ± 0.03^{e}	20.90 ± 0.01^{c}	7.49 ± 0.03^{h}	9.45 ± 0.02^{g}	11.33 ± 0.08^{f}	27.90 ± 0.022^{b}	34.82 ± 0.06^{a}	19.20 ± 0.11^{c}
Phosphorus	3.10 ± 0.02^{e}	2.78 ± 0.01^{f}	2.39 ± 0.00^{f}	3.04 ± 0.02^{e}	3.17 ± 0.00^{e}	3.72 ± 0.01^{d}	3.11 ± 0.01	2.58 ± 0.00	4.92 ± 0.05^{c}	7.00 ± 0.01^{b}	7.29 ± 0.03^{b}	24.83 ± 0.08^{a}	24.85 ± 0.07 ^{a}	23.75 ± 0.06^{a}
Zinc	0.10 ± 0.03^{g}	0.07 ± 0.00^{h}	0.12 ± 0.00^{f}	0.09 ± 0.00^{h}	0.16 ± 0.00^{f}	0.54 ± 0.01^{c}	0.43 ± 0.01^{d}	0.77 ± 0.01^{b}	0.25 ± 0.01^{e}	0.21 ± 0.01^{e}	0.47 ± 0.01^{d}	1.16 ± 0.01^{a}	0.54 ± 0.03^{c}	0.43 ± 0.01^{d}
Potassium	3.52 ± 0.00^{j}	3.21 ± 0.01^{j}	2.95 ± 0.01^{k}	3.66 ± 0.05^{j}	3.91 ± 0.01^{i}	4.84 ± 0.00^{h}	4.18 ± 0.00^{h}	6.37 ± 0.05^{f}	5.66 ± 0.01^{g}	7.16 ± 0.02^{e}	8.46 ± 0.02^{d}	35.83 ± 0.03^{a}	32.51 ± 0.05^{c}	33.41 ± 0.05^{b}
Calcium	8.00 ± 0.01^{k}	3.43 ± 0.01^{m}	12.74 ± 0.01^{i}	4.06 ± 0.07^{l}	9.81 ± 0.05^{j}	36.12 ± 0.00^{c}	34.13 ± 0.01^{d}	43.90 ± 0.097^{b}	13.19 ± 0.01^{i}	15.38 ± 0.09^{h}	22.35 ± 0.07^{f}	32.81 ± 0.07^{e}	54.38 ± 0.03^{a}	18.27 ± 0.00^{g}
Iron	0.41 ± 0.01^{f}	0.37 ± 0.03^{g}	0.48 ± 0.07^{f}	0.34 ± 0.02^{g}	0.29 ± 0.00^{h}	0.89 ± 0.011^{d}	0.86 ± 0.00^{d}	1.43 ± 0.01^{b}	1.14 ± 0.01^{b}	0.45 ± 0.05^{f}	5.38 ± 0.03^{a}	0.94 ± 0.04^{c}	0.98 ± 0.09^{c}	0.61 ± 0.001^{e}

[a] The results are expressed on a dry weight basis. Measurements with different letters are significantly different from each other (p <0.05). Measurements that share the same letter are not significantly different. [b] SD is zero.

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observed in the study of Mahajan *et al.*[27] The detailed variation has been discussed as follows.

3.3.1. Moisture content. Moisture content varied significantly ($p \leq 0.05$) across milling streams (Table 2), a critical factor influencing storage stability, technical functionality, and end-product quality. In the break streams, moisture showed a progressive reduction from B1 (12.12%) to B4 (10.03%), directly reflecting the efficient separation and removal of the inherently more hygroscopic bran and germ components.[26] Within the reduction streams, the initial stream C1 contained the highest moisture (12.84%) due to its origin in the central endosperm, where pure, intact starch granules readily absorb and retain water. Conversely, the later reduction stream C5 had the lowest moisture (9.02%), attributable to its higher concentration of less hygroscopic fiber from the proximity to the bran layer. The by-products confirmed these material-driven trends, where CB (8.00%) exhibited the lowest moisture due to its porous, fibrous structure, while the GE (11.83%) and FB (10.56%) demonstrated more stable moisture levels, governed by their high lipid content. Finally, the SRF (11.26%) fell within optimal commercial ranges, ensuring safe shelf-life and consistent baking performance.[4] These variations underscore that moisture is not merely a compositional metric but a direct indicator of a stream's physical structure and chemical composition, highlighting the necessity of precise moisture control to predict shelf-life, prevent microbial spoilage, and ensure desired functional properties in final food products.[14]

3.3.2. Ash and acid-insoluble ash (AIA) distribution. Ash content varied significantly (p <0.001) across streams, increasing progressively from B1 (0.61%) to B4 (1.29%) due to bran accumulation.[21] Reduction streams showed distinct patterns: C1–C2A (0.54–0.71%) contained cleaner endosperm, while C3–C5 (0.93–1.37%) retained more bran particles. By-products exhibited the highest ash levels (CB – 4.93%; GE – 4.60%; FB – 4.57%), confirming their mineral density. Similar trends were observed for AIA, with bran/germ fractions (0.19–0.23%) showing maximum values from silica deposition,[29] while C5 (0.214%) indicated husk contamination. The straight-run flour's low ash (0.6%) and AIA (0.01%) reflect effective bran separation (Table 2).

3.3.3. Fat content. Fat content varied significantly ($p \leq 0.05$) across milled streams (Table 2), increasing progressively from B1 (1.14%) to B4 (2.61%) due to germ and aleurone incorporation. Reduction streams showed greater variation, with C1–C2F2 (1.48–1.92%) containing core endosperm lipids, while C3–C5 (4.13–4.21%) accumulated germ materials.[30] By-products exhibited the highest fat levels, bran fractions (4.12–4.78%) from aleurone lipids and germ (4.31%) despite processing losses.[21] SRF (1.67%) reflected typical commercial lipid removal. These patterns enable targeted stream selection for product-specific fat requirements.

3.3.4. Protein content distribution. Protein content varied significantly (p <0.05) across the mill streams (Table 2), peaking in the B3 fraction (12.22%) due to peripheral endosperm enrichment. Reduction streams exhibited lower values (C1–C2B: 8.31–8.60%) originating from the central endosperm, with an increase observed in later stages (C3–C4: 9.42–11.18%) due to subaleurone layer incorporation. By-products contained the highest protein levels, with the GE (24.32%) rich in metabolic and storage proteins, and bran fractions (16.79–17.34%) containing structural proteins.[31] SRF (9.63%) represented a medium-protein blend suitable for diverse baking applications. These distinct protein gradients highlight the potential for targeted flour blending to achieve specific functional properties.

The protein composition of each stream directly dictates these functional properties. For instance, the germ's abundant non-gluten proteins, while detrimental to gluten network formation and loaf volume in conventional baking, provide significant benefits, such as improved water-binding, emulsification, and lysine fortification, for plant-based and gluten-free formulations.[21] Conversely, the insoluble structural proteins in bran typically impair dough strength and crumb texture by disrupting the gluten matrix. However, their inherent association with dietary fibers makes them a valuable ingredient for enhancing water retention and nutritional density in high-fiber and low-carbohydrate food systems.

3.3.5. Gluten characteristics. Gluten quality varied significantly (p <0.05) across the breaks (B1–B4) and reductions (C1–C5) (Table 2), with break flours showing peak wet gluten in B3 (43.79%) and dry gluten in B3 (15.47%), reflecting the biochemical gradient within the wheat kernel, where the peripheral endosperm is enriched with superior gluten-forming proteins compared to the central, starch-rich endosperm. The gluten index remained stable in B1–B3 (80–81) but declined in B4 (73.36), indicating weaker gluten strength in later breaks. Reduction streams exhibited decreasing gluten content from C1 (wet: 33.30%; dry: 11.32%) to C5 (wet: 26.05%; dry: 9.12%), consistent with central endosperm origin. Early reduction streams (C1 and C2A) showed the highest gluten index (84.34–84.36), suggesting strong dough functionality. This is attributed to their composition from the subaleurone layer, a protein-dense endosperm region beneath the bran. SRF demonstrated robust dry gluten (11.46%), suitable for bread-making.[32] These findings support targeted blending of B3 and C1/C2A for high-gluten applications and B4/C4–C5 for low-gluten products, optimizing flour functionality.

3.3.6. Essential mineral distribution. The anatomical segregation of essential minerals within the wheat kernel resulted in significantly different (p <0.001) distribution patterns across break (B1–B4), reduction (C1–C5), and bran streams (Table 2). By-products exhibited superior mineral concentrations, with CB containing the highest calcium content (54.38 mg/100 g) and FB showing peak levels of potassium (35.83 mg/100 g), phosphorus (24.83 mg/100 g), and magnesium (27.90 mg/100 g). The GE was particularly rich in potassium (33.41 mg/100 g) and phosphorus (23.75 mg/100 g), reflecting its metabolic activity in the kernel. Notably, C5 demonstrated selective iron accumulation (5.38 mg/100 g), a 13-fold increase compared to SRF (0.41 mg/100 g).

Break flours showed distinct mineral partitioning: B2 contained elevated calcium (12.74 mg/100 g) and sodium (9.07 mg/100 g) (p <0.05), while B4 was richer in potassium (3.91 mg/100 g) and zinc (0.16 mg/100 g). In the reduction streams, C2F2 and

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C5 stood out with significantly higher iron (1.43 and 5.38 mg/100 g, respectively) and sodium (27.84 mg/100 g in C2F2) (p <0.01) (Table 2), indicating progressive mineral retention during later milling stages. These distribution patterns highlight how modern milling effectively concentrates minerals in bran and germ fractions while producing relatively mineral-depleted endosperm flours,[5] creating a nutritional trade-off between refined and whole grain products.

3.3.7. Heavy metals and food safety considerations. Parallel to essential minerals, heavy metals followed a similar bran-concentration pattern. Lead (Pb) levels peaked in bran fractions (B4: 0.074 mg kg^{-1}; FB: 0.079 mg kg^{-1}), consistent with their accumulation in outer kernel layers,[33] while refined streams like CF2F2 (0.001 mg kg^{-1}) remained well below the EU limits (EC No. 1881/2006). Arsenic (As) showed comparable trends, with FB (0.064 mg kg^{-1}) and CB (0.049 mg kg^{-1}) containing 2–3 times the arsenic levels of early milling streams (C1: 0.025 mg kg^{-1}), suggesting soil-derived contamination.[34] Cadmium (Cd) was similarly enriched in bran (FB: 0.060 mg kg^{-1}; CB: 0.055 mg kg^{-1}) but remained within safe limits in refined flour (CF2F2: 0.008 mg kg^{-1}) (EU Regulation 2023/915). Transition metals like copper (FB: 8.86 mg kg^{-1}) and nickel (CB: 0.302 mg kg^{-1}) (Table 3) exhibited bran-specific accumulation, likely from both soil residues and equipment abrasion.[35]

When it comes to food safety, the concurrent concentration of both beneficial minerals and hazardous metals in bran fractions presents a challenge for food applications. While by-products are potent natural mineral fortifiers, their heavy metal loads necessitate rigorous quality control. The selective enrichment of iron in C5 and other nutritionally valuable minerals in specific streams supports tailored flour blending for nutritional interventions. However, the elevated metal content in bran underscores the need for mitigation strategies such as pre-milling grain washing to reduce soil-borne contaminants, selective bran removal for high-risk populations, and sourcing wheat from low-pollution regions.[36] Regular monitoring of minerals and heavy metals is essential to balance nutritional enhancement with food safety, particularly for whole grain products that retain the metal-rich bran and germ fractions.

3.3.8. Amino acid distribution. The patterns of amino acid distribution across wheat roller-milling streams reveal significant (p <0.001) metabolic gradients within the wheat kernel that are preferentially partitioned throughout the milling process (Table 4). Glutamic acid (36.87–41.40%) and proline (11.92–13.16%), which are indicative of gluten proteins that concentrate in the endosperm, were consistently high in the break streams (B1–B4).[37] Since glutamic acid-rich gliadins and glutenins are primarily responsible for dough viscoelasticity, B3's highest glutamic acid concentration (41.40%) is consistent with its previously noted better gluten functioning.[32] As cytoplasmic and membrane-bound proteins from the subaleurone layer were gradually incorporated, the reduction streams showed a growing gradient of aspartic acid (4.81–6.56%), glycine (4.00–6.15%), and alanine (3.19–4.98%) from C2B to C5.[21,38] This pattern is most noticeable in C5, which had the highest concentrations of these amino acids and the lowest glutamic acid content (34.75%), suggesting that it contains more metabolic proteins than storage proteins. With the germ having extraordinarily high quantities of critical amino acids lysine (6.28%), arginine (7.13%), and threonine (4.27%), which are generally limited in wheat endosperm proteins, the by-products displayed nutritionally complementary profiles.[39] The unique protein composition of the aleurone layer, which is abundant in albumin and globulin-type proteins, is confirmed by the amino acid spectrum of the coarse bran, which includes 5.73% lysine and 6.86% arginine.[31] Since careful fractional blending may raise the total amino acid score of flour products, these distribution patterns have significant results for nutritional optimization. Additionally, the data show how milling fractionation efficiently separates wheat proteins by type and nutritional value, supporting the targeted use of specific streams: break flours for gluten functionality, early reduction streams for balanced protein content, and by-products for nutritional fortification.[15] A nutritional restriction of wheat proteins that may need to be addressed by supplementation or blending with other protein sources is highlighted by the consistently low quantities of sulfur-containing amino acids (methionine and cysteine) throughout all fractions (0.14–1.97%).[5]

Due to their predominance in gluten-forming storage proteins, the break streams (B1–B4) had noticeably greater quantities of non-essential amino acids (NEAAs), especially

Table 3 Heavy metal distribution[a]

		Breaks				Reductions						By-products		
ppm kg^{-1}	SRF	B1	B2	B3	B4	C1	C2F1	CF2F2	C3	C4	C5	FB	CB	GE
Pb	0.0063049	0.011844	0.008265	0.01244	0.074	0.00513	0.00528	0.00124	0.01212	0.03019	0.0291	0.0788	0.01437	0.02587
As	0.0101955	0.011610	0.010263	0.01262	0.032	0.02546	0.00748	0.01627	0.01452	0.01135	0.00658	0.0645	0.04904	0.04601
Cd	0.0165131	0.014947	0.015458	0.00777	0.030	0.01263	0.01343	0.00773	0.00877	0.0216	0.01271	0.05956	0.05514	0.0464
Hg	−0.0198884	0.000435	0.000323	0.00115	−0.013	−0.0242	0.00054	−0.0226	−0.0008	−0.0006	0.00013	0.00121	−0.0226	0.00294
Cu	1.6324004	1.590507	1.406273	1.75318	2.995	1.43328	1.27883	1.45525	2.28801	3.16998	2.55566	8.85727	8.61579	8.15012
Sn	0.0100306	0.118370	0.054934	0.03734	0.100	0.0308	0.02446	0.00545	0.0436	0.14092	0.0617	0.1119	0.00672	0.05798
Cr	0.022149	0.082613	0.046571	0.0399	0.114	0.05893	0.03549	0.02027	0.02009	0.0417	0.03401	0.07269	0.0469	0.07515
Ni	0.047152	0.069315	0.045985	0.03737	0.06345	0.03071	0.03066	0.03043	0.05162	0.06358	0.06003	0.28821	0.30214	0.24786

[a] The results are expressed on a dry weight basis. Measurements with different letters are significantly different from each other (p <0.0001). Measurements that share the same letter are not significantly different.

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Table 4 Amino acid distribution in streams[a]

	Break				Reduction						By-products		
	B1	B2	B3	B4	C1F1/C1F2	C2A	C2B	C3	C4	C5	CB	FB	Germ
Non-essential amino acids													
Aspartic acid	5.58 ± 0.18^{e,f}	3.58 ± 0.02^{i}	3.85 ± 0.08^{i}	5.41 ± 0.05^{g}	5.53 ± 0.11^{f}	5.40 ± 0.19^{g}	4.81 ± 0.49^{h}	5.90 ± 0.03^{e}	6.13 ± 0.05^{d,e}	6.56 ± 0.24^{d}	10.53 ± 0.14^{b}	7.07 ± 0.08^{c}	11.37 ± 0.17^{a}
Serine	4.32 ± 0.00^{b}	4.39 ± 0.02^{b}	3.94 ± 0.01^{d}	3.98 ± 0.00^{d}	4.44 ± 0.05^{b}	4.27 ± 0.01^{b,c}	4.12 ± 0.05^{c}	4.12 ± 0.01^{c}	4.22 ± 0.16^{b,c}	4.59 ± 0.79^{b}	4.63 ± 0.02^{a}	4.62 ± 0.21^{a}	4.78 ± 0.08^{a}
Glutamic acid	39.47 ± 0.10^{b}	40.15 ± 0.23^{b}	41.40 ± 0.18^{a}	36.87 ± 0.06^{c,d}	37.53 ± 0.46^{c}	39.18 ± 0.14^{b}	39.23 ± 0.37^{b}	37.34 ± 0.07^{c}	36.28 ± 0.09^{c,d}	34.75 ± 2.60^{d}	22.97 ± 0.15^{f}	33.68 ± 1.56^{e}	23.63 ± 0.13^{f}
Glycine	4.20 ± 0.00^{e}	4.39 ± 0.03^{e}	4.04 ± 0.05	4.71 ± 0.00^{d}	4.17 ± 0.04^{e}	4.29 ± 0.04^{e}	4.00 ± 0.01^{f}	4.15 ± 0.03^{e,f}	5.14 ± 0.07^{c}	6.15 ± 2.31^{b}	6.86 ± 0.04^{a}	4.56 ± 0.17^{d}	6.97 ± 0.02^{a}
Alanine	3.51 ± 0.04^{f}	3.36 ± 0.04^{f,g}	3.11 ± 0.11^{g}	3.80 ± 0.03^{e}	3.65 ± 0.04^{f}	3.44 ± 0.08^{f}	3.19 ± 0.05^{g}	3.74 ± 0.12^{e}	4.26 ± 0.26^{d}	4.98 ± 1.28^{c}	7.34 ± 0.24^{b}	4.31 ± 0.11^{d}	8.12 ± 0.11^{a}
Cysteine	0.26 ± 0.02^{b}	0.24 ± 0.09^{b}	0.51 ± 0.30^{a}	0.17 ± 0.00^{d}	0.23 ± 0.14^{b}	0.14 ± 0.01^{g}	0.14 ± 0.02^{g}	0.16 ± 0.00^{e}	0.16 ± 0.02^{e}	0.18 ± 0.06^{c}	0.15 ± 0.01^{f}	0.21 ± 0.01^{b}	0.18 ± 0.01^{c}
Tyrosine	2.40 ± 0.13^{c}	2.42 ± 0.01^{c}	2.09 ± 0.00^{g}	2.35 ± 0.01^{d}	2.54 ± 0.05^{b}	2.21 ± 0.05^{f}	2.43 ± 0.00^{c}	2.46 ± 0.15^{c}	2.27 ± 0.00^{e}	1.99 ± 0.40^{h}	2.58 ± 0.01^{b}	2.27 ± 0.24^{e}	2.71 ± 0.04^{a}
Arginine	2.18 ± 0.04^{g}	2.67 ± 0.11^{f}	2.91 ± 0.11^{e}	3.18 ± 0.06^{d}	2.70 ± 0.18^{e}	2.58 ± 0.15^{f}	2.78 ± 0.03^{e}	3.21 ± 0.27^{d}	3.62 ± 0.11^{c}	2.18 ± 0.48^{g}	6.86 ± 0.05^{b}	3.55 ± 0.15^{c}	7.13 ± 0.07^{a}
Proline	12.74 ± 0.81^{c}	13.16 ± 0.55^{b}	11.92 ± 0.38^{d}	12.56 ± 0.21^{c}	12.65 ± 0.41^{c}	12.96 ± 0.60^{c}	12.54 ± 0.00^{c}	11.67 ± 0.33	11.38 ± 0.82	12.67 ± 0.35^{c}	6.78 ± 0.07^{e}	14.19 ± 4.22^{a}	6.07 ± 0.29
Essential amino acids													
Threonine	2.67 ± 0.01^{e}	2.65 ± 0.01^{e}	2.53 ± 0.00^{f}	2.66 ± 0.01^{e}	2.78 ± 0.03^{d}	2.65 ± 0.01^{e}	2.65 ± 0.03^{e}	2.75 ± 0.01^{d}	2.79 ± 0.07^{d}	2.85 ± 0.21^{d}	3.89 ± 0.02^{b}	3.00 ± 0.13^{c}	4.27 ± 0.06^{a}
Valine	3.10 ± 0.16^{f}	2.96 ± 0.03^{g}	3.62 ± 0.01^{c}	3.14 ± 0.03^{f}	3.40 ± 0.02^{d}	3.07 ± 0.03^{f}	3.44 ± 0.04^{d}	3.65 ± 0.04^{c}	3.14 ± 0.00^{f}	3.58 ± 0.22^{c}	3.77 ± 0.08^{b}	3.30 ± 0.13^{e}	4.68 ± 0.03^{a}
Methionine	1.01 ± 0.01^{f}	1.13 ± 0.02^{e}	1.23 ± 0.05^{d}	1.43 ± 0.01^{c}	1.04 ± 0.02^{e}	1.12 ± 0.00^{e}	1.30 ± 0.07^{d}	1.24 ± 0.06^{d}	1.14 ± 0.02^{e}	0.99 ± 0.07^{f}	1.64 ± 0.06^{b}	0.88 ± 0.05^{g}	1.97 ± 0.00^{a}
Isoleucine	2.28 ± 0.03^{e}	2.23 ± 0.01^{e}	2.77 ± 0.08^{a}	2.38 ± 0.01^{d}	2.54 ± 0.00^{c}	2.33 ± 0.03^{d}	2.64 ± 0.03^{b}	2.72 ± 0.00^{a}	2.14 ± 0.04^{f}	2.40 ± 0.01^{d}	2.40 ± 0.03^{d}	2.29 ± 0.11^{e}	2.58 ± 0.17^{c}
Leucine*	6.83 ± 0.06	6.77 ± 0.03	6.76 ± 0.23	6.93 ± 0.06	7.13 ± 0.03	6.87 ± 0.13	7.11 ± 0.07	7.09 ± 0.00	6.68 ± 0.00	6.92 ± 0.04	6.49 ± 0.10	6.38 ± 0.43	6.52 ± 0.02
Phenylalanine	4.83 ± 0.34^{b}	5.05 ± 0.03^{a}	4.75 ± 0.01^{b}	4.94 ± 0.00^{b}	5.19 ± 0.07^{a}	4.79 ± 0.28^{b}	4.97 ± 0.08^{b}	4.68 ± 0.42^{c}	4.81 ± 0.02^{b}	4.37 ± 0.74^{c}	3.78 ± 0.04^{d}	4.10 ± 0.58^{c}	3.69 ± 0.01^{d}
Histidine	2.86 ± 0.15^{c}	2.86 ± 0.10^{c}	2.53 ± 0.03^{f}	3.02 ± 0.03^{b}	2.51 ± 0.29^{f}	2.64 ± 0.10^{e}	2.60 ± 0.07^{e}	2.72 ± 0.05^{d}	3.06 ± 0.35^{b}	2.70 ± 0.31^{d}	3.59 ± 0.11^{a}	2.88 ± 0.12^{c}	3.76 ± 0.11^{a}
Lysine	1.77 ± 0.00^{f}	1.99 ± 0.03	2.05 ± 0.01^{e}	2.45 ± 0.06^{d}	1.97 ± 0.05^{e,f}	2.08 ± 0.01^{e}	2.05 ± 0.02^{e}	2.41 ± 0.00^{d}	2.77 ± 0.01^{c}	2.15 ± 0.21^{d}	5.73 ± 0.04^{b}	2.71 ± 0.15^{c}	6.28 ± 0.19^{a}

[a] Measurements with different letters are significantly different from each other (p <0.001). Measurements that share the same letter are not significantly different.

glutamic acid (36.87–41.40%) and proline (11.92–13.16%). On the other hand, the reduction streams (C1–C5) showed a progressive rise in aspartic acid, glycine, alanine, and essential amino acids (lysine, arginine, threonine), an amino acid signature indicative of structural and metabolic proteins rather than the storage proteins predominant in the subaleurone layer.[25] As a result of wheat's evolutionary adaptation for protein storage, these NEAAs together made up between 70 and 80% of the total amino acids in endosperm-derived fractions.

The generation of acrylamide during high-temperature processing (such as baking or frying) is affected by this distribution.[1] The higher percentage of NEAAs in break flours may theoretically raise the risk of acrylamide since the Maillard reaction between reducing sugars and free asparagine, a NEAA, is the primary mechanism by which acrylamide is formed.[21] However, this worry is lessened by wheat's low asparagine content (not quantified explicitly in this study, but usually less than 1–0.5% in flour) compared to other cereals.[40] Instead, if processed at high temperatures without optimization, the reduction streams (C3–C5) may present a marginally increased risk of acrylamide due to their higher protein diversity and possible residual sugars from damaged starch.[21]

Despite having a high quantity of essential amino acids, the germ and bran fractions include more free sugars and asparagine, which may increase acrylamide synthesis if utilized in products that have undergone thermal processing.[41] Therefore, break flours have a moderate risk of acrylamide despite their higher baking functionality, whereas by-products (if added to baked products) might need mitigation techniques (asparaginase treatment or optimal baking conditions). This emphasizes how tailored stream blending can help wheat flour

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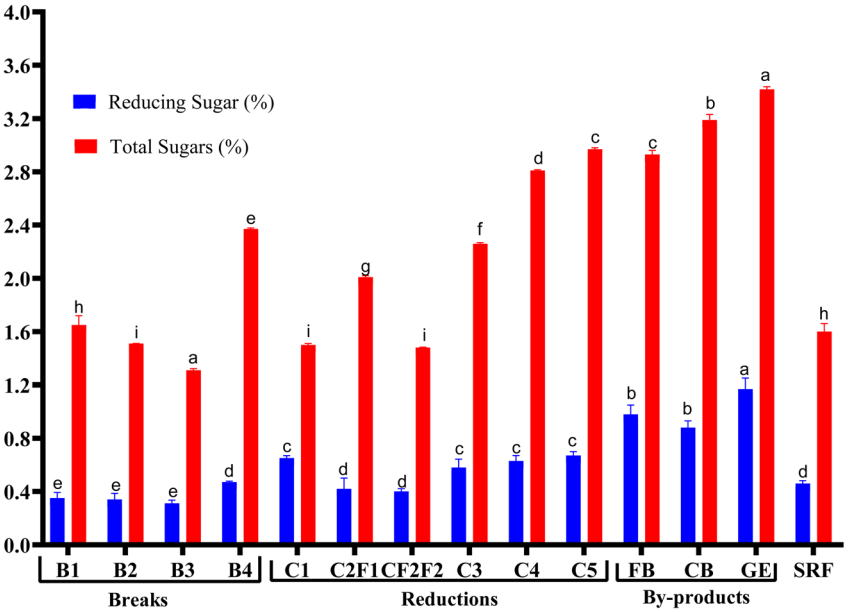

Fig. 1 Reducing sugar and total sugars. The results are statistically significant with $p \leq 0.05$. Measurements with different letters are significantly different from each other ($p < 0.05$). Measurements that share the same letter are not significantly different.

products strike the ideal balance between nutritional value and technological performance.

3.3.9. Distribution of reducing and total sugars. The distribution of reducing and total sugars across different streams reveals significant variations ($p < 0.05$), influenced by the milling intensity and anatomical composition of wheat kernels (Fig. 1). The break streams, which primarily separate endosperm from bran in the initial milling stages, exhibited relatively low sugar levels in early breaks (B1–B3), with reducing sugar ranging from 0.31% to 0.34% and total sugars between 1.31% and 1.65%. However, B4 showed a notable increase in reducing sugar (0.47%) and total sugars (2.37%), likely due to greater bran contamination as milling progresses.[21] This aligns with studies indicating that later break flours contain more aleurone layer residues, contributing to higher sugar content.[39]

The reduction streams, responsible for further refining endosperm particles, exhibited a rising trend in sugar content from C1 to C5. C1 (0.65% reducing sugar and 1.50% total sugar) had moderate levels, likely due to residual bran particles. C2A (0.42%) and C2B (0.40%) showed lower reducing sugars, possibly due to purer endosperm extraction. C3–C5 exhibited a sharp increase in reducing sugar (0.58–0.67%) and total sugars (2.26–2.97%), suggesting greater aleurone and germ incorporation in finer streams.[25]

On the other hand, the SRF exhibited 0.46% of reducing sugar and 1.60% of total sugar values between early break and reduction flours, reflecting a blend of multiple milling streams. Its sugar content is lower than bran-rich fractions but higher than purified patent flours, consistent with commercial milling practices.[26]

As expected, bran and germ fractions contained the significantly highest ($p < 0.05$) sugar levels (Fig. 1). The FB, CB, and GE exhibited 0.98%, 0.88%, and 1.17% of reducing sugars and 2.93%, 3.19%, and 3.42% of total sugars, respectively. This confirms that non-endosperm components are richer in free sugars, particularly reducing sugars like glucose and fructose, which are more in the germ and aleurone layer.[21] The germ is metabolically active and contains high soluble sugars for seedling development, explaining its peak values. These findings highlight how milling fractionation affects sugar distribution,[16] with bran and germ being the richest sources, while refined flours (early breaks, C2) contain minimal sugars. These sugar variations in the streams will directly affect acrylamide formation in the end products. So, optimizing flour selection based on sugar content can improve product quality and safety.

3.4. Phytochemical distributions

The distribution of phytochemicals varied significantly ($p < 0.05$) across all milling streams (Fig. 2), with distinct compositional profiles observed for carotenoids, phenolic content, flavonoids, and antioxidant activity. Notably, FB, CB, and GE fractions exhibited the highest concentrations of these bioactive compounds, consistent with their role as primary reservoirs of phytochemicals in wheat kernels.[22,42,43] However, select flour streams B4, C4, and C5 also contained significantly higher ($p < 0.05$) levels of bioactive compounds than other breaks and reductions, suggesting partial retention of nutrient-rich aleurone and subaleurone layers during milling.[15] The individual phytochemical components displayed unique distribution patterns. These compounds are directly associated with the color values of flour fractions.

3.4.1. Carotenoids. Wheat milling fractions' carotenoid content differed significantly ($p < 0.05$) among break (B1–B4), reduction (C1–C5), and by-product streams (Fig. 2a), indicating that these lipophilic pigments are not evenly distributed in the wheat kernel. According to Hidalgo and Brandolini,[28]

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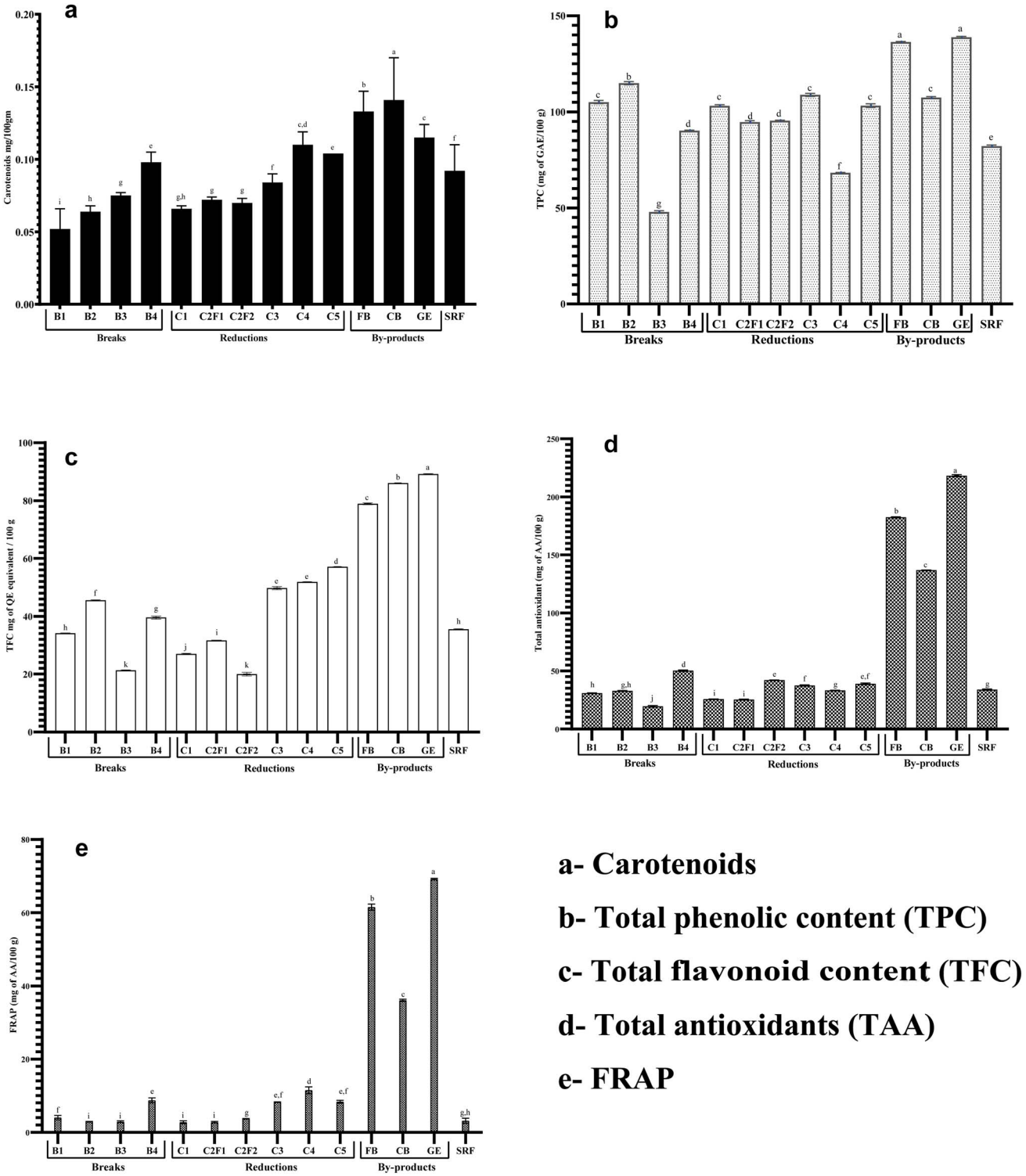

Fig. 2 Phytochemicals: the results are statistically significant with $p \leq 0.05$. Measurements with different letters are significantly different from each other ($p < 0.05$). Measurements that share the same letter are not significantly different.

carotenoid levels in the break flours increased gradually from B1 (0.052 mg/100 g) to B4 (0.098 mg/100 g), indicating higher retention of carotenoid-rich aleurone and subaleurone layers in subsequent break streams. The carotenoid concentrations in C4 (0.110 mg/100 g) and C5 (0.104 mg/100 g) were the greatest in the reduction system, which showed a similar pattern. This was probably because of leftover bran and germ particles.[25] Remarkably, C2F1 (0.072 mg/100 g) and C2F2 (0.070 mg/100 g) displayed similar values, suggesting that these mid-stream fractions received consistent grinding impacts.

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The by-products with the most significant (p <0.05) levels of carotenoid content were the GE (0.115 mg/100 g), FB (0.133 mg/100 g), and CB (0.141 mg/100 g), indicating that these fractions are essential sources of lutein and other xanthophylls in wheat.[42] According to Liu *et al.*,[15] the bran fractions' higher carotenoid content is consistent with their function in shielding the kernel from oxidative stress. In keeping with its blended nature, SRF (0.092 mg/100 g), a mixture of several streams, showed intermediate carotenoid levels. Break and reduction flours retain modest carotenoid levels. However, due to bran contamination, B4, C4, and C5 streams offer enhanced nutritional value for whole-grain applications.

3.4.2. Total phenolic content in wheat milling streams. The varied distribution of phenolic compounds in wheat kernel sections was reflected in the significant (p <0.05) variance in the total phenolic content (TPC) between streams. B1 (105.12 mg GAE/100 g), B4 (90.22 mg GAE/100 g), and B2 (114.96 mg GAE/100 g) had the significantly greatest (p ≤0.005) TPC of all the break flours, whereas B3 had much lower amounts (47.93 mg GAE/100 g), significantly not in the same group (p ≤0.005). According to this pattern, B3 reflected a significantly higher (p <0.05) percentage of endosperm fraction than others, whereas early break streams (B1–B2) retained more phenolic-rich aleurone material.[15] The reduction system showed variable TPC values; the highest concentrations were observed in C3 (108.85 mg GAE/100 g) and C5 (103.26 mg GAE/100 g), most likely as a result of leftover bran particles in these later reduction streams.[39]

With GE (138.80 mg GAE/100 g), FB (136.31 mg GAE/100 g), and CB (127.16 mg GAE/100 g) exhibiting 1.5–3 times greater TPC than typical flour streams, by-products had significantly higher (p <0.05) phenolic contents. These results are consistent with earlier studies that found that the bran and germ of wheat are the primary sources of phenolic acids, especially ferulic acid and other phenolics attached to cell walls.[42,43] The intermediate values displayed by the SRF (82.19 mg GAE/100 g) aligned with its composition as a combination of many streams.

These findings point to significant results for product development and milling practices. Compared to more refined streams like B3, the higher phenolic content in early break (B1–B2) and select reduction (C3, C5) streams indicates that these fractions may offer more nutritional value. According to Beta *et al.*,[45] the by-products' extraordinarily high TPC highlights their potential as beneficial dietary additives for phenolic fortification. The variance between streams highlights the planned use of milling fractionation to create flours with the desired phenolic content for particular nutritional uses.

3.4.3. Total flavonoid content (TFC). The TFC exhibited significant variation (p <0.05) across milling fractions (Fig. 2c), reflecting the heterogeneous distribution of these bioactive compounds in wheat kernels. Among break flours, B2 (45.57 mg CE/100 g) and B4 (39.58 mg CE/100 g) retained significantly higher (p <0.05) TFC than B1 (34.11 mg CE/100 g) and B3 (21.28 mg CE/100 g), suggesting incomplete separation of bran particles in later break stages. In the reduction system, TFC increased progressively from C1 (26.98 mg CE/100 g) to C5 (57.07 mg CE/100 g), with C3–C5 exhibiting 1.8–2.7 times higher flavonoids than early reduction streams (C2F1–C2F2) (Fig. 2c). This trend correlates with incorporating subaleurone material, which is richer in bound flavonoids.[22,23,42,43]

By-products demonstrated the highest TFC values, with GE (89.20 mg CE/100 g) and CB (86.11 mg CE/100 g) containing 2.5–4.2 times more flavonoids than most flour streams (Fig. 2c). These findings align with previous studies showing that wheat bran and germ are primary reservoirs of flavonoid glycosides, particularly apigenin and luteolin derivatives.[44] FB (78.93 mg CE/100 g) also showed elevated TFC, though marginally lower than CB and GE, likely due to partial loss of flavonoid-rich aleurone layers during milling.[39] SRF (35.53 mg CE/100 g) showed intermediate TFC, consistent with its composition as a blend of multiple streams. These results demonstrate how milling fractionation can strategically separate flavonoid-rich fractions while maintaining flour functionality, supporting the development of value-added wheat ingredients.

3.4.4. Total antioxidant activity and FRAP. Significant (p <0.05) differences in the distribution of phenolic and bioactive compounds were reflected in the total antioxidant activity and ferric reducing antioxidant power (FRAP) of wheat milling fractions across break, reduction and by-product streams (Fig. 2d and e). Due to higher bran particle retention in subsequent break streams, B4 had the highest overall antioxidant activity (50.20 μmol TE per g) and FRAP (8.68 μmol Fe^{2+} per g) among break flours.[44] B3, on the other hand, showed lower values (19.39 μmol TE per g and 2.90 μmol Fe^{2+} per g), indicating that the starchy endosperm in this fraction was separated more effectively.[23,39]

C4 had the highest FRAP (11.45 μmol Fe^{2+} per g) in the reduction system, presumably as a result of leftover germ and aleurone components, while C2F2 (42.03 μmol TE per g) and C5 (38.82 μmol TE per g) showed increased antioxidant activity. The highest antioxidant capacity was demonstrated by the by-products, which confirmed their richness in phenolic acids, flavonoids, and other redox-active compounds GE (218.28 μmol TE per g and 69.19 μmol Fe^{2+} per g), FB (182.37 μmol TE per g and 61.54 μmol Fe^{2+} per g), and CB (136.69 μmol TE per g and 36.08 μmol Fe^{2+} per g).[45] Sovrani *et al.*[43] and Giordano *et al.*[22] observed similar trends. In keeping with its composite nature, SRF had moderate values of TAA and FRAP (33.84 μmol TE per g and 3.18 μmol Fe^{2+} per g, respectively).

3.5. Anti-nutrient distribution

Anti-nutrient distribution across roller-milled wheat flour fractions varied significantly (p <0.001) (Fig. 3), reflecting their concentration in the outer grain layers.

3.5.1. Tannin content. Tannins were the highest in the bran streams, with CB containing 1.397 mg g^{-1} and FB containing 1.048 mg g^{-1}, consistent with their localization in the seed coat and aleurone layer.[46] Break flours (B1–B4) exhibited significantly (p <0.001) moderate tannin levels (0.207–0.380 mg g^{-1}), attributed to residual bran contamination, while reduction flours (C1–C5) showed a progressive increase (0.385–0.976 mg g^{-1}) due to FB particles persevering in later streams. Notably, SRF showed 0.686 mg g^{-1} tannins (Fig. 3), higher than the

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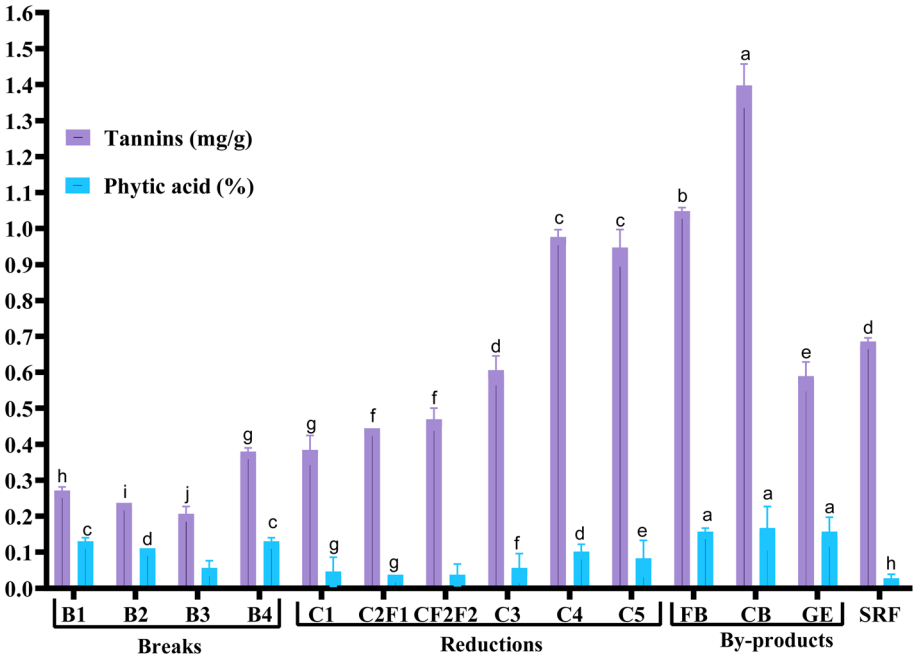

Fig. 3 Antinutrients: tannins and phytic acid. The results are statistically significant with $p \leq 0.01$. Measurements with different letters are significantly different from each other ($p < 0.01$). Measurements that share the same letter are not significantly different.

patent flour; however, it was lower than bran-contaminated streams, suggesting partial bran inclusion. GE had 0.59 mg g^{-1}, likely from phenolic compounds in the embryo.[47]

3.5.2. Phytic acid. Phytic acid followed a similar trend to tannins, peaking in CB (0.167%) and FB (0.157%), aligning with its aleurone-layer predominance.[44] Break flours significantly ($p < 0.001$) retained (0.056–0.13%) phytic acid, while reduction flours (C1–C5) had lower levels (0.037–0.102%), reflecting efficient endosperm separation. GE exhibited 0.157%, which is consistent with its role as a phosphorus store. Straight-run flour had minimal phytic acid (0.028%), confirming its refinement (Fig. 3).

The sharp decline in phytic acid from bran fractions (>0.15%) to refined flours (<0.1%) demonstrates milling's effectiveness in reducing anti-nutrients. However, tannins persist in reduction streams (0.385–0.976 mg g^{-1}) due to

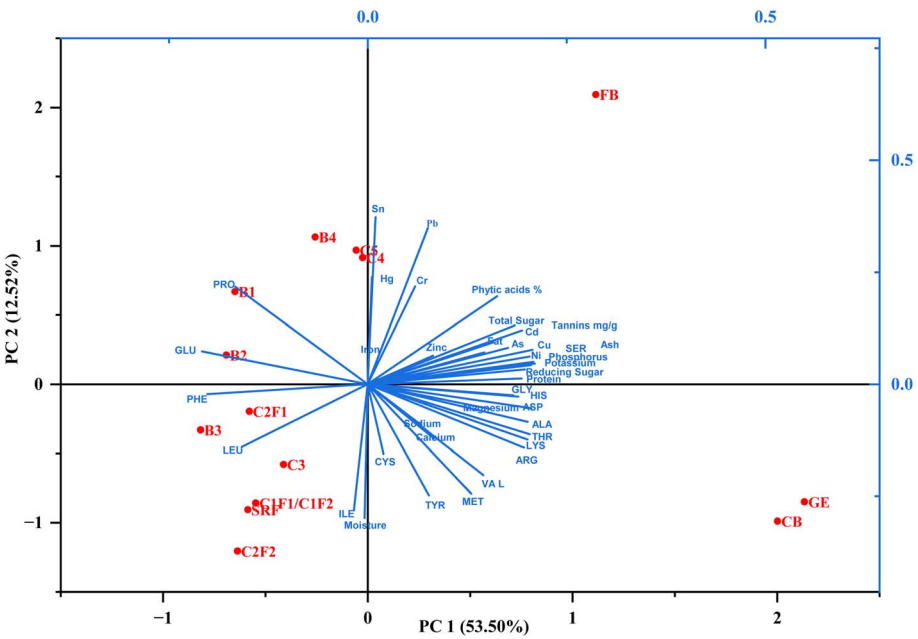

Fig. 4 Principal component analysis: biplot-compositional variability.

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satisfactory bran adhesion. Blending high-anti-nutrient streams (bran and germ) with others may deteriorate mineral chelation, requiring interventions like fermentation.[48] Our results align with studies reporting bran tannins at 0.3–0.8% (ref. 48) and phytic acid at 2–6% in aleurone.[44] The low phytic acid content in SRF (<0.03%) matches with refined flour data, supporting its nutritional advantage. Tannins and phytic acid are concentrated in bran and germ; milling reduces the challenges of eliminating them. For fortified bakery products, low-extraction flours minimize anti-nutrient effects, while bran-rich blends may need phytase activation (*e.g.*, fermentation) to improve mineral absorption.

4. Principal component analysis interpretation

Principal component analysis (PCA) of the chemical compositions of milled streams (Fig. 4) reveals distinct clustering based on compositional differences. The first two principal components (PC1: 53.50%; PC2: 12.52%) collectively explain 66.02% of the total variance. The PCA biplot clearly separates bran streams and break/reduction flours, indicating significant compositional differences. Bran streams are positioned at the far right along PC1 and are associated with higher levels of non-essential amino acids (NEAAs), heavy metals, anti-nutrients, sugars, and ash. In contrast, the break flour (B1–B4) cluster in the upper-left quadrant is closely aligned with C4 and C5, suggesting shared intermediate characteristics. The reduction flour (C1–C3) group in the lower-left quadrant, near SRF, reflects their refined endosperm-dominated composition.

Bran streams correlate strongly with ash, sugars, phenolics, and anti-nutrients. C4 and C5 show transitional properties, positioned near FB; however, they have lower bioactive content. Pure endosperm fractions (B1–B3 and SRF) cluster separately, indicating minimal bran contamination and higher gluten quality. This PCA highlights a nutritional functionality divide: bran streams are rich in micronutrients, but they are also anti-nutrients and heavy metals, while break/reduction flours offer superior baking functionality.

5. Conclusion

This study comprehensively maps the nutritional and compositional gradients in industrial wheat milling streams. A key finding is the inherent trade-off: refinement enhances technological functionality but reduces nutrients and bioactives. Significant variations were observed, with bran and germ streams identified as rich sources of lysine (6.28%), antioxidants (FRAP: 36–69 μmol Fe^{2+} per g), and minerals, albeit with concurrent higher levels of anti-nutrients and heavy metals. Break flours (B1–B4) demonstrated superior baking potential due to their high gluten-forming protein content, while later streams (B4, C4, and C5) offered greater nutrient density, making them ideal for whole-grain applications.

The observed 5–10-fold variation in essential nutrients underscores the potential of precision milling. Strategic applications include the targeted recombination of streams to create nutritionally enhanced flours and the specialized use of streams and break flours for premium baking, reduction streams for balanced blends, and by-products as functional fortifiers. These findings establish a groundwork for developing specialty flours with improved amino acid profiles, sustained baking quality, and potential for acrylamide mitigation in end products.

However, this study has certain limitations. The compositional analysis is primarily investigative; it does not include functional validation of the proposed stream recombination through baking tests or rheological studies. Furthermore, the chance of anti-nutrients during processing and their impact on nutrient bioavailability were not investigated. Future work should therefore focus on optimizing specific blending ratios and conducting comprehensive functional evaluations to balance nutritional enhancement and acrylamide mitigation with end-product performance.

Author contributions

Veeranna H: conceptualization, writing – original draft, investigation, methodology, formal analysis, data curation, graphics, statistics, visualization, validation. Swamy Gowda M. R. & Salony Azam Sheikh: formal analysis, data curation. Nandini P. Shetty & Sridevi Annapurna Singh: resource, supervision. A. A. Inamdar: conceptualization, funding acquisition, writing – review & editing, project administration, supervision, resources, validation.

Conflicts of interest

The authors declare no competing interests.

Data availability

Data will be made available on request.

Consent for publication: all authors agreed to the publication of the manuscript.

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