beverages

MDPI

Supplementary Material

A Modified Brewing Procedure Informed by the Enzymatic Profiles of Gluten-Free Malts Significantly Improves Fermentable Sugar Generation in Gluten-Free Brewing

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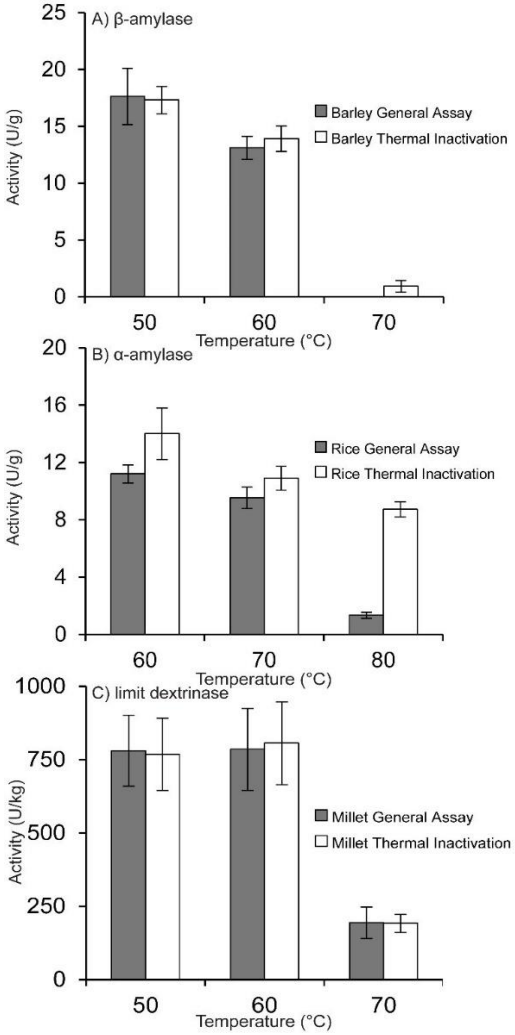

Figure S1. Activity of barley β-amylase, rice α-amylase, and millet limit dextrinase as measured using the general assay procedure and using the thermal inactivation method described in *SM1.1*. Data represent mean (n = 3) ± standard deviations.

Table S1. Enzyme analysis of the pooled teff samples used in the mashing experiments compared to the teff samples used for the enzyme profiling. Activity was measured at their pH optimum and at 40 °C using the general assay procedure. The major differences in the malt used for the mashing samples was that they were lower in β-amylase. While this demonstrates there were differences in the malting trials, the pooled ivory and brown teff samples will lower β-amylase activity were still successful in generating fermentable sugars when used with the ExGM procedure. Data represent mean (n=3) ± standard deviations.

Enzyme	Ivory Teff Enzyme Profile	Pooled Ivory Teff	Brown Teff Enzyme Profile	Pooled Brown Teff
β-amylase activity (U/g)	6.14 ± 0.63	2.42 ± 0.19	3.86 ± 0.48	1.83 ± 0.05
α-amylase activity (U/g)	29.57 ± 2.85	26.91 ± 0.57	27.34 ± 2.50	29.52 ± 0.68
limit dextrinase activity (U/kg)	147.31 ± 3.97	138.19 ± 1.43	302.62 ± 4.16	358.06 ± 7.09

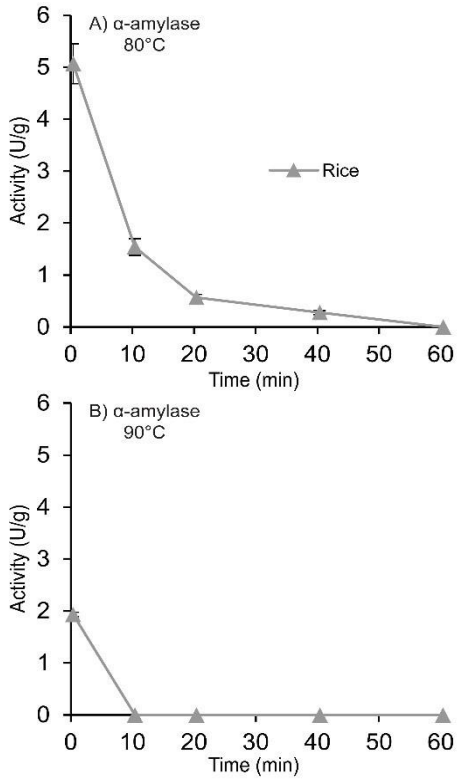

Figure S2. Rice α-amylase thermostability at 80 °C and 90 °C. Activity is expressed as the total remaining activity after incubation at each selected time point. Data represent mean (n = 3) ± standard deviations, some error bars are contained within data points.

Table S2. Measured SG and final wort pH of collected mash samples. Data represent mean (n = 2) ± standard deviations.

Specific Gravity @ 20 °C									
Treatment	**Barley**	**Ivory Teff**	**Brown Teff**	**Sorghum**	**Millet**	**Rice**	**Corn**	**Buckwheat**	**GFB1**
IE	-	1.045 ± 0.000	1.044 ± 0.001	1.043 ± 0.001	1.042 ± 0.001	1.037 ± 0.001	1.044 ± 0.001	1.033 ± 0.001	-
IM	1.046 ± 0.001	1.043 ± 0.002	1.042 ± 0.000	1.039 ± 0.001	1.037 ± 0.001	1.038 ± 0.001	1.043 ± 0.002	1.036 ± 0.001	-
pH-IM	-	1.046 ± 0.000	1.045 ± 0.002	1.040 ± 0.002	1.037 ± 0.002	1.038 ± 0.001	1.041 ± 0.001	1.032 ± 0.001	-
XT	-	1.045 ± 0.001	1.044 ± 0.004	1.044 ± 0.002	1.040 ± 0.001	1.038 ± 0.001	1.041 ± 0.001	1.017 ± 0.003	-
ExGM	-	1.052 ± 0.001	1.049 ± 0.001	1.048 ± 0.001	1.041 ± 0.000	1.038 ± 0.000	1.045 ± 0.001	1.043 ± 0.001	1.043 ± 0.001
Final pH									
IE	-	5.83 ± 0.01	5.68 ± 0.01	5.64 ± 0.01	5.44 ± 0.02	5.90 ± 0.03	5.24 ± 0.00	6.21 ± 0.04	-
IM	5.28 ± 0.01	5.90 ± 0.01	5.81 ± 0.01	5.57 ± 0.01	5.48 ± 0.04	5.87 ± 0.01	5.24 ± 0.01	6.15 ± 0.03	-
pH-IM	-	5.25 ± 0.00	5.28 ± 0.00	5.14 ± 0.01	5.19 ± 0.04	5.20 ± 0.01	5.18 ± 0.01	5.17 ± 0.09	-
XT	-	5.31 ± 0.00	5.26 ± 0.23	5.03 ± 0.04	5.31 ± 0.01	5.26 ± 0.01	5.17 ± 0.02	5.26 ± 0.04	-
ExGM	-	5.32 ± 0.01	5.37 ± 0.01	5.01 ± 0.00	5.21 ± 0.01	5.16 ± 0.00	5.13 ± 0.01	5.01 ± 0.01	5.12 ± 0.01

Table S3. Detailed step-by-step guide describing the processing of the five mashing treatments. Combined cells demonstrate processes that were held constant between the different treatments.

	Treatments				
	Inactivated Enzymes (**IE**)	Infusion Mash (**IM**)	pH-adjusted Infusion Mash (**pH-IM**)	Extended Time Infusion Mash (**XT**)	ExGM Decoction **(ExGM)**
Liquor-to-Grist Ratio (3:1)	15 mL MH_2O **:** 5 g Malt Flour				
Total Volume	30 mL				
	Mashing Schedule				
Strike Temperature (°C)	80	80	80	70	70
Initial Mashing Temp (°C)	65	65	65	55	55
pH adjustment	-	-	pH adjust to ~5.3 with lactic acid		
Stage 1	Bring to 99 °C for 1.5 min	1 h 65 °C	1 h 65 °C	2 h 45 min 55 °C	(enzyme extraction) 30 min 55 °C
Stage 2	Cool to 65 °C	-	-	-	a) Centrifuge then decant extract & hold at 55 °C b) Add 5 mL MH_2O to grain, gelatinize at 90 °C for 1.5 min c) Cool to 55 °C then recombine Enzyme Extract and grain
Stage 3	1 h 65 °C	-	-	-	2 h 55 °C
End Mash	Bring mash to 80 °C, centrifuge, and decant 1st wort into sterile 50 mL conical tube				
Sparge Sparge water and wort fractions held >70 °C	a) Add 10 mL MH_2O to grain, hold >70 °C for 10 min b) Centrifuge then combine 1st and 2nd worts c) Repeat: add 5 mL MH_2O to grain, hold 5 min d) Centrifuge then combine all 3 wort Fractions				a) Add 5 mL MH_2O to grain, hold >70 °C for 10 min b) Centrifuge then combine 1st and 2nd worts c) Repeat: Add 5 mL MH_2O to grain, hold 5 min d) Centrifuge then combine all 3 wort Fractions
Boil	a) Bring collected wort to 100 °C for 1.5 min b) Cool to room temperature in ice bath				