The Annals of the University Dunarea de Jos of Galati
Fascicle VI – Food Technology (2023), 47(2), 207-220

ORIGINAL RESEARCH PAPER

EFFECT OF COMBINED GERMINATION AND SPONTANEOUS FERMENTATION ON THE BIOACTIVE, MINERAL, AND MICROBIAL PROFILE OF RED SORGHUM AND PEARL MILLET FLOURS

SALIOU MAWOUMA[1], MIHAELA COTÂRLEȚ[2], NINA NICOLETA LAZĂR[2,3], NICOLETA STĂNCIUC[2], GABRIELA RÂPEANU[2*]

1 University of Maroua, Faculty of Science, Department of Biological Sciences, PO BOX 814, Maroua, Cameroon

2 Dunărea de Jos University of Galați, Faculty of Food Science and Engineering, 111, Domnească Street, 800201, Galați, România

3 Dunărea de Jos University of Galați, REXDAN Research Infrastructure, 98, George Coșbuc Street, 800385, Galați, Romania

* Corresponding author: gabriela.rapeanu@ugal.ro

Received on 19 September 2023
Revised on 18 December 2023

Abstract

The aim of the study was to evaluate the influence of combined germination and spontaneous fermentation on the phytochemical, mineral, and microbial profile of red sorghum (*Sorghum bicolor* (L.) Moench) and pearl millet (*Pennisetum glaucum* (L.) R.Br.) flours. The analysis of the studied flours were performed by molecular spectrophotometry and flame atomic absorption spectrophotometry, respectively. Bioavailability of the iron, zinc, and calcium was estimated using the phytates/minerals molar ratios. The microbial load was counted using culture media specific to total bacteria, lactic acid bacteria, yeast and moulds, and Gram-negative bacteria. Combined processing resulted in flours exhibiting the lowest ($p < 0.05$) total phenols, flavonoids, 3-deoxy-anthocyanidins, phytates contents, and DPPH scavenging activity and the highest ($p < 0.05$) ABTS inhibition, mineral contents, and bioavailable iron, zinc, and calcium contents. Combined processing also resulted in the highest ($p < 0.05$) microbial cell counts, with lactic acid bacteria being the most abundant species in both processed cereals. Combining germination and spontaneous fermentation of red sorghum and pearl millet flours could be a useful and simple processing technique to develop food products that alleviate mineral deficiencies and promote human health.

Keywords*:* combined biological processing; red sorghum; pearl millet; bioactive compounds; minerals; microorganisms

https://doi.org/10.35219/foodtechnology.2023.2.13

Introduction

Cereals are among the major constituents of diets in sub-Saharan Africa (Macauley and Ramadjita, 2015; Serna-Saldivar, 2016). In addition to high levels of minerals, cereals also contain bioactive compounds which are beneficial to health because of their antioxidant properties (Izadi *et al.*, 2012; Baniwal *et al.*, 2021). Antioxidants protect cells and tissues by acting as free radical scavengers and/or transition metal chelators (Rice-Evans *et al.*, 1996). Minerals have numerous health benefits including tissue maintenance, bone and teeth formation, and health, serving as enzyme cofactors, and participating in the immune system and other body functions (Soetan *et al.*, 2010; Gupta and Gupta, 2014). In the Sahelian part of Africa, sorghum and millet are among the most produced and consumed minor cereals by local populations (Taylor, 2019; Raheem *et al.*, 2021; Ramashia *et al.*, 2021). Our previous work on the chemical composition and antioxidant profile of four sorghum and two pearl millet cultivars grown and consumed in the Far-north region of Cameroon revealed a global nutritional superiority of the Mouri pearl millet cultivar which exhibited high amounts of minerals, while phytochemicals and antioxidant activity were noticeably higher in red sorghum (Mawouma *et al.*, 2022).

Before consumption, cereals are subjected to various processing techniques. These techniques include heat treatments, mechanical treatments, and biological processing such as soaking, fermentation, and germination (Serna-Saldivar, 2016). In addition to improving the sensory properties of cereal products, processing methods lower antinutrient levels and improve minerals and vitamins bioavailability (Ochanda *et al.*, 2010; Lestienne, 2004). Soaking, which is a preliminary step to mechanical and biological processing of cereals, can lead to the modifications of grains texture and the release of water-soluble compounds. Many studies showed soaking significantly decreased total phenols and minerals, while others revealed an increase in antioxidant activity (Li *et al.*, 2022). Germination results in the degradation of macronutrients, reduction of anti-nutritional factors, and increase of the bioactive potential of sorghum grains (Liu et al., 2019). Studies on fermentation revealed contradictory effects on total phenol contents and total flavonoid contents (Li *et al.*, 2022). A review done by Taylor and Duodu (2015) showed that biological processing methods also affect the antioxidant potential of sorghum and millet grains. It can be deduced that the effects of germination and fermentation on cereal grains depend on the type of processing method and processing conditions.

To the best of our knowledge, there are no research data reported on the combined germination and spontaneous fermentation on the chemical properties of red sorghum and pearl millet grains. Expected results would reveal possible synergetic or antagonist impacts on the processed cereal products composition, with nutritional and health implications. A great interest exist in nutraceutical industries to develop functional ingredients derived from red sorghum and pearl millet, by using different processing techniques that result in desired high-quality products.

The objective of this research was to study the effect of combined germination and spontaneous fermentation on the bioactive, mineral, and microbial profile of pearl millet and red sorghum flours.

Materials and methods

Chemicals

All solvents and reagents used in this study were purchased from Sigma Aldrich (Germany).

Biological material

The both grains red sorghum (*Sorghum bicolor* (L.) Moench) and Mouri pearl millet (*Pennisetum glaucum* (L.) R.Br) were provided from a local market of Maroua (Far-north region of Cameroon). The grain samples were cleaned and sorted in order to remove all impurities. After that, samples were sun dried, and further packed in polypropylene bags and kept at room temperature.

A part of the grain samples were ground using a domestic grinder (Bosch TSM6A014R, 180W) to obtain flours with particle size of 250 µm used as the unprocessed control, while the remaining part was kept for further processing.

Biological processing

Germination

Clean grains were soaked in distilled water in a ratio of 1:1 for 12 h at temperature of 30°C. After discarding the steep water, wet grains were allowed to germinate on a piece of wet cloth in an oven at temperature of 30°C for 48h. Water was sprinkled intermittently to moisten the cloth. After germination, the grains were dried at 50°C for 12 h using an oven. The rootlets of germinated and dried grains were removed by scrubbing manually over a sieve. The grains were then ground in a domestic grinder (Bosch TSM6A014R, 180W) for 30-sec to obtain flours (particle size 250 µm). Non-germinated grains were also ground in the same conditions.

Spontaneous fermentation

Aliquots of flours obtained from non-germinated and germinated grains were mixed with sterile distilled water (1:1 w/v) in sterile glass containers. The glass containers were then covered and incubated at 30°C for 48 hours. Aliquots of wet fermented mixtures were used for microbial analyses while the remaining parts were dried at 50°C to constant weight (over 22 hours) and kept at -20°C until further chemical analyses.

The unprocessed red sorghum and pearl millet flours, and the samples subjected only to germination, or fermentation, were used in this experiment for comparative purposes.

Microbial count in the fermented flours

During spontaneous fermentation, 10 g of the different samples were aseptically collected at the beginning and after 48 h of fermentation for culture-based enumeration of microorganisms. The collected samples were mixed with 90 mL of buffered peptone water (0.1% [w/v] peptone, 0.85% [w/v] NaCl; pH 7.2) and vortexed for 3 min to obtain homogenous mixtures. From these, serial dilutions were prepared and then surface inoculated onto the following selective media: MRS (De Man, Rogosa and Sharpe) agar for total lactic acid bacteria count (TLABC) (Cotârleț *et al*., 2019), Plate Count Agar (PCA) for total bacteria count (TBC) (Wang *et al*.,

2023), Potato Dextrose Agar (PDA) for total yeast-mould count (TYMC) (ISO 21527-2:2008; Nistor *et al*., 2021) and Violet Red Bile Agar (VRBA) for total Gram-negative count (TGNC) (ISO 21528-2:2017; Nistor *et al.*, 2021). Plates were incubated at 30°C for 48 h. The counts were recorded and expressed as colony-forming units (CFU/g).

Chemical analyses

Determination of phytochemicals

Phytochemicals were evaluated using an ultrasound-assisted extraction which was done by mixing 1g of each grain flour with a volume of 9 mL of 70 % methanol (v/v). The samples were mixed for period of 5 min and then sonicated for 30 min in an ultrasonic water bath (MRC Scientific Instruments) at 40 kHz, 30-35˚C. After that, the samples were centrifuged at 6000 rpm, at temperature of 10˚C (Universal 320R Hettich Zentrifugen) for 10 min, and finally the supernatants were concentrated to dryness using an AVC 2-18 concentrator (Christ, UK). For further analysis the dried samples were redissolved in 70 % methanol (v/v) solution to reach to have a concentration of 10 mg/mL.

Total polyphenol content (TPC). TPC was measured using the Folin–Ciocalteu method (Turturică *et al.*, 2016). A volume of 0.20 mL extract was mixed with 15.8 mL of distilled water and a volume of 1 mL of Folin–Ciocalteau reagent was added. After 10 min a volume of 3 mL of Na_2CO_3 20% was added, and samples were stored at room temperature in the dark for 60 min. The optical density was measured with a Biochrom Libra S22 spectrophotometer at 765 nm, and the results were expressed as mg gallic acid equivalents/g DE flour using a calibration curve (0.1–0.5 mg/mL, $R^2 = 0.984$).

Total Flavonoids Content (TFC). TFC of sorghum and pearl millet flours was measured using the aluminum chloride spectrophotometric method (Dewanto *et al.*, 2002). In brief, a volume of 0.25 mL extract was mixed with 2 mL of distilled water and 0.075 mL of $NaNO_2$ 5%. After 5 min, a volume of 0.15 mL of $AlCl_3$ was added to the mixture and kept for 6 minutes. After that a volume of 0.5 mL of NaOH 1M was added and the absorbance was measured at 510 nm with a spectrophotometer (Biochrom Libra S22). The results were estimated as milligrams of catechin equivalents /g DE flour using a calibration curve (0.1–0.5 mg/mL, $R^2 = 0.997$).

Total 3-Deoxyanthocyanidin Content (TDC). The TDC was measured by reading the absorbances of redissolved extracts at 480 nm, and was expressed as milligrams of 3-deoxyanthocyanidin /g DE flour, using the molar extinction coefficient of luteolinidin, which is 13,800 M^{-1} cm^{-1} (Kumar *et al*., 2015).

Phytate content. For phytate content determination a method described by Vaintraub and Lapteva (1988) was used with small modifications. In brief, a quantity of 0.5 g of flour was mixed with 10 mL of 2.4 % HCl for a period of 1 hour at room temperature under manual shaking and then centrifuged at 3000 rpm at room temperature.for 30 min. Further, a volume of 3 mL of the supernatant was mixed with 1 mL of Wade reagent (0.03 % $FeCl_3$ solution with 0.3 % sulfosalicylic acid in distilled water) and and after the mixture was centrifuged at 3000 rpm for 10 min at

room temperature. The absorbance of supernatant was measured at 500 nm, and and phytate concentration was expressed using a phytic acid standard curve (5–40 mg/mL, R^2 = 0.980) as phytic acids in milligrams /100 g DW).

Antioxidant activity

DPPH scavenging method. The extracts' antiradical activity was evaluated using the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) compound. Briefly, a volume of 3.9 mL of DPPH solution 0.1 M was mixed with 0.1 mL of each extract, and stored for 30 min at room temperature in the dark before the absorbance at 515 nm were recorded (Af) with a spectrophotometer (Biochrom Libra S22). The blank consisted of 3.9 mL DPPH solution 0.1 M (in methanol) and 0.1 mL methanol instead of the extract, was kept for 30 min at room temperature in the dark and the absorbance was measured at 515 nm using (A0).

The inhibition percentage was calculated using following ecuation:

$$\% I_{DPPH} = (A0 - Af)/A0 \times 100$$

ABTS scavenging method. To estimate the ABTS scavenging activity, a volume of 2.85 mL of ABTS solution 7 mM, having the absorbance of 1.1 at wavelength of 734 (A0), was treated with a volume of 0.15 mL of extract (Af). The mixture was kept for 2 hours in the dark, and the absorbance was measured at 734 nm with a spectrophotometer (Biochrom Libra S22). The inhibition percentage was estimated as follows:

$$\% I_{ABTS} = (A0 - Af)/A0 \times 100$$

Determination of mineral content

The mineral content of red sorghum and pearl millet flours was estimated using an Atomic Absorption Spectrophotometer (3400 AAS Agilent Technologies). Briefly, a quantity of 1g of flour was burned at 500°C and further the ash was mixed with 2.5 mL nitric acid (1N). The mixture was filtrated and diluted using ultrapure water before analysis.

Estimation of mineral bioavailability

The relative bioavailability of iron, zinc, and calcium was estimated by the molar ratios of phytate to mineral, that is, Phy:Fe, Phy:Zn, Phy:Ca, and Phy·Ca:Zn (Zhang *et al.*, 2022). The molar ratios were calculated using the following formula:

$$\text{Phy:mineral molar ratio} = \frac{\text{Number of moles of phytates in the sample}}{\text{Number of moles of mineral in the sample}}$$

For Phy·Ca:Zn molar ratio, the numerator was multiplied by the number of moles of calcium in the sample.

Statistical analysis

The results are presented as means and standard deviation. Data were subjected to ANOVA and significant differences between means were revealed by post-hoc Duncan's multiple range test ($p < 0.05$), using IBM SPPSS Statistics 20.0 software.

Results and discussion

Bioactive profile of processed flours

Red sorghum and pearl millet contain various phytochemicals such as phenolic compounds and phytates that are secondary plant metabolites having bioactive and also anti-nutrient properties (Girard and Ramadjita, 2018; Mawouma *et al.*, 2022). Phenolic compounds in cereals include phenolic acid, flavonoid, and procyanidin, (Espitia-Hernandez *et al.*, 2022).

Analysing the results presented in Table 1, significant variations ($p < 0.05$) of the concentrations of TPC, TFC, TDC, and phytates among processed flours can be observed.

Table 1. Phytochemical content of processed red sorghum and pearl millet flours.

Samples	TPC (mg GAE/g DE)	TFC (mg CE/g DE)	TDC (mg/g DE)	Phytates (mg/100g DW)
		Red sorghum		
Control	82.23 ± 3.29^{d}	23.83 ± 1.28^{c}	9.06 ± 0.32^{d}	209.74 ± 0.89^{c}
Germinated (G)	63.70 ± 1.77^{c}	29.84 ± 0.51^{d}	0.69 ± 0.00^{ab}	144.61 ± 4.62^{b}
Fermented (F)	30.84 ± 0.25^{b}	15.56 ± 1.60^{b}	0.58 ± 0.01^{a}	133.84 ± 10.77^{b}
Combined (G+F)	25.49 ± 1.68^{a}	10.93 ± 0.43^{a}	0.98 ± 0.40^{c}	20.76 ± 3.85^{a}
		Pearl millet		
Control	19.15 ± 0.80^{b}	8.85 ± 0.07^{d}	1.01 ± 0.54^{c}	258.46 ± 7.69^{b}
Germinated (G)	25.02 ± 0.29^{d}	7.31 ± 0.92^{c}	0.08 ± 0.04^{a}	241.53 ± 9.23^{b}
Fermented (F)	23.02 ± 1.54^{c}	6.24 ± 0.43^{b}	0.10 ± 0.00^{ab}	228.46 ± 28.46^{b}
Combined (G+F)	14.11 ± 0.28^{a}	3.81 ± 0.16^{a}	0.14 ± 0.00^{b}	38.46 ± 10.77^{a}

TPC: total polyphenols content; TFC: total flavonoids content; TDC: total 3-deoxyanthocyanidin content; GAE: gallic acid equivalent; CE: catechin equivalent; DW: dry weight of flour; DE: dry weight of extract. For each investigated flour, mean values in the same column with different superscript letters are significantly different ($p < 0.05$).

It can be observed that in general, biological processing reduces the concentration of phytochemicals in red sorghum flours. Except for TDC, the most important reduction was observed in combined processing, followed by fermentation and then germination. As far as pearl millet flour is concerned, a similar tendency of reduced concentrations of phytochemicals in processed flours compared to unprocessed flour (control) was observed, except for total phenols content that was lower in unprocessed flour, compare to germinated and fermented flours. As with sorghum flours, the combined processing globally resulted in the greatest reduction of phytochemical contents. A review done by Li *et al*. (2022) presenting previous experimental studies revealed a contradictory effect of germination and fermentation on the phytochemicals content of grains. Hithamani and Srinivasan (2014) found that germination did not cause any significant change in the total phenol content and

significantly reduced total flavonoid content of cereal and pulse grains, while James *et al.* (2022) reported that germination and fermentation significantly increased the amount of bioactive phytochemicals. The reduction of phytochemical contents observed in the present study may be due to leaching during grain hydration prior to germination and to biochemical breakdown through activities of favourable enzymes occurring during biological processing.

The radical scavenging activities of unprocessed and processed red sorghum and pearl millet flours are presented in Table 2, with DPPH and ABTS being used as free radicals.

Table 2. Antioxidant activity (inhibition percentage) of processed red sorghum and pearl millet flours extracts.

Samples	DPPH (%)	ABTS (%)
Red sorghum		
Control	81.16 ± 0.52^{b}	89.99 ± 5.51^{a}
Germinated (G)	82.85 ± 0.35^{b}	93.67 ± 1.37^{b}
Fermented (F)	82.76 ± 2.51^{b}	92.95 ± 0.72^{b}
Combined (G+F)	56.80 ± 1.81^{a}	92.71 ± 0.54^{b}
Pearl millet		
Control	69.28 ± 5.12^{c}	84.39 ± 2.61^{a}
Germinated (G)	64.90 ± 0.57^{c}	94.30 ± 0.19^{b}
Fermented (F)	47.38 ± 1.46^{b}	93.92 ± 0.09^{b}
Combined (G+F)	21.38 ± 1.57^{a}	93.64 ± 0.54^{b}

For each investigated flour, mean values in the same column with different superscript letters are significantly different ($p < 0.05$).

Values revealed that DPPH antioxidant activity was globally higher in red sorghum flours compared to pearl millet flours. This result is in accordance with our previous investigation which revealed a net superiority of red sorghum on *Mouri* pearl millet as far as DPPH scavenging activity was concerned (Mawouma *et al.*, 2022). When carried on separately, germination and fermentation did not have a significant effect; but when combined, these two biological processes significantly reduced the DPPH scavenging activity. Although a general reduction in antioxidant phytochemicals was observed during processing, the formation of new compounds with similar radical scavenging activity would have occurred, suggesting that phenolic compounds can be transformed into other health-beneficial molecules during sprouting (Adebo *et al.*, 2020). As far as pearl millet flours are concerned, biological processing reduced the DPPH scavenging activity of flours, the greatest reduction being observed in fermentation and combined processing germination and fermentation. The reduction of antioxidant phytochemicals in processed pearl millet flours, as shown in Table 1, may explain this result. A synergetic effect of germination and fermentation may be

the greatest reduction of DPPH scavenging activity observed in combined processing.

In terms of ABTS, a slight significant increase was observed in the inhibition activity of processed red sorghum and pearl millet flours ($p < 0.05$). This result can also be explained by the contribution of newly formed antioxidant compounds during biological processing. Previous studies mentioned an increase in antioxidant activity in germinated and fermented cereal grains (Taylor and Duodu, 2015; Liu *et al.*, 2019; Li *et al.*, 2022).

It can be noticed that combined processing drastically reduced the amount of the investigated phytochemicals and could compromise the bioactive potential of processed red sorghum and pearl millet flours. However, this outcome may be advantageous from a nutritional point of view. Phenolic compounds and phytates are also known as antinutritional factors that reduce proteins digestibility and inhibit the intestinal absorption of dietary bivalent cations such as iron, zinc, and calcium (Hassan *et al.*, 2006; Platel and Srinivasan, 2016; Popova and Mihaylova, 2019).

Mineral profile of processed flours

Mineral content

The mineral content of the studied flours is detailed in Table 3. As shown in Table 3, separately processed flours (germination and fermentation) exhibited significantly higher ($p < 0.05$) mineral contents compared to unprocessed flours, while the combined processing exhibited significantly lower mineral contents. The only exception recorded was copper (Cu) content with no significant variation ($p > 0.05$) in sorghum flours and significant variation ($p < 0.05$) for pearl millet flour. Fermentation was the processing technique that provoked the highest increase in mineral contents, followed by combined processing, and germination. This finding is in agreement with many recent studies that reported germination and fermentation to increase the amount of minerals in processed sorghum, finger millet, and pearl millet flours (Coulibaly *et al.*, 2011; Mohapatra *et al.*, 2021; Davana *et al.*, 2021; Mudau *et al.*, 2022; Nkhata *et al.*, 2018). Loss in the dry matter by the breakdown of anti-nutritional factors and other complex organic compounds during germination and fermentation could lead to the concentration of minerals in processed flours (Nkhata *et al.*, 2018). The limited increase in mineral content displayed by germinated flours may be due to the leaching of minerals in soaking water (Li *et al.*, 2022).

Iron, Zinc, and Calcium bioavailability in processed flours

Molar ratios of phytate:iron, phytate:zinc, and phytate:calcium are used to estimate the relative bioavailability of these minerals in plant-based foods. A Phytates:Iron molar ratio below 1 is an indicator of good iron bioavailability (Hurell, 2004), while Phytates:Zinc molar ratio below 10 (Sandberg *et al.*, 1987; Morris and Ellis, 1989) and Phytates.Calcium:Zinc below 200 (Bindra *et al.*, 1986) is an indicator of good zinc bioavailability. As far as the Phytates:Calcium molar ratio is concerned, a value below 0.24 is predictive of good calcium bioavailability (Morris and Ellis, 1985).

The calculated molar ratios are shown in Table 4. Except for the flours obtained from combined processing, all the remaining studied flours had Phy:Fe and Phy:Ca molar

ratios above the threshold that estimates a good iron bioavailability. All the Phy:Zn and Phy.Ca:Zn molar ratios are below the critical threshold suggesting a desirable zinc bioavailability. Globally, it can be noticed that all the flours obtained from combined processing exhibited desired molar ratios for iron, zinc, and calcium. This result could be attributed to the maximal reduction of phytate contents in flours obtained from combined germination and fermentation.

Table 3. Macro-elements and race elements content of processed red sorghum and pearl millet flours (mg/100g DW).

Macro-elements					
Samples	**Ca**	**Na**	**K**	**Mg**	**P**
Red sorghum					
Control	9.86 ± 0.03^{a}	3.85 ± 0.01^{a}	254.02 ± 0.16^{a}	132.81 ± 0.56^{a}	275.07 ± 0.37^{ab}
Germinated (G)	16.27 ± 0.08^{c}	4.52 ± 0.57^{b}	302.12 ± 0.55^{c}	167.24 ± 8.35^{b}	283.95 ± 3.66^{bc}
Fermented (F)	18.15 ± 0.09^{d}	6.68 ± 0.09^{d}	330.23 ± 7.53^{d}	180.43 ± 11.26^{b}	338.58 ± 5.56^{e}
Combined (G+F)	17.60 ± 0.29^{d}	5.09 ± 0.17^{c}	306.32 ± 6.19^{c}	163.95 ± 2.10^{b}	299.84 ± 5.13^{d}
Pearl millet					
Control	14.41 ± 0.27^{b}	3.65 ± 0.04^{a}	282.25 ± 1.73^{b}	130.97 ± 0.40^{a}	269.02 ± 0.16^{a}
Germinated (G)	20.84 ± 1.01^{e}	6.07 ± 0.23^{e}	383.40 ± 1.02^{e}	246.53 ± 4.31^{d}	287.55 ± 4.57^{c}
Fermented (F)	22.95 ± 0.83^{f}	7.25 ± 0.13^{d}	422.63 ± 13.97^{f}	135.43 ± 3.50^{a}	401.820 ± 2.23^{f}
Combined (G+F)	21.83 ± 0.40^{ef}	6.69 ± 0.21^{d}	336.04 ± 12.62^{d}	224.61 ± 19.70^{c}	331.98 ± 7.83^{e}
Trace elements					
Samples	**Cu**	**Fe**	**Mn**	**Zn**	
Red sorghum					
Control	0.30 ± 0.01^{a}	2.80 ± 0.01^{a}	1.43 ± 0.02	1.95 ± 0.06^{a}	
Germinated (G)	0.28 ± 0.03^{a}	5.69 ± 0.31^{b}	2.89 ± 0.17^{c}	3.24 ± 0.25^{b}	
Fermented (F)	0.36 ± 0.04^{a}	6.29 ± 0.13^{d}	4.75± 0.11^{e}	3.63 ± 0.54^{b}	
Combined (G+F)	0.29 ± 0.11^{a}	5.90 ± 0.01^{cd}	3.63 ± 0.30^{d}	3.43 ± 0.23^{b}	
Pearl millet					
Control	0.55 ± 0.06^{b}	4.53 ± 0.04^{b}	0.85 ± 0.01^{a}	1.82 ± 0.01^{a}	
Germinated (G)	0.87 ± 0.05^{c}	7.72 ± 0.12^{e}	1.56 ± 0.11^{b}	4.34 ± 0.18^{c}	
Fermented (F)	1.66 ± 0.04^{e}	8.38 ± 0.38^{f}	1.27 ± 0.22^{b}	6.38 ± 0.13^{d}	
Combined (G+F)	1.45 ± 0.11^{d}	8.16 ± 0.18^{ef}	1.48 ± 0.16^{b}	5.99 ± 0.111^{d}	

DW: dry weight of flour. For each investigated flour, mean values in the same column with different superscript letters are significantly different ($p < 0.05$).

The enhanced mineral contents and some mineral bioavailability of processed flours imply that both germination and fermentation used separately or in combination can be promoted as household or industrial processing techniques to produce cereal-

based food products that can address mineral deficiencies in vulnerable groups of the population, especially children and women.

Table 4. Phytates:minerals molar ratios in processed sorghum and pearl millet flours.

Samples	Phy:Fe	Phy:Zn	Phy.Ca:Zn	Phy:Ca
		Red sorghum		
Control	6.34 ± 0.00^{d}	4.90 ± 0.12^{d}	1.20 ± 0.03^{c}	1.30 ± 0.00^{d}
Germinated (G)	2.15 ± 0.02^{c}	3.15 ± 0.06^{c}	0.83 ± 0.03^{b}	0.54 ± 0.03^{c}
Fermented (F)	1.80 ± 0.18^{b}	1.68 ± 0.06^{b}	0.76 ± 0.03^{b}	0.45 ± 0.05^{b}
Combined (G+F)	0.30 ± 0.08^{a}	0.27 ± 0.05^{a}	0.12 ± 0.03^{a}	0.07 ± 0.02^{a}
		Pearl millet		
Control	4.82 ± 0.16^{b}	6.45 ± 0.22^{d}	2.32 ± 0.04^{d}	1.09 ± 0.07^{c}
Germinated (G)	2.65 ± 0.18^{b}	2.54 ± 0.24^{c}	1.32 ± 0.0p6^{c}	0.70 ± 0.07^{b}
Fermented (F)	2.30 ± 0.30^{b}	1.63 ± 0.32^{b}	0.94 ± 0.22^{b}	0.60 ± 0.08^{b}
Combined (G+F)	0.40 ± 0.11^{a}	0.29 ± 0.12^{a}	0.16 ± 0.06^{a}	0.11 ± 0.04^{a}

For each investigated flour, mean values in the same column with different superscript letters are significantly different ($p < 0.05$).

Microbiological profile of processed flours

The microbial population of unprocessed and processed flours was monitored for total lactic acid bacteria count (TLABC), total bacteria count (TBC), total yeast-mould count (TYMC), and total Gram-negative count (TGNC). Values shown in Table 5 suggested a microbial diversity in the studied samples, indicating that germination and fermentation resulted in a competitive growth of different endogenous microorganisms. This microbial growth could be attributed to available nutrients and other simple organic compounds such as organic acids produced from the breakdown of complex molecules during biological processing. The highest microbial load was observed in samples from the combined processing of red sorghum and pearl millet flours. In the present study, the predominant microorganisms were LAB, followed by yeast and moulds. Previous studies reported the co-existence of lactic acid bacteria and yeasts in fermented food matrixes (Wood and Hodge, 1995; Ottogalli *et al.*, 1996; Ewuoso *et al.*, 2020). In the association between lactic acid bacteria and yeasts, the yeast provides vitamins that enhance the LAB growth, instead, the LAB produces acids for yeast growth (Odunfa and Adeyele, 1985). Acidic conditions generated by LAB are unfavourable to the development of food-spoiling microorganisms and harmful enterobacteria (Ayivi *et al.*, 2020). Lactic acid bacteria have many other probiotic properties such as boosting and strengthening the human immune system and preventing colon cancers, and many other diseases (Ayivi *et al*., 2020).

The growth of microorganisms in processed flours could explain the reduction of phytochemicals observed in this study. Microorganisms are known to produce

enzymes that degrade secondary metabolites found in plant-based food products (Nkhata *et al*., 2018).

Table 5. Microbial counts (CFU/g) of processed sorghum and pearl millet flours

Samples	TBC	TLBC	TYMC	TGNC
		Red sorghum		
Control	1.5×10^3	1.5×10^2	8.0×10^3	2.0×10^3
Germinated (G)	1.2×10^8	8.6×10^6	2.4×10^6	3.2×10^5
Fermented (F)	2.6×10^7	5.5×10^7	3.0×10^5	2.0×10^4
Combined (G+F)	6.5×10^{10}	1.4×10^8	2.4×10^7	2.8×10^8
		Pearl millet		
Control	3.4×10^5	1.4×10^4	1.6×10^5	4.5×10^3
Germinated (G)	1.2×10^8	4.8×10^6	4.0×10^6	4.4×10^5
Fermented (F)	6.9×10^7	4.6×10^{10}	4.8×10^6	1.6×10^4
Combined (G+F)	4.2×10^9	2.6×10^{10}	9.3×10^6	2.0×10^6

TBC: total bacteria count; TLBC: total lactic acid bacteria count; TYMC: total yeast-mould count; TGNC: total Gram-negative count.

Conclusions

Biological processing, such as germination and spontaneous fermentation, are common techniques applied to minor cereals before their consumption. Combining germination and spontaneous fermentation had the highest impact on the bioactive, mineral, and microbial profiles of red sorghum and pearl millet flours. The content of phytochemicals was drastically reduced while there was a global improvement in the mineral status. The enhanced mineral content and bioavailability in processed flours suggest the potential of combined processing as a useful and simple means to develop food products suitable for alleviating the mineral deficiencies in vulnerable groups of the population and promote health.

Acknowledgments

AUF (Agence Universitaire de la Francophonie) is acknowledged for granting a postdoctoral fellowship (Bourse postdoctorale Eugen-Ionescu, Edition 2020). The Integrated Center for Research, Expertise, and Technological Transfer in the Food Industry is acknowledged for providing technical support.

References

Adebo, O.A., Gabriela Medina-Meza, I. 2020. Impact of fermentation on the phenolic compounds and antioxidant activity of whole cereal grains: A mini review. *Molecules*, **25**(4), 927.

Ayivi, R.D., Gyawali, R., Krastanov, A., Aljaloud, S.O., Worku, M., Tahergorabi, R., Claro da Silva, R., Ibrahim, S. A. 2020. Lactic acid bacteria: Food safety and human health applications. *Dairy*, **1**(3), 202-232.

Baniwal, P., Mehra, R., Kumar, N., Sharma, S., Kumar, S. 2021. Cereals: Functional constituents and its health benefits. *The Pharma Innovation Journal*, **10**(2), 343-349.

Bindra, G.S., Gibson, R.S. Thompson, L.U. 1986. [Phytate][calcium]/[zinc] ratios in Asian immigrant lacto-ovo vergetarian diets and their relationship to zinc nutriture. *Nutrition Research*, **6**, 475-483.

Cotârleț, M., Maftei, N.M., Bahrim, G.E. 2019. Increasing the fermentation efficiency of *Lactobacillus paracasei* ssp. *paracasei* MIUG BL6 in a rye flour sourdough. *Turkish Journal of Biochemistry*, **44**(3), 307-315.

Coulibaly, A., Kouakou, B., Chen, J. 2011. Phytic acid in cereal grains: structure, healthy or harmful ways to reduce phytic acid in cereal grains and their effects on nutritional quality. *American Journal of Plant Nutrition and Fertilization Technology*, **1**(1), 1-22.

Davana, T. V., Revanna, M.L., Begum, S.S. 2021. Effect of malting on the nutritional composition, anti-nutrition factors and mineral composition on sorghum (*Sorghum bicolor*). *Asian Journal of Dairy and Food Research*, **40**(4), 451-455.

Dewanto, V., Wu, X., Adom, K.K., Liu, R.H. 2002. Thermal Processing Enhances the Nutritional Value of Tomatoes by In-creasing Total Antioxidant Activity. *Journal of Agricultural and Food Chemistry*, **50**(10), 3010–3014.

Espitia-Hernández, P., Chavez Gonzalez, M.L., Ascacio-Valdés, J.A., Dávila-Medina, D., Flores-Naveda, A., Silva, T., Chacón X.R., Sepúlveda, L. 2022. Sorghum (*Sorghum bicolor L.*) as a potential source of bioactive substances and their biological properties. *Critical Reviews in Food Science and Nutrition*, **62**(8), 2269-2280.

Ewuoso, M.O., Animashaun, O.H., Adejumo, A.A. 2020. Lactic acid bacteria and yeasts in spontaneously fermented sorghum sourdough. *American Journal of Microbiological Research*, **8**(2), 63-72.

Girard, A.L., Awika, J.M. 2018. Sorghum polyphenols and other bioactive components as functional and health promoting food ingredients. *Journal of Cereal Science*, **84**, 112-124.

Gupta, U. C., Gupta, S. C. 2014. Sources and deficiency diseases of mineral nutrients in human health and nutrition: a review. *Pedosphere*, **24**(1), 13-38.

Hassan, A.B., Ahmed, I.A.M., Osman, N.M., Eltayeb, M.M., Osman, G.A., Babiker, E.E. 2006. Effect of processing treatments followed by fermentation on protein content and digestibility of pearl millet (*Pennisetum typhoideum*) cultivars. *Pakistan Journal of Nutrition*, **5**(1), 86-89.

Hithamani, G., Srinivasan, K. 2014. Effect of domestic processing on the polyphenol content and bioaccessibility in finger millet (*Eleusine coracana*) and pearl millet (*Pennisetum glaucum*). *Food Chemistry*, **164**, 55-62.

Hurrell, R.F. 2004. Phytic acid degradation as a means of improving iron absorption. *International Journal of Vitamin and Nutrition Research*, **74** (6), 445–452.

ISO 21527-2:2008 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and moulds — Part 2: Colony count technique in products with water activity less than or equal to 0,95.

ISO 21528-2:2017 Microbiology of the food chain — Horizontal method for the detection and enumeration of *Enterobacteriaceae* — Part 2: Colony-count technique.

Izadi, Z., Nasirpour, A., Izadi, M., Izadi, T. 2012. Reducing blood cholesterol by a healthy diet. *International Food Research Journal*, **19**(1), 29–37.

James, S., Bagirei, S.Y., James, S.A., Maina, C.Y., Ibrahim-Baba, A., Ilemona, A., Ozovehe, A. 2022. Total phenolic, flavonoid and antioxidant capacities of processed pearl millet and sorghum flours. *Croatian Journal of Food Science and Technology*, **14**(2), 272-281.

Kumar, V.G., Viswanathan, R., Malathi, P., Nandakumar, M.P., Sundar, A.R 2015. Differential Induction of 3-deoxyanthocyanidin phytoalexins in relation to *Colletotrichum falcatum* resistance in sugarcane. *Sugar Tech*, **17**, 314–321.

Lestienne, I. 2004. *Contribution à l'étude de la biodisponibilité du fer et du zinc dans le grain de mil et conditions d'amélioration dans les aliments de complément*. Montpellier: Université Montpellier II.

Li, Z., Zhao, X., Zhang, X., Liu, H. 2022. The effects of processing on bioactive compounds and biological activities of sorghum grains. *Molecules*, **27**(10), 3246.

Liu, H., Kang, Y., Zhao, X., Liu, Y., Zhang, X., Zhang, S. 2019. Effects of elicitation on bioactive compounds and biological activities of sprouts. *Journal of Functional Foods*, ***53***, 136-145.

Macauley, H., Ramadjita, T. 2015. Les cultures céréalières: riz, maïs, millet, sorgho et blé. *Africa Rice Center*, Benin.

Mawouma, S., Condurache, N. N., Turturică, M., Constantin, O. E., Croitoru, C., Rapeanu, G. 2022. Chemical composition and antioxidant profile of sorghum (*Sorghum bicolor (L.) moench*) and pearl millet (*Pennisetum glaucum* (L.) R. Br) grains cultivated in the far-North region of Cameroon. *Foods*, **11**(14), 2026.

Mohapatra, D., Patel, A. S., Kar, A., Deshpande, S. S., Tripathi, M. K. 2021. Effect of different processing conditions on essential minerals and heavy metal composition of sorghum grain. *Journal of Food Processing and Preservation*, **45**(1), e14909.

Morris, E. R., Ellis, R. 1989. Usefulness of the dietary phytic acid/zinc molar ratio as an index of zinc bioavailability to rats and humans. *Biological Trace Element Research*, **19**, 107-117.

Morris, E.R., Ellis, R. 1985. *Bioavailability of dietary calcium-effect of phytate on adult men consuming non-vegetarian diets*. In: ACS Symposium Series 75: Nutritional Bioavailability of Calcium. Kies C (eds). American Chemical Society: Washington, DC, USA.

Mudau, M., Ramashia, S.E., Mashau, M.E. 2022. Mineral content, functional, thermo-pasting, and microstructural properties of spontaneously fermented finger millet flours. *Foods*, **11**(16), 2474.

Nistor, O.V., Bolea, C.A., Andronoiu, D.G., Cotârleț, M., Stănciuc, N. 2021. Attempts for developing novel sugar-based and sugar-free sea buckthorn marmalades. *Molecules*, **26**, 3073.

Nkhata, S.G., Ayua, E., Kamau, E.H., Shingiro, J.B. 2018. Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. *Food Science & Nutrition*, **6**(8), 2446-2458.

Ochanda, S. O., Akoth, O. C., Mwasaru, A. M., Kagwiria, O. J., Mathooko, F. M. 2010. Effects of malting and fermentation treatments on group B-vitamins of red sorghum, white sorghum and pearl millets in Kenya. *Journal of Applied Biosciences*, **34**, 2128-2134.

Odunfa, S.A., Adeyele, S. 1985. Microbiological changes during the traditional production of ogi-baba, a West African fermented sorghum gruel. *Journal of Cereal Science*, **3**(2), 173-180.

Ottogalli, G., Galli, A., Foschino, R. 1996. Italian bakery products obtained with sourdough: characterization of the typical microflora. *Advances in Food Sciences*, **18**(5), 131-144.

Platel, K., Srinivasan, K. 2016. Bioavailability of micronutrients from plant foods: an update. *Critical Reviews in Food Science and Nutrition*, **56**(10), 1608-1619.

Popova, A., Mihaylova, D. 2019. Antinutrients in plant-based foods: A review. *The Open Biotechnology Journal*, **13**(1), 68-76.

Raheem, D., Dayoub, M., Birech, R., Nakiyemba, A. 2021. The contribution of cereal grains to food security and sustainability in Africa: Potential application of UAV in Ghana, Nigeria, Uganda, and Namibia. *Urban Science*, **5**(1), 8.

Ramashia, S.E., Mashau, M.E., Onipe, O.O. 2021. Millets cereal grains: nutritional composition and utilisation in Sub-Saharan Africa. London: IntechOpen.

Rice-Evans, C.A., Miller, N.J., Paganga, G. 1996. Structure-antioxidant activitiy relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*, **20**, 933-956.

Sandberg, A.S., Anderson, H., Carlesson, N.G., Sandstrom, B. 1987. Degradation products of bran phytate formed during digestion in the human small intestine: effects of extrusion cooking on digestibility. *The Journal of Nutrition*, **117**, 2061-2065.

Serna-Saldivar, S.O. 2016. *Cereal grains: properties, processing, and nutritional attributes*. CRC Press.

Soetan, K. O., Olaiya, C. O., Oyewole, O. E. 2010. The importance of mineral elements for humans, domestic animals and plants: A review. *African journal of food science*, **4**(5), 200-222.

Taylor, J. R. 2019. *Sorghum and millets: Taxonomy, history, distribution, and production*. In Sorghum and millets. AACC International Press.

Taylor, J. R., Duodu, K.G. 2015. Effects of processing sorghum and millets on their phenolic phytochemicals and the implications of this to the health-enhancing properties of sorghum and millet food and beverage products. *Journal of the Science of Food and Agriculture*, **95**(2), 225-237.

Turturică, M., Stănciuc, N., Bahrim, G., Râpeanu, G. 2016. Effect of thermal treatment on phenolic compounds from plum (prunus domestica) extracts – A kinetic study. *Journal of Food Engineering*, **171**, 200–207.

Vaintraub, I. A., Lapteva, N. A. 1988. Colorimetric determination of phytate in unpurified extracts of seeds and the products of their processing. *Analytical Biochemistry*, **175**(1), 227-230.

Wang, M., Bai, Z., Liu, S., Liu, Y., Wang, Z., Zhou, G., Gong, X., Jiang, Y., Sui, Z. 2023. Accurate quantification of total bacteria in raw milk by flow cytometry using membrane potential as a key viability parameter. *LWT-Food Science and Technology*, **173**, 114315.

Wood, B. J., Hodge, M. M. 1985. Yeast-lactic acid bacteria interactions and their contribution to fermented foodstuffs. *Microbiology of fermented foods*, **1**, 263-293.

Zhang, Y. Y., Stockmann, R., Ng, K., Ajlouni, S. 2022. Revisiting phytate-element interactions: implications for iron, zinc and calcium bioavailability, with emphasis on legumes. *Critical reviews in food science and nutrition*, **62**(6), 1696-1712.