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RESEARCH ARTICLE

MALTING CHARACTERISTICS OF SOME NEPALESE FINGER MILLET (*Eleusine coracana*) VARIETIES

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ABSTRACT

Finger millet is the fourth most important food crop of Nepal. In many countries millet has been used as a major ingredient in the manufacture of malt. Millet malt is extremely used in the preparation of weaning foods, infant foods and beverages. Today there is a growing demand for gluten free food and beverages from people with celiac disease. Information regarding the malting of finger millet in Nepalese context is scarce. The aim of this study was to investigate the malting characteristics of some Nepalese finger millet varieties. Six finger millet varieties were germinated at 28±1 ºC for 48 to 84 h, kilned at 50±2 ºC and analyzed for amylase and carboxypeptidase activities, total free amino acids and free amino nitrogen contents in the malts. All native millets had negligible α-amylase activity (0.05–0.11 units) while 72 h germinated Juwain millet showed the highest α-amylase activity (22.96 units). Beta amylase activity in native millets were in the range of 0.1–2.4 units and it reached maximum value on 48h of germination in all millet varieties with a highest value of 385 units in Kabre millet. Diastatic activity ranged from 8.3 to 17.3 ºDP (db) in 48 h germinated millet malts. Forty-eight hour germinated Juwain millet exhibited the highest carboxypeptidase activity (242.5 units), total free amino acids (310 mg glycine/100 g dry malt) and free amino nitrogen (57.8 mg glycine/100 g dry malt) contents of all the millet malts. Although Juwain millet variety seemed best for malting, further investigation on malt extract and wort analyses are suggested for their brewing quality assessment.

INTRODUCTION

Millet is an annual grass that is extensively used in tropical and sub-tropical areas of the world. It is the fifth most important cereals in the world after wheat, maize, rice and barley (Shayo *et al*, 2001). In East African countries, among other uses, millet is malted and used to brew various traditional beers (Ekundayo, 1996). Malting is simply a biochemical modification of the grain to produce malt that has enhanced nutritional quality than normal grain. During malting the grain develops amylolytic enzymes, which hydrolyze starch to fermentable sugars. Some advantages brought about by malting include the improvement of grain nutritional quality by reducing the antinutritinal factors responsible for poor digestibility and increasing palatability of the grains. Use of millet to replace extensive imported barley malt in beer brewing has been tried (Skinner, 1976; Nout and Davies, 1982). Sorghum has substituted barley malt in the production of lager beer in Nigeria (Koleoso and Olatunji, 1988). The malting of finger millet is superior to other millet and it is ranked next to barley (Malleshi and Desikchar, 1986). Taylor *et al*. (2006) also reported that finger millet malt has a higher level of β-amylase activity than that of sorghum and much less than that of barley. Millet in the form of malt is probably a much better option than using unmalted grain as adjunct in brewing (Taylor, 2009).

In many countries, millet has been used successfully as a substitute for barley, for instance, finger millet has been used in the East Africa (Kenya) and India as a major ingredient in the traditional manufacture of malt (Nout, 1981). Local maltsters and brewers in Kenya claim that millet malts are preferred locally because of their higher enzymatic activity and flavor (Nout, 1981; Singh *et al*. (1988). Millet is also a good source of α- and β-amylases and malted millet is extremely used in weaning food, infant food and supplementary food formulations (Malleshi, 2005). Pearl millet is widely home malted in sub-Saharan Africa for small scale brewing of traditional African beer. A small amount is industrially malted in Zimbabwe for commercial opaque beer brewing to supplement sorghum malt (Pelembe *et al*., 2004). Finger millet is important millet and its malting has been practiced both at home and industrial level in India and in some of the African countries (Ravindran, 1991). Malted ragi flour or extracts derived from it are extensively used in the preparation of weaning and infants' foods, beverages or other pharmaceutical preparations (Narayanaswamy *et al*., 1971).

Finger millet (*Eleusine coracana*) is the fourth most important food crops of Nepal. It is a vital crop for mid-hills where it is commonly grown as a relay crop in maize/millet system and as a mono crop in the hilly areas of mid-western region. Finger millet occupied 265,496 ha of land and produced 291,098 MT with the productivity of 1,096 kg/ha (NARC, 2010). In Nepal, there are 5 breweries in operation at present and produce about 500,000 hL of beer annually utilizing about 7,000 MT of barley malt that are imported from abroad. This proves that about 3,000 m Nepalese rupees (40m US$) is being used to purchase barley malt for Nepalese breweries annually. Use of finger millet is limited only to some traditional food preparations like *jand* (a traditional alcoholic fermented undistilled alcoholic beverage), *rakshi* (distilled alcoholic drink), *roti* and *dhido* (traditional staple food preparations in Nepal). Hence, finger millet malt could substitute barley malt, partly if not totally, that helps to pause overflow of a huge Nepali currency thereby making benefit to both industrialists and farmers. Moreover, in the developing countries, today there is a growing demand for gluten-free food and beverages from people with celiac disease and other intolerances who cannot eat products from wheat, barley or rye (Fasano and Catassi, 2001). The aim of this work, therefore, was to analyze the malting characteristics of Nepalese finger millet varieties with the view to determining their suitability in brewing lager beer.

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MATERIALS AND METHODS

Malting of finger millet

Six finger millet varieties (GPU 0025, GE 5016, Dalle, Okhle and Kabre varieties from Hill Crops Research Program, Dolakha, and Juwain millet from Khotang district of Nepal) were collected. They were cleaned and washed thoroughly to remove all immature grains, light materials and dirt. They were then steeped in surplus water at room temperature (28º±2ºC) for overnight. After soaking, the grain was drained, spread on aluminum tray (7±1 mm bed thickness), covered with moistened muslin cloth and germinated for different time (48, 72 and 84 h) in a BOD chamber maintained at 28º ±1 ºC and 93±2% RH. During germination, the millet was turned, sprayed with water and covered with wetted muslin twice a day. The germinated millet (green malt) was kilned in a mechanical dryer (REICO Drying Chamber, India) at 50º±2ºC for 24 h and rootlets were removed by rubbing and winnowing.

Enzyme analyses

Enzyme extraction

One gram of powdered sample was ground in a pestle and mortar with distilled water, volume made up to 50 mL, filtered through Whatman No. 1 filter paper and the filtrate was used as enzyme source for α- and β-amylase activity determination. For carboxypeptidase activity , 2 g of powdered malt was extracted with 20 mL of 0.1M citrate-phosphate buffer (pH 7.0), filtered through Whatman No 1 filter paper and volume made up to 25 mL with 0.1 M citrate-phosphate buffer.

Alpha-amylase activity

Alpha-amylase activity was determined as per Malik and Singh (1980). Briefly, a reaction mixture containing 2 mL of starch (150 mg starch, 600 mg KH_2PO_4, 20 mg anhydrous $CaCl_2$ dissolved in 100 mL distilled water, boiled for 1 min, cooled and filtered) and 1 mL of diluted enzyme were mixed in a test tube and incubated at 40ºC for 30 min. The reaction mixture and enzyme were brought to 40ºC before mixing. At zero and 30 min of incubation, 0.2 mL of the aliquot of reaction mixture was mixed with 3 mL of IKI solution (254 mg iodine and 4 g KI dissolved in 1 L of water) and absorbance was measured at 620 nm using a digital spectrophotometer (M.S.Electronics, Pvt., Ltd, India, model 305). The instrument was adjusted to 100% transparency with distilled water. Enzyme activity was expressed in terms of decrease in optical density per min at 620 nm per gram of dry sample.

Beta-amylase activity

Beta-amylase activity was determined as per Malik and Singh (1980). Briefly, one mL of starch solution (1% in 0.067 M phosphate buffer, pH 6), 1 mL of undiluted enzyme extract and 1 mL of 0.1M EDTA were mixed in a test-tube and incubated for 30 min at 37ºC (the substrate and enzyme were brought to 37ºC before mixing) and the reducing sugar contents in the control (0 min incubation) and sample (30 min incubation) were determined by Nelson-Somogyi method as per Sadasivam and Manickam (1996) using a standard curve prepared from maltose sugar (Sisco Research Lab Pvt., Ltd., Mumbai, India)). Enzyme activity was expressed in terms of mg maltose produced per g of dry malt over 30 min of incubation.

Carboxypeptidase activity

Carboxypeptidase activity was determined as per Okolo and Ezeogu (1995) with slight modifications. Two mL of the enzyme extract and 2 mL of 2.5 mg/mL bovine serum albumin (Central drug house, Pvt. Ltd., India) prepared in 0.1 M citrate-phosphate buffer of pH 7, were pipetted in a test tube, mixed and incubated for 1 h at 40 ºC after which the reaction was terminated by adding 2 mL of 15% TCA. The mixture was centrifuged at 3000 rpm (Remi lab centrifuge, India), the supernatant separated and the residue washed with 2 mL of TCA followed by centrifugation. The supernatants were pooled together and the final volume made up to 10 mL with distilled water. Free amino nitrogen (FAN) content in the filtrate was determined as per AOAC Official Method 945.30 (AOAC, 2005) and carboxypeptidase activity was calculated as the difference between the FAN (glycine equivalent) contents (µg FAN/h. g dry malt) of the filtrate from 1 and 0 h incubated reaction mixtures.

Diastatic power

Malts were ground in a coffee grinder and were used for diastatic activity determination by ferricyanide modification method as per AOAC Official Method 935.31 (AOAC, 2005) using extra pure soluble starch (Merck Ltd., Mumbai, India).

Total free amino acids (TFAA) and free amino nitrogen (FAN)

Five hundred milligram of powdered sample was extracted with 10 mL of 80% ethanol for overnight at room temperature, centrifuged and the supernatant separated. The residue was re-extracted with 5 mL of 80% ethanol for 12 h, centrifuged, the supernatants pooled together and volume made up to 15 mL with 80% alcohol. TFAA and FAN contents were determined as per AOAC Official method 945.30 (AOAC, 2005).

Statistical analyses

The experiment was conducted in a Completely Randomized Design (CRD) with three replications. The data were analyzed using two-way ANOVA as per Buysse *et al*. (2007) at 5% level of significance and the means were compared by LSD method.

RESULTS

Different finger millet varieties were germinated for 0, 48, 72 and 84 h at 28±1ºC, kilned at 50±2 ºC and analyzed for α-amylase, β-amylase, total diastatic and carboxypeptidase activities and total free amino acids (TFAA) and free amino nitrogen (FAN) contents of millet malts. Alpha amylase activity of millet malts was significantly affected ($p<0.05$) by millet varieties and germination times and the results are shown in Fig. 1

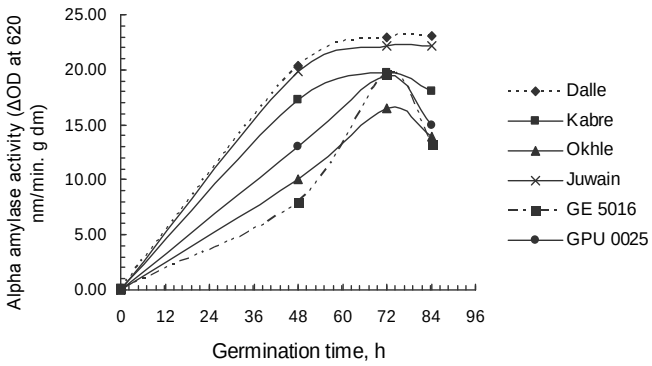

Fig. 1. Effect of millet variety and germination time on α-amylase activity of millet malts[#]

#: values are the means of three replications.
1 unit of α-amylase activity was defined as 1 unit decrease in OD at 620 nm per min by 1 g of dry malt.

Alpha amylase activities of ungerminated millets were in the range of 0.05 – 0.11 units but the values were not significantly different ($p>0.05$). Statistical analysis showed that both millet variety and germination time had a significant effect on α-amylase activity of malts. Increasing germination time up to 72 h significantly increased the α-amylase activity in all millet varieties. Alpha amylase activities in 48 h germinated malts were 20.41, 17.23, 10.02, 19.80, 7.95 and

13.03 units in Dalle, Kabre, Okhle, Juwain, GE 5016 and GPU 0025 millet varieties respectively. While the values in 72 h germinated malts were 22.96, 19.73, 16.55, 22.16, 19.54 and 19.47 units for the respective millet varieties. Germination beyond 72 h significantly reduced α-amylase activity in Kabre, Okhle, GE 5016 and GPU 0025 millet varieties while no remarkable changes were found in Dalle and Juwain millet malts. In Dale, Kabre and Juwain millets, large increment in α-amylase activity was observed during 48 h of germination and further germination up to 72 h increased α-amylase activity by about 2.5 units. In Okhle, GE 5016 and GPU 0025 varieties, a greater extent of α-amylase activity was found during 72 h of germination with about 6.5, 11.6 and 6.4 units higher α-amylase activity compared to the respective 48 h germinated millet malts. Alpha amylase activity of 72 h germinated Kabre, GE 5016 and GPU 0025 malts were similar (19.6 ± 0.1 units). Similarly, the values for Dalle and Juwain malts were also similar (22.6 ± 0.4 units). It was found that 72 h germination of either Dalle or Juwain could give the maximum α-amylase activity in malts of all the treatment combinations studied. Like α-amylase activity, β-amylase activity was also significantly affected by both millet variety and germination time and their interaction effect was also significant ($p<0.05$). Except for Juwain millet, β-amylase activity was highest at 48 h of germination and further germination significantly reduced β-amylase activity in all millet varieties.

Beta amylase activity in native millets was in the range of 0.1 – 2.4 units but the values were not significantly different. Beta amylase activities in 48 h germinated millet malts were 357.4, 385, 360.2, 244.9, 349.2 and 355.3 units in Dalle, Kabre, Okhle, Juwain, GE 5016 and GPU 0025 millet varieties respectively. Statistical analysis showed that β-amylase activity of Kabre malt was highest (385.0 units) of all the treatment combinations used while the values among Dalle, GPU 0025 and Okhle malts were not significantly different ($p>0.05$). From Fig. 2, it can be observed that Kabre and Juwain millet had an intermediate interaction with germination time while the rest of the millet varieties showed a strong interaction with germination time on malt β-amylase activity. Although, α-amylase activity was higher in 72 h of germination, β-amylase activity, TFAA and FAN contents (Fig. 5 and 6) were significantly higher in 48 h germinated millet malts. Therefore, total diastatic power of 48 h germinated millet malts were determined and the results were found to be 12.3, 17.3, 8.3, 11.3, 8.3 and 15.0 ºDP (dry basis) for Dalle, Kabre, Okhle, Juwain, GE 5016 and GPU 0025 millet malts respectively (Fig. 3). Statistical analysis revealed that Kabre millet malt had the highest diastatic power followed by GPU 0025 of all the malts but the values between GE 5016 and Okhle and between Juwain and Dalle malts were not significantly different. Hence, based on amylase activity, Kabre millet germinated for 48 h could give the best millet malt of all the treatment combinations studied. Effect of millet variety and germination time on carboxypeptidase activity is depicted in Fig. 4. Millet variety, germination time and their interaction all significantly affected carboxypeptidase activity of millet malt.

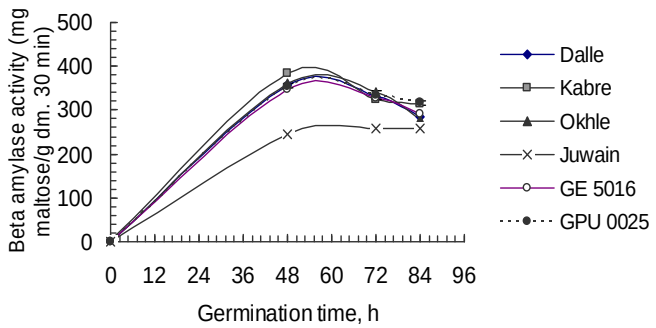

Fig.2. Effect of millet variety and germination time on β-amylase activity of millet malts#

#: Values are the means of three replications.
1 unit of β-amylase activity was defined the production of 1 mg maltose by 1 g dry malt over 30 min of incubation under the experimental conditions.

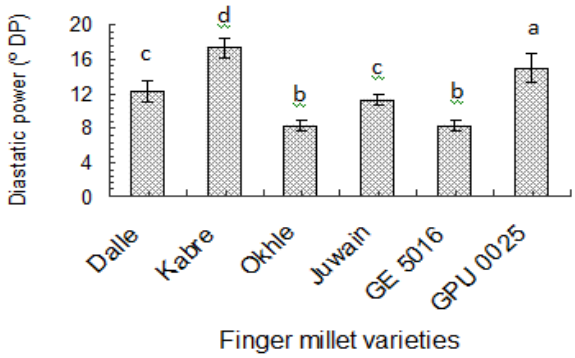

Fig. 3. Total diastatic power (DP) of millet malts germinated at 28±1 ºC for 48 h#

#: values are the means (n=3) ± SD. Bars sharing similar letters are not significantly different ($p>0.05$) by LSD.

Carboxypeptidase activity increased with germination time in Kabre millet variety with a maximum value of 134.2 units on 84 h of germination while in Juwain millet a maximum activity of 242.5 units was found on 48 h of germination. In the case of GPU 0025 variety, carboxypeptidase activity ranged from 66.6 to 73.4 units over the germination time of 48 – 84 h, and the values were statistically not different. Carboxypeptidase activity was maximum at 72 h of germination and decreased thereafter in Okhle and GE 5016 millet with maximum activities of 60.1 and 42.4 units for the respective millet varieties. No remarkable difference in carboxypeptidase activity between 48 and 72 h germinated Dalle malt was found with a maximum value of 73.4 units on 84 h of germination. On the whole, maximum carboxypeptidase activity of 242.5 units was found in 48 h germinated Juwain millet malt followed by 134.2 units in 84 h germinated Kabre millet malt. Fig. 5 and 6 show the effect of millet variety and germination time on total free amino acids (TFAA) and free amino nitrogen (FAN) contents in millet malts. Both the millet variety and germination time showed a significant effect on the TFAA contents in millet malts. Except for Juwain native millet, the TFAA contents were not different and the values were in the range of 53.9 – 57.9 mg glycine equivalent/100 g dm while Juwain millet variety had the highest TFAA content (87.1 mg glycine/100g dm) of all the millet varieties. TFAA contents reached maximum at 48 h of germination and decreased significantly on further germination in all millet varieties.

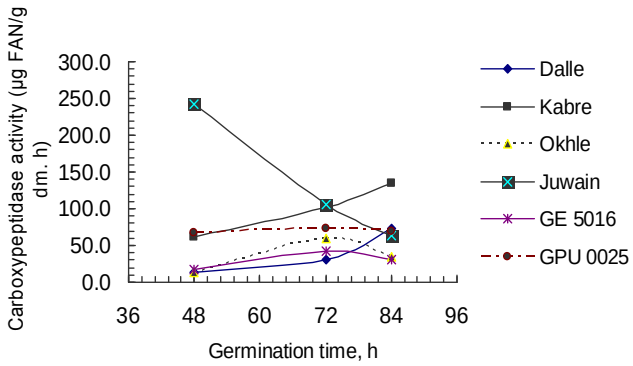

Fig.4. Effect of millet variety and germination time on carboxypeptidase activity of millet malts#

#:values are the means of three replications.
One unit of carboxypeptidase activity was defined as the production of 1 µg of free amino nitrogen (FAN) as glycine by 1 g of dry malt over 1 h of incubation at 40 ºC.

TFAA contents in 48 h germinated millet malts were 188.7, 209.0, 178.8, 310.0, 195.3 and 186.2 mg glycine/ 100g dm in Dalle, Kabre, Okhle, Juwain, GE 5016 and GPU 0025 millet varieties respectively. Statistical analysis indicated that Juwain malt had the maximum TFAA content (310.0 mg glycine/100g dm) of all the malts while the values among Dalle, Okhle, GE 5016 and GPU 0025 and between Kabre and GE 5016 malts were not significantly different ($p>0.05$). Juwain millet was found to have an intermediate interaction while rest of the millet varieties showed a strong interaction with germination time on TFAA contents of malts (Fig 5). A similar trend to that of TFAA contents was also found for FAN contents in millet malts (Fig. 6). Except in Juwain millet, FAN contents in other native millet varieties were in the range of 10.1 – 10.9 mg glycine/100g dm and the values were statistically not different while a maximum FAN content of 16.4 mg glycine/100g dm was found in Juwain millet malt. FAN content reached maximum in 48 h of germination and further germination resulted a significant reduction in all millet varieties. FAN contents in 48 h germinated malts were 35.2, 39.0, 33.4, 57.8, 36.4 and 34.7 mg glycine/100g dm in Dalle, Kabre, Okhle, Juwain, GE 5016 and GPU 0025 millet varieties respectively. Juwain malt had the highest FAN contents of all the malts while the values among Dalle, Okhle, GE and GPU and between Kabre and GE malts were not different. Juwain millet showed an intermediate interaction while all other millet varieties showed a strong interaction with germination time on FAN contents of malt. Hence, 48 h germinated Juwain millet resulted the maximum TFAA and FAN contents in the malt.

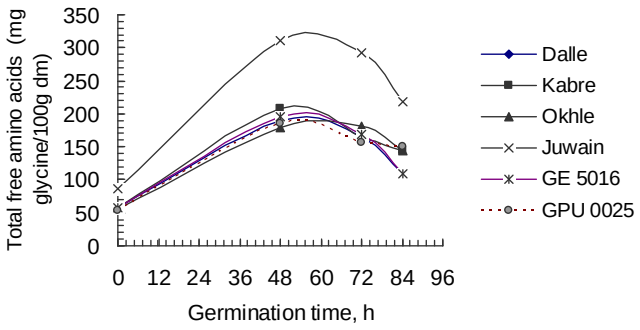

Fig.5. Effect of millet variety and germination time on total free amino acids (TFAA) contents of millet malts#

#: values are the means of three replications

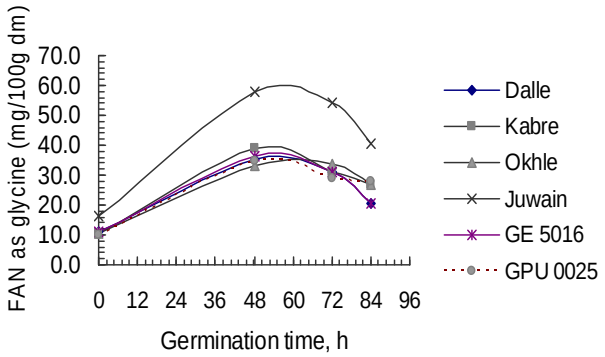

Fig. 6. Effect of millet variety and germination time on free amino nitrogen (FAN) contents of millet malts#

#: values are the means of three replications.

DISCUSSION

Amylase and carboxypeptidase activities

Nic Phiarais *et al*. (2005) reported 0.1 and 5.3 units/g of α- and β-amylase activities in native and malted buckwheat respectively. Similarly, Nithya *et al*. (2006) also reported the presence of α-and β-amylase activities (5.33 and 8.0 µM maltose/mL·min respectively) in native pearl millet. Absence of β-amylase activity in native pearl millet (Pelembe *et al*., 2004) and sorghum (Taylor and Robbins, 1993) were also reported. Beta-amylase activity of commercial barley malts was reported to be 354.8 mg maltose/g dry matter (Tiwari, 2010) which was slightly higher than that of Dalle millet malt found in this study. Tiwari (2010) also reported α-and β-amylase activities in the range of 49.6 - 55.5 and 194.5 - 215.5 units/g dry matter in Kabre-1 and ACC#523-1 finger millet varieties respectively. Seed germination triggers several metabolic changes and in turn increases the activity of various enzymes including amylases. According to Bewley and Black (1983), β-amylases were present in an inactive latent form and were activated during seed germination. Daussant *et al*. (1994) reported that two distinct categories of cereal β-amylases have recently been recognized to exist. One is the classical endosperm β-amylase of the *Triticeae* species, barley, wheat and rye; which is present in high amounts and at high activities in the ungerminated seeds.

Another "tissue ubiquitous" form of enzymes present in much lower amounts and activities in all cereals appear to represent the entire enzyme complements of the seed of the non-*Triticeae* species. Negligible α-amylase found in our study was also supported by Zeigler (1995) who reported the absence of this enzyme in native grains and its synthesis is the primary task of malting. β-amylase activity in Indian finger millet malts were in the range of 156-300 units (Shukla *et al*., 1986a) which agreed to our findings. Pelembe *et al*. (2004) reported that both diastatic activity and β-amylase activity in pearl millet malt increased with germination time. Diastatic activity was reported to increased with germination time in SE composite and SE 13 pearl millet varieties up to 6 days of germination at 28º–30ºC and decreased afterwards, while in the case of SE 2124 millet variety, diastatic power reached maximum in 2 days and decreased afterwards. Similar trend was also reported for α-amylase activity in the three millet varieties. For SE composite pearl millet variety, β-amylase activity was reported to be maximum on day 2, while for SE 13 and SE 2124 millet varieties it reached at maximum on 4 and 8 days of germination respectively (Nzelibe, 1995). According to Gimbi and Kitabatake (2002) the highest α-amylase activity in African finger millet was exhibited in finger millet malt flour germinated at 15 ºC for 9 days, and at 20 ºC for 6 days while the highest β-amylase activity being displayed in the malt flour germinated for 5 days at 30 ºC. But in our experiment there was excessive root growth beyond 84 h of germination at 28º ±1 ºC.

Wide variations in α- and β-amylase activities and total diastatic power (DP) among different millet malts could be due their varying ability to produce gibberellins, the hormones which are produced during germination of grain and diffuse into the endosperm and aleurone layer. In the endosperm, they induce the synthesis of amylases (Dendy, 1995). Amylases are responsible for the generation of extract and fermentable extract during the conversion of starch to alcohol. There is no universally accepted specification for the sorghum and millet malts for their diastatic activity. However, a minimum specification of DP of 28 sorghum diastqtic unit (SDU)/g for malt for industrial sorghum or millet brewing appears to be widely used (Dewar *et al*., 1995). Dewar *et al*. (1997) reported that the diastatic activity of sorghum malt germinated at 30 ºC was highest (45 SDU/g) on day 4 and decreased to 39 SDU/g on day 6. The diastatic activity of millet malts obtained in this study cannot be directly compared with other reported values since the extent of DP is widely affected by millet variety, germination time and temperature, steep-out moisture content and method used in its determination. However, the total diastatic power of millet malts obtained in this study were lower to those reported for other cereal malts. Carboxypeptidases are known to be responsible for the release of FAN from solubilized proteins during malting. Carboxypeptidase activity in ICSV 400 and KSV 8 sorghum malts prepared using 1 h of

air-rest period during germination were 428 and 222.5 mg FAN/3h/g dry malt respectively (Okolo and Ezeogu, 1995) which were quite higher than those of Dalle, Okhle, GPU 0025 and GE 5016, malts but were similar to those of Kabre and Juwain finger millet malts found in this study (Fig.4).

The amino acid content is an important malt parameter for the of growth and metabolism of sugar in the wort. To increase brewing fermentability and efficiency, malts with high levels of free amino nitrogen and amino acids are essential. Total soluble nitrogen and free amino acids increase with increasing germination time due to more extensive protein hydrolysis (Nie *et al*., 2010). Amino acid content plays a crucial role in yeast nutrition (Clapperton, 1971). FAN contents in millet malts were reported to be 87 to 155 mg/100g by Morral *et al*. (1986); 96-108 mg/100g by Shayo *et al*. (2001) and the results were quite higher than those found in our experiment (maximum value of 57.8 mg glycine equivalent/100g dry malt). The FAN content of the malt is the product of the catabolic processes, which degrade the storage proteins into new proteins in roots and shoots. Adequate FAN content is necessary to support yeast growth during fermentation (Shayo *et al*., 2001). Okolo and Ezeogu (1995) reported that the FAN content in ICSV 400 and KSV 8 sorghum varieties were 181.1 and 140.8 mg% dry malt using 1 h of air rest period during malting which were quite higher than those found in millet malts in this study. According to Pelembe *et al*. (2004) malt FAN increased with germination time in pearl millet. Similar trend was also reported in sorghum malt (Dewar *et al*., 1997; Morrall *et al*., 1986; Nout and Davis, 1982) and in finger millet malt (Nout and Davis, 1982).

Conclusions

Alpha and β-amylase activities were found maximum on 72 and 48 h of germination respectively at 28±1ºC in all millet varieties. Total free amino acids and FAN contents were maximum on 48 h germination. All millet varieties behaved differently with germination time with respect to carboxypeptidase activity and no relationship existed between malt's carboxypeptidase activity and FAN contents. Based on α- amylase and carboxypeptidase activities, total free amino acids and FAN contents, 48 h germinated Juwain millet malt could be regarded as the best of all malts. While based on β-amylase activity and total diastatic power, 48 h germinated Kabre millet malt seemed superior. Hence, in order to obviate the dilemma in selecting the best finger millet variety for malting, further investigation on malt extract and wort analyses is suggested.

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