Sorghum Malts for the Production of a Lager Beer

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ABSTRACT

The major difficulties encountered when producing a lager beer with sorghum malt were investigated. Forty-nine sorghum cultivars of various origins were evaluated for *Aspergillus* contamination, mycotoxins, α- and β-amylases, and β-glucanases after malting. More than 60 sorghum malt samples were used in a laboratory-scale brewery, and the major wort characteristics were analyzed. The data clearly demonstrated that the cultivar (and, presumably, the growth location) had an effect in determining saccharification, filtration speed, diastatic power, and wort amino acid content. Saccharification problems were attributable to the great variability of starch gelatinization temperature and viscosity and to the lack of β-amylase in most of the cultivars. The level of amino acids in the wort depended strongly on the water content at steep-out. Also important was the wort amino acid profile, which is quite different from the amino acid profile of barley malt worts. A much higher first group-second group amino acid ratio was observed in sorghum wort. Under these conditions, the uptake of amino acids by yeast was disturbed, and a concomitant high production of vicinal diketones occurred during fermentation. All of these drawbacks can be remedied by selecting "brewing" sorghum cultivars, adapting the brewing procedure, and using appropriate brewing equipment.

Keywords: Aflatoxin, Barley, Enzymes, Malt, Sorghum, Wort composition

Barley cultivation in tropical areas currently is not feasible. Beer production in Africa thus requires the costly import of barley malt from temperate regions. Sorghum malt is a potential substitute for barley malt (3,46,85). Currently, sorghum malt is produced for brewing traditional African beverages (67–69,82,88). These products are turbid and invariably exhibit a high residual starch content (38,39,42,68,88,93).

Although there is increasing interest in the germination and malting properties of sorghum in relation to the manufacture of lager beers and as a possible replacement for barley malt (2,4–8,24,33,35,36,47,56,65,70–74,76,87,90), the major objectives of sorghum malting have not yet been reached (3,75). One of the more serious problems frequently pointed out is insufficient enzyme levels. Low β-amylase activity especially makes for slow and incomplete saccharification of the mash. Another obstacle is limited endosperm cell wall degradation, with the inherent economical and technical drawbacks of low extract yield, poor wort separation, and poor beer filtration (2,3,11,13,32,33,37, 41,46,47,62,65,73).

Although brewers' specifications for barley malt and methods for producing the appropriate malt are fairly well established, this is far from the case with sorghum malt.

The aim of this article is to focus on the malt properties and wort characteristics of sorghum and to compare them with those of barley, in relation to the manufacture of a lager beer. Sorghum cultivars from very different geographical locations were screened on a laboratory scale before industrial scale-up.

EXPERIMENTAL

Sorghum Grain and Malt

To examine the cultivar effect, grains from 49 sorghum cultivars were used (three from Kenya, two from Nigeria, four from South Africa, five from Burundi, 14 from Cameroon, 12 from Rwanda, three from Sudan, one from Taiwan, one from Ghana, and four from Mali). These were provided by B. P. Louant of the Laboratoire de Phytotechnie Tropicale et Sub-Tropicale, Université Catholique de Louvain, Louvain-la-Neuve, Belgium. The malts were prepared by the engineering division of Unibra (Brussels, Belgium). After steeping at 30°C for a period adapted to each cultivar, the grains were germinated at 30°C (rh 95%) for periods of up to five days and then dried at 50°C for 24 hr to approximately 7% humidity. The dried roots and shoots were rubbed off by hand in a sieve.

Fungal Analysis of Sorghum Grain and Malt

The external fungal contamination was monitored after the grains were washed with sterile water (100 ml per 100 kernels) and the washed grains were plated on 4.8% Czapek-Dox agar (Merck, Darmstadt, Germany) in the presence of chloramphenicol at 500 mg/L (Fluka, Buchs, Switzerland). Plates were incubated for six days at 27°C. The fungal contamination of the subsurface layers (the internal infection) was estimated after treating the grain for 1 min with a solution of either 0.1% $HgCl_2$ (w/v) or 1% HClO (v/v). Both chemicals inactivated the fungi present on the kernel surface (external infection), but $HgCl_2$ was more effective. After these treatments, the grain was rinsed five times with sterile water and incubated on the same medium as above. One hundred kernels were analyzed for each treatment (10 kernels per 90-mm petri dish).

Aflatoxin B_1 Analysis

Flour (25 g) (Buhler-Miag [Minneapolis, MN] laboratory disk mill type DLFU at setting 0.1) was mixed with 125 ml of chloroform, 8 g of Celite (Hyflo Supercell, Union Chimique Belge, Leuven, Belgium), and 12 ml of water. After a 30-min extraction under magnetic stirring at room temperature, the mixture was centrifuged at 6,000 rpm (Sorvall RC2B with GSA rotor), and the supernatant was filtered (no. 597 1/2 filter paper, Schleicher et Schuell, Dassel, Germany). Wort samples (25 g) were mixed with 125 ml of chloroform. After stirring and centrifugation as described above, the chloroform layer was decanted by means of a 250-ml separatory funnel. The chloroform extract was further purified on a glass column (23 × 400 mm) containing anhydrous sodium sulfate and Kieselgel 60 (Merck) as described by Blanc (17). The aflatoxin extract was quantitatively transferred to a centrifuge tube and evaporated to dryness in a Vortex evaporator (Buchler). The residue was dissolved in 250 μl of eluent in an ultrasonic bath and filtered through a Millipore HV filter (0.45 μm) (Millipore Corp., Bedford, MA). A 20-μl sample was applied to the column. The high-performance liquid chromatography (HPLC) system consisted of an HPLC pump (model 5000, Varian Associates, Palo Alto, CA), an autosampler (Millipore-Waters, WISP 710 B), and a Novapak C-18 column (8 × 100 mm, 5 μm) from Millipore-Waters. Aflatoxin B_1 was detected with a fluorescence detector (model 650S, Perkin-Elmer Corp., Norwalk, CT). Excitation and emission wavelengths were 366 and 430 nm, respectively. The column was eluted with a mixture of acetonitrile, methanol, and water (20:20:60, v/v) at a flow rate of 0.8 ml/min. Aflatoxin B_1 standard (Makor, Jerusalem, Israel) was used to calibrate the method. Aflatoxin B_1 standard added to the flour was recovered to the extent of 94% by this method. For screening the grain and malt samples, the aflatoxin B_1 content was measured by thin-layer chromatography (16).

Amylase Assays

Bendelow's procedure (15) was used to determine the total amylase and α-amylase activities. β-Amylase activity was calculated as the difference between total amylase and α-amylase activities. A saturated stock solution of *p*-chloromercuribenzoic acid was used to inhibit β-amylase. All activities were recorded in the same units (milligrams of maltose per milligram of malt).

β-Glucanase Assays

Three methods were used to measure β-glucanase activity: the viscometric method (14), the radial diffusion assay (58), and the azo-barley glucan method (Biocon assay kit) (60). For the radial diffusion assay, extraction of malt flour was carried out as described by Bathgate (14).

Pasting Viscosity of Barley and Sorghum Flours

The Brabender Amylograph (Brabender Corp., New Rochelle Park, NJ) was used to determine the pasting viscosity of barley and sorghum flours (10). Milling was carried out with the Buhler-Miag laboratory disk mill type DLFU at setting 0.2.

Grain and Malt Polyphenol Content

After milling (at setting 0.1), the polyphenol content was determined as described by Jerumanis (49).

Wort Fermentable Sugar Analysis

The wort fermentable sugar composition was determined by HPLC. The HPLC system consisted of an HPLC pump (LKB 2150), a Rheodyne injector (model 7120) equipped with a 20-μl loop, a 2-μm in-line stainless steel prefilter (Millipore-Waters), a guard-pak module containing a silica precolumn (RCSS silica guard-pak insert) (Millipore-Waters), a radial compression module (Millipore-Waters, model RCM 100) containing a silica column cartridge (8 × 100 mm, 10 μm) (Millipore-Waters), and a refractive index detector (Laboratory Data Control, refractomonitor model 1107). Wort (1.8 ml) was diluted with 0.2 ml of 33% rhamnose (w/v) as internal standard, and the mixture was passed through a C-18 Sep-Pak cartridge (Millipore-Waters) activated by flushing with 10 ml of methanol followed by 10 ml of ultrapure water (Milli-Q water purification system, Millipore). The last 1 ml was collected and used for HPLC analysis. The turbid sample was filtered on an HAWP 0.22-μm filter (Millipore). A 20-μl wort sample was applied to the in situ amino-derivatized silica column (17) after a preliminary column washing with 500 ml of methanol. Analyses were carried out at a flow rate of 1.5 ml/min at room temperature (20–25°C). The eluent was recirculated in a bottle under magnetic stirring. If a loss of resolution was observed due to the column, derivatization was repeated. Tetraethylenepentamine (20% solution, w/v) and sugar standards were from Merck. Fructose, glucose, and sucrose were of chromatography standard grade, maltose monohydrate was for biochemical usage, and rhamnose monohydrate was for biochemical and microbiological usage. Maltotriose (~ 90%) was from Boehringer (Manheim, Germany).

Wort Amino Acid Analyses

Sample preparation and wort amino acid analysis were carried out as described by Dethier et al (27).

Wort β-Glucan Measurement

Soluble β-glucan concentrations were determined in the worts by precipitation with 30% (w/v) ammonium sulfate (16 hr at 4°C), three washings of the precipitate with 70% (v/v) ethanol, digestion with sulfuric acid, and colorimetric assay of β-glucans as glucose with anthrone (54). The method for determining β-glucans by means of the anthrone reagent was adapted from Hall (44) and Yadav et al (95). Two milliliters of β-glucan solution was mixed with 10 ml of anthrone reagent (0.2%, w/v, in concentrated sulfuric acid) at room temperature. The tubes containing the reaction mixture were transferred to a 100°C water bath, heated for 10 min, and transferred to an ice bath for 5 min. The tubes finally were transferred to a water bath at 20°C for 20 min, after which the optical densities were measured at 620 nm.

Examination by Light Microscopy

After overnight steeping at room temperature, the grains were cut longitudinally with a hand-held razor blade mounted on metal stubs. Thin sections were stained with an iodine solution (1%, w/v) and examined under a light microscope (×70).

Wort Characteristics

Analyses of the viscosity, extract, and saccharification were performed as recommended by the European Brewery Convention (34).

Brewing with Barley Malt

Mashing of industrial samples was performed as described in *Analytica EBC* (34).

Brewing with Sorghum Malt

Mashing was carried out by Unibra, using a three-step mashing program adapted from Ogundiwin and Tehinse (71), including preparation of an enzymatic extract, gelatinization and partial liquefaction of the starch residue, and complete liquefaction and saccharification of the mash.

RESULTS AND DISCUSSION

Sanitary Conditions

Sorghum malting requires drastic control of the sanitary state of the kernels. Indeed, although sorghum is not considered a "high-risk" cereal with regard to its microorganism and toxin contents (80,83,84), unless produced in hot and humid regions (9,26,45,61,91), the high temperature (around 30°C) and relative humidity (>85%) required to produce the malt are ideal for the development of fungi often present on the kernel surface (16,21,28,48,52,64,78,81). Among these, special attention must be paid to *Aspergillus flavus*, the growth of which leads to the production of aflatoxins. Aflatoxins are thermostable molecules reportedly responsible for several diseases, including liver cancer (18,20,28,94). As shown in Table I, of 50 samples of sorghum of various origins, 80% were positive for *A. flavus*. Among these, 10% were contaminated by aflatoxin B_1. During malting, however, the proportion of samples positive for aflatoxin B_1 reached 52%, and the average aflatoxin B_1 content was 42.5 μg/L. Concentrations as high as 175 μg/L were observed.

Given the thermoresistance of aflatoxin B_1 (16,28,57), it is likely that part of the toxin molecules will pass through the brewing process into the beer unless some other decomposition or removal mechanism occurs. The data presented in Table II illustrate the evolution of aflatoxin B_1 during a brewing process using naturally contaminated sorghum malt (aflatoxin B_1, 104 μg/L). More than 60% of the toxin was removed in the spent grains. Another 15.8% was recovered in the sweet wort. Subsequent boiling of the wort did not affect its toxin content (15% recovery) (Table II). Using

TABLE I
Natural Occurrence of *Aspergillus flavus* and Aflatoxin B_1 in Sorghum Grain Samples and in the Corresponding Sorghum Malts

Sample	*A. flavus* Positive (%)	Aflatoxin B_1 Positive (%)	Range of Toxin Content (μg/L)
Sorghum grain[a]	80	19	2–12
Sorghum malt[a]		52	2–175[b]

[a] $n = 50$.
[b] $x = 42.5$.

artificially contaminated sorghum, Chu et al (22) found 14 and 18% malt toxin in the final beer with respective initial malt contaminations of 1 and 10 mg/L. Although the toxin content was drastically reduced from its initial level during the brewing process, it must be stressed that 23% of the aflatoxin B_1 was not recovered in the present study (Table II). Moreover, the identity and toxicity of potential degradation products were not known. No other aflatoxin was detected.

To reduce fungal growth, formaldehyde (0.15%, v/v) was added to the steep water. However, this treatment was not likely to be effective against microorganisms present in the subsurface layers of the kernel (internal contamination, see Experimental) (51). Under these conditions, moreover, the growth of some fungi (*Fusarium*) was even stimulated (*data not shown*). Formaldehyde concentrations above 0.3% (v/v) reduced the germinative capacity of the kernels (*data not shown*). Sorghum samples contaminated by *A. flavus* in the subsurface layers of the kernel should be discarded, as should samples containing more than 5 μg/L of aflatoxin B_1 (92). Rabie and Thiel (78) pointed out that apart from the fungi that produce known mycotoxins, other highly toxic fungi that produce unknown toxins occur fairly regularly in high numbers in certain samples. As a safety rule, the best solution to the mycotoxin problem in sorghum malt does not lie in detection and/or determination of mycotoxins but rather in the use of sorghum grains in a good sanitary state. Indeed, the elimination or reduction of all fungi eventually present on the grains to safe levels does not totally rule out the presence of toxins in the malt.

Liquefaction and Saccharification of the Mash

A major difficulty when dealing with sorghum malt concerns liquefaction and saccharification of the mash. These technical problems can be attributed in part to the peculiar biochemical characteristics of sorghum malt, especially its α- and β-amylase contents (5,47,63,66,73). The α- and β-amylase activities were measured on 30 different cultivars. Bendelow's method was used to discriminate between β- and α-amylase (15). Industrial barley malts were included in the test for comparison. The α-amylase activities of sorghum and commercial barley malts are illustrated in Figure 1A. Eighty percent of the sorghum malts exhibited α-amylase activities very similar to or even higher than those usually found in typical industrial lager barley malts. The high α-amylase potential of sorghum has been pointed out by Palmer and Bathgate (77). Unfortunately, β-amylase activities in sorghum malts were not nearly so satisfactory. As shown in Figure 1B, all sorghum malts were characterized by a low β-amylase activity: nearly 60% of the sorghum malts contained a very low enzyme activity. For a few sorghum malts, β-amylase activity was not even detectable.

Typical amylase activity data for several sorghum cultivars are summarized in Table III. Group A includes some of the best sorghum malts produced. These were all white sorghums. Group B includes sorghum malts from red cultivars that behaved poorly during the brewing process (*data not shown*). Although the presence of polyphenols may have a negative effect on the enzymes (23,63), other factors should be considered. The data for white sorghums MD 4.1, MF 4.2, and ML 7.2 (group C) indicated very low β-amylase activity even when polyphenol levels were low. The causes of such behavior are not understood and are

TABLE II
Fate of Aflatoxin B_1 from Sorghum During the Brewing Process[a]

Sample	Aflatoxin B_1 (μg/L)	Recovery of Aflatoxin B_1 (%)
Sorghum malt	104	100.0
Mashing (calculated)	12.2	
Wort before filtration	11.5	81.2
Sweet wort	3.8	15.8
Spent grains	22.5	62.4
Pitching wort	2.6	15.0

[a]Total recovery of alfatoxin B_1 (spent grains + sweet wort) was 77.4%.

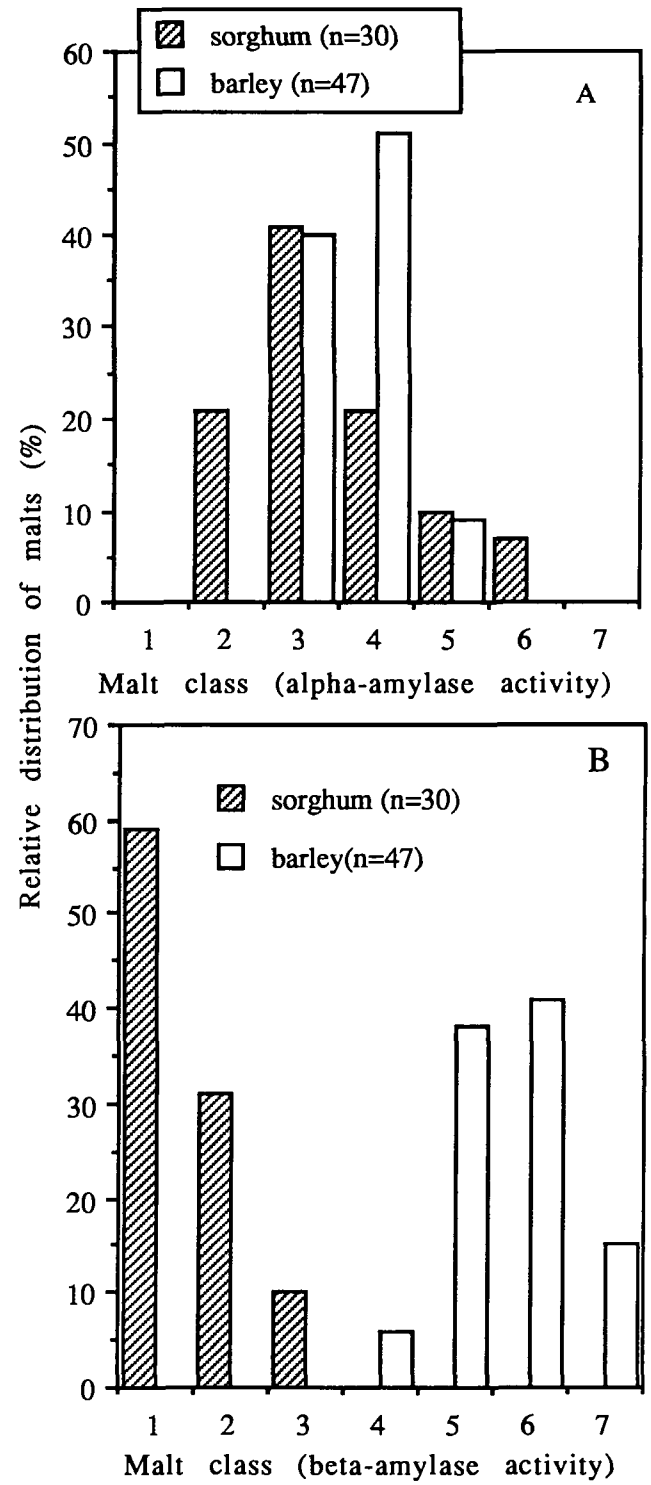

Fig. 1. Classification of barley and sorghum malts according to their amylase activity levels. α-Amylase **(A)** and β-amylase **(B)** activities were assayed as described under Experimental. The malts were classified into seven classes according to their enzymatic level (in milligrams of maltose per milligram of malt) as follows: class 1 ($0.0 \leq$ amylase activity ≤ 0.1), class 2 ($0.1 <$ amylase activity ≤ 0.2), class 3 ($0.2 <$ amylase activity ≤ 0.3), class 4 ($0.3 <$ amylase activity ≤ 0.4), class 5 ($0.4 <$ amylase activity ≤ 0.5), class 6 ($0.5 <$ amylase activity ≤ 0.6), class 7 ($0.6 <$ amylase activity ≤ 0.7).

under investigation. Several hypotheses are being explored, such as the presence of an inhibitor or inhibitors other than polyphenols, a low level of soluble enzyme, partial insolubilization of the enzyme, or a low yield of enzyme production during malting. Interestingly, unlike the barley enzyme, which is already present in the ungerminated kernel, sorghum β-amylase develops during germination of the grain, alongside α-amylase (31,73) (L. Mélotte and J. P. Dufour, *unpublished data*).

The ratio of β- to α-amylase was calculated for sorghum and barley malts from the data presented in Figure 1. For sorghum, the ratio was 0.31 ± 0.16 (n = 29). For barley, it was 1.64 ± 0.28 (n = 47). Similar ratios have been reported by others (31,73). This five- to sixfold difference in ratios is mainly due to the low β-amylase activity in sorghum malt. Under such conditions, of course, saccharification of the mash was disturbed and worts low in fermentable extracts were obtained. Such behavior has been pointed out previously (5,11,65,75).

Starch Characteristics

Slow and incomplete saccharification of the mash also was attributable to the basic chemical and physical characteristics of sorghum. Factors to be considered when starch hydrolysis is troublesome include the high starch gelatinization temperature and/or the high viscosity of the starch solution (19,86). Typical Brabender viscosity curves for barley and various sorghum flours are illustrated in Figure 2. Gelatinization of sorghum starch started at around 75°C (a much higher temperature than that for barley) and reached completeness at temperatures far exceeding the thermoresistance of the amylolytic enzymes (90°C for SD2, 94°C for SK2 and SK3, and above 97°C for SP1) (Fig. 2A). A few sorghum cultivars exhibited a lower starch gelatinization temperature very near that of barley (Fig. 2B). For most of the sorghums, the viscosity of the gelatinized starch was higher than that for barley. For example, with sorghum SB5, the high viscosity of the gelatinized starch caused technical problems (*data not shown*). Such variability in sorghum starch behavior could be due to varying percentages of branching in starch amylopectin and to varying bonding forces in the granules (higher resistance to swelling and rupture) (1).

Another point of interest in relation to starch was its distribution in the sorghum kernel. Under the microscope, it was possible to distinguish the pericarp, the embryo, and the endosperm on a section of mature sorghum kernel (Fig. 3). Iodine staining of the thin section indicated the presence of starchy material in the central layer (the mesocarp) of the pericarp of some sorghum cultivars (in addition to the endosperm). Sanders reported the presence of starch in this area as early as 1955 (79). Figure 3 shows microscopic iodine-stained thin sections of sorghums representative of the absence (Fig. 3A), moderate levels (Fig. 3B), and high levels (Fig. 3C) of starch in the mesocarp. Under our conditions, sorghum cultivars with a high starch level in the mesocarp always behaved poorly during brewing (*data not shown*). The absence of starch in the mesocarp is thus a desirable feature to be included among the specifications for selecting sorghum cultivars for brewing.

TABLE III
Amylase Activities and Polyphenol Contents of Sorghum Malts from Various Cultivars

Cultivar[a]	Polyphenol Content, %		Amylase Activities (mg of maltose/mg of malt)	
	Grain	Malt	α-Amylase	β-Amylase
Group A				
MC 08.4 (w)	0.44	0.31	0.388	0.109
ML 01.1 (w)	0.34	0.07	0.517	0.237
MK 03.2 (w)	0.53	0.34	0.424	0.230
Group B				
MS 07.1 (r)	2.58	0.41	0.247	0.052
MS 17.4 (r)	2.23	0.26	0.245	0.037
MS 16.4 (r)	3.39	0.49	0.235	0.070
Group C				
MD 04.1 (w)	0.21	0.04	0.222	0.035
MF 04.2 (w)	0.19	0.04	0.235	0.026
ML 07.2 (w)	0.34	0.11	0.220	0.013

[a] w = white, r = red.

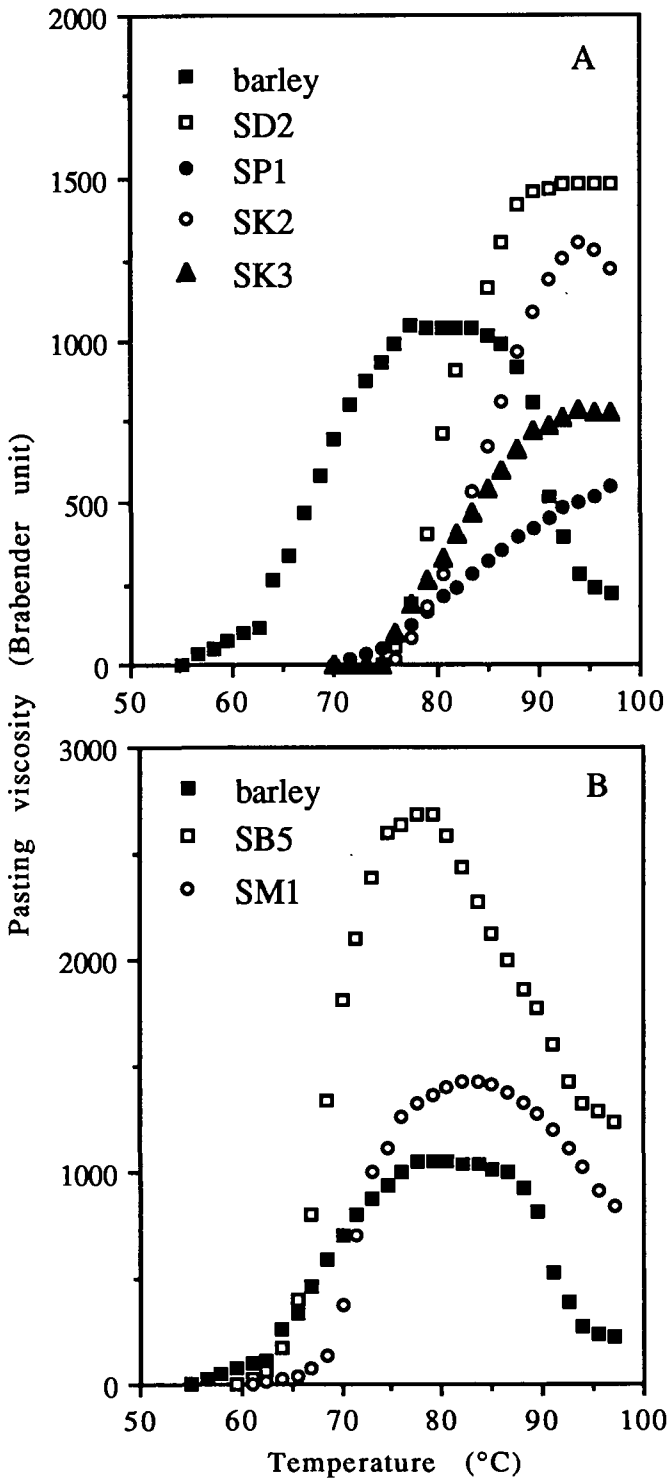

Fig. 2. Brabender viscosity curves for barley and sorghums SD2, SP1, SK2, and SK3 (A) and SB5 and SM1 (B).

Wort Filtration

Wort filtration problems may naturally arise from the various above-mentioned features: incomplete saccharification of the mash due to a high starch gelatinization temperature, low starch susceptibility to amylolysis, low starch dispersibility, and low amylolytic activities. Poor wort separation may also be attributable to high wort viscosity, to which several factors might contribute, including a high level of nonstarch polysaccharides (β-glucans, pentosans, etc.) of polyphenol-protein complexes or of cysteine-rich proteins, the presence of incompletely dispersed gelatinized starch, and/or a high dextrin content. Under our conditions, the measured viscosities of sorghum malt worts could not account for the observed differences in wort filtration behavior. This is illustrated in Table IV. The average viscosity of (iodine negative) sorghum malt wort was around 1.36 cP (± 0.1) (n = 14, five sorghum cultivars), much lower than the corresponding value for normal barley malt wort under the same conditions (1.55–1.60 cP).

Nor did the soluble β-glucan concentration appear to cause poor wort filtration behavior. The data presented in Table IV show no correlation between wort β-glucan levels and the wort filtration speed. Moreover, the β-glucan content of sorghum malt wort was relatively low compared with the corresponding average value for barley malt wort (around 350 mg/L). Although quantities of soluble β-glucans in the sorghum malt worts were presumably too low to affect wort filtration, the molecular characteristics of β-glucans extracted in the wort might be of prime importance. It has been shown that cell wall breakdown is rather limited during sorghum malting (33,76). As pointed out by Palmer (75), it is quite possible that these partly degraded β-glucans, pentosans, and proteins are extracted into the wort during mashing. Such molecules could clog the filter bed.

Limited cell wall breakdown in some sorghum cultivars could be attributable to the high level of proteins and/or to the low level of cell wall hydrolyzing enzymes, especially endo-β-1,3-1,4-glucanase (75). Figure 4 shows β-glucanase activities (viscometric method) for barley and sorghum malts. Most sorghum malts were characterized by very low β-glucanase activities: 77% of the

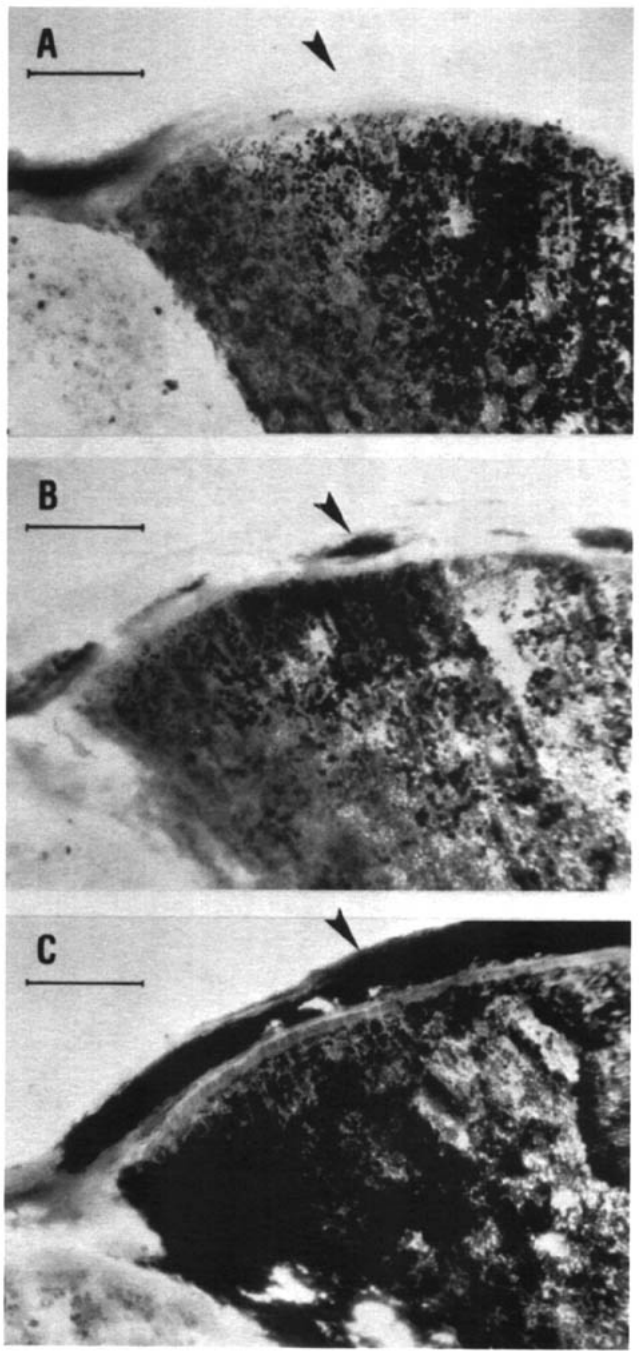

Fig. 3. Iodine-stained longitudinal thin sections of sorghums SK3 **(A)**, SD4 **(B)**, and SD3 **(C)**. Arrows indicate the absence or the presence of starch in the mesocarp. Scale bar = 215 μm.

TABLE IV
Influence of Wort Viscosity and β-Glucan Content on the Filtration Speed

Sorghum Malt	Wort Extract (°P)	Viscosity (cP)	β-Glucans (mg/L)	Filtration Speed[a] (min)
MK 2.5	8.52	1.24	196	32
MK 4.1	8.99	1.24	45	110
MK 2.3	8.67	1.40	187	30
MK 1.1	8.81	1.40	44	>120

[a]The wort filtration speed was measured as the time elapsed between recirculation of the initial turbid wort (100 ml) and complete filtration.

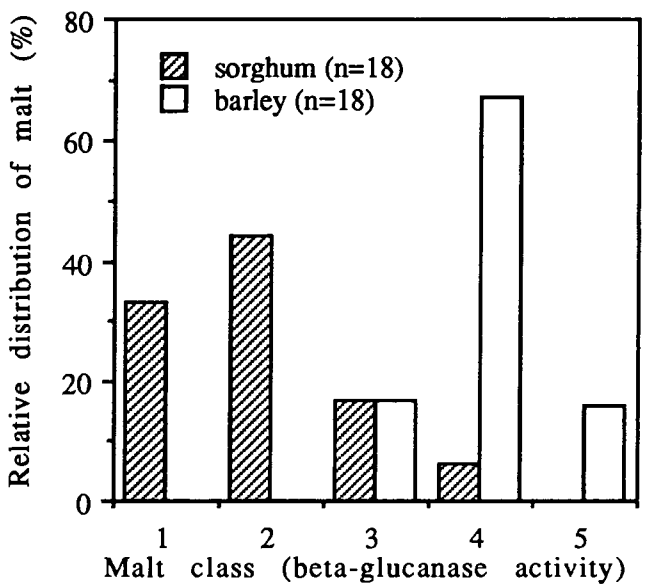

Fig. 4. Classification of barley and sorghum malts according to their β-glucanase activity levels. β-Glucanase activity was assayed as described by Bathgate (14). The malts were classified in five classes according to their enzymatic levels (in IRVU per hour per 100 g of malt) as follows: class 1 ($0 \leq$ β-glucanase activity ≤ 100), class 2 ($100 <$ β-glucanase activity ≤ 200), class 3 ($200 <$ β-glucanase activity ≤ 300), class 4 ($300 <$ β-glucanase activity ≤ 400), class 5 ($400 <$ β-glucanase activity ≤ 500).

samples exhibited activities below the lowest values observed for barley malts.

At this point, the β-glucanase assay deserves a comment. Very strange data were obtained for sorghum malts with the Biocon assay kit. For this reason, two other methods (the viscometric method and the radial diffusion technique) were used to measure β-glucanase activities. Figure 5 shows the correlation between the radial diffusion technique and the viscometric method. The correlation between these methods was good. It was almost identical for the barley and sorghum malts. The Biocon method, on the other hand, produced a completely different pattern for barley and sorghum malts (see the comparison of this method with the viscometric method in Fig. 6). It would appear that the Biocon assay does not respond to the β-glucanases of sorghum malts in the same way as it does to those of barley malts. This behavior may be related to the low level of endo-β-1,3-1,4-glucanase in sorghum malt (75).

Sorghum Malt Wort

A three-step mashing procedure was used to investigate sorghum malt wort characteristics (see Experimental). The average extract yield was 82.7 (n = 55), with a maximum of 89.3% and a minimum of 74.3% (Fig. 7). These values contrast with previously reported data, which were demonstrably low (5,73). Recently, Palmer reported (75) that high-starch extracts could be produced with sorghum malts by means of a similar three-step mashing procedure. Nevertheless, the corresponding fermentable extracts were significantly lower than they were in barley malt worts. Low fermentable sugar content also was obtained for a significant number (36%) of samples tested in the present study (Fig. 7). However, considering only the worts that saccharified (iodine negative), the average apparent limit attenuation was excellent, 83.3% (±4%, n = 17) (*data not shown*). As far as these two parameters are concerned, the results suggest that it is feasible to produce sorghum malts of a quality similar to barley malt if the right sorghum cultivars (i.e., cultivars with the appropriate biochemical and physicochemical characteristics and adapted to the microclimate of the growing area) are selected. Our data confirm the work of Glennie et al, showing that the amount of

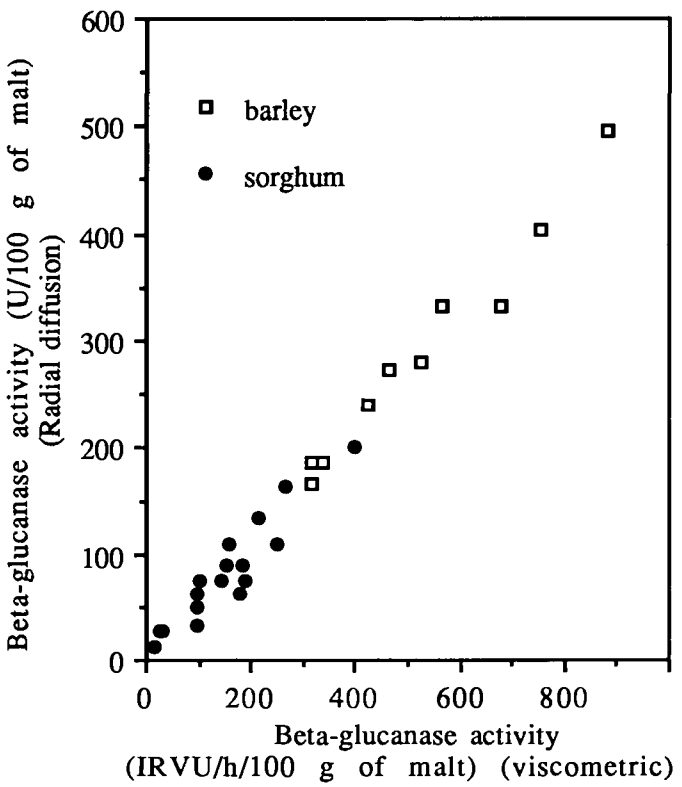

Fig. 5. Comparison of radial diffusion technique and viscometric method for β-glucanase activity in barley and sorghum malts. IRVU = increase in reciprocal viscosity unit.

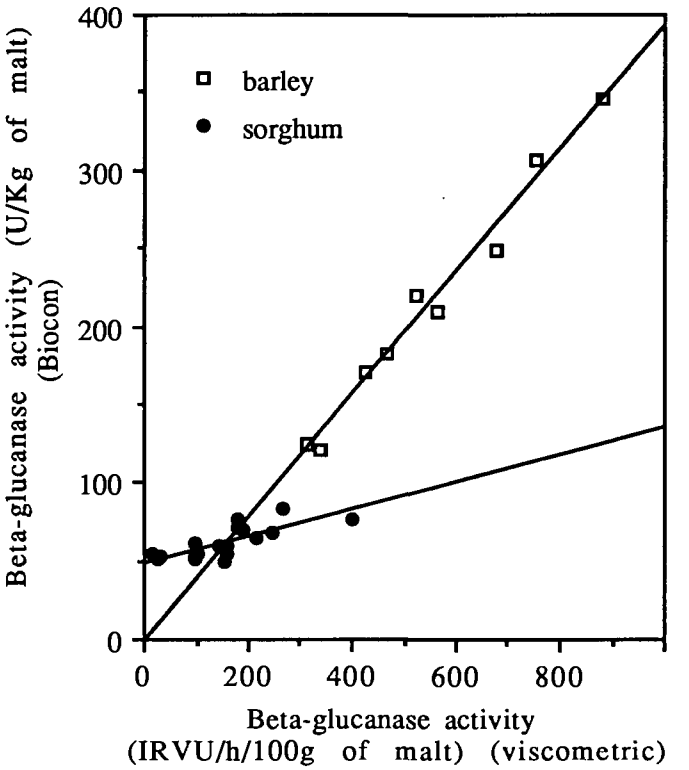

Fig. 6. Comparison of Biocon assay kit and viscometric method for β-glucanase activity in the barley and sorghum malts. IRVU = increase in reciprocal viscosity unit.

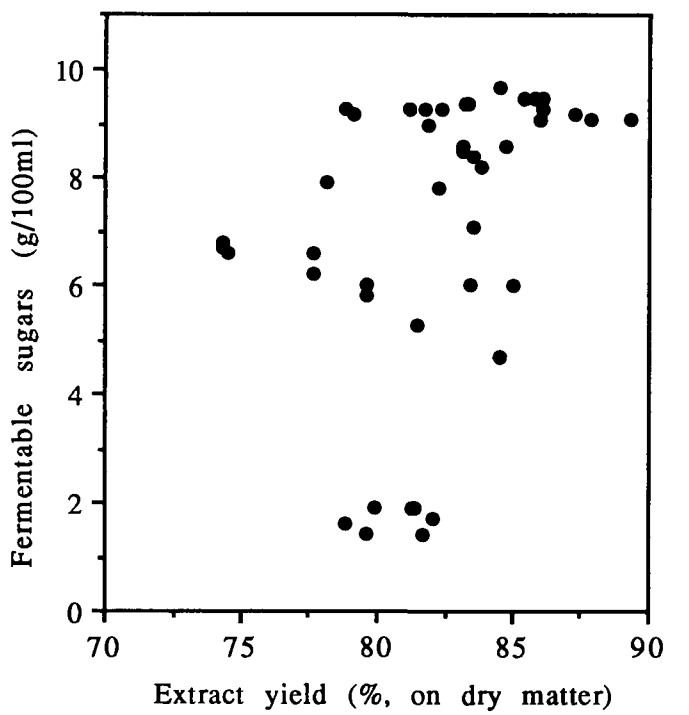

Fig. 7. Relationship between extract yield and wort fermentable sugar content for sorghum malts. Values for fermentable sugar contents are for 12° P original gravity.

hot water extract in sorghum is cultivar related (40). Preliminary data from an ongoing study on selected sorghum cultivars grown together on different locations reveal that the growing environment might also play a critical role on the malting and brewing potential of the sorghum cultivar (J.-L. Van Haecht, *personal communication*).

Wort fermentable sugar and amino acid profiles deserve some attention. Relative amounts of fermentable sugars are presented in Table V. The major difference between sorghum and barley malts was found to reside in the glucose content: in the sorghum malt wort, glucose accounted for nearly 30% of the fermentable sugars, as opposed to only 15% in the barley malt wort. At such high glucose concentrations, some yeasts may lose their capacity to ferment maltose (43,53). The risk is real, especially if sugar is used to improve the sorghum wort extract.

Wort amino acid content is another item to be considered with regard to beer quality (29). The amino acid content of the sorghum malt wort can be modulated by selecting an appropriate sorghum cultivar and adjusting the germination conditions (time, temperature, and moisture). These parameters have been shown to considerably affect proteinase and carboxypeptidase activities (35). We also investigated, under otherwise identical conditions, the effect of water content at steep-out on the free α-amino nitrogen of wort. It was found that the higher the water uptake, the higher the α-amino nitrogen content of the wort (Fig. 8). Similar behavior has been reported by Morral et al (62). High moisture content presumably favors enzyme penetration into the compact (steely) starchy endosperm (12). Enzyme diffusion is especially critical in sorghum as these enzymes are produced only by the scutellum (89). Contrary to the case of barley, the period required to reach the optimal water uptake for appropriate malt modification differs considerably from one sorghum cultivar to another (*data not shown*). In contrast to previously reported data, there is no doubt that adequate α-amino nitrogen can be obtained with sorghum malt. This major difference is presumably attributable to the lower steep-out moisture frequently used for sorghum malting (33–35%) (62). The limited amino acid content, one of the major drawbacks of recent beer production from maize and/or sorghum adjuncts using commercial enzymes (25,29,55), can thus easily be circumvented when using sorghum malt.

The wort amino acid profile also was considered. On the basis of their sequential uptake by yeast cells, wort amino acids have been divided into three groups (50). For the brewer, one of the important consequences of this sequential uptake of amino acids by yeast is the formation of diacetyl as a side effect of the regulation of valine synthesis (59). This phenomenon was intensified with the sorghum malt wort. Table VI illustrates the relative distribution of each group of amino acids for sorghum and barley malt worts. Relative amounts of the three groups in sorghum wort differed significantly from the known values for barley (30). Group I was significantly (about 10%) enriched, whereas groups II and III were correspondingly reduced (by about 5 and 6%, respectively).

In addition to the above, the amino acid profiles of groups I and II were also different in sorghum and barley worts (Table VII). In particular, the relative threonine (+ asparagine) content increased from 8.2% in barley malt wort up to almost 19% in

TABLE V
Relative Amount of Fermentable Sugars in Barley and Sorghum Malt Worts

Sugar (%)	Sorghum[a]	Barley[b]
Fructose	3.5 (1.42)	1.8 (0.19)
Glucose	29.9 (5.34)	11.9 (0.72)
Sucrose	0.41 (1.35)	4.2 (0.43)
Maltose	52.5 (6.22)	70.5 (1.23)
Maltotriose	13.7 (2.42)	11.7 (1.14)

[a] $n = 50$. Figures in parentheses represent the standard deviation.
[b] $n = 20$.

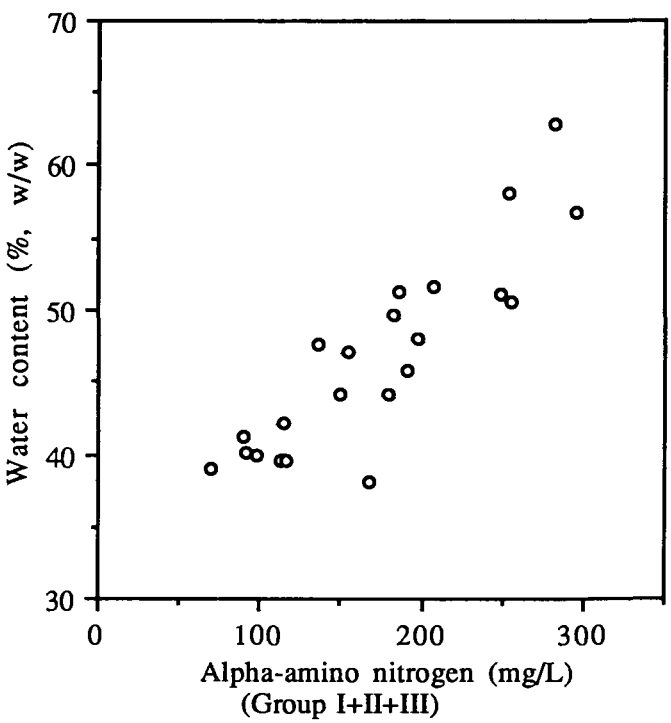

Fig. 8. Effect of the water content at steep-out on the amino acid content of wort (12°P). Malts were germinated for three days. The amino acid content (groups I, II and III in milligrams per liter of NH_2) of wort samples was calculated from amino acid analyses as described under Experimental.

TABLE VI
Relative Amount (%) of Each Group of Amino Acids in Barley and Sorghum Malt Worts

Group[a]	Sorghum[b]	Barley[c]
I	49.4 (3.32)	38.6 (1.58)
II	26.2 (1.56)	31.0 (1.30)
III	24.3 (2.01)	30.4 (0.73)

[a] Individual amino acid contents were obtained after analysis by high-performance liquid chromatography as described under Experimental. According to Jones and Pierce (50), group I includes Asp, Glu, Thr, Ser, Lys, Arg; group II, Val, Met, Leu, Ile, His; group III, Trp, Gly, Ala, Tyr, Phe. Group IV (Pro) was not included in the calculation.
[b] $n = 50$. Figures in parentheses represent the standard deviation.
[c] $n = 40$.

TABLE VII
Relative Amount of Some Amino Acids of Groups I and II in Barley and Sorghum Malt Worts

Amino Acid	Sorghum[a] (%)	Barley[b] (%)
Group I		
Threonine + glutamine	18.7 (3.90)	8.2 (1.08)
Serine + asparagine	5.2 (1.11)	4.8 (0.39)
Lysine	5.3 (0.85)	6.7 (0.52)
Arginine	8.8 (3.32)	9.8 (0.73)
Group II		
Valine	5.0 (0.68)	8.1 (0.41)
Isoleucine	2.7 (0.26)	5.0 (0.23)

[a] $n = 50$. Figures in parentheses represent the standard deviation.
[b] $n = 40$.

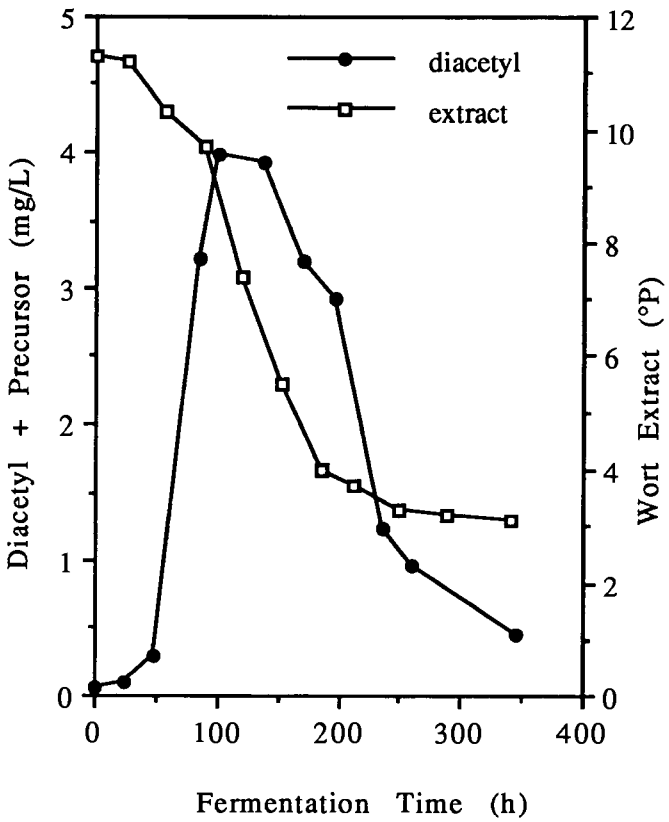

Fig. 9. Evolution of diacetyl (including precursor) and wort extract during fermentation of sorghum malt wort (11.3°P, free amino nitrogen = 337 mg/L).

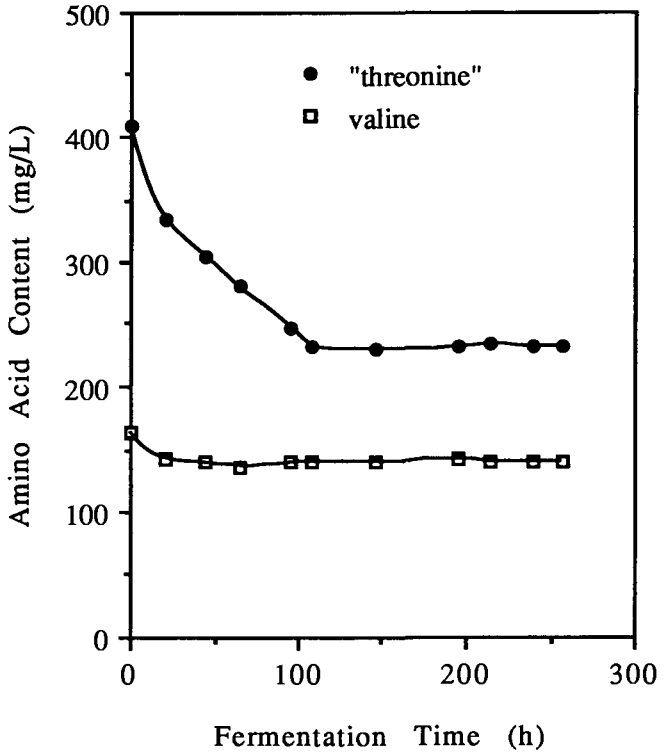

Fig. 10. Evolution of threonine (+ asparagine) and valine during fermentation of sorghum malt wort (11.3°P, free amino nitrogen = 337 mg/L).

sorghum malt wort. This enrichment was not general. On the contrary, the relative levels of lysine and arginine, two essential amino acids, were reduced. As for the second group, attention must be paid to the valine and isoleucine contents, which tended to be lower in sorghum malt. In keeping with the mechanisms regulating valine synthesis, a high level of total vicinal diketones was expected. In practice, a peak as high as 4 mg/L was observed (Fig. 9) in a wort containing an abnormally high level of free α-amino nitrogen (337 mg/L). (The free α-amino acid content for groups I, II, and III, in milligrams per liter of NH_2, was calculated from amino acid analyses as described under Experimental) (Fig. 10). Under these conditions, valine was not taken up by yeast As a result, valine inhibition of α-acetolactate synthesis did not operate, which explains the higher vicinal diketone value observed during fermentation. Although less acute, this phenomenon occurred in most sorghum worts. It was nevertheless possible to adjust the fermentation conditions (temperature profile) so as to reach an acceptable diacetyl level at the end of fermentation (*data not shown*).

CONCLUSIONS

Our data confirm and extend previous reports (5,11,65,75,86) of difficulties encountered in saccharifying sorghum malt wort. Such problems are mainly attributable to starch characteristics and to the unusually low diastatic power. The above data indicate that sorghum polyphenols are not necessarily responsible for the saccharification problems. Furthermore, poor filtration behavior is not directly related to the soluble β-glucan content of the filtered wort. Under our malting and mashing conditions, the extract yields are usually slightly higher than the corresponding values for barley malt. The amino acid content can be modulated easily, but the amino acid profile differs drastically from that of barley malt wort. We can expect this to affect the synthesis of flavor compounds, notably diacetyl.

The conventional EBC mashing procedure is not applicable to sorghum malt. Previously reported data based on this technique should be interpreted with caution. New analytical methods are needed to accurately assess sorghum's malting and brewing potential. The performance of the malt during the brewing process depends heavily on the cultivar used and where it is grown. The use of an appropriate cultivar makes it feasible to produce, with minor modifications to the brewing apparatus, a lager beer with characteristics very similar to a typical African lager (50% barley malt, 50% adjunct).

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