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STUDIES ON AMYLOLYSIS OF DIFFERENT RICE PRODUCTS DURING A LIQUEFACTION PROCESS USING THERMOSTABLE ALPHA-AMYLASE

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ABSTRACT

Amylolysis of different starchy rice products (starch and flour from amylose and glutinous-rice) were studied and compared with that of purified starch from potato, corn and tapioca during a liquefaction process using thermostable alpha-amylase. Addition of Ca^{2+} greatly improved enzyme stability. Increasing the concentration of Ca^{2+} from the normal level of 70 ppm at 105°C to 250 or 500 ppm improved the liquefaction temperature by 2°C and increased the rate of liquefaction to DE-10 by 16.667% and 21.528%, respectively. Maximum reducing sugar production was obtained at 107°C with rice amylose (30% w/w). Residual activity of alpha-amylase after 10 min liquefaction at 105°C was in the range of 90-96%. For liquefaction of different starchy substrates (30% w/w), increasing order of reducing sugar production and enzyme stability correlated with increasing amylose content. For example purified rice-starch gives higher enzyme stability than rice-flour. The lower stability and reducing sugar production may have resulted from the higher content of protein in the flour (6.5%) than in the starch (0.7%).

INTRODUCTION

Rice is one of the main food crops in Thailand and rice flour, rice starch and processed starch products are manufactured from it on an industrial scale. Liquefaction and subsequent saccharification of rice products upgrades their economic value since there is a great demand in the confectionary and fermentation industries for a wide spectrum of syrups[1,2]. Many studies have been carried out on the hydrolysis of corn, wheat, tapioca and potato products[3,4,5], but few comparable studies have been done on rice products. To fulfill the need for detailed studies on liquefaction and saccharification of rice products, this study focused on amylolysis of different rice products during liquefaction using thermostable alpha-amylase. The aim was to examine hydrolysis of flour and purified starch (amylose) from normal rice and glutinous rice and to compare the results to those obtained using starch from potato, corn and tapioca. In addition to product formation, the aim was to examine enzyme stability during liquefaction.

The use of Ca^{2+} concentrations up to 70 ppm[6,7] in processing has been reported to increase the thermal stability of alpha-amylase from *B. licheneformis* and *B. subtilis*. In those reports, enzyme stability at different Ca^{2+} concentrations increased up to 70 ppm. High reducing sugar production during amylolysis depends on good initial enzyme activity and good enzyme stablilty for an extended period at a relatively high reaction temperature. Increase in temperature increases the kinetic energy of reactant molecules and also decreases the activation energy, thereby favouring enzyme-substrate (E-S) complex formation[8,9]. However, high temperature also increases the vibrational energy of enzyme molecules and this can lead to conformational alterations which ultimately result in denaturation[8]. Reaction time must also be considered in these phenomena[9]. Thus, the various factors need to be optimized in such a way that the enzyme can be stabilized and reactivity increased. In this study, the effects of high Ca^{2+} concentration on enzyme activity and stability were examined during liquefaction.

MATERIALS AND METHODS

Materials

Thermostable alpha-amylase of *Bacillus licheneformis* (Termamyl 60L) was purchased from Novo industries, Denmark. Purified starch from amylose-rice, glutinous-rice, potato, corn and tapioca and finely ground flour of amylose-rice and glutinous-rice were purchased from the local market.

Analytical Methods

Reducing sugar was estimated by the DNS method[10]. Moisture, starch, protein (N x 6.25), fat, fibre and ash content of different substrates were analysed by AOAC methods[11]. Amylose content of starch was determined using the iodine-complex method[12]. Alpha-amylase activity was measured as follows. Enzyme (0.1 ml)and substrate mixture (0.9 ml) in 1M Tris-maleate buffer, pH 7.0 containing 500 ppm Ca^{2+} for enzyme stabilization were combined at 98°C. The resulting assay mixture consisted of 12.5 mg starch and 0.87-4.33 µg of enzyme protein in one ml. Enzyme reactions were terminated at 0 and 5 min by changing the pH to alkali with the addition of DNS. Reducing sugar production was then measured and amylolytic activity was expressed in terms of reducing sugar released (mg) per min.

Liquefaction Process

Enzyme substrates (30% w/w) consisted of slurries of 15 g of different substrates (purified starch from potato, corn, tapioca, amylose-rice and glutinous-rice, and flour from amylose-rice and glutinous-rice) mixed with 35g tris-maleate buffer (pH 7.0). The final pH of the slurry (45 ml) was adjusted to 7.0.

Slurries were placed in thin-walled microtubes and 0.01 ml enzyme (30 µl ml^{-1}) was added with complete mixing. Then microtubes were incubated at appropriate temperatures on an oil bath. Amylolytic action was terminated by chilling the microtubes in an ice-bath. The chilled hydrolysate was then homogenized in Tris-maleate buffer, pH 7.0 at 0°C at a final volume of 10 ml. Then the reducing sugar in the homogenate was measured as enzyme activity using soluble starch at 98°C described above.

Enzyme stability was calculated in terms of percentage residual activity or as half life period. Half life was calculated according to the Arrhenius equation assuming that enzyme activity decay followed a first order reaction scheme[6]. A_t and A_o are activities at time t and 0 respectively.

$$A_t = A_o e^{(-\ln(2/t1/2) \times t)}$$

RESULTS AND DISCUSSIONS

Effect of Enzyme Concentration on the Amylolytic Activity

The properties of the alpha-amylase enzyme from *B. licheneformis* are given in Table 1. It was necessary to determine a suitable enzyme concentration for measurement of enzyme activity. Reducing sugar production profiles at various enzyme concentrations are shown in Figure 1. Amylolytic action can be monitored in terms of rate of reducing sugar released (or production)[13], rate of starch degradation[13], or rate of reduction in viscosity of gelatinized starch[5]. In this work, amylolytic activity was monitored using the rate of reducing sugar released. At enzyme concentrations from 0.005-0.1 µl (0.433 - 8.66 µg protein) ml^{-1}, initial activity was directly proportional to enzyme concentration in the range of 0.005 to 0.05 µl ml^{-1}. Deviation from direct proportionality thereafter may have been due to substrate limitation (Figure 1b). Thus, under our experimental conditions, 0.005-0.05 µl (0.433 -4.33 µg protein) ml^{-1} of enzyme was considered suitable to measure enzyme activity.

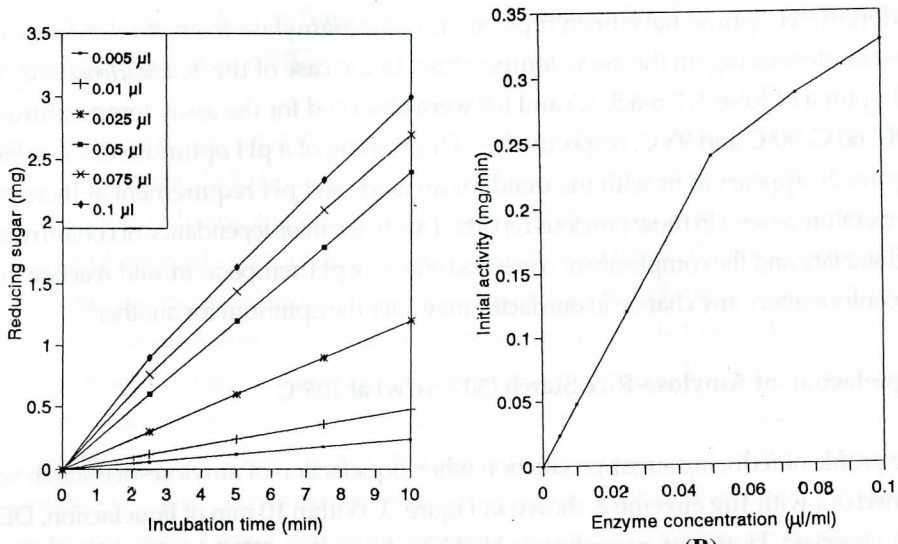
TABLE 1
PROPERTIES OF ALPHA-AMYLASE FROM *BACILLUS LICHENEFORMIS*

PROPERTIES	
Density	1.2 g ml^{-1}
Protein content	72.3 ± 7 mg protein g^{-1}
Specific acitivity	55.325 micro g glucose min^{-1} micro g $protein^{-1}$

Enzyme activity was assayed with a starch solution (12.5 mg ml^{-1}) at 98°C and at pH 7.0 containing 500 ppm Ca^{2+} for enzyme stabilization.

Effect of pH on Enzyme Activity

Tests on pH optimum were carried out at 98°C for 5 min and results were compared to those reported in the literature for alpha-amylase[6,7]. Relative activities of 0%, 100% and 90%, were obtained at pH 3.5, 7 and 8 respectively (Figure 2). There are several possible reasons for these changes in activity. Variations in pH can affect the conformation of enzyme proteins and thereby influences the access of substrate to the active site[8]. Further more, pH can influence the ionization of amino acid moieties including the two histidine residues found at the active site of alpha-amylase and thought to take part in E-S complex formation. In addition carboxylic and imidazole groups are essential for subsequent breakdown of the E-P complex for product during the double displacement mechanism of the alpha-amylase family of enzymes[13,14,15].



(A) (B)

FIGURE 1. EFFECT OF ENZYME CONCENTRATION ON (A) THE PROFILE OF REDUCING SUGAR PRODUCTION AND (B) THE INITIAL ACTIVITY DURING THE HYDROLYSIS OF SOLUBLE STARCH (12.5 mg/ml) AT 98°C AND AT pH 7.0

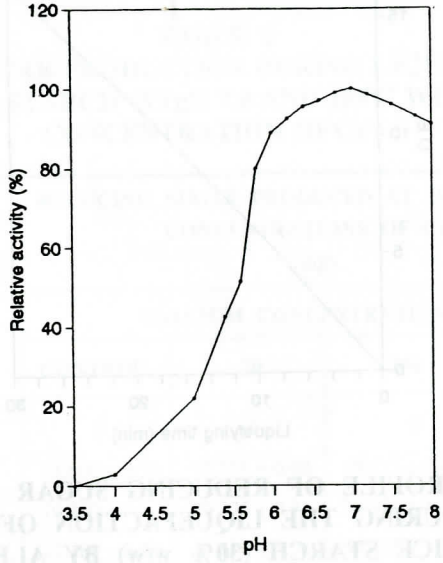


FIGURE 2. EFFECT OF pH ON THE AMYLOLYTIC ACTIVITY DURING THE HYDROLYSIS OF SOLUBLE STARCH (12.5 mg/ml) AT 98°C

Different pH optima have been reported for alpha-amylase from *B. licheneformis* and *B. subtilis* depending on the assay temperature. In the case of the *B. licheneformis* enzyme pH optima of 5.6-6.3, 5.6-6.5, 6.5 and 6.8 were observed for the assay temperatures of 37°C, 60°C, 90°C and 95°C, respectively[6,7]. Our finding of a pH optimum of 7.0 at 98°C (Figure 2), appears to fit with the trend for an increased pH requirement at increased temperature as seen in these previous reports. Due to the interdependancy of conformation and stability and the complexity of combined effects of pH, temperature and reaction time on conformation, any change in one factor may alter the optimum for another[8].

Liquefaction of Amylose-Rice Starch (30% w/w) at 105°C

The profile of reducing sugar production when liquefaction of amylose-rice starch was carried out with 1µg enzyme is shown in Figure 3. Within 10 min of liquefaction, DE-6 was observed. However, according to NOVO information, DE 8-10 should have been obtained. The unexpected low reducing sugar production could have been due to loss of enzyme activity during storage before purchase.

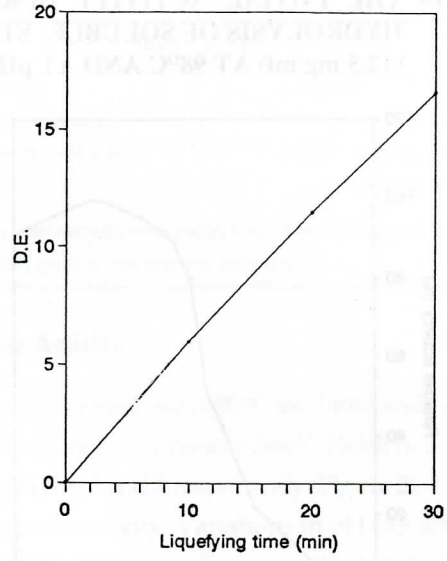


FIGURE 3. PROFILE OF REDUCING SUGAR PRODUCTION DURING THE LIQUEFACTION OF AMYLOSE-RICE STARCH (30% w/w) BY ALPHA-AMYLASE (1 µg/g SOLIDS) AT 105°C IN THE PRESENCE OF 70 ppm Ca^{2+}

Effect of Ca^{2+} on Reducing Sugar Production and on Stability of Alpha-amylase during Liquefaction at 105°C

Much higher reducing sugar was produced from amylose-rice starch in the presence of Ca^{2+} in the range of 70-500 ppm when compared to the control with no added Ca^{2+} (Table 2). In addition, reducing sugar production at 70-500 ppm Ca^{2+} was still ascending at 30 min of incubation whereas it had reached a plateau at 20 min in the control. Stability of the enzyme also increased with increasing Ca^{2+} concentration (Table 3). The requirement of Ca^{2+} for the amylolytic action has been shown by reversible EDTA deactivation[13,14]. For example, prolonged dialysis against EDTA inactivates amylase from *B. substilis* and *Aspergillus oryzae* activity can be restored by addition of Ca^{2+}. In addition to enzyme stabilization, Ca^{2+} can help to prevent proteolyses of alpha-amylase by contaminant proteases[13,14,15] and it can reduce the denaturating action of some inhibitors[13,14]. Although it can be seen from Table 2, that reducing sugar production continued to increase as the concentrations of Ca^{2+} above 70 ppm. By contrast, the stability of the enzyme was profoundly increased by higher concentrations of Ca^{2+} since half lives for the enzyme were 8.01 ± 0.10, 87.09 ± 0.79, 167.08 ± 0.72 and 225 ± 4.22 min, respectively, at 0, 70, 250 and 500 ppm. Thus, even if a substrate contained sufficient Ca^{2+} for full enzyme activity (in the absence of chelating agents), addition of excess Ca^{2+} would be warranted to improve enzyme stability.

TABLE 2
REDUCING SUGAR PRODUCTION DURING LIQUEFACTION OF AMYLOSE-RICE STARCH ATpH 7.0 AND 105°C WITH DIFFERENT CONCENTRATION OF Ca^{2+}

INCUBATION TIME (MIN)	REDUCING SUGAR PRODUCED AT DIFFERENT CONCENTRATIONS OF Ca^{2+} (mg)			
	CALCIUM CONCENTRATION (ppm)			
	CONTROL	70	250	500
0	0	0	0	0
10	11.560 ± 0.56	15.310 ± 0.60	15.625 ± 0.48	15.780 ± 0.70
20	16.250 ± 0.60	29.375 ± 0.95	30.625 ± 1.30	31.250 ± 250
30	17.180 ± 0.70	42.500 ± 1.03	44.375 ± 1.50	46.250 ± 1.05

The data were done in triplicates.

TABLE 3
THERMAL STABILITY OF ALPHA-AMYLASE DURING LIQUEFACTION OF AMYLOSE-RICE STARCH AT pH 7.0 AND 105°C WITH DIFFERENT CONCENTRATION OF Ca^{2+}

INCUBATION TIME (MIN)	HALF LIFE OF ENZYME AT DIFFERENT CONCENTRATIONS OF Ca^{2+} (MIN)			
	CALCIUM CONCENTRATION (ppm)			
	CONTROL	70	250	500
10	8.10	86.51	168.00	230.60
20	8.06	86.55	166.25	224.00
30	7.86	88.22	167.00	220.41
mean	8.01 ± 0.1	87.09 ± 0.79	167.08 ± 0.72	225.00 ± 4.22

Effect of Temperature on Reducing Sugar Production and Enzyme Stability at High Ca^{2+} Concentration.

Since high enzyme stability was observed at 105°C with high Ca^{2+} addition, the effect of temperature on reducing sugar production and thermal stability of the enzyme were re-examined in the event that increased temperature could lead to a beneficial increase in reaction rate. The data of reducing sugar production at different temperatures in the presence of 250 ppm Ca^{2+} (Table 4) and 500 ppm Ca^{2+} (data not shown) are quite similar. Thermal stabilities under the same conditions are shown in Tables 5 and 6, respectively. The highest reducing sugar production was obtained at a liquefaction temperature of 107°C. In general practise, 105°C with 70 ppm Ca^{2+} are recommended in liquefaction processes to obtain a hydrolysate of DE-10[6]. Under these conditions, DE-10 was obtained in 17.5 min (Figure 3). At the same temperature, 250 ppm Ca^{2+} gave DE-10 in 16.8 min, while at 107°C it gave DE-10 in 15 min (Table 4). This represented a 16.667% increase in the liquefaction rate when compared to that obtained at the conditions (70 ppm Ca^{2+} and 105°C) adopted in general practise. Increasing Ca^{2+} to 500 ppm at 107°C increased the liquefaction rate by 21.528% (data not shown). The enzyme half lives at 107°C for 250 and 500 ppm Ca^{2+} were 90.83 ± 1.66 and 113.6 ± 1.56 min, respectively (Tables 5 and 6). Thus, increasing the temperature and Ca^{2+} concentration could be exploited to increase the liquefaction rate. Furthermore, the significant stability of the enzyme at high Ca^{2+} concentrations might be exploited in its recovery rather than denaturation, which is

normal practise at the end of liquefaction. When considering relative activity and residual activity at 10 min, they were in the range of 90-96% of those at 105°C (Figure 4).

TABLE 4
EFFECT OF TEMPERATURE ON REDUCING SUGAR PRODUCTION DURING LIQUEFACTION OF AMYLOSE-RICE STARCH AT pH 7.0 WITH 250 ppm Ca^{2+}

INCUBATION TIME (MIN)	REDUCING SUGAR PRODUCED AT DIFFERENT TEMPERATURES (mg)				
	TEMPERATURES (°C)				
	98	103	105	107	110
0	0	0	0	0	0
10	14.375 ± 0.75	15.313 ± 0.51	15.938 ± 0.78	17.340 ± 1.08	13.750 ± 0.55
20	27.500 ± 0.95	29.375 ± 1.35	30.625 ± 1.05	33.405 ± 0.90	25.000 ± 0.50
30	40.000 ± 1.60	41.250 ± 1.05	43.750 ± 0.65	46.875 ± 0.81	28.250 ± 0.45

At 105°C, DE-10 was reached in 16.8 min whereas at 107°C DE-10 was reached in 15 min.

The data were done in triplicates.

TABLE 5
EFFECT OF TEMPERATURE ON STABILITY OF ENZYME DURING LIQUEFACTION OF AMYLOSE-RICE STARCH AT pH 7.0 WITH 250 ppm Ca^{2+}

INCUBATION TIME (MIN)	HALF LIFE OF ENZYME AT DIFFERENT TEMPERATURES (MIN)				
	TEMPERATURES (°C)				
	98	103	105	107	110
10	564.68	220.80	164.74	89.52	41.13
20	565.88	224.00	162.00	89.80	41.32
30	567.80	222.00	166.50	93.18	40.05
mean	556.12 ± 1.28	222.26 ± 1.32	164.41 ± 1.85	90.83 ± 1.66	40.83 ± 0.559

TABLE 6
EFFECT OF TEMPERATURE ON STABILITY OF ENZYME DURING LIQUEFACTION OF AMYLOSE-RICE STARCH AT pH 7.0 WITH 500 ppm Ca^{2+}

INCUBATION TIME (MIN)	HALF LIFE OF ENZYME AT DIFFERENT TEMPERATURES (MIN)				
	TEMPERATURES (°C)				
	98	103	105	107	110
10	663.00	245.83	213.00	114.90	55.96
20	666.00	249.32	216.00	111.40	54.38
30	682.00	249.30	220.00	114.50	56.00
mean	670.33 ± 8.33	248.16 ± 1.63	216.33 ± 2.867	113.60 ± 1.564	55.45 ± 0.75

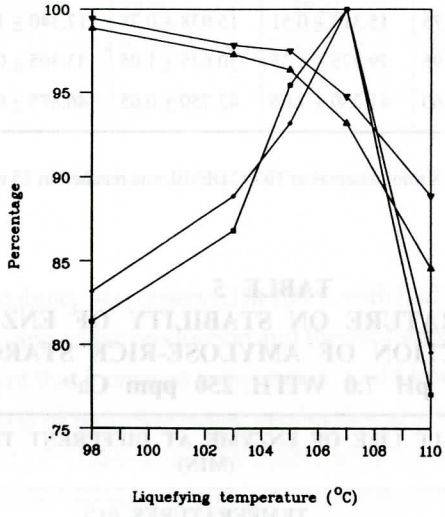


FIGURE 4. EFFECT OF LIQUEFYING TEMPERATURE ON RELATIVE INITIAL AND RELATIVE RESIDUAL ACTIVITY DURING 10 MIN. INCUBATION OF AMYLOSE-RICE STARCH (30% W/W)

Amylolysis of Different Rice Substrates during Liquefaction

Results for comparative liquefaction of amylose-rice starch, glutinous-rice starch, potato starch, corn starch and tapioca starch (30 %w/w) are shown in Tables 8, 9, 10 and 11.

Using 250 ppm Ca^{2+}, amylolysis of amylose-rice starch was greater than that of glutinous-rice starch, but it was comparable to that of tapioca starch and lower than that of potato starch and corn starch. Thus, the highest reducing sugar production was obtained with potato starch and the lowest with glutinous-rice starch (Table 8). Using 500 ppm Ca^{2+} the same pattern was observed (Table 10).

To understand the reasons for different production rates, the physico-chemical properties of the tested substrates were analysed. The results are presented in Table 7. The greatest differences were found for amylose contents while other properties were nearly same amongst the different starch substrates. Amylose-rice starch had a significantly higher amylose content (26%) than glutinous-rice-starch (5%). As alpha-1,4 linkages are most susceptible to alpha-amylase action while alpha-1,6 linkages are resistant, reducing sugar production is higher with amylose than amylopectin[13,14,15], this may explain the difference in production between the two substrates. In support to this argument, the amylose contents of potato starch and corn starch were greater than that of amylose-rice starch and also gave better amylolysis results.

TABLE 7
PROPERTIES OF DIFFERENT SUBSTRATES

PROPERTIES	PERCENTAGE						
	PURIFIED STARCH					FLOUR	
	Potato	Corn	Tapioca	A-rice	G-rice	A-rice	G-rice
Moisture	13.5	13.5	13.5	13.5	13.5	12.5	12.5
Protein	0.5	0.4	0.3	0.7	0.7	6.5	6.5
Fat	0.2	0.2	0.3	0.3	0.3	0.3	0.3
Fibre	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Ash	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Starch	85.3	85.4	85.4	85.0	85.0	80.2	80.2
Amylose in starch	36	32	28	26	5	26	5

When considering the thermal stability of the enzyme, increase in half life was also observed with increase in amylose content (Tables 7, 9 and 11). It has been reported that alpha-1,6 linkages in amylopectin molecules exert steric hindrance for access of alpha-amylase near alpha-1,4 linkages[13,14] and that the enzyme may act either by random or by successive cleavage[15]. Linear amylose molecules would give a better probability for formation of the E-S complex with both patterns of enzyme action when compared to branched amylopectin molecules. Therefore, with amylose, the active site of alpha-amylase would be protected for more time than it would with amylopectin during a similar time of hydrolysis.

This protection could extend against heat denaturation and thereby improve the thermal stability of the enzyme.

TABLE 8
REDUCING SUGAR PRODUCTION WITH DIFFERENT SUBSTRATES DURING LIQUEFACTION AT pH 7.0 AND 105°C IN THE PRESENCE OF 250 ppm Ca^{2+}

INCUBATION TIME (MIN)	REDUCING SUGAR PRODUCED FOR DIFFERENT SUBSTRATES (m g)						
	PURIFIED STARCH					FLOUR	
	Potato	Corn	Tapioca	A-rice*1	G-rice*2	A-rice*1	G-rice*2
0	0	0	0	0	0	0	0
10	16.563±0.80	16.406±0.5	16.094±0.55	15.938±0.45	14.219±0.81	12.969±0.45	11.875±0.50
20	32.186±0.55	32.031±0.60	31.250±0.65	30.937±0.78	27.187±0.50	25.000±0.89	22.500±0.90
30	46.875±1.50	46.250±0.95	45.313±0.55	45.000±0.80	39.375±0.75	35.938±0.65	32.500±0.53

*1 - A-rice = Amylose-rice

*2 - G-rice = Glutinous-rice

The data were done in triplicates.

TABLE 9
THERMAL STABILITY OF THE ENZYME WHEN LIQUEFYING DIFFERENT SUBSTRATES AT 105°C AND AT pH 7.0 IN THE PRESENCE OF 250 ppm Ca^{2+}

INCUBATION TIME (MIN)	HALF LIFE OF ENZYME DURING LIQUEFACTION OF DIFFERENT SUBSTRATES (MIN)						
	PURIFIED STARCH					FLOUR	
	Potato	Corn	Tapioca	A-rice	G-rice	A-rice	G-rice
10	171.79	176.68	168.56	168.56	161.00	95.51	85.90
20	176.59	171.80	168.45	168.45	154.16	95.58	89.80
30	174.23	170.21	162.60	162.60	155.26	94.77	82.21
mean	174.20 ± 1.95	170.89 ± 0.67	166.54 ± 2.87	166.54 ± 2.87	156.81 ± 2.99	95.28 ± 0.37	85.97 ± 3.10

TABLE 10
REDUCING SUGAR PRODUCTION WITH DIFFERENT SUBSTRATES DURING LIQUEFACTION AT pH 7.0 AND 105°C IN THE PRESENCE OF 500 ppm Ca^{2+}

INCUBATION TIME (MIN)	REDUCING SUGAR PRODUCED FOR DIFFERENT SUBSTRATES (m g)						
	PURIFIED STARCH					FLOUR	
	Potato	Corn	Tapioca	A-rice	G-rice	A-rice	G-rice
0	0	0	0	0	0	0	0
10	16.563±0.65	16.563±0.50	16.094±0.71	15.938±0.45	14.531±0.40	13.251±0.35	12.188±0.70
20	32.813±0.51	32.500±0.55	32.406±0.59	31.250±0.41	28.436±0.53	25.625±0.59	23.436±0.63
30	47.813±0.91	46.875±0.85	45.938±0.93	45.625±1.05	41.250±1.08	37.188±0.95	34.063±0.85

The data were done in triplicates.

TABLE 11
THERMAL STABILITY OF THE ENZYME WHEN LIQUEFYING DIFFERENT SUBSTRATES AT 105°C AND pH 7.0 IN THE PRESENCE OF 500 ppm Ca^{2+}

INCUBATION TIME (MIN)	HALF LIFE OF ENZYME WHEN LIQUEFYING DIFFERENT FEEDS (MIN)						
	PURIFIED STARCH					FLOUR	
	Potato	Corn	Tapioca	A-rice	G-rice	A-rice	G-rice
10	227.50	220.00	217.96	217.96	206.56	137.96	122.53
20	230.78	224.00	220.25	218.40	203.03	134.40	122.50
30	231.24	220.00	220.00	220.00	203.80	137.87	123.40
mean	229.84	221.33	218.78	218.78	204.46	136.74	122.81
	±	±	±	±	±	±	±
	1.67	1.88	0.87	0.87	1.52	1.66	0.42

A comparison of amylolysis of amylose-rice starch and amylose-rice flour showed better production of reducing sugar with the starch than the flour during liquefaction with 250 and 500 ppm Ca^{2+}. Proximal analysis of the starch and flour showed that there was a significantly higher amount of protein (6.5%) in the flour than in the starch (0.7 %). Further more, obvious precipitation of protein was observed during liquefaction of the flour. Precipitation of protein during the flour liquefaction process at 105°C may have entrapped some enzyme molecules and thereby reduced its availabilty for reaction with amylose. The precipitated protein may also have trapped some Ca^{2+} and reduced its availability for enzyme stabilization.

In conclusion, the lower sugar production (Table 8 and 10) and lower enzyme stability (Table 9 and 11) observed with amylose rice-flour when compared to amylose-rice starch were most likely caused by a lower amylose content and higher protein content in the former. Similar can be applied to the comparison of glutinous-rice flour and starch. Abraham et.al.[5] have reported that rice flour showed lower liquefaction than corn starch during liquefaction (20% w/w at 90°C), and proximal analysis showed higher protein and fat, and lower amylose in the rice flour than in the corn starch.

CONCLUSIONS

Liquefaction of different rice substrates showed that amylose-rice performed better than glutinous rice. Higher liquefaction rates and higher enzyme stabilities were observed with purified starches than with comparable flours. Protein precipitation flours at the liquefaction temperature may entrap some of free enzyme and Ca^{2+} leading to lower performance. Application of higher concentrations of Ca^{2+} than previously practised allows for an increased in the temperature and rate of liquefaction. In addition, high Ca^{2+} enhanced enzyme stability and opened the possibility of enzyme recovery instead of denaturation at the end of liquefaction.

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