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EXTRUSION-COOKING OF CASSAVA STARCH AS A PRE-TREATMENT FOR ITS SIMULTANEOUS SACCHARIFICATION AND FERMENTATION FOR ETHANOL PRODUCTION

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Single screw extrusion of cassava starch was evaluated as a pre-treatment for the enzymatic hydrolysis of the extrudate and fermentation to yield alcohol. The acid concentration, barrel temperature and moisture content showed that all the variables were significant. Increasing acid concentration or barrel temperature induced starch depolymerisation with a higher water solubility index and lower water absorption index. At 20 and 24% moisture contents the cold paste viscosity decreased. As a result of the addition of acid during extrusion cooking the degree of starch hydrolysis resulted in low hot paste viscosity. Acid concentration was significant in the production of reducing sugars. At concentrations above 0.024 N, as the temperature increased, the reducing sugar content also increased. Nevertheless, at concentrations below 0.024 N, the reducing sugar content showed the opposite result. The best yield of alcohol obtained from the extruded starch was 98.7% (0.56 g of ethanol/g starch), which, on average, was 5.7% and 6.8% higher than that obtained from starch extruded without acid and from starch gelatinized by the conventional method, respectively.

Keywords: cassava starch, ethanol, extrusion, fermentation

The conversion of carbohydrates present in grains and tubers into fermentable substrates for the production of alcohol is an old process. Extrusion-cooked substrates for fermentation into alcohol produce a high alcohol yield, and have a short conversion time. Extrusion cooking of cereals has lower energy requirements than conventional cooking methods (KORN, 1982; BEN-GERA et al., 1984). The thermo-mechanical liquefaction of starch under elevated conditions of temperature, pressure and shear, was demonstrated in the early 1970s (SUZUKI, 1970). Cereal starches can be extrusion cooked to allow for direct saccharification by glucoamylase. Enzymatic liquefaction can be realized during and after the extrusion cooking process. Combining enzymatic conversion with gelatinisation and thermo-mechanical liquefaction also solves the problem of high extrudate paste viscosity, allowing downstream enzymatic processing (LINKO et al., 1978; HAKULIN et al., 1983; 1980; 1983a; 1983b). KERVINEN and co-workers (1984) cited that phosphoric acid or sodium hydroxide, rather than

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thermostable enzymes, can be added during extrusion to hydrolise the starch. KORN (1982) used raw corn as the starting material for alcohol production and reported that the cost of ethanol produced by the extrusion process was lower than that obtained by the conventional process. Extrusion is an efficient pre-treatment for the simultaneous saccharification and fermentation of cassava starch, resulting in 92.4% ethanol conversion (GROSSMANN & EL-DASH, 1988). Compared to other starchy materials, cassava offers a high potential, because it is possible to obtain 180 l alcohol/t of raw material, which represents 2.6 times the production of alcohol from 1 ton of sugar cane (MENEZES, 1978). The application of extrusion-cooking to the thermal-mechanical and chemical modification of native starch offers numerous perspectives to significantly vary the properties of starch. However, far too little is known about alcohol production from extruded acid modified cassava starch. The objective of the present work was to investigate the effect of some variables such as acid concentration, moisture content and extruder barrel temperature on some physico-chemical properties of extruded cassava starch. Also, extruded samples were subsequently evaluated for the simultaneous saccharification and fermentation to alcohol without α-amylase treatment.

1. Materials and methods

1.1. Raw material

Raw commercial cassava starch was obtained from Lorenz National Ind. Ltd., Cianorte, PR, Brazil. Thermostable *Bacillus licheniformis* α-amylase, Thermamyl 60 L, and Amyloglucosidase Novo (AMG 300 L) were obtained from Novo Industry Ltd. (SP, Brazil). The compressed commercial yeast (*Saccharomyces cerevisiae*) was obtained from Fleischmann SP, Brazil.

1.2. Extruder and extrusion conditions

Cassava starch was processed using a laboratory scale, single screw extruder constructed by EMBRAPA-Brazil. The extruder barrel (380 mm length and 19 mm diameter) contained three independently controlled sections, die assembly electric heaters and a 3:1 compression ratio screw. The feed rate was kept constant at 65.0 g min^{-1} and the die diameter was 4 mm. The processing temperature was fixed at 80 and 100 °C in zones 1 and 2, respectively. The screw speed was held at 100 r.p.m. Sulphuric acid ($6–74\times10^{-3}$ N) was dissolved in distilled water and added to the cassava starch to obtain the desired moisture content and acid concentration. The temperature in zone 3 and the die were kept constant for the assays, and the acid concentration (H_2SO_4) and moisture content of the cassava starch varied according to the experimental design (Table 1).

Table 1. Operating ranges of the extruder process

	Extruder conditions				
Independent variables	–a	–1	0	+1	+a
Acid concentration (N)	0.006	0.02	0.04	0.06	0.074
Barrel temperature (°C)	93	120	160	200	227
Moisture content (%)	13.3	16.0	20.0	24.0	26.7

$\pm | \alpha | = 1.681$

1.3. Experimental design for extrusion variables

A central composite response surface design was used, with the overall ranges and selected variables shown in Table 1. The data obtained were analysed using the SAS program (1987).

1.4. Analytical methods

The water absorption index (WAI) and water solubility index (WSI) were determined as described by LINKO and co-workers (1980). Cold and hot paste viscosity was measured in a 10% (w/w, d.b.) aqueous suspension using a Brabender Viscoamylograph. The reducing sugar content was determined according to the methods of SOMOGY (1945) and NELSON (1944).

1.5. Saccharification and fermentation

Saccharification and fermentation were conducted at the same time according to the method of PARK and RIVERA (1982). Twenty-five grams of substrate were dissolved in 210 ml tap water containing amyloglucosidase (900 A.G.) in a 500 ml Erlenmeyer flask. Ten ml of 15% (w/w) yeast suspensions were added to the flask. All flasks were protected from the atmosphere by a tube containing concentrated sulphuric acid and incubated at 30 °C with frequent stirring for 35 h. The ethanol concentration was measured by gas chromatography using the Model 37 D (Scientific Instruments Co.) with isobutanol as internal standard and a 3.5 m length Chromosorb W column. The temperature of the equipment was set at 115 °C, 175 °C and 225 °C for the column, detector and vaporizer, respectively. The air flow was 350 cm^3 min^{-1} and the H_2 flow was 35 cm^3 min^{-1}.

2. Results and discussion

2.1. Effect of extrusion variables on some properties of extruded cassava starch

The effect of the extrusion variables, temperature (°C), feed moisture (%) and acid concentration (N), on the functional properties of the extruded cassava starch are shown in Table 2. The contribution of each factor (independent variables) can be evaluated by their significance value (P). Table 3 shows regression equation of all responses (independent variables) studied.

Table 2. Significance of extruder variables on the properties of extruded cassava starch

Independent variables	Significance (P)				
	Cold paste viscosity	Hot paste viscosity	Water absorption index	Water solubility index	Reducing sugar content
Acid concentration (N)	0.0189	0.0001	0.0050	0.0083	0.0001
Barrel temperature (°C)	0.0190	0.0348	0.0248	0.0165	0.0070
Moisture content (%)	0.0007	0.7128	0.0747	0.8401	0.2240

Table 3. Regression equation coefficients for hot paste viscosity, cold paste viscosity, water absorption index, water solubility index and reducing sugar content

Response	Equation	R^2	P
Hot paste viscosity	$-13.40+4.46M+0.11T-73.87A-0.06M^2+0.02\times10^{-1}T^2+11.361A^2-0.02MT-1.65MA-9.38TA$	0.8902	0.001
Cold paste viscosity	$-440.76+56.33M-0.48T+830.97A-0.35M^2+0.75\times10^{-2}T^2+29{,}012.2A^2-0.15MT-359.4MA+10.94TA$	0.8793	0.0015
Water absorption index	$-6.78+0.81M+0.03T+32.55A-0.02M^2-0.01\times10^{-2}T^2-1{,}021.5A^2-0.44\times10^{-3}MT-0.39MA+0.17TA$	0.8497	0.042
Water solubility index	$83.1-0.82M-0.26T-1{,}356.3A-0.21\times10^{-1}M^2+0.11\times10^{-2}T^2+19{,}438A^2+0.09MT-15.55MA+2.76TA$	0.8214	0.0089
Reducing sugar content	$850.6-27.69M-2.59T-30{,}331A+0.48M^{-2}+0.33\times10^{-2}T_2+214{,}198.65A^2-0.95\times10^{-2}MT+385.94MA+72.97TA$	0.9382	0.0001

A: acid concentration (N); M: moisture content (%), T: barrel temperature (°C)

The extent of macromolecular degradation in starch is known to be a function of the extrusion parameters. Research has improved the extent of polymerization by adding thermostable α-amylase to a "wet" starch (up to 65% moisture content on a wet basis) during extrusion at 123 °C, producing maltodextrins with final degrees of polymerization of 3–4 using corn (CHOUVEL et al., 1983), barley (LINKO et al., 1983a; 1983b) and wheat (HAKULIN et al., 1983) starches. Extrusion was suggested as an efficient pre-treatment, followed by saccharification and alcoholic fermentation (KORN & HARPER, 1982; BEN-GERA et al., 1984; LINKO et al., 1984). These authors had obtained the same yield in alcohol as in the traditional method, with an energy reduction

of up to 75%. The contribution of acid treatment during extrusion-cooking on the properties of the cassava starch, as a pre-treatment for its simultaneous saccharification and fermentation to ethanol, was shown to be significant.

2.2. Effects of the independent variables on the water absorption index, water solubility index, cold and hot paste viscosities and reducing sugar content

The water solubility index, water absorption index and viscosity are parameters that have been used to monitor changes that occur in starch as a result of extrusion. These parameters have been widely used to measure depolymerisation and/or decomposition reactions of starch in which the activation energy is applied by mechanical forces, temperature, moisture and thermostable enzymes. The use of the water solubility index and water absorption measurements is justified at the practical level by the fact that they readily yield the "degree of cook" without requiring time-consuming biochemical extractions, isolations and the characterization of complex mixtures. Viscosity has commonly been used to give a measure of macromolecular degradation in extruded starches (COLONNA & MERCIER, 1983). It is useful in fundamental studies, since for linear polymers it can be directly related to average molecular weight. Extrusion cooking of starch led to a degradation of amylose and amylopectin by chain splitting, and the more energy applied or the addition of acid or alkali, the greater of degradation of starch. The extent of the starch modification due to the effects of the extrusion parameters and the acid, can be efficiently measured by applying the referred methods of analysis.

2.2.1. Effect of extrusion conditions on water absorption index (WAI). Figure 1 shows the effect of acid concentration and extrusion conditions on the water absorption index (WAI) of cassava starch at 16, 20 and 24% moisture content. The water absorption index was found to be influenced by the acid concentration, temperature and moisture. In the low range of acid concentration (<0.04 N), the water absorption index decreased as the temperature increased. At a given extrusion temperature, as the acid concentration increased, the water absorption index of the extrudate increased up to a maximum, and then decreased. WAI was strongly affected by the acid concentration during the extrusion process. Increasing barrel temperatures showed a similar effect on WAI. However, higher acid concentrations or barrel temperatures increased starch depolymerisation, associated with the soluble molecules, increasing WSI and decreasing WAI. Microstructural studies have revealed that with an increasing severity of treatment, there is an initial increase in water absorption which is related to the proportion of gelatinized material in the product (KIRBY et al., 1988). The water absorption passes through a maximum, which coincides with an essentially complete particle disruption, and the gelatinisation and disruption of the constituent maize starch. The WAI gives a measure of the volume of the swollen gelled particles, which maintain their integrity in aqueous dispersion. The index is found to correlate with the dispersion viscosities measured at room temperatures.

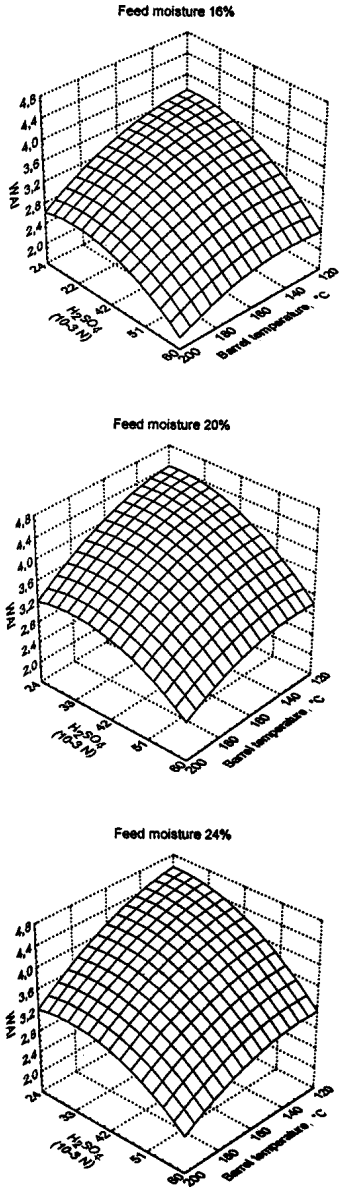

Fig. 1. Effects of acid concentration, barrel temperature and feed moisture on the water absorption index of extruded cassava starch

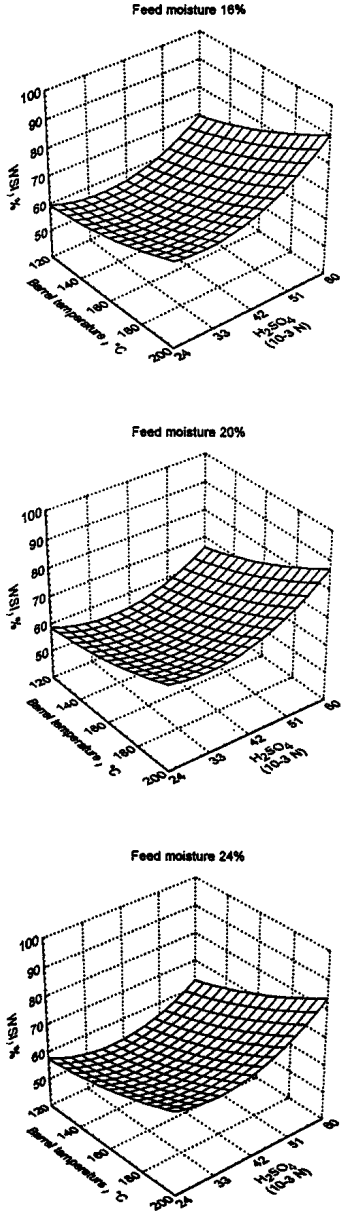

Fig. 2. Effects of acid concentration, barrel temperature and feed moisture on the water solubility index of extruded cassava starch

The interaction between barrel temperature and acid concentration was the most significant variable. When this is removed, the temperature becomes the next most significant variable. The highest WAI were obtained at the highest moisture content (24%). Similar trends were reported by KERVINEN and co-workers (1984). These authors cited that the moisture content influences the water absorption index less than other factors. PÉREZ-SIRA and GONZÁLEZ-PARADA (1997) reported that the moisture content and temperature reduction caused an increase in water absorption capacity, solubility percentage and swelling power values of the cassava starch.

2.2.2. Effect of extrusion conditions on water solubility index (WSI). A regression analysis of the water solubility index with respect to acid and temperature, indicated that these variables were highly significant ($P<0.01$). Solubility studies showed that water solubility increases with increasing acid concentration and barrel temperature (Fig. 2). However, this effect was more noticeable with increases in the acid concentration.

Increasing acid concentration, increased the WSI. This can be explained by higher starch degradation as a result of starch hydrolysis. The severity of the treatment in the extruder was increased with the addition of acid, resulting in increasing quantities of water soluble molecules. WSI values ranged from 55 to 90% higher than those reported by COLONNA and MERCIER (1983) for tapioca starch (82.0–86.4) extruded at 125–200 °C and 22% moisture content. As the water solubility increased there was a decrease in viscosity which reflects a decrease in the average molecular weight of the amylose and amylopectin chains. This means that water solubility can be regarded as an index of the degree of macromolecular degradation. KERVINEN and co-workers (1984) found that an increase in phosphoric acid content generally decreased water absorption index and increased the water solubility index. DARNOKO and ARTZ (1988) showed that the effect of pH on the water solubility index was temperature dependent. KERVINEN and co-workers (1984) extruded wheat starch with the addition of acid or alkali in a twin-screw Creusot-Loire BC extrusion cooker, and showed that at a mass temperature of 160 °C, increasing the feed moisture generally increased WAI and decreased WSI, except at high levels of phosphoric acid.

2.2.3. Effect of extrusion conditions on cold-paste viscosity (25 °C). Figure 3 illustrates the effect of the extrusion temperature (93–227 °C), the moisture content (13.3–26.7%), and the acid concentration (0.006–0.074 N) on the cold-paste viscosity (25 °C) of the extrudates. Evidently, all the variables influenced the cold paste viscosity. At 20 and 24% moisture content, as the temperature and acid concentration increased, the cold-paste viscosity decreased. As expected, the combined action of moisture content and acid concentration increased the extent of starch hydrolysis. Cold paste viscosity is an important property in determining the usefulness of extruded starch for alcohol production by a simultaneous saccharification and fermentation process, because temperatures in the range of 30–32 °C are used and low viscosity is required at these temperatures to avoid technical problems. Extruded cereal starches used for the direct saccharification by glucoamylases form very viscous pastes, which are difficult to handle at the high solids levels desirable for subsequent enzymatic processing.

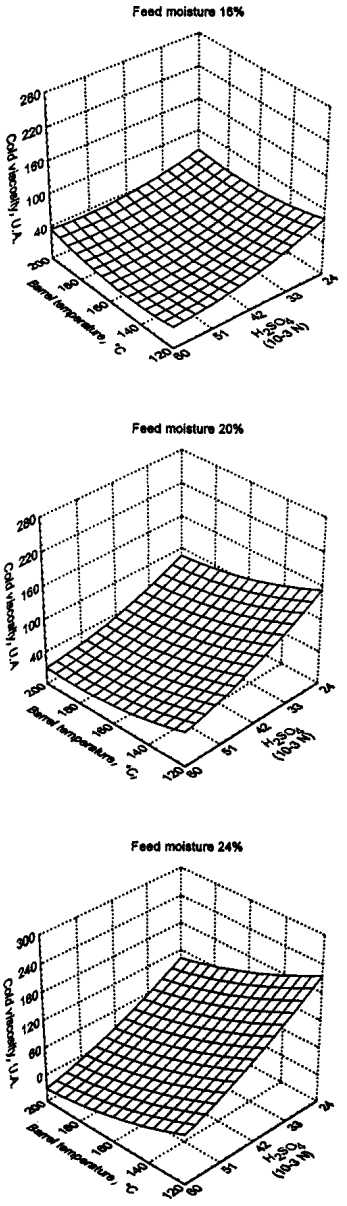

Fig. 3. Effects of acid concentration, barrel temperature and feed moisture on the cold viscosity (25 °C) of extruded cassava starch

They also retrograde rapidly, unless kept at above 60 °C for downstream enzymatic processing (LINKO, 1989). It would be advantageous to use a moisture content of 24% combined with a higher temperature and medium acid concentration (approximately 0.05 N), because in this range the viscosity is almost at zero. In the traditional process of alcohol production thermostable α-amylase is used to reduce gel viscosity, but the results of this work showed that extrusion could substitute α-amylase in obtaining products with similar properties. Evidently the addition of acid during the extrusion-cooking of cassava starch decreased cold paste viscosity, which corresponded to a molecular weight decrease as a result of starch hydrolysis. DARNOKO and ARTZ (1988) reported similar results to those found in this study. A decrease in pH from 4.5 to 2.0 at any extrusion temperature resulted in a great decrease in the apparent viscosity of the extruded starch slurry, suggesting that acid hydrolysis had occurred. Below pH 3.5, the lower the extrusion pH and the greater the extrusion temperature, the lower the apparent viscosity of the extrudate slurry. However, above pH 3.5–4.0, the effect of extrusion temperature was less pronounced. The viscosity of the slurry from cassava starch extruded at pH 4.5 was much greater than that of a slurry made from cassava starch extruded at pH 2.0. This occurred at all the extrusion temperatures studied and indicated that hydrolysis occurred during extrusion, although no small molecular weight materials (degree of polymerization ≤7) were detected by HPLC.

2.2.4. Effect of extrusion conditions on hot-paste viscosity (95 °C). The effects of the extrusion temperature (93–227 °C), the moisture content (13.3–26.7%) and the acid concentration (0.006–0.074 N) on the hot-paste viscosity (95 °C) of the extrudates are shown in Fig. 4. The hot paste viscosity was influenced by the acid concentration and extrusion temperature ($P<0.0001$ and $P<0.04$, respectively). Increasing the acid concentration at high temperatures decreased the hot paste viscosity, while the hot paste viscosity increased at low acid concentrations and with continuous increasing of the barrel temperature. The addition of acid during cassava starch extrusion induced starch hydrolysis, resulting in low hot paste viscosity. DARNOKO and ARTZ (1988) reported that extrusion temperature and pH affected the key parameters associated with the hydrolysis of starch, such as the water solubility index, the water absorption index, the apparent viscosity and the susceptibility of the extruded starch to glucoamylase.

2.2.5. Effect of extrusion conditions on the reducing sugar content (RSC). Acid concentration and temperature were highly significant ($P<0.01$) on the reducing sugar content. The regression equation accounted for 93.8% of the total variation (R^2). At acid concentrations above 0.024 N, as the temperature increased, the reducing sugar content also increased (Fig. 5). However, at concentrations below 0.024 N, the reducing sugar content showed the reverse effect. In the higher temperature range, as the acid concentration increased, the reducing sugar content increased. Similar trends were observed by other authors such as KERVINEN and co-workers (1984). These authors reported that acid treatment remarkably decreased the average molecular weight of the starch and the organized structure of the starch was destroyed to a great extent.

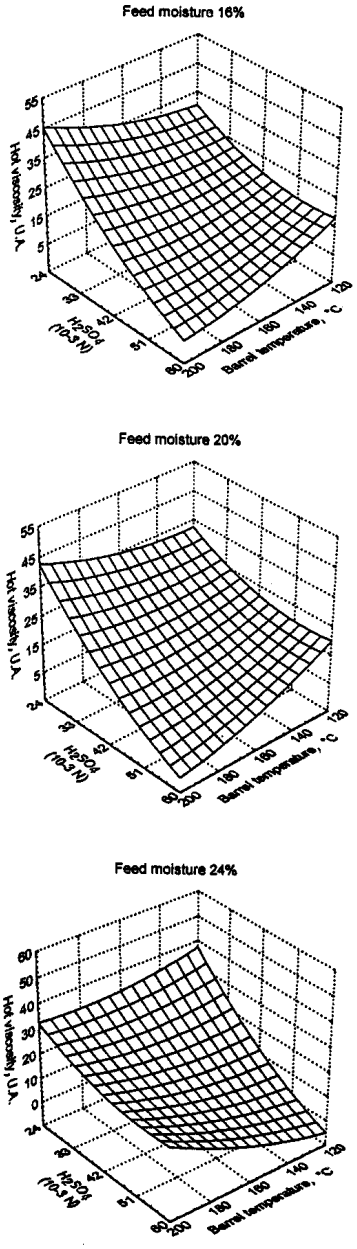

Fig. 4. Effects of acid concentration, barrel temperature and feed moisture on the hot viscosity (95 °C) of extruded cassava starch

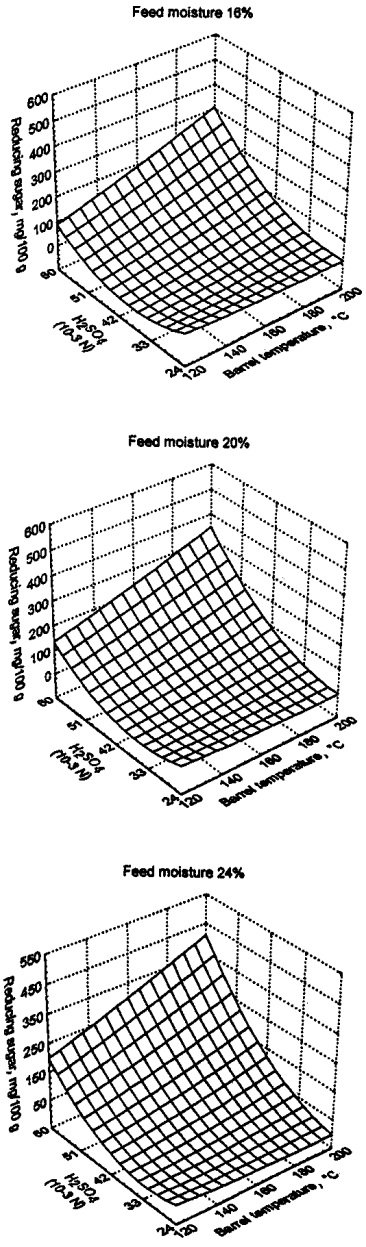

Fig. 5. Effects of acid concentration, barrel temperature and feed moisture on the reducing sugars contents of extruded cassava starch

Starch molecules containing low-moisture contents can be depolymerised easily by heat or heat-shear treatments, and the shearforce, in addition to the heat treatment, contributed significantly to molecular cleavage (FUJIO et al., 1995). MERCIER and FEILLET (1975) observed no formation of maltodextrins during the extrusion of cereal starches. Also LINKO and co-workers (1983a) reported low DE-values after barley starch extrusion. The moisture, feed rate, and discharge temperature of the extruder affected glucose yields after enzymatic hydrolysis, and the best corn extruded samples for alcohol production gave higher glucose yields (BEN-GERA et al., 1984). TOMASIK (1989) reported that depolymerisation occurs in dry starch below 300 °C, forming dextrins. However, the time required for such thermal dextrinisation is longer (min or h, depending on the use of catalyst) than required for processing in extruders. Also RODIS and co-workers (1993) indicated that the absence of glucose or maltodextrins indicates that fragmentation sites are largely internal, rather than adjacent to the reducing or non-reducing terminals of the amylopectin molecule. In addition, the amount of fragmentation is highly affected by the chemical nature of the extrudate, design and configuration of the extruder and extruder operating conditions. GROSSMANN and EL-DASH (1988) reported that the mildest extrusion conditions tested were sufficient to allow for high levels of cassava starch hydrolysis. Also, the cassava starch granule is so fragile that it breaks up easily during thermal treatment (MERCIER & FEILLET, 1975). However, when the water concentration is too low, starch gelatinisation is incomplete. Only partial disappearance of the crystalline structure of the starch takes place, the protein-starch matrix remaining partially intact, and protein membranes may mask the starch granules. In such cases, subsequent conversion of the starch into maltose or glucose will be incomplete and slow, wasteful and costly (BEN-GERA et al., 1984). DARNOKO and ARTZ (1988) reported that starch hydrolysis is increased with an increase in extrusion temperature and a decrease in pH. KERVINEN and co-workers (1984) extruded wheat starch in a twin screw extruder with phosphoric acid (1.5%), and observed that the degree of hydrolysis with alkali was smaller than with acid, and an increase in starch depolymerisation was obtained with an increase in acid concentration.

2.3. Alcohol production by simultaneous saccharification and fermentation

The regression equation did not account for the low value of total variation and showed that the mathematical model to explain the experimental result was not reliable. However, the different operational conditions during extrusion showed an average of 87.5% fermentation efficiency (Fig. 6). With the combination of two variables, such as 20% of moisture content and 217 °C of barrel temperature, it was possible to obtain 93.4% (0.53 g of ethanol/g starch) fermentation efficiency. Comparing this result with that of starch gelatinized by the conventional method (92.4%) the former substrate yielded a slightly higher fermentation efficiency. For starch extruded with acid, the average fermentation efficiency increased up to 93.8%. However, combining the variables such as 24% of moisture content, 200 °C barrel temperature, and 0.06 N acid concentration, the fermentation efficiency was as high as 98.7% (0.56 g of ethanol/g starch) (Fig. 6). Therefore, the fermentation efficiency of cassava starch extruded with

added acid, was, on average, 5.6% and 6.8% higher than those of starch extruded without acid and starch gelatinized by the conventional method, respectively. Also, combining the parameters of the extruder conditions, it was possible to obtain even higher ethanol yields. The improved ethanol yields as compared to extrusion cooking alone, or with the use of enzymes occurred due to the combined action of barrel temperature and acid as achieved in the extruder, resulting in more gelatinisation and dextrinisation, thus hydrolyzing more starch to reducing sugars. GROSSMANN and EL-DASH (1988) reported 92.4% ethanol conversion from extruded cassava starch.

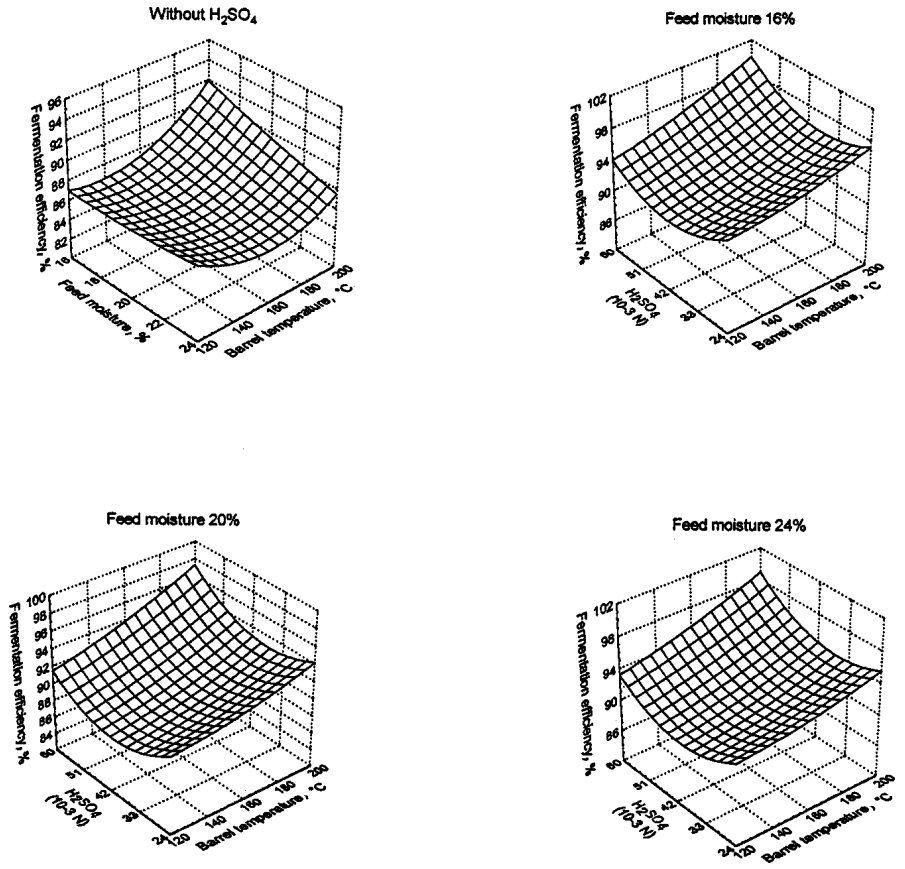

Fig. 6. Effects of acid concentration, barrel temperature and feed moisture on the fermentation efficiency of extruded cassava starch

KORN (1982) studied the extrusion process of corn for alcohol production, and found that the highest glucose and ethanol yields and lowest energy inputs were obtained with a feed rate of 5.2 kg min^{-1}, temperature of 160 °C and feed moisture of 15.1%. Cereal extrusion for alcohol production had the same yield in alcohol as in the conventional method, with a reduction in energy consumption of up to 75% (LINKO et al., 1978; BEN-GERA et al., 1984).

3. Conclusion

Based on the changes in cassava starch during extrusion-cooking with added acid, it is feasible to use single screw extrusion as a pre-treatment for simultaneous saccharification and fermentation to ethanol. With acid concentrations above 0.024 N and high barrel temperatures, the starch molecules were significantly depolymerised to reducing sugars under shear. However, at concentrations below 0.024 N, this trend was reversed. The molecular cleavage of starch was revealed by the liberation of reducing sugars.

Cassava starch extruded with acid showed a greater fermentation efficiency when comparing with that of starch extruded without acid and that of starch gelatinized by the conventional method. By controlling the extrusion conditions, it was possible to obtain products with a higher degree of hydrolysis and lower product viscosity, without the use of thermostable enzymes. The best alcohol yield from the extruded starch (98.7%) was obtained by combining the following variables: 24% moisture content, 200 °C barrel temperature and 0.06 N acid concentration. On average the yield was 5.7% and 6.8% higher than with starch extruded without acid and starch gelatinized by the conventional method, respectively.

Acid concentration followed by barrel temperature, markedly affected the performance of the extruded product, and were found to be the most significant variables with respect to the water solubility index, water absorption index, cold and hot paste viscosities and reducing sugar content. The starch extruded with acid showed greater fermentation efficiency when comparing with those starches extruded without acid or gelatinized by the conventional method. This study confirmed the efficiency of extrusion as a pre-treatment for cassava starch to be used in the simultaneous saccharification and fermentation for ethanol production. By controlling the extrusion conditions, it was possible to obtain products with a higher degree of hydrolysis and lower product viscosity without the use of thermostable enzymes, especially, α-amylases.

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