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Full Length Research Paper

Evaluation of locally produced *Saccharomyces cerevisiae* pectinase enzyme for industrial extraction of starch from cassava in Ghana

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Enzymes are widely used in industries to improve upon process parameters such as product yield and rate of product formation. Such biotechnological applications are not currently exploited by industries in Ghana. The objective of the study was to evaluate the suitability of a crude pectin enzyme for the industrial extraction of cassava starch. Pectin enzymes produced by culturing *Saccharomyces cerevisiae* ATCC 52712 in an appropriate medium for 6 days was used as a crude enzyme extract and tested for its ability to enhance the extractability of cassava starch. An enzyme dosage of 0.02% with a reaction time of 30 min gave the optimum increase in rate of starch extraction (60%) and increase in yield of starch extracted (53%). When these optimum conditions were applied on a scale-up extraction process with a traditional starch processor, the corresponding values were 50 and 45.6%, respectively. These results indicate the possible use of enzyme technology to improve starch processing by starch producers in Ghana if the enzymes are made available.

Key words: Pectin enzymes, enzyme dosage, extraction rate, reaction time, traditional extraction, yield.

INTRODUCTION

Starch is an important industrial product, being widely used in the paper, wood, textiles, food and other Industries owing to its viscosity and gelling properties (Hegenbert, 1996). An important source of starch is from cassava *(Manihot esculenta* Cranz) which is a widely cultivated starchy root crop in many developing countries, especially in Africa, Latin America and Asia (FAO, 1991). In 1996, the starch market in Ghana was about 4,200 tons of which most was imported (Graffham, 1997). The importance of cassava starch production in Ghana for foreign exchange was emphasized in what is known as the President's Special Initiative (PSI) for boosting cassava cultivation for starch production (A Briefing Document, 2001), which later on led to the establishment of a starch production factory in Bawjiase in the Central Region of Ghana in mid – 2003. Prior to the PSI however, starch was mainly produced for local consumption by small scale traditional processors which are still the major producers for the local market. Both traditional and mechanized processes for starch extraction usually result in significant amount of the starch not released from the boundaries of the cell wall because the efficiency of rasping which sets free the starch granules is not more than 90% of the starch present in the roots (Kordylas, 1990). Regarding the traditional starch processors, the loss could even be more. The traditional extraction process also takes a long time to be accomplished. Thus the need to assist such local starch industries to realize the maximum yield of starch with their traditional methods through technological improvements is important.

Commercial pectin enzymes have been employed in starch extraction from sweet potato (Rahman and Rakshit, 2003), yam (Daiuto et al., 2005) and cassava (Sriroth et al., 2000).

The use of such commercial enzymes for the same application in Ghana will be difficult due to the cost involved in importation, but more importantly the difficulty to be faced with keeping the enzymes active because of unreliable power supply. An alternative approach is for the enzyme to be produced locally and made available to the starch processors on demand.

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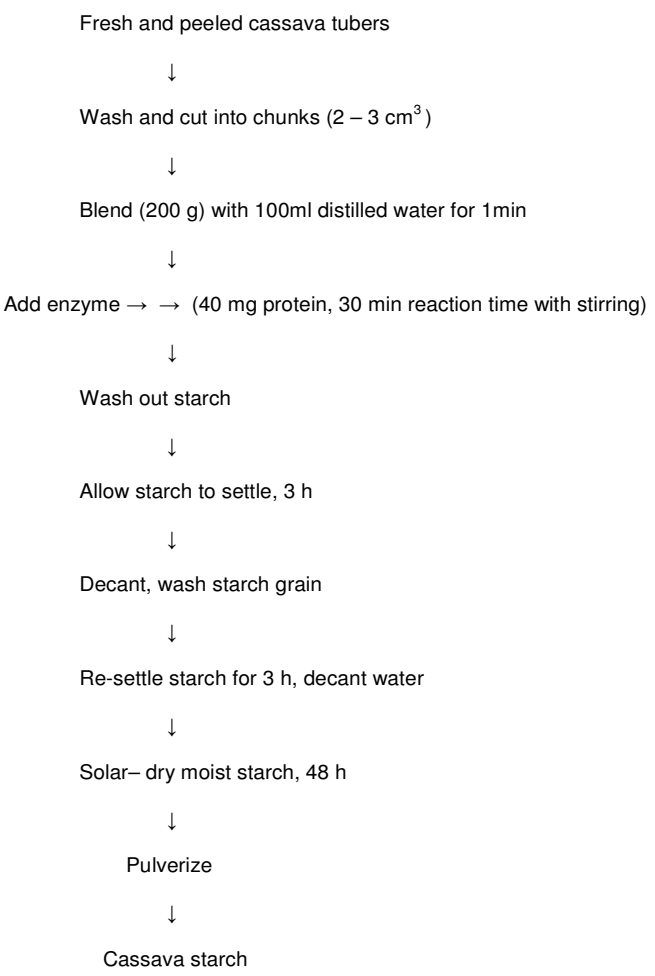

Figure 1. Flow chart for enzyme – assisted extraction of cassava starch.

In a previous study, pectinase enzyme activity from *Saccharomyces chevalieri* (ATCC52712) now renamed *S. cerevisiae* ATCC52712 was reported (Dzogbefia et al., 1999). Furthermore, the suitability of this crude yeast pectinnnase for fruit juice extraction was reported (Dzogbefia and Djokoto, 2006; Djokoto et al., 2006; Dzogbefia et al., 2001). In the present study, the suitability of this enzyme for starch production at a local factory in Ghana was evaluated.

METHODOLOGY

Cassava *(M. esculenta* Cranz) 'Afisiafi' variety was obtained from the Crops Research Institute (CRI), Council for Scientific and industrial Research (CSIR) at Fumesua, a suburb of Kumasi and from the Agricultural Station in Wenchi, Ghana.

Culturing of *S. cerevisiae* for pectin enzyme production

S. cerevisiae (ATCC 52712) was obtained from American Type Culture Collection, Maryland, USA, and had been maintained on agar slants in the freezer for use. It was pre-cultured in malt extract broth for 3 days at room temperature in a UV sterilized room to a cell density of 4.95×10^6 cells ml^{-1}.

The medium used for enzyme production consisted of a 1% pectin medium made up of (gl^{-1}): pectin (10.0); $NaNO_3$ (2.0); KH_2PO_4 (1.0); $MgSO_4.7H_2O$ (0.5); $FeSO_4.7H_2O$ (0.01); NaCl (10.0) and adjusted to a pH of 3.5 as previously reported (Sanchez et al., 1984; Buamah et al., 1997). This medium was dispensed in 100 ml aliquots into 250 ml conical flasks, sterilized at 121°C for 15 min and allowed to cool. The flasks were then inoculated with 4 ml of the malt-extract broth containing 4.95×10^6 *S. cerevisiae* cells ml^{-1} and incubated at room temperature (25 – 28°C) without shaking for 6 days (Attipoe, 1999). At the end of incubation, the culture medium was centrifuged at 3,600 g for 10 min in a Centrikon T – 42 K centrifuge (Kontron Instruments, ALC International Srl, Italy) maintained at 4°C. The supernatant constituted a crude pectin enzyme preparation which was used for the starch extraction studies.

The total protein content and pectin enzyme activity of the extract were determined, respectively, by the methods of Lowry et al. (1951) and McComb and McCready (1952).

The enzyme activity was expressed in units and defined as the amount of crude extract that liberated one micromole of galacturonic acid per minute from apple pectin under the assay conditions. This was computed to be 0.062 units with a specific activity of 0.62 units/ ml extract (0.344 units/mg total proteins).

Effect of enzyme dosage and reaction time on rate of starch extraction

The method described by Kordylas (1990) was adopted for the general extraction of starch with the exception of the enzyme addition as shown in Figure 1. Five different dosages of enzyme extract (10 – 50 mg total protein) were added to 200 g portions of cassava mash, thoroughly mixed and allowed to stand for specified reaction times of 30, 60, and 90 min. After treatment, the mash was transferred into a funnel (Pyrex Brand 100) lined with cheese cloth and the starch milk collected in a graduated cylinder and measuring the volume of starch draining off at 30 s intervals for 6 min. The results were used to determine the extraction rate, as well as enzyme dosage and reaction time combination that gave optimum rate and yield of starch. This optimum combination was utilized for extraction of starch from 1 Kg cassava tuber for further analysis.

Each determination was replicated. Control reactions contained distilled water in place of enzyme extract. The enzyme aided extraction process is illustrated in Figure 1.

Large – scale extraction of starch at a traditional starch processing site

The optimum enzyme dosage and reaction time that was established from the laboratory scale and which did not negatively affect the properties of the native starch was adopted for large scale extraction with a traditional processor at Amakom, a suburb of Kumasi. Twenty-five (25) kilograms of cassava was milled using a local cassava mill and processed as in Figure 1. The mash was then treated with 2842 ml enzyme extract (5000 mg total protein) and left to stand for 30 min before extracting the starch. After the reaction period, the mash was put in a basket covered with a clean white cloth and supported on a bucket. The cassava mash was then washed with 30,000 ml water, filtered through the white cloth and filtrate allowed to stand for 8 h in the plastic bucket. After starch sedimentation and removal of supernatant, the wet starch was then sun-dried for 4 days. The yield of starch was calculated based on dry weight. The control was processed by the traditional method without any enzyme addition. Results were analyzed statistically using Analysis of Variance (ANOVA) test with a CoStat programme (CoHort Software, 2003).

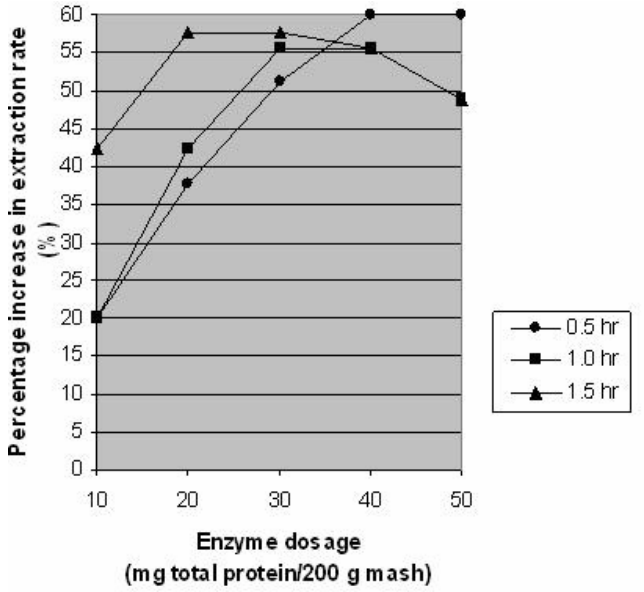

Figure 2. Effect of enzyme dosage and reaction time on percentage increase in starch extraction rate over controls.

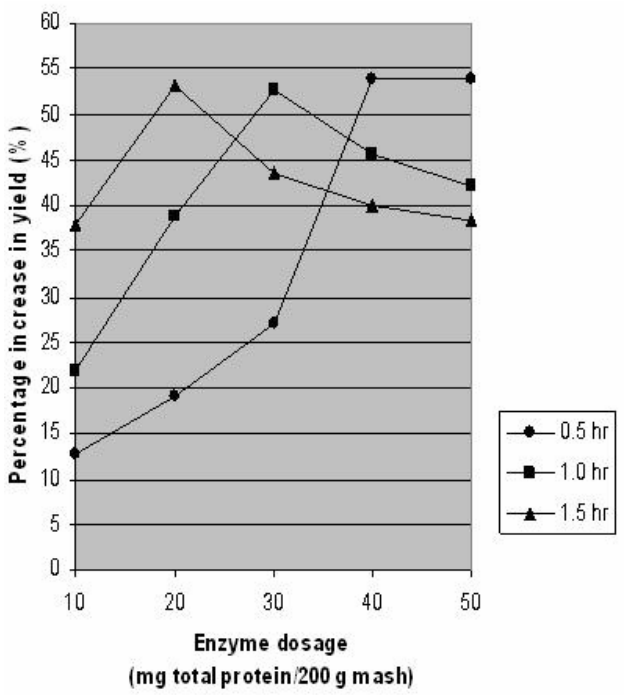

Figure 3. Effect of enzyme dosage and reaction time on yield of starch extracted.

RESULTS AND DISCUSSION

The combined effect of enzyme dosage and reaction time on rate of starch extraction and yield of starch are shown in Figures 2 and 3. Using initial rates for flow of starch, it was found that for 30 min reaction time, significant differences ($P<0.05$) occurred between the various enzyme dosages employed except for the 40 and 50 mg total protein dosages, each of which gave the highest percent-tage increase in flow rate of 60% (Figure 2). With 1 and 1.5 h reaction times, lower enzyme dosages were required to attain maximum increases in extraction rates (Figure 2). The corresponding percentage increases in yield of starch at different enzyme dosages and reaction times were however not significantly different (Figure 3). Our interest was in identifying the enzyme dosage-reaction time combination that gave maximum rate of extraction with maximum starch yield without any adverse effect on the properties such as granule size and shape, solubility, swelling power, moisture, water binding capacity, color, etc. of the resulting starch. This was identified, based on the properties of the analysed starch, to be 30 min with 40 mg protein/200 g mash (0.02%). In all cases considered, the enzyme dosage – reaction time combination that produced maximum percentage increase in yield enzymes was mainly to increase the rate of starch extraction and the yield of the resulting starch. The combined effect of enzyme dosage and reaction time that to avoid the formation of undesirable products was also considered important in this technology. Thus the results of 30 min reaction time with 0.02% enzyme dosage being the optimum conditions to achieve these two objectives were quite exciting.would optimize both objectives, but with no major negative effect on the starch itself was critical.

Additionally, a minimum enzyme dosage that saves cost of enzyme and minimum reaction time that cuts down on processing time also coincided with the highest percenttage increase in extraction rate over the untreated samples (Figures 2 – 3).

The objective of treating cassava mash with pectin enzymes was mainly to increase the rate of starch extraction and the yield of the resulting starch. The combined effect of enzyme dosage and reaction time that would optimize both objectives, but with no major negative effect on the starch itself was critical. Additionally, a minimum enzyme dosage that saves cost of enzyme and minimum reaction time that cuts down on processing time to avoid the formation of undesirable products was also considered important in this technology. Thus the results of 30 min reaction time with 0.02% enzyme dosage being the optimum conditions to achieve these two objectives were quite exciting. Treatment of pulp or mash with pectolytic enzymes is undertaken for different reasons such as cell wall disintegration, de-pectinisation, reduction of pulp viscosity leading to increased flow rate, release of cellular components and increase in yield of product (Demir et al., 2001; Rai et al., 2004; Whitaker, 1990).

In cassava starch extraction, the combination of mechanical rasping and use of hydrolytic enzymes such as pectinases to disintegrate the pulp and cause better release of starch is possible. This has been found to be cost effective as it saves on energy cost due to increased rasping (Rahman and Rakshit, 2003). The pectolytic enzymes break down the pectate network of the cell wall

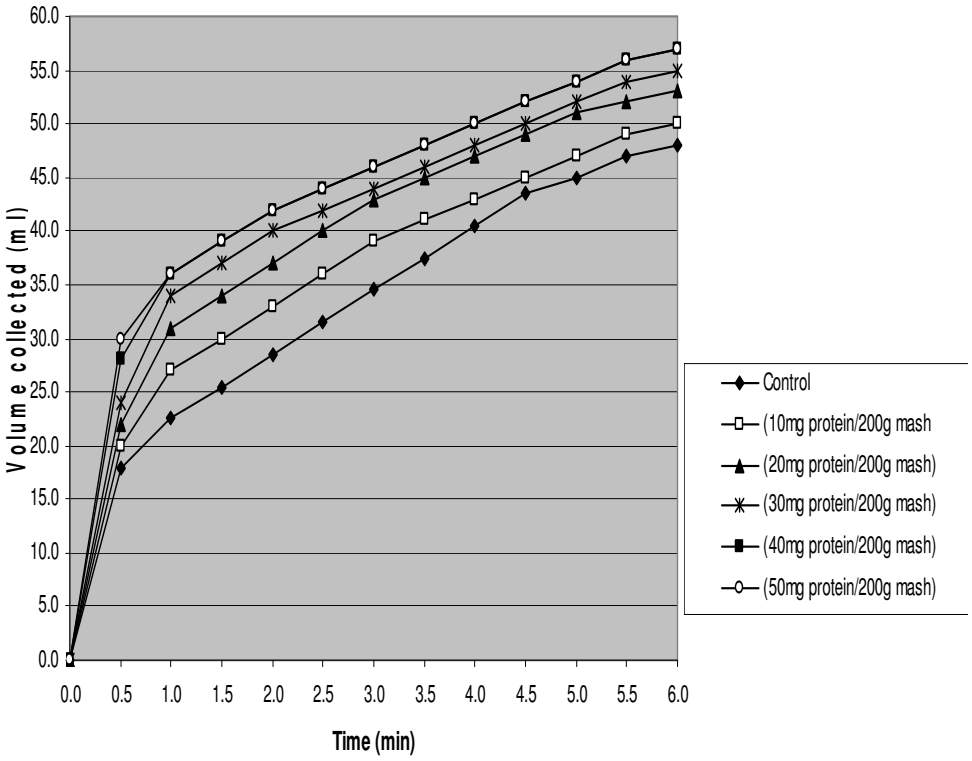

Figure 4. Flow pattern of starch milk using different enzyme dosages and 30 min reaction time.

leading to release of the starch granules (Herron et al., 2000; Rahman and Rakshit, 2003). Thus the increased rate of starch extracted, coupled with increased yield observed in this work are consistent with these reports.

Figure 4 shows the flow pattern of starch milk using the reaction time of 30 min with different enzyme dosages. It can be seen that at all the dosages employed, the flow rates were higher than the control and the optimum flow rate (extraction rate) was observed at 40 mg total protein (0.02%). Thus during the starch processing, the application of pectolytic enzymes enhanced the breakdown of pectic substances, resulting in decrease in viscosity leading to increased flow rate with the release of the starch granules. Daiuto et al. (2005), whilst employing various extraction methods for yam starch, noted that treatments which reduced viscosity made the separation of the starch slurry from the residual mass easier.

The use of pectic enzymes in this case was intended to degrade the pectin which is mostly present in the cell wall in order to disintegrate the cell wall structure and facilitate the extraction of starch. The initial pectin content of the variety of cassava used was 0.3 g 100 g $^{-1}$. This decreased significantly ($P< 0.05$) to 0.06 g 100 g $^{-1}$ with the enzyme dosage of 0.02%. The pectin content of the control reaction was 0.13 g 100 g^{-1} thus indicating reduction in the pectin content by about 80% with the enzyme treatment. Attipoe (1999), in a preliminary study using this crude pectin enzyme extract, observed that the addition of 72 mg total protein extract to 200 mg cassava mash (0.036%) for 30 min reaction time produced about 61% increase in extraction rate over the controls. The data however did not establish the optimum amount of enzyme required in the reaction. The results obtained in this study therefore clearly established the usefulness of enzyme technology to starch extraction with respect to reduction in extraction time as well as increase in the yield of starch extracted.

The optimum conditions established in the laboratory scale extraction (combination of 0.02% enzyme dosage and 30 min reaction time) were applied to starch extraction at a small – scale starch processing industry in Amakom, Kumasi. Figure 5 illustrates the results of this treatment. The enzyme treated samples gave signifycantly higher flow rates compared to controls ($P< 0.05$). When the flow rate was followed for 6 min it was realized that the volume of starch milk collected for the untreated samples in 6 min could equally be collected in 4 min for the enzyme treated samples (Figure 5). Simultaneously, the yield of starch also increased significantly ($P< 0.05$) from 13.0 to 18.9% upon enzyme treatment, translating into a percentage increase in yield of 45.6% and an increase in rate of extraction of 50.0% over the controls. Comparing these values to those obtained at the laboratory scale (60 and 53.9%) for increase in extraction rate and yield respectively, one can conclude that the technology could be effectively applied to small – scale starch

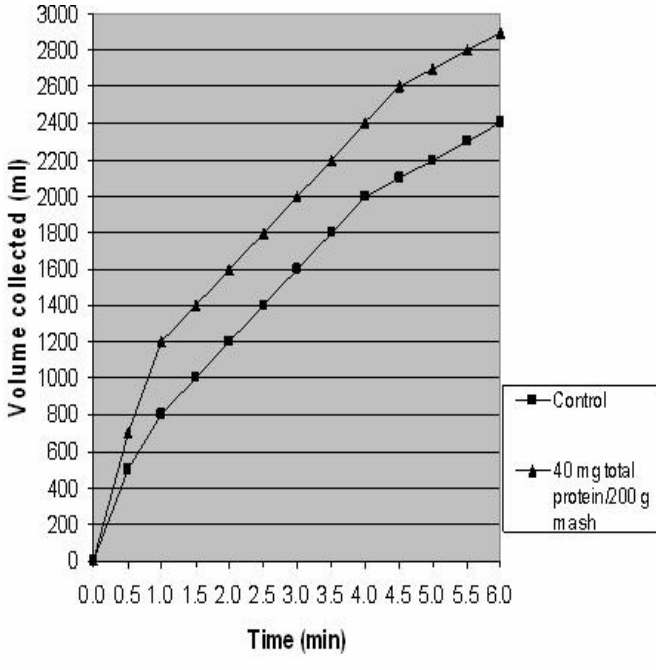

Figure 5. Flow pattern of starch milk during large – scale extraction under optimal conditions of enzyme dosage and reaction time of 30 min.

processing industries. The differences observed in the values for the laboratory scale and the large scale extraction could be attributed to differences in the processing conditions employed; for example, differ-rent raspers were used as well as different screens to wash out the starch. Kordylas (1990) observed that the efficiency of the rasping operation determines to a large extent, the overall yield of starch during processing.

Commercial pectic enzymes have been used to enhance cassava starch extraction (Sriroth et al., 2000; Dauito et al., 2005). The combined application of 15 Novo cellulase units of cellulase and 122.5 polygal-acturonase units of pectinase per g dry pulp for 60 min resulted in 40% starch recovery (Sriroth et al., 2000). In this report, an enzyme dosage of 0.02% (equivalent of 40 mg total protein [13.8 units] crude enzyme extract per 200 g mash for 30 min led to 45.6% increase in starch recovery at the industrial level. These results therefore revealed that a crude pectin enzyme preparation from *S. cerevisiae ATCC 52712* could equally be effectively used for starch extraction, thus dispensing with dependence on imported enzymes.

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