academicJournals

Vol. 12(23), pp. 3738-3744, 5 June, 2013
DOI: 10.5897/AJB2013.12300
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Isolation of α-amylase from malted rice (Wita 7) extract using cassava starch column procedure

E. Asante[1], A. A. Adjaottor [2] and M. Y. Woode[3]

[1]Department of Oil and Gas Engineering, All Nations University College, Ghana.
[2]Department of Materials Engineering, Kwame Nkrumah University of Science and Technology, Ghana.
[3]Department of Chemical Engineering, Kwame Nkrumah University of Science and Technology, Ghana.

Accepted 27 May, 2013

The current study investigated the production and purification of α-amylase from malted rice (Wita 7) extract. In the course of the study, malting parameters such as germination energy, malt yield as well as malt loss of rice (Wita 7) were estimated. An appraisal of α-amylase production during malting with rice (Wita 7) revealed that, the production level peaked on the 8th day of germination with an estimated activity of 19.436 U/ml. The crude α-amylase from the 8th day malt extract was subsequently purified using starch column procedure. The purified extract recorded an estimated activity of 75.549 U/ml. The project further examined the effects of varying the ratios of starch adsorbent height to column diameter (L/D) during the purification step. For the columns employed (ID = 2.45 cm; 4.15 cm), the 2nd elution fractions from the experiments which were conducted with L/D ratios greater than 0.33 contained the highest amount of α-amylase. In summary, we have revealed that, the 8th day malt was most effective (optimum malting time) for starch hydrolysis. Again, the purification process produced a 4-fold enzyme activity ratio of 19:75. Finally, the greater the L/D ratio, the higher the efficacy for purification.

Key words: Alpha Amylase, Wita 7 rice, Malting, Starch adsorbent, Elution fraction

INTRODUCTION

α-Amylases are hydrolytic enzymes which specifically break the α-1, 4-glucosidic bonds in starch. Possible sources of α-amylase in plants, animals and microbes have been reported (Aiyer, 2005). The search for an alternative source as a substitute for the costly imported microbial α-amylase enzymes has led to the studies in cereal malting (Hammond and Ayernor, 2000). Malting is a process involving steeping, germination and drying of cereal seeds with the prime objective of promoting the development of hydrolytic enzymes that are not active in the raw seeds (Dewar et al., 1997). In Ghana, the outcome of studies on the various cereals has suggested that, rice has the highest α-amylase production during malting (Hammond and Ayernor, 2000). Research has indicated that, the *PSB. RC 34* and *PSB.RC 14 Rio Grande* varieties of rice exhibited the highest starch conversion rate when malted for 9 and 10 days, respectively (Hammond and Ayernor, 2001; Ayernor and Ocloo, 2007). The choice for the *Wita 7* variety of rice in the current study was influenced by the need to investigate other varieties of rice for their malting characteristics.

The sequential and interactive activities of α-amylase, beta amylase and other starch degrading enzymes lead to an absolute completion of starch hydrolysis (Osman, 2002). Most of these enzymes are thermostable. As a result, their combined use for starch hydrolysis at a given temperature reduces their overall effectiveness due to the differences in optimum working temperature conditions.

*Corresponding author. E-mail: allincopapa@yahoo.com

Consequently, the exclusive use of a pure α-amylase solution for starch hydrolysis at a given temperature increases its hydrolytic potency. The choice of an enzyme purification process is mainly influenced by factors such as the potential market, the processing cost, the final quality required and the available technology (Amritkar et al., 2004). In developed countries, a number of costly enzyme purification techniques such as expanded bed and ion exchange chromatography that ensure fast and efficient purification have evolved which are not available in developing countries. α-Amylases from various species have been purified to homogeneity by binding to cross linked starch (Rozie et al., 1991). This requires the need to explore available local materials including cassava starch whose surface area provides a strong affinity ligand for α-amylase enzymes in its purification process. In this paper, we report the malting characteristics of *Wita 7* variety of rice and the subsequent isolation of α-amylase from the rice extract using alcohol-treated cassava starch in a column.

MATERIALS AND METHODS

Paddy rice (*Wita 7*) was obtained from the Crop Research Institute (CRI) at Fumesua near Kumasi and was stored at an average room temperature of 28°C. The cassava dough used for the preparation of starch was obtained from a local market. Reagents like ammonium sulfate, ethanol, calcium chloride and potassium hydrogen diphosphate were obtained from the laboratory stores of the Departments of Chemistry, Chemical and Materials engineering, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi. However, reagents such as malic acid and dinitrosalicyclic acid were obtained from local chemical suppliers.

Malting of rice grains

400 g of rice seeds were cleaned by washing in pipe-borne water. The cleaned grains were subsequently soaked in fresh pipe-borne water in a plastic container at a temperature of 28±1°C for 24 h. The soaked grains were allowed to germinate in a cardboard box lined with a sterilized jute sack for 12 days at a temperature of 26±3°C and watered two to three times daily. Germinating seeds were taken from the cardboard box on each day of the germination period and dried at a temperature range of 40 to 50°C in an oven (Gallenhamp hotbox oven; size 1) for 24 h. The dried samples for each day were milled to finer particles using a blender machine (Double-m-cucina blender model No: DM-1731). The powder was packaged in small transparent polythene pouches and stored at 4°C in a refrigerator.

Germination energy determination

Germination energy was determined by using a modified form of the method reported by Hammond and Ayernor (2001). In the current experiment, it was determined by randomly selecting 50 rice seeds from the germinating chamber daily. The ratio of germinated seeds to the total number of seeds selected was evaluated as the germination energy for the day of interest.

Malt yield and malting loss determination

Malt yield is the residual grain content after the malting process. Malt yield for each day of the malting period on percentage dry basis was determined by taking the dry weights of an equal number of grains (50 rice seeds) before and after malting in the absence of any developed roots and shoots. The ratio of the dry weights of the malted grains to the rice grains before malting was expressed as a percentage to give the malt yield.

$$\text{Malt yield (\% on dry basis)} = \frac{Malt(dry.wt)}{Ricegrain(wt)} \times 100$$

Malt loss indicates the material loss expressed as a percentage of dry weight in converting the rice seeds to malt. The malt loss for each day of the malting period was evaluated by noting the difference in the dry weights of rice grains (50) before and after malting. The difference was further divided by the dry weight of 50 rice grains before malting and expressed as a percentage.

$$\text{Malt loss (\% on dry basis)} = \frac{Ricegrain(wt.) - Malt(dry.wt)}{Ricegrain(wt)} \times 100$$

Enzyme extraction

In the current research, two different extraction media were used. Initially, 0.05 M of sodium phosphate buffer of pH 8.0 was used as the extraction medium during the quantitative assessment of α-amylase enzymes developed during the 12 day malting period. The choice of sodium phosphate buffer of pH 8.0 as the extraction medium was based on its high extractive potential of α-amylase from rice malt (Osman, 2002). However, following the α-amylase purification procedure as reported by Beleia and Varriano-Marston (1981), sodium acetate 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 12 day malting period, 3 g of the milled malt sample was initially weighed into centrifuge tubes and 12 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 4500 rpm for 15 min. The pH of the supernatant obtained was reduced to 5.5 by using 0.2 M hydrochloric acid before testing for the activity of the α-amylase present. During the purification step, the same amount of milled sample from the 8th day malt was weighed into 8 separate centrifuge tubes and 12 ml of 0.05 M sodium acetate buffer having a pH of 5.4 was used. The malt suspension was allowed to sit with intermittent stirring for 2 h and later centrifuged at a speed of 2000 rpm for 15 min. The supernatants from the eight different tubes were collected into a conical flask and the pH was adjusted to 6.0

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

The level of α-amylase produced on each day of the malting period was inferred 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. 0.3 g of calcium chloride salt was added 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 color following the boiling process. The absorbance of the red color

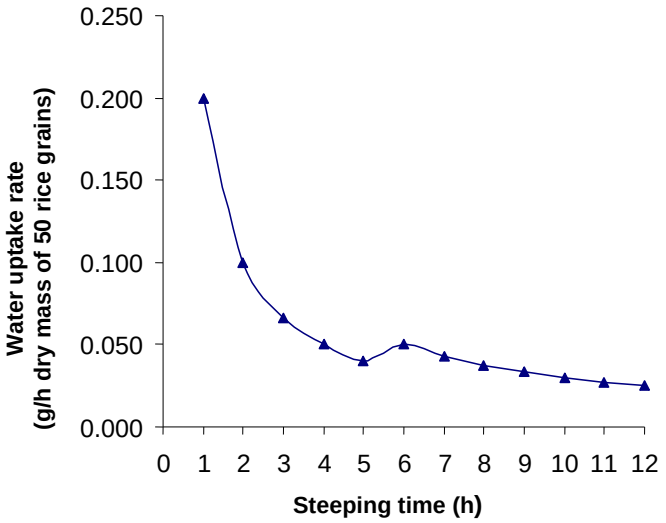

Figure 1. Water uptake rate per mass of 50 dry rice grains during the first 12 h of steeping.

developed was read from a spectrophotometer set 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). 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.

$$\text{Activities (U/ml)} = \left(\frac{mg/ml(maltose) \times 10^3}{Mw.maltose \times time(\min)} \right) \times 2$$

α-Amylase isolation and purification by cassava starch adsorbent column

The results from the DNSA test for α-amylase activity revealed that the extract from the 8th day malt had the highest α-amylase level. Subsequently, fresh rice malt malted for 8 days was prepared and its extract (supernatant) was treated to isolate the α-amylase present using cassava starch column procedures. The pH of the supernatant contained in a conical flask was adjusted to 6.0 and the beta amylase present was denatured by heating in a water bath at an average temperature of 70°C for 15 min. The heated extract was allowed to cool to a temperature of 10°C. To precipitate out the α-amylase present in the extract, ammonium sulfate was added in minute quantities to establish a saturation of 0.45. The basis for the addition was determined using the relation below:

$$\text{Ammonium sulfate per liter of cooled extract} = \frac{533(S_2 - S_1)}{100 - 0.3S_2}$$

Where, S_1 and S_2 refer to the initial and final salt saturation respectively.

The solution obtained was left to stay overnight at a low temperature of 4°C in a refrigerator. The amount of ammonium sulfate added to the extract varied according to the volume obtained. The α-amylase precipitate formed was separated from the solution by pouring out the supernatant after centrifuging at a speed of 4500 rpm for 15 min. 0.5 ml of sodium acetate buffer of pH 5.6 was added to dissolve the partially purified amylase precipitate. Moreover, an alcohol solution of concentration 80% (v/v) was added to establish a volume ratio of 5:1 with respect to the amylase and the alcohol. Finally, 0.2 g of calcium was added to maintain the structural integrity of the α-amylase solution.

The cassava starch adsorbent was prepared by measuring a known quantity of the starch powder into a given volume of alcohol solution. The mixture was allowed to stay in a refrigerator for 30 min and the alcohol was poured out leaving behind the starch precipitate. In the current study, different amounts of starch which had been weighed and precipitated in alcohol solution were carefully transferred into two modified glass columns with internal diameters 2.45 and 4.15 cm fitted with sieve-like metallic support plates clogged with cotton wool. These amounts of precipitated starch (adsorbent) in the modified glass columns helped to established length to diameter (L/D) ratios of 0.08, 0.16, 0.33, 0.41 and 0.49. Upon drying, the starch precipitate served as the adsorbent bed. The partially purified amylase solution was carefully poured on the surface of the adsorbent and the α-amylases present were adsorbed. The adsorbed α-amylases were then eluted with 5 ml of 0.6% (w/v) calcium acetate solution. The elution process was repeated five times for each L/D ratio obtained in the two types of columns used.

RESULTS AND DISCUSSION

Water uptake by rice grains

The rate of water uptake by the rice seeds was rapid during the first hour of steeping reaching an uptake rate of 0.2 g/h per the mass of 50 rice grains as indicated in Figure 1. Thereafter, there was a progressive decline in the rate of water uptake to about 0.04 g/h per the mass of 50 rice grains at the fifth hour after which there was a gradual rise in uptake rate to 0.05 g/h per the mass of 50 rice grains at the sixth hour and again the decline in water uptake rate continued gradually until saturation point of the seed was established. Owing to the concentration difference established between the surrounding water and internal structures of the rice seeds such as the endosperm and embryo, the surrounding water moved by osmosis through the bran (husks) which acted as a semi permeable membrane.

The unexpected rise during the 6th hour could be attributed to the point that, the bran which comprises of substructures such as the epidermis, mesocarp, cross and tube cells initially absorbed the water during the first 5 h until it became fully saturated. By the 6th hour, the endosperm having a relatively low amount of moisture content compared to the bran led to the creation of a high driving force. The high driving force caused a greater amount of water to be absorbed at the 6th hour and gradually declined in the subsequent hours as the endosperm became fully saturated as indicated in Figure 1.

Germination energy of rice grains

As indicated in Figure 2, the first day of germination recorded 34% as the germination energy. The number

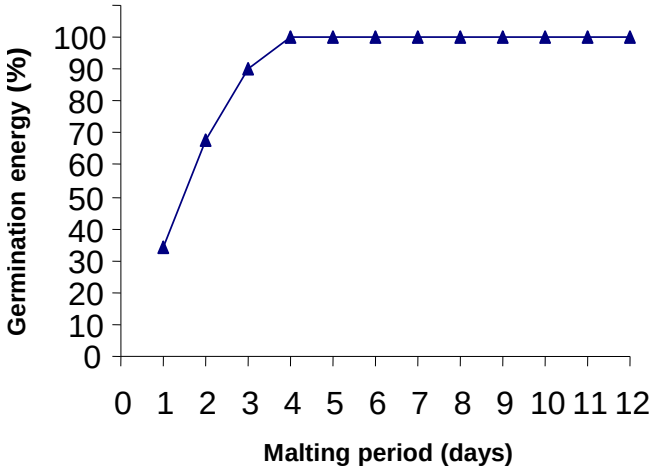

Figure 2. The germination energy of the rice grains during the malting period.

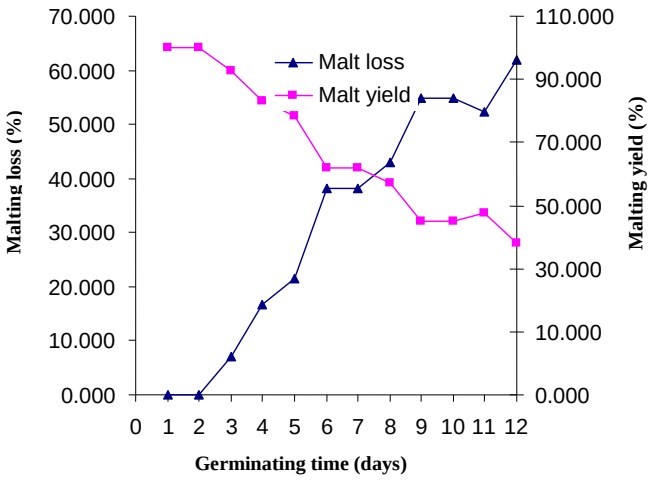

Figure 3. Malting loss and malting yield of Wita 7 rice variety.

doubled to 68% during the second day. By the end of the fourth day, most of the grains had germinated reflecting germination energy of approximately 100%. Germination energy is the ratio of the germinated grains to the total number of grains within a specific time of germination. It is also an indication of the availability of reducing sugars which is the main source of energy for growth by the embryo. The germination energy is greatly dependent on the method of estimation with respect to the availability of water, air, temperature and the germination time allowed.

According to the results, the Wita 7 variety of rice exhibited high germination energy. Ayernor and Hammond (2001) reported that, during germination, the proportion of the grains germinating varied with the quantity of water supplied and with the degree of maturity of the grain. Moreover, further works have revealed that, since malting was a time-limited process, grains were considered suitable for malting only if more than 90% attained maximum germination within 3 days (Agbale et al., 2007). As shown in Figure 2, 90% of the rice (Wita 7) grains attained the maximum germination by the third day which made it suitable for malting.

Malting loss and malt yield determination

The rapid breakdown of the starch content in the *Wita 7* rice seeds between the 2nd and 9th day of germination as seen in Figure 3 contributed to malting losses. The results suggest that as the malting loss increased with increasing time of germination, malt yield which is the residual grain content after malting decreased. Moreover, an estimated average rate of malt loss between the 3rd and 6th day of malting was determined as 10.319% per day. However, a lower value of 4.772% per day was obtained as the average malt loss between the 7th and 12th day. These observations could be attributed to the chemical changes that occur during the malting process. Suhasini and Mellashi (1995) reported that, malting loss was caused by metabolic activity and it increased with increasing duration of germination. The relatively high value of average rate of malt loss recorded between 3rd and 6th days suggested a high rate of α-amylase production which led to a high rate of starch degradation to other reducing sugars. The availability of the reducing sugars was necessary to serve as energy sources for tissue development. During the latter part of the malting period, the rate of α-amylase production generally decreased which led to a decline in the rate of starch degradation and the subsequent malt loss.

α-Amylase activities

According to Figure 4, the level of α-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. Subsequently, there was a gradual rise in reducing sugars production to the 8th day where the highest enzyme activity (19.436 U/ml) was registered. Afterwards there was a gradual decline in enzyme activities through to the 12th day. The trend established for α-amylase production during the first and second days of malting could be attributed to the need to produce enough reducing sugars from the reserve starch required to initiate germination as reported by Azakawa et al. (1968, 1969). In the subsequent days after the second day, the level of α-amylase increased 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 produced to meet the purpose of starch breakdown. After the optimum day for α-amylase production, the decline in producing the enzyme

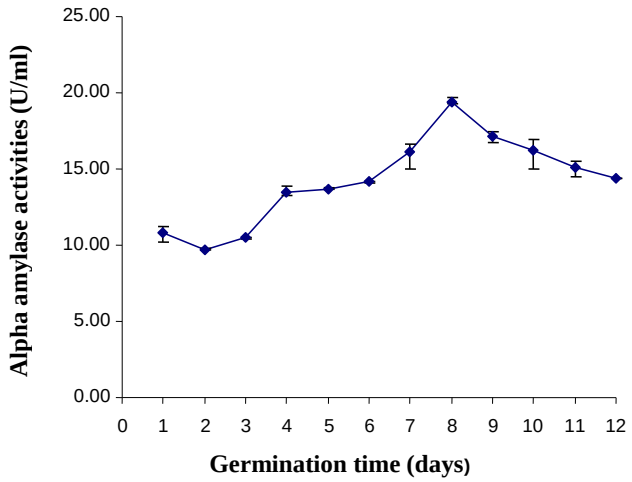

Figure 4. Trend of α-amylase production in Wita (7) rice variety.

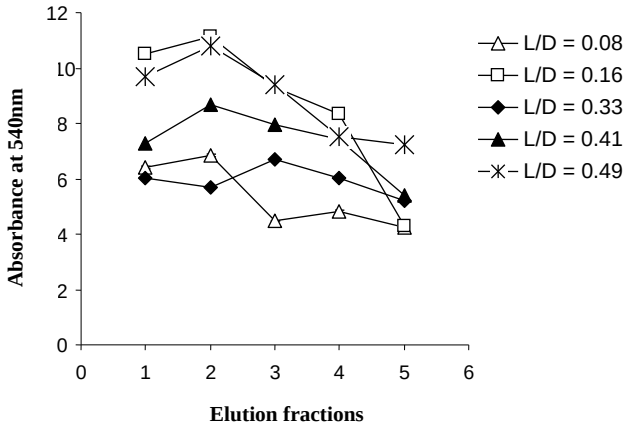

Figure 5. Absorbance readings of elution fractions obtained from varying adsorbent height (L) in a column having a constant internal diameter (D) of 4.15 cm.

enzyme could be attributed to the fact that during the latter time of germination most of the starch reserve in the endosperm had already been hydrolyzed to reducing sugars hence the biosynthesis of α-amylase production was controlled.

Further, the trend of α-amylase production in cereals could be attributed to the relative contributions of the production sites. Ranki and Sopanen (1984) 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. 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%. Accordingly, the decline in the amount of reducing sugars obtained between the first and second days malt extracts were as a result of the reduction in the production of α-amylase from the scutellum site. Following the decline in enzyme production from the scutellum site, the aleurone layers became 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.

The effect of adsorbent height to diameter ratios (L/D) on α-amylase concentration in elution fractions

Figure 5 shows the trend of absorbance readings established by varying the amount of starch adsorbent in a column having an internal diameter of 4.15 cm. The variation in the amounts of starch adsorbent used in a constant internal diameter column created (L/D) ratios of 0.08, 0.16, 0.33, 0.41 and 0.49. The current observation shows a general pattern of improved absorbance reading which reflected an increase in enzyme activities from the 1st to 2nd elution fractions. This is followed by a gradual decline from the 3rd through to the 5th elution fraction. Again, it was observed that, the amount of enzyme adsorbed and eluted generally increased with increasing L/D ratios except for the L/D ratio of 0.16 which showed an anomaly.

The 2nd elution fraction from the L/D ratio of 0.49 which had the highest bed height (2.03 cm) showed a fairly high absorbance reading of 10.805. This was followed by L/D ratios of 0.41, 0.33 and 0.08 in a decreasing order of absorbance reading. The progressive increase in bed height as reported increased the contact between the enzymes and the adsorbent particles (Toledo et al., 2007). As a result, more enzymes were adsorbed as they moved down along the increased height of the starch adsorbent. Though the L/D ratio of 0.16 with a relatively small bed height registered an unreasonably high absorbance reading, experimental errors, including the inability to determine the actual amount of α-amylase precipitate that was introduced initially to the adsorbent bed may have accounted for the current observation.

In Figure 6, the L/D ratios of 0.08 and 0.16 show relatively low absorbance readings as compared to those of L/D ratios of 0.33, 0.41 and 0.49. Admittedly, the relatively small bed heights created by these ratios minimized the extent of enzyme - adsorbent interaction as reported by Somers et al. (1995). This led to a reduction in enzyme adsorption and subsequent elution from the starch adsorbent bed. The highest absorbance reading was recorded by the L/D ratio of 0.33, followed by the ratios of 0.41 and 0.49. The slight difference in the readings observed (10.118, 10.012 and 9.955) respectively showed that, the increase in adsorbent height facilitated the enzyme adsorption and elution.

Figure 7 represents elution fractions in a column with a constant diameter of 2.45 cm. It shows that, the L/D ratios of 0.33, 0.41 and 0.49 show a general pattern of improved absorbance readings from the 1st to the 2nd elution fraction before there was a gradual decline from the 3rd through to the 5th elution fractions. The impact of

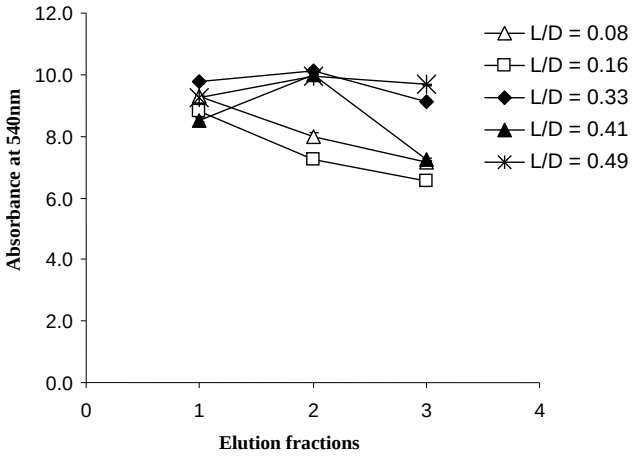

Figure 6. Absorbance readings of 3 elution fractions obtained from varying adsorbent height (L) in a column having a constant internal diameter (D) of 2.45 cm.

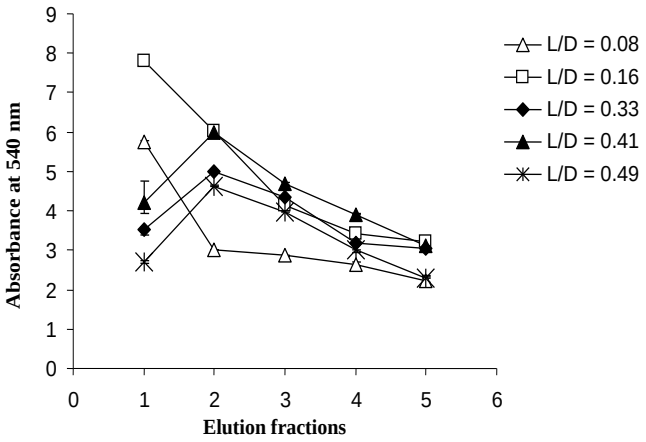

Figure 7. Absorbance readings of 5 elution fractions obtained from varying adsorbent height (L) in a column having a constant internal diameter (D) of 2.45 cm. Translating the absorbance readings of α-amylase concentration in the various purified elution fractions into activities, the highest activity was 75.549 U/ml.

L/D ratios on enzyme adsorption and elution from starch adsorbents was not clearly shown by Figure 7. The L/D ratio of 0.16 with a bed height of 0.4 cm registers the highest absorbance reading. The current observation could not be explicitly accounted for on the basis of adsorbent height. This was because, the adsorbent height created by the L/D ratio of 0.16 was not enough to favor maximum enzyme - adsorbent interaction as compared to the heights formed by the L/D ratios of 0.33, 0.41 and 0.49. It was a case of an anomaly that could not be explicitly explained now, however, possible reasons leading to the irregular trend might be attributed to enzyme losses though poor handling and unknown amounts of α-amylase solutions that were initially introduced to the various adsorbent bed heights prior to the elution processes.

Conclusion

The outcome of the investigation into the trend of α-amylase and reducing sugars formation in Wita 7 variety of rice during malting suggested that, α-amylase production peaked on the 8th day of germination when performed at a temperature range of 26±3°C. The crude α-amylase in the extract from the 8th day malt exhibited an estimated activity of 19.436 U/ml. Considering malting characteristics such as germination energy, malt yield as well as malt loss, we have revealed that, the Wita 7 rice variety could be accredited for having good germination energy since nearly 100% of the rice seeds sprouted on the 4th day of germination under the prevailing conditions. The malt yield was 57.14% with a corresponding malt loss of 42.86% on the 8th day of germination. The purified α-amylase expressed an estimated activity of 75.549 U/ml which showed a remarkable increase from 19.436 U/ml for the crude state. In our pursuit to investigate the various elution fractions for α-amylase concentrations using varying adsorbent height to column diameter (L/D) ratios, the results portrayed that, for L/D ratios greater than 0.33, the 2nd elution fraction usually contained the highest enzyme concentration. Though some anomalies were registered in our attempt to investigate the effects of increasing the starch adsorbent height to diameter ratio (L/D), the outcome generally suggested that, the extent of enzyme adsorption could be enhanced by increasing the L/D ratios but the concentration of enzymes in a given elution fraction will depend on the ability of the elution solvent to dislodge the trapped enzymes from the adsorbent. The outcome of the current work shows that, crude α-amylase from rice malt extracts can be purified by using locally treated cassava starch as adsorbent in a column to attain higher activities.

REFERENCES

Agbale MC, Adamafio NA, Agyeman KOG, Sackey ST (2007). Malting and brewing properties of selected cereals cultivated in Ghana. J. Ghana Sci. Assoc. 9(2):146-155.

Aiyer PV (2005). Review on amylases and their applications. Afr. J. Technol. 4(13):1525-1529.

Amritkar N, Kamat M, Lali A (2004). Expanded bed affinity purification of bacterial α-amylase and cellulase on composite substrate analogue cellulose matrices. Process Biochem. 39:565-570.

Ayernor GS, Hammond TK (2001). Characteristics of malted rice for the production of sugar syrup, J. Ghana Sci. Assoc. 3(3):91-99.

Ayernor GS, Ocloo FCK (2007). Physico-chemical changes and diastatic activity associated with germinating paddy rice (PSB.Rc 34). Afr. J. Food Sci. 1:37-41.

Azakawa T, Fukuchi S, Murata T (1968). Enzymic mechanism of starch breakdown in germinating rice seeds 1. analytical study; Plant Physiol. 43:1899-1905.

Azakawa T, Nomura T, Kono Y (1969). Enzymic mechanism of starch breakdown in germinating rice seeds 2, scutellum as the site of

sucrose synthesis; Plant Physiol. 44:765-769.
Beleia A, Varriano-Marston E (1981). Properties of partially purified α-amylase. Cereal Chem. 58(5):433-437.
Dewar J, Taylor JRN, Berjak P (1997). Determination of improved steeping conditions for sorghum malting, J. Cereal Sci. 26: 129-136.
Osman AM (2002). Advantages of using natural substrate-based methods in assessing the roles and synergistic and competitive interactions of barley malt starch degrading enzymes. J. Inst. Brew. 108(2):204-214.
Ranki H, Sopanen T (1984). Secretion of α-amylase by the aleurone layer and the scutellum of germinating barley grain. Plant Physiol. 75(3): 10-715.
Rozie H, Somers W, Van't RK, Rombouts FM, Visser J (1991). Cross-linked potato starch as an affinity adsorbent for bacterial α-amylase. Carbohydr. Polym. 15:349-365.
Somers WAC, Koenen PHM, Rozie HJ, Visser J, Rombouts FM, Riet KV (1995). Isolation of α-Amylase on cross-linked starch. Enzyme Microbiol. Technol. 17:56-92.
Suhasini A, Melleshi NG (1995). Influence of malting conditions on the amylase activity, physical characteristics and nutrient composition of wheat malt. J. Food Sci. Technol. 32(2):98-103.
Toledo AL, Severo JB, Souza RR, Campos EC, Santana JCC, Tambourgi EB (2007). Purification by expanded bed adsorption and characterisation of an α-amylase FORILASE NTL from *Aspergillus niger*. J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci. 846:51-56.