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ROLE OF ALPHA-GLUCOSIDASE IN THE FERMENTABLE SUGAR COMPOSITION OF SORGHUM MALT MASHES*

BY J. R. N. TAYLOR

University of Pretoria, Cereal Foods Research Unit, Department of Food Science, Pretoria 0002, South Africa

AND J. DEWAR

CSIR Food Science and Technology, P.O. Box 395, Pretoria 0001, South Africa

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The cause of the high glucose to maltose ratio in sorghum malt worts was studied. Mashing temperature and pH strongly affected both the amount of glucose and the proportion of glucose relative to total fermentable sugars. The relative proportion of glucose was higher when mashing was performed. at pH 4.0, close to the pH optimum for sorghum alpha-glucosidase, than at the natural pH of the mash (pH 5.0–5.5). Mashing according to the EBC procedure using an enzymic malt extract with pre-cooked malt insoluble solids producing a wort containing maltose and glucose in an approximately 4:1 ratio, whereas mashing with a malt extract without pre-cooking the malt insoluble solids resulted in a wort with approximately equal amounts of maltose and glucose. Both treatments gave the same quantity of total fermentable sugars and amount of wort extract. Sorghum alpha-glucosidase was confirmed to be highly insoluble in water. All or virtually all activity was associated with the insoluble solids. Hence, it appears that the high amount of glucose formed when sorghum malt is mashed conventionally is due to alpha-glucosidase activity. Pre-cooking the malt insoluble solids inactivates the alpha-glucosidase, preventing the hydrolysis of maltose to glucose.

Key Words: *Sorghum malt, mashing, malt extract, alpha-glucosidase*

INTRODUCTION

Malted sorghum (*Sorghum bicolor* (L.) Moench) is used to brew traditional African sorghum (opaque) beer. Sorghum, unlike barely, can be cultivated in the semi-arid and tropical regions. Consequently, countries in these regions have in recent years been interested in brewing European-type lager beer using sorghum malt[10,11,14].

A number of researchers have observed that when mashing is carried out using sorghum malt, the resulting wort contains different proportions of the various fermentable sugars when compared with barley malt wort[4,6,13,16]. Sorghum malt worts have been found to contain similar levels of glucose and maltose[4,16], whereas barley malt worts contain several times more maltose than glucose[3]. Palmer[13] attributed the difference to the low beta-amylase activity in sorghum malt relative to barley malt, whereas Byrne and co-workers[4] suggested that the high level of glucose in sorghum malt worts was due to alpha-glucosidase activity. Beta-amylase (E.C. 3.2.1.2.) catalyses the hydrolysis of the penultimate alpha-(1->4) glucosidic bond at the non-reducing end of polysaccharides to release maltose. Alpha-glucosidase (maltase) (E.C. 3.2.1.20.) catalyses the hydrolysis of terminal, non-reducing alpha-(1->4) linked glucose residues with the release of glucose, oligosaccharides being hydrolysed rapidly, whereas polysaccharides are hydrolysed relatively slowly, or not at all.

The objective of this study was to determine the cause of the different fermentable sugar composition in sorghum malt mashing compared with barley malt mashing.

EXPERIMENTAL METHODS

Sorghum malts

Seven different sorghum malts were used in this study. The effect of mashing conditions was investigated using five malts, three prepared in the laboratory and two from industrial sorghum maltsters. The diastatic power of the malts ranged from 19.0 to 56.6 sorghum diastatic units (SDU)/g. To investigate mashing with malt extracts, two malts were used: a laboratory-prepared malt of cultivar Barnard Red, diastatic power 29.9 SDU/g and an industrial malt (cultivar unknown), diastatic power 23.5 SDU/g. Laboratory malting was carried out as described[5].

Mashing

Mashing was performed in a BRF laboratory mashing bath (Crisp Malting Ltd, Great Ryburgh, UK). To investigate the effect of mashing conditions, malt was mashed in distilled water at a concentration of 14% (w/w) dry solids basis. To help conserve alpha-amylase activity[18], calcium chloride was added to the distilled water before mashing to give a calcium ion concentration of 0.02% (w/w) in the mash. Constant temperature infusion mashing was performed for 2 h at 60, 65, 70, 75 and 80°C at the natural pH of the mash (pH 5.0–5.5) and at 60 and 70°C at pH 4.0. The pH of the mash was adjusted by the addition of 1 M HCl. Wort separation was by centrifugation at 10 000 × g for 10 min at 4°C.

Mashing was also performed using enzymic malt extracts. Milled malt (25 g) was weighed into 250 ml centrifuge bottles and distilled water (100 ml) was added. No calcium chloride was added. The malt was extracted for 30 min with continuous stirring at 4°C. A clear supernatant (enzymic malt extract) was obtained by centrifugation at 10 000 × g for 10 min at 4°C. The residue (insoluble solids) was transferred to a stainless steel mashing beaker and re-suspended in 50 ml distilled water. It was then pressure cooked at 100 kPa for 10 min. During this time the supernatant was stored at 4°C. A control where both the supernatant and the residue were stored at 4°C, then the residue re-suspended in 50 ml distilled water was also prepared. The beakers containing the re-suspended residues were placed in the mashing bath and attemperated to 45°C. The supernatants were then added and mashing was carried out according to the European Brewery Convention (EBC) Congress procedure[7]. This comprises a

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temperature programme of 45°C for 30 min, raised to 70°C over 30 min, then held at 70°C for 60 min. After mashing, the contents of the beakers were made up to 225 g with distilled water. Wort separation was then performed by centrifugation as described above.

Analyses

Diastatic power was determined by the standard method for sorghum malt[15], except that distilled water and not a peptone solution was used as the enzyme extractant.

Fermentable sugars (maltotriose, maltose and glucose) were determined by first specifically hydrolysing both maltotriose and maltose to glucose using alpha-glucosidase[16], then measuring glucose by the glucose oxidase method[8]. Free glucose was determined separately by omitting the alpha-glucosidase hydrolysis step.

Maltotriose, maltose and glucose were also determined individually by HPLC using a Bio-Rad HPX-42A column, as described[9].

Wort extract (soluble solids) was determined using a pycnometer[7] and calculated using the Plato table.

Alpha-glucosidase activity was assayed as described[19]. Enzymic malt extract, malt insoluble solids (prepared as above for mashing) and whole milled malt were incubated with maltose for 90 min at 30°C, pH 3.75 with continuous stirring. Appropriate controls without the maltose substrate were included. The reaction was terminated by adjusting the pH of the incubation mixture to pH 7.0. Glucose was determined by the glucose oxidase method[8].

Results and Discussion

Table I shows that both the temperature and pH of mashing affected the amount of total fermentable sugars (maltotriose, maltose and glucose) and free glucose produced during mashing. When mashing was carried out at the natural pH of the mash (pH 5.0–5.5) both total fermentable sugars and free glucose increased with mashing temperature to a maximum at 70°C, then declined at higher temperatures. The proportion of glucose, however, declined with increasing mashing temperature, from 58.6% at 60°C to 23.1% at 80°C. In contrast, when mashing was conducted at pH 4.0 the amounts of total fermentable sugars and of free glucose produced were less at 70°C than at 60°C. However, as when mashing at the natural pH, the proportion of glucose relative to total fermentable sugars decreased at higher mashing temperature. The fact that both mashing temperature and pH affected both the total amount of free glucose and the proportion of glucose relative to total fermentable sugars indicated strongly that the differences in sugar composition between sorghum and barley malt worts are as a result of enzyme action.

It is of interest that the proportion of glucose relative to total fermentable sugars was higher when mashing was performed at pH 4.0 than at pH 5.0–5.5, 80.3% as against 58.6% at 60°C. This suggests that it is the enzyme alpha-glucosidase in sorghum which is responsible for the difference in the sugar composition of worts produced from sorghum and barley malts. Sorghum alpha-glucosidase has a rather acidic pH optimum (pH 3.75)[19]. Hence, it is likely that when mashing at pH 4.0 a higher proportion of maltose would have been hydrolysed into glucose than at the natural mash pH of pH 5.0–5.5. Less total fermentable sugars were produced at pH 4.0 than at pH 5.0–5.5 because the pH optima for sorghum alpha- and beta-amylase are pH 4.72[1] and pH 5.2–5.5[2], respectively.

It has been suggested that an effective way of mashing with sorghum malt is to prepare an aqueous malt extract[4,11,13]. This can be done by mashing at 45 or 50°C then removing some of the supernatant liqour. The starch-rich mash residue is cooked and then cooled. It is then recombined with the extract and mashing performed at a constant temperature of 60–65°C or using a rising temperature programme up to 75°C. Palmer[13] claimed that as a result of gelatinising the starch, worts with extract (soluble solids) levels comparable to those from barley malt could be obtained. Byrne and co-workers[4] showed that by mashing with a malt extract equivalent to approximately 20% of mash volume there was an increase in maltotriose and maltose and a reduction in glucose, compared with a conventional mashing procedure. Furthermore, the quantity of fermentable sugars obtained was almost double that of a conventional mashing procedure.

In this study an enzymic malt extract was prepared at a temperature of 4°C to limit enzyme action during extraction and to conserve enzyme activity. Centrifugation was performed at 10 000 × g which enabled a quantity of extract equivalent to approximately 50% of mash volume to be produced, as opposed to the 20% obtained by Byrne and co-workers[4]. Table II shows that with both a laboratory and an industrially produced malt, mashing with an enzyme extract in combination with pre-cooking the insoluble solids did not result in more extract or fermentable sugars than mashing with a malt extract but without pre-cooking the insoluble solids. This finding appears to contradict the previously published work[4,13]. However, the different findings can be accounted for by the different mashing conditions employed. To obtain high levels of soluble solids when mashing with sorghum malt it is necessary to gelatinise and enzymatically

TABLE I. Effect of mashing conditions on the fermentable sugar content of worts prepared from sorghum malts[a]

Mashing conditions	Fermentable sugars (%)	Glucose (%)	
60°C, Natural pH	5.05 ± 1.37[b]	2.96 ± 0.92	(58.6)[c]
65°C, Natural pH	6.56 ± 1.42	3.27 ± 1.02	(49.8)
70°C, Natural pH	7.41 ± 0.71	3.50 ± 1.79	(47.2)
75°C, Natural pH	5.44 ± 1.26	1.40 ± 0.53	(25.7)
80°C, Natural pH	2.81 ± 0.60	0.65 ± 0.30	(23.1)
60°C, pH 4.0	2.84 ± 1.22	2.28 ± 0.78	(80.3)
70°C, pH 4.0	1.18 ± 0.55	0.60 ± 0.27	(50.8)

[a]Values are means from five different malts
[b]Standard deviation
[c]Glucose as a percentage of fermentable sugars

TABLE II. Effect of cooking the insoluble malt solids on extract and wort fermentable sugar composition when mashing with enzymic extracts from sorghum malt[a]

Sample	Extract (%)	Maltotriose (%)	Maltose (%)	Glucose (%)	Total (%)
Barnard Red uncooked insol. solids	86.8 ± 0.8	2.04 ± 0.21[b] (23.2)[c]	3.33 ± 0.45 (38.0)	3.39 ± 0.52 (38.7)	8.76
Barnard Red pre-cooked insol. solids	86.5 ± 0.3	2.25 ± 0.08 (27.9)	4.43 ± 0.27 (55.0)	1.38 ± 0.11 (17.1)	8.06
Industrial malt uncooked insol. solids	81.5 ± 0.5	1.75 ± 0.47 (30.2)	2.16 ± 0.56 (37.2)	1.89 ± 0.54 (32.6)	5.80
Industrial malt pre-cooked insol. solids	80.8 ± 3.3	1.75 ± 0.21 (26.7)	4.00 ± 0.06 (61.0)	0.80 ± 0.01 (12.2)	6.55

[a]Values are the means from two replicate experiments
[b]Standard deviation
[c]Relative percentage

hydrolyse the sorghum malt starch[16]. The gelatinisation temperature of sorghum malt starch is in the range 64–68°C[17]. It appears that Palmer[13], in his conventional mashing system, employed a constant mashing temperature of 65°C. This temperature probably did not facilitate complete starch gelatinisation as it has been shown at a constant mashing temperature much higher levels of soluble solids are obtained at 70°C compared with 65°C[16]. The conventional mashing system used by Byrne and co-workers[4] was a rising temperature programme with holding points at 50, 65, 72 and 78°C. The periods at 72 and 78°C would facilitate starch gelatinisation. However, 72°C was held for only 10 minutes which may have been insufficient to enzymically hydrolyse the gelatinised starch before the amylase enzymes would have been inactivated as the temperature increased. The EBC Congress mash procedure used in this study involves holding the mash at 70°C for 60 min, which judging by the high level of extract obtained appears to permit simultaneous gelatinisation and hydrolysis of the starch.

Whilst there was no difference in extract and total fermentable sugars between pre-cooking and not pre-cooking the malt insoluble solids, there was a great difference in the fermentable sugar composition of the two treatments (Table II). As with previous studies of mashing sorghum malt[4,16], not pre-cooking the insoluble solids produced worts with a high proportion of glucose. The ratio of maltotriose to maltose to glucose was approximately 1:1.4:1.4. Similar results were obtained with both the malts examined despite their difference in diastatic power. In contrast, pre-cooking the insoluble malt solids resulted in worts with a much lower proportion of glucose and a higher proportion of maltose. The ratio of maltotriose to maltose to glucose was approximately 1:2.1:0.5, the relative amounts of maltose and glucose being similar to the 4:1 ratio in barley malt worts[3]. Again, similar results were obtained for both malts. These results show clearly that a change in the relative amounts of maltose and glucose was brought about by the action of cooking the insoluble solids. That similar amounts of extract were obtained with both treatments discounts the possibility that the change in fermentable sugar ratio was due to the pre-cooking process gelatinising the starch. The concept that the low amount of beta-amylase in sorghum malt is responsible for the low levels of maltose in sorghum malt worts[13] can also be discounted. The pre-cooking treatment which resulted in an increase in maltose, would in fact further reduce the amount of beta-amylase in the mash. Approximately 50% of the malt enzyme compliment would have remained in the liquid phase associated with the insoluble solids and thus would have been inactivated by the heat treatment.

The results, however, can be explained as a consequence of sorghum malt alpha-glucosidase activity. Sorghum alpha-glucosidase is highly insoluble in water[19]. Table III shows that at most only 2–3% of the total malt alpha-glucosidase activity was in the enzymic extract. In fact the amount measured in the residue was equivalent to all the activity in the malt. The slight anomaly is due presumably to the difficulty in assaying an insoluble enzyme. As all or virtually all the alpha-glucosidase was in the residue means that when the residue was cooked the enzyme would have been inactivated. Thus, unlike the treatment where the insoluble solids were not pre-cooked, hydrolysis of maltose to glucose could not take place during the mashing process. Hence, the fermentable sugar composition of the wort was as a result of the action of the alpha- and beta-amylase enzymes alone. Where the insoluble-solids were not pre-cooked, the alpha-glucosidase would have been active during mashing and thus some of the maltose was hydrolysed into glucose.

Barley malt also contains alpha-glucosidase[12]. However, it does not lead to high levels of glucose in the wort. This is probably because its activity is rather low. The alpha-glucosidase activity of barley grain is approximately 10 times less than in sorghum grain[19].

TABLE III. Alpha-glucosidase activity of sorghum malt extract, residue and whole malt (g glucose released per 100 g malt (dry basis) under the conditions of the assay)[a]

Sample	Malt extract	Residue	Whole malt
Barnard Red malt	0.15 ± 0.07[b] (3.2)[c]	5.65 ± 0.54	4.69 ± 0.28
Industrial malt	0.06 ± 0.08 (2.3)	3.23 ± 0.92	2.66 ± 0.21

[a]Values are means from two replicate experiments
[b]Standard deviation
[c]Percentage of activity in whole malt

CONCLUSIONS

Conventional mashing with sorghum malts produces worts with high levels of glucose. This is due to the action of the sorghum alpha-glucosidase enzyme which hydrolyses maltose into glucose. As the alpha-glucosidase is insoluble in water it can be separated from the other diastatic enzymes (alpha- and beta-amylase) which are water soluble. The alpha-glucosidase can then be inactivated by heat treatment. Mashing the heat-treated, insoluble malt solids with an aqueous malt extract, which contains alpha- and beta-amylase, will then produce a wort with a ratio of maltose to glucose similar to that found in barley malt wort.

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