African Journal of Agricultural Research Vol. 3 (1), pp. 007-012, January 2008
Available online at http://www.academicjournals.org/AJAR
ISSN 1991-637X © 2008 Academic Journals

Full Length Research Paper

Influence of malting time on α and β Amylases secretions in Nigerian Amylolytic maize Cultivars

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Accepted 1 December, 2007

Screening for alpha and beta Amylases were carried out in one hundred malted Nigerian maize cultivars. The trend of secretions of alpha and beta amylases was observed for eleven day germination period. Results obtained showed that third to fifth day germination periods could be taken as optimum periods for Amylolytic activities. Hence overall malt from 9071 and TZE $COMP_4C_2$ varieties were the most α Amylolytic on third day germination with the values of 134.48 and125.68 U. mg^{-1} protein respectively. While KU 1409 STR and TZUTSR SGY-$SYNF_2$ malt were the most α Amylolytic on fifth day germination with the values 29.29 U and 31.00 U.mg^{-1} protein respectively. These findings showed that α amylase activity is dependent on types of cultivars and decreases after fourth day of germination. Malt from SPMAT variety was the most β amylolytic closely followed by MAKA $SRBC_5$. Results obtained showed that the malt β Amylase activity was also markedly ($P \leq 0.05$) influenced by germination days and types of cultivar. These findings revealed that β amylase development in maize is dependent on the cultivars and the days of germination with over 80% of the cultivars progressively increasing in β amylase activity as days of germination increased up to five days of germination.

Key words: Maize, Amylolytic, α Amylase, β Amylase, Cultivars.

INTRODUCTION

Plants in their natural states contain enzyme that they made for their own use. These enzymes are often separated from the plant product and used to accelerate chemical reactions in industry. (Boyer, 1970). Examples of such enzyme are α and β Amylases. The former hydrolyze alpha 1, 4 glycosidic linkages, randomly yielding dextrin, oligosaccharides and monosaccharide hence are referred to as endoamylase. The latter which is exoamylase hydrolyse the 1,4- glycosidic linkage from the non reducing outer polysaccharide chain ends to yield beta-limit dextrins and maltose (Hopkins, 1946; Bernfeld 1951; Bailey and Whelan 1957; Voet and Voet, 2000) These enzymes are found naturally cereal plants such as barley, sorghum, rye and wheat. Chief among these is barley that is most commonly used on an industrial scale to saccarify starch in food and beverage industries because of the presence of high activity of α and β Amylase (Kneen, 1944; Engel, 1947; Norris and Lewis, 1965; Palmer, 1989)

Earlier works have shown that cereal grains could differ significantly in their modes of Amylases development (Kneen, 1944; Lauriere, 1992; Ziegler,1999) The low activity of amylases in most cereal other than barley malt has been defined as one of the most serious obstacles to their use as replacement for barley malt (Takaku, 1988; Okungbowa et al., 2002) However the gluten – free characteristics of maize makes it a good substitute for barley malt especially for people suffering from Celiac disease (Sweeney, 2004)

The conventional malting process involving the use of maize instead of barley remains the same but maize has low concentration of enzymes needed for proper conversion of the grain into malt during steeping (Watson, 1987; Sweeney, 2001). In the Industry large amount of maize and longer mashing time are always employed to compensate for the low diastatic power or combine effect of α and β amylase in maize (Sweeney, 2004). Against

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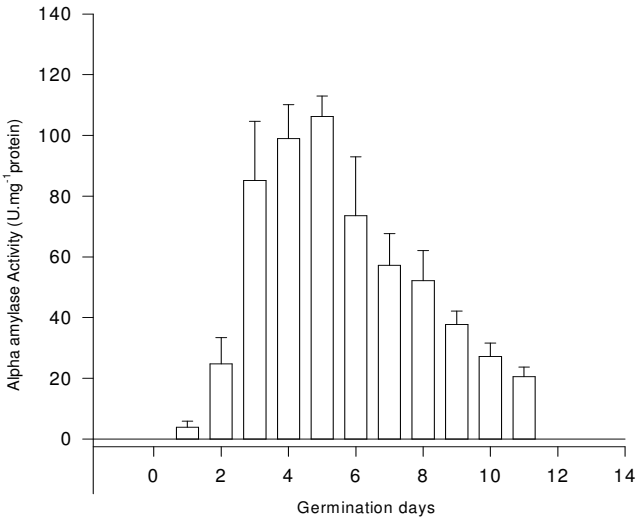

Figure 1. Trend of Alpha Amylase secretion up to eleven days germination in TZEE*TZEE- W*DEMARSCUS*TZEE-W.

the foregoing this work was to compared effect of malting time on the Amylolytic activities of Nigerian maize cultivars and established the optimum period for their malting.

MATERIALS AND METHOD

One hundred different maize cultivars were obtained from the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. Two hundred and fifty grams of each grain was surfaced sterilized by immersion in 1% sodium hypochlorite for thirty minutes as described by Morral et al. (1962).

In order to counteract water sensitivity and accelerate germination the method described by Okolo and Odibo (2003) was utilized. The cultivars were steeped to give a grain / water ratio 3:4 for 65 h in a cycle comprising 6 h wet and 3 h dry. After the steeping regime the cultivars were spread in germination boxes in the dark and allowed to grow for eleven consecutive days in an atmosphere of near water saturation at room temperature to encourage sprouting as described by Morral et al. (1962). During the period 0.1 M of Potassium bromide was sprinkled every day on the grains in order to prevent root growth and malting loss. One percent formaldehyde solution was also sprinkled every day to reduce phenol and improve growth. At the end of the germination period samples were dried in oven (Leader, UK) at 45^{o}C for 24 h as described by Nouvelle (1960).

Enzyme extraction

Each sample of malted maize cultivar was blended in either phosphate or acetate buffer with buffer solution with the use of a Binatone homogenizer (Model BLG 400, UK). Selective inactivation as reported by Sarivastona et al. (1962) was modified such that the medium for α amylase extraction was in phosphate buffer pH 6.8 while β amylase was in sodium acetate buffer pH 4.8 The homogenate was centrifuged at a speed of 3200 g for 30 min at 4^{o}C using a cold centrifuge (Model GL – 16G 11, Shung Hai An Ting KE) and the supernatant fraction was collected for enzyme assay.

Enzyme assay

The α and β amylases activities were carried out as outlined by Sun and Henson (1991) Two percent starch was incubated with 0.4 ml aliquot of the crude enzyme for 30 min. Reducing sugar was quantitated by the Nelson method (1944). Enzyme activities expressed in milligram glucose equivalent per minutes per milliter of enzymes extract.

Estimation of protein content

As outlined by Lubran (1978), the Biuret method of assay was adapted to quantify the total protein in all of the analyzed samples.

Statistical analyses

The data were subjected to statistical analysis of variance using SAS (1999) software. The DUNCAN option was used to find means of significant treatment. Sigma Plot software (1994) was used to present the trend of α and β amylases secretions.

RESULTS AND DISCUSSION

The exploitation of maize for Industrial large scale brewing and the likes are generally known in all parts of the World (Watson, 1988). The empirical data highlighted in this study could be a working baseline upon which the Nigerian Amylolytic maize cultivars under study could be classifies. This idea is necessary and germane to the development of a novel maize cultivars that can compete favorably well with other prefer cereal such as Sorghum, Wheat and Barley malt.

Figure 1 described the trend of α Amylase secretion while Figure 2 described also the trend of β Amylase secretion in TZEE*TZEE-W*DEMARSCUS*TZEE-W from the resting period up to eleven day germination period. The graphs (Figure 1 and 2) depict general trend of α and β amylases secretions as discovered in this study. It is on this premise that optimum period for Amylolytic secretion in all the maize cultivars was chosen to be between 3rd to 5th day germination period.

Both Figures 3 and 4 highlighted the top twenty cultivars that are rich in each respective amylase. This quailfies them to be classified as “high alpha amylolytic” and “high beta amylolytic” respectively. In this investigation it was found that thirteen out of the twenty cultivars selected (60%) were strictly either “alpha amylolytic” or “beta amylolytic” throughout the germination periods. This discovery suggests that there could be some varieties that are rich in both α and β amylase. Base on this assumption, 26.67 and 8.16 U.mg^{-1}protein were set as the baseline for α and β amylase respectively on third day germination (Figure 5). A total of twenty-five cultivars were found to be above this baseline. This also suggests that one quarter of the whole population of the maize cultivars under investigation is highly rich in both alpha and beta amylase. It also shows that all the cultivars produce more of α -amylase than β- amylase on third day germination.

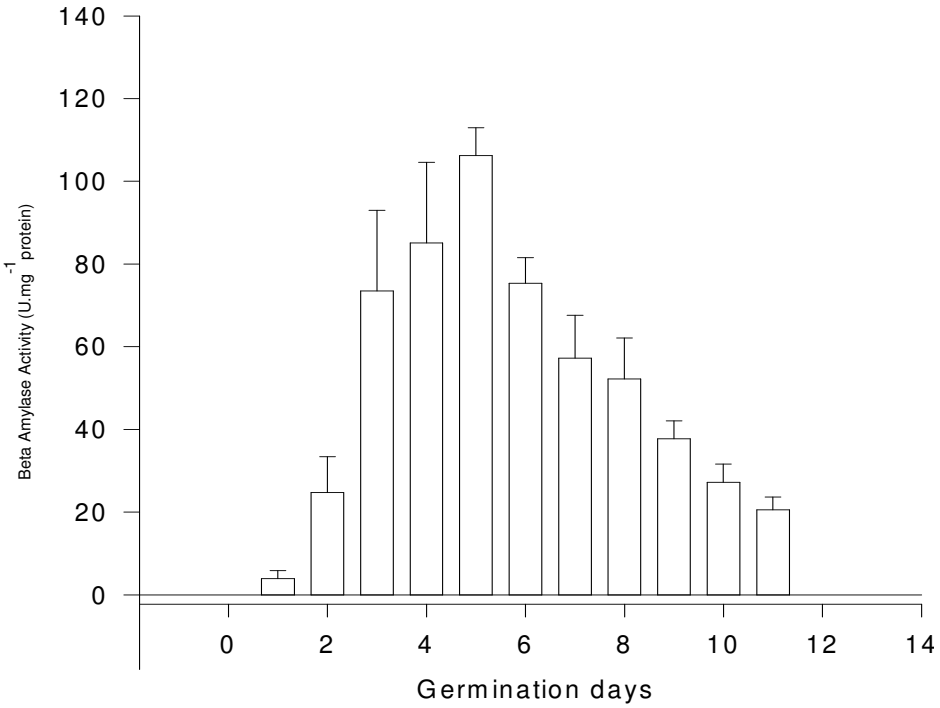

Figure 2. Trend of beta amylase secretion up to eleven days in TZEE*TZEE- W*DEMARSCUS*TZEE-

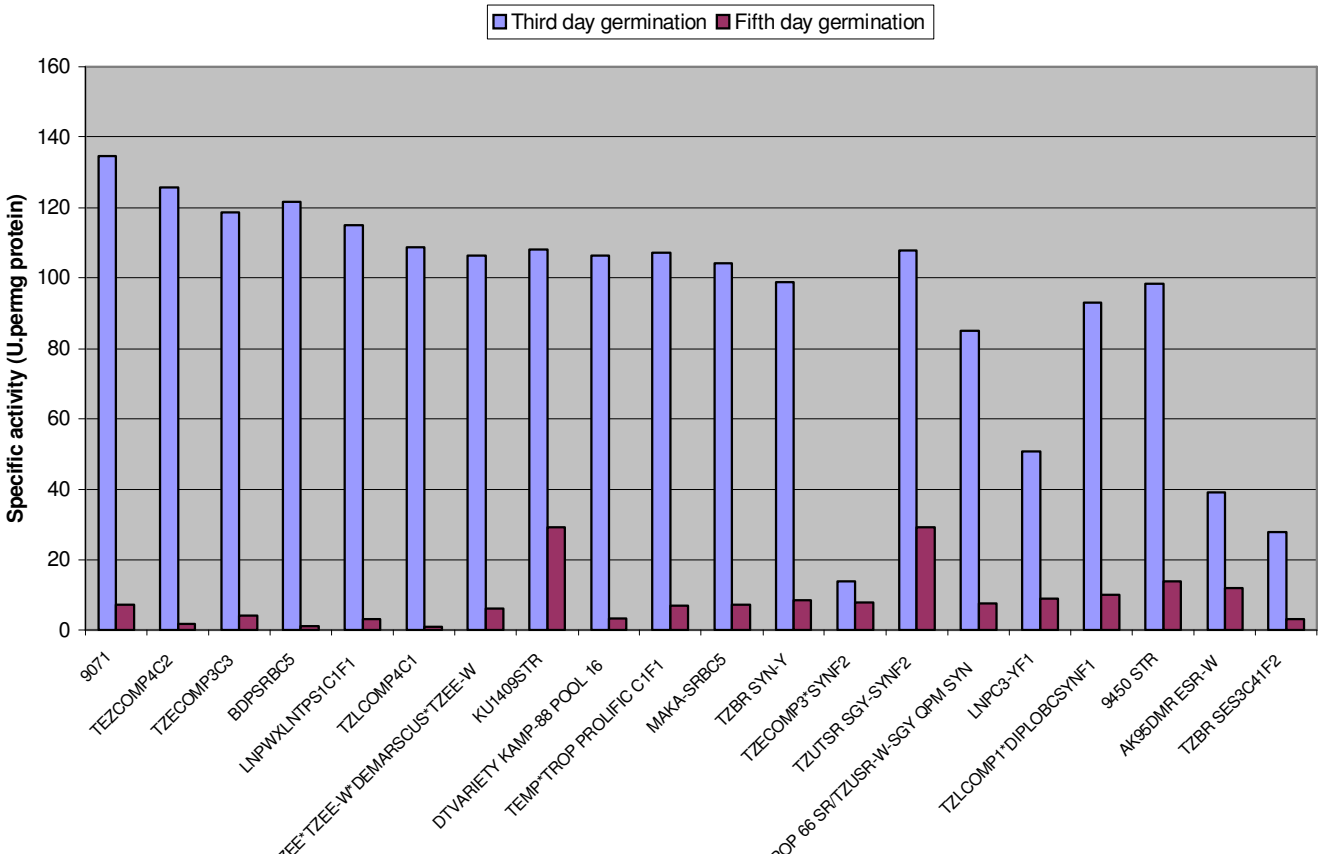

Figure 3. Alpha “Amylolytic” activity as influenced by cultivar variety and malting period.

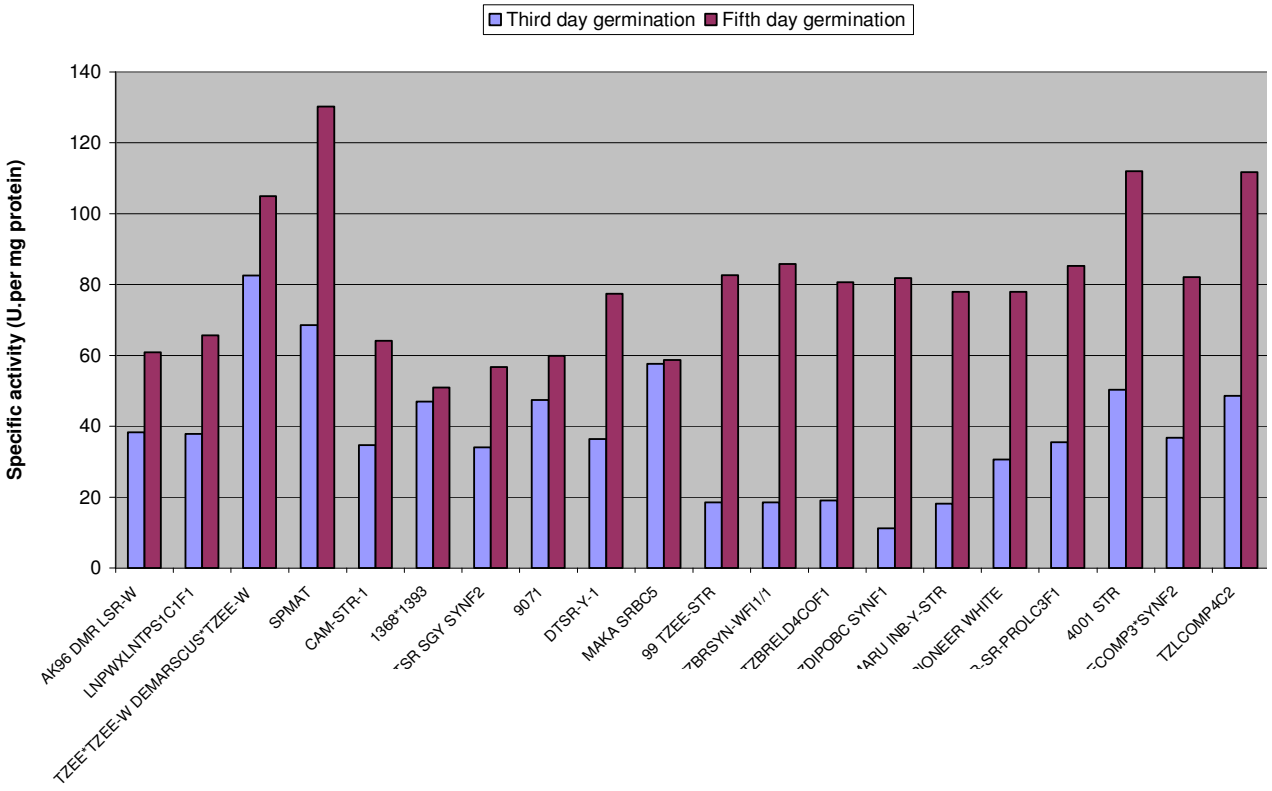

Figure 4. Beta "Amylolytic" activity as influenced by cultivar variety and malting period.

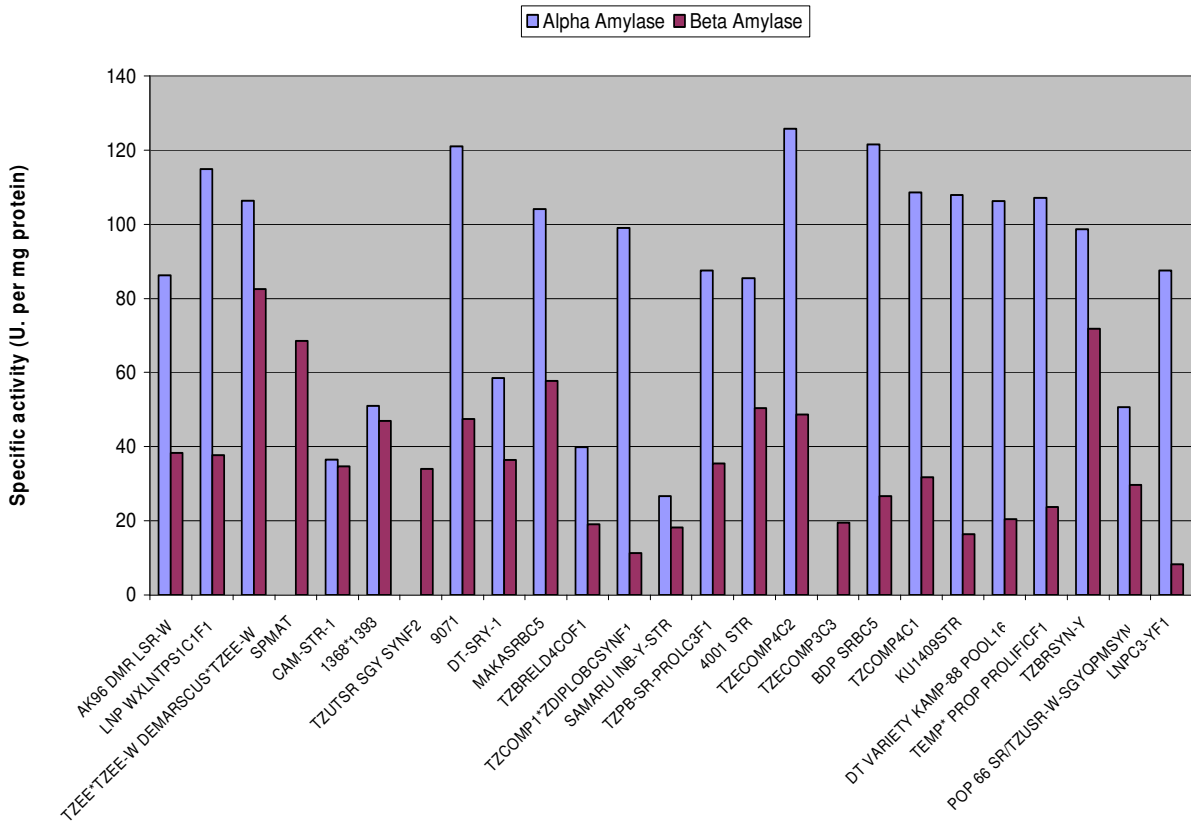

Figure 5. Activities of the amylotic cultivars on the third day of germination.

Table 1. Physical characteristics of the recommended cultivars.

Name of cultivar	Colour	Kernel classification	Number of grains per 10 g	Average shoot length(cm) on third day germination	Average shoot length(cm) on fifth day germination	% of kernel growth on day 3 of germination	% of kernel growth on day 5 of germination not clear
TZEE*TZEE-W*DEMARSCUS*TZEE-W	White	Dent	27	3.9	5.4	100	100
SPMAT	Light-Yellow	Dent	28	0.7	1.9	49	96
TZBR SYN-Y	White	Floury	21	1.4	2.7	93	100
TZE $COMP_4C_2$	Dull-White	Floury	19	0.7	2.0	32	75
MAKA-$SRBC_5$	White	Flint	36	1.7	3.3	86	100
4001STR	Yellow	Pop	47	1.2	1.9	91.6	100

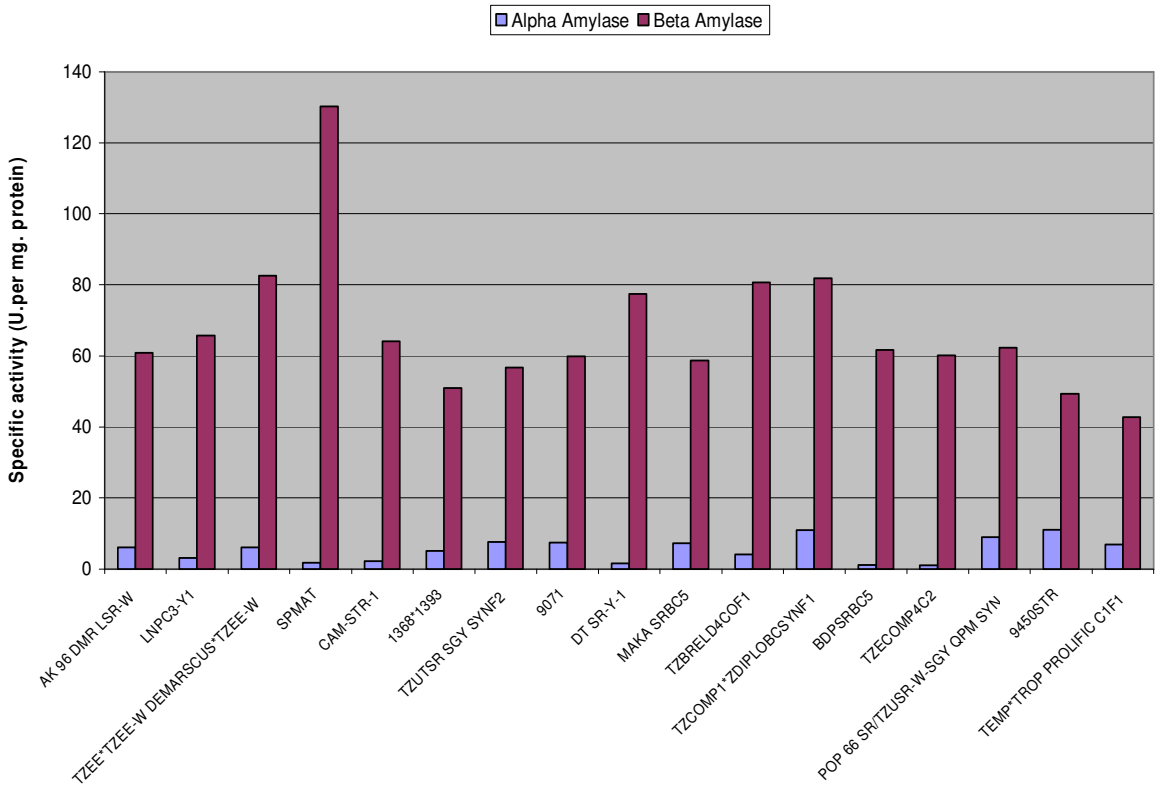

Figure 6. Activities of the amylotic cultivars on the fifth day of germination.

Similar reports were also made earlier by Kneen (1944) and Bernfeld (1951) on wheat and barley respectively while Dure (1960) also reported same on the extent of α-amylases activity in relation to days of maize germination. At the fifth day germination, seventeen percent of the whole cultivars were qualified to be called “Amylolytic” as 1.15 and 42.8 $U.mg^{-1}$protein were set as baseline for α and β amylases respectively (Figure 6). It also shows a reduction of thirty-two percent in number of “Amylolytic” cultivars at the fifth day germination. There was also increase in the amount of beta amylase secretion and decrease in the amount of alpha amylase on fifth day of germination. This discovery supports the evidence of Dure (1960) that β- amylase accounts for only one-tenth of the total amylolytic activity in the maize endosperm at the peak of amylolytic activity during germination. The general trend that appears in all the cultivars investigated shows that during germination α –Amylase production were favoured before β-Amylase. This observation strikingly corresponds to the works of Peat, (1951); Crocker and Barton (1953); Lauriere et al. (1992) they all reported that during germination both α and β amylase activities increase in the maize endosperm, but the former becomes by far the chief amylolytic enzyme. The feature of these recommended “Amylolytic” cultivars is shown in Table 1. It is clearly seen that the breed TZEE*TZEE-W*DEMARSCUS*TZEE-W showed high growth rate compared to others closely followed is TZBR SYN-Y. Perhaps this could also be an indication of the rate of Amylolytic enzyme production. The cultivars 4001 STR followed by MAKA-$SRBC_5$ have the highest base on the volume of space occupied by the grains. This could be a determining factor in their choice for source of Amylolytic enzyme in the industry. However from the Table it can also be inferred that the presence of the Amylolytic enzyme is not kernel discriminatory.

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