

## Effect of Germination Temperatures on Proteolysis of the Gluten-Free Grains Rice and Buckwheat during Malting and Mashing

R. C. Agu,<sup>\*,†</sup> Y. Chiba,<sup>‡</sup> V. Goodfellow,<sup>‡</sup> J. MacKinlay,<sup>‡</sup> J. M. Brosnan,<sup>†</sup> T. A. Bringhurst,<sup>†</sup> F. R. Jack,<sup>†</sup> B. Harrison,<sup>†</sup> S. Y. Pearson,<sup>†</sup> and J. H. Bryce<sup>‡</sup>

<sup>†</sup>The Scotch Whisky Research Institute, Research Avenue North, Riccarton, Edinburgh EH14 4AP, Scotland, United Kingdom

<sup>‡</sup>International Centre for Brewing and Distilling, School of Life Sciences, Heriot-Watt University, Riccarton, Edinburgh EH14 4AS, Scotland, United Kingdom

**ABSTRACT:** This study examined the performance of rice and buckwheat when malted under various temperature conditions and for different lengths of time. The mashed malts produced from both rice and buckwheat contained a wide spectra of sugars and amino acids that are required for yeast fermentation, regardless of malting temperature. At the germination temperatures of 20, 25, and 30 °C used, production of reducing sugars and free amino nitrogen (FAN) followed similar patterns. This implies that 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, from these grains. Such consistency in the availability of yeast substrates is likely to reduce differences in processing when these malts are used for brewing. This study revealed that, while rice malt consistently produced more maltose than glucose, buckwheat malt gave several times more glucose than maltose, across all germination temperatures. Buckwheat malt also produced more soluble and free amino nitrogen than rice malt. Unlike sorghum, which has gained wide application in the brewing industry for the production of gluten-free beer, the use of rice and buckwheat is minimal. This study provides novel information regarding the potential of rice and buckwheat for brewing. Both followed similar patterns to sorghum, suggesting that they could play a similar role to sorghum in the brewing industry. Inclusion of rice and buckwheat as brewing raw materials will increase the availability of suitable materials for use in the production of gluten-free beer, potentially making it more sustainable, cheaper, and more widely available.

**KEYWORDS:** Decantation mashing, buckwheat, free amino nitrogen, germination temperature, hot water extract, malting, proteolysis, rice

### ■ INTRODUCTION

In a previous study,<sup>1</sup> we examined the malting performance of sorghum and millet at various temperatures selected to mimic the conditions where these cereals are grown. The malting temperature can be difficult to control, especially in tropical countries.<sup>2</sup> Results showed that, under these various temperature conditions, malt produced from sorghum and millet contained adequate levels of sugars and amino acids required for yeast fermentation and, hence, beer production.<sup>1</sup> In this paper, we have extended our research to examine two other cereals, namely, rice and buckwheat. The main reason in both of these studies is to look into the performance of novel raw materials for use in the food and beverage industries, in response to the current economic climate, which is putting pressure on the sourcing of affordable cereal supplies. In addition, the increasing demand for cereal-derived bioethanol, in either partial or total replacement of fossil fuels, is beginning to impact the supply chain. The use of sorghum as a brewing raw material has been studied extensively in the past, and research on millet has progressed reasonably well. However, studies on rice and buckwheat and, in particular, their performance under different malting conditions are still very scant when compared to studies on barley and malt.

Another important aspect this research is that all four grains (sorghum, millet, rice, and buckwheat) are gluten-free. Gluten protein found in many cereals, such as wheat, rye, triticale, barley, and oats, and their derivatives is damaging to the health of those that suffer from celiac disease. This disease, which can

be difficult to detect,<sup>3,4</sup> is a dietary intolerance to gluten that results in damage to the lining of the small bowel, such that food is not absorbed properly.<sup>3</sup> At present, no cure has been found for celiac disease; therefore, sufferers must adhere to a lifelong gluten-free diet.<sup>3,5</sup> This can be difficult because many staples of the Western diet are based on wheat flour.<sup>5</sup> The problems associated with cereals that contain gluten protein has led researchers to look into the suitability of using gluten-free cereals (for example, sorghum and millet) or pseudo-cereals (for example, buckwheat) for food processing, including the brewing of gluten-free beers suitable for consumption by celiac sufferers.<sup>4,5</sup>

Similar to sorghum and millet, rice is a well-defined cereal. Buckwheat is not; it belongs to the pseudo-cereals. Buckwheat noodles are eaten by people from Tibet and northern China, and they play a major role in the cuisines of Japan (*soba*).<sup>6</sup> In recent years, research studies into the use of buckwheat in brewing gluten-free beers have been on the rise.<sup>7–14</sup> While acknowledging the fact that buckwheat is not a cereal, it can be used in the same way as other traditional cereals, such as barley, to produce malt that can form the basis of a mash that will brew a beer suitable for celiac sufferers or others sensitive to certain

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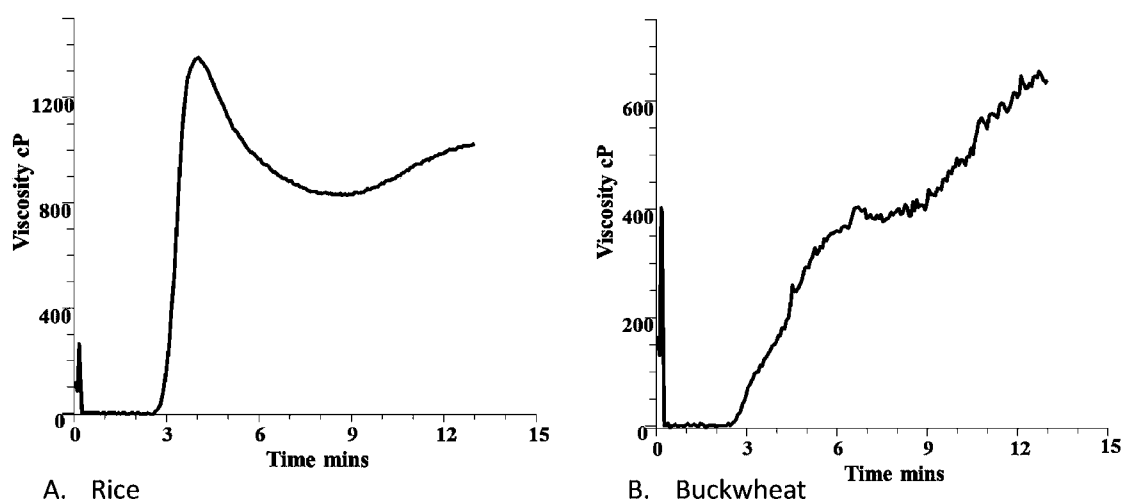
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A. Germination test

B. Enlargement

**Figure 1.** Germination test experiments for buckwheat: (A) grains that germinated during the test and (B) physiological pattern of germination, with rootlet growth and no visible shoot.



A. Rice

B. Buckwheat

**Figure 2.** RVA pasting characteristics of rice and buckwheat: (A) rice and (B) buckwheat.

glycoproteins.<sup>15</sup> Indeed, a Canadian microbrewery's gluten-free *La Messagere* beer is brewed from rice and buckwheat. In terms of brewing, buckwheat is considered an important raw material because it has some additional health benefits, such as high amino acid contents, in particular a high level of lysine, when compared to other cereals.<sup>6</sup> In addition, buckwheat contains antioxidants, such as rutin.<sup>7</sup>

As far as we are aware, no systematic physiological studies have been carried out comparing buckwheat and rice malted at various tropical temperatures. The results of this study and of our previous work on sorghum and millet<sup>1</sup> will help to increase the knowledge and understanding of the malting behavior of different gluten-free cereals, with a view to assess their suitability and flexibility in brewing gluten-free beers. The work was undertaken as a result of the interest of an international brewing company who carried out this study at Heriot-Watt University, with some supporting research input from The Scotch Whisky Research Institute.

## MATERIALS AND METHODS

**Grain Samples.** Rice and buckwheat were purchased from Boston Seeds.

**Preliminary Analyses of Rice and Buckwheat.** Moisture contents were determined according to the Institute of Brewing method for barley,<sup>16</sup> as described earlier.<sup>1</sup> Thousand grain weights were determined by counting up to 1000 grains and then weighing them.<sup>17</sup>

**Table 1.** Numerical Data Generated by the RVA Instrument

sample	peak viscosity (cP)	holding strength (cP)	final viscosity (cP)	peak time (min)
rice	1351	827	1022	4.07
buckwheat	403	358	638	6.73

Determinations were performed in triplicate, and mean values were used in this report.

**Germination Tests.** A modified germination test based on the Institute of Brewing method<sup>16</sup> was performed on the samples prior to malting and described previously.<sup>1</sup> Germinated grains were then counted.

**Rapid Visco Analysis (RVA) Program for Unmalted Cereals (Rice and Buckwheat).** A RVA was carried out using a standard program for unmalted cereals, as described elsewhere.<sup>18</sup> The RVA peak and final viscosities give an indication of the viscous load encountered by the RVA analyzer during the run and represent a very important parameter for cereal processing.<sup>18</sup>

**Steeping and Malting of Rice and Buckwheat.** Samples of rice and buckwheat (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.<sup>18</sup> Details of this procedure have been reported in our earlier publication.<sup>1</sup>

**Malt Analyses.  $\alpha$ -Amylase Activity.** The activity of  $\alpha$ -amylase of malted rice and buckwheat was determined using the Megazyme Assay Kit,<sup>19</sup> as reported previously.<sup>20</sup>

**Mashing of Rice and Buckwheat Malts.** The decantation mashing system that was described previously<sup>20</sup> was used in mashing of malted samples of rice and buckwheat. Rice or buckwheat malt was milled in a

Buhler Miag Mill at a setting of 2, and the mashing procedure is as described earlier.<sup>1</sup>

**Hot Water Extract (HWE) of All Malts.** HWE was determined by feeding the wort sample obtained after mashing rice or buckwheat malt into a density meter (calculating digital density meter, Stanton Redcroft

PAAR DMA 46, London, U.K.). After conversion to specific gravity (SG), the HWE was calculated.<sup>20</sup>

**Determination of Total Soluble Nitrogen (TSN) and  $\alpha$ -Amino Nitrogen of Rice and Buckwheat Malt Worts.** TSN present in the HWE was determined using the standard method described in the

**Table 2. Properties of Day 4 and Day 5 Germinated Rice and Buckwheat**

parameters	germination temperature (20 °C)		germination temperature (25 °C)		germination temperature (30 °C)	
	rice	buckwheat	rice	buckwheat	rice	buckwheat
Day 4 Germinated Rice and Buckwheat						
HWE (L deg/kg)	322	315	306	226	225	223
TSN (%)	0.31	0.40	0.38	0.54	0.55	0.60
FAN (mg/L)	67	101	89	146	139	135
Day 5 Germinated Rice and Buckwheat						
HWE (L deg/kg)	325	315	302	227	224	223
TSN (%)	0.46	0.50	0.52	0.57	0.65	0.69
FAN (mg/L)	133	144	121	152	177	188

**Table 3. Sugar Profiles of Rice and Buckwheat Germinated at Different Temperatures for 4 Days**

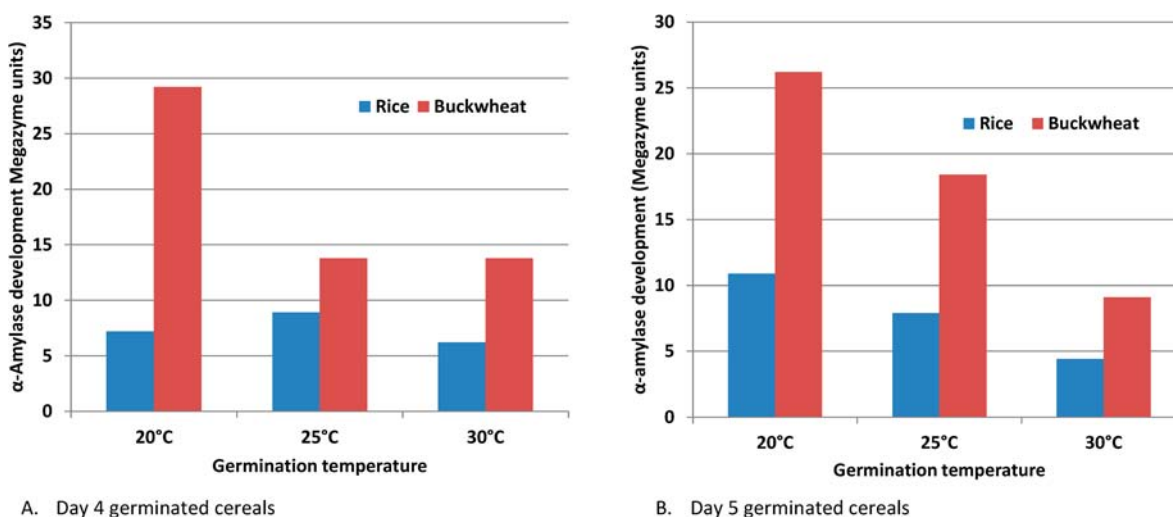
sugar profile (g/L)	germination temperature (20 °C)		germination temperature (25 °C)		germination temperature (30 °C)	
	rice	buckwheat	rice	buckwheat	rice	buckwheat
glucose	16.2	20.2	18.1	20.1	17.1	19.6
fructose	0.5	1.8	0.5	1.2	0.4	1.2
sucrose	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>
maltose	29.6	5.5	34.7	5.7	30.4	5.3
maltotriose	13.1	3.5	12.2	4.1	11.9	4.0
glucose/maltose ratio	1:1.8	3.7:1	1:1.9	3.5:1	1:3.1	3.7:1

<sup>a</sup>ND = not detected.

**Table 4. Sugar Profiles of Rice and Buckwheat Germinated at Different Temperatures for 5 Days**

sugar profile (g/L)	germination temperature (20 °C)		germination temperature (25 °C)		germination temperature (30 °C)	
	rice	buckwheat	rice	buckwheat	rice	buckwheat
glucose	21.9	21.2	22.3	21.7	21.3	21.9
fructose	0.7	2.1	0.7	1.4	0.6	1.4
sucrose	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>
maltose	29.2	6.0	30.6	4.5	24.7	4.1
maltotriose	13.8	3.8	11.9	3.1	11.4	3.1
glucose/maltose ratio	1:1.3	3.5:1	1:1.4	4.8:1	1:1.2	5.3:1

<sup>a</sup>ND = not detected.



**Figure 3.**  $\alpha$ -Amylase development during germination of rice and buckwheat at 20, 25, and 30 °C: (A)  $\alpha$ -amylase development after 4 days of germination and (B)  $\alpha$ -amylase development after 5 days of germination.

Institute of Brewing method.<sup>1,16</sup> The  $\alpha$ -amino nitrogen was determined using the ninhydrin assay method, as described in the Institute of Brewing method.<sup>1,16</sup>

**High-Performance Anion Exchange (HPAE) of Sugar Composition of Rice and Buckwheat Worts.** Separation was performed using HPAE chromatography, and detection was performed by a pulsed amperometric detector (PAD), as described previously.<sup>1</sup>

**High-Performance Liquid Chromatography (HPLC) of Amino Acid Composition of Sorghum and Millet Worts.** Analysis of amino acids present in the HWE was performed by gradient elution, HPLC, using fluorescence as a means of detection, as described previously.<sup>1</sup>

**Statistical Analyses.** Analysis of variance (ANOVA) was used to determine whether or not there were any significant difference in amino acid levels in the worts depending upon germination times and temperatures (Unistat, version 5.0, Unistat, Ltd.). A *p* value of less than 0.05 was considered significant.

## RESULTS AND DISCUSSION

The germination test results showed that both rice and buckwheat germinated effectively under these conditions. Buckwheat showed 97% germination, and rice showed 98% germination. The germinating buckwheat grains are shown in Figure 1. It was interesting to observe that buckwheat produced a single long rootlet with a conspicuous absence of a shoot (Figure 1B). A similar single rootlet system is found in sorghum, although germinating sorghum also produces a well-defined shoot.<sup>21</sup> This is different from the 3–4 rootlet system found in germinating barley.

The RVA pasting characteristics of rice and buckwheat are shown in Figure 2. Buckwheat followed a pattern similar to that observed for some sorghum or maize varieties, which is different from the pasting profile of rice. Table 1 shows the numerical

**Table 5. Amino Acid Profiles of Rice and Buckwheat Germinated at Different Temperatures for 4 Days**

amino acid profile ( $\mu\text{mol/mL}$ )	germination temperature (20 °C)		germination temperature (25 °C)		germination temperature (30 °C)	
	rice	buckwheat	rice	buckwheat	rice	buckwheat
aspartic	0.282	0.227	0.281	0.216	0.277	0.208
glutamic	0.190	0.651	0.207	0.671	0.222	0.671
asparagine	0.031	0.032	0.033	0.022	0.035	0.026
glutamine	0.448	0.222	0.463	0.229	0.457	0.230
serine	0.222	0.297	0.230	0.292	0.230	0.285
arginine	0.313	0.371	0.337	0.362	0.337	0.348
threonine	0.158	0.226	0.147	0.222	0.147	0.238
glycine	0.382	0.341	0.356	0.347	0.351	0.330
alanine	0.833	1.232	0.840	1.070	0.834	1.142
proline	0.373	0.331	0.380	0.311	0.379	0.329
valine	0.609	0.567	0.644	0.550	0.623	0.527
methionine	0.133	0.151	0.122	0.160	0.136	0.160
isoleucine	0.363	0.370	0.348	0.380	0.354	0.374
leucine	0.817	0.845	0.822	0.862	0.834	0.829
tryptophan	0.122	0.146	0.131	0.143	0.131	0.145
phenylalanine	0.411	0.410	0.420	0.380	0.415	0.383
lysine	0.357	0.401	0.355	0.377	0.346	0.378
histidine	0.183	0.212	0.191	0.204	0.186	0.208
tyrosine	0.602	0.665	0.593	0.628	0.522	0.654

**Table 6. Amino Acid Profiles of Sorghum and Millet Germinated at Different Temperatures for 5 Days**

amino acid profile ( $\mu\text{mol/mL}$ )	germination temperature (20 °C)		germination temperature (25 °C)		germination temperature (30 °C)	
	rice	buckwheat	rice	buckwheat	rice	buckwheat
aspartic	0.293	0.211	0.318	0.202	0.339	0.196
glutamic	0.215	0.642	0.213	0.632	0.214	0.473
asparagine	0.040	0.030	0.037	0.025	0.034	0.028
glutamine	0.452	0.202	0.460	0.218	0.463	0.232
serine	0.225	0.280	0.231	0.300	0.214	0.305
arginine	0.310	0.315	0.347	0.383	0.334	0.342
threonine	0.132	0.205	0.131	0.230	0.130	0.231
glycine	0.362	0.323	0.369	0.330	0.352	0.315
alanine	0.828	1.106	0.845	1.266	0.815	1.181
proline	0.380	0.314	0.389	0.384	0.386	0.362
valine	0.608	0.551	0.617	0.538	0.597	0.572
methionine	0.151	0.158	0.146	0.171	0.144	0.167
isoleucine	0.380	0.356	0.371	0.370	0.391	0.360
leucine	0.809	0.838	0.803	0.864	0.802	0.860
tryptophan	0.108	0.128	0.109	0.145	0.120	0.141
phenylalanine	0.438	0.373	0.436	0.392	0.431	0.389
lysine	0.382	0.385	0.384	0.390	0.378	0.379
histidine	0.191	0.243	0.194	0.230	0.190	0.222
tyrosine	0.591	0.613	0.632	0.605	0.607	0.610

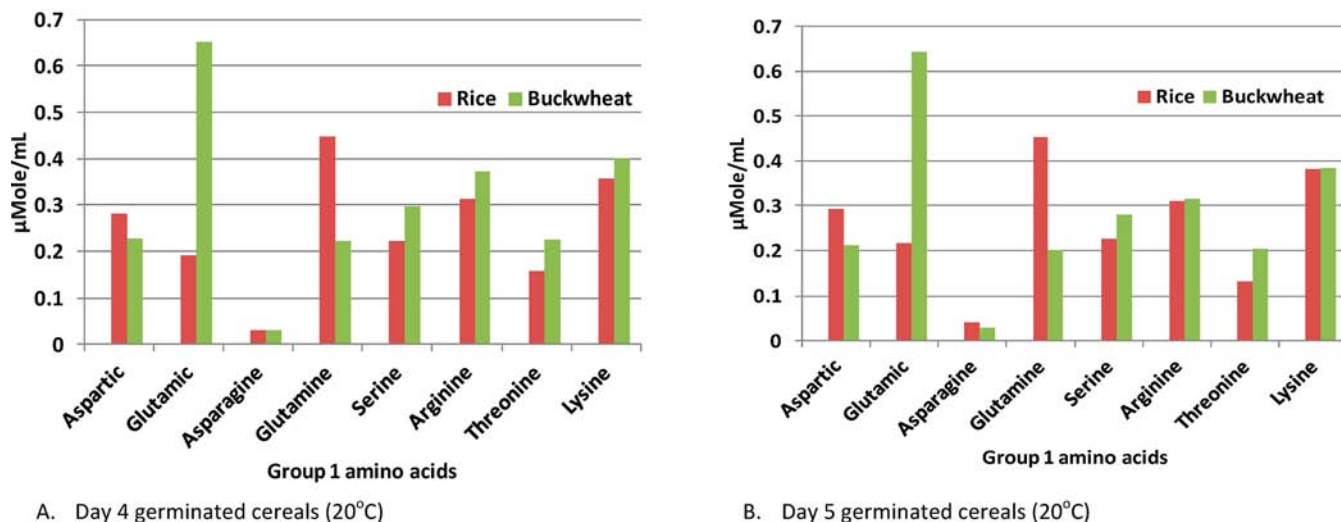
data generated by the RVA instrument. This clearly shows that rice gave peak and final viscosities that were approximately 2.5 times higher than the values obtained for the buckwheat. In general, RVA peak and final viscosities (obtained when cereal flours are run through the RVA instrument) are indicative of the viscous load to be encountered when processing cereals.<sup>18</sup>

Table 2 shows the properties of day 4 and day 5 malts of rice and buckwheat malted at 20, 25, and 30 °C. The HWEs were higher for the rice than for the buckwheat, but no differences were observed in extract levels between day 4 and day 5 for either grain type. The results suggest that, under the malting conditions used in this study, the germination temperature will significantly affect HWE production from both cereals, with the highest levels being produced at the lowest germination temperature (20 °C).

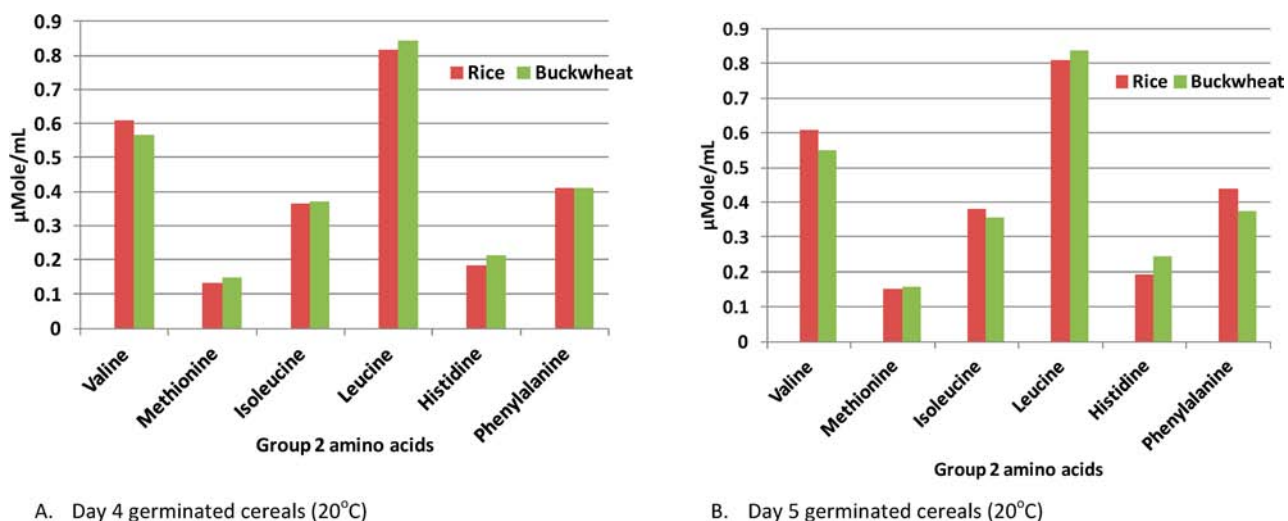
In contrast to the observation for extract yield, higher amounts of TSN were produced at higher germination temperatures (Table 2). Again, both rice and buckwheat malts produced lower levels of soluble nitrogen on day 4 than on day 5, with overall levels being higher in buckwheat than in rice.

Both rice and buckwheat released sufficient free amino nitrogen (FAN) products to support yeast fermentation after 4 days of germination,<sup>22,23</sup> with levels increasing further on day 5 (Table 2). Buckwheat released higher FAN products than rice, except at the highest temperature (30 °C) after 4 days, when rice released higher amounts.

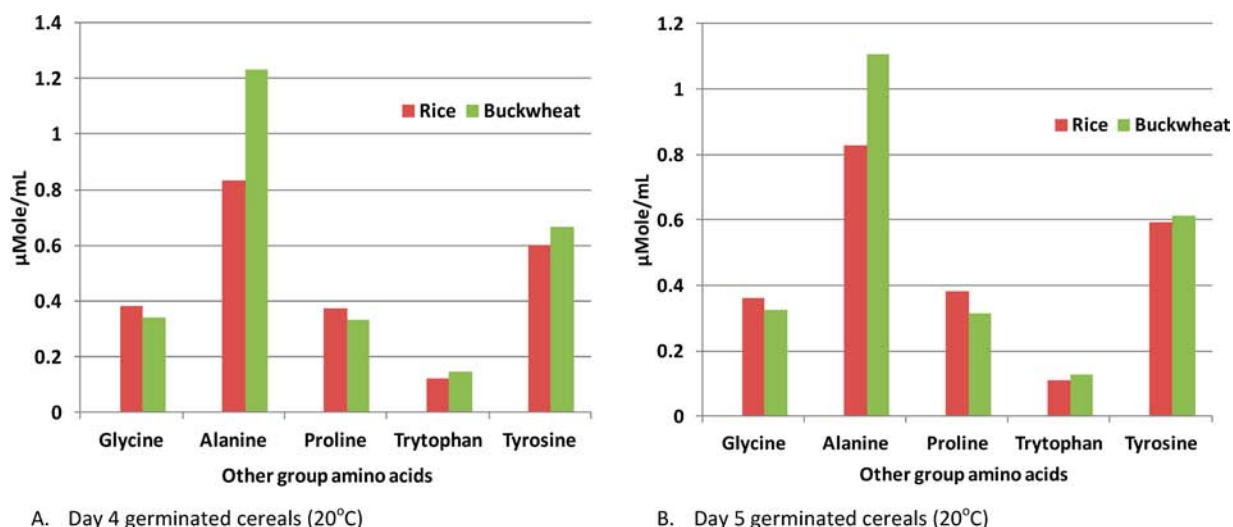
Table 3 shows the sugar profile found in the HWE from mashed malts of rice and buckwheat (day 4 malt). Both malts produced a wide range of sugar spectra. It is interesting to observe that no sucrose was produced, whereas sorghum and millet malts produced under the same conditions in our previous study<sup>1</sup> did contain this sugar. Similar trends in sugar spectra were obtained when the germination time was extended to day 5 (Table 4). Rice, sorghum, and millet all produced more maltose than glucose. The reverse was the case for buckwheat, as the amount of glucose was 3–5 times that of maltose. These results highlight differences in the sugar profiles of these gluten-free cereals, which are important because they will impact the ability of yeast to metabolize these sugars during fermentation. The exact implications of this are not clear at present. However,



**Figure 4.** Pattern of the release of group 1 amino acids during germination of rice and buckwheat at 20 °C: (A) release of amino acids after 4 days of germination and (B) release of amino acids after 5 days of germination.



**Figure 5.** Pattern of the release of group 2 amino acids during germination of rice and buckwheat at 20 °C: (A) release of amino acids after 4 days of germination and (B) release of amino acids after 5 days of germination.



**Figure 6.** Pattern of the release of other group amino acids during germination of rice and buckwheat at 20 °C: (A) release of amino acids after 4 days of germination and (B) release of amino acids after 5 days of germination.

it has been reported that in a fermentation medium where glucose is produced in much excess to maltose, some yeast strains may lose their ability to ferment maltose.<sup>26,27</sup> Other fermentability experiments (results not shown) did not indicate processing issues during the fermentation of the mash obtained from using buckwheat as an adjunct. Hence, more studies are required in this area.

Within the individual grain types, the ratio of maltose/glucose (Tables 3 and 4) was consistent across the different malting temperatures. This observation is, again, important because these results show that variations in the malting temperature will not alter the balance of sugar substrates in the derived wort.<sup>24</sup>

With regard to  $\alpha$ -amylase development during the malting of rice and buckwheat (Figure 3), it is again worth noting that both samples followed similar trends to sorghum.<sup>25</sup> Interestingly, buckwheat developed higher levels of  $\alpha$ -amylase enzymes than rice when they were malted, which may explain why buckwheat produced a much higher amount of glucose in its wort.<sup>28,29</sup> Higher levels of  $\alpha$ -amylase were generated at the lower germination temperature of 20 °C for both rice and buckwheat, as observed in our earlier study on sorghum and millet. These results are contrary to earlier publications, where it has been reported that higher temperatures favored the development of  $\alpha$ -amylase in tropical sorghum.<sup>20,30–36</sup> Notwithstanding, results of the present study further confirm that these cereals could be readily used to produce beer.

Amino acid production when rice and buckwheat were malted is shown in Tables 5 and 6. Both rice and buckwheat malts produced a similar range of the essential amino acids required during fermentation. Production of a broad spectrum of amino acids has been linked with sorghum producing nutritious wort.<sup>38</sup> By extension, the worts of rice and buckwheat are also likely to produce nutritious wort, with excellent brewing performance in terms of the parameters measured. In general, buckwheat produced higher amounts of some amino acids, such as glutamine, alanine, and methionine than rice, which confirms the results of other studies.<sup>13</sup> Methionine, an important amino acid required for adequate and effective yeast performance during fermentation, was produced in both rice and buckwheat malts at significant levels, regardless of the malting temperature.<sup>1,37</sup> In general, while rice malt produced higher amounts of amino acids on day 5 germination, buckwheat produced

**Table 7.** ANOVA Examining Differences in Different Amino Acid Production at 20, 25, and 30 °C for Germinated Rice and Buckwheat, Taking the Length of Germination and Temperature into Account

amino acid	rice		buckwheat	
	temperature effect	germination effect	temperature effect	germination effect
aspartic	0.6061	0.1304	0.0135 <sup>a</sup>	0.0067 <sup>a</sup>
glutamic	0.5330	0.6068	0.5654	0.2969
asparagine	0.9615	0.3001	0.1105	0.6799
glutamine	0.1250	0.4825	0.2503	0.2693
serine	0.5697	0.5762	0.8431	0.7686
arginine	0.0617 <sup>a</sup>	0.7874	0.5841	0.6062
threonine	0.3669	0.0252 <sup>a</sup>	0.3673	0.5094
glycine	0.3986	0.8549	0.0089 <sup>a</sup>	0.0028 <sup>a</sup>
alanine	0.3019	0.4589	0.9974	0.7336
proline	0.0189 <sup>a</sup>	0.0075 <sup>a</sup>	0.7248	0.3726
valine	0.2642	0.1685	0.8348	0.8007
methionine	0.4851	0.0702 <sup>a</sup>	0.0374 <sup>a</sup>	0.0247 <sup>a</sup>
isoleucine	0.3347	0.0494 <sup>a</sup>	0.0345 <sup>a</sup>	0.0109 <sup>a</sup>
leucine	0.7960	0.1051	0.4210	0.5286
tryptophan	0.2266	0.0412 <sup>a</sup>	0.6475	0.3774
phenylalanine	0.6050	0.0330 <sup>a</sup>	0.9466	0.7213
lysine	0.1412	0.0050 <sup>a</sup>	0.4946	0.9441
histidine	0.1694	0.0820 <sup>a</sup>	0.2974	0.0425 <sup>a</sup>
tyrosine	0.4911	0.3072	0.2973	0.0444 <sup>a</sup>

<sup>a</sup>Germination time and temperature showed significant difference ( $p < 0.05$ ) for buckwheat.

higher levels on day 4, although the differences were marginal (Tables 5 and 6).

Rice and buckwheat followed similar patterns in the production of different groups of amino acids. Figure 4 shows the results of the production of group 1 amino acids for rice and buckwheat germinated at 20 °C for 4 and 5 days. Both malted cereals released very small amounts of asparagine on day 4 and day 5 germination times (Figure 4). Figure 5 shows the results of group 2 amino acids of rice and buckwheat malts germinated at 20 °C. Again, both cereals released lower amounts of methionine and histidine on day 4 and day 5 germination times (Figure 5). Figure 6 shows the results of other groups of amino acids of rice and buckwheat

germinated at 20 °C for 4 and 5 days, with both cereals releasing lower amounts of tryptophan on day 4 and day 5 germination times (Figure 6). From these results, it can be seen that the production of these amino acids followed a similar trend in both cereals and the patterns of amino acid production are similar to those found in sorghum malt wort.<sup>1</sup>

ANOVA was used to determine whether or not there were any significant differences in amino acid levels in the worts depending upon germination times and temperatures (Table 7). Results of ANOVA indicated that, while the germination temperature showed no significant difference with regard to isoleucine released into the wort from rice malt, there was a significant difference resulting from the germination time ( $p = 0.3347$  and  $0.0494$ , respectively; Table 8). This is different from buckwheat malt, where ANOVA results indicated that both germination temperature and time showed a significant difference

**Table 8. ANOVA Examining Differences in Isoleucine Production at 20, 25, and 30 °C for Germinated Rice, Taking the Length of Germination into Account**

as a result of	sum of squares	degree of freedom	mean square	F statistic	significance
main effects	0.001	3	0.000	7.579	0.1188
germination temperature	0.000	2	0.000	1.987	0.3347
day	0.001	1	0.001	18.763	0.0494
explained	0.001	3	0.000	7.579	0.1188
error	0.000	2	0.000		
total	0.001	5	0.000		

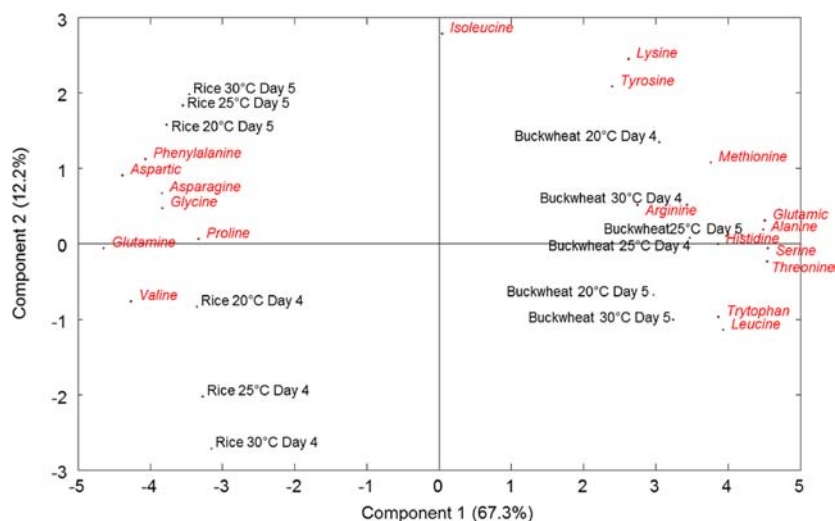
**Table 9. ANOVA Examining Differences in Isoleucine Production at 20, 25, and 30 °C for Germinated Buckwheat, Taking the Length of Germination into Account**

as a result of	sum of squares	degree of freedom	mean square	F statistic	significance
main effects	0.000	3	0.000	48.750	0.0202
germination temperature	0.000	2	0.000	28.000	0.0345
day	0.000	1	0.000	90.250	0.0109
explained	0.000	3	0.000	48.750	0.0202
error	0.000	2	0.000		
total	0.000	5	0.000		

for isoleucine ( $p = 0.0345$  and  $0.0109$ , respectively; Table 9). These ANOVA results (Tables 7–9) are important observations highlighting how variations in the germination temperature and time will affect the production of these amino acids in malted rice and buckwheat even when they were malted under similar conditions.

Principal component analysis (PCA), a multivariate statistical analysis, was carried out to summarize the main compositional differences, in terms of amino acid content, between the various samples (Figure 7). The largest difference, shown by the horizontal separation along component 1, was in terms of grain type, irrespective of the germination time or temperature. Therefore, it can be concluded that grain type has the greatest influence on the amino acid composition. The rice samples contained relatively high levels of phenylalanine, aspartic acid, asparagine, glycine, proline, glutamine, and valine, while the buckwheat samples contained relatively high levels of lysine, tyrosine, methionine, arginine, glutamic acid, alanine, histidine, serine, threonine, tryptophan, and leucine. Isoleucine was located in the middle of component 1, being present at similar levels in rice and buckwheat. Component 2 showed a separation of the rice samples depending upon the germination time. Isoleucine was found to be an important contributor to this separation, being found at relatively high levels in day 5 compared to day 4. These results suggest that the germination time has more influence on amino acid composition in rice than in buckwheat.

Overall, this study showed that both rice and buckwheat followed a similar pattern when they were malted under equivalent conditions. Both cereal types produced a 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 the germination temperature will be minimal. This is important because the malting temperature can be difficult to control in industrial practice.<sup>2</sup> Furthermore, both cereals showed additional good quality with regard to consistency, as reported recently for sorghum and millet,<sup>1</sup> with regard to malting and mashing. Although both cereals showed similarity in terms of malting response, subtle differences were found with regard to the effects of the germination temperature and time in the production of some amino acids. While sorghum has gained wide application in the brewing industry, rice and buckwheat have not. More studies would be required to find out how the subtle differences observed



**Figure 7.** PCA of day 4 and day 5 germinated rice and buckwheat malts.

in the release of amino acids as well as excessive release of glucose in buckwheat caused by the effect of the germination temperature will affect yeast fermentation. In the future, rice and buckwheat may become significant raw materials in the brewing industry and could increase the choice of grain types suitable for the production of gluten-free beer.

## AUTHOR INFORMATION

### Corresponding Author

\*E-mail: reginald.agu@swri.co.uk.

### Notes

The authors declare no competing financial interest.

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