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Damirga: A traditional fermented flour for rancidity reduction and revitalizing the popularity of pearl millet

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ABSTRACT

Pearl millet is a nutritious cereal and a staple food security crop in many developing countries. However, its flour has a short shelf life due to rapid rancidity. This study evaluated the effects of the traditional Damirga processing method on the physicochemical properties, colorimetric parameters, and rancidity indicators of flour from two cultivars, Aziz and Baladi Yellow (BY). Damirga-processed flour demonstrated lower fat content (3.6 %), higher carbohydrate content (78.2 %), improved bulk density (1.93 g/cm^3), and greater swelling power compared to raw flour. It exhibited an enhanced lightness index (L*), higher whitening index, and lower browning index, as well as reduced lipase and lipoxygenase activities, peroxide value, and comprehensive acid value, indicating lower rancidity. Aziz exhibited higher ash content, fat content, and susceptibility to rancidity, while BY showed higher fiber content, oil absorption capacity, and lightness index. Aziz also had greater water absorption capacity, while BY exhibited significantly higher values for blueness (b), whitening index (WI = 72.06), and a more appealing visual profile. Despite similar bulk density and swelling power, Aziz exhibited higher water absorption capacity, while BY showed greater oil absorption capacity. Optimizing Damirga and cultivar selection offers promising solutions to extend pearl millet flour shelf life.

1. Introduction

Pearl millet (*Pennisetum glaucum*) is a resilient C4 cereal and a nutritious staple food crop relevant to food security in marginal agricultural areas. Approximately 90 million people across sub-Saharan Africa rely on pearl millet for their livelihood and nutritional needs (Serba et al., 2020). Pearl millet is rich in carbohydrates (including fiber and starch), proteins, vitamins, and minerals, making it an important daily source of essential nutrients (Abdulrahman and Omoniyi, 2016; Duodu and Awika, 2019). In addition, it contains various bioactive compounds, such as flavonoids, which contribute to its antioxidant activity (Elsafy et al., 2024). Because of its nutritional properties and health benefits, pearl millet is recognized as a novel "well-being" food. In 2023, the United Nations General Assembly declared the 'International Year of Millets' to promote awareness of its potential to improve nutrition, enhance food security, and reduce malnutrition and poverty on a global scale.

Pearl millet is gluten-free and has a lower glycemic index than other cereals, making it particularly beneficial for individuals with diabetes or celiac disease (El Khoury et al., 2018). However, despite its advantages as a staple food crop, the shelf life of pearl millet flour is limited due to its rapid onset of rancidity. This issue is primarily attributed to the grain's high lipid content in the grain and the increased activity of enzymes, such as lipoxygenase, peroxidase, and polyphenol oxidases, during storage (Goyal and Chugh, 2017).

Pearl millet has a higher lipid content than other cereals, making it particularly susceptible to rancidity. Its grain structure consists of pericarps, germs, and endosperms, with germ and bran layers containing triglycerides rich in unsaturated fatty acids (FAs) (Serna-Saldivar and Espinosa-Ramírez, 2019). When ground into flour, these lipids are exposed to lipolytic enzymes and oxygen. Lipases break down triglycerides, releasing free fatty acids (FFA), which induced flour acidity and contribute to a rancid off-flavor. FFAs are more prone to oxidation by lipoxygenase and peroxidase than esterified lipids (Rosentrater, 2022). These enzymes degrade unsaturated fatty acids into peroxides, triggering peroxidation chain reactions that promote rancidity. Oxygen

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exposure accelerates this process, leading to undesirable aromas, off-flavors, and nutritional losses in pearl millet-based products, negatively affecting food quality and consumer satisfaction (Selvan et al., 2022). Innovative strategies to regulate enzymatic activity in pearl millet are urgently required to enhance food safety and product quality.

Goswami et al. (2020) developed a rancidity matrix that incorporates key biochemical indicators, such as the comprehensive peroxide value (CPV), comprehensive acid value (CAV), and enzymatic activities of lipase and lipoxygenase, to assess the rancidity of lipid-rich products. This matrix provides a detailed assessment of lipid-containing foods' oxidative stability and quality. The acid value reflects the presence of free fatty acids formed through lipid hydrolysis and oxidation, while the peroxide value measures primary oxidation products, specifically hydroperoxides. These indicators enable researchers and food technologists to understand better and mitigate the effects of rancidity in pearl millet products, ultimately enhancing their stability and quality (Bhargavi et al., 2024).

Traditional Indigenous Sudanese food processing methods for pearl millet, such as Damirga, may help prevent rancidity. Damirga is a fermented flour made from dehulled pearl millet grains that are fermented for up to 72 h and then sun-dried (Abdalla et al., 1998; Ahmed et al., 2010; Sulieman et al., 2022). This method, widely practiced by women in Western Sudan, significantly extends the shelf life of pearl millet flour. The process primarily relies on lactic acid fermentation, in which bacteria produce lactic acid, inhibiting microbial growth and enzymatic activity and stabilizing the flour's lipids (Akinola et al., 2017). A preliminary survey conducted in the Gadarif state of Sudan revealed that most respondents reported that Damirga could preserve pearl millet flour for up to six months without rancidity (unpublished results).

Previous studies have assessed the agronomic performance and nutritional composition of the two target pearl millet cultivars, Aziz, a biofortified variety with high yield and micronutrient content, and Baladi Yellow, a resilient local landrace with higher fiber and lower fat content. However, no prior research has investigated their rancidity profiles, physical characteristics, or colorimetric traits following traditional processing methods such as *Damirga*. This lack of comparative rancidity assessment underscores the novelty of the present study, which uniquely integrates cultivar-specific responses with biochemical and functional flour quality under traditional postharvest processing conditions.

This study was based on the hypothesis that dehulling, fermentation and sun drying enhance the oxidative stability of pearl millet flour, as demonstrated by traditional Damirga processing and natural fermentation methods. The aim was to evaluate the rancidity matrix of fresh and Damirga-processed flour from two pearl millet cultivars, the local landrace Baladi Yellow and the iron-enriched biofortified cultivar Aziz. To investigate the effects of these processing methods on rancidity and overall quality, we measured vital parameters, including the rancidity matrix, fatty acid composition, colorimetric characteristics, chemical properties (proximate analysis), and physical properties. These comparisons elucidate how dehulling, fermentation, and sun-drying in the Damirga process influence fresh pearl millet flour's oxidative stability and quality.

2. Materials and methods

2.1. Plant materials

This study used two pearl millet genotypes to produce raw and Damirga flours. Baladi Yellow (BY) is a local landrace favored in Sudan for its yellow pericarp color (Bashir et al., 2014). It is commonly used to prepare Damirga porridge in the Darfur region of Western Sudan. The second, Aziz, is a high-yielding, early-maturing, iron-enriched, biofortified pearl millet cultivar released in Sudan in 2022 by the Sudan National Variety Release Committee. The Aziz cultivar was introduced to Sudan from ICRISAT, India, to address anemia in the Sudanese population.

2.2. Damirga and raw flour

Pearl millet grains from two genotypes, Aziz and BY, were obtained from the Gadarif Research Station. The grains were thoroughly cleaned to remove foreign materials, stones, impurities, and broken grains. The cleaned grains were then divided into two groups. The first group was milled into fine flour using a grain mill and sieved through a 0.25-mm sieve to ensure uniform particle size. The flour was stored in airtight plastic zip bags at 4 °C until chemical analysis. The second group of grains was used to prepare Damirga flour.

Damirga flour was prepared at the household level using traditional methods described by Abdalla et al. (1998); Ahmed et al. (2010). The process began by soaking the pearl millet grains in water (approximately 20 % of the grain weight) for one hour. The soaked grains were then hand-pounded using a wooden mortar and pestle for approximately 30 min, followed by winnowing to separate the bran from the kernel. The kernels were collected and soaked in water at a 1:2 grain-to-water ratio, allowing them to ferment naturally for 72 h at an average temperature of 30 °C. After fermentation, the soaking water was drained, and the fermented grains were washed twice with tap water, sun-dried, and milled into fine flour using an electric grinder. The flour was then sieved through a 0.25-mm sieve. Damirga flour was stored in airtight plastic zip bags at 4 °C until analysis.

Both raw milled and Damirga flour were analyzed for rancidity-related enzyme activities, including lipase and lipoxygenase. Additional tests were conducted to evaluate the comprehensive peroxide value (CPV), comprehensive acid value (CAV), fatty acid composition (FA), physicochemical properties, and color attributes.

2.3. Chemical analysis

The chemical compositions of raw and Damirga pearl millet flours were analyzed using standard methods.

Moisture content was determined using the ICC Standard Method (Rasper and Walker, 2000). For the analysis, 5 g of each flour sample was placed in a pre-dried, pre-weighed aluminum pan and oven-dried at 105 °C until a constant weight was achieved. The dried samples were cooled in a desiccator, and the moisture content was calculated based on the weight loss and expressed as a percentage of the initial sample weight.

Crude fat content was measured using Soxhlet extraction according to ICC Standard Method No. 136 (1994) (Rasper and Walker, 2000). Approximately 2 g of dried sample was extracted with petroleum ether for 6 h. After extraction, the solvent was evaporated, and the remaining fat was dried to a constant weight. The fat content was calculated as a percentage of the initial sample weight.

The nitrogen content was quantified using the Dumas combustion method on a Flash 2000 N.C. Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) (Moț et al., 2022). Nitrogen content was converted to crude protein content using a factor of 6.25. Each sample was analyzed in triplicate.

Crude fiber content was determined using the AOAC (Ac, 1984) official method. The samples were first digested in 1.25 % sulfuric acid, followed by 1.25 % sodium hydroxide. The residue was filtered, washed, dried at 105 °C, and weighed again. The ash weight was subtracted to calculate the fiber percentage.

Ash content was assessed using a modified UNE-EN ISO 2171: 2023 method. Three grams of the sample were ignited in a muffle furnace at 550 °C to incinerate all organic matter into ash. The remaining ash was cooled in a desiccator, weighed, and expressed as a percentage of the initial sample weight.

The carbohydrate content was calculated as the difference. The moisture, fat, protein, and ash percentages were subtracted from 100 %, assuming that the remaining fraction consisted primarily of

carbohydrates, including sugars, starches, and fibers.

2.4. Physical properties of raw and Damirga pearl millet flour

2.4.1. Bulk density

The bulk densities (BD) of the raw and Damirga flour samples were determined using the method described by Wang and Kinsella (1976). Ten grams of each sample were placed into a 50-mL graduated cylinder. The cylinder was gently tapped on the benchtop ten times to compact the sample and achieve a consistent volume. Once the sample had settled, the final volume was recorded. Bulk density was then calculated as the mass per unit volume of the settled sample and expressed in grams per milliliter (g/mL).

2.4.2. Water and oil absorption capacities

The water absorbance capacity (WAC) and oil absorption capacity (OAC) of the Damirga and raw pearl millet flour samples were determined using the method described by Lin and Zayas (1987). One gram of each flour sample was placed in a pre-weighed centrifuge tube, and 10 mL of distilled water or refined sunflower oil was added. The mixture was allowed to stand for 1 h to ensure thorough interaction between the flour and the liquid.

The dispersion was centrifuged at 1500×g for 30 min to separate the unabsorbed liquid. After centrifugation, the supernatant was carefully decanted, and residual droplets were removed from the tube. The tubes were reweighed to determine the water or oil retained in the flour samples. WAC and OAC were expressed as grams of water or oil absorbed per gram of flour.

2.4.3. Swelling power

Swelling power was determined following the method outlined by the American Association of Cereal Chemists (AACC) method 56-21-01. Briefly, 1 g of raw and Damirga flour was placed in a centrifuge tube, and 10 mL of distilled water was added. The mixture was vigorously stirred to create a homogenous slurry. The tube was then heated in a water bath at 80 °C for 30 min with gentle stirring to ensure uniform heat distribution and prevent localized overheating.

After heating, the tube was cooled to room temperature (25 °C) and centrifuged at 3000 rpm for 20 min to separate the swollen flour from the supernatant. The supernatant was carefully decanted without disturbing the settled flour pellet. The volume of the swollen flour was measured, and swelling power was expressed as the volume of swollen flour per gram of dry flour (mL/g).

2.5. Color measurements

The color attributes of raw and Damirga pearl millet flours were measured using a Chroma Meter CR 400 (Konica Minolta, Japan). Before measurement, the colorimeter was calibrated with a standard white reference plate to ensure accuracy. The assessed color parameters included lightness (L*) and chromaticity coordinates of red/green (a*) and yellow/blue (b*) based on the CIE-LAB color space system (Sawant et al., 2013).

The total color difference (ΔE, Eq. 1) was calculated to quantify the overall color difference between Damirga and raw flour. The whiteness index (WI, Eq. 2) was computed to evaluate the degree of similarity with pure white, while the saturation index (SI, Eq. 3) assessed the intensity or purity of the color. The hue angle (hue in Eq. 4) describes the perceived color type (Pathare et al., 2013). Additionally, the browning index (BI, Eqs. 5 and 6) was calculated to indicate the extent of browning, potentially resulting from Maillard reactions or caramelization during processing (Rani et al., 2020). These indices were computed using standard formulas based on the CIE-LAB system:

$$\Delta E = \sqrt{(L_0 - L^*)^2 + (a_0 - a^*)^2 + (b_0 - b^*)^2} \quad (1)$$

Where subscript "0" refers to the color reading of the fresh raw flour used as the reference.

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (2)$$

$$S.I. = \sqrt{a^{*2} + b^{*2}} \quad (3)$$

$$Hue = \tan - 1 \quad (b^*/a^*) \quad (4)$$

$$BI = \frac{100(x - 0.31)}{0.17} \quad (5)$$

Where x,

$$x = \frac{(a^* + 1.75L^*)}{(5.645L^* + a^* - 0.012 \quad b^*)} \quad (6)$$

2.6. Fatty acid composition (FA)

The fatty acid (FA) compositions of raw and Damirga pearl millet flours were analyzed using a modified gas chromatography (GC) protocol described by Snell et al. (2022). The samples were first freeze-dried, and 50 mg of each sample was methylated in 2 mL of 2 % sulfuric acid in methanol at 90°C for 60 min, with heptadecenoic acid methyl ester (17:0-ME) used as an internal standard. Phase separation was achieved by adding 1 mL of heptane and 2 mL of water, followed by vortexing and centrifugation at 3000 rpm for 2 min. The upper heptane layer, containing the fatty acid methyl esters (FAMEs), was collected for further analysis.

Final separation was performed using an Intuvo DB-23 column (30 m, 0.25 mm inner diameter, 0.25 μm film thickness) on a gas chromatograph. The sample was split between a mass spectrometer for FAME identification and a flame ionization detector for quantification (Intuvo GC and MSD5977, Agilent Technologies, Santa Clara, CA, USA). Fatty acids were expressed as percentages of total fatty acids. FAMEs were identified and quantified by comparing their peak retention times with those of known fatty acid standards.

2.7. Rancidity -caused enzyme activity assay

2.7.1. Enzyme extract preparation

Lipase and lipoxygenase were extracted from raw and Damirga pearl millet flours using a modified method based on Goswami et al. (2020). For the extraction, 2 g of flour was mixed with 20 mL of 0.2 M potassium phosphate buffer (pH 7.5) in a pre-chilled mortar and pestle, maintaining a solid-to-liquid ratio of 1:10 (w/v). The mixture was centrifuged at 10,000 rpm for 20 min at 4 °C. The supernatant was filtered through four cheesecloth layers to obtain a clear enzyme extract, which was used for enzyme activity assays. The total soluble protein content of the extract was quantified using the Lowry method (Lowry et al., 1951).

2.7.2. Lipase activity assay

Lipase activity in Damirga and raw pearl millet flours was measured using the method initially reported by Itaya and Ui (1966) with modification by Goswami et al. (2020). A reaction mixture containing 10 μL of enzyme extract and 1 mL of substrate (0.98 % [w/v] NaCl, 200 μL Tween 20, olive oil, and water in a 1:3 ratio) was incubated at 37 °C for 15 min. The reaction was stopped by placing the mixture in a water bath at 90 °C for 5 min. Subsequently, 2 mL of copper triethanolamine reagent (1 M triethanolamine, 1 N acetic acid, and 6.45 % cupric nitrate) and 5 mL of chloroform were added, followed by thorough stirring. Then, 0.1 mL of 11 mM diethyl dithiocarbamate (DDC) was added to the lower yellow layer of the reaction mixture. The absorbance of the yellow layer was measured at a wavelength of 440 nm using a UV-Vis spectrophotometer (*Multiskan Go, Model 1510, Thermo Fisher Scientific, Vantaa, Finland*). A standard curve was prepared using oleic acid, and

lipase activity was expressed as mM free fatty acids per minute per milligram of soluble protein.

2.7.3. Lipoxygenase (LOX) activity

Lipoxygenase (LOX) activity in Damirga and raw pearl millet flours was measured using the method described by Surrey (1964). The substrate was prepared by mixing 50 μL of Tween 20 and 50 μL of linoleic acid in 10 mL of borate buffer (pH 9.0). Subsequently,1.3 mL of 1 N NaOH and 90 mL of borate buffer (pH 9.0) were added, followed by distilled water to adjust the final volume to 200 mL, yielding a linoleic acid concentration of approximately 7.5 mM.

For the reaction, 2.5 mL of 0.05 M acetate buffer (pH 4.2), 60 μL of 7.5 mM linoleic acid substrate, and 25 μL of enzyme extract were mixed. Absorbance at 234 nm was recorded continuously for 60 s against a blank. The molar extinction coefficient of linoleic acid (25 mM^{-1} cm^{-1}) was used to calculate the LOX activity, which was expressed as nanomoles of hydroperoxide produced per minute per gram of flour.

2.7.4. Hydrolytic and oxidative rancidity markers

2.7.4.1. Estimation of comprehensive peroxide value (CPV). The comprehensive peroxide value (CPV) of raw and Damirga pearl millet flour was determined using the AOAC Aoac (1990) method, with modifications from Goswami et al. (2020). For this analysis, 6 mL of an acetic acid-chloroform mixture (3:2) was added to 1 g of the flour sample. The mixture was shaken thoroughly and allowed to settle at room temperature for 5 min. Next, 0.1 mL of freshly saturated potassium iodide solution and 6 mL of deionized water were added, followed by 0.2 mL of 1 % starch solution, which caused the reaction mixture to turn dark blue. The solution was titrated with 0.1 N sodium thiosulfate until the dark blue color disappeared. The CPV was calculated using the following equation (Eq. 1),

$$\text{CPV} = \frac{(\text{A} - \text{B})\text{x N x1000}}{\text{W}} \tag{7}$$

Where:

CPV is the comprehensive peroxide value; A and B represent the titration volumes of sodium thiosulfate $Na_2S_2O_3$ for the sample (in mL) and blank, respectively; W is the weight of flour in grams; and N is the normality of the sodium thiosulfate ($Na_2S_2O_3$) standard solution.

2.7.4.2. Estimation of comprehensive acid value (CAV). The comprehensive acid value (CAV) of raw and Damirga pearl millet flours was determined using the standard acid value estimation method outlined by the Association of Official Analytical Chemists (Aoac, 1990), with slight modifications based on Goswami et al. (2020). In this procedure, 1 g of flour was mixed with 10 mL of freshly neutralized 95 % ethyl alcohol and heated on a hot plate at 60 °C until boiling. After cooling, 0.1 mL of the phenolphthalein indicator was added to the mixture and titrated with 0.1 N NaOH. The CAV was calculated using the following equation (Eq. 2),

$$\text{CAV} = \frac{40 \quad x \quad A \quad x \quad N}{W} \tag{8}$$

Where CAV is the comprehensive acid value, A is the volume of NaOH used (in mL), 40 is the constant value equivalent mass of 0.1 N NaOH, W is the weight of the sample in grams, and N is the normality of the standard NaOH solution.

2.8. Statistical analysis

The data were evaluated for homogeneity of variance using Levene's test and for normality using the Shapiro-Wilk test, with a significance threshold of $P < 0.05$. The effects of pearl millet genotypes, the Damirga processing procedure, and their interactions were evaluated using two-way ANOVA in XLSTAT software. Tukey's Honestly Significant Difference (HSD) post-hoc test was applied to identify significant treatment differences. Each treatment was replicated three times to ensure statistical reliability. Multivariate analysis was performed using Principal Component Analysis (PCA) and partial least squares regression (PLS) using XLSTAT (Tenenhaus et al., 2005). Correlation plots were generated using the *prcomp* function in R, and visualizations were created using *factoextra*, *pls*, *ggcorrplot, ggplot2*, and *ggrepel* packages.

3. Results and discussion

3.1. Chemical analysis

Chemical analysis revealed significant differences in ash, fiber, and fat content between the cultivars, while protein, moisture, and total carbohydrate content showed no significant variation. Aziz had significantly higher ash and fat content than BY, whereas BY exhibited a higher fiber content (Table 1 & Supplementary 1). These findings align with those of Jandu and Kawatra (2019), who reported notable variations in proximate composition and mineral content among pearl millet genotypes, highlighting the role of genetic diversity in enhancing nutritional profiles and end-product quality. This is particularly relevant for regions such as Sudan, where pearl millet is believed to have been domesticated (Beldados et al., 2018). While environmental conditions such as soil fertility, rainfall, and management practices are known to influence grain composition, all cultivars in this study were grown under uniform agro-ecological and agronomic conditions. This controlled setting reduces the confounding influence of external factors, thereby increasing confidence that the observed differences largely reflect inherent genotypic variation. Nonetheless, we acknowledge that subtle genotype-by-environment interactions may still contribute to trait expression.

Flour processing methods also had significant effects on all proximate parameters. Raw flour displayed higher values for most proximate components, except carbohydrates, whereas Damirga flour showed a considerable increase. Interactions between cultivar and flour processing methods significantly influenced only the fiber and fat content (Table 1 & Supplementary 1). These differences can be attributed to Damirga processing, which involves decortication and fermentation, both known to affect the nutrient composition of pearl millet. For instance, Babiker et al. (2018) reported that decortication significantly reduces pearl millet ash, protein, oil, and crude fiber content. Similarly, previous studies have shown that fermentation reduces lipid and fiber content in millet (Abioye et al., 2021; Kumari et al., 2022). The reduction in protein, fat, fiber, ash, and moisture in *Damirga*-processed flour results from the combined effects of fermentation and decortication. Fermentative microbes consume macronutrients for growth, breaking down proteins, lipids, and fibers (Gänzle, 2014), while their metabolic activity also contributes to moisture loss. Decortication, on the other hand, physically removes the bran and germ, rich in fat, fiber, and minerals, leading to further depletion (Babiker et al., 2018). These processes together explain the compositional changes observed and support the functional benefits of traditional *Damirga* processing.

In our study, Damirga flour exhibited a notably higher carbohydrate content compared to raw flour. This finding contrasts with the results of Chinenye et al. (2017), who reported reduced total carbohydrate content and increased soluble sugar content following decortication and fermentation. However, Akinola et al. (2017) observed that fermentation loosens starch granules, thereby enhancing nutrient bioavailability and improving the digestibility of carbohydrates and proteins. These observations suggest that the traditional Damirga processing method applied in our study may promote the retention or even a relative increase of complex carbohydrates, likely due to specific fermentation conditions and microbial activity that limit excessive starch hydrolysis. This nuanced effect of processing underscores the importance of optimizing traditional methods to enhance the nutritional profile of pearl

Table 1
Proximate analysis of the two pearl millet cultivars, flour processing methods, and their interaction effects on protein, ash, moisture, fiber, fat, and carbohydrate contents.

	Proximate analysis (%)					
Cultivars	**Protein content**	**Ash content**	**Moisture content**	**Fiber content**	**Crude fat content**	**Carbohydrates content**
Aziz	11.9 (±3.1)[a]	1.5 (±0.4)[a]	6.0 (±0.6)[a]	3.1 (±0.6)[b]	4.5 (±0.8)[a]	73.1 (±5.5)[a]
Baladi yellow	11.8 (±3.1)[a]	1.4 (±0.4)[b]	6.1 (±0.7)[a]	3.4 (±0.9)[a]	4.1 (±0.5)[b]	73.3 (±5.6)[a]
Flour processing						
Damirga	9.0 (±0.1)[b]	1.1 (±0.1)[b]	5.5 (±0.2)[b]	2.6 (±0.1)[b]	3.6 (±0.1)[b]	78.2 (±0.3)[a]
Raw flour	14.7 (±0.1)[a]	1.8 (±0.1)[a]	6.6 (±0.1)[a]	3.9 (±0.3)[a]	4.9 (±0.4)[a]	68.1 (±0.2)[b]
Two-Way ANOVA						
R^2	1.00	0.99	0.95	1.00	1.00	1.00
F	3159.1	411.7	50.9	1554.5	1251.7	1585.9
Pr > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cultivars, C	3.1	14.5	0.3	209.3	357.3	2.9
	0.12	0.005	0.62	< 0.0001	< 0.0001	0.13
Flour processing, FP	9474.1	1219.8	151.6	4281.2	3215.6	4754.6
	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
C*F	0.02	0.99	0.66	172.97	182.29	0.30
	0.88	0.35	0.44	< 0.0001	< 0.0001	0.60

Values represent the means of three replicates, with standard deviations presented in parentheses. Within each column, values followed by different superscript letters (a, b) indicate statistically significant differences at $P < 0.05$, as determined by Tukey's Honest Significant Difference (HSD) test. Statistical significance is denoted as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and NS indicates no significant difference ($P \geq 0.05$). Pr > F: Probability of observing the F-statistic under the null hypothesis (p-value). Values < 0.05 indicate statistically significant differences. R^2 is the proportion of variance explained by the model.

millet flour.

The interaction between the cultivar and flour processing method highlights how different pearl millet genotypes respond to traditional Damirga processing. Although the processing generally reduced protein, fiber, and fat content, the extent of these reductions varied between cultivars. This variability is supported by Abdalla et al. (1998), who found that traditional processing significantly reduced phytate and mineral (iron, calcium, zinc) content in pearl millet, with the degree of reduction depending on the grain's composition. Ahmed et al. (2010) further demonstrated that decortication and fermentation reduced total phosphorus and antinutritional factors such as phytates and polyphenols, with cultivar-dependent variation. Similarly, Sulieman et al. (2022) highlighted that Sudanese fermented cereal products, including *Damirga*, exhibit diverse nutritional outcomes depending on grain type, fermentation duration, and microbial composition. This aligns with our findings, where cultivar-specific differences influenced the extent of nutrient loss. Understanding these changes can guide households in optimizing processing methods to maximize nutrient retention, enhance taste, and extend shelf life (Gowda et al., 2022).

3.2. Physical properties of Damirga and raw flour

Significant varietal differences were observed in water absorption capacity (WAC) and oil absorption capacity (OAC). Aziz exhibited significantly higher WAC, and BY showed significantly higher OAC (Table 2 & Supplementary 2). These findings are consistent with those of Falade and Kolawole (2013), who reported notable differences among pearl millet cultivars, highlighting their suitability for specific food applications. The higher WAC of Aziz may be attributed to its flour structure, which likely has a greater proportion of polar hydrophilic components such as starch and soluble fibers. These constituents can bind and retain more water, thus improving hydration capacity. In contrast, BY's higher OAC could be linked to its higher fiber content and flour matrix composition, which may include more surface-active components interacting with lipids. These cultivar-specific functional traits reflect their processing potential in different food applications, with Aziz being better suited for moisture-retentive preparations, while BY may perform well in products where fat absorption enhances texture or flavor. Understanding these properties is essential for optimizing

Table 2
Physical properties of the two pearl millet cultivars, flour processing methods, and their interaction effects on bulk density, water absorption capacity (WAC), oil absorption capacity (OAC), and swelling power.

	Physical properties			
Cultivars	**Bulk density (g/cm3)**	**Water Absorption Capacity**	**Oil Absorption Capacity**	**Swelling power (mL/g)**
Aziz	1.88 (±0.13)[a]	1.15 (±0.05)[a]	0.92 (±0.05)[b]	6.22 (±1.06)[a]
Baladi yellow	1.80 (±0.11)[a]	1.04 (±0.02)[b]	1.00 (±0.06)[a]	6.30 (±0.81)[a]
Flour processing				
Damirga	1.93 (±0.08)[a]	1.07 (±0.06)[b]	0.95 (±0.07)[a]	7.09 (±0.07)[a]
Raw flour	1.75 (±0.08)[b]	1.12 (±0.08)[a]	0.97 (±0.07)[a]	5.43 (±0.35)[b]
Two-way ANOVA				
R^2	0.76	0.85	0.44	0.94
F	8.61	14.85	2.06	45.31
Pr > F	0.007	0.001	0.185	< 0.0001
Cultivars, C	4.17	37.30	5.66	0.31
	0.08	0.00	0.04	0.59
Flour processing, FP	20.17	6.57	0.30	133.65
	0.00	0.03	0.60	< 0.0001
C*FP	1.50	0.68	0.20	1.98
	0.26	0.43	0.66	0.20

Values represent the means of three replicates, with standard deviations presented in parentheses. Within each column, values followed by different superscript letters (a, b) indicate statistically significant differences at $P < 0.05$, as determined by Tukey's Honest Significant Difference (HSD) test. Statistical significance is denoted as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and NS indicates no significant difference ($P \geq 0.05$). Pr > F: Probability of observing the F-statistic under the null hypothesis (p-value). Values < 0.05 indicate statistically significant differences. R^2 is the proportion of variance explained by the model.

Damirga flour use in traditional foods, such as stiff porridge, where moisture retention and oil absorption influence the final product (Sunagar and Sreerama, 2024).

Bulk density and swelling power did not differ significantly between the cultivars (Table 2 & Supplementary 2). Bulk density is a crucial physical property that influences storage and packaging efficiency. The similar values observed for these two properties suggest comparable particle sizes and starch hydration behavior between Aziz and BY. However, as shown in Table 2, significant differences were observed in water absorption capacity (WAC), with Aziz exhibiting a higher WAC than BY (1.16 vs. 1.04 g/g, $p < 0.001$). This indicates that Aziz flour may contain a greater proportion of hydrophilic constituents, such as soluble fibers or damaged starch, which facilitate a stronger water-binding capacity. This cultivar-specific functional property is particularly relevant for traditional food preparations, such as porridges, where water retention influences consistency and sensory quality. These characteristics make both cultivars well-suited for applications requiring consistent density and swelling properties, such as extruded products (Zhang et al., 2014).

Except for the oil absorption capacity, flour processing significantly affected all physical properties measured. Damirga flour exhibited higher bulk density than raw flour, while raw flour showed a higher WAC than Damirga flour. No significant interactions existed between cultivars and flour processing methods for physical properties (Table 2 & Supplementary 2). Swelling power was significantly affected by flour processing ($p < 0.0001$), with *Damirga* flour showing higher values than raw flour (7.09 vs. 5.43 mL/g). This increase may be attributed to the partial breakdown of the starch matrix during fermentation and decortication, which enhances the exposure of starch granules to water. The lack of significant difference between the cultivars suggests that the genotype had limited influence on this trait, while the processing method played a more dominant role. Enhanced swelling power in *Damirga* flour indicates improved gelatinization potential, which is desirable in products requiring thickening or water retention.

The increased bulk density of *Damirga* flour likely resulted from bran removal during decortication, which created a more compact flour structure by reducing the proportion of fibrous, less dense outer layers. Adebo and Kesa (2023) reported similar findings in sorghum, where dehulling led to higher bulk density due to increased carbohydrate concentration and reduced moisture. This densification effect is associated with the removal of bran and germ, which typically have lower packing efficiency compared to the endosperm. Furthermore, although Makinde and Abolarin (2020) focused on cowpea, they found that dehulling improved hydration-related properties by making starch granules more accessible, which may also indirectly support denser flour matrices.

The WAC of Damirga flour was significantly lower than that of raw flour. This finding is consistent with that of Olajire et al. (2021), who observed that fermentation in pigeon peas reduced WAC owing to the breakdown of structural carbohydrates into simpler sugars, thereby diminishing the flour's ability to retain water. In the context of this study, the lower WAC may result from the enzymatic degradation of insoluble fibers and complex polysaccharides during fermentation, which weakens the flour's matrix and reduces the number of hydrophilic sites available for water binding. Additionally, fermentation may partially solubilize starch and lower molecular weight components, leading to a less cohesive structure with reduced water-holding capacity. Furthermore, the microorganisms involved in fermentation can influence WAC, highlighting the complex interplay between microbial activity and the substrate's physical properties (Qi et al., 2023). In the context of this study, the reduced WAC in *Damirga* flour compared to raw flour may be attributed to structural changes induced by fermentation, such as the enzymatic breakdown of insoluble fibers and complex polysaccharides. This degradation likely reduces the number of hydrophilic sites available for water binding and weakens the flour matrix. As observed in cereal-based substrates like glutinous rice (Qi et al., 2023), microbial fermentation can alter starch structure and hydration behavior, further contributing to lower water absorption capacity. These results emphasize the variability in fermentation outcomes, which depends on the substrate and specific fermentation conditions.

3.3. Color measurements

Both cultivars and flour processing methods significantly affected the measured color parameters (Table 3 & Supplementary 3). BY exhibited significantly higher lightness (L*), blue-yellow chromaticity (b*), whitening index (WI), browning index (BI), and saturation index (SI) compared to Aziz. This trend suggests that BY flour has a brighter and more vivid appearance, likely due to its lower pigment concentration and finer endosperm characteristics. In contrast, the relatively darker tone and lower L and WI values in Aziz may be attributed to higher concentrations of polyphenols or other colored phytochemicals in the bran layer, which influence the visual appearance of the flour after processing. In contrast, Aziz had significantly higher red-green chromaticity (a*), total color difference (**ΔE**), and hue angle than BY. Damirga flour showed significantly higher L* and WI values compared to raw flour, whereas raw flour had higher values for a*, b*, **ΔE**, BI, SI, and hue angle (Table 3 & Supplementary 3).

The interaction between cultivar and processing was substantial for nearly all color parameters. The genotypic variation in color measurements between the two pearl millet cultivars is likely due to genetic makeup and intrinsic chemical composition differences. The higher L*, b*, WI, BI, and SI values in BY indicate that this cultivar produces flour with greater lightness and visual appeal. These traits are advantageous for products such as flatbreads and porridges, where brighter color is often associated with freshness and quality.

In contrast, Aziz exhibited higher a*, ΔE, and hue angles, indicating a more intense red hue. This makes Aziz suitable for darker-colored products, such as fermented and roasted foods. The darker hue of Aziz flour may be attributed to its higher levels of polyphenolic compounds, which offer antioxidant properties and potential health benefits (Suma Pushparaj and Urooj, 2014; Yarrakula et al., 2022). However, these pigments can also contribute to bitterness and rancidity, emphasizing the importance of maintaining the flour quality during storage (Onyeoziri et al., 2021).

Our findings further revealed that Damirga flour had significantly higher L* and WI compared to raw flour, consistent with the observation of Huang et al. (2021), who reported increased L* and WI values in dehulled pearl millet due to the removal of dark pigments from the bran. Also, through biochemical transformations, lactic acid fermentation during Damirga processing enhances lightness and WI (Ashaolu and Reale, 2020). These results underscore the potential of tailored processing techniques, such as dehulling, fermentation, and sun drying, to optimize visual and functional attributes, such as lightness and WI, thereby broadening the applications of fermented products.

In addition to these increases, Damirga flour also showed a significant decrease in a, b, total color difference (ΔE), browning index (BI), and saturation index (SI) compared to raw flour (Table 3 & Supplementary 3). This trend may be attributed to pigment loss during decortication and the degradation of polyphenolic compounds and Maillard reaction products during fermentation. The reduction in ΔE and BI indicates a lighter and more visually uniform flour, while the lower a* and b* values reflect reduced red and yellow intensity. The drop in SI further confirms diminished color saturation. Collectively, these changes suggest improved flour brightness and visual appeal, which may enhance acceptance in food applications requiring refined color attributes.

3.4. Fatty acid composition

Significant differences were observed between the cultivars in the content of monounsaturated fatty acids (MUFAs) myristoleic and oleic

Table 3
Colorimetric properties of the two pearl millet cultivars, flour processing methods, and their interaction effects on lightness (L), greenness/redness (a), blueness/yellowness (b*), total color difference (ΔE), whiteness index (WI), browning index (BI), saturation index (SI), and hue angle (H*).

Cultivars	Color measurements							
	L*	a*	b*	Total color difference (ΔE)	Whiteness index (WI)	Browning index (BI)	Saturation index (SI)	Hue angle (Hue)
Aziz	70.8 (±6.5)[b]	0.8 (±0.3)[a]	12.6 (±0.8)[b]	26.6 (±5.4)[a]	67.9 (±5.5)[b]	21.0 (±6.5)[b]	12.6 (±0.9)[b]	1.50 (±0.02)[a]
Baladi yellow	83.4 (±8.1)[a]	0.3 (±0.1)[b]	22.0 (±2.3)[a]	23.4 (±5.8)[b]	72.1 (±6.6)[a]	30.7 (±7.4)[a]	22.0 (±2.3)[a]	−0.01 (±0.00)[b]
Flour processing								
Damirga	87.5 (±3.7)[a]	0.25 (±0.1)[b]	15.9 (±4.5)[b]	16.2 (±2.0)[b]	79.1 (±1.2)[a]	19.5 (±4.9)[b]	15.9 (±4.5)[b]	−0.02 (±0.00)[b]
Raw flour	66.8 (±8.5)[b]	0.92 (±0.2)[a]	18.7 (±5.9)[a]	33.8 (±5.6)[a]	60.8 (±5.8)[b]	32.3 (±5.8)[a]	18.7 (±5.9)[a]	1.51 (±0.03)[a]
Two-Way ANOVA								
R^2	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00
F	84355.4	704.1	79949.4	52263.7	53909.3	20183.1	73939.2	1314632.7
Pr > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cultivars, C	64800.0	797.5	216456.8	4344.5	7228.3	22113.0	199881.5	1272589.9
	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Flour processing, FP	173993.8	1289.0	19343.7	131836.2	137884.4	38283.8	18250.3	1303308.3
	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
C*FP	14272.5	25.8	4047.8	20610.5	16615.1	152.4	3685.8	1368000.0
	< 0.0001	0.001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Values represent the means of three replicates, with standard deviations presented in parentheses. Within each column, values followed by different superscript letters (a, b) indicate statistically significant differences at $P < 0.05$, as determined by Tukey's Honest Significant Difference (HSD) test. Statistical significance is denoted as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and NS indicates no significant difference ($P \geq 0.05$). R^2 is the proportion of variance explained by the model.

acids, whereas no differences were found for gadoleic and erucic acids. Aziz had significantly higher myristoleic and oleic acids than BY (Table 4.1 & Supplementary 4), highlighting the importance of cultivar selection in determining MUFA content. Oleic acid, a key MUFA, promotes cardiovascular health and enhances the shelf life of food products owing to its oxidative stability (Gillingham et al., 2011). The higher oleic acid content in Aziz makes it particularly valuable for nutritional and industrial applications, especially in baking and frying, where oxidative stability is critical for an extended shelf life.

Damirga flour had significantly higher myristoleic acid content than raw flour, while raw flour exhibited significantly higher oleic acid content than Damirga flour. No significant differences were observed in gadoleic and erucic acid content between the two flour processing methods. The interaction between cultivars and flour processing methods was significant for myristoleic acid content (Table 4.1 & Supplementary 4), indicating that Dmirga processing techniques such as dehulling, wet fermentation, and sun drying can influence specific fatty acid levels. Nguyen et al. (2015) found that removing grain hulls increases beneficial fatty acids' availability, enhancing their dietary and industrial applications. Similarly, Adrio (2017) found that microbial fermentation can enhance MUFA biosynthesis in certain plant oils, offering nutritional and industrial benefits.

The oleic acid content of the raw flour was significantly higher, suggesting that fermentation may alter fat composition through biochemical changes. This reduction in oleic acid during *Damirga* fermentation can be attributed to microbial-mediated lipid metabolism

Table 4.1
Fatty acid composition (%) of the two pearl millet cultivars, flour processing methods, and their interaction effects on monounsaturated (myristoleic, oleic, gadoleic, erucic) and polyunsaturated (linoleic, linolenic) fatty acids.

Cultivars	Mono- and polyunsaturated fatty acid compositions (%)					
	Myristoleic 14:1	Oleic 18:1	Gadoleic 20:1	Erucic 22:1	Linoleic 18:2	Linolenic 18:3
Aziz	0.06 (±0.01)[a]	30.1 (±0.2)[a]	0.15 (±0.01)[b]	0.05 (±0.01)[b]	41.0 (±0.2)[b]	3.1 (±0.06)[b]
Yellow Baladi	0.05 (±0.01)[b]	27.1 (±0.2)[b]	0.16 (±0.01)[b]	0.04 (±0.01)[b]	44.0 (±0.6)[a]	3.6 (±0.03)[a]
Flour processing						
Damirga	0.06 (±0.01)[a]	28.4 (±1.7)[b]	0.15 (±0.02)[a]	0.03 (±0.01)[a]	42.3 (±2.0)[b]	3.3 (±0.3)[b]
Flour	0.05 (±0.00)[b]	28.8 (±1.6)[a]	0.16 (±0.01)[a]	0.06 (±0.01)[a]	42.7 (±1.3)[a]	3.4 (±0.3)[a]
Two-Way ANOVA						
R^2	0.98	1.00	0.45	0.40	0.99	0.99
F	124.46	890.93	2.20	1.76	507.92	272.41
Pr > F	< 0.0001	< 0.0001	0.166	0.233	< 0.0001	< 0.0001
Cultivars, C	168.78	2636.83	2.40	1.07	1434.55	803.24
	< 0.0001	< 0.0001	0.160	0.331	< 0.0001	< 0.0001
Flour processing, FP	37.15	34.35	1.32	4.19	19.07	13.44
	0.000	0.000	0.285	0.075	0.002	0.006
C*FP	167.45	1.61	2.88	0.00	70.12	0.55
	< 0.0001	0.240	0.128	0.956	< 0.0001	0.480

Values represent the means of three replicates, with standard deviations presented in parentheses. Within each column, values followed by different superscript letters (a, b) indicate statistically significant differences at $P < 0.05$, as determined by Tukey's Honest Significant Difference (HSD) test. Statistical significance is denoted as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and NS indicates no significant difference ($P \geq 0.05$). Pr > F: Probability of observing the F-statistic under the null hypothesis (p-value). Values < 0.05 indicate statistically significant differences. R^2 is the proportion of variance explained by the model.

and oxidative degradation. Oleic acid, being a monounsaturated fatty acid, is more susceptible to oxidation than saturated fats. During fermentation, lipase enzymes, either endogenous or produced by fermentative microbes, can hydrolyze triglycerides, releasing free oleic acid, which is then further oxidized or metabolized by microbial pathways. These biochemical transformations lead to a measurable decline in oleic acid levels in the final fermented product. Moreover, prolonged fermentation may shift the lipid balance by favoring the preservation of more oxidation-resistant saturated fatty acids, thereby reducing the relative proportion of oleic acid in the overall fatty acid profile. This is consistent with Liu et al. (2016), who noted that oleic acid is an effective co-substrate, enhancing microbial activity during aerobic fermentation and increasing citric acid production (Andres and Dunne, 2022). Similarly, Yan et al. (2022) demonstrated that adding oleic acid during submerged fermentation significantly boosts the biosynthesis of bioactive compounds such as ganoderic acids in *Ganoderma lucidum*.

Significant varietal differences were observed in the content of the polyunsaturated fatty acids linoleic and linolenic acids, with BY showing higher levels than Aziz. Aher et al. (2022) reported that the genetic background of pearl millet plays a crucial role in determining the rancidity matrix and fatty acid composition, as genetic variations influence fatty acid biosynthesis pathways in grains. The higher concentrations of PUFAs in BY may appeal to health-conscious consumers seeking grains rich in essential fatty acids (EFAs).

The interaction between cultivar and flour processing method was significant only for linoleic acid (Table 4.1 & Supplementary 4), emphasizing the combined influence of genetic factors and traditional processing methods on the fatty acid composition of pearl millet. Understanding these variations is critical for optimizing food products' nutritional and functional properties (Ali et al., 2023).

Our findings further confirmed that genetic background significantly influences lipid profiles, as evidenced by the differences in saturated fatty acid content among pearl millet cultivars. The iron-enriched, biofortified cultivar Aziz had higher concentrations of stearic, arachidic, and behenic acid than Baladi Yellow, indicating that biofortification enhances fatty acid biosynthesis. Biofortification improves both fatty acid biosynthesis and nutrient absorption in fortified crops (Celorrio et al., 2016). Stearic acid is noteworthy for its neutral effects on blood cholesterol levels, making Aziz a potentially healthier choice for consumers seeking to minimize saturated fat intake while enhancing the lipid functionality of food products (Hunter et al., 2010).

The saturated fatty acids stearic, arachidic, and behenic acids varied significantly between cultivars, with Aziz having considerably higher levels than BY. Significant differences were also observed in palmitic and stearic acid content based on flour processing, whereas arachidic, behenic, and lignoceric acids showed no notable variation between flour processing (Table 4.2 & Supplementary 4).

Damirga flour had a significantly higher palmitic acid content than raw flour, whereas raw flour exhibited significantly higher stearic acid levels. This difference may be attributed to changes in lipid metabolism during fermentation, which affect fatty acid hydrolysis and transport (Wang et al., 2020). The higher stearic acid content in raw flour indicates that the lipid profile remained less altered than that of Damirga flour.

The interaction between cultivar and flour processing was significant for all measured saturated fatty acids (Table 4.2 & Supplementary 4), highlighting the complexity of lipid metabolism in pearl millet. These interactions reveal that Damirga processing affects the fatty acid profiles of different cultivars. For instance, the stearic acid content in raw flour reflects the absence of fermentation. In contrast, the increased palmitic acid content in Damirga flour can be attributed to the effect of flour processing. During fermentation and decortication, selective degradation of unsaturated fatty acids, such as linoleic and linolenic acids, may occur due to their higher susceptibility to oxidative breakdown and microbial metabolism. This shift can lead to a relative increase in the proportion of saturated fatty acids, such as palmitic acid, which are more stable and less prone to oxidation. Furthermore, microbial activity during fermentation may activate endogenous lipases or introduce exogenous enzymes that hydrolyze complex lipids, altering the fatty acid composition in favor of more saturated fractions.

3.5. Rancidity matrix measurements

Significant differences were observed between cultivars for rancidity matrix parameters, with Aziz showing a higher comprehensive acid value (CAV) than BY. In contrast, lipase activity, lipoxygenase activity, and other rancidity-related measures did not differ significantly between cultivars (Table 5). Higher CAV levels in Aziz indicate greater susceptibility to lipid hydrolysis, likely due to its distinct biochemical properties. CAV is recommended as a reliable indicator of lipid rancidity to assess flour quality and shelf life stability better. These findings are consistent with previous studies showing variability in rancidity

Table 4.2
Saturated fatty acid composition (%) of the two pearl millet cultivars, flour processing methods, and their interaction effects on palmitic (16:0), stearic (18:0), arachidic (20:0), behenic (22:0), and lignoceric (24:0) acids.

	Saturated fatty acid content (%)				
Cultivars	**Palmitic 16:0**	**Stearic 18:0**	**Arachidic 20:0**	**Behenic 22:0**	**Lignoceric 24:0**
Aziz	20.1 (±0.4)^{b}	4.1 (±0.03)^{a}	0.90 (±0.01)^{a}	0.27 (±0.01)^{a}	0.23 (±0.02)^{b}
Yellow Baladi	20.1 (±0.1)^{b}	3.7 (±0.19)^{b}	0.81 (±0.06)b	0.25 (±0.02)^{b}	0.22 (±0.02)^{b}
Flour processing					
Damirga	20.2 (±0.2)^{a}	3.8 (±0.30)^{b}	0.84 (±0.08)^{a}	0.26 (±0.03)^{a}	0.22 (±0.02)^{a}
Raw flour	19.9 (±0.2)^{b}	4.0 (±0.09)^{a}	0.88 (±0.03)^{a}	0.27 (±0.01)^{a}	0.23 (±0.02)^{a}
Two-Way ANOVA					
R^2	0.86	0.99	0.83	0.78	0.61
F	16.75	180.99	12.83	9.71	4.13
Pr > F	0.001	< 0.0001	0.002	0.005	0.048
Cultivars, C	0.05	358.50	26.68	14.46	0.77
	0.824	< 0.0001	0.001	0.005	0.405
Flour processing, FP	25.44	77.14	4.72	2.04	0.15
	0.001	< 0.0001	0.062	0.191	0.708
C*FP	24.76	107.32	7.08	12.64	11.46
	0.001	< 0.0001	0.029	0.007	0.010

Values represent the means of three replicates, with standard deviations presented in parentheses. Within each column, values followed by different superscript letters (a, b) indicate statistically significant differences at $P < 0.05$, as determined by Tukey's Honest Significant Difference (HSD) test. Statistical significance is denoted as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and NS indicates no significant difference ($P \geq 0.05$). Pr > F: Probability of observing the F-statistic under the null hypothesis (p-value). Values < 0.05 indicate statistically significant differences. R^2 is the proportion of variance explained by the model.

Table 5
Rancidity matrix parameters of the two pearl millet cultivars, flour processing methods, and their interaction effects on lipase activity, lipoxygenase activity, comprehensive acid value (CAV), and comprehensive peroxide value (CPV).

	Rancidity matrix parameters			
Cultivars	**Lipase activity**	**Lipoxygenase activity**	**Comprehensive Acid Value (CAV)**	**Comprehensive Peroxide Value (CPV)**
Aziz	9.3 (±2.6)[a]	41.7 (±9.6)[a]	4.3 (±1.6)[a]	45.8 (±11.6)[a]
Baladi yellow	8.2 (±2.2)[a]	41.2 (±8.9)[a]	3.6 (±1.4)[b]	42.6 (±12.8)[a]
Flour processing				
Damirga	3.6 (±0.5)[b]	19.4 (±0.1)[b]	1.5 (±0.3)[b]	8.5 (±0.5)[b]
Raw flour	13.9 (±1.6)[a]	63.4 (±1.2)[a]	6.4 (±0.5)[a]	79.9 (±6.3)[a]
Two-way ANOVA				
R^2	0.98	1.00	0.99	0.99
F	127.9	2464.4	464.0	305.2
Pr > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cultivars, C	4.7	0.7	25.0	1.8
	0.06	0.42	0.001	0.22
Flour processing, FP	368.9	7391. 9	1366.0	911.5
	< 0.0001	< 0.0001	< 0.0001	< 0.0001
C*FP	10.20	0.51	1.02	2.18
	0.01	0.50	0.34	0.18

Values represent the means of three replicates, with standard deviations presented in parentheses. Within each column, values followed by different superscript letters (a, b) indicate statistically significant differences at $P < 0.05$, as determined by Tukey's Honest Significant Difference (HSD) test. Statistical significance is denoted as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and NS indicates no significant difference ($P \geq 0.05$). Pr > F: Probability of observing the F-statistic under the null hypothesis (p-value). Values < 0.05 indicate statistically significant differences. R^2 is the proportion of variance explained by the model.

susceptibility among cultivars, where certain genotypes display reduced rancidity due to specific genetic traits (Aher et al., 2022; Bhargavi et al., 2024; Goswami et al., 2020).

For example, Moin et al. (2024) identified contrasting rancidity profiles among pearl millet genotypes such as ICMB-863 (high rancidity) and ICMB-95222 (low rancidity), highlighting their potential for breeding programs aimed at improving flour quality. This study also identified five genes associated with grain quality traits, including rancidity regulation, marking a significant step in understanding the role of phospholipases in enhancing the agronomic and quality characteristics of pearl millet.

Flour processing methods significantly influenced all rancidity matrix parameters, with Damirga flour exhibiting lower values. The interaction between cultivar and flour processing methods was significant only for lipase activity, indicating that traditional processes such as dehulling, fermentation, and sun drying can slow lipid rancidity. The reduction in rancidity indicators observed in Damirga flour may be partly due to decreased crude fiber during decortication. Studies have shown that decortication levels of 1–3 % by weight can reduce rancidity in millet flour by lowering lipoxygenase activity and lipid oxidation (Goswami et al., 2024).

Fermentation has also been reported to extend the shelf life of pearl millet flour by promoting the growth of lactic acid bacteria (LAB) and yeast, which preserve the flour by converting sugars to lactic acid (Kumar et al., 2024). This process creates an acidic environment, lowers the pH, and inhibits the growth of harmful bacteria and spoilage organisms that compromise product quality. Furthermore, fermentation reduces moisture content by metabolizing carbohydrates through microbial and enzymatic activities, which minimizes undesirable odors, such as the mousy smear caused by polyphenol oxidase activity (Sharma et al., 2015).

When combined with rapid drying and proper packaging, fermentation effectively prolongs the shelf life of pearl millet flour without compromising its quality. Dehulling is another effective method for mitigating rancidity by removing the outer bran layer, which contains significant amounts of lipids and anti-nutrient compounds (Chauhan, 2018; Pallavi et al., 2023). However, while dehulling reduces the fat content, it may result in the loss of beneficial minerals and phytochemicals. These trade-offs must be carefully considered when designing processes to optimize the shelf life and nutritional value of pearl millet flour.

3.6. Principal component analysis, partial least squares regression, and correlations between the rancidity matrix-related parameters

Principal Component Analysis (PCA) of the Damirga and raw pearl millet flour samples revealed significant insights into the effects of processing methods and cultivar variations on flour characteristics. The first two principal components (PC1 and PC2) explained 53.6 % and 36.3 % of the total variance, respectively, effectively capturing the underlying structure (Fig. 1.1). PC1 primarily separated the samples based on the processing methods, with positive PC1 values associated with Damirga-processed flours and negative values corresponding to raw flours. This separation highlights the influence of processing on enhancing specific flour properties, such as lightness and carbohydrate content, crucial for consumer acceptance and nutritional value (Onyeoziri et al., 2021).

Positive PC1 loadings for Damirga-processed flours indicated higher levels of lightness (L*), carbohydrate content (CAR), swelling power (SP), bulk density (BD), and specific fatty acids (myristic acid 14:0 and palmitic acid 16:0) (Figure 1–1). These traits improve the suitability of flour for diverse food applications. Conversely, negative PC1 loadings for raw flours reflected less desirable attributes, such as increased rancidity and lower nutritional quality.

PC2, however, highlighted cultivar-specific differences. Positive PC2 values corresponded to BY, indicating higher levels of blueness/yellowness (b*), oil absorption capacity (OAC), saturation index (SI), browning index (BI), whiteness index (WI), fiber content (FI), lipoxygenase activity (LOA), lipase activity (LIA), protein content (PC), and moisture content (MC) (Fig. 1.1). Additionally, BY exhibited higher levels of beneficial fatty acids, including linoleic acid (18:2), α-linoleic acid (18:3), and eicosenoic acid (20:1), underscoring its nutritional advantages. In contrast, the negative PC2 values for Aziz suggest comparatively lower levels of these beneficial traits, which may affect its overall utility in food applications.

Partial Least Squares Regression (PLS) analysis provided further insights into the clustering patterns among the samples, demonstrating solid model performance for the Aziz-Damirga (Az-D) combination, which exhibited minimal distance from the Y-axis (Fig. 1.2). This alignment with the predicted values highlights the potential of Az-D to be a high-quality product. In contrast, the Baladi-Damirga (BY-D), Baladi-raw (BY-R), and Aziz-flour (Az-R) samples showed greater distances from both axes, indicating a poorer fit to the model and suggesting a lower overall quality. These findings emphasize the critical

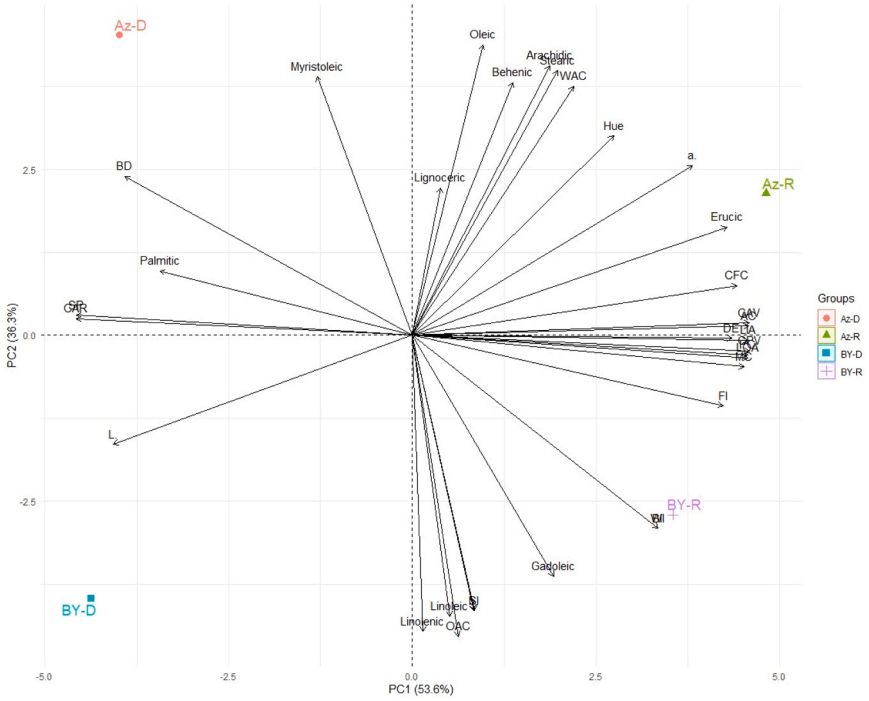

Fig. 1.1. Principal components analysis for rancidity-related matrix and chemical composition, physical properties, color attributes, and fatty acid composition. BD (Bulk Density), WI (Whiteness Index), L* (Lightness), a* (Redness), b* (Blue/Yellow), H* (Hue angle), SP (Swelling power), CAR (Carbohydrates content), WAC (Water Absorption Capacity), OAC (Oil Absorption Capacity), SI (Saturation index), BI (Browning Index), FI (Fiber content), PC (Protein content), MC (Moisture Content), CPV (Comprehensive Peroxide Value), CFC (Fat content), CAV (Comprehensive Acid Value), AC (Ash content), LIA (Lipase activity), LOA (Lipoxygenase activity). Groups are indicated as Az-D (Aziz Dameriga), Az-R (Aziz Flour), BY-D (Baladi Dameriga), and BY-R (Baladi Flour).

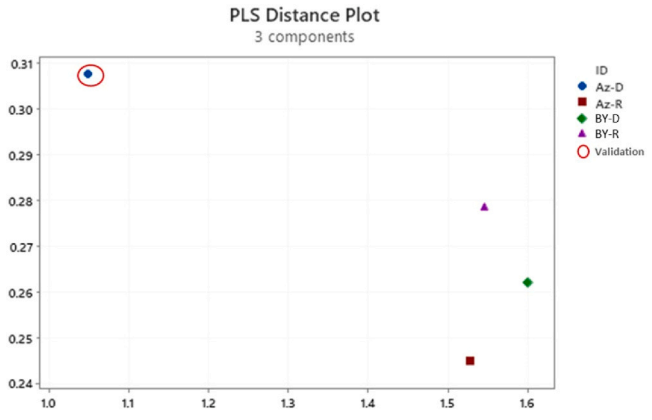

Fig. 1.2. Partial Least Squares (PLS) distance plot. (Az-D) Aziz-Damriga, (Az-R) Aziz-flour, (BY-D) Baladi-Damirga, (BY-R) Baladi-flour. The plot illustrates the distance of each sample from the X and Y axes based on 3 PLS components. The validation sample, marked with a red circle, closely aligns with the (Az-D) sample.

role of selecting appropriate processing techniques and cultivars to enhance pearl millet flour's nutritional and functional attributes, expanding its applicability to various food products.

Our results revealed positive correlations between lipase activity and crude fat, CAV, CPV, ash, crude protein, and moisture content (Fig. 1.3), indicating the central role of lipase in lipid breakdown and its influence on related physicochemical properties. The strong correlation with the total color difference ($R = 0.98$ ($P < 0.05$) further emphasized the impact of lipase-induced lipid oxidation on starch stability and carbohydrate polymers.

Both CAV and CPV were positively correlated with crude fat, protein, and moisture contents (Fig. 1.3), indicating a clear link between enzymatic activity and product quality, particularly regarding stability and composition. Goswami et al. (2020) noted that high moisture levels accelerated microbial growth and enzymatic processes, leading to lipid oxidation and protein denaturation. This process results in the hydrolysis of triglycerides into free fatty acids, thereby intensifying rancidity (Pande et al., 2024). Consequently, maintaining low moisture levels is crucial for extending the shelf life of pearl millet flour.

The susceptibility of pearl millet to rancidity is influenced mainly by lipid content and oxygen exposure during storage. With a crude fat content of 7.1 %, pearl millet is prone to oxidation, resulting in undesirable flavors and aromas (Dias-Martins et al., 2018). Rancidity is a multifaceted process driven by lipid composition and enzymatic activity. Tools such as the rancidity matrix provide valuable insights into assessing flour quality, whereas proximate analysis underscores the nutritional value of grains. Future improvements to the Damirga processing technique and genetic modifications will be vital for enhancing storage stability and expanding the applicability of pearl millet in food systems. Oxidation affects not only the flavor and aroma but also the protein stability and overall nutritional value of pearl millet flour (dos Santos et al., 2024).

4. Conclusions

This study demonstrated that Damirga processing, involving dehulling, fermentation, sun-drying, and milling, significantly reduced rancidity indicators such as lipase and lipoxygenase activities, comprehensive peroxide value (CPV), and comprehensive acid value (CAV), effectively extending the shelf life of millet flour. Additionally, the process enhanced attributes such as lightness and carbohydrate content while reducing protein and fiber levels, highlighting its impact on nutritional and physical properties. Cultivar selection also influenced flour characteristics, with Aziz exhibiting higher ash and fat contents and BY showing superior fiber content. These findings underscore the need for further research on the mechanisms driving changes in fatty

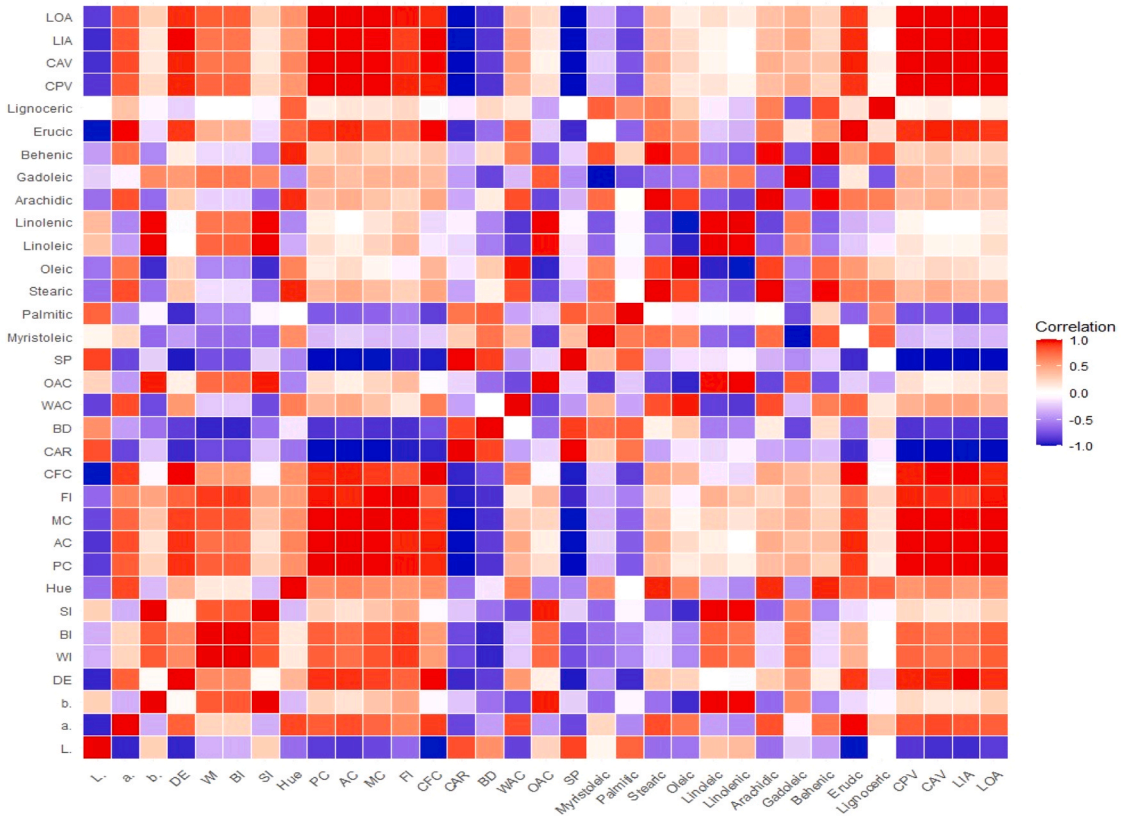

Fig. 1.3. A correlation matrix where the colors indicate Pearson correlation coefficients among rancidity-related matrix parameters, chemical composition parameters, physical properties parameters, color attributes, and fatty acid composition. BD (Bulk Density), WI (Whiteness Index), L* (Lightness), a* (Redness), b* (Blue/Yellow), H* (Hue angle), SP (Swelling power), CAR (Carbohydrates content), WAC (Water Absorption Capacity), OAC (Oil Absorption Capacity), SI (Saturation index), BI (Browning Index), FI (Fiber content), PC (Protein content), MC (Moisture Content), CPV (Comprehensive Peroxide Value), CFC (Fat content), CAV (Comprehensive Acid Value), AC (Ash content), LIA (Lipase activity), LOA (Lipoxygenase activity).

acid composition and rancidity to guide breeding and processing strategies that improve the quality and application of pearl millet in diverse food systems.

CRediT authorship contribution statement

Elkhatim Khitma: Writing – original draft, Methodology, Data curation. **Eva Johansson:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Mohammed Elsafy:** Writing – review & editing, Visualization, Validation, Formal analysis. **Tilal Abdelhalim:** Writing – review & editing, Visualization, Funding acquisition, Conceptualization. **Idris Yousif:** Writing – review & editing, Validation. **Hamid Manhal:** Writing – review & editing, Methodology, Investigation, Data curation. **Mahbubjon Rahmatov:** Writing – review & editing, Validation, Funding acquisition. **Faraz Muneer:** Writing – review & editing, Investigation, Funding acquisition, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Hereby, we declare that no conflict of interest for the study titled '**Damirga: A Traditional Fermented Flour for Rancidity Reduction and Revitalizing the Popularity of Pearl Millet'**. Additionally, we state that this manuscript has not been published previously and is not under consideration for publication elsewhere in English or in any other language.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2025.108263.

Data availability

Data will be made available on request.

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