Effect of Amyloglucosidase on Wort Composition and Fermentable Carbohydrate Depletion in Sorghum Lager Beers

David Del Pozo-Insfran[1,2], Diana Urias-Lugo[1],
Carmen Hernandez-Brenes[1] and Sergio O. Serna Saldivar[1,3]

ABSTRACT

J. Inst. Brew. 110(2), 124–132, 2004

A three-factorial experiment with a level of confidence of $P < 0.05$ was performed to study fermentable carbohydrate depletion and ethanol production during 144 h fermentations of lager beers produced with barley malt (BM), sorghum malt (SM), refined maize (MZ) or waxy sorghum (WXSOR) grits treated during mashing with or without amyloglucosidase (AMG). The percentage glucose, maltose and maltotriose, based on total fermentable carbohydrates for the BM wort was 20, 68 and 13% and for the SM wort 35, 48 and 17% respectively. Treatment with AMG increased wort glucose from 9.3 to 24.5 g/L wort and total fermentable sugar equivalents, expressed as g glucose/L, from 59.2 to 72.6 g/L wort. The SM worts had approximately 50% more glucose and 40% less initial maltose content respectively compared to the BM worts. The WXSOR grits produced worts and beers with similar properties to those produced from the MZ adjuncts. AMG addition led to a >2.5 fold increment in wort glucose and 23% in total fermentable carbohydrate content. Linear regression analysis determined that the consumption rate of fermentable carbohydrates during fermentation followed first order reaction kinetics. Depletion times to reach 50% of the initial concentrations of glucose, maltose and maltotriose were 49, 128 and 125 h, respectively, clearly indicating that the fermenting yeast preferred glucose. Maltose and maltotriose depletion times of the AMG treated worts were significantly faster and lower, respectively, when compared with the untreated worts. At the end of the fermentation, the BM beers contained higher ethanol levels (5.1% v/v) than the SM beers (3.9% v/v). For AMG treated beers, no significant differences in ethanol content were observed among samples mashed with BM and beers produced from SM and MZ grits. The results demonstrated that AMG could be used to increase the initial concentration of glucose and total fermentable carbohydrates thus decreasing dextrin levels, especially from sorghum mashes.

Key words: Amyloglucosidase, carbohydrates, sorghum.

INTRODUCTION

Worldwide the brewing industry is becoming more competitive and is constantly looking for ways to improve beer quality and reduce manufacturing costs. Barley malt and brewing adjuncts are a main factor contributing to overall production costs; thus, the utilization of alternative raw materials and innovative processes can increase profitability. According to Bajomo and Young[7], sorghum is a leading contender for replacing barley malt in beer production, particularly in tropical countries where barley cultivation is unsuccessful. The utilization of sorghum for malting and grits is thus a potential feasible option to reduce costs, as well as reducing the imports of barley and other cereal products (maize and/or rice grits).

Brewers can utilize enzymes such as proteases, glucanases and amylases to improve the production and final characteristics of beer. Amyloglucosidase is one of the most popular amylolytic enzymes utilized during mashing to assist with the hydrolysis of dextrins into glucose, consequently increasing the content of fermentable carbohydrates and reducing dextrins. Amyloglucosidase (α-1,4-D-glucan glucohydrolases) is an exoamylase which cleaves both α,1-4 and α,1-6 glycosidic bonds, acting on the external glucose residues of amylose or amylopectin, producing mainly glucose and in lesser concentrations maltose and α-limited dextrins[17,29].

Sorghum (*Sorghum bicolor* L. Moench) is a cereal with remarkable genetic variability, having an important advantage over temperate grains because it can yield crops under harsh environmental conditions. Among the different varieties of sorghum, there are those with waxy endosperm that contain more than 95% amylopectin and a relatively weak protein matrix[23,25]. These sorghums are more susceptible to hydrolysis by amylolytic and proteolytic enzymes, and also to degradation in the ruminal system of domestic animals, properties that increase their processing potential and nutritional value[22]. All these characteristics plus the tendency to gelatinize more rapidly than regular endosperm types, make waxy sorghums more attractive as potential sources of industrial brewing material[19,20]. In contrast to barley, sorghum malt has less diastatic, β-amylase and glucanase activities and requires a higher temperature for starch gelatinization[3,4,15]. Thus, when sorghum malt is used, starch may not be sufficiently hydrolyzed, causing difficulties during wort filtration and beer hazi-

---

[1] ITESM-Campus Monterrey, Department of Food Science and Technology and Center of Biotechnology, Av. Eugenio Garza Sada 2501 Sur, C.P. 64849 Monterrey, N.L. México.

[2] Current address: Department of Food Science and Human Nutrition, University of Florida. P.O. Box 110370, Gainesville, FL 32611-0370.

[3] Corresponding author. E-mail: sserna@itesm.mx

Publication no. G-2004-0520-208

ness. Osorio-Morales *et al.*[21] studied the characteristics of worts obtained from different types of refined sorghum grits, and concluded that white waxy grits were the most viable option in terms of brewing grit yield and filtration rate. Barredo *et al.*[8] determined that the fermentation kinetic profiles for worts produced from barley malt and waxy sorghum grits were comparable, in terms of pH, reducing sugar and number of yeast cells. In previous research[28] it was concluded that sorghum lagers had lower ethanol when compared with control barley beers and that amyloglucosidase addition increased wort fermentable carbohydrate levels and the beer ethanol concentration. The objectives of the present research were to study the effects of amyloglucosidase addition on wort composition and fermentable carbohydrate utilization during fermentation of lager beers produced with different malts (barley or sorghum) and brewing adjuncts (maize or waxy sorghum grits).

MATERIALS AND METHODS

Barley and sorghum malts

Commercial barley malt (BM) was obtained from Cervecería Cuauhtemoc Moctezuma (Monterrey, México). Sorghum malt (SM) was obtained from a white sorghum genotype (ICVS 400) recognized to have high diastatic activity. Sorghum was manually cleaned to remove stones, sticks, glumes, damaged and broken kernels and other foreign material. Broken kernels were removed with sieves 8 and 10. The cleaned kernels were soaked in a distilled water solution containing 0.01% (v/v) formaldehyde for 24 h in a germination cabinet (Seedburo Equipment Co., Chicago IL) set at 25°C. Grains were continuously aerated with an aquarium pump (Elite 800, Rolf C. Hagen Corp.). The soaking solution was discarded and grains bleached for 10 min with a 2% (v/v) chlorine solution. Excess chlorine was removed with a distilled water wash. The resulting kernels were placed on germination trays and germinated for 120 h with controlled relative humidity (88–92%) and temperature (26 ± 1°C). The relative humidity was maintained using a KNO_3 saturated solution. In order to obtain a homogenous germination, grains were stirred every 12 h. Malts were dehydrated in a convection oven at 45–50°C for 12 h. Before mashing, sorghum and barley malts were milled through the break rolls of a pilot plant roller mill (Chopin, Model CD 1, Villenueve, La Garenne, France) and all fractions were recovered and blended.

Maize and waxy sorghum grits

The yellow maize (MZ) grits were obtained from the corn dry milling plant of Agroinsa (Guadalupe, N.L. México). Waxy grits were obtained after decortication of a white waxy sorghum using an IDRC mill at Texas A&M University. The decorticated kernels were milled into brewing grits with a pilot plant roller mill (Chopin Model CD1, Villanueve France). Kernels were ground through the break rolls and milled fractions classified with mesh sieves 45 and 100. Particles retained by the 100 mesh and throughs of the 45 mesh sieve were considered brewing grits.

Other brewing ingredients

Pelleted hops (*Humulus lupulus*) and a strain of *Saccharomyces cerevisiae* suitable for lager fermentations were obtained from Cerveceria Cuauhtemoc-Moctezuma, S.A. de C.V (Monterrey, Mexico). Calcium chloride (125 mg/L) dissolved in distilled water was used as the calcium source to ensure amylase activity.

Amyloglucosidase

Commercial amyloglucosidase (AMG, Novo Nordisk, Denmark) extracted from *Rhizopus* sp. was used. This starch-degrading enzyme yields 0.33 mg glucose per minute at pH 4.5 and 55°C.

Treatments

A 2 × 2 × 2 trifactorial experiment was designed to examine the effect of AMG addition on fermentable carbohydrate depletion throughout the 144 h fermentation of lagers mashed with different malts (barley or sorghum) and refined brewing adjuncts (maize or sorghum grits).

Wort and beer production

Control worts were produced following the double-mashing process previously researched by Osorio Morales *et al.*[21] Worts were produced with 128 g malt, 211 g brewing adjuncts and 1.5 L water. When used, AMG was added during the second mashing step at a concentration of 0.591 g per L of wort. The AMG had 30 min at its optimum temperature of 55–60°C before the temperature was gradually increased to 96°C. Resulting worts were filtrated as recommended by Osorio Morales *et al.*[21] and adjusted to 12°P with water. The wort was hopped with commercial pelleted hops by heating to 96°C ± 1°C on a hot plate (Barnstead/Thermolyne model SP46925) with magnetic agitation for 1 h. Hops were added at a concentration 1 g/L (0.7 g added at the beginning and 0.3 g 10 min before the end of the heat treatment). Upon cooling, the hopped wort was stored at 4°C. Spent hops and other materials precipitated during refrigeration were separated by centrifugation (Beckman Avanti J-251) for 10 min at 10°C at 7,500 rpm (10,395 × *g*). The liquid phase was decanted and then vacuum filtered through No. 1 Whatman paper.

Then 900 mL of hopped wort was inoculated with *Saccharomyces cerevisiae* in a sterile laboratory bioreactor constructed from an autoclavable 1000 mL Erlenmeyer flask containing sampling ports designed by Barredo-Moguel *et al.*[8] The original yeast strain was stored in sterilized test tubes with yeast and malt agar (100 mL contained 1 g glucose, 0.5 g casein peptone, 0.3 g yeast extract, 0.3 g malt extract and 2 g agar) under refrigeration at 4°C. Yeast was propagated in yeast-malt broth and incubated at 30°C for 24 h. The inoculum was prepared by transferring propagated yeast to previously autoclaved (121°C for 20 min) 10 mL yeast and malt broth (100 mL contained 1 g glucose, 0.5 g casein peptone, 0.3 g yeast extract and 0.3 g malt extract). After 12 h incubation the medium was placed into 50 mL malt broth for 24 h. The 50 mL culture was finally placed in 300 mL wort and incubated for 24 h. Yeast cells were counted using the 40× objective of a light microscope (Zeiss bifocal) equipped

Table I. Effects of malt type, adjunct type and amyloglucosidase on initial wort pH and content of glucose, maltose, maltotriose and total fermentable carbohydrates expressed as glucose equivalents[a,b].

Treatment	pH	Initial glucose (g/L)	Initial maltose (g/L)	Initial maltotriose (g/L)	Initial sugar equivalents (g glucose/L)
BM + MZ	5.00 a	7.3 c	50.5 a	10.4 b	71.6 ab
BM + MZ + AMG	5.06 a	20.3 b	45.6 ab	8.1 d	77.0 a
BM + WXSOR	5.50 a	7.4 c	43.7 b	9.6 c	63.7 b
BM + WXSOR + AMG	5.54 a	18.9 b	45.7 ab	7.2 e	74.6 ab
SM + MZ	5.23 a	11.7 c	25.9 c	12.4 a	49.7 c
SM + MZ + AMG	5.33 a	27.2 a	28.7 c	8.6 d	69.1 ab
SM + WXSOR	5.27 bc	10.9 c	29.3 c	9.4 c	51.8 c
SM + WXSOR + AMG	5.39 ab	31.7 a	27.7 c	7.2 e	69.7 ab
Malt type					
BM	5.40 a	13.5 b	46.4 a	8.8 a	71.7 a
SM	5.20 b	20.4 a	27.9 b	9.6 a	60.1 b
Adjunct type					
MZ	5.28 a	16.6 a	37.7 a	9.9 a	66.9 a
WXSOR	5.32 a	17.2 a	36.6 a	8.6 b	64.9 a
Amyloglucosidase addition					
Without AMG	5.26 b	9.3 b	37.4 a	9.9 a	59.2 b
With AMG	5.36 a	24.5 a	36.9 a	8.6 b	72.6 a

[a] Mixed and individual effects values represent means of two and eight replicates respectively. BM= barley malt; SM = sorghum malt; MZ = maize grits; WXSOR = waxy sorghum grits; AMG = amyloglucosidase.

[b] Mean comparisons with different letter(s) within columns are statistically different (LSD test, $P < 0.05$).

with a Neubauer (Levy de C.A. Hausser & Son) counting chamber. Experimental worts were inoculated with 1.5×10^7 yeast cells/mL. At the beginning of fermentation worts were equilibrated to 11°C and every 24 h the temperature increased 1°C to 16°C. After fermentation, yeast was separated from beer by centrifugation (5000 × *g* for 10 min at 10°C). Absence of yeast cells in the supernatant was corroborated using the microscopic procedure described by Ernandes *et al.*[12] Samples for subsequent analysis were immediately stored in a –20°C freezer (Fisher Scientific Isotemp, Conway, AR).

Chemical analysis

The diastatic activity of the malts was determined according to Method 22-16 of the American Association of Cereal Chemists[5]. The pH measurements were performed using a potentiometer (Beckman Model PHI φ™50, Palo Alto, CA) in CO_2 free samples equilibrated at 25°C. Ethanol was quantified via gas chromatography–flame ionization detector according to AOAC method 984.14[6]. The gas chromatograph (Hewlett Packard 6890, Wilmington, DE) was equipped with a 30 m long, 530 µm internal diameter capillary column with cross-linked polyethylene glycol (HP-INNOWax, Hewlett Packard, Wilmington, DE). Ethanol (99.5% pure, Sigma Aldrich, Inc, St Louis, MO) mixed at different concentrations with hexane was used to build the standard curve. The spectrophotometric AOAC method No. 976.08 was used to determine color in degassed beer samples[6], where color (Lovibond) was directly related to absorbance at 430 nm. The concentrations of glucose (RT = 6.6 min), maltose (RT = 11.5 min) and maltotriose (RT = 19.1 min) in fermented worts were quantified using external standards (Supelco, Bellafonte, PA) by HPLC using a Hewlett Packard 1100 system equipped with a refractive index (RI) detector. The total fermentable carbohydrate content was determined by adding the glucose, maltose and maltotriose content of each sample. Carbohydrate separation was performed at ambient temperature in a Zorbax-NH2 column (250 × 4.6 mm) (Supelco, Bellafonte, PA), using acetonitrile–water (75:25) as the mobile phase at a flow rate of 1.5 mL/min. Prior to HPLC analysis, samples were purified using pre-conditioned Diaion WA-30 weak ionic exchange columns (Supelco, Bellafonte, PA) in order to remove organic acids and other interfering organic compounds as described by Howard *et al.*[16] The first mL eluting from the column was discarded, the remainder volume collected and diluted to adjust for each carbohydrate concentration, filtered through a 0.45-µm PTFE Millipore filter, and analyzed by HPLC-RI.

Statistical analysis

Data represents the mean of two replications analyzed as a multi-factorial experimental design comparing two types of malt (BM or SM), two types of grits (MZ or WXSOR), in the presence or absence of AMG, and evaluated at six sampling points during wort fermentation. Multiple linear regression, analysis of variance, and Pearson correlations were conducted using JMP software Version 5[24], and mean separation performed by the LSD test ($P < 0.05$).

RESULTS AND DISCUSSION

Diastatic activity of barley and sorghum malts

BM (118.8°L or Lintner, or 475 maltose equivalents) had a diastatic activity six times higher than SM (19.8°L, or 79 maltose equivalents). Other authors have also reported similar differences[9,11,13,17]. Etokakpan and Palmer[13,14] observed that BM (75°L) had 5 times more diastatic power and its endosperm cell walls were degraded more extensively during malting than SM (15°L). Taylor[27] and Daiber and Taylor[11] concluded that BM has a 6°C lower gelatinization temperature, about 3 and 4 times more diastatic and β-amylase activities than SM. However, Beta *et*

Table II. Effects of malt type, adjunct type and amyloglucosidase on beer ethanol, pH and residual content of glucose, maltose, maltotriose and total fermentable carbohydrates expressed as glucose equivalents[a,b].

Treatment	pH	Final glucose (g/L)	Final maltose (g/L)	Final maltotriose (g/L)	Final sugar equivalents (g glucose/L)	Carbohydrate utilization (%)	Ethanol (% v/v)
BM + MZ	4.10 a	1.22 a	21.89 ab	6.59 a	31.32 a	55.9 a	5.14 a
BM + MZ + AMG	4.17 a	2.64 a	25.36 a	2.69 cd	32.22 a	57.8 a	5.10 a
BM + WXSOR	4.22 a	0.84 a	13.25 bc	3.74 bcd	18.80 a	70.4 a	5.07 a
BM + WXSOR + AMG	4.27 a	1.54 a	17.29 abc	1.72 d	21.57 a	71.1 a	5.14 a
SM + MZ	4.08 a	0.91 a	11.74 c	2.89 cd	16.36 a	67.0 a	3.76 b
SM + MZ + AMG	4.00 a	6.02 a	14.73 bc	4.26 bc	26.09 a	62.4 a	4.44 ab
SM + WXSOR	4.20 a	1.31 a	14.60 bc	5.75 ab	22.83 a	56.1 a	3.60 b
SM + WXSOR + AMG	4.19 a	6.05 a	11.02 c	3.33 cd	21.22 a	70.5 a	3.98 b
Malt type							
BM	4.19 a	1.56 a	19.45 a	3.69 a	25.98 a	66.3 a	5.12 a
SM	4.12 a	3.57 a	13.02 b	4.05 a	21.62 a	60.9 a	3.95 b
Adjunct type							
MZ	4.08 a	2.70 a	18.43 a	4.11 a	26.50 a	60.8 a	4.61 a
WXSOR	4.21 a	2.43 a	14.04 a	3.63 a	21.11 a	67.0 a	4.45 a
Amyloglucosidase addition							
Without AMG	4.16 a	1.07 b	15.37 a	4.74 a	22.33 a	62.3 a	4.40 a
With AMG	4.15 a	4.06 a	17.10 a	3.00 b	25.28 a	65.5 a	4.67 a

[a] Mixed and individual effects values represent means of two and eight replicates respectively. BM = barley malt; SM = sorghum malt; MZ = maize grits; WXSOR = waxy sorghum grits; AMG = amyloglucosidase.

[b] Mean comparisons with different letter(s) within columns are statistically different (LSD test, $P < 0.05$).

al.[9], after studying 16 different sorghums, determined that two genotypes had amylase levels similar to that of commercial BM. The former study also showed that the β-amylase activity of SM was low (11–41 U/g) when compared with BM (52 U/g). Aisien *et al.*[4] indicated that one important difference between sorghum and barley malts is that alpha amylase in sorghum is synthesized in the embryo whereas in barley it is in both the aleurone and scutellar tissue. Furthermore, addition of different sorts of gibberellins was totally ineffective in increasing alpha amylase in SM.

Wort changes and properties throughout fermentation

pH. Malt type and AMG addition had a significant effect on initial wort pH values. BM and AMG treated worts had 0.1–0.2 higher pH's than their corresponding counterparts (Table I). However, non-significant differences were observed after fermentation (Table II). The initial wort pH of 5.4–5.8 was approximately 1 unit higher than the optimum for AMG activity. As expected, a pronounced decrease in pH was observed during the first 48 h fermentation caused mainly by the efflux of H^+ ions as a by-product of the transport system and by organic acid production; however only gradual decreases in pH were observed subsequently (Fig. 1). At the end of fermentation pH values reached the range of pH 4.1–4.2, slightly higher than those in commercial beers[19]. Differences in final pH values could be explained in terms of initial levels; pH levels in this study were not initially adjusted whereas commercial worts are normally adjusted in the range of pH 5–5.4[19]. Initial wort pH's results are in agreement with Agu and Palmer[2] who also obtained sorghum and barley worts of 5.4–5.5; however, when worts were fermented into beer the pH dropped to 4.6 and 4.0 for barley and sorghum beers, respectively. As occurred in this study, the high pH drop occurred during the first day fermentation.

Fermentable carbohydrate depletion and ethanol production. The percentage glucose, maltose and maltotriose based on total fermentable carbohydrates for the BM wort were 20, 68 and 13% and for the SM wort 35, 48 and 17% respectively (Table I). Most noteworthy differences were in glucose and maltose content likely due to the higher β-amylase activity of BM. As a result of the lower β-amylase activity, SM worts contained approximately 40% less maltose than counterparts produced from BM (Table I, Fig. 2). Beta *et al.*[9] and Taylor[27] concluded that SM has a low ratio of β- to α-amylase, which limits the conversion of dextrins into maltose and simpler sugars. This limitation adversely affects worts properties, as worts rich in dextrins and low in fermentable sugars are usually obtained when SM is used. In a recent study, Mugula *et al.*[18] also found that SM worts contained significantly less maltose than BM worts.

WXSOR grits produced worts with similar ($P > 0.05$) glucose, maltose and total fermentable carbohydrates contents as those produced from MZ adjuncts. In previous research Osorio-Morales *et al.*[21] concluded that among different types of sorghums, the WXSOR was the most suitable for use as brewing adjunct and Barredo Moguel *et al.*[8] reported that worts obtained from BM and WXSOR grits had almost identical properties as their counterparts produced from commercial adjuncts.

AMG addition led to a > 2.5 fold increment in wort glucose and > 20% in total fermentable carbohydrate content without affecting maltose concentration. AMG presumably complemented the intrinsic malt amylases with subsequent increments in both glucose and total fermentable sugars (Table I, Fig. 2). SM worts were the most favored by AMG addition, as the glucose content increased from 11.3 to 29.5 g/L wort. Interestingly, AMG treated SM worts achieved similar levels of fermentable carbohydrates to those observed for BM worts (Table I). AMG addition resulted in similar maltotriose breakdown for all treat-

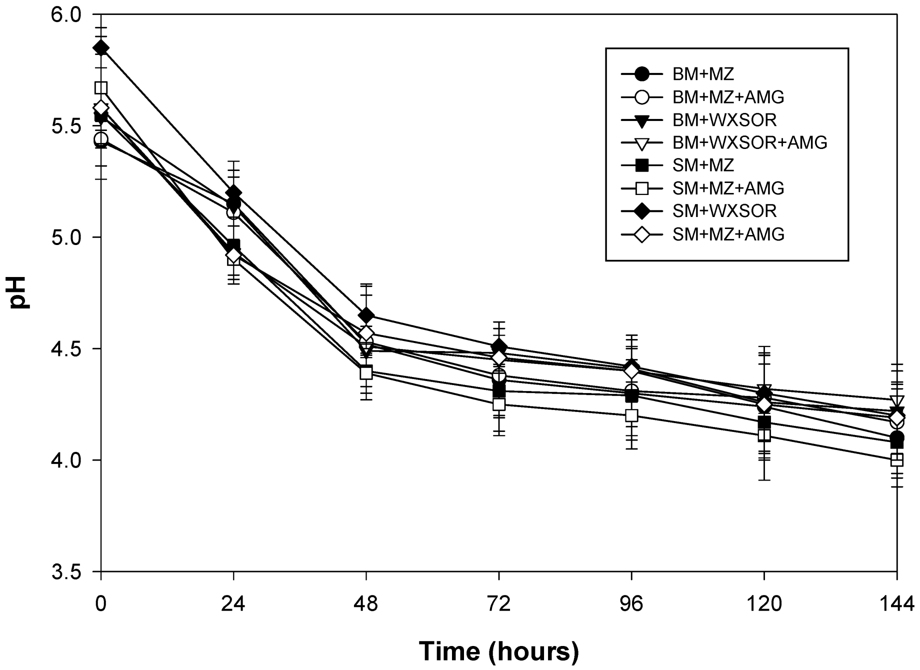

Fig. 1. Effects of malt type, adjunct type and amyloglucosidase addition on pH profiles during fermentation of worts. BM = barley malt; SM = sorghum, MZ = maize grits; WXSOR = waxy sorghum grits; AMG = amyloglucosidase.

ments (Fig. 2). For this carbohydrate, differences observed were independent of malt type. The most striking difference due to AMG addition was this enzymatic treatment increased by approximately 20% the initial content of fermentable carbohydrates in all treatments and presented a more pronounced effect for treatments containing SM (up to 18.6 g/L). The enzyme, therefore, can be used as an alternative to produce sorghum lager beers with less dextrins and a higher ethanol concentration.

After fermentation, BM extracts produced beers with approximately 1% more ethanol than their counterparts produced from SM (Table II). The lower ethanol content is due to the lower initial total fermentable carbohydrate content of the SM worts. BM beers contained more residual maltose when compared to SM beers whereas lager beers produced from MZ grits contained similar amounts of residual glucose, maltose, maltotriose, total sugar equivalents and ethanol to counterparts produced from WXSOR grits (Table II, Fig. 2). Beers produced from AMG treated mashes also contained significantly higher amounts of residual glucose and 0.27% more ethanol ($P <$ 0.07) than beers from untreated worts (Table II, Figs. 2 and 3).

Linear regression analyses were conducted and served to determine the adequacy of the model describing the kinetics of fermentable carbohydrate depletion during fermentation, and confirmed that they followed first order kinetics ($P < 0.05$) after one-day fermentation (Table III). First order rate constants (β_1) and depletion times ($DT_{50\%}$), the latter defined as the time needed to reach 50% substrate degradation for each type of sugar, were calculated using the following equations[26]:

$$\ln(S_t/S_0) = -\beta_1 t; \qquad DT_{50\%} = (\ln 0.5)/\beta_1$$

where S_0 was the initial substrate concentration and S_t was the substrate concentration at a given fermentation time (t). Rates were calculated using the initial substrate concentration of each treatment.

Results indicated that DT's$_{50\%}$ for glucose, maltose and maltotriose were 49, 128 and 125 h respectively clearly indicating that glucose was the preferred substrate for the fermenting yeast. Depletion times to reach half of the initial glucose and maltose were similar when BM and SM fermenting worts were compared; however, the maltotriose of BM was consumed at a faster rate (Table III). The most significant difference between DTs$_{50\%}$ of fermentable carbohydrates was observed in maltose. Maltose from WXSOR grits mashes were consumed at a faster rate (higher β_1 and lower $DT_{50\%}$) than maltose from MZ grits mashes. The 45 h difference in $DT_{50\%}$ occurred despite that the initial concentration of all fermentable sugars was similar in these treatments (Table I). Interestingly, depletion times to reach half of the initial maltose and maltotriose for AMG treated mashes were higher and lower respectively than their untreated counterparts (Table III). AMG-treated mashes had a lower rate of maltose depletion probably because these worts contained higher amounts of total fermentable carbohydrates that were more readily available for the fermenting yeast. It is known that if a wort has an excessively high proportion of glucose, then maltose and maltotriose fermentation may be slow and a "sticking" fermentation may result. This is because the synthesis of yeast maltase, which is required for maltose and maltotriose fermentation, is inhibited[10].

Approximately 85, 56, 58 and 64% of the glucose, maltose, maltotriose and fermentable carbohydrates respectively, were consumed by the fermenting yeast throughout fermentation. SM and AMG-treated beers contained 2.3

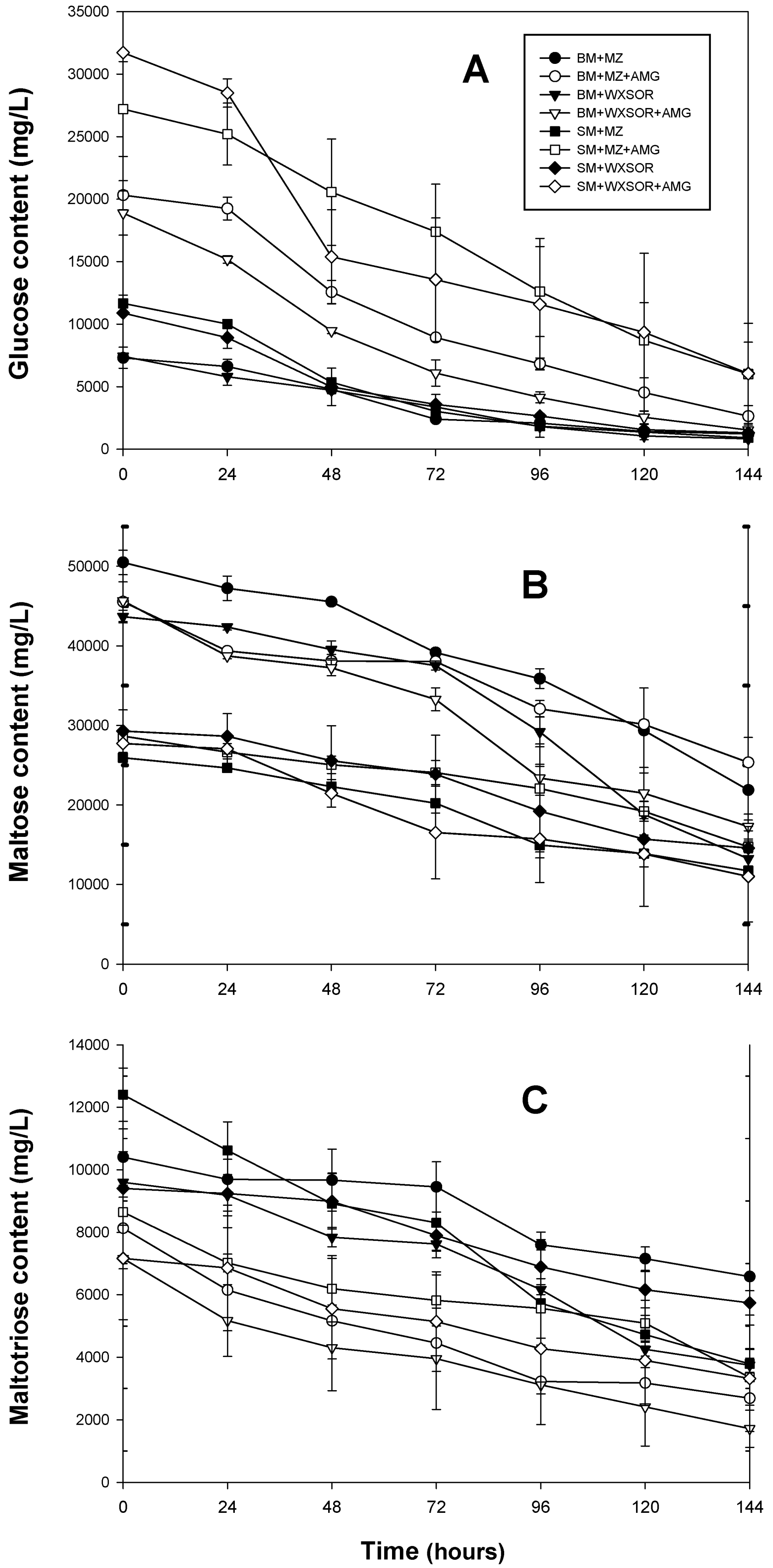

Fig. 2. Effects of malt type, adjunct type and amyloglucosidase addition on glucose (A), maltose (B) and maltotriose (C) consumption trends during fermentation of worts. BM = barley malt; SM = sorghum, MZ = maize grits; WXSOR = waxy sorghum grits; AMG = amyloglucosidase.

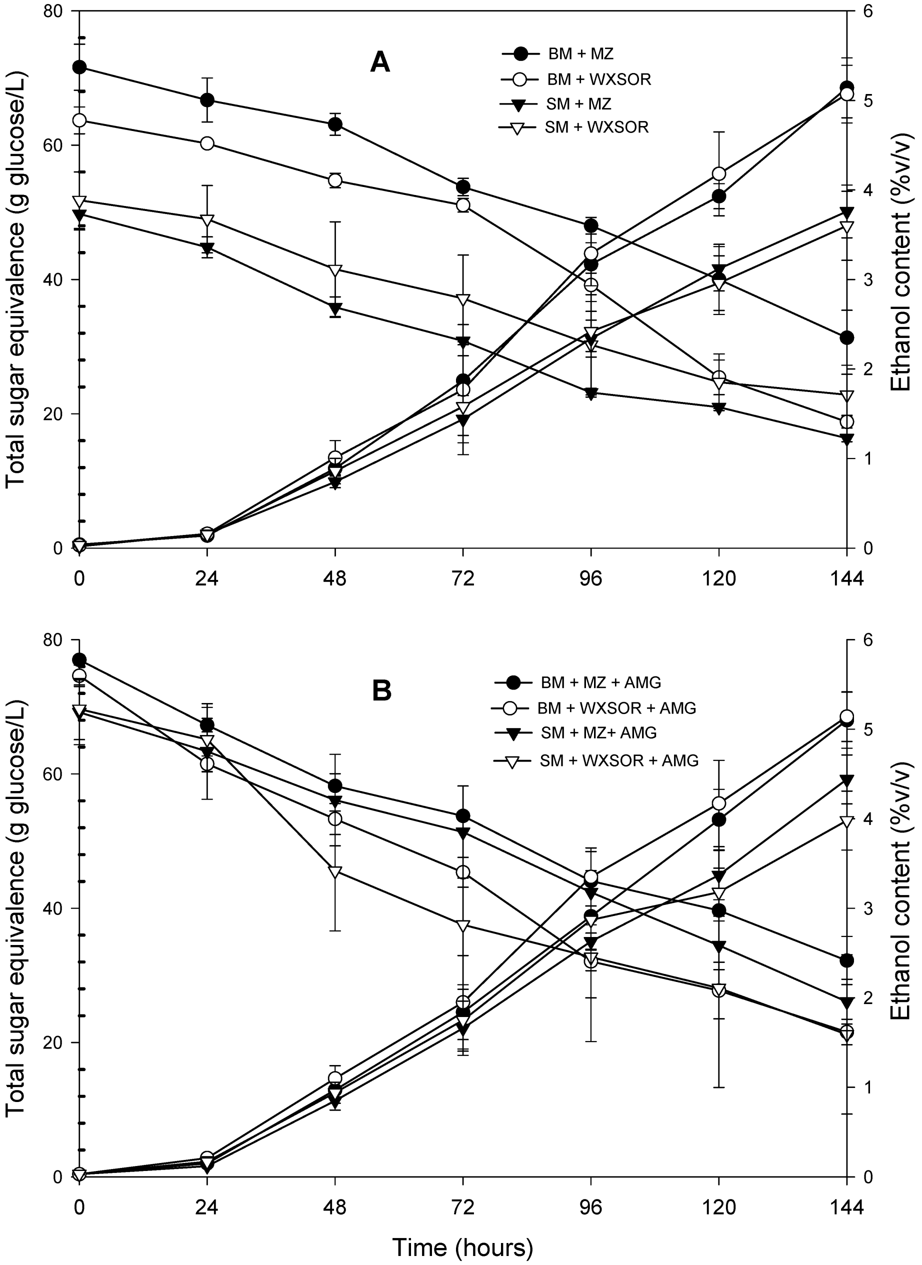

Fig. 3. Effects of malt type, adjunct type and amyloglucosidase addition on total fermentable carbohydrates depletion and ethanol production during fermentation of worts. BM = barley malt; SM = sorghum, MZ = maize grits; WXSOR = waxy sorghum grits; AMG = amyloglucosidase.

and 3.8 times more residual glucose than their corresponding counterparts. This is important because the residual glucose can affect the organoleptic beer properties and color via formation of Maillard compounds. BM beers finished fermentation with more residual maltose than SM beers (Figs. 2 and 3). A longer fermentation can further deplete glucose, maltose and fermentable carbohydrates and increase ethanol concentration.

Similar ethanol generation trends were observed for all treatments during the first 24 h fermentation (Fig. 3), independently of wort type and AMG addition. During the first hours of the lager fermentation, the yeast used fermentable carbohydrates, mainly glucose, to yield organic acids that rapidly lowered the pH (Fig. 1) but yielded little ethanol (< 0.2% v/v) (Fig. 3). Ethanol was produced at a rate of approximately 1 and 0.8% for the BM and SM beers during the subsequent 5 days of fermentation (Fig. 3). After 144 h fermentation, BM beers contained higher ethanol content (5.12% v/v) than analogs produced from SM (3.95% v/v). Mugula *et al.*[18] also observed lower ethanol content in sorghum beers when compared with commercial barley beers. Agu[1] obtained barley malt beers with higher alcohol (3.65%) than sorghum (3.09) and millet (2.55%) beers. Additional fermentable carbohydrates

Table III. Effects of malt type, adjunct type and amyloglucosidase addition on first order depletion kinetic parameters of glucose, maltose and maltotriose[a].

	Glucose			Maltose			Maltotriose		
Treatment	β_1[b]	R^2	$DT_{50\%}$[c]	β_1	R^2	$DT_{50\%}$	β_1	R^2	$DT_{50\%}$
BM + MZ	13.8 ab	0.97	50.2 ab	5.5 bc	0.92	126 cd	3.3 d	0.92	210 a
BM + MZ + AMG	14.3 ab	0.98	48.5 ab	3.7 d	0.94	187 a	7.6 b	0.98	91.2 d
BM + WXSOR	16.2 a	0.97	42.8 b	8.2 a	0.86	84.5 d	6.8 ba	0.93	101 d
BM + WXSOR + AMG	17.7 a	0.99	39.2 b	6.8 b	0.96	102 c	9.1 a	0.98	76.1 d
SM + MZ	18.9 a	0.99	36.7 b	5.8 b	0.96	120 c	8.4 b	0.98	82.5 d
SM + MZ + AMG	10.6 b	0.96	65.4 a	4.1 d	0.90	169 a	5.3 c	0.89	131 c
SM + WXSOR	15.6 a	0.99	44.4 a	5.3 c	0.95	131 b	3.8 d	0.95	182 b
SM + WXSOR + AMG	11.1 b	0.96	62.4 a	6.6 b	0.97	105 c	5.5 c	0.99	126 c
Malt type									
BM	15.5 a		45.2 a	6.1 a		124.9 a	6.7 a		119.6 a
SM	14.1 a		52.2 a	5.5 a		131.3 a	5.8 a		130.4 a
Adjunct type									
MZ	14.4 a		50.2 a	4.8 a		150.5 a	6.2 a		128.7 a
WXSOR	15.2 a		47.2 a	6.7 a		105.6 b	6.3 a		121.3 a
Amyloglucosidase addition									
Without AMG	16.1 a		43.5 a	6.2 a		115.4 b	5.6 a		143.9 a
With AMG	13.4 b		53.8 a	5.3 b		140.8 a	6.9 a		106.1 b

[a] BM = barley malt; SM = sorghum malt; MZ = maize grits; WXSOR = waxy sorghum grits; AMG = amyloglucosidase.

[b] Indicates the first order consumption rate of each substrate ($\beta_1 * 10^3$, hours^{-1}) with its correspondent regression coefficient (R^2).

[c] Depletion times ($DT_{50\%}$), defined as the time needed to reach 50% of substrate degradation for each type of sugar.

due to AMG supplementation did not increase final ethanol content of BM beers but significantly increased it in SM beers (Table II, Fig. 3). Correlation analysis showed that the variation in ethanol production was highly associated to changes in maltose ($r = 0.85$) and total fermentable carbohydrates ($r = 0.80$).

BM beers had higher color scores (1.85 Lovibond color) than counterparts produced with SM (1.55 Lovibond color). The differences can be attributed to the higher content of reducing sugars and alpha amino nitrogen[28] that favored Maillard reactions during the heat treatments used for mashing and hop addition. A similar trend was observed when comparing beers produced from different grits, as those containing WXSOR grits had significantly higher Lovibond color than counterparts produced from MZ grits. This difference can be attributed to the higher "b" CIE color attribute of the former adjuncts. AMG treated beers had similar color to untreated beers even though AMG addition increased glucose and total fermentable carbohydrates content.

CONCLUSIONS

This study demonstrated that the fermentation potential of a wort is strongly influenced by its initial concentration of total fermentable carbohydrate and its composition. Addition of AMG increased >20% the amount of fermentable carbohydrate with a two fold glucose content especially in SM worts. AMG helped in the degradation of dextrins into simpler fermentable carbohydrate moieties and complemented intrinsic α-amylase activity. Glucose, maltose, maltotriose and total fermentable carbohydrates followed a first order depletion kinetics during fermentation. Glucose was consumed at a higher rate during fermentation than maltose and maltotriose. Depletion times to reach half of the initial glucose, maltose and maltotriose were 49, 128 and 125 h respectively indicating that the fermenting yeast favored glucose.

Among SM beers, the maximum alcohol content was obtained in beers produced from mashes treated with AMG because they contained more fermentable carbohydrates and a higher proportion of glucose. These beers contained 0.5% more ethanol than their untreated counterparts and contained similar ethanol contents to commercial lager beers. This study clearly demonstrated that is feasible to produce 100% sorghum beers when a proper sorghum malt and source of adjunct is selected and the mashes are treated with AMG. The utilization of AMG increased ethanol concentration without significantly affecting beer pH and color. These beers can be produced to satisfy the increasing demands of light beers with lesser amounts of dextrin.

ACKNOWLEDGMENTS

This research was supported by grant CAT-005 from the Instituto Tecnologico y de Estudios Superiores de Monterrey. Partial support form the US Agency for International Development through the International Sorghum and Millet Collaborative Research Support Program is acknowledged.

REFERENCES

1. Agu, R.C., Comparative study of experimental beers brewed from millet, sorghum and barley malts. *Proc. Biochem.,* 1995, **30**, 311–315.
2. Agu, R.C. and Palmer, G.H., Comparative development of soluble nitrogen in the malts of barley and sorghum. *Proc. Biochem.,* 1999, **35**, 497–502.
3. Aisien, A.O. and Muts, G.C.J., Microscale malting and brewing studies of some sorghum varieties. *J. Inst. Brew.,* 1987, **93**, 328–331.
4. Aisien, A.O., Palmer G.H. and Stark, J.R., The development of enzymes during germination and seedling growth in Nigerian sorghum. *Starch/Starke,* 1983, **35**, 316–320.

5. American Association of Cereal Chemists. Approved Methods of the AACC, 10th ed. The Association: St. Paul, MN, 2000
6. Association Official Analytical Chemists. Official Methods of Analysis. The Society: Washington, 15th ed., 1990.
7. Bajomo, M.F. and Young, T.W., The properties, composition and fermentabilities of worts from 100% raw sorghum and commercial enzymes. *J. Inst. Brew.,* 1993, **99**, 153–158.
8. Barredo Moguel, L.H., Rojas de Gante C. and Serna Saldivar, S.O., Comparison between a commercial wort and a waxy sorghum wort fermented into lager beer, with emphasis on yeast growth and ethanol production. *J. Am. Soc. Brew. Chem.,* 2001, **59**, 24–27.
9. Beta, T., Rooney L.W. and Waniska, R.D., Malting characteristics of sorghum cultivars. *Cereal Chem.,* 1995, **72**, 533–538.
10. Briggs, D.E., Hough, J.S., Stevens, R. and Young, T.W., Malting and Brewing Science, Second Edition, Chapman and Hall: London, 1981.
11. Daiber, K.H. and Taylor, J.R.N., Opaque beers. In: Sorghum and Millets: Chemistry and Technology. Dendy, D.A.V., Ed., American Association of Cereal Chemists: St. Paul, 1995, pp. 299–324.
12. Ernandes, J.R., Williams, J.W., Russell I. and Stewart, G.G., Effect of yeast adaptation to maltose utilization on sugar uptake during the fermentation of brewer's wort. *J. Inst. Brew.,* 1993, **99**, 67–71
13. Etokakpan, O.U. and Palmer, G.H., A simple diamylase procedure for the estimation of alpha amylase and diastatic activity. *J. Inst. Brew.,* 1990, **96**, 89–91.
14. Etokakpan, O.U. and Palmer, G.H., Comparative studies of the development of endosperm degrading enzymes in malting sorghum and barley. *World J. Microbiology and Biotechnology,* 1990, **6**, 408–417.
15. Hallgren, L., Lager beers from sorghum. In: Sorghum and Millets, Chemistry and Technology. Dendy, D.A.V., Ed., American Association of Cereal Chemists: St. Paul, MN, USA, 1995, pp. 283–298.
16. Howard, L.R., Braswell, P.D. and Aselage, J., Chemical composition and color of strained carrots as affected by processing. *J. Food Sci.,* 1996, **61**, 327–330.
17. Martinez-Anaya, M.A., Enzymes and bread flavor. *J. Agric. Food Chem.,* 1996, **44**, 2469–2480.
18. Mugula, J.K., Nnko, S.A.M., Narvhus, J.A., and Sørhaug, T., Microbiological and fermentation characteristics of *togwa,* a Tanzanian fermented food. *Int. J. of Food Microb.,* 2003, **80**, 187–199.
19. Munroe, J.H., Fermentation. Chapter 13 in: Handbook of brewing. Hardwick, W., ed. Marcel Dekker: New York, 1995.
20. Odibo, F.J.C., Nwankwo L.N. and Agu, R.C., Production of malt extract and beer from Nigerian sorghum varieties. *Proc. Biochem.,* 2002, **37**, 851–855.
21. Osorio-Morales, S., Serna-Saldívar, S.O., Chávez Contreras, J., Almeida Domínguez, H.D. and Rooney, L.W., Production of brewing adjuncts and sweet worts from different types of sorghum. *J. Am. Soc. Brew. Chem.,* 2001, **58**, 21–25.
22. Rooney, L.W. and Pflugfelder, R.L., Factors affecting starch digestibility with special emphasis on sorghum and corn. *J. Anim. Sci.,* 1986, **63**, 1607–1623.
23. Rooney, L.W. and Serna-Saldivar, S.O., Sorghum. In: Handbook of Cereal Science and Technology. 2nd Edition. K. Kulp and Ponte, J.G., Eds., Marcel Dekker Inc.: New York, 2000, pp. 149–176.
24. SAS Institute, Inc. SAS Institute Inc., SAS Campus Drive, Cary, NC, 2002.
25. Serna Saldivar, S.O. and Rooney, L.W., Structure and chemistry of sorghum and millets. In: Sorghum and Millets, Chemistry and Technology, Dendy, D., Ed., American Association of Cereal Chemists: St. Paul, 1995, pp. 69–124.
26. Taoukis, P.S., Labuza, T.P. and Saguy, I.S., Kinetics of food deterioration and shelf-life prediction. In: Valentas, K.J., Rotstein, E. and Singh, R.P., eds. Handbook of Food Engineering Practice. New York: CRC Press, 1997, pp. 361–403.
27. Taylor, J.R.N., Mashing with malted grain sorghum. *J. Am. Soc. Brew. Chem.,* 1992, **50**, 1–34.
28. Urias Lugo, D., Efecto de amiloglucosidasa en la cinética de fermentación de cervezas tipo Lager elaboradas a partir de maltas de sorgo o cebada. Tesis de Maestría en Biotecnología. Instituto Tecnológico y de Estudios Superiores de Monterrey. Monterrey, N.L., México, 2001.
29. Van der Maarel, M.J.E.C., van der Veen, B., Uitdehaag, J.C.M., Leemhuis, H. and Dijkhuizen, L., Properties and applications of starch-converting enzymes of the α-amylase family. *J. of Biotech.,* 2002, **94**, 137–155.

(Manuscript accepted for publication March 2004)