THE COMBINATION OF RICE MALT AND AMYLOGLUCOSIDASE FOR THE PRODUCTION OF SUGAR SYRUP FROM CASSAVA FLOUR

*G.S. Ayemon, *T.K. Hemmond and **A. Griffith

*Dept. of Nutrition and Food Science, University of Ghana, Legon, P.O. Box LG 134, Legon, Ghana
**Natural Resources Institute, University of Greenwich, U.K.

ABSTRACT—Hydrolysis of cassava flour by the combination of rice malt extract (RME) and amylloglucosidase (AMG) on yield and type of sugars were investigated. RME was prepared by malting paddy rice for 10 days at 28°C and diastatic power determined. Effect of rice-malt enzyme concentration on rate of liquefaction and AMG concentration on rate of saccharification were studied. The combined effect of RME and AMG on yield of sugars was investigated. Simultaneous consideration of temperature, pH and time was studied using response surface methodology. Types of sugars present in syrup were identified and quantified by HPLC. Results indicated that the RME had a diastatic power of 91.46 degree Lintner. The highest RME concentration that could liquefy 10% w/w of cassava flour was 8% w/w within an hour. The combination of 8% w/w of RME with 100-unit/ml AMG or 10% w/w of RME with 200-unit/ml AMG resulted in the highest yield of sugars. Simultaneous consideration of temperature, pH and time indicated that the highest yield of sugars occurred at 60°C, pH 4.5 in 4.5 hours of liquefaction and saccharification. HPLC analysis on syrup produced by RME alone, identified glucose, maltose and other sugars, but the combination of RME and AMG produced glucose and maltose only. In conclusion the combination of rice-malt extract with Amyloglucosidase under the specified conditions of temperature, pH and time could increase yields of sugars up to DE 80 from cassava flour.

INTRODUCTION

The industrial hydrolysis of starch into glucose syrup can be a fully enzymatic process. The depolymerization is performed in two steps (liquefaction and saccharification) owing to the incompatibility of the amylolytic enzymes involved (Legen et al., 1998). Enzymes capable of hydrolysing starch are many and their properties are considerably different (Karanazis et al., 1997). In the industrial production of high maltose syrup with exo-maltogenic enzymes, debranching enzymes are always employed, both to increase the yield of maltose and shorten the reaction time (Jahua, 1999). Martensson (1974) investigated the effect of pullulanase concentration on the time course of maltose formation in hydrolysates of 1% starch at a fixed α-amylase concentration. Kinetic expression for the combined action of α-amylase and debranching enzymes has been proposed (Shirasaki et al., 1987). Hoge et al. (1992) developed a kinetic model for the enzymatic hydrolysis of starch to high maltose syrup by simultaneous use of α-amylase and isoamylase with an ultrafiltration reactor system. Fujii et al. (1988) reported the synergistic action of α-amylase and glucoamylase on starch hydrolysis.

Cassava flour has advantages such as complete and faster hydrolysis compared to other flours (Vasungolpe et al., 1984) and its use is being encouraged. Logos and Tazembohung (1978) studied the saccharification of both cassava starch and cassava meal using dual enzymes. They achieved approximately 100% conversion. Zanin and De Morais (1998) reported the modelling of cassava starch saccharification. Traditional malt sugar has only 30-40% maltose and the majority of the remaining sugar is dextrin (Wang, 1998).

The study was conducted to investigate the hydrolysis of cassava flour by the use of rice malt and amylloglucosidase combination on yield and type of sugar syrup produced.

MATERIALS AND METHODS

Paddy Rice (PSR BC 14) Rio Grande type was purchased from Irrigation Development Authority (IDA) at Ashaiman.
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near Accra. Fresh cassava roots were purchased from the Council for Scientific and Industrial Research (CSIR) farms at Teshie near Accra.

Amyloglucosidase (AMG) (EC 3.2.1.2) from \textit{aspergillus niger} with activity 6000 unit/ml in solution in 1m glucose containing 0.5% sodium bicarbonate as preservative. One unit will liberate 1.0 mg of glucose in 3 hours at pH 4.5 in 55°C.

Preparation of rice malt extract (RME).

The fresh ground malt was weighed into 100ml of citric acid-sodium phosphate buffer (pH was adjusted using 0.1M HCl and 0.1M NaOH) and allowed to stand for an hour with intermittent stirring, after which the mixture was centrifuged for 15 minutes at 3000 rpm. The supernatant was used as rice malt extract (RME) for the hydrolysis of the cassava flour in all experimental runs.

 Determination of Diastatic power in rice malt

This was done using the Association of Official Analytical Chemist AOAC (1990) Approved Method 935.31.

Effect of rice malt extract (RME) concentration on rate of liquefaction

Ten (10%) percent of cassava flour was gelatinised and hundred (100) parts of it was hydrolysed using different concentrations of RME (2, 4, 6, 8 and 10% w/v). Ten (10) parts of enzyme concentration was used to hydrolyse the cassava flour. The hydrolysis was done in a thermostatted water bath with stirring at pH 5.5, temperature 55°C. The hydrolysates were carried out for 2.0 hours in triplicates. The yields of sugars were measured as reducing sugars by Lane and Fyson’s method and expressed as dectrose equivalent (DE).

Effect of amyloglucosidase (AMG) concentration on rate of saccharification

This was done by adding concentrations of 200, 300, 400, 500, and 600-AMG unit/ml to cassava flour hydrolysate of (DE 7%) obtained from RME hydrolysed cassava flour. The mixtures were saccharified at a temperature of 55°C and pH 4.5 for 2 hours and stopped by raising the temperature to boiling point in 10 minutes. Total reducing sugars produced was determined as above.

Effect of enzyme combinations and concentrations on the yield of sugars

This was investigated using RME alone and a combination RME and AMG to hydrolyse gelatinised cassava flour. Ten (10) parts of enzyme concentrations (2, 4, 6, 8 and 10%) of RME were each used to hydrolyse 100% samples of 10% cassava flour in a citric acid-sodium phosphate buffer in triplicates at pH 5.5, temperature 55°C in a thermostatted water bath for 2 hours. A second batch was hydrolysed as before for 1 hour and the pHs of the cassava flour hydrolysates were then changed to pH 4.5 and temperatures raised to 60°C to continue the hydrolysis for another 1 hour after the addition of 20 parts of AMG (600-200 AMG unit/ml) to (2-10% w/v) of RME. The yield of sugars was determined as reducing sugars.

Experimental Design and Statistical Analysis

Table 1. Summary of process variables used in central composite rotatable design

<table>
<thead>
<tr>
<th>Levels</th>
<th>Yields</th>
<th>+0.66</th>
<th>0</th>
<th>-0.66</th>
<th>-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature X</td>
<td>55.00</td>
<td>53.62</td>
<td>60.00</td>
<td>62.97</td>
<td>65.00</td>
</tr>
<tr>
<td>pH</td>
<td>5.00</td>
<td>4.20</td>
<td>5.40</td>
<td>5.79</td>
<td>6.00</td>
</tr>
<tr>
<td>Time (hrs) X</td>
<td>3.00</td>
<td>3.66</td>
<td>4.50</td>
<td>5.39</td>
<td>6.00</td>
</tr>
</tbody>
</table>

Twenty variable combinations were generated as shown in the above table 1 for the experimental run. The effects of the independent variables on yield of sugars, were investigated. Stepwise regression analysis and analysis of variance (ANOVA) were performed on the data using Statgraphics computer software (Statistical Graphical Corporation, STSC Inc., USA). Models developed for each index were examined for lack of fit and response surface plots generated.

Determination of sugar profiles in cassava flour hydrolysate by High Performance Liquid Chromatograph (HPLC) Method

This was done using a Shimadzu HPLC system which consisted of SIL-6B auto-injector, SCL-6B system controller, C-R7A chromatopac, CLC-CDS, M (15cm) column, (Shimadzu Corp., Japan). Samples (cassava flour hydrolysate) were diluted with distilled water at a dilution factor of 1:25 and automatically injected in HPLC. The peak retention time, peak height, and peak areas of the individual sugars were compared with those of standard glucose and maltose. The mobile phase was acetonitrile and water (70:30) at a flow rate of 1ml/min. and column temperature of...
40°C

RESULTS AND DISCUSSION

Effect of enzyme concentration on reaction rate:

Figure 1 shows the effect of rice malt concentration on reaction rate of liquefaction of cassava flour. At a constant cassava flour concentration of 10% (w/w) the concentration of RME was increased from 2% (w/v) to 10% (w/v) to hydrolyse the gelatinized cassava flour. The reaction rate of liquefaction increased with increasing enzyme concentration from 2% (w/v) to 8% (w/v) then began to level off after 8% (w/v) concentration to 10% (w/v). For most enzymatic reactions the speed of the reaction is proportional to the concentration of the enzyme at least during the earliest stages of the reaction (Reed, 1975). The reduction in activity could obviously be due to the depletion of substrate. Other reasons could be a limitation of reaction mixture, which becomes saturated at higher concentrations or inhibition by product (Tucker and Woods, 1991).

Figure 2 shows the reaction rate of saccharification by the enzyme amyloglucosidase on RME-hydrolysed cassava flour hydrolysate DE 37. The reaction rate of saccharification increased with increasing AMG concentration.

Fig. 1. Effect of RME concentration on rate of liquefaction of cassava flour

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Effect of enzyme concentration and combination on yield of sugars

Figure 3 shows the effects of enzyme concentration and combination on yields of sugars. The pattern of yield followed the enzyme concentration. The highest yields of 31.88 and 32.35 DE occurred at enzyme concentrations 8% and 10%(w/v) respectively using RME after 2 hours of liquefaction. Nebesyn (1990) reported a DE of 36.60 after 4 hours of hydrolysis using 8 mg/ml of maltogluaminase. When RME was used in combination with AMG at concentration of 2% (600 AMG unit/ml) 4% (480 AMG unit/ml) 6% (360 AMG unit/ml) 8% (300 AMG unit/ml) and 10% (200 AMG unit/ml) the yield of sugars were higher than when RME was used alone. The combination of RME and AMG at concentration 8%(w/v) plus 200 AMG unit/ml resulted in the highest yields of 53.81 and 54.08 DE respectively after 2 hours of hydrolysis.

The combination of 2% RME with 600-AMG unit/ml had a lower yield. Nebesyn (1990) reported a DE of 36.60 after 8 hours of hydrolysis using 15 MANU maltogluaminase in combination with 0.4 PUN pullulanase. Nebesyn (1990) reported a DE of 22.60 after 4 hours of hydrolysis with 0.120 FAU Fungamyl α-amyrase and a DE of 33.60 after 4 hours of hydrolysis using 0.080 FAU Fungamyl α-amyrase in combination with 0.0375 AG amylogluaminase. There are a number of enzymes involved in the complete hydrolysis of starch to yield very high levels of sugars and each of these enzymes contribute only to some extent to the final yield and type of sugars produced. Comparing the yields when RME alone was used, to when used in combination with AMG, the lower yield of sugars produced by RME might be due to the enzyme type α-amylase present in the RME which was able to hydrolyse only the α-1, 4 glucosidic bonds in amylpectin and related polysaccharides of the starch but by-passed the (2->1, 6 glucosidic bonds which it could not hydrolyse. The major
products of hydrolysis could then be oligosaccharides of varying chain lengths with little amount of glucose in the α-amylase randomly split one molecule of starch into smaller molecules. The increase observed on addition of AMG could be attributed to another type of activity. AMG is an exoacting enzyme, rapidly breaks starch down starting from the non-reducing end to release glucose and increase the yield of sugars. This enzyme is also weakly hydrolytic towards α-1 linkages, a fact of commercial importance since this activity permits the production of high glucose syrup (Tucker and Woods, 1991). Greater activity towards those linkages can be achieved by addition of more enzymes. However, this can result in the unwanted side reaction termed "reversion", in which the glucose molecules produced repolymerize to form isomaltose, and hence the final yield of sugars is lowered. Selecting appropriate enzyme dosages and combinations is important to achieving the desired sugars yield. Niagam and Singh (1995) reported that with a careful balance of the ratio of AMG to α-amylase, high-glucose syrup (30–40% glucose, 30–40% isomaltose) or high-maltose syrup (30–50% maltose, 6–10% glucose) could be achieved.

When RME was used in combination with AMG in hydrolysis cassava flour, the regression equation generated for yield was:

\[ Z = -2965(1-9-7235) + 1218891X_1 - 512.78(47X_2 + 0.50168X_3 - 502.76X_2X_3 + 2.61166X_2 + 1.29436X_1X_3 - 1.86614X_2X_3 \]

(1)

(were: \( X_1 \) = temperature, \( X_2 \) = pH, \( X_3 \) = time; \( Z \) = yield of sugars and \( R \) = 0.82, 77%). The model showed significant effect (P < 0.05) (Table 2).

The model had no significant “lack of fit” (P < 0.05) and F-ratio > 1. This implies that the model is sufficient for predicting yield of sugars from cassava flour using RME and AMG combinations. The model could explain 80% of the variations in yield. Further ANOVA for variables in the order fitted indicated that pH had both linear and quadratic effect on the yield, temperature and time had only quadratic effect on yield. The combined effects of temperature and time, temperature, pH and time were also significant (P < 0.05).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of squares</th>
<th>Degree of freedom</th>
<th>Mean sum of squares</th>
<th>F-ratio</th>
<th>P-value</th>
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<td>Model</td>
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<tr>
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<td>Lack of fit</td>
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<td>7</td>
<td>3.564</td>
<td>0.0279</td>
<td>0.002</td>
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<td>Pure error</td>
<td>5.74</td>
<td>5</td>
<td>1.148</td>
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<td></td>
</tr>
</tbody>
</table>

Table 2. ANOVA for the full regression on yield of sugars using wet rice malt and amylase combination.
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Figure 4: Response surface plots for (a) Effect of temperature and pH, (b) Effect of temperature and time, (c) Effect of pH and time on yield of sugars from cassava flour using RME alone and RME–AMG.

The HPLC analysis of cassava flour hydrolysate showed that the HMF identified glucose, maltose, and other sugars in the syrups produced by RME alone. Sugar syrups produced by RME and AMG combination contained glucose and maltose mainly. The sugar distribution in any one type of syrup, of course, depended upon its method of manufacturing or production and the temperature and quantity of enzyme used (Jank and Penycost, 1973). The sugars produced when RME alone was used could be due to the presence of the different enzyme types that might be in the rice malt. Rice malt has been said to be rich in many enzymes especially -amylase, amylglucosidase, and dextrinase (Akazawa, 1972). The -amylase and dextrinase might be responsible for the production of maltose. The glucose might also have been produced through the action of AMG which is an exo-acting enzyme hydrolyzing the 1–4 and 1–6 linkages from the nonreducing end of the starch polymer (Akazawa, 1972). Table 3 shows the approximate quantities of maltose, glucose, and other sugars in syrups produced from cassava flour by different enzyme systems.

Table 3: Dextrin equivalent and types of sugars identified and quantified by HPLC in syrups produced from cassava flour by amylase extract (RME) alone and RME and AMG combination.
The quantity of glucose or maltose present is a function of the enzyme type being used and the conditions. Cecil (1995) reported that syrups produced in Vietnam using rice malt alone contained 60% maltose, 16% glucose and 24% of higher polymers.

**CONCLUSION**

On enzyme concentration and combination, 8% or 10% w/v RME in combination with 300 AMG undiluted or 200 AMG undiluted resulted in the highest yield of sugars. On the simultaneous consideration of temperature, pH, and time on yield of sugars (DE 60-80), highest yields occurred at 60ºC, pH 4.5 in 4.5 hours of hydrolysis and saccharification using RME and AMG. Production of sugars from cassava flour using RME could be increased by the addition of AMG under the specified experimental conditions.

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**REFERENCES**


