

Properties of Three Sorghum Cultivars Used for the Production of Bili-Bili Beverage in Northern Cameroon

E.J. Nso¹, P.E. Ajebesome¹, C.M. Mbofung¹ and G.H. Palmer^{2,3}

ABSTRACT

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The malting characteristics of sorghum malts produced locally in Cameroon for Bili-Bili brewing were compared with those of malts produced in a laboratory. The analytical values of both malts were similar but the brewing potential of the laboratory malts were marginally better than those of the locally produced malts. Of the three cultivars examined, Madjeru had the lowest levels of β -amylase, maltose levels and fermentability. The worts of the Madjeru filtered the slowest of the three malts. During malting β -glucanase developed rapidly and development was temperature-dependent.

Key words: α -Amylase, β -amylase, β -glucanase, Bili-Bili, fermentability, FAN, sorghum.

INTRODUCTION

Bili-Bili is a traditional beer of Northern Cameroon. It is produced by fermenting the aqueous extract of malted sorghum. Bili-Bili was regarded originally as a daily drink for the elderly but it is now consumed by the adult population. Its growing popularity has encouraged developments in production and commercialisation in both rural and urban areas of Northern Cameroon. Bili-Bili is mainly consumed by the poor and the production techniques are rudimentary. Malted sorghum is the major raw material used and it is malted locally. The efficiency of local malting techniques has never been assessed. Since the production process of beer is linked to the quality of the raw material used^{4-6,16-19}, initial studies were carried out to compare the malting and brewing qualities of sorghum grains harvested in Cameroon with sorghum malts produced by traditional malting methods in Cameroon. The purpose of this study was to compare the brewing potential of malts produced by modern malting methods, at the International Centre for Brewing and Distilling (ICBD) Heriot Watt, with those of malts produced locally in Cameroon, so that defects in local production techniques could be identified and eliminated.

¹National Advanced School of Agro-Process Industries (ENSAI), University of Ngaoundere, P.O. Box 455, Ngaoundere, Cameroon.

²International Centre for Brewing and Distilling (ICBD), Heriot Watt University, Riccarton, Edinburgh, Scotland, EH14 4AS.

³Corresponding author. E-mail: g.h.palmer@hw.ac.uk

MATERIALS AND METHODS

Sorghum grains

Sorghum grains (cultivars Safrari, Madjeru and S.35) were supplied by the Institute of Agronomic Research and Development (IRAD) Marona. In the (ICBD) laboratory, grains of each cultivar were weighed and picked clean of debris, broken grains and other contaminating materials. The weights of “clean” samples were recorded. In general, variations in duplicate analyses did not exceed 7%.

Moisture

Moisture content was determined according to the recommended methods of Analytica-EBC¹².

Bulk density, thousand corn weight, corn size index^{7,9,10,19}

The bulk density of the sorghum grains was determined using a known volume of grains and results expressed as g/cm³. Thousand corn weight (TCW) was performed according to the recommended methods of Analytica-EBC. Corn size index was determined by two methods, firstly using a caliper rule as described by De Clerck⁵ and secondly by the Sieving Test Method as described in the recommended methods analysis of IOB¹⁰.

Germination tests

All germination tests were carried out as described in the IOB recommended methods of analysis¹⁰.

Total starch determination

The total starch of the sorghum grains was determined according to the Megazyme amyloglucosidase/ α -amylase method¹⁴.

Total nitrogen determination

The total nitrogen of sorghum grains was determined using the Kjeldahl method as described by recommended methods of analysis of IOB¹⁰. A Tecator System 2020 digester block was used for digestion. Distillation was performed using a Tecator Kjeltac System 1002 distillation unit. Titration was carried out using the Metrohm Herisau Multiburette E485 System.

Malting of sorghum samples in the laboratory

For the cultivar, Safrari and Madjeru grains, greater than 2.8 mm in diameter, were selected for malting. For

S.35, grains greater than 2.2 mm in diameter were selected for malting because of generally small corn size. About 1 kg of each grain sample was washed using 750 mL of distilled water by shaking at 120 rpm for 2 h at 24°C in a translational circular shaker (Gallenkamp Orbital Incubator). The grains were then rinsed repeatedly with distilled water three times and transferred to Seegar germination boxes (Seegar Maschinen-Fabrik, Fellbach, Germany) for steeping, germination and kilning¹⁻³. Unless stated otherwise, steeping was carried out at 24°C for 24 h, followed by germination for four days at 24°C, kilning was at 30°C for 48 h^{1-3,7,18}.

Traditional method of producing local Cameroon malts

Sorghum samples were manually picked clean of foreign bodies, washed twice and steeped for 24 h using well water. The steep water was then drained off and the grains spread on plastic sheets at bed-depths of about 5 cm at ambient temperature (~38°C during the day and ~31°C at night at Mindjil, and ~38°C during the day and ~29°C at night at Yagoua). The grains were covered with woollen bags, and germination was carried out for three days, with intermittent sprinkling of water 3 times a day. Kilning was carried out under direct sunlight for three days.

Total soluble nitrogen (TSN)

Total soluble nitrogen of the sorghum malts was determined using the Kjeldahl method as described in the IOB recommended methods of analysis¹⁰.

Free α -amino nitrogen

Wort α -amino nitrogen was determined by the Ninhydrin method¹⁰.

The α - and β -amylase extraction and assay

The extraction and assay of α -amylase and β -amylase was done using the Megazyme method for these amylolytic enzymes^{13,15}.

The β -glucanase extraction and assay

The Megazyme assay procedure for β -glucanase and cellulase enzymes was employed^{11,12}.

Mashing of sorghum malts

Mashing was carried out by the decantation process where separated, enzyme-active worts were used to convert the gelatinized starch of the mash^{2,3,8,18}. Duplicates of about 52 g of sorghum malt were ground (disc setting at 0.7 mm) using a Buhler Universal Laboratory Disc Mill (type DLFU) and 50 g weighed into a mashing beaker which was placed into a mashing bath attemperated to 45°C. Distilled water (300 mL) at 45°C was added and stirred to avoid balling. Mashing temperature was maintained at 45°C for 1 h. The mash was taken off and allowed to settle for 5 min. The supernatant was decanted from the sedimented mash and kept. Distilled water (150 mL) was added to the sediment and boiled for 20 min with intermittent stirring at intervals of 5 min. The boiled mash was cooled to 60°C, the enzyme-rich supernatant was added and the mash incubated at 60°C for 1 h. The mash was cooled to 20°C for 20 min. The stirrer was washed

with small amounts of distilled water and the mash brought to a final volume of 515 mL. Filtration time was assessed as recommended in Analytica-EBC⁹.

Extract determination

Hot water extract was determined using the Calculating Digital Density Meter, Stanton Redcroft PAAR DMA 46. After conversion to specific gravity, hot water extract was calculated¹⁰.

Fermentable sugars in wort by HPLC

The separation of fermentable sugars (glucose, fructose, sucrose, maltose and maltotriose) of the worts was achieved at high pH (500 mM NaOH) by High Performance Anion Exchange (HP AE). The instrumentation consisted of a Dionex PAD, Gilson 302 and 305 pumps, Gilson 802 manometric module, Gilson 811B dynamic mixer, Hewlett Packard 1050 autoinjector, Dionex eluent degas module and Hewlett Packard Chemstation data handling (HP3365). Fermentation percentages and fermentable extracts were determined as recommended by the IOB¹⁰.

Wort viscosity

Wort viscosity was determined using a Beckman (UK) Digital Membrane Viscometer.

RESULTS AND DISCUSSION

The grains of three sorghum cultivars commonly used in Northern Cameroon for human consumption and for the production of Bili-Bili were studied by examining some of the brewing properties of their malts. These properties are presented in Table I. It is difficult to explain the 80% Germination Energy (GE) of Madjeru. However, it is possible that water limitation may be the cause because the grains germinated well in the 8 mL water sensitivity test. However, storage periods of up to 18 months had no effect on the viability of the grains of both Safrari and S.35 cultivars (GE = 98%). Germination Capacities (GC) of the cultivars were: 99% for Safrari, 97% for Madjeru and 98% for S.35. The 80% Germination Energy of Madjeru may reflect unknown aspects of dormancy in sorghum.

Other important brewing properties of the grains analysed were Total Nitrogen (TN), Total Soluble Nitrogen

TABLE I. Some properties of three Cameroonian sorghum cultivars.

Property	Cultivar type		
	Safrari	Madjeru	S.35
Colour	Yellow	White	Cream
* G.E. (%) (4 ml test)	98	97	98
+ G.E. (%) (4 ml test)	98	80	98
* G.E. (%) (8 ml test)	95	96	96
Water sensitivity (%)	3	1	3
+ G.C. (%)	99	97	98
Moisture (%)	12.4	13.7	12.4
1000 grain weight (g)	49.9	40.5	28.3
Bulk density (g/cm ³)	0.907	0.877	0.969
Total starch (%)	74.3	80.2	73.1
Total nitrogen (%)	1.5	1.3	1.4
TSN (%)	0.54	0.62	0.56
Total protein (%)	9.7	7.9	8.5

* Tests conducted in 2000 (6 months after harvest)

+ Tests conducted in 2001 (18 months after harvest)

(TSN) and Total Starch (TS). The results obtained are presented in Table I. The Safrari cultivar grains were richest in TN while Madjeru were richest in TS. The TSN values are comparable to those reported for other studies on sorghum¹⁻³. Thousand corn weight (TCW) and bulk density of the grains were also determined. The mean values for these properties are presented in Table I. The TCW was highest for Safrari and lowest for S.35. The grains of Safrari and Madjeru showed mean values of TCW similar to those generally reported for barley^{18,19}. It is worth noting that although the TCW for S.35 is low, its bulk density is slightly higher than those obtained for Safrari and Madjeru, suggesting a high potential yield from starch as fermentable extract from this cultivar. Finally, grain size index was de-

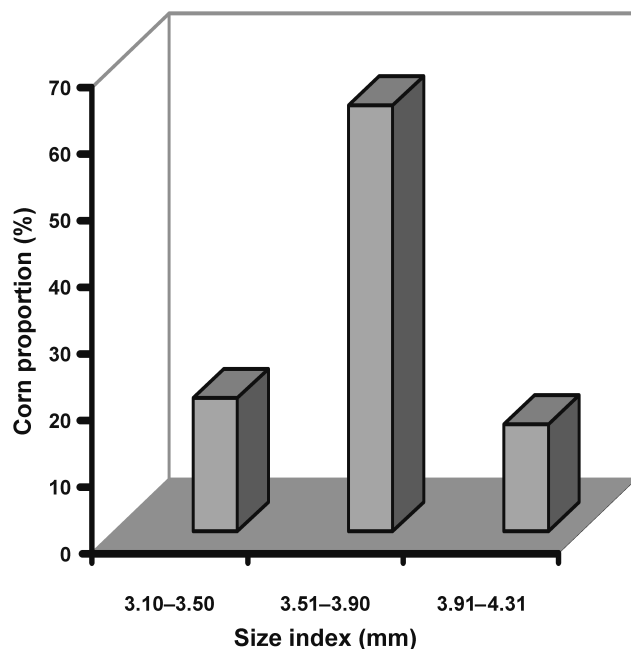


Fig. 1. Proportion (%) of sorghum grains of Safrari cultivar type as a function of size index (mm).

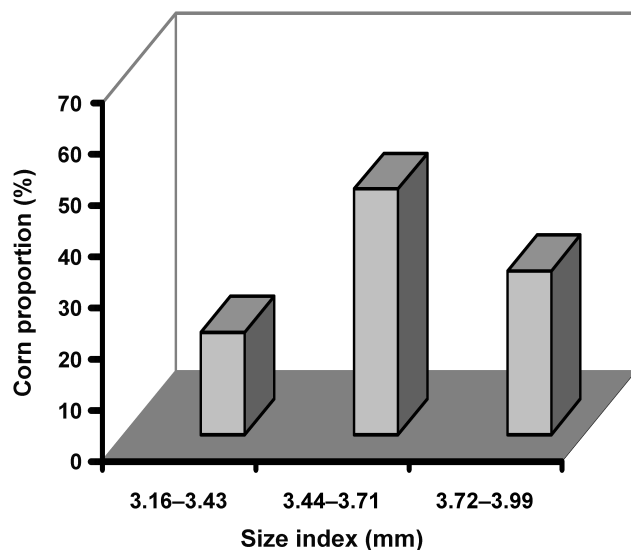


Fig. 2. Proportion (%) of sorghum grains of Madjeru cultivar type as a function of size index (mm).

termined first by using a caliper rule, and second by the Sieving Test Method. Results obtained are presented in Figs. 1, 2, 3 and Table II respectively. Generally, the size index is expected to have a bearing on the extract of the grains following malting. For brewing purposes, corns of proportions/size index ranges of 65%/3.51-3.90 mm for Safrari, 52%/3.44-3.71 mm for Madjeru, and 58%/2.55-2.58 mm for S.35, should reflect yields in extract for each cultivar. When analysed using the Sieving Test Method, more than 98% of Safrari and Madjeru grains were retained by the 2.8 mm sieve, and 56% and 28% of the S.35 grains were retained by the 2.5 and 2.2 mm sieves respectively (see Table II). These were the fractions used for laboratory studies.

Table III compares the HWE of local Bili-Bili malts, as produced in two important Bili-Bili-producing localities of Northern Cameroon (Mindjil and Yagoua), with those of malts obtained in the laboratory at ICB/Heriot-Watt. The extracts obtained from laboratory malts were marginally higher than those of the local malts, indicating that the latter may have undergone higher malting losses than laboratory malts (~20%). This may relate to the higher ambient malting temperatures or longer malting times. The results obtained for fermentable sugars after HPLC

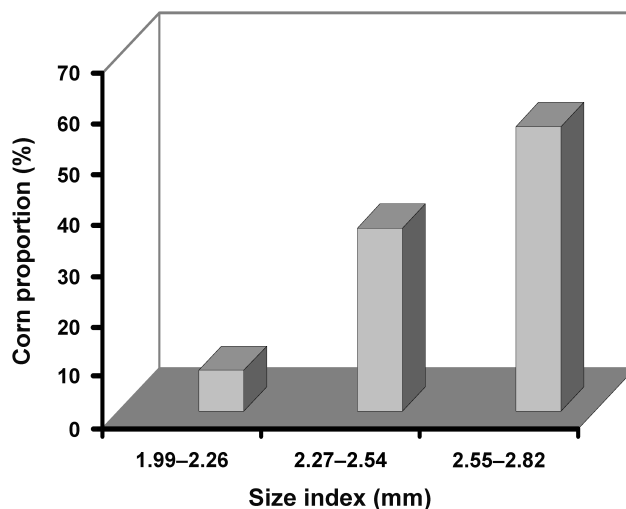


Fig. 3. Proportion (%) of sorghum grains of S.35 cultivar type as a function of size index (mm).

TABLE II. Corn size and evenness of sorghum.

Width of sieve slots (mm)	Weight of fraction (%)		
	Safrari	Madjeru	S.35
< 2.2	–	0.1	7.5
2.2 to 2.5	0.1	0.1	28.4
2.5 to 2.8	0.6	1.0	56.4
>2.8	99.2	98.5	7.1

TABLE III. Hot Water Extract (HWE) (1°/kg; dry basis) of sorghum malts from different localities.

Cultivar type	ICBD	Mindjil	Yagoua
Safrari	334	319	303
Madjeru	300	293	302
S.35	334	288	289

analysis (Table IV) show the same trends as observed for the HWE analysis. Fermentable sugars were higher for the laboratory malts (Table IVa) than for the Mindjil and Yagoua malts (Table IVb). Although the Madjeru cultivar had the highest TS, it contained the lowest levels of fermentable sugars. As previously reported for barley and sorghum malts⁴, maltose (~57%) was for each cultivar the most important of the fermentable sugars, followed by glucose or maltotriose (~20%), fructose (~2%) and finally sucrose (~0.5%), which in some cases, such as the Madjeru cultivar, was not detected. The results in Table IV also indicate that Madjeru produced half as much maltose as the other two cultivars, irrespective of malting for Bili-Bili fermentation in Yagoua (Fig. 4). While the worts of Safrari and S.35 fermented down to 2°P, that of Madjeru never went below 3.5°P. Since the TSN of this cultivar was the highest of the three, its low levels of fermentable sugars and its low fermentability are likely linked to very low β -amylase levels. In terms of malting conditions in the three localities, the α -amylase potential of the malts was better under laboratory conditions at ICBD than under local conditions at Mindjil and Yagoua (Tables V, VI). The low β -amylase potential of sorghum has been confirmed by these studies^{7,8,18}.

Poor degradation of β -glucans during malting and poor hydrolysis of starch during mashing can lead to slow wort filtration, reduced extraction efficiency, and haze formation in beers¹⁸. Fig. 5 shows that the worts of Safrari and S.35 filtered much faster than those of Madjeru. The Bili-Bili worts of Madjeru and Yagoua malts however, filtered faster than the worts of malts produced in the laboratory, suggesting that the former worts were less viscous than the latter. This was confirmed by the results in Tables VII, VIII. The ease with which worts of Bili-Bili malts filtered as compared with worts obtained from laboratory malts, further suggests that the Mindjil and Yagoua malts made at higher malting temperatures were probably better modified than those made in the laboratory. Efficient grain modification during germination is mainly due to the combined action of cell wall degrading enzymes such as β -glucanase and proteases which help to break down the protein matrix. These enzymes expose the starch granules to amylase attack during mashing¹⁸. Germination temperature had

TABLE IVa. Fermentable sugars (g/100 mL) in worts of three Cameroonian sorghum cultivar types malted in laboratory at ICBD/Heriot-Watt (Scotland).

	Glucose	Fructose	Sucrose	Maltose	Malto-triose
Safrari	1.39	0.15	0.11	4.64	1.41
Madjeru	1.20	0.08	ND	2.98	1.57
S.35	1.44	0.22	0.03	4.36	1.37

TABLE IVb. Fermentable sugars (g/100 mL) of worts of *Bili-Bili* malts obtained in two different localities in Northern Cameroon for three sorghum cultivar types.

	Mindjil					Yagoua				
	Glucose	Fructose	Sucrose	Maltose	Malto-triose	Glucose	Fructose	Sucro	Maltose	Malto-triose
Safrari	1.13	0.12	0.03	3.56	0.94	0.98	0.14	0.02	3.51	0.97
Madjeru	1.29	0.01	ND	1.85	1.14	0.68	0.08	ND	1.81	0.84
S.35	1.13	0.17	0.03	3.54	1.01	1.25	0.21	ND	3.00	0.81

a significant effect on the development of β -glucanase activities of the three malts obtained at the various localities. The enzyme potential for the Bili-Bili malts from Mindjil and Yagoua was higher than that for the laboratory malts. These results suggest that the development of β -glucanase during germination is probably temperature-dependent. This was checked for germination temperatures of 26°C and 32°C. Fig. 6 confirms the temperature-dependence of β -glucanase development. Increase in germination temperatures between 24°C and 32°C seemed to favour β -glucanase development only in Safrari and Madjeru. The reasons for these differences are not known.

Another important brewing property study was on the α -amino nitrogen (FAN) potential of the Bili-Bili malts. Table IX compares the FAN of the local Bili-Bili malts of Mindjil and Yagoua to those obtained in the laboratory. The results were once more higher under laboratory conditions than those in Bili-Bili malts produced locally in Cameroon. The results are however similar to those reported in the literature for sorghum¹⁻³. These FAN levels are sufficient to sustain yeast growth and development during fermentation.

CONCLUSION

Local Bili-Bili malts made in Cameroon have similar properties to those made in the laboratory at ICBD/Heriot-Watt. The local malting procedure for Bili-Bili malts is less controlled and this diminishes the brewing potential of the malts, particularly in terms of fermentable extracts and FAN. The Madjeru cultivar produced poorer

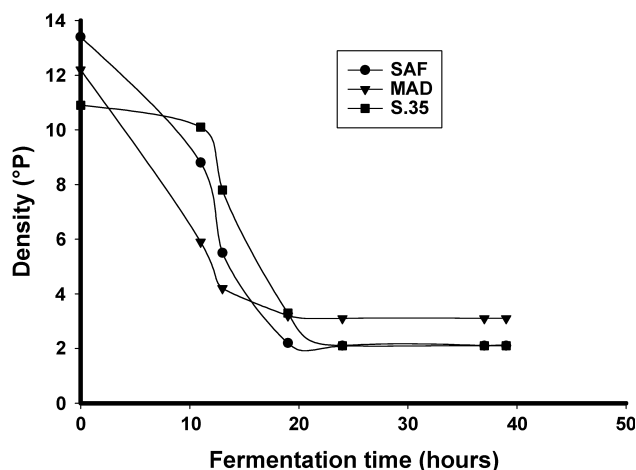


Fig. 4. Time-course of wort density during *Bili-Bili* fermentation in Yagoua for Safrari (SAF), Madjeru (MAD) and S.35 sorghum cultivars. The same yeast preparation was used in the three cases.

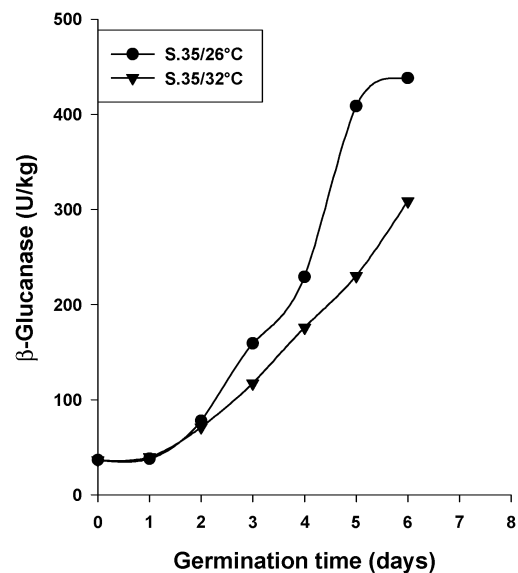
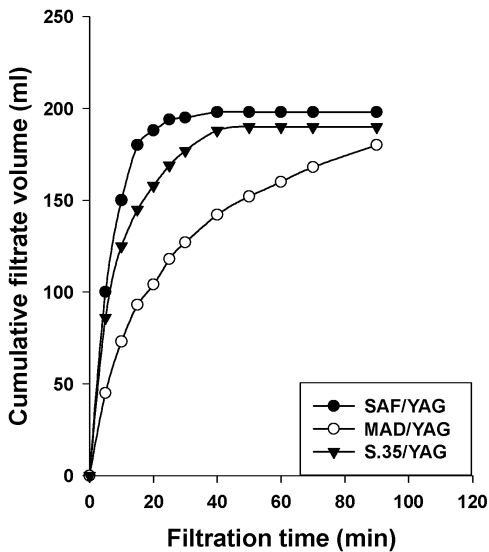
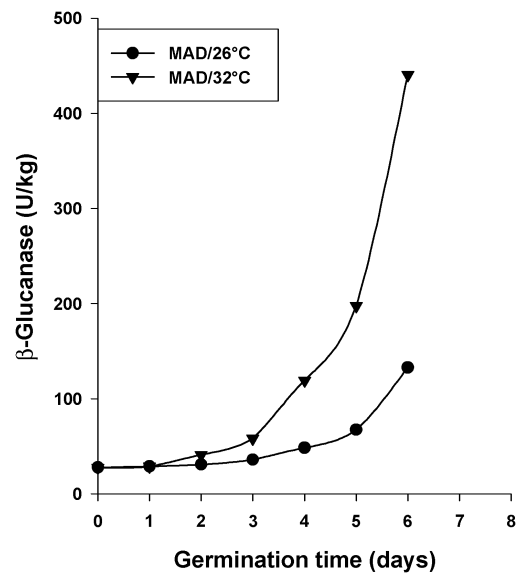
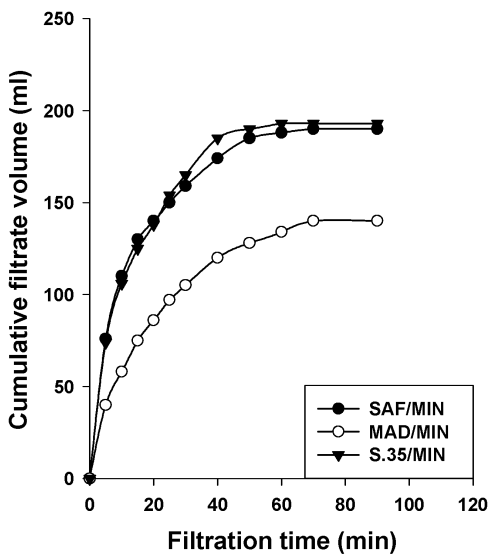
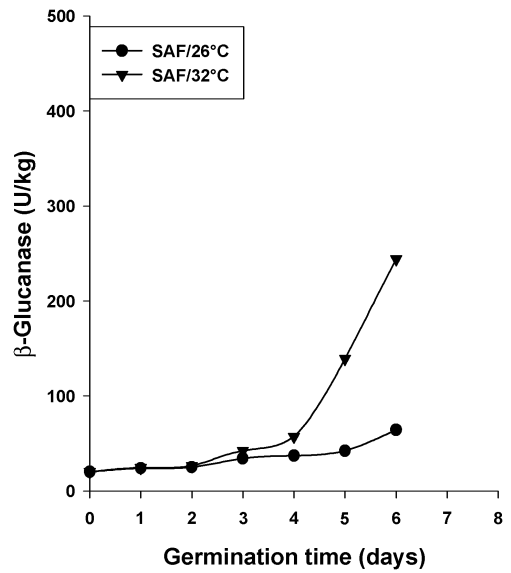
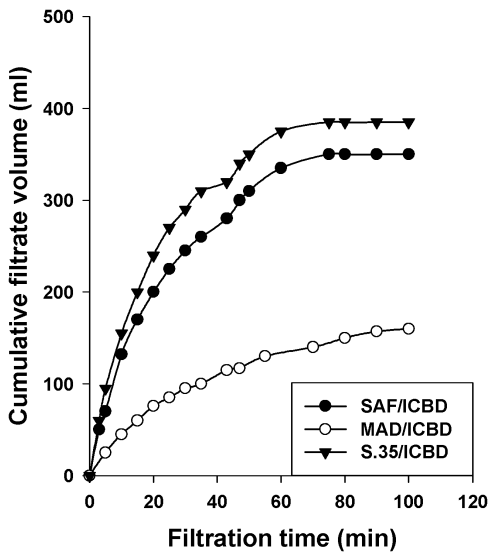


Fig. 5. Cumulative filtrate volumes as a function of time for worts of the sorghum cultivars Safhari, Madjeru and S.35 after mashing by decantation. Worts of laboratory malts were obtained using 50 g grist, while the *Bili-Bili* worts were obtained using 25 g grist of malts.

Fig. 6. Time-course for β -glucanase development during germination at 26 and 32°C for Safhari, Madjeru and S.35 cultivars respectively.

TABLE V. α -Amylase activity (units/g) of sorghum malts for different localities.

Cultivar type	ICBD	Mindjil	Yagoua
Safrari	94.56	21.62	28.95
Madjeru	56.59	12.03	10.34
S.35	51.70	22.18	20.49

TABLE VI. β -Amylase activity (units/g) of sorghum malts for different localities.

Cultivar type	ICBD	Mindjil	Yagoua
Safrari	43.02	27.84	27.36
Madjeru	2.15	3.94	3.70
S.35	30.11	32.50	27.36

TABLE VII. Viscosity (cP) of sorghum worts for different localities.

Cultivar type	ICBD	Mindjil	Yagoua
Safrari	1.72	1.48	1.39
Madjeru	2.43	1.54	1.44
S.35	1.56	1.39	1.32

TABLE VIII. β -Glucanase activity (U/Kg) of sorghum malts for different localities.

Cultivar type	ICBD	Mindjil	Yagoua
Safrari	62	536	209
Madjeru	159	853	211
S.35	179	211	419

TABLE IX. Total soluble nitrogen (TSN % dry weight) and α -amino nitrogen (FAN in mg/L) of sorghum malts from different localities.

	TSN	FAN		
		ICBD	Mindjil	Yagoua
Safrari	0.54	127	104	111
Madjeru	0.62	113	114	97
S.35	0.56	133	103	114

quality malts than the cultivars of Safrari and S.35, when malted either locally in the Cameroon, or in the laboratory. The β -amylase development is very low in this cultivar. Both the malting conditions and the genetics of the different cultivars influenced the malting performance of the sorghums studied.

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