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Sorghum Beer Brewing Using *Eleusine coracana* "Finger Millet" to Improve the Saccharification

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ABSTRACT

Three *Eleusine coracana* "finger millet" varieties (Musama, N161 and Mwamba) from Rwanda were studied with a view to improve the saccharification during the mashing of red sorghum malt (Kigufi, variety of Rwanda). Traditional mashing procedure (infusion) and decantation mashing procedure were employed and the produced worts were assessed for their brewing qualities. The findings prove that β-amylase activities of Musama (301.6 U mL^{-1}), N161 (227.2 U mL^{-1}) and Mwamba (112.6 U mL) malts were much higher than in Kigufi sorghum malt (73.3 U mL^{-1}). The mashing of the mixture of sorghum malt with Rwandan finger millet (Musama variety) malt allowed to produce more fermentable sugars, particularly maltose, reaching amounts 2-fold higher than when pure sorghum malt was used. Moreover, when the decantation method is applied, the fermentable sugars content draws a 100% increase by comparison to the traditional mashing method (infusion). However, the free amino nitrogen content of the worts obtained by decantation mashing procedure was slightly lower than that of the worts obtained by traditional mashing procedure, but was still within the range needed for yeast growth. In African context, *Eleusine coracana* malt could be used for improve the conversion of sorghum starch into fermentable sugars during sorghum beer brewing.

Key words: Sorghum, *Eleusine coracana*, enzyme, saccharification, fermentation, beer

INTRODUCTION

Unlike barley, classified in the same *Graminea* family, sorghum is very well adapted to the semi-arid and sub-tropical conditions prevailing over most of the African continent (Agu and Palmer, 1998). The resulting beers are known as *Ikigage* in Rwanda (Lyumugabe *et al*., 2010), as tchoukoutou in Benin or Togo (Kayode *et al*., 2005), as dolo in Burkina-Faso (Dicko *et al*., 2006) and many other equivalents in Nigeria, Ghana, Sudan, Chad, South Africa (Lyumugabe *et al*., 2010) with variations in manufacturing processes (Haggblade and Holzapfel, 2004).

In Rwanda, *Ikigage* beer is appreciated in various festivals and Rwandese ceremonies (e.g., marriage, birth, baptism, dowery, etc) and constitutes a source of economic return for the women manufacturers. However, like the most of African sorghum beers, *Ikigage* is less attractive as convenience beverage than western beers brewed with barley malt because of poor hygienic quality and low ethanol content (Lyumugabe *et al*., 2010). The low conversion of sorghum starch into

fermentable sugars for yeast (*Saccharomyces cerevisiae*) is incriminated. The low levels of β-amylase activity in sorghum malt can explain the low saccharification rate (Palmer, 1989; Dufour *et al*., 1992). Other studies showed that the main reason is likely to be inadequate gelatinization of sorghum starch rather than inadequate levels of hydrolytic enzymes (Agu and Palmer, 1997a; Dufour *et al*., 1992).

Decantation mashing procedure is widely applied in West Africa. Some authors have proved that the process of removing the "enzymatic supernatant" and rising gelatinization temperature helps enhancing starch gelatinization while preserving the enzymatic activity pool as well. This method proved relative high levels of starch extracts in comparison to those of barley malts (Palmer, 1989; Igyor *et al*., 2001). Still lower fermentable sugars yield of these sorghum worts suggested insufficient β-amylase levels (Palmer, 1989). The use of commercial enzymes (Dale *et al*., 1989; Bajomo and Young, 1994; Goode *et al*., 2003) or the mixtures of malted barley with sorghum (Okafor and Aniche, 1980; Goode and Arendt, 2003) during mashing were proposed as a solution to increase the β-amylase activity level. However, these optimal solutions for reducing the levels of non-fermentable sugars in sorghum wort are incongruous in an African traditional brewing context because tropical climate is not conducive to barley cultivation and commercial enzymes are not sustainable for reasonable production cost. Further, researches on the mixture of sorghum with local cereals have still not been extensively investigated.

Eleusine coracana, named commonly finger millet because the plant head resembles the hand fingers, is an annual cereal cultivated in Eastern and Southern Africa as well as Southern Asia (De Wet *et al*., 1984). Since ancient time, the grains are used or brewing African opaque beers (Nour and Davies, 1982; Gadaga *et al*., 1999; Muyanja *et al*., 2003; Lyumugabe *et al*., 2010), but little is known about its real interest in brewing. This study assesses the use of *Eleusine coracana* (called "Uburo" in Rwandan language) in sorghum brewing in order to increase the fermentable sugars of sorghum wort and ethanol content of traditional sorghum beer. In this study α- and β-amylase levels, free amino nitrogen and proteins content of three *Eleusine coracana* (Musama, N161 and Mwamba varieties) and red sorghum (Kigufi variety) from Rwanda were evaluated. Traditional mashing procedure (infusion) and decantation mashing procedure developed for sorghum malt were employed and the produced worts were assessed for their brewing qualities.

MATERIALS AND METHODS

Plant materials: The red sorghum grains (Kigufi variety) and *Eleusine coracana "*uburo*"* grains (Musama, N161 and Mwamba varieties) used in this study were obtained from Rubona and Musanze stations of Rwanda Agriculture Board (RAB). Several varieties of sorghum occur in Rwanda, among them, Kigufi is mostly used to prepare Rwandan traditional sorghum beer "*Ikigage*".

Malting procedure: After removal of broken kernels and debris, the selected grains (1 kg) were steeped in distilled water (2 L) at 25°C for 24 h. Before and after steeping, grains were sterilized by immersion in sodium hypochlorite solution (1% wt/v). After rinsing with sterile distilled water as described elsewhere (Ezeogu and Okolo, 1995), the grains were germinated at 30°C for 72 h and then kilned at 50°C for 24 h. The shoots and rootlets were removed manually and the malt kernels were fine milled.

Malt analyses

Alpha and beta amylase activities: Alpha and Beta amylase activities were determined on malt by using specific colorimetric methods developed by Megazyme international (Ireland Ltd, Irlande):

AMYLAZYME (Azurine-crosslinked amylose = AZCL-Amylose) for α-amylases and BETAMYL for β-amylase. The results are expressed in Cerapha units per gram corresponding to enzymes quantity necessary to release a p-nitrophénol (μM min^{-1} g^{-1} of dry matter). Dry Matter (DM) contents were determined by oven drying at 105°C to constant weight.

Protein and free amino nitrogen: Protein content in grain was determined with the Kjeldahl method using a Vapodest 30 sec (Gerhardt, Königswinter, Germany) and by multiplying the results with the 6.25 coefficient.

The extraction of free amino nitrogen in malt was carried out as described by Pelembe *et al*. (2002). The free amino nitrogen was determined by European Brewery Convention (EBC., 1998a) using Ninhydrin method and glycine as reference amino acid.

Wort production*:* The wort was produced by Rwandese traditional mashing procedure described elsewhere (Lyumugabe *et al*., 2010) and by decantation mashing procedure developed for sorghum (Palmer, 1989; Agu and Palmer, 1996; Igyor *et al*., 2001).

Traditional mashing procedure: This technique is a slight modification of Rwandese traditional procedure. Fifty gram milled malt were added to 1500 mL distilled water and heated up to at 80°C for 30 min and then cooled below 65°C before addition of 250 g milled malt were added. The mash was stirred and the temperature is maintained at 65°C for 60 min and then cooled to 30°C.

Decantation mashing procedure: Three hundred grams of milled malt were mixed with 1500 mL distilled water at 45°C and left in decantation during 30 min. Thereafter, 750 mL of the clear "enzymatic supernatant" was removed while mash residues were heated at 90°C for 30 min to gelatinize malt starch. After cooling below 50°C, the clear "enzymatic supernatant" was re-added and then the mixture was brewed according to the following mashing program: the 60 min at 63°C, 10 min at 75°C and cooled to 30°C.

Wort analyses

Extract, fermentability and free amino nitrogen: The specific gravity was measured in triplicate using the pycnometer method at 20°C. Wort extracts was calculated according to method 8.3 of European Convention of Brewing (EBC., 1998b). Fermentability was determined also according to method 8.6 of European Convention of Brewing (EBC., 1998d). The Ninhydrin method was used to estimate free amino nitrogen present in wort according to method 8.10 of European Convention of Brewing (EBC., 1998a).

Sugars and starch: The sugars composition of wort samples was performed by HPLC on an Agilent 1100 series apparatus (Agilent Technologies, Massy, France) equipped with a refractometric detector. The samples, previously filtered through a 0.2 μm acetate membrane, were eluted with 0.1% H_3PO_4 at 0.5 mL min^{-1}. Sugars were separated on a C-610-H ion exchange column (300×7.8 mm, supelco, Bellefonte, PA) preceded by a pre-column H (5 cm×4.6 mm, supelco, Bellefonte, Pennsylvania, United States). Qualitative analysis of starch was done on wort using the iodine colour complex reaction. The iodine starch complex leading to blue-black colour, indicate the presence of starch.

Viscosity and colour: Viscosity was determined by method 8.4 of European Convention of brewing (EBC., 1998c) using glass capillary viscometer. Colour was also determined using procedure recommended by European Convention of Brewing.

Fermentation: Filtered wort (500 mL) was pitched with yeast *Saccharomyces cerevisiae* (RV6 strain) in a sterile 1000 mL Erlenmeyer flask equipped with a gas trap. The RV6 strain was obtained from the Walloon Center of Industrial Biology (CWBI, Gembloux, Belgium). Yeast was propagated on 10 mL of YPD (Yeast extract, Peptone, Dextrose) broth and incubated at 30°C for 24 h. The inoculum was prepared by transferring propagated yeast to sterilized 10 mL YPD (15 h incubation) then placed into 50 mL YPD broth (24 h incubation). Fifteen milliliters of the resulting culture were placed in 100 mL wort and incubated for 24 h. Finally, experimental worts (500 mL) were pitched with 10^6 yeast cells mL^{-1} and the fermentation was carried out at 30°C for 72 h. Ethanol was determined by enzymatic method using the Boehringer Kit (R-Biopharm AG,D-64293 Darmastadt).

Statistical analysis: The experiments were conducted in triplicate and results of biochemical properties of malts and sugar profile of worts were expressed as mean with standard deviation. The means were compared using epi-info software version 6.04. Statistical significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Biochemical properties of sorghum and finger millet malts from rwanda: Starch hydrolytic activities of grains assessed by colorimetric methods show important differences between malts from Rwandan sorghum (Kigufi variety) and finger millet (Musama, N161 and Mwamba varieties) (Fig. 1). The higher level of α-amylase activity is given for Kigufi sorghum variety (268.2 U mL^{-1}) and the lowest levels in the Mwamba eleusine variety (43 U mL^{-1}). The high α-amylase potential of sorghum has been observed by several authors (Aisien, 1982; Aisien and Palmer, 1983). The α-amylase levels here reported for the Kigufi sorghum reveal to be in similar ranges than the levels previously pointed out by other authors for sorghum malts (Dufour *et al*., 1992; Beta *et al*., 1995; Agu and Palmer, 1997b; Letsididi *et al*., 2008; Ba *et al*., 2010) and barley malt (Brennan *et al*., 1997; Georg-Kraemer *et al*., 2001).

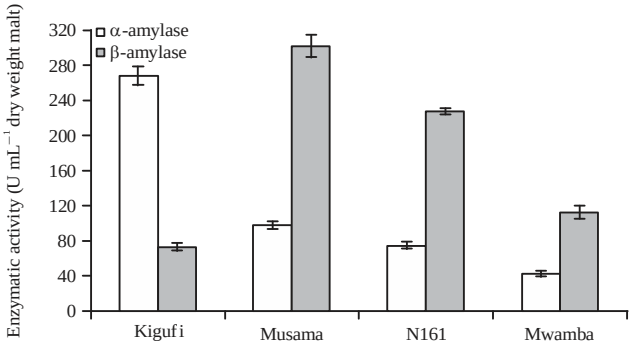

Fig. 1: Enzymatic activity of sorghum (Kigufi variety) and finger millet (Musama, N161 and Mwamba varieties) malts from rwanda

Table 1: Free amino nitrogen and proteins contents of sorghum (Kigufi variety) and finger millet (Musama, N161 and Mwamba varieties) from rwanda

Parameters	Kigufi	Musama	N161	Mwamba
Proteins (%)	10.9±0.5	10.5±0.3	10.8±0 .4	9.6±0.3
Free amino nitrogen (mg L^{-1})	76.7±2.7	121.0±3.6	98.9±5.8	128.0±7.2

Concerning the β-amylase activity, Kigufi sorghum variety (73.3 U mL^{-1}) exhibited a lower level than in finger millet malts from the varieties of Musama (301.6 U g^{-1}), N161 (227.2 U mL^{-1}) and Mwamba (112 U mL^{-1}) varieties. Similar trends were previously reported in comparison of β-amylase activity levels in finger millet malts (81-608 U mL^{-1}) and sorghum malt (17-57 U mL^{-1}) (Taylor and Robins, 1993; Taylor, 2009). The findings prove that the β-amylase activity level of sorghum Kigufi variety is slightly higher than the activity levels reported by Taylor and Robins (1993) while using same method. However, remains lower once compared to the kind of sorghum cultivars from Botswana (Letsididi *et al*., 2008) and Nigeria (Agu and Palmer, 1996).

However, β-amylase activity of finger millet malts used in this work was considerably higher than the reported for sorghum and sometimes slightly lower than reported for barley (414 U mL^{-1}) by Taylor and Robins (1993). The generation of maltose in wort being essential for a good fermentation (Ziegler, 1999), the high saccharification activity potential of those Rwandan finger millet varieties is very significant with respect to its potential for beer brewing.

The results in Table 1 did not show a great difference between total protein contents of sorghum (11%) and finger millet malts (10-11%). However, finger millet malts contained larger amounts of free amino nitrogen (99-128 mg L^{-1}) when compared to sorghum malt (77 mg L^{-1}). In sorghum malt, the lower nutrient content can be explained by insufficient proteolysis due to the vitreous nature of sorghum endosperm structure (Klopfenstein and Hoseney, 1995) or by the too high specificities of endopeptidase in the proteins decomposition (Agu and Palmer, 1999). As a consequence, low protein hydrolysis into free amino nitrogen and short chain peptides is obtained (Taylor and Evan, 1989).

Use of finger millet malt in sorghum beer brewing: Decantation mashing procedure involves the partition of the wort by removal of enzymatic supernatant in order to conduct adapted gelatinization temperature to those tropical cereals (80-100°C) while preserving the hydrolytic enzyme pool integrity. Enzymatic part is then reintroduced in the cooled wort for infusion mashing (65°C for 60 min and 75°C for 10 min). In order to assess the interest of this method with the Rwandan varieties and the introduction of finger millet malts in sorghum malt for brewing, the decantation mashing and Rwandan traditional mashing (infusion at 65°C) are here compared with different combination of the two malted cereals.

The results of effect of mashing method and Rwandan finger millet on sorghum wort properties are shown in Table 2. The higher β-amylase activity of finger millet malt was confirmed by a significant increase of wort extract when used 30% finger millet (9.5% sugar density (w/w) with traditional infusion mashing versus 8.3% sugar density (w/w) with pure sorghum malt). Those values were even higher when decantation mashing was applied (13% sugar density (w/w) with mixture of sorghum and uburo malt versus 9.4% sugar density (w/w) with pure sorghum malt). Other studies (Igyor *et al*., 2001) reported also the higher extract rate of worts from decantation mashing procedure most probably due to better gelatinization of sorghum starch.

However, the results in Table 2 showed also that the free amino nitrogen contents in worts (70% sorghum mixed with 30% of Rwandan finger millet) obtained by traditional infusion mashing procedure is slightly higher (192.3 mg L^{-1}) compared to those obtained by decantation mashing

Table 2: Effect of mashing method and finger millet on sorghum wort properties

	Traditional infusion mashing				Decantation mashing			
Parameters	100% sorghum	90 % sorghum and 10% finger millet	80 % sorghum and 20% finger millet	70% sorghum and 30% finger millet	100% sorghum	90 % sorghum and 10% finger millet	80 % sorghum and 20% finger millet	70% sorghum and 30% finger millet
Extract	8.30	8.30	8.70	9.50	9.40	9.70	11.40	13.00
Fermentability (%)	68.80	nd*	77.70	76.20	72.00	nd	77.80	78.80
Free amino nitrogen (mg L^{-1})	138.20	145.20	172.00	192.30	129.90	141.00	163.40	183.10
Colour (°EBC)	7.10	7.70	11.10	11.20	9.10	9.00	18.20	18.90
Viscosity (cp)	1.32	1.32	1.34	1.37	1.46	1.47	1.52	1.57
Iodine test	+	+	+	+	+	+	+	+

*Not determined, +: Positive iodine reaction indicating the presence of starch

Table 3: Sugar profile (g L^{-1}) of worts from sorghum and finger millet malts

	Traditional infusion mashing		Decantation mashing	
Parameters	100% sorghum	70% sorghum and 30% finger millet	100% sorghum	70% sorghum and 30% finger millet
Glucose	9.3±0.05	7.0±0.07	18.5±0.77	15.1±1.03
Maltose	13.4±0.70	25.7±1.21	28.2±0.82	53.3±1.33
Maltotriose	5.0±0.05	12.4±0.62	9.9±1.01	23.5±0.51
Fructose	1.3±0.02	1.8±0.07	2.1±0.02	3.6±0.30

Table 4: Characteristics of beers made with sorghum and finger millet malts

	Traditional infusion mashing		Decantation mashing	
Parameters	100% sorghum	70% sorghum and 30% finger millet	100% sorghum	70% sorghum and 30% finger millet
pH	4.60	4.50	4.50	4.40
Specific gravity	1.006	1.009	1.007	1.013
Ethanol (% v/v)	2.30	3.10	3.30	4.70
FAN (mg L^{-1})	51.00	64.20	48.00	41.40
Colour (°EBC)	6.20	6.00	11.00	19.70

procedure (183.1 mg L^{-1}). This can be explained by the temperature of infusion mashing (65°C) increasing the activity of proteolytic enzymes (Briggs *et al*., 2004) while the decantation mashing method probably denatured and precipitated the proteins of wort, rendering them less susceptible to proteolytic enzyme degradation (Agu and Palmer, 1997c).

The preservation of the enzymatic pool from high temperature treatment results into higher fermentable sugars level, maltose particularly (Table 3). These results confirm also the influence of β-amylase, from Rwandan finger millet malt, in the production of maltose with a twofold increase with respect of the same mash composition (e.g. 13.4 g L^{-1} with pure sorghum versus 25.7 g L^{-1} with 30% of Rwandan finger millet malt in infusion mashing method (Table 4).

The decantation mashing method permitted a twofold gain in maltose content from the same malted cereal combination, leading to a fourfold increase when this method is applied with 30% of Rwandan finger millet malt (53.3 g L^{-1}) in comparison with pure sorghum in traditional infusion method (13.4 g L^{-1}). However, a slight diminution of glucose content is observed with the use of finger millet malt. The dilution of the a-amylase pool by diminution of sorghum part can explain this observation.

CONCLUSION

This study shows that the β-amylase activity is higher in finger millet "*Eleusine coracana*" malts than in sorghum malt. The mixture of sorghum malt (70%) with finger millet (Musama variety) malt (30%) mashed by decantation method produced sufficient extract,

fermentable sugars, free amino nitrogen and ethanol during fermentation than 100% sorghum malt. Although, the decantation mashing procedure proved able to increase by two-fold the fermentable sugars of wort (maltose), free amino nitrogen was found slight low level than traditional infusion mashing method, but still in sufficient level for a proper fermentation (leading to a 50% increase in ethanol). To improve brewing of traditional sorghum beer in Africa context, finger millet malt could be used in place of barley or extraneous enzyme. More research would be needed to establish the flavor of the final beer produced from this study.

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