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Effect of Germination Temperatures on Proteolysis of the Gluten-Free Grains Sorghum and Millet during Malting and Mashing

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ABSTRACT: Our study showed that sorghum and millet followed a similar pattern of changes when they were malted under similar conditions. When the malt from these cereals was mashed, both cereal types produced wide spectra of substrates (sugars and amino acids) that are required for yeast fermentation when malted at either lower or higher temperatures. At the germination temperatures of 20, 25, and 30 °C used in malting both cereal types, production of reducing sugars and that of free amino nitrogen (FAN) were similar. This is an important quality attribute for both cereals because it implies that variation in temperature during the malting of sorghum and millet, especially when malting temperature is difficult to control, and also reflecting temperature variations, experienced in different countries, will not have an adverse effect on the production and release of amino acids and sugars required by yeast during fermentation. Such consistency in the availability of yeast food (substrates) for metabolism during fermentation when sorghum and millet are malted at various temperatures is likely to reduce processing issues when their malts are used for brewing. Although sorghum has gained wide application in the brewing industry, and has been used extensively in brewing gluten-free beer on industrial scale, this is not the case with millet. The work described here provides novel information regarding the potential of millet for brewing. When both cereals were malted, the results obtained for millet in this study followed patterns similar to those of sorghum. This suggests that millet, in terms of sugars and amino acids, can play a role similar to that of sorghum in the brewing industry. This further suggests that millet, like sorghum, would be a good raw material for brewing gluten-free beer. Inclusion of millet as a brewing raw material will increase the availability of suitable materials (raw material sustainability) for use in the production of gluten-free beer, beverages, and other products. The availability of wider range of raw materials will not only help to reduce costs of beer production, but by extension, the benefit of reduced cost of production can be gained by consumers of gluten-free beer as the product would be cheaper and more widely available.

KEYWORDS: *decantation mashing, free amino nitrogen, germination temperature, hot water extract, malting, millet, proteolysis, sorghum*

INTRODUCTION

Sorghum as a brewing raw material has been studied extensively in the past, whereas millet has not. This work provides a comparison of the malting performance of sorghum with that of millet, which has not been studied to the same extent as sorghum. Comparing the properties of these two major cereals under similar conditions will increase our knowledge and understanding of the physiology and malting behavior of millet and provide a basis for comparison with sorghum and other cereals such as buckwheat and rice. For the purposes of this Article, we have focused our work specifically on millet and sorghum.

Gluten is a generic name for certain types of proteins contained in the cereal grains wheat, rye, triticale, barley, and oats and derivatives from these.[1] It is a storage protein, and it is the main structure-forming protein in the flour of these cereals.[2] The protein fractions of gluten are glutenin and gliadin, and due to these two components, gluten shows cohesive, elastic properties. These properties are important for food, and therefore gluten is used in many foods as a food additive, especially bread making. Gluten removal results in major problems for bakers, and, currently, many gluten-free products available on the market are of low quality and short shelf life, and exhibit poor mouthfeel and flavor.[2]

On the other hand, gluten is a harmful substance for a patient who suffers from celiac disease (CD). CD is a genetic immune media enteropathy, which is triggered by the ingestion of gluten.[2] It is a life-long dietary intolerance to gluten resulting in damage to the lining of the small bowel such that food is not absorbed properly.[1] Even small amounts of gluten in foods may affect those with celiac disease and result in health problems, and damage can occur to the small bowel even in the absence of symptoms.[1] CD is the result of an interaction between genetic and environmental factors, and the only medical treatment is lifelong dedication to a gluten-free diet at present.[2,3] This is not easy because many staples of Western diet are based on wheat flour.[3] In recent years, the labeling of gluten-free foods and beverages is increasing as diagnosis of CD increases. Because of the development of sensitive serodiagnosis, it is now possible to evaluate the prevalence of CD. Screening tests showed that

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there is a high prevalence of CD among both healthy children and adults.[2] The screening tests show that CD is one of the most frequent diseases, as a genetic-based disease, and occurs in a ratio of one person to 130−300 persons in the European population.[2]

There is no international agreement over the term "gluten-free" or universal symbol that makes gluten-free products distinguishable. However, in the international Codex standard that is used by many countries in Europe, the revision of gluten-free standards is progressing, but it is hard to realize under present conditions because there is no agreement on the detection method of gluten and the permissible amount of gluten. The international Codex Alimentarius Commission (2006) defines gluten as "a protein fraction from wheat, rye, barley, oats, or their crossbred varieties and derivatives thereof, to which some persons are intolerant and that is insoluble in water and 0.5 M NaCl". Prolamins are protein fractions from gluten, which can be extracted by 40−70% (v/v) of ethanol. According to the International Codex Alimentarius Commission (2006), gluten-free products are defined in various ways.[2]

Because of their chemical composition, maize, rice, sorghum, and the pseudocereals (amaranth, buckwheat, and quinoa) are suitable raw materials for the production of gluten-free beer. With regard to the brewing industries, many "gluten-free beers" are available in the market, and this type of beer broadens the range of beverages that is consumable for a patient who suffers from CD. Some examples of such beers include Baird's Tale Dragon's Gold gluten-free lager, made from 100% sorghum; Ramapo Valley Brewery's gluten-free honey lager; Anheuser-Busch's gluten-free Redbridge beer; Milwakee microbrewery's Mbege ale and Shakparo ale, both African style beers brewed from sorghum and millet; and a Canadian microbrewery's gluten-free La Messagere brewed from rice and buckwheat.

Earlier studies carried out on these gluten-free cereals[4−6] and the follow-up research studies,[7−43] have enabled the production of gluten-free beer from them on an industrial scale in different countries. The general focus of most research regarding gluten-free brewing technologies is mainly on malting and mashing studies using a single brewing material. Although comparative studies on millet, sorghum (gluten-free cereals), and barley (nongluten-free cereal) have been reported,[44,45] as far as we are aware, there are few research reports that evaluate and compare specific gluten-free materials together under similar conditions. In the current economic situation where manufacturing industries (for example, brewing and distilling industries) are looking to extend their options for various types of raw materials in an effort to address sustainability issues, research into the use of different raw materials is becoming of increasing importance. This is encouraging researchers to investigate novel raw materials and is even more crucial now that the demand for cereal derived bioethanol production is beginning to impact on cereals. In our overall research, we have studied four gluten-free raw materials (sorghum, millet, rice, and buckwheat) under similar conditions, and in this Article we are presenting our results for millet and sorghum, which have proved to be successful brewing materials. While some aspects of these have been studied previously,[9−15,18,19,21,46−48] this work provides a new more detailed perspective on these cereals. The original work was commissioned on behalf of an international brewing company who have a strong interest in the commercial application of these cereals.

MATERIALS AND METHODS

Grain Samples. Sorghum and millet were purchased from Bostonseeds on their website, and the work was undertaken as a result of the interest of a Brewing Co. who carried out this study at Heriot-Watt University, with some supporting research input from the Scotch Whisky Research Institute. The sorghum sample was a hybrid-mix cultivate, described as PEN 110DWARF sorghum, while the millet was white (*Proso* millet).

Preliminary Analyses of Sorghum and Millet. Moisture content of sorghum and millet was determined according to the Recommended Methods of the Institute of Brewing for barley.[49] The Kjeldahl total nitrogen was determined using Recommended Methods of the Institute of Brewing[49] and the Tecator equipment as described previously.[50] Thousand grain weights of sorghum and millet were determined by counting up to 1000 grains and then weighing them.[50] Determinations were performed in triplicate, and mean values given were used in this report.

Germination Tests. A modified germination test based on the Institute of Brewing Recommended Method of Analysis of the Institute of Brewing[49] method was performed on the samples prior to malting. In these tests, 100 grains were transferred to a Petri-dish containing 2 layers of Whatman No. 1 filter paper. Exactly 3, 4, and 6 mL of water were added, and samples were incubated at room temperature (∼20 °C) for 72, 96, and 120 h. Germinated grains were then counted.

Steeping and Malting of Sorghum and Millet. Samples, sorghum and millet (600 g) each, were steeped in water at 20 °C for 20 h, followed by a 4 h air-rest and further 22 h wet-steep.[7] After steeping, samples were germinated at 20, 25, and 30 °C for 4 and 5 days. Steeping and germination were performed by using the Custom Laboratory Products micromalting equipment (Keith, Banffshire) at Heriot-Watt University. Germinated grain samples were kilned at 50 °C for 24 h in a Seeger kiln.[7] Dried malt was rubbed by hand and sieved to remove rootlets and shoots, and the samples were used for analysis.

Malt Analyses. *α-Amylase Activity.* The activity of α-amylase of malted sorghum and millet was determined using the Megazyme Assay Kit (McCleary and Sheehan[51] as reported previously.[8]

Mashing of Sorghum and Millet Malts. Decantation mashing system that was described previously[8] was used in mashing of malted samples of sorghum and millet. In brief, sorghum or millet malt was milled in a Buhler Miag Mill at setting 2. The flour (50 g) was extracted with 360 mL of distilled water in a 500 mL Erlenmeyer flask at 30 °C for 30 min after which the enzymatically active wort was decanted. The mash residue was then heated to 100 °C in a water bath (Grant E3, Grant Instrument Ltd., Barrington Cambridge) to gelatinize the malt starch. After being cooled, the decanted supernatant was returned to the boiled mash, and the volume was adjusted to 360 mL (stainless mashing beaker) and then mashed in the BRF mashing bath (Crisp Malting Ltd., Great Ryburgh, UK) at 65 °C for 1 h.

Hot Water Extract of All Malts. Hot water extract (HWE) was determined by feeding the wort sample obtained after mashing sorghum or millet malt into a density meter (Calculating Digital Density Meter, Stanton Redcroft PAAR DMA 46, London, UK). After conversion to specific gravity (SG), the hot water extract was calculated.[8]

Determination of Total Soluble Nitrogen and α-Amino Nitrogen of Sorghum and Millet Malt Worts. Total soluble nitrogen (TSN) present in the hot water extract (HWE) was determined using the standard method described in the Recommended Methods of Analysis of the Institute of Brewing.[49] The α-amino nitrogen was determined using the Ninhydrin assay method as described in the Recommended Methods of Analysis of the Institute of Brewing.[49]

HPAE of Sugar Composition of Sorghum and Millet Worts. Separation was performed using high performance anion exchange (HPAE), and detection was performed by a pulsed amperometric detector (PAD). A detailed description of the HPAE instrument and column used for sugar profile tests is as follows.

Table 1. Properties of Day 4 and Day 5 Germinated Sorghum and Millet

	germination temperature (20 °C)		germination temperature (25 °C)		germination temperature (30 °C)	
parameters	sorghum	millet	sorghum	millet	sorghum	millet
			Day 4 Germinated Sorghum and Millet			
HWE (L°/kg)	230	274	221	274	198	266
TSN (%)	0.74	0.50	0.63	0.53	0.52	0.48
FAN (mg/L)	205	125	181	134	133	130
			Day 5 Germinated Sorghum and Millet			
HWE (L°/kg)	209	258	190	217	174	227
TSN (%)	0.68	0.55	0.61	0.57	0.49	0.57
FAN (mg/L)	244	143	220	140	144	147

Table 2. Sugar Profiles of Sorghum and Millet Germinated at Different Temperatures for 4 days

	germination temperature (20 °C)		germination temperature (25 °C)		germination temperature (30 °C)	
sugar profile (g/L)	sorghum	millet	sorghum	millet	sorghum	millet
glucose	14.5	14.0	15.3	15.6	14.5	15.6
fructose	2.2	2.4	3.2	2.8	2.0	2.8
sucrose	0.2	0.8	0.3	0.9	0.3	0.9
maltose	21.0	36.3	22.1	38.3	20.3	38.3
maltotriose	8.3	13.2	9.1	13.4	8.0	13.4
glucose:maltose ratio	1:1.4	1:2.6	1:1.4	1:2.5	1:1.4	1:2.5

Table 3. Sugar Profiles of Sorghum and Millet Germinated at Different Temperatures for 5 days

	germination temperature (20 °C)		germination temperature (25 °C)		germination temperature (30 °C)	
sugar profile (g/L)	sorghum	millet	sorghum	millet	sorghum	millet
glucose	16.5	23.0	15.7	21.1	15.3	20.8
fructose	2.6	2.8	2.6	4.0	2.1	2.8
sucrose	0.2	0.2	0.2	0.3	0.1	0.2
maltose	15.4	17.0	12.5	14.2	13.6	15.8
maltotriose	7.2	8.5	5.8	6.8	6.0	6.9
glucose:maltose ratio	1:0.9	1:0.7	1:0.8	1:0.7	1:0.9	1:0.8

Instrumentation. This included a Dionex PAD (pulsed electrochemical detector) with gold electrode, Gilson 302 and 305 pump, Gilson 802 Manometric Module, Gilson 811 B Dynamic mixer, Hewlett-Packard 1050 auto injector, Dionex eluent degas module, and Hewlett-Packard Chemstation data handling (HP3365).

Column. This included a Dionex Carbopac PA-100 Guard column, 4 × 50 mm, Dionex Carbopac PA-100 column, 4 × 250 mm.

HPLC of Amino Acid Composition of Sorghum and Millet Worts. Analysis of amino acids present in the HWE was performed by gradient elution, high performance liquid chromatography (HPLC), using fluorescence as a means detection. Detailed description of the HPLC instrument and column used for amino acid profile test is as follows.

Instrumentation. This included a Gilson 231 autoampler with 40 L dilutor, Rheodyne 7010 injector with 20 uL loop, Gilson 302 and 306 pumps with 5SC pump head, Gilson 802 Manometric controller, Gilson 811 C dynamic mixer, Gilson 715 data handling package, Phenomenex Degassex (degassing unit) model DG4400, and Jasco FP 1520 fluorescent detector.

Column. A Phenospere Next, 5u, C18, 150 × 4.6 mm, from Phenomenex UK Ltd., Queens Avenue, Hurdsfield Estate, Macclesfield, Cheshire, SK10 2YF column was placed in an oven unit at 270 °C.

RESULTS AND DISCUSSION

The sorghum and millet samples used in this study were not bred for malting and may not meet all of the criteria for malting quality cereal. This notwithstanding, the results emerging from the study highlight important aspects of these cereals both in terms of their value for food and in brewing particularly when they have been studied under the same conditions. Malting was performed on these cereals at a range of different temperatures to establish their brewing behavior under different temperature conditions, which were chosen to reflect temperature variations experienced in different countries. Table 1 shows the properties of day 4 and day 5 sorghum and millet malted at 20, 25, and 30 °C. The results in Table 1 show that day 4 germinated samples produced higher hot water extract (HWE) yield than day 5 germinated samples for both cereal types. This suggests that under the malting conditions used in this study, day 4 germination was better in producing extract yield from both cereals. In contrast to the similar pattern observed for extract yield, both cereals developed different levels of soluble nitrogen and free amino nitrogen. While sorghum malt produced higher levels of soluble nitrogen on day 4, malted millet produced higher levels of soluble nitrogen on day 5. In general, both samples (sorghum and millet) produced higher levels of FAN products on day 5, as compared to the previous day. They also released sufficient FAN products to support yeast fermentation after both day 4 and day 5 germinations,[52,53] regardless of germination temperature. Protein hydrolysis (solubilization and amino acid release) in sorghum and millet malts is discussed in more detail below.

Table 2 shows the sugar profiles found in the hot water extract (HWE) obtained from mashed malts of sorghum and millet (day 4 malt). Worts from both cereals produced a wide range of sugar spectra. Both sorghum and millet produced similar amounts of wort glucose in day 4 malt, with millet malt

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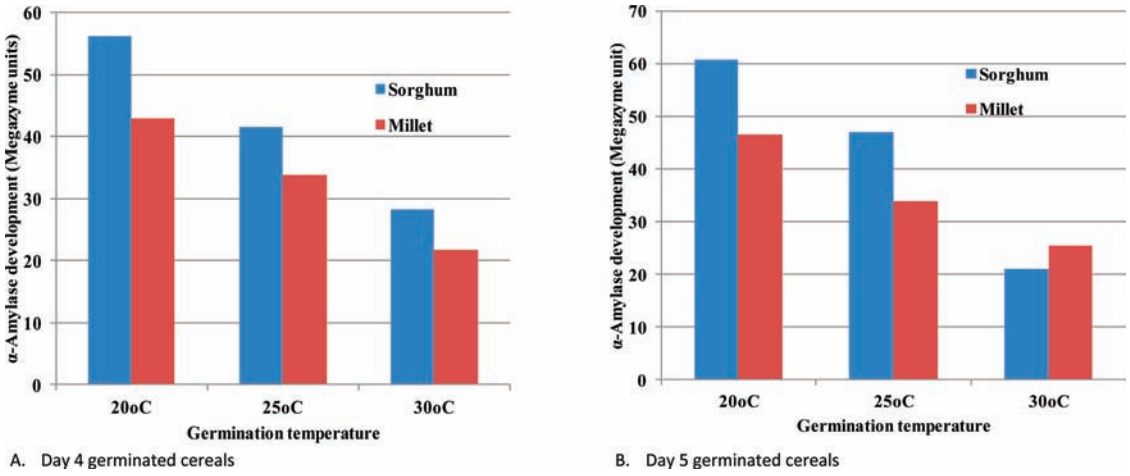

Figure 1. α-Amylase development during germination of sorghum and millet at 20, 25, and 30 °C: A = α-amylase development after 4-day germination; B = α-amylase development after 5-day germination.

Table 4. α-Amino Acid Profiles of Sorghum and Millet Germinated at Different Temperatures for 4 days

	germination temperature (20 °C)		germination temperature (25 °C)		germination temperature (30 °C)	
amino acid profile (μmol/mL)	sorghum	millet	sorghum	millet	sorghum	millet
aspartic	0.328	0.291	0.360	0.312	0.350	0.308
glutamic	0.468	0.059	0.408	0.062	0.435	0.059
asparagine	1.160	0.567	1.345	0.495	1.282	0.506
glutamine	2.353	1.192	2.156	1.138	2.268	1.114
serine	0.368	0.352	0.364	0.356	0.379	0.357
arginine	0.331	0.403	0.342	0.383	0.339	0.384
threonine	0.240	0.158	0.229	0.165	0.235	0.171
glycine	0.531	0.339	0.490	0.332	0.500	0.341
alanine	1.362	0.865	1.092	0.876	1.181	0.843
proline	2.380	1.383	2.250	1.178	2.326	1.286
valine	0.680	0.515	0.657	0.500	0.689	0.501
methionine	0.170	0.165	0.176	0.164	0.185	0.155
isoleucine	0.437	0.378	0.408	0.358	0.450	0.359
leucine	1.083	0.814	0.987	0.803	0.992	0.810
trytophan	0.190	0.152	0.167	0.141	0.173	0.140
phenylalanine	0.680	0.415	0.612	0.411	0.650	0.422
lysine	0.478	0.404	0.452	0.409	0.474	0.430
histidine	0.270	0.250	0.384	0.248	0.284	0.239
tyrosine	1.052	0.470	0.992	0.480	1.003	0.480

producing a marginally higher level of maltose than sorghum malt. On the other hand, while day 5 germinated sorghum maintained a similar level of wort glucose production, millet produced higher amounts of glucose and lower amounts of maltose when germination was extended to day 5 (Table 3). The sugar profiles found in sorghum and millet malt worts are important as they will impact the ability of yeast to metabolize these sugars during fermentation. The ratios of maltose to glucose reported in Tables 2 and 3 are consistent at the different malting temperatures. This observation is important as these results show that sorghum and millet will produce consistent levels of soluble nitrogen and sugar substrates in the derived wort when malted at different temperatures.[54] The consistency in the patterns of glucose and maltose production observed in this work is in agreement with earlier reports.[55] This means that these cereals are likely to show some degree of consistency when they are used in food processing and brewing applications.

The ratio of maltose to glucose sugars released into the wort of sorghum malt is well documented.[7,8,55] The pattern of maltose to glucose ratio obtained for millet malt, which is similar to that found in sorghum malt, suggests that millet is likely to behave like sorghum. Some studies have linked the maltose to glucose ratios in different cereals (barley and sorghum) to the levels of α-amylase and β-amylase developed in cereals during malting.[46,56] In these studies, the ratio of β-amylase to α-amylase developed during malting of cereals appeared to influence maltose to glucose ratio found in their worts.[10,46,56] It is therefore worth noting that both sorghum and millet followed similar trends in the development of α-amylase when they were malted for 4 or 5 days (Figure 1). Sorghum, however, developed marginally higher levels of α-amylase than millet on day 5 germination time. It is also worth noting that both the sorghum and the millet samples used in this study developed higher levels of α-amylase at the lower germination temperature of 20 °C rather than at higher temperature of 30 °C. These results are contrary to earlier communications where

Table 5. α-Amino Acid Profiles of Sorghum and Millet Germinated at Different Temperatures for 5 days

	germination temperature (20 °C)		germination temperature (25 °C)		germination temperature (30 °C)	
amino acid profile (μmol/mL)	sorghum	millet	sorghum	millet	sorghum	millet
aspartic	0.386	0.336	0.397	0.309	0.360	0.320
glutamic	0.421	0.073	0.410	0.068	0.401	0.066
asparagine	1.290	0.520	1.325	0.531	1.306	0.503
glutamine	2.662	1.086	2.752	1.062	2.502	1.072
serine	0.372	0.359	0.363	0.354	0.366	0.370
arginine	0.348	0.349	0.364	0.358	0.372	0.384
threonine	0.225	0.165	0.232	0.176	0.223	0.232
glycine	0.510	0.342	0.527	0.344	0.503	0.335
alanine	1.318	0.865	1.065	0.887	1.110	0.898
proline	2.440	1.415	2.226	1.392	2.186	1.249
valine	0.650	0.490	0.630	0.490	0.612	0.508
methionine	0.196	0.166	0.183	0.174	0.192	0.160
isoleucine	0.428	0.333	0.410	0.334	0.459	0.337
leucine	1.090	0.848	0.933	0.830	0.982	0.835
trytophan	0.193	0.129	0.171	0.125	0.171	0.136
phenylalanine	0.716	0.430	0.676	0.439	0.669	0.431
lysine	0.478	0.412	0.418	0.439	0.478	0.429
histidine	0.295	0.273	0.310	0.268	0.317	0.264
tyrosine	1.136	0.471	0.959	0.454	0.992	0.471

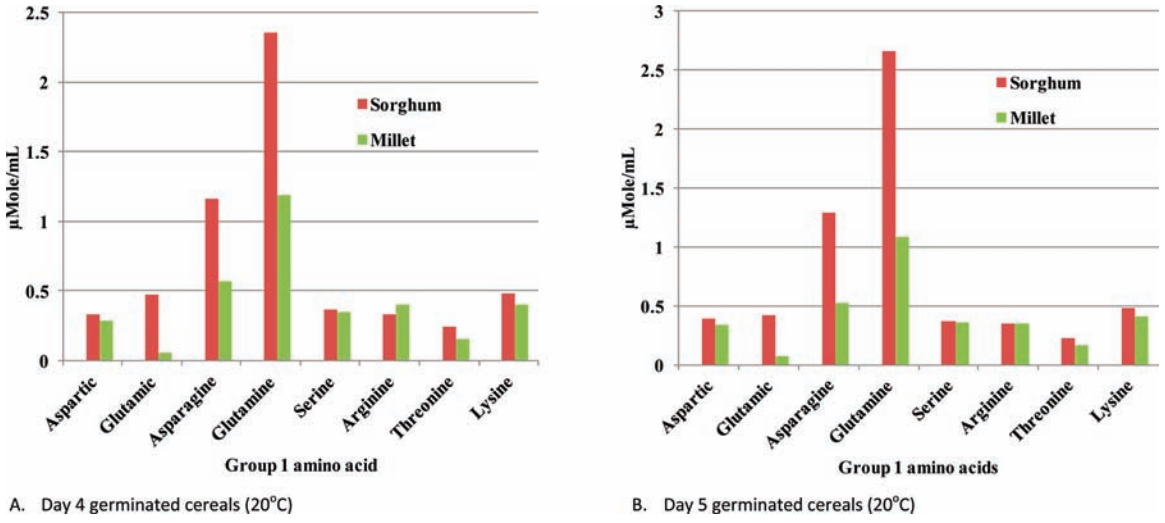

Figure 2. Pattern of release of group 1 amino acids during germination of sorghum and millet at 20 °C: A = release of amino acid after 4-day germination; B = release of amino acid after 5-day germination.

it was reported that higher temperature favored the development of α-amylase in tropical sorghum.[8,13,28,57−61] However, this result for α-amylase development should be treated with caution because the samples of sorghum and millet studied were of feed quality rather than malting grade. This may have some implications for the results of enzyme development obtained in this study and highlights the advantage of using malting grade cereals, which, if commercially sound, could also be used to produce beer. These results confirm that, although these cereals are feed grade rather than malting quality, they could be readily used to produce beer.

Interesting results were found with regard to amino acid production when malted sorghum and millet were mashed. Both malted cereals released a broad spectrum of amino acids at the different malting days and temperatures. This observation is important because it suggests that malting time or temperature is not likely to affect significantly the amino acid profile of malted sorghum and millet, especially with regard to methionine production. Yeast requires sulfur principally for the biosynthesis of sulfur-containing amino acids. Methionine, which is the most effectively used amino acid in yeast nutrition,[62] that is also an important amino acid required for adequate and effective yeast performance during fermentation was produced in sorghum and millet malts at similar levels regardless of malting temperature. Production of a wide spectrum of amino acids from sorghum malt may, in part, explain why sorghum malt is associated with producing nutritious wort.[63] It is also important to observe that millet also followed similar patterns in their production of amino acids on day 4 (Table 4) and day 5 germination time (Table 5). Again, it is worth noting that for the day 4 and day 5 germination periods both malted sorghum and millet samples produced high levels of asparagine, glutamine, alanine, proline, leucine, and tyrosine, with sorghum malt producing approximately twice the amount of these found in millet malt (Tables 4 and 5). These results show that sorghum and millet malts

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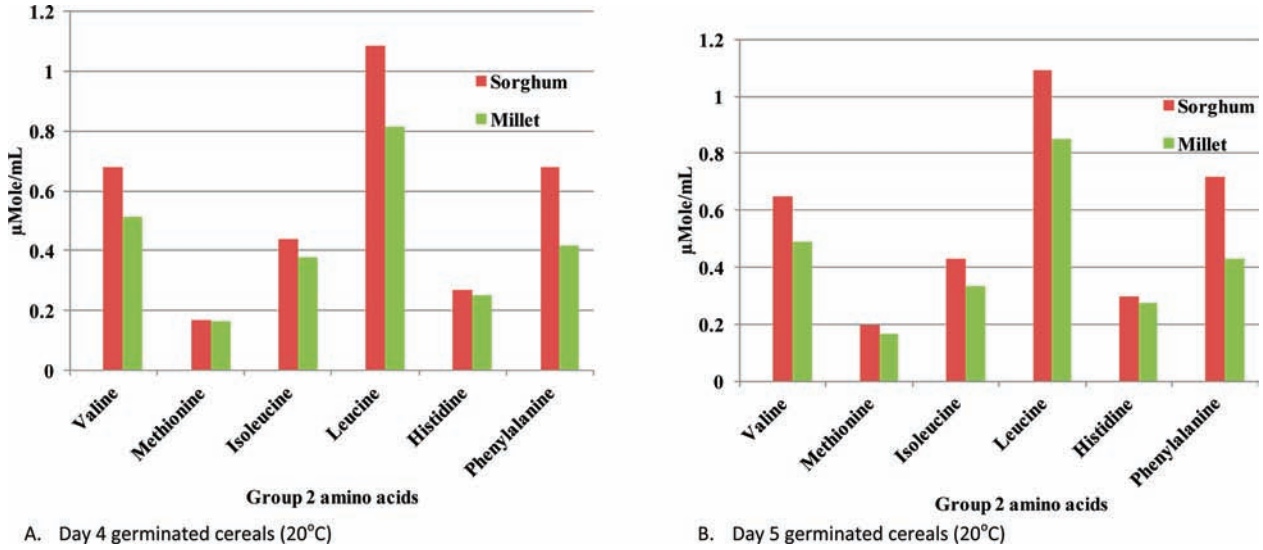

Figure 3. Pattern of release of group 2 amino acids during germination of sorghum and millet at 20 °C: A = release of amino acid after 4-day germination; B = release of amino acid after 5-day germination.

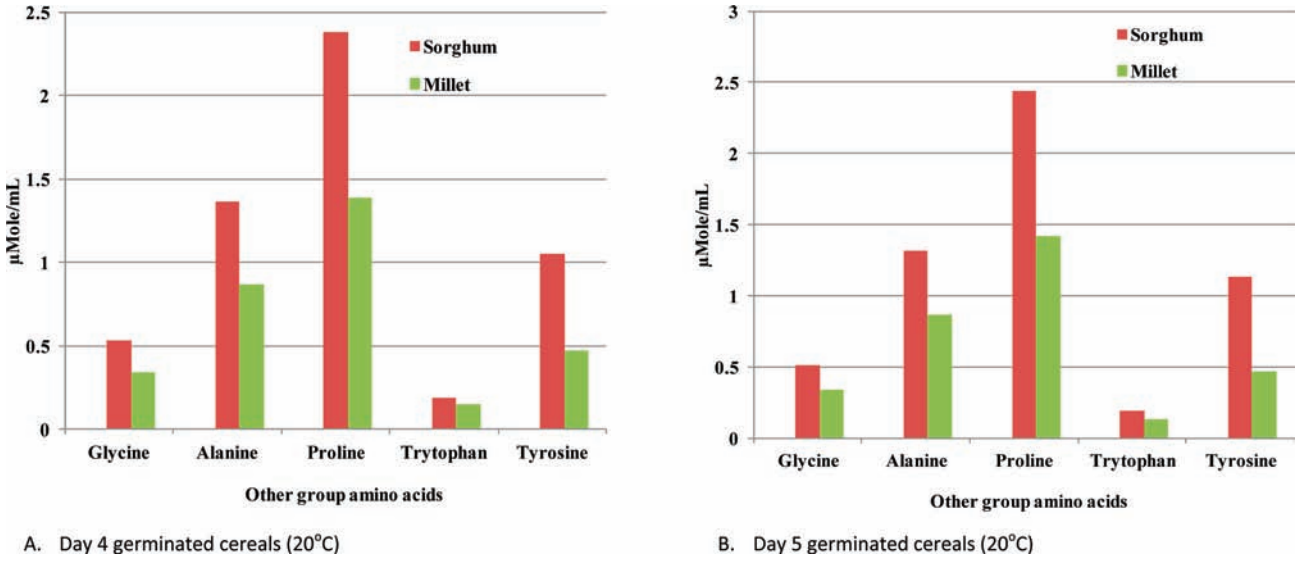

Figure 4. Pattern of release of other group amino acids during germination of sorghum and millet at 20 °C: A = release of amino acid after 4-day germination; B = release of amino acid after 5-day germination.

produce a similar range of essential amino acids distribution required during fermentations.

Similar patterns of release are again seen in the production of the different groups of amino acids. Figure 2 shows the results of production of group 1 amino acids for sorghum and millet germinated at 20 °C for 4 and 5 days. Figure 3 shows the results of group 2 amino acids of sorghum and millet malts germinated at 20 °C, while Figure 4 shows the results of other groups of amino acids of sorghum and millet germinated at 20 °C for 4 and 5 days. From these results, it can be seen that the production of these amino acids followed a similar trend in both cereals. Because sorghum malt produces nutritious wort,[63] by extension, millet is likely to behave in a manner similar to that of sorghum, with regard to producing nutritious wort with excellent brewing performance in terms of the parameters measured.

To investigate further the pattern of formation of amino acids in the worts of sorghum and millet malts, the data were tested by using analysis of variance (ANOVA). ANOVA showed there was no significant difference for the vast majority of the amino acids at the different germination times and temperatures. However, results of ANOVA indicated that, while germination temperature showed no significant difference with regard to isoleucine released into the wort from millet malt, there was a significant difference resulting from germination time ($p > 0.05$ and $p = 0.05$, respectively; Table 6). The reverse was the case for sorghum malt where ANOVA results indicated that germination temperature showed a significant difference, while germination time showed no significant difference ($p = 0.03$ and $p > 0.05$, respectively; Table 7). A similar observation was made for leucine with the ANOVA, indicating that germination temperature showed no significant difference for millet malt, while germination time showed a significant difference ($p > 0.05$ and $p = 0.0089$, respectively; Table 8). Again, the reverse was the case for sorghum malt where ANOVA indicated that germination temperature showed

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Table 6. ANOVA Examining Differences in Isoleucine Production at 20, 25, and 30°C Germinated Millet Taking Length of Germination into Account

due to	sum of squares	DoF	mean square	F-stat	signif
main effects	0.001	3	0.000	6.080	0.1445
germination temperature	0.000	2	0.000	0.618	0.6180
day	0.001	1	0.001	17.004	0.0541
explained	0.001	3	0.000	6.080	0.1445
error	0.000	2	0.000		
total	0.002	5	0.000		

Table 7. ANOVA Examining Differences in Isoleucine Production at 20, 25, and 30 °C Germinated Sorghum Taking Length of Germination into Account

due to	sum of squares	DoF	mean square	F-stat	signif
main effects	0.002	3	0.001	16.775	0.0568
germination temperature	0.002	2	0.001	25.154	0.0382
day	0.000	1	0.000	0.016	0.9104
explained	0.002	3	0.001	16.775	0.0568
error	0.000	2	0.000		
total	0.002	5	0.000		

Table 8. ANOVA Examining Differences in Leucine Production at 20, 25, and 30 °C Germinated Millet Taking Length of Germination into Account

due to	sum of squares	DoF	mean square	F-stat	signif
main effects	0.001	3	0.000	43.134	0.0227
germination temperature	0.000	2	0.000	9.507	0.0952
day	0.001	1	0.001	110.388	0.0089
explained	0.001	3	0.000	43.134	0.0227
error	0.000	2	0.000		
total	0.002	5	0.000		

significant difference, while germination time showed no significant difference (p = 0.0071 and p > 0.05, respectively; Table 8). These ANOVA results for isoleucine and leucine (group 2 amino acids) are important observations and highlight how variations in germination temperature of sorghum and millet will affect the production of these amino acids in these malted cereals.

Other differences worth mentioning between sorghum and millet malts in terms of amino acids release into their worts are arginine and alanine production. While no significant difference was found for these amino acids with regard to germination temperature and time for millet malt, this was different for sorghum malt. ANOVA showed that while germination temperature showed no significant difference with regard to arginine released into the wort of sorghum malt, germination time showed a significant difference (p > 0.05 and p = 0.0367, respectively; Table 9). In contrast, the results of ANOVA showed that germination temperature showed a significant difference with regard to alanine released into the wort of sorghum malt, and germination time showed no significant difference (p = 0.0066 and p > 0.05, respectively; Table 10).

Principal component analysis (PCA) was carried out to summarize the main compositional differences between the various samples (Figure 5). Component 1 separated samples due to grain type, irrespective of germination time or temperature, and described the large majority of variance between samples (78.3%). Therefore, grain type was the factor found to have the greatest influence on sample composition. The millet samples contained relatively high levels of arginine, while the sorghum samples contained relatively high levels of all other amino acids.

Table 9. ANOVA Examining Differences in Leucine Production at 20, 25, and 30 °C Germinated Sorghum Taking Length of Germination into Account

due to	sum of squares	DoF	mean square	F-stat	signif
main effects	0.013	3	0.004	93.889	0.0106
germination temperature	0.013	2	0.006	140.817	0.0071
day	0.000	1	0.000	0.033	0.8727
explained	0.013	3	0.004	93.889	0.0106
error	0.000	2	0.000		
total	0.013	5	0.003		

Table 10. ANOVA Examining Differences in Arginine Production at 20, 25, and 30 °C Germinated Sorghum Taking Length of Germination into Account

due to	sum of squares	DoF	mean square	F-stat	signif
main effects	0.001	3	0.000	11.546	0.0808
germination temperature	0.000	2	0.000	4.423	0.1844
day	0.001	1	0.001	25.791	0.0367
explained	0.001	3	0.000	11.546	0.0808
error	0.000	2	0.000		
total	0.001	5	0.000		

This study showed that both sorghum and millet followed a similar pattern when they were malted under similar conditions. Both cereal types produced wide spectra of substrates (sugars and amino acids) when malted and mashed at all temperatures. This is an important quality feature of these cereals because it shows that the influence of temperature will be minimal on these cereals when they are malted when malting temperature is not adequately controlled in industrial practice.[64] Furthermore, both cereals showed additional good quality with regard to consistency as was reported recently for millet[54] in terms of growing environment. Although both cereals showed great similarity in terms of malting quality, subtle differences were found with regard to the effects of germination temperature and time in the production of some amino acids. Overall, results of ANOVA revealed that grain type caused a significant difference for the vast majority of amino acids except for serine. While sorghum has gained wide application in the brewing industry, millet has not. More studies would be required to find out how these subtle differences observed in the release of amino acids caused by the effect of germination temperature will affect yeast fermentation of wort produced from malted millet and sorghum. This study shows that millet exhibited similarity to sorghum when malted under the same conditions. In the future, millet may become a significant raw material in the brewing industry and could increase the choice of raw material suitable for the production of gluten-free beer.

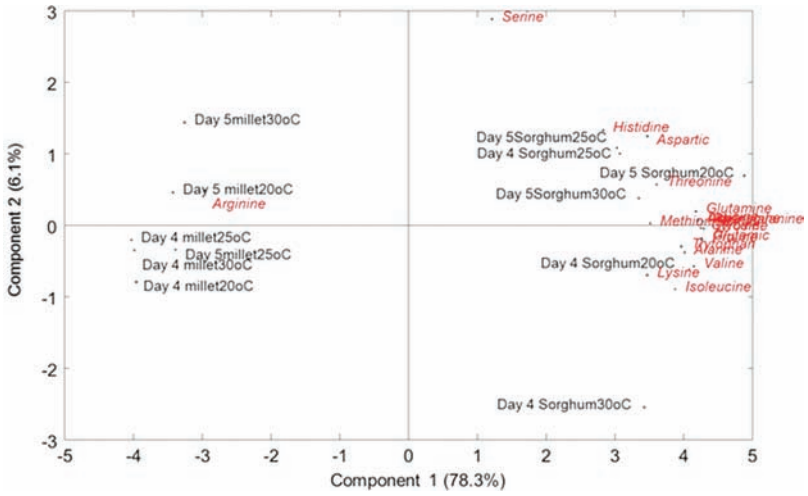

Figure 5. Principal component analysis of day 4 and day 5 germinated sorghum and millet malts.

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Notes

The authors declare no competing financial interest.

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