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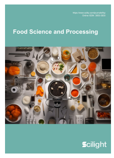

Article

The Effect of Fermentation, Malting, and Cooking on Protein Content and Fractions of Sorghum Cultivars

Wedad H. Abdelhaleem [1], Almujtaba H. M. Abdallh [2], Abdullahi H. El Tinay [2] and Elfadil E. Babiker [2,3,*]

[1] Ministry of Science and Technology, Industrial Research and Consultancy Centre, Khartoum North 11111, Sudan
[2] Department of Food Science and Technology, Faculty of Agriculture, University of Khartoum, Khartoum North 11111, Sudan
[3] Department of Food Science and Nutrition, College of Food and Agricultural Sciences, King Saud University, Riyadh 11451, Saudi Arabia
* Correspondence: elfadilbabiker@yahoo.com

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Abstract: This study examined the effects of fermentation, malt addition, and cooking on the protein content and protein fractions of low- (Mugud) and high-tannin (Karamaka) sorghum cultivars. The flour of the seeds of both cultivars was mixed with 5% malt. Then, the flour, with or without malt, was fermented for 16 h. Samples were taken every 4 h during fermentation to monitor changes in pH, titratable acidity, protein content, and protein fractions. Additionally, the fermented flour, with or without malt, was cooked to analyze changes in the protein fractions of the cultivars. The fermentation of flour, regardless of malt addition, elevated crude protein content and titratable acidity while reducing pH in both cultivars. A significant ($p < 0.05$) increase in the (globulin + albumin) percentage was noted during the fermentation of both cultivars' flour, while other fractions exhibited variability. Cooking the fermented dough significantly ($p < 0.05$) reduced the fractions of both cultivars, except for G3-glutelin and insoluble proteins, which were increased significantly ($p < 0.05$). Malting followed by fermentation had a slight effect on the fractions, except for G3-glutelin and insoluble proteins, which were significantly ($p < 0.05$) reduced for both cultivars. The results revealed that adding malt to sorghum flour and fermenting it is a valuable method for improving the nutritional quality even after cooking.

Keywords: Sorghum; fermentation; malt; cooking; protein fractions

1. Introduction

As a versatile and profitable grain, sorghum (*Sorghum bicolor* L.) is cultivated widely in dry areas of Africa, Asia, Australia, and North and South America [1] because it is adaptable to different environmental conditions. Sorghum ranks sixth among cereals worldwide, with a total production of 59 million tons [2]. Sorghum is among the most healthful and nutrient-dense food crops, characterized by its high levels of minerals, energy, protein, vitamins, fiber, phenolic compounds, and gluten-free properties. It serves as a significant supply of minerals, including iron and zinc, in low-income communities. Sorghum, while healthy, exhibits a nutritional profile that is significantly influenced by genotype and environmental factors [3].

Grain sorghum matches the protein content of traditional food crops such as millet, roots, tubers, pulses, and vegetables, and is gluten-free, making it appropriate for individuals with celiac disease. Sorghum lacks an inedible husk, unlike many other cereals [4]. This indicates that it supplies the body with additional fiber and various essential nutrients and has a lower glycemic index, which is particularly advantageous for individuals with blood sugar issues, including diabetes. The proteins of crops, such as sorghum, exhibit disparities in amino acid content, solubility, and digestibility. Consequently, crop quality is evaluated not only by protein content but also by its utility, informed by analysis of its fractional composition [5].

Sorghum is a substantial source of carbohydrates and protein. The majority of the proteins are concentrated in the endosperm, specifically prolamins and glutelin, while the germ is predominantly composed of albumins and globulins. Milling of the grain removes the outer pericarp, increasing protein content while reducing components such as cellulose, fat, and mineral content [6]. Several proteins were present in sorghum grain, particularly the primary storage proteins known as kafirins, along with high levels of important amino acids, including leucine, phenylalanine, isoleucine, valine, and methionine, making it a valuable source of protein in areas where protein-rich foods are limited or expensive [7].

The grain bran and the germ of sorghum contained both albumins (10%) and globulin (30%) proteins and such fractions have been reported to contain high lysine concentrations compared to the major storage proteins (kafirins) in the grain [8] with molecular weights ranging from 14 to 80 kDa [9]. Glutelin is predominantly located in the sorghum endosperm, has a lysine concentration higher than that of albumins and globulins, and contains elevated levels of charged amino acids such as glutamic acid and aspartic acid. The glutelin content and composition of sorghum vary with variety and lysine level, as well as development conditions; however, endosperm type does not influence its content [10].

The fermentation of chemically modified strains enhanced protein digestibility by altering protein structure via lactic acid and by increasing the total proportions of globulins and albumin in grain; however, it constrained the impact on grain nutritional quality [11]. Fermentation can modify the structural composition of kafirin and glutelin proteins, enhancing their susceptibility to enzymatic hydrolysis. Glutelins may be polluted due to inadequate isolation of prior fractions, resulting in variability in their properties and necessitating method modification [12]. Wet processing procedures, including soaking, malting, and fermentation, are cost-effective approaches for diminishing the antinutritional components of sorghum. Such processing methods can enhance the functional properties, nutritional value, bioavailability, and sensory qualities of sorghum grain [13]. Therefore, this research investigated the impact of fermentation, malt incorporation, and cooking on the protein content and protein fractions of low- and high-tannin sorghum cultivars.

2. Materials and Methods

2.1. Source of Sorghum Grains

This study utilized two sorghum cultivars: Mugud (low tannin), sourced from the Agricultural Research and Technology Corporation in Khartoum, Sudan, and Karamaka (high tannin), a local cultivar from Western Sudan. All chemicals utilized in this study were of analytical grade.

2.2. Sample Preparation

The cultivars grains were thoroughly hand-cleaned to remove husks, broken grains, and other extraneous impurities. The refined grains of each cultivar were classified into two categories. One group was milled into fine flour using a hammer mill (Gibbons Electric, Essex, UK) and then sieved through a 0.4 mm mesh. The alternate group was malted using the methods described by Bhise et al. [14], with minor adjustments. The grains were immersed in water three times their volume for 10 h, followed by a 1-h air exposure after 6 h of soaking. For each air rest, the infusion water was substituted. Following steeping (10 h), the grains were sterilized by immersion in 1% sodium hypochlorite for 20 min, then drained before germination. The saturated grains were spread on damp jute bags, covered with a wet cotton cloth, and permitted to germinate at ambient temperature (28 ± 3 °C) for six days. Following germination, the grains were desiccated in a Gallenkamp oven (BS type OV-160; Manchester, UK) at 50 °C for 24 h. The rootlets and shoots of the grains were separated from the kernels by passing the grains through a 0.6 mm sieve (Endecotts Ltd., London, UK). The malted grains were milled into fine flour using a hammer mill (Gibbons Electric, Essex, UK) and passed through a 0.4 mm sieve. Approximately 5% (5 g) of the malt was amalgamated with 95% (95 g) of the flour in 200 mL of distilled water. The flour, whether containing malt or not, was fermented according to the method of Ibrahim et al. [15], with a minor modification. A starter from previously fermented doughs of the same cultivar was used. The dough was prepared with a 5% starter-to-sorghum flour ratio and fermented for 24 h. Approximately 200 mL of distilled water was added and thoroughly mixed with a glass rod. The slurry was allowed to ferment at room temperature (24 ± 1 °C). Samples were obtained at 0 h and then every 4 h till reaching 16 h. The pH was measured every 4 h during fermentation using a pH meter (PUSL München 2, Karl Kolb, Germany). The samples were subsequently dried in a Gallenkamp oven (BS type OV-160; Manchester, UK) at 50 °C for 24 h. The dehydrated samples were groundh into fine flour using a hammer mill (Gibbons Electric, Essex, UK) and subsequently sieved through a 0.4 mm screen for the evaluation of crude protein. The fermented flour, with or without malt, was placed in a water bath for 20 min. The viscous material was allocated in petri dishes and desiccated in a Gallenkamp oven (BS type OV-160; Manchester, UK) at 50 °C for 24 h. The desiccated flakes were ground to a fine flour using a hammer mill (Gibbons Electric, Essex, UK) and then filtered through a 0.4 mm screen for the study of protein fractions.

2.3. Determination of Crude Protein

Crude protein (Nx6.25) was assessed according to the AOAC [16] protocol 984.13.

2.4. Determination of pH and Titratable Acidity

The pH of the fermenting dough was measured initially, then every 4 h for 16 h, using a glass electrode pH meter (PUSL, MUNCHENZ, KARL KOLB, Germany). Titratable acidity, quantified as lactic acid, was assessed using titration with 0.1 N NaOH to a pH of 8.1.

2.5. Protein Fractionation

Three independent samples, each weighing 3.5 g, were suspended in 35 mL of each extractant using magnetic stirring in 50 mL centrifuge tubes. Nitrogen was extracted from a defatted sample in a sequential manner utilizing a variety of solvents, following the Landry and Moureaux method [17]. To isolate salt-soluble globulin, 0.5 M NaCl was added to the sample powder, and the mixture was agitated for 60 and 30 min at 4 °C. The residue was extracted twice with an equal volume of distilled water for 15 min at 4 °C to get water-soluble albumin. Subsequently, the residue was agitated with 60% ethanol twice for 30 min at 20 °C, followed by treatment at 60 °C for 30 min, and then extracted with 55% isopropanol (Pr-OH) at 20 °C to isolate alcohol-soluble prolamin. The residue was extracted using a solution of 60% ethanol and 0.6% 2-mercaptoethanol (2-ME), stirred for 30 min at 20 °C, and subsequently extracted with 55% propanol containing 0.6% 2-ME at the same temperature for an additional 30 min to isolate G1-glutelin. G2- and G3-glutelins were obtained through treatment with 0.0125 M borate buffer (pH 10), 0.6% 2-ME, and 0.5 M NaCl, and with 0.0125 M borate buffer (pH 10), 0.6% 2-ME, and 0.5 M sodium dodecyl sulfate (SDS), respectively. The residual solid after extraction was separated from the extractants via centrifugation using a bench centrifuge (BTL Warwickshire, CV22 7DH, England) at 30,000 g for 15 min. The nitrogen content of each fraction was assessed using the micro-Kjeldahl method [16].

2.6. Statistical Analyses

Each sample was analyzed in triplicate, and the results were subsequently averaged. Data were analyzed using analyses of variance (ANOVA) and the Duncan multiple range test, with a significance level of $p < 0.05$.

3. Results

3.1. pH, Titratable Acidity, and Crude Protein Changes during Fermentation and Malting

Figure 1 illustrates the impact of fermentation (Figure 1a) and malting and fermentation (Figure 1b) on pH, titratable acidity (TA), and crude protein of the flour from Mugud and Karamaka cultivars. The unfermented flour of the cultivars exhibited pH values of 6.12 for Mugud and 5.91 for Karamaka. Fermentation progressively decreased the pH of the flour from both cultivars with time. Fermentation of the flour for 16 h significantly ($p < 0.05$) reduced the pH to 3.07 and 3.39 over time for the cultivars, respectively. Subsequent decreases in pH were noted during fermentation of malted flour throughout time, with a minimum pH attained after 16 h in both cultivars. During fermentation, the TA of the Mugud cultivar increased ($p < 0.05$) from 0.36% to 1.60%, whereas that of Karamaka increased ($p < 0.05$) from 0.36% to 1.80%. The increase in TA was more pronounced after 16 h of malted flour fermentation, with TA of 1.95% for Mugud and 1.89% for the Karamaka cultivar. After 16 h of fermentation, the total protein content considerably increased ($p < 0.05$) from 10.58% to 13.11% in the Mugud cultivar and from 10.85% to 12.25% in the Karamaka cultivar. Fermenting malted flour for 16 h increased total protein content: from 10.36% to 14.82% for the Mugud cultivar and from 11.02% to 12.74% for the Karamaka cultivar.

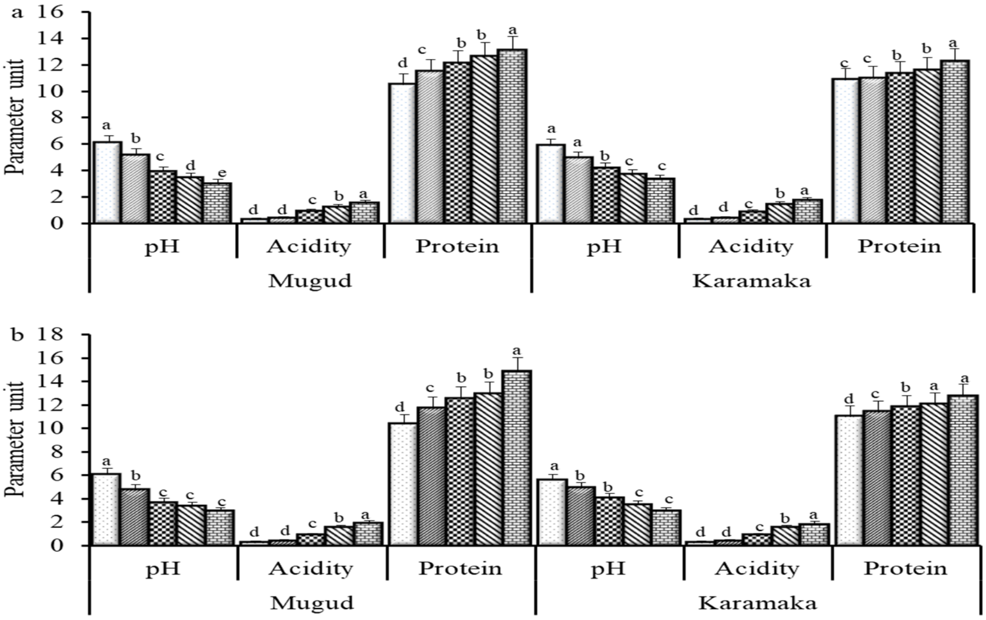

Figure 1. pH, titratable acidity (%), and crude protein content (%) of sorghum cultivars' flour after fermentation (**a**) and malting and fermentation (**b**). Columns from left to right depict fermentation periods of 0, 4, 8, 12, and 16 h. Values are the mean (±SD) of three replicates. Small letters "a, b, c, d, e" indicate a significant difference ($p < 0.05$) between fermentation periods (0, 4, 8, 12, and 16 h) for each parameter.

3.2. Changes in Protein Fractions during Fermentation and Cooking of Sorghum Flour

Tables 1 and 2 indicate variations in the percentage protein fractions throughout fermentation and cooking for the Mugud and Karamaka cultivars, respectively. The albumin plus globulin fraction was observed to be 15.50% at zero time and 17.55% at the end of the fermentation period (16 h) for Mugud cultivar (Table 1), while that of Karamaka cultivar (Table 2) was 15.32% at zero time and 17.37% at the end of the fermentation period. After cooking of the fermented dough the fraction was decreased from 15.50% to 12.43% at zero time and from 17.55% to 13.40% at the end of fermentation period for Mugud cultivar (Table 1) while that of Karamaka cultivar (Table 2) was decreased from15.32% to 11.55% at zero time and from 17.38% to 12.85% at the end of fermentation period.

The prolamin fraction (Kafirin) was significantly increased from 21.63% at zero time to 24.35% at the end of the fermentation period for the Mugud cultivar (Table 1), while that of the Karamaka cultivar (Table 2) was increased from 23.62% at zero time to 26.30% at the end of the fermentation period. Cooking of fermented flour from both cultivars significantly ($p < 0.05$) decreased Kafirin content to 8.48% and 8.90% at the end of fermentation for the Mugud and Karamaka cultivars, respectively. For both cultivars, all other fractions fluctuated during fermentation and continued to fluctuate after cooking. However, cooking was found to affect the content of these fractions adversely. G_3-glutelin increased after 4 h of fermentation and then decreased in the Mugud cultivar, whereas in Karamaka it decreased over the fermentation period. Cooking of the fermented dough significantly ($p < 0.05$) increased G3-glutelin in both cultivars, with values of 37.30% and 32.65% for the zero-time fermented dough of Mugud and Karamaka, respectively, and 37.35% and 32.65% for the 16 h fermented dough, respectively.

Table 1. Effect of fermentation followed by cooking on protein fractions of Mugud cultivar.

Fermentation Period (h)	Globulin + Albumin		Kafirin		G1-Glitelin		G2-Glutelin	
	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
0	15.50 ^{c} (±0.18)	12.43 ^{b} (±0.38)	21.63 ^{c} (±0.18)	7.52 ^{c} (±0.30)	32.53 ^{a} (±0.38)	32.72 ^{a} (±0.00)	6.00 ^{a} (±0.00)	5.40 ^{a} (±0.56)
4	15.57 ^{c} (±0.43)	12.19 ^{b} (±0.16)	23.30 ^{b} (±0.43)	9.09 ^{a} (±0.16)	30.60 ^{c} (±0.28)	31.45 ^{b} (±0.28)	5.20 ^{c} (±0.28)	4.70 ^{b} (±0.14)
8	16.73 ^{b} (±0.04)	12.50 ^{b} (±0.34)	24.51 ^{a} (±0.28)	8.70 ^{a} (±0.00)	31.45 ^{b} (±0.50)	32.70 ^{a} (±0.14)	5.17 ^{c} (±0.18)	4.95 ^{b} (±0.21)
12	17.39 ^{a} (±0.16)	13.43 ^{a} (±0.47)	24.23 ^{a} (±0.28)	8.28 ^{b} (±0.14)	31.81 ^{b} (±0.28)	32.22 ^{a} (±0.14)	4.82 ^{c} (±0.00)	4.55 ^{b} (±0.21)
16	17.55 ^{a} (±0.49)	13.40 ^{a} (±0.28)	24.35 ^{a} (±0.35)	8.48 ^{b} (±0.32)	30.35 ^{c} (±0.35)	32.71 ^{a} (±0.14)	5.63 ^{b} (±0.00)	5.50 ^{a} (±0.14)

Table 1. *Cont.*

Cultivar/Fermentation Period (h)	G3-Glutelin		Insoluble Protein		Protein Recovered	
	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
0	22.11 b (±0.15)	37.30 a (±0.42)	2.35 a (±0.35)	8.50 b (±0.28)	100.12	103.85
4	23.51 a (±0.14)	36.45 b (±0.22)	2.00 b (±0.28)	10.35 a (±0.35)	100.17	102.23
8	21.10 c (±0.21)	36.35 b (±0.01)	1.55 c (±0.35)	6.01 d (±0.14)	100.50	102.1
12	20.13 d (±0.10)	37.30 a (±0.28)	1.05 d (±0.07)	7.41 c (±0.28)	100.42	103.16
16	21.32 c (±0.42)	37.35 a (±0.21)	0.92 e (±0.00)	4.62 e (±0.28)	100.05	102.03

Values are means (±SD) of three replicates. Means that do not share a common letter "a, b, c, d" in a column are significantly different at $p < 0.05$ according to the DMR test.

Table 2. Effect of fermentation followed by cooking on protein fractions of Karamaka cultivar.

Fermentation Period (h)	Globulin + Albumin		Kafirin		G1-Glitelin		G2-Glutelin	
	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
0	15.32 c (±0.30)	11.55 b (±0.09)	23.60 d (±0.16)	9.01 b (±0.14)	29.82 c (±0.16)	29.90 c (±0.14)	6.70 a (±0.14)	6.50 a (±0.14)
4	15.65 c (±0.07)	11.55 b (±0.21)	23.15 d (±0.12)	8.50 b (±0.14)	30.60 a (±0.00)	31.61 b (±0.21)	6.50 a (±0.14)	5.85 b (±0.07)
8	15.60 c (±0.28)	11.60 b (±0.14)	24.65 c (±0.21)	9.65 a (±0.35)	29.81 c (±0.14)	30.75 d (±0.21)	6.05 ed (±0.07)	5.70 b (±0.14)
12	16.15 b (±0.22)	11.28 b (±0.04)	25.50 b (±0.14)	9.25 a (±0.35)	31.22 a (±0.26)	32.03 a (±0.21)	5.31 b (±0.02)	4.65 c (±0.21)
16	17.38 a (±0.32)	12.85 a (±0.11)	26.30 a (±0.42)	8.90 b (±0.00)	31.38 a (±0.07)	32.30 a (±0.11)	6.20 a (±0.00)	5.75 b (±0.21)

Fermentation Period (h)	G3-Glutelin		Insoluble Protein		Protein Recovered	
	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
0	23.83 a (±0.04)	32.65 d (±0.21)	1.45 a (±0.21)	10.61 a (±0.14)	100.70	100.20
4	22.72 b (±0.14)	35.75 b (±0.21)	1.50 a (±0.15)	9.85 b (±0.21)	100.10	103.15
8	22.45 b (±0.21)	34.60 c (±0.28)	1.50 a (±0.14)	7.80 c (±0.14)	100.05	100.10
12	22.87 b (±0.01)	36.75 a (±0.21)	1.40 b (±0.00)	9.50 b (±0.14)	102.45	103.46
16	20.31 c (±0.13)	32.65 d (±0.07)	1.45 a (±0.21)	10.85 a (±0.21)	103.02	103.32

Values are means (±SD) of three replicates. Means that do not share a common letter "a, b, c, d" in a column are significantly different at $p < 0.05$ according to the DMR test.

3.3. Changes in protein fractions during malting and fermentation of sorghum flour

Tables 3 and 4 show changes in the percentage protein fractions during fermentation and cooking of malted flour from the Mugud and Karamaka cultivars, respectively. About 5% of sorghum malt was mixed with 95% of the flour before fermentation, and the mixture was fermented for varying lengths of time. Each time, the fermented dough was cooked into a slurry and then dried. The albumin plus globulin fraction of the malted flour was significantly increased after fermentation. It was found to be 15.96% at zero time and 19.95% at the end of the fermentation period for Mugud cultivar (Table 3), while that of Karamaka cultivar (Table 4) was also significantly increased and was found to be 16.30% at zero time and 19.25% at the end of the fermentation period. The fraction increased slightly during the first 8 h and then increased significantly ($p < 0.05$) at the end of the fermentation period. After cooking, the albumin plus globulin fraction was decreased from 15.96% to 12.03% at zero time and from 19.95% to 16.65% at the end of the fermentation period for Mugud cultivar (Table 3) while that of Karamaka cultivar (Table 4) was decreased from16.30% to 11.68% at zero time and from 19.25% to 15.18% at the end of fermentation period. The prolamin fraction (Kafirin) was 19.58% at zero time and 22.90% at the end of the fermentation period for Mugud cultivar (Table 4), while that of Karamaka (Table 5) was 20.25% at zero time and 24.40%% at the end of the fermentation period. Cooking malted and fermented flour from both cultivars significantly ($p < 0.05$) decreased Kafirin content to 13.11% and 12.71% at the end of fermentation for Mugud and Karamak, respectively.

Table 3. Effect of malting and fermentation, followed by cooking, on protein fractions of Mugud cultivar.

Fermentation Period (h)	Globulin + Albumin		Kafirin		G1-Glitelin		G2-Glutelin	
	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
0	15.96 [d] (±0.63)	12.03 [c] (±0.08)	19.58 [c] (±0.54)	13.21 [c] (±0.28)	32.92 [b] (±0.14)	33.73 [ab] (±0.11)	5.30 [a] (±0.14)	4.30 [a] (±0.42)
4	17.68 [c] (±0.15)	12.19 [c] (±0.16)	20.78 [c] (±0.07)	13.75 [b] (±0.07)	33.30 [a] (±0.42)	33.72 [ab] (±0.28)	5.40 [a] (±0.16)	3.80 [b] (±0.14)
8	19.20 [b] (±0.14)	13.45 [b] (±0.01)	20.95 [c] (±0.35)	14.42 [a] (±0.28)	32.21 [b] (±0.28)	33.35 [b] (±0.35)	4.85 [a] (±0.21)	3.80 [b] (±0.00)
12	19.42 [b] (±0.14)	16.91 [a] (±0.14)	21.95 [b] (±0.21)	13.70 [b] (±0.14)	33.25 [a] (±0.21)	34.95 [a] (±0.50)	4.15 [c] (±0.07)	3.00 [b] (±0.14)
16	19.95 [a] (±0.21)	16.65 [a] (±0.07	22.92 [a] (±0.14)	13.11 [c] (±0.13)	32.90 [a] (±0.28)	33.85 [ab] (±0.21)	4.12 [c] (±0.14)	2.75 [c] (±0.07)

Fermentation Period (h)	G3-Glutelin		Insoluble Protein		Protein Recovered	
	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
0	22.85 [a] (±0.21)	22.29 [a] (±0.16)	2.40 [a] (±0.16)	15.71 [d]. (±0.14)	99.00	101.25
4	20.71 [b] (±0.42)	20.30 [b] (±0.14)	2.25 [a] (±0.35)	16.92 [c] (±0.28)	100.11	100.64
8	21.15 [b] (±0.21)	19.35 [c] (±0.07)	2.25 [a] (±0.21)	19.30 [b] (±0.42)	100.60	103.65
12	19.15 [c] (±0.22)	17.85 [d] (±0.21)	2.30 [a] (±0.14)	16.82 [c] (±0.28)	100.20	103.20
16	18.25 [c] (±0.35)	18.02 [d] (±1.7)	2.20 [a] (±0.14)	19.61 [a] (±0.14)	100.30	103.96

Values are means (±SD) of three replicates. Means that do not share a common letter "a, b, c, d, e" in a column are significantly different at $p < 0.05$ according to the DMR test.

Table 4. Effect of malting and fermentation followed by cooking on protein fractions of Karamaka cultivar.

Fermentation Period (h)	Globulin + Albumin		Kafirin		G1-Glitelin		G2-Glutelin	
	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
0	16.30 [c] (±0.28)	11.68 [b] (±0.04)	20.25 [e] (±0.21)	12.36 [a] (±0.06)	32.45 [a] (±0.63)	33.20 [a] (±0.14)	6.15 [a] (±0.07)	4.90 [a] (±0.14)
4	17.30 [b] (±0.42)	13.68 [b] (±0.03)	21.35 [d] (±0.07)	11.60 [b] (±0.00)	31.50 [b] (±0.00)	32.05 [b] (±0.07)	6.20 [a] (±0.28)	4.25 [a] (±0.07)
8	18.90 [a] (±0.14)	14.33 [b] (±0.04)	22.45 [c] (±0.21)	12.55 [a] (±0.35)	31.65 [b] (±0.21)	33.61 [a] (±0.16)	4.85 [b] (±0.21)	3.25 [b] (±0.07)
12	19.05 [a] (±0.08)	15.65 [a] (±0.07)	23.35 [b] (±0.22)	12.55 [a] (±0.21)	32.20 [a] (±0.28)	33.26 [a] (±0.23)	4.60 [b] (±0.14)	3.30 [b] (±0.42)
16	19.25 [a] (±0.21)	15.18 [a] (±0.03)	24.40 [a] (±0.28)	12.71 [a] (±0.13)	31.05 [b] (±0.07)	30.80 [a] (±0.42)	4.65 [b] (±0.07)	4.50 [a] (±0.14)

Fermentation Period (h)	G3-Glutelin		Insoluble Protein		Protein Recovered	
	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
0	21.58 [a] (±0.03)	19.10 [b] (±0.10)	2.60 [a] (±0.14)	19.50 [b] (±0.10)	99.33	100.74
4	21.65 [a] (±0.21)	19.45 [b] (±0.10)	2.30 [b] (±0.28)	21.55 [a] (±0.21)	100.30	101.58
8	20.20 [b] (±0.16)	19.60 [b] (±0.14)	2.40 [b] (±0.07)	18.00 [c] (±0.14)	100.45	101.34
12	21.35 [a] (±0.21)	21.06 [a] (±0.14)	2.30 [b] (±0.35)	14.00 [e] (±0.14)	102.85	99.82
16	19.90 [b] (±0.14)	18.98 [c] (±0.02)	2.30 [b] (±0.28)	16.94 [d] (±0.23)	101.55	102.11

Values are means (±SD) of three replicates. Means that do not share a common letter "a, b, c, d, e" in a column are significantly different at $p < 0.05$ according to the DMR test.

4. Discussion

This study examined the effects of fermentation, malt addition, and cooking on the protein content and fractions of low- and high-tannin sorghum cultivars. Fermentation is among the oldest food processing methods, historically employed in many nations [18]. Enzymatic activity of microorganisms during the process mitigates off-flavors, synthesizes exopolysaccharides, breaks down proteins into smaller peptides and bioactive amino acids, alters techno-functional properties, improves protein digestibility by reducing antinutritional factors, produces nutritional components like vitamins, and preserves food [19,20]. Malting is the regulated germination of cereal grains in humid air under controlled conditions, which generates hydrolytic enzymes that alter the structure of cereal grains, enhancing their solubility during mashing and converting grains into nutritious meals for both children and adults [21].

Fermentation of both cultivars' flour lowered the slurry pH, and a further drop was observed when malted flour was fermented. pH drops after fermentation primarily because yeast metabolizes sugars into alcohol, producing organic acids and releasing carbon dioxide, which forms carbonic acid, creating a more acidic environment. The results also indicated that adding malt to flour generally results in a lower pH after fermentation, particularly with active malt due to its significant enhancement of food availability for yeast and lactic acid bacteria [22]. While fermentation itself lowers the dough's pH by making it more acidic, adding malted flour accelerates this process [23]. The drop in pH during fermentation of malted and unmalted flour is consistent with the gradual increase in TA. The results are similar to those reported by Alhaag et al. [24], who found that during fermentation the pH decreased and was accompanied by an increase in acidity as lactic acid accumulated due to microbial activity. The fermentation duration and malting enhanced the crude protein content of both cultivars. This increase could be attributed to the breakdown of complex proteins into peptides and amino acids by proteolytic enzymes produced during fermentation [25]. Also, Bello et al. [26] found that higher protein content at higher malt concentrations could be attributed to enhanced proteolytic activity in germinated seeds. Moreover, the observed increase in protein content after fermentation or malting was probably due to a shift in dry matter content, resulting from the depletion of carbohydrates during germination and fermentation by fermenting microorganisms. It may thus appear, but not constitute a real increment. However, cells of the fermenting microorganisms could have contributed to the protein; therefore, germination or fermentation of sorghum results in an observable increase in crude protein content. However, a combination of the two treatments may generally result in an additional increment. In most human diets, protein is more limiting than carbohydrates. Therefore, the application of any process that appears to increase protein content, even at the expense of carbohydrates, may be advantageous nutritionally. The loss in dry matter, especially carbohydrates, is likely due to the physiological activities of fermenting organisms, which utilize part of the meal's nutrients, thereby reducing its dry matter content.

The albumin-globulin fraction increased significantly ($p < 0.05$) during the first 8 h and then increased slightly at the end of the fermentation period. High lysine content characterizes the albumin-globulin fraction because these proteins differ from prolamins (storage proteins) and frequently perform metabolic or transport functions that require a different, more balanced amino acid composition [27]. Thus, the nutritional value of sorghum flour would be expected to increase after fermentation. Although the fraction of cooked samples from both cultivars increased with the fermentation period, the amount was lower than that of uncooked fermented flour. Breaking the disulfide link during cooking resulted in a rougher, looser cross-section and surface, as well as poor cooking and textural qualities [28]. Thus, the lower disulfide bond composition of the cultivars' fraction would explain the reduced albumin-globulin ratio and poor functionality of the flour. The results obtained showed that cooking reduced the nutritional quality of the flour. The prolamin fraction (Kafirin) was significantly increased after fermentation but decreased after cooking. According to Shah et al. [29], when sorghum grain is used for bioethanol production, it enriches kafirin, the hydrophobic prolamin storage protein, resulting in dry distillers' grain with solubles as a byproduct. The prolamin fraction (kafirin) in sorghum frequently appears to increase after fermentation. This is because fermentation hydrolyzes starch and degrades other non-prolamin proteins, thereby increasing the concentration of the highly hydrophobic, alcohol-soluble kafirin in the remaining material. The prolamin fraction (kafirin) in sorghum reduces after cooking primarily because the heat induces extensive polymerization of the kafirin proteins through the formation of intermolecular disulfide bonds [30]. The Glutenin fractions fluctuated, while insoluble proteins increased even after cooking. The fluctuations in Glutenin fragments during fermentation and after cooking may be due to changes in protein structure, solubility, and enzymatic activity. Cooking increases insoluble protein levels primarily by denaturing and aggregating proteins, reducing their solubility. When food is cooked, the complex, folded structures of proteins unfold, and the exposed protein strands bond together (coagulation), reducing their solubility [31]. Fermentation increased the albumin plus globulin fraction, but after cooking, it was decreased. However, the fraction of cooked malted and fermented samples from both cultivars increased with the fermentation period, but the amount was lower than that of uncooked malted and fermented flour. The results showed that cooking reduced the nutritional quality of flour, but malting slightly alleviated this effect. Kaur & Prasad [32] studied the effect of germination and roasting on the water-soluble albumin fraction, salt-soluble globulin fraction, alcohol-soluble prolamin fraction, and alkali-soluble glutelin fraction. They found that globulin content decreased significantly, whereas the albumin fraction increased significantly after 6 days of germination. Also, they reported that malting improved the protein quality by activating enzymes that hydrolyze (break down) insoluble storage proteins (prolamins and glutelins) into soluble, smaller protein components, notably albumin and globulin, to aid embryo growth. Chusova et al. [33] found that during germination, the total level of albumins and globulins in oats increased dramatically, but prolamins and glutelins reduced significantly when compared to ungerminated grain. For both cultivars, all other fractions of the malted flour fluctuated during fermentation and even after cooking. However, cooking was found to affect the content of these fractions adversely. The malting and fermentation of sorghum flour were observed to elevate the prolamin fraction (kafirin), but at a lower rate, potentially due to residual antinutrients that impede the activity of proteolytic enzymes responsible for protein hydrolysis. Moreover, these protein structures containing kafirin generally display a spherical or ellipsoidal morphology [34]. In these protein bodies, kafirin molecules are tightly aggregated, creating a matrix-like structure that envelops lipid droplets. This configuration offers stability and safeguards the kafirin molecules, protecting them from proteolytic enzymatic activity, various environmental challenges, and solvent extraction. Fermentation of malted flour was found to adversely affect G3-glutenin, which was greatly reduced before and after cooking in both Mugud and Karamaka cultivars. Malting of the flour increased insoluble protein levels during fermentation after cooking in both cultivars. Cooking induces protein denaturation, disrupting the weak interactions that maintain proteins' natural, folded conformations. Upon unfolding, these proteins typically aggregate, reconfigure, and establish new, more robust linkages (such as disulfide bonds and hydrophobic interactions) that exhibit reduced solubility in water and are frequently more challenging for the body to digest [35]. The results showed that malting the flour alleviates the effect of cooking, but at the expense of insoluble protein.

5. Conclusions

Malting and fermentation are essential processes utilized to improve food quality during processing. The malting of sorghum grains modifies their biochemical characteristics, thereby improving protein content and fractions through controlled germination. During sorghum malting, endogenous proteases hydrolyze proteins, breaking down complex structures into simpler, soluble components, thereby enhancing protein content and fractions. On the other hand, fermentation significantly enhances protein content and fractions. Moreover, this method facilitates product preservation. However, cooking the fermented flour reduced protein content and fractions, whereas malting the flour alleviated the effect of cooking on these fractions. These methods are anticipated to enhance the culinary applications of grains, including sorghum ball gruel, alcoholic beverages, non-alcoholic drinks like "nasha," and sorghum bread slices (Kisra), thereby highlighting the substantial benefits of sorghum as a versatile and nutritious food source, especially in Africa.

Author Contributions

W.H.A.: Methodology, Data curation, Formal analysis, Conceptualization, Investigation, Writing—original draft. A.H.M.A.: Formal analysis, Conceptualization, Investigation, Writing—original draft. A.H.E.T.: Validation, Visualization, Writing—review & editing, Supervision. E.E.B.: Validation, Visualization, Writing—review & editing, Supervision. All authors have read and agreed to the published version of the manuscript.

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Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

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