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Studies on the α-Amylase and Total Amylase Activity during Malting Period of Some Nigerian Local Grains

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Abstract: ***The need for indigenous raw materials for malting industries necessitates the search for local cereals with high diastatic potentials. The current study investigated the production and determination of α-amylase from malted selected local cereals malt extract. In the course of the study, Alpha amylase and total amylase activities were determine in some selected Nigerian local grains (Rice (Oryza glaberrima), Wheat (Triticum aestivum), and Fonio millet (Digitaria exilis)) during the malting period (Days) and the highest alpha amylase activities were recorded. The grains were separately steeped, allowed to germinate for 8th days in a cupboard during which the alpha amylase activity were determine at two days interval using 3,5-dinitrosalicyclic acid method. The alpha amylase and total amylase activity were determined using 0.1M Sodium phosphate buffer at pH of 5.5 under constant temperature of 60ºC and substrate concentration of 1.5 %w/v. An appraisal of α-amylase production and determination revealed that, the production level peaked on the 8th day of germination in all the three (3) local cereals used, with an estimated activity of 19.39 U/ml in fonio millet, 20.66 U/ml in wheat and 22.58 U/ml in Rice while that of total amylase activity was 16.21 U/ml for fonio millet, 18.55 U/ml for Wheat and 20.25 U/ml in Rice. The results shows that all the millet varieties shows good malting properties in terms of alpha amylase activity except for fonio millet which show the lowest enzymatic activity signifying that it contains less starch content compare to the others and thus, do not have a good malting properties, while rice with the highest malting properties can be recommended as a substitute for barley and sorghum in the Nigeria brewing industry.***

1. Introduction

Malting is a biochemical process applied to cereals grains in which the grains are made to germinate and then quickly dried before shoot development. This process involves germination of grains until the food store (endosperm), which is available to support the development of the germ of the grain, has suffered some degradation from enzymes (Okafor, 1987, Asante E *et al*, 2013). During malting, the germination of the grains facilitate the production/release of enzymes which helps to modify the grain (i.e. attach the starch) to an optimal level of brew's extract. Both Alpha-amylase and Beta-amylase acts to increase the reducing sugar formation through a process known as saccharification (Dewar *et al*., 1997). According to Dewar *et al*., (1997) seeds during malting have been found to undergo various changes of modification such as increase in the quantities of alpha and beta-amylases in the grain and the partial degradation (by residual hydrolytic enzymes) of reserve substances such as cell wall, gums, protein, starch in the starchy endosperm. The most important characteristic of good malt are high enzyme levels to degrade starch and obtain high extract yield (Subramanian *et al*., 1995).

Barley (*Hordeum vulgare* L.) is the traditional cereal used in the production of malt; the principal material for both alcoholic and non-alcoholic beverages (Suhasini and Malleshi, 1995). Attempts have been made by scientists around the world particularly Africa to malt other cereal grains to partially or completely substitute barley. Also, economic situation with its consequent shortage of foreign exchange has made it necessary for many developing countries to examine the possibility of replacing imported industrial raw materials with local once (Dewar *et al*., 1997). In Nigeria, the brewing industry is one of the largest in the economic sector. Unfortunately, its chief raw material, Barley malt, is always imported because barley is not grown in Nigeria. However, In Nigeria, following the ban on the import of barley malt in 1988, the brewing industry has been utilizing sorghum and maize as raw materials for lager beer production. Sorghum can be used as raw grains, grits, or malted material (Ratnavathi and Chavan, 2016),but there is a great need to determine if locally available grains like rice, wheat, fonior millet in Kaduna State, Nigeria can safe as substitute raw materials for the malting industries. Malting grains develops the enzymes required for modifying the grain's starch into sugars, including the monosaccharide glucose, the disaccharide maltose, the trisaccharide maltotriose, and higher sugar called maltodextrines (Ayernor and Ocloo, 2007).. It also develops other enzymes, such as proteases, which break down the proteins in the grains into forms that can be used by yeast. Depending on when the malting process is stopped one gets a preferred starch enzymes ratio and partly converted starch into fermentable sugars. This research aim to determine the enzymatic activity of alpha amylase in some local malted grains (Fonio millet, wheat, and Rice) during the malting period.

2. Materials and Method

Equipment/Apparatus

UV-Spectrometer (UV-752, PEC Medical, USA), Centrifuge (KA-100, Zenith Lab.co), Water Bath (Equitron Ltd, Mumbai India), and Weighing balance (XL 191, Whatman, Jenway),

Chemicals

Dinitrocyliciclic acid solution (DNS), Potatoes starch, Calcium Chloride solution (CaCl, JHD), Sodium Hydroxide solution (NaOH), Sodium Acetate buffers and Distilled water.

Methodology

Sample Collection and Preparation

Four hundred grams of each of the grains were obtain from Sabo Market, Chikun Local Government Area of Kaduna State, Nigeria in the month of September. The Samples were washed thoroughly using running pipe-borne water. The cleaned grains were subsequently soaked in fresh pipe-borne water in a plastic container at standard room temperature for.

Malting of the grains

Malting of grains was done as described from American Association of Cereal Chemists, Inc. (Donn *et. al*., 1991). The soaked grains were allowed to germinate in a cardboard box line with a sterilized jute sack for 8 days at standard room temperature and watered three times daily. Germinating seeds were then taken from the cardboard box at two days interval of the malting period and dried at a temperature range of 40ºC -50ºC in an oven for 1 hour.

Crude Enzymes Extraction

Ten grams (10g) of each geminating malted grain was collected, the crude enzyme essay was carried out using the root and endosperm. The root and endosperm were grounded using mortar and pestle. Twenty (20 ml) of 20 *mM* sodium phosphate buffer, pH 5.5 and 1 *mM* $CaCl_2$ were added to the grounded malted grain and the mixture was sieved to obtain the crude enzyme extract. The extract was centrifuged at 4000 rpmfor 20 min, and the supernatant was collected as enzymes source. The residue was washed with a second portion of buffer,re-centrifuged and the combined supernatants were collected as enzyme source.

Choice of extraction media for Enzyme extraction

In this research, the choice of sodium phosphate buffer (0.01M, pH 5.5) as the extraction medium was based on its high extractive potential of α-amylase from cereal malt (Osman, 2002). In addition, following the α-amylase purification procedure as reported by Beleia and Varriano-Marston (1981), sodium phosphate buffer of pH 5.4 was used as the extraction medium during the isolation step.

In the initial assessment of α-amylase developed during the 8 day malting period, 10g of the milled malt sample was initially weighed into centrifuge tubes and 20 ml of the phosphate buffer was added. The enzymes were allowed to extract into the extraction medium for 30 min after which the enzyme suspension was centrifuged at a speed of 4000 rpm for 20 min. The pH of the supernatant obtained was maintain at 5.5 before testing for the activity of the α-amylase present. The crude enzymes extraction was done at two days interval of the malting periods to the 8th day

Determination of alpha amylase enzymes activity

α-amylase assay using 3,5-dinitrosalicylic acid (DNSA) method

The level of α-amylase produced on each day of the malting period was deduced from the amount of reducing sugars produced upon its reaction on soluble starch. The supernatants were heated at a temperature of 70°C for 15 min to denature all the beta amylase present. Calcium chloride salt (0.3g) was added before heating to maintain the structural integrity of the α-amylase. 1 ml of the heated extract was made to react with 5 ml of equilibrated cassava starch for 10 min. The reaction was terminated by adding 2 ml of 0.1 M sodium hydroxide solution. The reducing sugars formed after the reaction was measured by adding 1 ml of 3,5- dinitrosalicylic acid reagent to the mixture and further boiled for 5 min. The reducing sugars (maltose) formed changed the initial yellowish color of the 3,5-dinitrosalicylic acid reagent to red and further to a reddish black colure following the boiling process. The absorbance of the red color developed was read from a spectrometer at 540 nm. The absorbance read from the spectrophotometer was converted into α- amylase activities using a modified form of the formula used by Beleia and Varriano-Marston, (1981) and Asante E. *et al,* (2013). One unit of α-amylase was defined as the amount of micromoles of maltose produced per milliliter of α-amylase solution per minute under the conditions of test.

The optimum malting period (Days) was determined by determining the alpha amylase activity at two (2) days interval tilled the eight day of malting.

3. Result and Discussion

3.1 Result

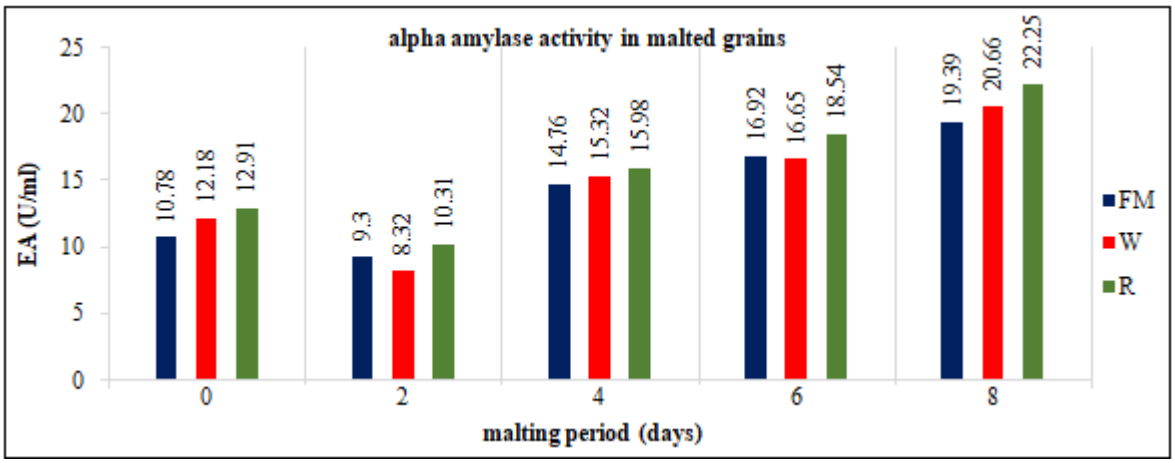

Figure 1: Graph of Alpha amylase activity against Malting period of Fonio millet (FM), Wheat (W) and Rice (R)

Alpha amylase activity in the three (3) malted grains
Figure 1 reveals the result for alpha amylase activity. The result indicates that Rice malt extract has the highest alpha amylase enzyme activity of 22.58 U/ml at the 8th day, followed by Wheat malt extract with 20.66 U/mlwhile Fonio Millet malt extract shows the lowest enzyme activity of 19.39 U/ml.

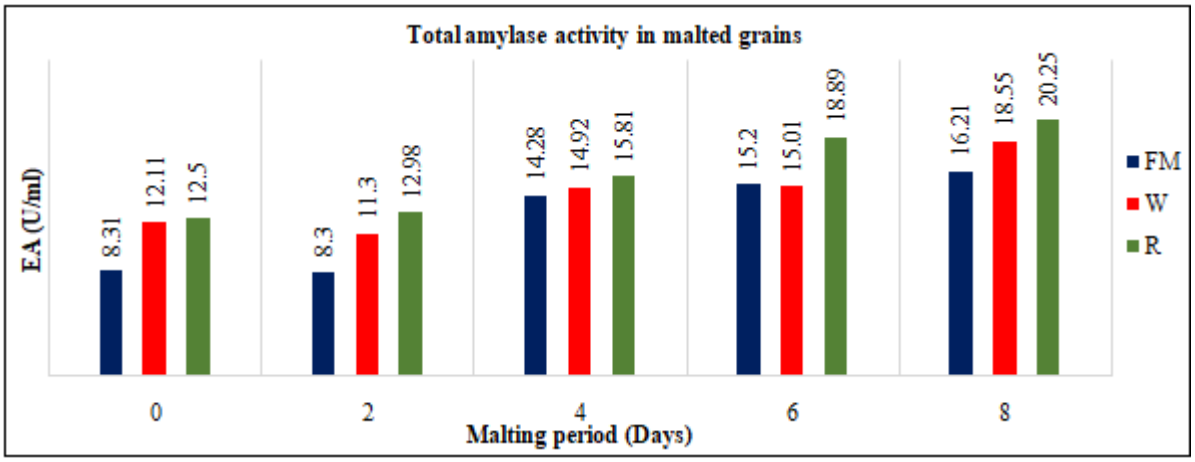

Figure 2: Graph of Amylase activity against Malting period of Fonio millet (FM), Rice (R) and Wheat (W)

Total Amylase activity in the three (3) malted grains
Fig.2 show the result for total amylase activity. From the result, Rice malt extract possessed the highest enzyme activity of 20.25 U/ml at the 8th day, followed by Wheat malt extract with 18.55 U/mlwhile Fonio Millet malt extract stilled shows the lowest enzyme activity of 16.21 U/ml.

3.2 Discussion

From the result above, the result indicated that Rice malt extract has the highest alpha amylase enzyme activity of 22.58 U/ml on the 8th day, followed by Wheat malt extract with an enzyme activity of 20.66 U/mlwhile Fonio Millet malt extract shows the lowest enzyme activity of 19.39 U/ml due to its very low starch content leading to the production of low alpha amylase during germination. According to the graphs, the level of alpha amylase present in the extract from the malt collected on day 1 was relatively higher than the amount present in the extract from the second day malt, this is because during malting, the α-amylase enzymes or total amylase enzymes production begins from the scutellum site. At germination begins, the aleurone layers become activated and took over the production of α-amylase for the hydrolysis of the reserved starch in the endosperm during the subsequent days of germination. Ranki and Sopanen (1984) also identified that α-amylase was largely secreted by the scutellum during the first and second days of germination in a depreciating manner before the subsequent activation of the aleurone layers leading to more enzymes production.The high alpha amylase and total amylase activities at the first day after soaking the grains for 24 hours is attribute to the need to produce enough reducing sugars from the reserve starch in the grains, which is required to initiate germination as reported by Azakawa *et al*., (1968, 1969) Although it was concluded that, the scutellum secreted α-amylase during the initial stages of germination, its contribution to the total activity in the starchy endosperm was only 5 to 10%. Furthermore, the ////////decline in the amount of reducing sugars obtained between the first and second days malt extracts can further be attributed as a result of the reduction in the production of α-amylase from the scutellum site. In the subsequent days, the level of α-amylase remarkably increases for the re-synthesis of sucrose to keep pace with its great demand for tissue (shoots and roots) development. This trend continued until the 8th day where, as a result of the high demand for reducing sugars (sucrose) for tissue development, the highest amount of α-amylase was recorded to meet the purpose of starch breakdown. After the eighth day the enzymes activities is expected to decline as plant development progresses

4. Conclusion

At the end of this study, substantial enzymatic activities were recorded in all the three different varieties of local grains used (Fonio millet, Wheat and Rice), and thus had good malting potentials. The enzymatic activity from the eighth day of germination of rice malt (22.58 U/ml) means that rice malt can served as a good replacement for barley malt to our Nigeria local brewery industries, this will further diversify the use of these local cereals adding more value to our local grains thereby creating a ready market for farm produce. This revealed that, the rice cereals variety in this region of Nigeria (Chikun local government, Kaduna State) could be accredited for having good malting potential with high enzymatic activity in U/ml.

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Appendix 1

Raw Result Data

Alpha amylase enzyme activity in the three (3) grains

	EA (U/ml)	EA (U/ml)	EA (U/ml)
Malting period (Days)	Fonio millet	Wheat	Rice
0	10.78	12.18	12.91
2	9.30	8.32	10.31
4	14.76	15.32	15.98
6	16.92	16.65	18.54
8	19.39	20.66	22.58

Amylase enzyme activity in the three (3) grains

	EA (U/ml)	EA (U/ml)	EA (U/ml)
Malting period (Days)	Fonio millet	Wheat	Rice
0	8.31	12.11	12.50
2	8.30	11.30	12.98
4	14.28	14.92	15.81
6	15.2	15.01	18.89
8	16.21	18.55	20.25