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Exploring Ultrasonic Energy Followed by Natural Fermentation Processing to Enhance Functional Properties and Bioactive Compounds in Millet (*Pennisetum glaucum* L.) Grains

Mohammed Saeed Alkaltham, Akram A. Qasem, Mohamed A. Ibraheem and Amro B. Hassan *

Department of Food Science and Nutrition, Faculty of Food and Agricultural Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia; malkaltham@ksu.edu.sa (M.S.A.); aqasem@ksu.edu.sa (A.A.Q.); mfadol@ksu.edu.sa (M.A.I.)

* Correspondence: ahassan2ks.c@ksu.edu.sa; Tel.: +966-500204967

Abstract: This study explores the effect of ultrasonic treatment followed by fermentation on the in vitro protein digestibility, protein solubility, functional properties, antioxidant activity, total carotenoid content, and gamma-aminobutyric acid (GABA) levels in millet grains. Ultrasonic treatment was applied at different temperatures (20, 40, and 60 °C). The findings indicated significant improvements in phenolic and flavonoid contents and antioxidant activity in terms of the results of the DPPH, FRAP, and ABTS assays of millet grains after ultrasonic treatment alone or combined with fermentation. Moreover, the carotenoid and GABA contents were found to be significantly higher in the ultrasonic-treated grains. The protein solubility functional properties of the millet grains were also improved after the ultrasonic treatment alone or coupled with the fermentation process. Principal component analysis (PCA) revealed that the combined ultrasonic treatment and fermentation of the millet grains could enhance their antioxidant activity, functional characteristics, and vital compounds. Furthermore, the partial least squares (PLS) validation model emphasised that the ultrasonic treatment of millet at 40 °C, followed by fermentation, is the most optimal treatment among the other treatments. Hence, the conclusions highlight the potential of combined ultrasonic (40 °C) and fermentation treatments to improve grains' nutritional value and functional properties, making millet more suitable for use in health-promoting food products.

Keywords: ultrasonic; fermentation; functional properties; antioxidants; protein digestibility; phytochemical compounds; millet grain

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1. Introduction

Pearl millet (*Pennisetum glaucum* (L.) is vital to agriculture and food in many developing countries due to its ability to thrive in hot and dry conditions. Millet, in particular, is recognised for its nutritional and medicinal benefits [1,2]. Millet is a small-seeded cereal from the Poaceae family. The various types of millet include pearl millet, foxtail millet, finger millet, kodo millet, proso millet, little millet, and barnyard millet [3,4]. It was initially domesticated in arid and semi-arid climates, with minimal water and nutrient involvement, making it a more drought-resistant cereal crop in contrast to the other millet varieties Foxtail millet (*Setaria italica*) and Proso millet (*Panicum miliaceum*). Due to this, pearl millet offers a rich nutritional profile. It is protein-rich, containing a significant amount of essential amino acids. Prolamin, the primary storage protein in millet, makes up over 55% of its protein content. High in hydrophobic amino acids, this protein can release bioactive peptides during digestion [5]. Also, millet contains phytochemicals, phenolic acids, flavonoids, amino acids, vitamin B, and minerals such as iron, potassium, phosphorus, calcium, and zinc [3,4,6,7]. Grains, including millet, must be processed to enhance nutrients' bioavailability, bio-accessibility, and digestibility [8]. This processing can involve both traditional and innovative methods. The processing ways, such as cooking, soaking, sprouting, and

fermentation, are used to create various millet-based dishes [9,10]. Processing also boosts enzymatic activity, reduces the dry mass, changes the microstructure, improves water and oil retention, and enhances nutrients' bio-accessibility and bioavailability [11]. Several food processing methods impact the nutritional and sensory qualities of grains, which cause a substantial impact on the physicochemical content and functional properties of proteins [12]. Many processing methods have been explored to improve protein formation and functionality, enhancing food quality.

Numerous research studies have demonstrated that applying ultrasonic treatment as a source of non-thermal energy can affect grains' nutritional, chemical, and functional characteristics. Recently, ultrasound technology has been explored as a promising and eco-friendly technology for modifying the structure and optimising the function of biological macromolecules, particularly proteins [13–16]. Ultrasonic energy can modify food components, particularly proteins, through several actions, including cavitation, shear, crushing, and stirring. Additionally, unlike traditional food processing methods, ultrasound reduces the processing costs, speeds up processing, preserves the heat-sensitive ingredients, and simplifies the food processing operations [17].

The fermentation processing of grains enhances the nutritional and functional aspects of various foods. Particularly, fermenting with sourdough, due to its diverse microbial community, increases the number of bioactive compounds, enhances protein digestibility, improves mineral bioavailability, lowers the glycemic index, boosts the dietary fibre content, and removes antinutritional factors [18,19]. During fermentation processing, beneficial compounds and organic substances are formed as a result of microorganism action in the breakdown of carbohydrates and proteins in cereal flour [20,21]. It has been reported that the fermentation of millet flour significantly increases its digestibility, the bioavailability of nutrients, and the available bioactive compounds and improves the antioxidant properties and functional properties [22–24]. In contrast, unfermented cereals often have natural antioxidants, such as phenolic compounds, bound or polymerized, reducing their bio-accessibility [25,26].

Combining ultrasonication with other treatments (e.g., germination) has been shown to optimise the nutritional profile of grains by reducing the amount of antinutrients and enhancing the quantity of beneficial compounds, like antioxidants in kodo and little millet [27]. Although several techniques have been applied to enhance the nutritional value and functional quality of millet, the evidence of the use of ultrasonication subsequent to fermentation on millet to improve its nutritional and functional properties is very scant. Hence, in this study, we aim to investigate the impact of ultrasonic treatments at different temperature levels, followed by fermentation, on the protein digestibility, solubility, functional properties, antioxidant capacity, and γ-aminobutyric acid (GABA) levels of pearl millet grains.

2. Materials and Methods

2.1. Sample Preparation and Ultrasonic Treatment

The local pearl millet genotype (baladi) used in this study was obtained from a local market in Saudi Arabia. The grains were cleaned and then kept in plastic bags in a refrigerator at 4 °C until they were used for experiments. Ultrasonic treatments were performed using 250 W and 40 kHz ultrasonic equipment. Whole millet grains were held in a glass jar and treated at 20, 40, and 60 °C temperatures for 20 min. The control and treated grains were ground and passed through a 0.4 mm sieve. The fermentation process was conducted naturally by mixing millet flour with H_2O (1:3 w/v) and incubated at 37 °C for 14 h until the pH dropped to 3.9. In millet fermentation, common LAB species, including Lactobacillus, Lactococcus, Pediococcus, and Leuconostoc species, are involved [28]. The dried fermented flour was then ground into a fine powder and kept at 4 °C for analysis.

2.2. Phytochemical Content and Antioxidant Activity

Methanolic (100%) extracts of the control and treated samples were prepared according to Talhaoui et al. [29]. The Folin–Ciocalteu's reagent method of Waterhouse [30] was applied to estimate the total phenolic content of millet. The absorbance of the extract was assessed at 765 nm using a UV–Vis spectrophotometer (UVIKON 930, BIO-TEK Kontron, Eching, Germany). The gallic acid standard curve was set at different absorptions, and the samples' TPC was stated as mg gallic acid equivalents mg GAE/g dry weight (R^2 = 0.9974).

The flour's total flavonoid content (TFC) was calculated as designated by Kim et al. [31]. The absorbance of the extracts was read using a UV–Vis spectrophotometer (510 nm). The standard curve was set at different concentrations of catechin (R^2 = 0.99316), and the TFC was expressed as mg catechin equivalents (mg CE/g) dry weight.

The scavenging activity of the DPPH radicals of the pearl millet extracts was measured as described by Chang et al. [32]. The spectrophotometer was used to calculate the DPPH scavenging at the absorbance of 517 nm. The calculated DPPH scavenging was expressed as Trolox equivalents per g (mg Trolox/g). The ferric-reducing antioxidant power (FRAP) assay of pearl millet extracts were measured according to the procedure of Oyaizu, [33]. The absorbance of the FRAP solution was detected at 593 nm against a blank using a UV–visible spectrophotometer. Trolox was used as a standard for setting the calibration curves, and the values were expressed as mg Trolox equivalent/g. Re et al. determined the ABTS radical scavenging activity of the samples as described by [34]. The ABTS mixture solution was produced and added to the extracted samples. Then, ABTS cation radical scavenging activity was detected using an ultraviolet–visible spectrophotometer (734 nm) and expressed as Trolox equivalent (mgTE/g).

2.3. γ-Aminobutyric Acid Content

The γ-aminobutyric acid (GABA) content was measured by the method of Sansenya et al. [35]. The absorbance of the resulting solution was detected at 645 nm using a spectrophotometer. The GABA content of the test sample was calculated by comparing the absorption value with the standard GABA content curve (R^2 = 0.9923).

2.4. Determination of Carotenoids

Carotenoids were measured according to the method of Jacques et al. [36]. The carotenoids were extracted with acetone (25 mL) and then fractionated using petroleum ether (20 mL) and H_2O (100 mL). The absorbance was measured at 450 nm using an ultraviolet–visible (UV–VIS PD-303 UV) spectrophotometer, and the total carotenoid content was expressed as μg/g of DM.

2.5. In Vitro Protein Digestibility and Protein Solubility

The in vitro protein digestibility (IVPD) of the samples was determined using the enzymatic method (pepsin—0.1 N HCl;1 mg:15 mL) following the procedure of Maliwal [37]. The enzyme digestible protein was assessed using the AOAC [38] method. Abd Elmoneim and Bernhardt's [39] protocol was followed to estimate the protein solubility in millet samples. The soluble protein content in the clear supernatant was quantified using the AOAC [38] method.

2.6. Holding Capacities

The holding capacities, such as the water-holding capacity (WHC) and oil-holding capacity (OHC) of samples of flour, were measured following the procedure of Sudha et al. [40]. Flour samples were suspended in water for the WHC and in sunflower oil for the OHC in a ratio of 1*w*:10*v*. The WHC was assessed as the amount of water taken per gram of the sample, whereas the OHC was expressed as the amount of oil bound per gram of dry matter of the sample.

2.7. Emulsification Properties

The emulsifying properties, such as the emulsifying activity index (EAI) and emulsion stability index (ESI), of the flour samples were measured using the method described by Klompong et al. [41]. About 0.3 g of the sample was mixed with deionised water (30 mL) and then 10 mL of sunflower vegetable oil. The mixture was homogenised at a speed of 20,000 rpm for 1 min. The aliquot of the emulsion, 50 µL, was transferred from the bottom of the container at 0 and 10 min after homogenisation and dissolved with 0.1% sodium dodecyl sulphate (5 mL) solution. The absorbance of the diluted solution was measured at 500 nm using a spectrophotometer (UVIKON 930, BIO-TEK Kontron, Eching, Germany).

$$\text{EAI}\left(\frac{\text{m}^2}{\text{g}}\right) = \frac{2 \times 2.303 \times \text{A}_0}{0.25 \times \text{sample wieght}}$$

$$\text{ESI (min)} = \frac{\text{A}_{10} \times \Delta\text{t}}{\Delta\text{A}}$$

where A_0 is the absorbance at 0 min; A_{10} is the absorbance at 10 min after; Δt = 10 min; and $\Delta A = A_0 - A_{10}$.

2.8. Foaming Properties

The foaming properties, such as the foaming capacity (FC) and foam stability (FS) of the samples, were determined according to Wang et al. [42]. A blend of flour (0.7 g) and H_2O (100 mL) was whipped in a high-speed laboratory blender for 5 min and then decanted, and the foam volume was recorded. The FS was calculated based on the decrease in the foam's volume after one hour.

2.9. Statistical Analysis

All analysis measurements were done in triplicate. Two-way ANOVA statistically evaluated the data in the XLSTAT premium software package (XLSTAT 2023.2). The significance levels were compared using the least significant difference test (LSD). The HJ-biplot PCA algorithms test and the partial least squares regression test (PLS) were achieved through XLSTAT software, as described by Vidal et al. [43] and Tenenhaus et al. [44].

3. Results and Discussion

3.1. Effect of Ultrasonic Treatment Followed by Fermentation on the Total Phenolic Content and Total Flavonoid Content of Millet Grains

The results of the total phenolic content (TPC) and total flavonoid content (TFC) of the millet grains after ultrasonic treatment alone or followed by fermentation are shown in Table 1. The findings indicated that ultrasonic treatment of millet grains at different temperatures caused a significant ($p < 0.001$) increment in the TPC. The highest value of the TPC was obtained when the grains were treated at 60 °C. Interestingly, the fermentation process of the ultrasonic-treated millet significantly ($p < 0.001$) enhanced its TPC.

It was also shown in Table 1 that the content of the total flavonoid of the millet grains was significantly ($p < 0.001$) influenced by the ultrasonic treatment, fermentation, and the interaction between both processes. It was stated that the TFC of millet increased significantly ($p < 0.001$) as the ultrasonic temperature rose, and the higher value of 4.44 mg CE/g was detected at 60 °C ultrasonic treatment. Interestingly, fermentation alone or ultrasonication followed by fermentation was found to enhance the TFC of millet grains ($p < 0.001$).

The significant increment of the TPC and TFC in the millet grains after ultrasonic treatment alone or coupled with fermentation might be due to the mechanisms of the cell structure disruption, microbial activity, and improved extraction efficiency [45]. Similar to our findings, Swieca et al. [46] found that the content of total phenolics and flavonoids in lentil seeds increased significantly due to fermentation. Interestingly, Zhao et al. [47] stated that a combination of ultrasonic treatment and fermentation enhanced the phenolic content

of soybeans. Hence, ultrasonic treatment alone or coupled with fermentation can increase the phenolic compounds of millet, making them more valuable for human health.

Table 1. Effect of ultrasonic treatment followed by fermentation on the total phenolic content (TPC) and total flavonoid content (TFC) of millet grains.

Ultrasonic Treatment	TPC (mg GAE/g)		TFC (mg CE/g)	
	Raw	Fermented	Raw	Fermented
Control	3.7 ± 0.35 d	6.0 ± 0.20 c	0.6 ± 0.08 g	4.4 ± 0.13 c
20 °C	5.6 ± 0.14 c	5.8 ± 0.03 c	1.4 ± 0.13 f	4.0 ± 0.08 cd
40 °C	7.5 ± 0.11 b	7.6 ± 0.05 ab	2.3 ± 0.08 e	5.1 ± 0.08 b
60 °C	8.3 ± 0.28 a	7.7 ± 0.46 ab	4.4 ± 0.23 c	5.8 ± 0.27 a
Two-way ANOVA				
Ultrasonic (U)	***		***	
Fermentation (F)	***		***	
U * F	***		***	
SE± (U)	0.101		0.062	
SE± (F)	0.071		0.044	
LSD	0.426		0.185	

Letters indicate that values do not differ significantly at $p < 0.05$ according to LSD; (*) indicate significant variation: ***, significant at $p < 0.001$ level. Each value represents the average of 3 replications.

3.2. Effect of Ultrasonic Treatment Followed by Fermentation on the Antioxidant Activity of Millet Grains

The antioxidant activity of control and treated grains was described in terms of DPPH, FRAP, and ABTS (Table 2). As shown in the table, the antioxidant activity of DPPH and FRAP of millet gain was found to be significantly ($p < 0.001$) affected by ultrasonic treatment, fermentation, and the interaction of both treatments. The DPPH of the untreated millet sample was 3.16 mg Trolox/g. Ultrasonic treatment of millet grain for 20, 40, and 60 °C significantly ($p < 0.001$) increased the DPPH to 3.36, 5.07, and 6.95 mg Trolox/g, respectively. Interestingly, the DPPH of the fermented millet grains was significantly higher than that of unfermented grains. The highest DPPH value, 10.4 mg Trolox/g, was observed in the grain treated with ultrasonication for 60 °C, followed by fermentation (Table 2). The ultrasonic treatment and fermentation impact the ferric-reducing antioxidant power (FRAP) similarly to DPPH. Increasing the temperature of the ultrasonic treatment significantly ($p < 0.001$) increased the FRAP from 3.7 to 4.2, 4.7, and 4.7 mg Trolox/g. The fermentation process also caused significant ($p < 0.001$) increments to 5.9 mg Trolox/g. Moreover, the FRAP of the fermented grains after ultrasonic treatment was higher than that of those treated at 60 °C (Table 2). On the contrary, the ABTS of the grains was found to be significantly reduced after ultrasonic treatment. However, fermentation of untreated millet grains significantly ($p < 0.001$) increased the ABTS to 5.39 mg Trolox/g. Additionally, fermentation of ultrasonic-treated grains increased the ABTS antioxidant, and the highest ABTS value, 6.03 mg Trolox/g, was obtained in the fermented millet grains treated with ultrasonication at 60 °C (Table 2). The reduction in the ABTS values of millet after ultrasonication might be associated with several mechanism actions. It has been stated by Chemat et al. [48] that ultrasonication may cause a structural change in certain antioxidants, which can decrease the ABTS scavenging ability.

In this study, the increases in the antioxidant activity (DPPH, FRAP, and ABTS) are principally due to several factors associated with ultrasonic and fermentation treatments of the grains. These factors include the metabolic actions of microorganisms during fermentation and a breakdown of cell walls during ultrasonic treatment [49,50]. Hence, both processing treatments may enhance the extraction and bioavailability of antioxidant compounds in millet. Moreover, combining the ultrasonic treatment with fermentation can dramatically increase the antioxidant capacity of grains; however, they increase the phenolic compounds' content and availability, which results in increased antioxidant activity.

Tiwari et al. [50] reported that ultrasonic treatment significantly increases the DPPH and FRAP values due to the increased content of phenolic compounds in fruit and vegetable extracts. The combination of ultrasonic treatment and fermentation resulted in a more efficient release of antioxidants, contributing to the higher DPPH, FRAP, and ABTS values. The increase in antioxidant activity in soybeans was also observed due to the combination of ultrasonic treatment and fermentation, as Zhao et al. [47] reported.

Table 2. Effect of ultrasonic treatment followed by fermentation on the antioxidant activity of millet grains.

Ultrasonic Treatment	DPPH (mg Trolox/g)		FRAP (mg Trolox/g)		ABTS (mg Trolox/g)	
	Raw	Fermented	Raw	Fermented	Raw	Fermented
Control	3.2 ± 0.14 f	7.9 ± 0.47 c	3.7 ± 0.00 g	5.9 ± 0.00 c	4.9 ± 0.12 c	5.4 ± 0.11 b
20 °C	3.4 ± 0.33 f	8.5 ± 0.14 bc	4.2 ± 0.00 f	6.2 ± 0.04 b	4.1 ± 0.00 d	4.8 ± 0.04 c
40 °C	5.1 ± 0.26 e	8.8 ± 0.07 b	4.7 ± 0.01 e	6.0 ± 0.01 c	4.2 ± 0.02 d	5.5 ± 0.02 b
60 °C	6.9 ± 0.26 d	10.4 ± 0.14 a	4.7 ± 0.00 d	6.9 ± 0.05 a	4.3 ± 0.04 d	6.0 ± 0.05 a
Two-way ANOVA						
Ultrasonic (U)	***		***		***	
Fermentation (F)	***		***		***	
U * F	***		***		***	
SE± (U)	0.106		0.018		0.037	
SE± (F)	0.075		0.013		0.018	
LSD	0.317		0.073		0.055	

Letters indicate that values do not differ significantly at $p < 0.05$ according to LSD; (*) indicate significant variation: ***, significant at $p < 0.001$ level. Each value represents the average of 3 replications.

3.3. Effect of Ultrasonic Treatment Followed by Fermentation on the In Vitro Protein Digestibility and Protein Solubility of Millet Grains

Table 3 describes the in vitro protein digestibility (IVPD) and protein solubility of millet grains as affected by ultrasonic treatment with or without fermentation. The IVPD was found to be significantly ($p < 0.001$) increased after ultrasonic treatment, particularly at 20 and 40 °C. However, treatment of millet grain with ultrasonication at 60 °C reduced the IVPD to 31.4%. Also, the lowest IVPD value, 28.7%, was detected in fermented millet grains treated with ultrasonication at 60 °C (Table 3). The protein solubility of untreated grains was found to be 19.7%. Ultrasonic treatment caused a significant ($p < 0.001$) increment in the protein solubility of the grains, particularly at the highest treated temperature, 60 °C (41.9%). Compared to unfermented millet samples, the protein solubility was found to be significantly higher in the fermented samples ($p < 0.001$), particularly in those samples treated with ultrasonication at 20 and 40 °C (Table 3).

Changes in the protein solubility and digestibility in millet grains due to ultrasonic and fermentation treatments might be attributed to the breakdown of protein aggregates and the partial hydrolysis of protein structures. The protein solubility of soybeans was found to be significantly improved after ultrasonic treatment [47]. Due to the fermentation process of grains, the reduction in the antinutritional factor and increased enzyme activity improved protein digestibility [51]. Moreover, coupled ultrasonication and fermentation cause a significant enhancement in protein solubility and digestibility of grain [52]. The combination of ultrasonication and fermentation has a synergistic impact on the protein solubility and digestibility of grains, which may improve protein functionality.

Table 3. Effect of ultrasonic treatment followed by fermentation on the in vitro protein digestibility and protein solubility of millet grains.

Ultrasonic Treatment	IVPD (%)		Protein Solubility (%)	
	Raw	Fermented	Raw	Fermented
Control	31.8 ± 0.31 d	33.6 ± 0.29 b	19.7 ± 0.49 f	33.6 ± 0.29 e
20 °C	32.8 ± 0.00 c	30.4 ± 0.15 f	39.0 ± 0.09 d	50.3 ± 0.15 a
40 °C	38.5 ± 0.51 a	31.0 ± 0.40 e	37.7 ± 0.06 d	47.0 ± 0.40 b
60 °C	31.4 ± 0.15 de	28.7 ± 0.15 g	41.9 ± 0.17 c	40.1 ± 0.15 cd
Two-way ANOVA				
Ultrasonic (U)	***		***	
Fermentation (F)	***		***	
U * F	***		***	
SE± (U)	0.118		0.367	
SE± (F)	0.084		0.259	
LSD	0.820		1.484	

Letters indicate that values do not differ significantly at $p < 0.05$ according to LSD; (*) indicate significant variation: ***, significant at $p < 0.001$ level. Each value represents the average of 3 replications.

3.4. Effect of Ultrasonic Treatment Followed by Fermentation on the Gamma-Aminobutyric Acid and Total Carotenoids of Millet Grains

The results of the total carotenoids and Gamma-aminobutyric acid (GABA) of the millet grains after ultrasonic treatment alone or followed by fermentation are shown in Table 4. The total carotenoid of the control sample was 41.2 µg/g. Ultrasonic treatment of millet grains at 20, 40, and 60 °C caused a significant ($p < 0.001$) increment in the total carotenoids to 45.0, 55.3, and 69.4 µg/g, respectively. Stimulatingly, the fermentation process of the control and ultrasonic-treated millet significantly ($p < 0.001$) enhanced its total carotenoids. The highest value of the total carotenoid was 78.5 µg/g, observed in the ultrasonic-treated grains at 60 °C. The increment of the carotenoids in millet due to the ultrasonication alone or combined with fermentation might be due to the disruption of the cellular matrix and increasing microbial breakdown during the process. An increase in carotenoids in grains due to the ultrasonication and fermentation processes has also been observed in corn and fermented food [53,54], respectively.

Table 4. Effect of ultrasonic treatment followed by fermentation on the gamma-aminobutyric acid and total carotenoids of millet grains.

Ultrasonic Treatment	Total Carotenoid (µg/g)		GABA (mg/g)	
	Raw	Fermented	Raw	Fermented
Control	41.2 ± 2.23 e	54.0 ± 6.68 cd	4.7 ± 0.17 e	3.8 ± 0.04 f
20 °C	45.0 ± 2.27 de	59.2 ± 8.03 bc	4.6 ± 0.04 d	4.9 ± 0.04 d
40 °C	55.3 ± 2.23 cd	69.4 ± 0.00 ab	6.0 ± 0.42 c	6.2 ± 0.00 b
60 °C	69.4 ± 0.00 ab	78.5 ± 4.46 a	6.8 ± 0.07 a	4.9 ± 0.00 d
Two-way ANOVA				
Ultrasonic (U)	***		***	
Fermentation (F)	***		***	
U * F	ns		***	
SE± (U)	2.448		0.029	
SE± (F)	1.224		0.021	
LSD	7.005		0.119	

Letters indicate that values do not differ significantly at $p < 0.05$ according to LSD (*) indicate significant variation: ns, not significant; ***, significant at $p < 0.001$ level. Each value represents the average of 3 replications.

The GABA content of the millet grains was found to be 4.86 mg/g. Ultrasonic treatment of millet grains at 20, 40, and 60 °C significantly ($p < 0.001$) increased its GABA content

to 4.59, 6.04, and 6.75 mg/g, respectively (Table 4). However, the GABA content was found to be significantly ($p < 0.001$) lower in the fermented millet grains compared to unfermented samples (Table 4). The enhancement of GABA in millet grains might result from the increasing activity of glutamate decarboxylase (GAD) during the ultrasonic treatment. Stimulation of the GAD catalyses the conversion of glutamate to GABA due to ultrasonication, which has been observed in various grains like brown rice [55]. Moreover, combining ultrasonic energy, particularly at temperatures up to 40 °C, with fermentation stimulates the synthesis of GABA in millet due to the action of the microbial decarboxylation of glutamate [56]. Regarding the obtained results, the increment of the GABA and carotenoid contents in millet due to the combination of both treatments offers a synergistic consequence, leading to higher bioavailability and content of these imperative bioactive substances, improving the nutritional quality of grains.

3.5. Effect of Ultrasonic Treatment Followed by Fermentation on the Functional Properties of Millet Grains

Figure 1A–F illustrates the effect of the ultrasonic treatment and ultrasonic treatment followed by fermentation on the millet's functional properties in terms of the water-holding capacity (WHO), oil-holding capacity (OHC), emulsion activity index (EAI), emulsion stability index (ESI), foaming capacity (FC), and foaming stability (FS). The WHC of the millet grains was found to be significantly ($p < 0.001$) influenced by ultrasonic treatment, particularly at the treatment temperatures of 20 and 40 C. Fermentation of the raw millet samples or fermentation of the ultrasonic-treated grains significantly ($p < 0.001$) increased its WHC (Figure 1A). The OHC was found to be similar in the control sample and ultrasonic samples. However, the OHC of fermented millet and ultrasonic-treated samples followed by fermentation was significantly higher ($p < 0.001$). Fermentation of the control sample increased the OHC to 1.17 mL/g, while the fermentation process of ultrasonic-treated samples for 20, 40, and 60 °C caused increments of the OHC to 1.38, 1.38, and 1.53 mL/g, respectively (Figure 1B).

The EAI of the millet sample was found to be 7.02 m^2/g. Ultrasonic treatment of millet at 20, 40, and 60 °C significantly ($p < 0.001$) increased the EAI to 8.46, 8.11, and 8.11 m^2/g, respectively (Figure 1C). However, the fermentation process caused a significant reduction in the EAI of the millet. The ESI stability of the millet grains was found to be 2.81 min (Figure 1D). The ESI of the millet was significantly ($p < 0.001$) increased due to the ultrasonic treatment, particularly at 20 °C (6.91 min). The ESI of the fermented millet was found to be 8.87 min. Fermentation of the ultrasonic-treated millet at 20 and 40 °C significantly ($p < 0.001$) increased the ESI to 9.82 and 10.20 min, respectively.

Figure 1E describes the foaming capacity of raw and processed millet. As shown in the figure, ultrasonic treatment caused no significant ($p < 0.001$) changes in the FC of millet grains, particularly at 20 and 40 °C. However, ultrasonic treatment at 60 °C caused an increment in the FC to 3.83%. The fermentation process was found to have a significant ($p < 0.001$) influence on the millet FC. It was found to be significantly lower in the fermented raw samples.

Interestingly, fermentation of ultrasonic-treated millet at 20 and 40 °C sharply increased the FC to 4.77 and 4.39%, respectively. However, fermentation of ultrasonic-treated grains at 60 °C reduced the FC to 2.20% ($p < 0.001$). The foaming stability of millet grains was found to be significantly similar in the raw and processed samples, as illustrated in Figure 1F.

The changes in the millet's functional properties, such as the action of ultrasonic treatment and fermentation processes, might be due to the interference of several actions, such as physical disruption, chemical modification, and enzymatic activity. It has been stated that ultrasonication enhances the functional properties of the grain since it causes the breakdown of the component structure of the grain, which may lead to modified protein and starch structure conformation. These changes enhance the functional performance of the macromolecules that are required in food production [57]. The solubility and functional

properties of whey protein were improved after ultrasonic treatment due to the mechanisms of cavitation and protein unfolding [52]. Moreover, Yeo and Liong [51] stated that fermentation improves soy-based foods' solubility emulsification properties and water-holding capacity. Similar to our findings, combining ultrasonication and fermentation significantly improved cereal grains' protein solubility and emulsification characteristics [47]. The significant enhancement of the functional properties of millet grain due to ultrasonic treatment, either alone or followed by fermentation, may result in grains with superior functional and nutritional qualities.

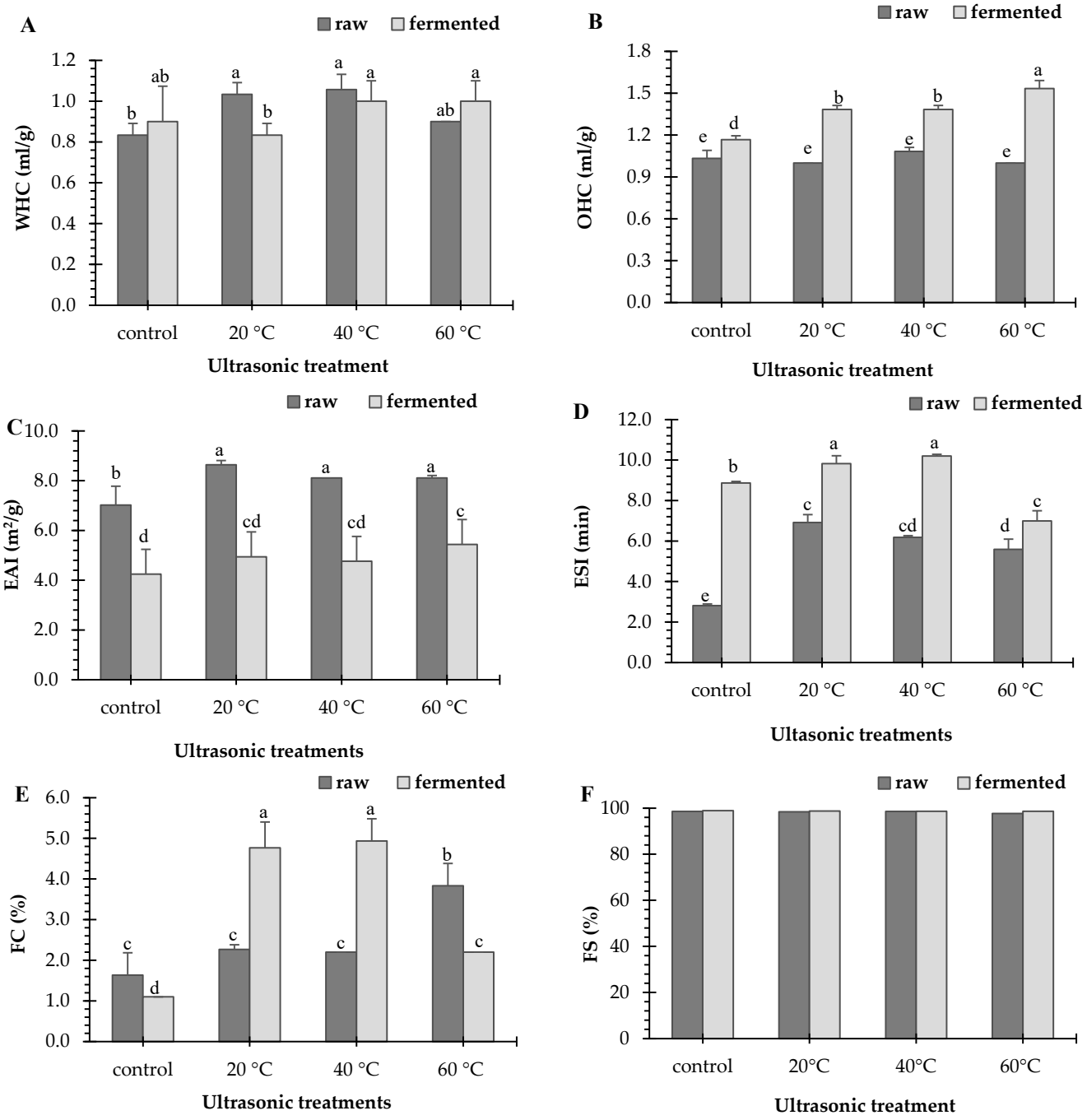

Figure 1. Effect of ultrasonic treatment followed by fermentation on the water holding capacity (**A**), oil holding capacity (**B**), emulsion activity index (**C**), emulsion stability index (**D**), foaming capacity (**E**), and foaming stability (**F**) of millet grains. Values with the same letter are not significantly different at $p < 0.05$ according to LSD. Each value represents the average of 3 replications.

3.6. Principal Component and Partial Validation Squares Regression Analyses

The principal component analysis (PCA) shows the interrelationships between ultrasonic treatment at different temperatures (0, 20, 40, and 60 °C) or ultrasonic treatment followed by fermentation and the protein digestibility and solubility, total carotenoids, GABA, functional properties, and antioxidants of the millet grains in Figure 2A. The axes contribution of the PC1 and PC2 are explained as 50.14% and 19.82%, which resulted in a variability (69.97%) of the plotted components. A high positive correlation was also observed between ultrasonic treatments at 20, 40, and 60 C of the grains, followed by fermentation and grains' quality parameters. The PCA biplot revealed a clear clustering of the untreated millet samples (control and control-fermented) in the third quadrant of the plot compared to the ultrasonic-treated samples. Moreover, ultrasonication combined with fermentation samples scattered in the first and second quarters is perfectly separated based on their interactions with the bioactive compounds (GABA and carotenoid), phenolic compounds (TPC and TFC), antioxidant activity (DPPH, FRAP, and ABTS) and the functional properties (WHC, OHC, ESI, FC, and FS). Concerning Yan et al. [58], the eccentricity and observation of the variables that appear at a < 90° angle are positively connected, whereas that of angels > 90° are linked with a negative association, and those with a 90° angle do not show a connexion in the biplot. Therefore, observations revealed that combined ultrasonic treatment and fermentation of millet grains could enhance their bioactive content, antioxidant activity, functional characteristics, and vital compounds.

The partial least squares regression analysis (PLS) was described in Figure 2B. The PLS demonstrated the interactive effect of the ultrasonic treatments at different temperatures alone or followed by natural fermentation (x variables) on the assessed quality parameters of the millet grains. As shown in the model, the PLS model validated that the treatments, particularly ultrasonic treatment at 40 °C followed by fermentation, achieved the most suitable treatment for nutritional and functional applications for the millet grains, which might be considered for further food industry requests.

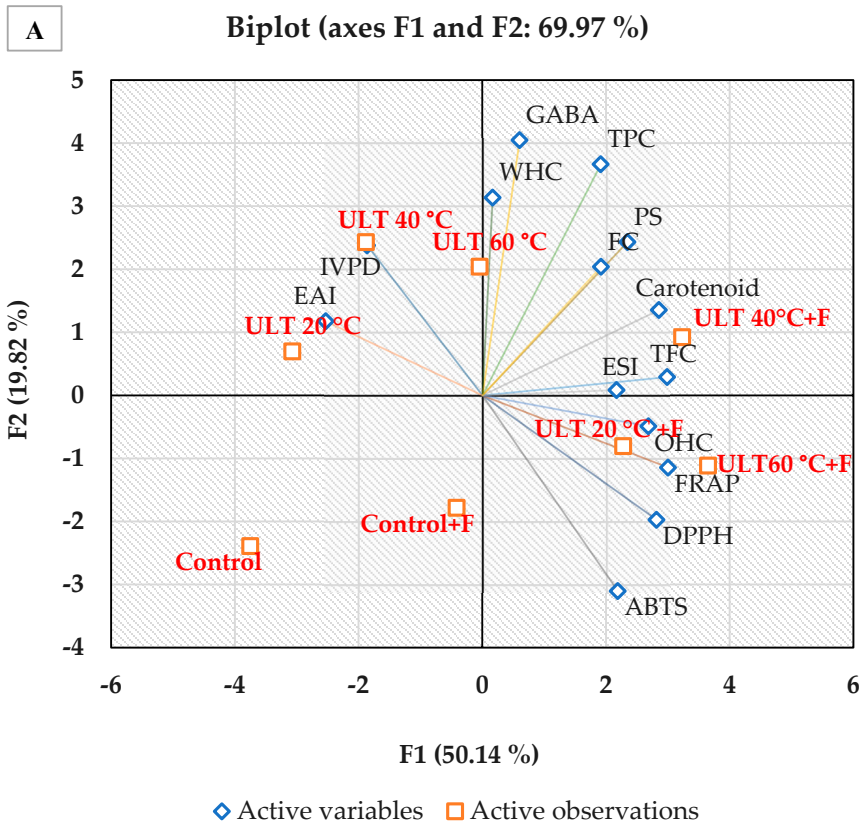

Figure 2. *Cont.*

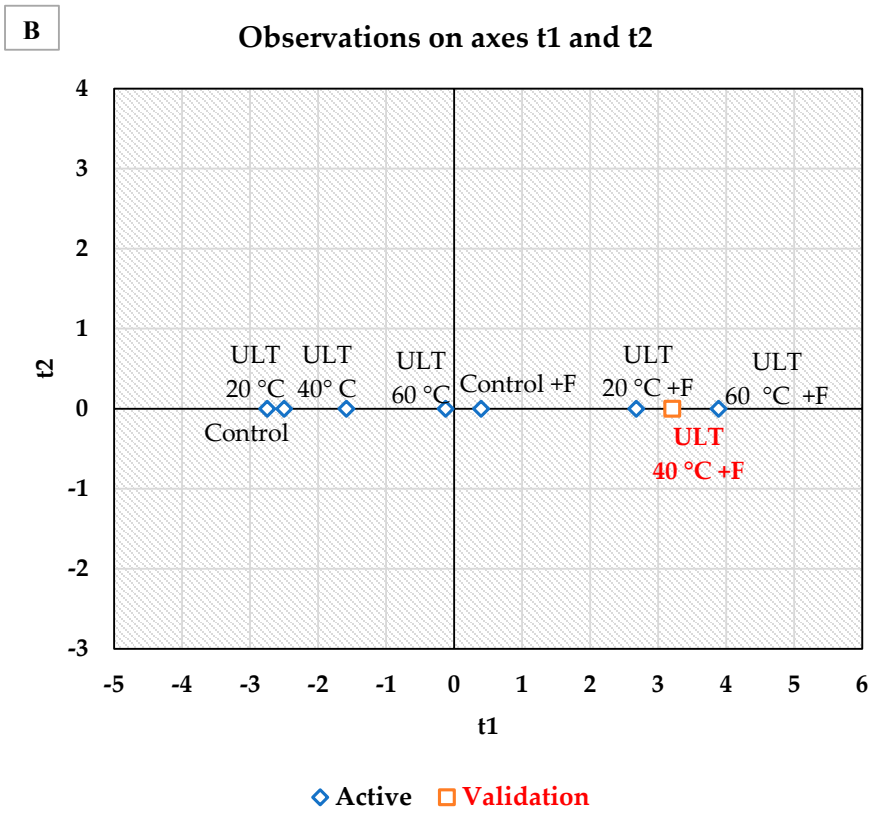

Figure 2. Principal component analysis (**A**) and partial least squares regression analysis (**B**) for the parameters determined of control and treated millet grains.

4. Conclusions

In conclusion, this study demonstrates how fermentation and ultrasonic treatment improve millet's nutritional makeup and practical qualities; however, a more thorough comprehension of the underlying mechanics would be beneficial. By causing cavitation, which damages cell walls and improves nutrient accessibility, ultrasonic treatment affects protein conformation and cell structure. Additionally, partially unfolding proteins and changing their structure improves the solubility and digestibility of proteins. Certain bacteria, such as Lactobacillus, Lactococcus, Pediococcus, and Leuconostoc species, are essential for fermentation because they change millet's nutrient profile and functionality. Hence, future studies should concentrate on measuring these impacts and investigating how the regulated application of ultrasonic treatment and particular microbial strains could provide millet's nutritional and functional advantages, offering a more reliable and useful method of processing millet-based foods.

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References

1. Amadou, I.; Le, G.W.; Shi, Y.H. Evaluation of antimicrobial, antioxidant activities, and nutritional values of fermented foxtail millet extracts by Lactobacillus paracasei Fn032. *Int. J. Food Prop.* **2013**, *8*, 1179–1190. [CrossRef]
2. Yousaf, L.; Hou, D.; Liaqat, H.; Shen, Q. Millet: A review of its nutritional and functional changes during processing. *Food Res. Int.* **2021**, *142*, 110197. [CrossRef] [PubMed]
3. Saleh, A.S.; Zhang, Q.; Chen, J.; Shen, Q. Millet grains: Nutritional quality, processing, and potential health benefits. *Compr. Rev. Food Sci. Food Saf.* **2013**, *12*, 281–295. [CrossRef]
4. Abedin, M.J.; Abdullah, A.T.M.; Satter, M.A.; Farzana, T. Physical, functional, nutritional and antioxidant properties of foxtail millet in Bangladesh. *Heliyon* **2022**, *20*, 11186. [CrossRef]
5. Zhang, Y.; Jing, X.; Chen, Z.; Wang, X. Purification and identification of antioxidant peptides from millet gliadin treated with high hydrostatic pressure. *LWT-Food Sci. Technol.* **2022**, *164*, 113654. [CrossRef]
6. Singh, K.P.; Mishra, A.; Mishra, H.N. Fuzzy analysis of sensory attributes of bread prepared from millet-based composite flours. *LWT-Food Sci. Technol.* **2012**, *48*, 276–282. [CrossRef]
7. Bandyopadhyay, T.; Jaiswal, V.; Prasad, M. Nutrition potential of foxtail millet in comparison to other millets and major cereals. In *The Foxtail Millet Genome*; Springer: Cham, Switzerland, 2017; pp. 123–135. [CrossRef]
8. Samtiya, M.; Soni, K.; Chawla, S.; Poonia, A.; Sehgal, S.; Dhewa, T. Key anti-nutrients of millet and their reduction strategies: An overview. *Act. Sci. Nutr. Health* **2021**, *5*, 68–80. [CrossRef]
9. Yadav, P.; Shukla, A.K.; Kumari, A.; Dhewa, T.; Kumar, A. Nutritional evaluation of probiotics enriched rabadi beverage (PERB) and molecular mapping of digestive enzyme with dietary fibre for exploring the therapeutic potential. *Food Humanit.* **2024**, *2*, 100221. [CrossRef]
10. Neeru Choudhary, P.; Dhewa, T.; Kumar, A. Process optimization of pearl millet-based fermented beverage components using response surface methodology and evaluation of its physicochemical parameters. *J. Food Sci. Technol.* **2024**, *61*, 1557–1568. [CrossRef]
11. Gowda, N.A.N.; Siliveru, K.; Prasad, P.V.V.; Bhatt, Y.; Netravati, B.P.; Gurikar, C. Modern Processing of Indian Millets: A Perspective on Changes in Nutritional Properties. *Foods* **2022**, *11*, 499. [CrossRef]
12. Wang, S.; Xu, X.; Wang, S.; Wang, J.; Peng, W. Effects of Microwave Treatment on Structure, Functional Properties and Antioxidant Activities of Germinated Tartary Buckwheat Protein. *Foods* **2022**, *11*, 1373. [CrossRef] [PubMed]
13. Jiang, L.; Wang, J.; Li, Y.; Wang, Z.; Liang, J.; Wang, R.; Chen, Y.; Ma, W.; Qi, B.; Zhang, M. Effects of ultrasound on the structure and physical properties of black bean protein isolates. *Food Res. Int.* **2014**, *62*, 595–601. [CrossRef]
14. Higuera-Barraza, O.A.; Del Toro-Sanchez, C.L.; Ruiz-Cruz, S.; Márquez-Ríos, E. Effects of high-energy ultrasound on the functional properties of proteins. *Ultrason. Sonochem.* **2016**, *31*, 558–562. [CrossRef] [PubMed]
15. Flores-Jiménez, N.T.; Ulloa, J.A.; Silvas, J.E.U.; Ramírez, J.C.R.; Ulloa, P.R.; Rosales, P.U.B.; Carrillo, Y.S.; Leyva, R.G. Effect of high-intensity ultrasound on the compositional, physicochemical, biochemical, functional and structural properties of canola (*Brassica napus* L.) protein isolate. *Food Res. Int.* **2019**, *121*, 947–956. [CrossRef]
16. Jin, J.; Okagu, O.D.; Yagoub, A.E.A.; Udenigwe, C.C. Effects of sonication on the in vitro digestibility and structural properties of buckwheat protein isolates. *Ultrason. Sonochem.* **2021**, *70*, 105348. [CrossRef]
17. Téllez-Morales, J.A.; Hernández-Santo, B.; Rodríguez-Miranda, J. Effect of ultrasound on the techno-functional properties of food components/ingredients: A review. *Ultrason. Sonochem.* **2020**, *61*, 104787. [CrossRef]
18. Arora, K.; Ameur, H.; Polo, A.; Di Cagno, R.; Rizzello, C.G.; Gobbetti, M. Thirty years of knowledge on sourdough fermentation: A systematic review. *Trends Food Sci. Technol.* **2021**, *108*, 71–83. [CrossRef]
19. Gabriele, M.; Pucci, L. Fermentation and germination as a way to improve cereals antioxidant and antiinflammatory properties. In *Current Advances for Development of Functional Foods Modulating Inflammation and Oxidative Stress*; Academic Press: Cambridge, MA, USA, 2022; Volume 1, pp. 477–497.
20. Hur, S.J.; Lee, S.Y.; Kim, Y.C.; Choi, I.; Kim, G.B. Effect of fermentation on the antioxidant activity in plant-based foods. *Food Chem.* **2014**, *160*, 346–356. [CrossRef]
21. Shiferaw Terefe, N.; Augustin, M.A. Fermentation for tailoring the technological and health related functionality of food products. *Crit. Rev. Food Sci. Nutr.* **2020**, *60*, 2887–2913. [CrossRef]
22. Martinez-Villaluenga, C.; Peñas, E.; Frias, J. Bioactive peptides in fermented foods: Production and evidence for health effects. In *Fermented Foods in Health and Disease Prevention*; Academic Press: Cambridge, MA, USA, 2017; Volume 1, pp. 23–47.
23. Abah, C.R.; Ishiwu, C.N.; Obiegbuna, J.E.; Oladejo, A.A. Nutritional composition, functional properties and food applications of millet grains. *Asian Food Sci. J.* **2020**, *14*, 9–19. [CrossRef]
24. Yang, T.; Ma, S.; Liu, J.; Sun, B.; Wang, X. Influences of four processing methods on main nutritional components of foxtail millet: A review. *Grain Oil Sci. Technol.* **2022**, *5*, 156–165. [CrossRef]
25. Verni, M.; Verardo, V.; Rizzello, C.G. How Fermentation Affects the Antioxidant Properties of Cereals and Legumes. *Foods* **2019**, *8*, 362. [CrossRef] [PubMed]
26. Balli, D.; Bellumori, M.; Pucci, L.; Gabriele, M.; Longo, V.; Paoli, P.; Melani, F.; Mulinacci, N.; Innocenti, M. Does Fermentation Really Increase the Phenolic Content in Cereals? A Study on Millet. *Foods* **2020**, *9*, 303. [CrossRef] [PubMed]

27. Dey, S.; Saxena, A.; Kumar, Y.; Maity, T.; Tarafdar, A. Optimizing the effect of ultrasonication and germination on antinutrients and antioxidants of kodo (*Paspalum scrobiculatum*) and little (*Panicum sumatrense*) millets. *J. Food Sci. Technol.* **2023**, *60*, 2990–3001. [CrossRef]
28. Ray, R.C.; Ward, O.P. *Microbial Biotechnology in Horticulture*; Science Publishers: New York, NY, USA, 2004; Volume 1.
29. Talhaoui, N.; Gómez-Caravaca, A.M.; León, L.; De la Rosa, R.; Segura-Carretero, A.; Fernández-Gutiérrez, A. Determination of phenolic compounds of 'Sikitita'olive leaves by HPLC-DAD-TOF-MS. Comparison with its parents 'Arbequina'and 'Picual'olive leaves. *LWT-Food Sci. Technol.* **2014**, *58*, 28–34. [CrossRef]
30. Waterhouse, A.L. Determination of total phenolics. In *Current Protocols in Food Analytical Chemistry*; John Wiley & Sons: New York, NY, USA, 2002; Volume 6, pp. I1.1.1–I1.1.8.
31. Kim, D.O.; Jeong, S.W.; Lee, C.Y. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chem.* **2003**, *81*, 321–326. [CrossRef]
32. Chang, S.T.; Wu, J.H.; Wang, S.Y.; Kang, P.L.; Yang, N.S.; Shyur, L.F. Antioxidant activity of extracts from Acacia confusa bark and heartwood. *J. Agric. Food Chem.* **2001**, *49*, 3420–3424. [CrossRef]
33. Oyaizu, M. Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn. J. Nutr. Diet.* **1986**, *44*, 307–315. [CrossRef]
34. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]
35. Sansenya, S.; Hua, Y.; Chumanee, S.; Phasai, K.; Sricheewin, C. Effect of Gamma Irradiation on 2-Acetyl-1-pyrroline Content, GABA Content and Volatile Compounds of Germinated Rice (Thai Upland Rice). *Plants* **2017**, *10*, 18. [CrossRef]
36. Jacques, A.C.; Pertuzatti, P.B.; Barcia, M.T.; Zambiazi, R.C. Scientific note: Bioactive compounds in small fruits cultivated in the southern region of Brazil. *Braz. J. Food Technol.* **2009**, *12*, 123–127. [CrossRef]
37. Maliwal, B.P. In vitro methods to assess the nutritive value of leaf protein concentrate. *J. Agric. Food Chem.* **1983**, *31*, 315–319. [CrossRef] [PubMed]
38. AOAC. *Official Methods of Analysis of Association of Official Analytical Chemists*, 16th ed.; Association of Official Analytical Chemists: Rockville, MD, USA, 2005.
39. Abd Elmoneim, O.E.; Bernhardt, R. Influence of grain germination on functional properties of sorghum flour. *Food Chem.* **2010**, *121*, 387–392.
40. Sudha, M.L.; Baskaran, V.; Leelavathi, K. Apple pomace as a source of dietary fiber and polyphenols and its effect on the rheological characteristics and cake making. *Food Chem.* **2007**, *104*, 686–692. [CrossRef]
41. Klompong, V.; Benjakul, S.; Kantachote, D.; Shahidi, F. Antioxidative activity and functional properties of protein hydrolysate of yellow stripe trevally (*Selaroides leptolepis*) as influenced by the degree of hydrolysis and enzyme type. *Food Chem.* **2007**, *102*, 1317–1327. [CrossRef]
42. Wang, X.S.; Tang, C.H.; Li, B.S.; Yang, X.Q.; Li, L.; Ma, C.Y. Effects of high-pressure treatment on some physicochemical and functional properties of soy protein isolates. *Food Hydrocoll.* **2008**, *22*, 560–567. [CrossRef]
43. Vidal, N.P.; Manful, C.F.; Pham, T.H.; Stewart, P.; Keough, D.; Thomas, R. The use of XLSTAT in conducting principal component analysis (PCA) when evaluating the relationships between sensory and quality attributes in grilled foods. *MethodsX* **2020**, *22*, 100835. [CrossRef]
44. Tenenhaus, M.; Pagès, J.; Ambroisine, L.; Guinot, C. PLS methodology to study relationships between hedonic judgements and product characteristics. *Food Qual. Prefer.* **2005**, *16*, 315–325. [CrossRef]
45. Barba, F.J.; Parniakov, O.; Pereira, S.A.; Wiktor, A.; Grimi, N.; Boussetta, N.; Saraiva, J.A.; Raso, J.; Martin-Belloso, O.; Witrowa-Rajchert, D.; et al. Current applications and new opportunities for the use of pulsed electric fields in food science and industry. *Food Res. Int.* **2015**, *77*, 773–798. [CrossRef]
46. Świeca, M.; Gawlik-Dziki, U.; Kowalczyk, D.; Złotek, U. Impact of germination time and type of illumination on the antioxidant compounds and antioxidant capacity of Lens culinaris sprouts. *Sci. Hortic.* **2012**, *140*, 87–95. [CrossRef]
47. Kong, Y.; Sun, L.; Wu, Z.; Li, Y.; Kang, Z.; Xie, F.; Yu, D. Effects of ultrasonic treatment on the structural, functional properties and beany flavor of soy protein isolate: Comparison with traditional thermal treatment. *Ultrason. Sonochem.* **2023**, *101*, 106675. [CrossRef] [PubMed]
48. Chemat, F.; Huma, Z.E.; Khan, M.K. Applications of ultrasound in food technology: Processing, preservation, and extraction. *Ultrason. Sonochem.* **2011**, *18*, 813–835. [CrossRef] [PubMed]
49. Wang, H.; Tao, Y.; Li, Y.; Wu, S.; Li, D.; Liu, X.; Han, Y.; Manickam, S.; Show, P.L. Application of ultrasonication at different microbial growth stages during apple juice fermentation by Lactobacillus plantarum: Investigation on the metabolic response. *Ultrason. Sonochem.* **2021**, *73*, 105486. [CrossRef] [PubMed]
50. Tiwari, B.K.; Muthukumarappan, K.; O'donnell, C.P.; Cullen, P.J. Inactivation kinetics of pectin methylesterase and cloud retention in sonicated orange juice. *Innov. Food Sci. Emerg. Technol.* **2009**, *10*, 166–171. [CrossRef]
51. Yeo, S.K.; Liong, M.T. Effect of prebiotics on viability and growth characteristics of probiotics in soymilk. *J. Sci. Food Agric.* **2010**, *90*, 267–275. [CrossRef]
52. Jambrak, A.R.; Mason, T.J.; Lelas, V.; Herceg, Z.; Herceg, I.L. Effect of ultrasound treatment on solubility and foaming properties of whey protein suspensions. *J. Food Eng.* **2008**, *86*, 281–287. [CrossRef]

53. Suo, G.; Zhou, C.; Su, W.; Hu, X. Effects of ultrasonic treatment on color, carotenoid content, enzyme activity, rheological properties, and microstructure of pumpkin juice during storage. *Ultrason. Sonochem.* **2022**, *84*, 105974. [CrossRef]
54. Rodriguez-Concepcion, M.; Stange, C. Biosynthesis of carotenoids in carrot: An underground story comes to light. *Arch. Biochem. Biophys.* **2013**, *539*, 110–116. [CrossRef]
55. Tsou, S.F.; Hsu, H.Y.; Chen, S.D. Effects of Different Pretreatments on the GABA Content of Germinated Brown Rice. *Appl. Sci.* **2024**, *14*, 5771. [CrossRef]
56. Cataldo, P.G.; Villegas, J.M.; Savoy de Giori, G.; Saavedra, L.; Hebert, E.M. Enhancement of γ-aminobutyric acid (GABA) production by Lactobacillus brevis CRL 2013 based on carbohydrate fermentation. *Int. J. Food Microbiol.* **2020**, *333*, 108792. [CrossRef]
57. Wang, Y.; Li, B.; Guo, Y.; Liu, C.; Liu, J.; Tan, B.; Guo, Z.; Wang, Z.; Jiang, L. Effects of ultrasound on the structural and emulsifying properties and interfacial properties of oxidized soybean protein aggregates. *Ultrason. Sonochem.* **2022**, *87*, 106046. [CrossRef] [PubMed]
58. Yan, W.; Frégeau-Reid, J. Breeding line selection based on multiple traits. *Crop Sci.* **2008**, *482*, 417–423. [CrossRef]