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Evaluation of Different Parameters Effect on Maltodextrin Production by α-amylase Termamyl 2-x

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Abstract: The aim of this study was application of α-amylase termamyl 2-x for maltodextrin production from corn starch and evaluation of its industrial uses. The effective parameters were studied in laboratory and pilot plant scale. This process includes preparation of starch suspension, pH adjustment, addition of enzyme and heating under stirring. Before enzyme inactivation, DE and°Brix were controlled continuously. After separation of soluble sections by centrifuge, maltodextrin liquid was dried by spray drier. In this study, DE values at dry matter, were calculated under three enzyme concentrations (0.2, 0.25 and 0.3 ml of α-amylase termamyl 2-x per Kg of starch) and in three different hydrolysis temperatures (60, 65 and 70°C) at constant pH (6). A completely randomized design with factorial arrangement and 5 replications was conducted. Multiple linear regression was used for relationship between Dextrose Equivalent (DE) and different parameters (enzyme concentration, temperature and hydrolysis time). The results from different enzyme dosage at the same temperature and time of hydrolysis do differ significantly ($p = 0.05$). Meanwhile the best enzyme concentration and hydrolysis temperature for maltodextrin (DE between 18 and 19) production after 300 minutes were 0.25 ml of enzyme per kg of starch and 70°C, respectively. In these conditions, the least residual starch concentration and α-amylase activity were observed.

Key words: Maltodextrin · dextrose equivalent · enzymatic hydrolysis · α-amylase · spray drying

INTRODUCTION

Today, modified starch derivatives produced, have wide applications in food industries. These products are produced by physical, chemical [1] or specific enzymatic [2] methods. The wide range of hydrolyzates available are described in terms of their 'Dextrose Equivalent' (DE) value, is defined as measure of the total reducing power of all sugars present relative to glucose expressed on a dry weight basis [3].

Maltodextrin '$(C_6H_{10}O_5)$ $n.H_2O$' is a mixture of saccharides with a molecular weight between polysaccharides and oligosaccharides with DE lower than 20 (not sweet), which is available as white powders mostly or concentrated solution [4]. Maltodextrin is more soluble in water than native starches, also is cheaper in comparison with other major edible hydrocolloids and its solutions have a bland flavour and smooth mouthfeel [5].

Maltodextrin as a food additive has been applied for about 35 years [3]. It performs multifaceted functions in food systems, including buxlking, caking resistance, texture and body improvement, films formation, binding of flavour and fat, serving as oxygen barriers, giving surface sheen, aiding to dispersion and solubility, increasinge of soluble solids, crystallization inhibition and control of freezing point, fillings and as product extenders. Maltodextrin has been studied as a plasticizer to reduce glass transition temperature in materials. It has been proven useful to reduce Maillard reactions and is used in microencapsulation of food components such as vitamins, minerals and colourants [3, 5, 6].

Corn starch has been extensively used as a raw material in maltodextrin production [7]. The structure of maltodextrins depends on botanical sources, which determine their physicochemical properties [8].

Nowadays, acid hydrolysis of starch is limited for maltodextrin production andparticularly recommended for production of glucose syrups [4]. Enzymatic hydrolysis of starch has distinct advantages compare to acid process. There is no need to remove salts formed during acid neutralization and due to wider pH range and lower temperatures this process is more economic and control

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of process is easier too [9]. Enzyme-catalyzed conversion with α-amylase from *Bacillus* strains is commonly used for production of maltodextrins [10].

The maximum activities of the α-amylases are usually in the pH range between 4.8 and 6.5, but the activity-pH profile and location of the pH optima differ depending on the enzyme source [11]. For production of low DE maltodextrins, BAN 480 L (a type of α-amylases) may be used [12] and now pullulanases are used for production of gelling maltodextrins [13].

The major steps in the enzymatic conversion of starch are liquefaction and saccharification. In liquefaction, the enzyme hydrolyses the α-1, 4-glycosidic bonds in starch, whereby the viscosity of the gel rapidly decreases and maltodextrins are produced. When maltodextrins are saccharified by further hydrolysis using glucoamylase or fungal α-amylase, a variety of sweeteners can be produced [12, 14].

Maltodextrin production by various α-amylases has been studied in many recerches. Slomiska *et al.* [15] studied liquefaction of starch by thermostable α-amylase. Placido *et al.* [14] identified the maltooligosaccharides of hydrolyzed starch with α-amylase thermamyl 120 L (DE = 18 to 20) by HPLC. The drying kinetics of maltodextrin (DE = 12) in a convection oven were modeled using Fick's second law of diffusion and following the William, Landel and Ferry (WLF) equation [16].

In all enzymatic starch hydrolysis, the most important problem is to sure from enzyme inactivation. Ozbek and Yu¨ceer [17] studied α-amylase inactivation during wheat starch hydrolysis process. Apar and Ozbek, investigated the effects of operating conditions on the enzymatic hydrolysis of corn [18] and rice starches [19]. They also studied α-amylase inactivation by temperature during starch hydrolysis [20]. In the mentioned works, some inactivation models were tested to determine the relationship between process variables and enzyme stability during the hydrolysis process.

The purpose of present study was determining the possibility of maltodextrin production by α-amylase termamyl 2-x, from corn starch in laboratory and pilot plant scale and evaluation of different parameters in the process (enzyme concentrations, temperatures and hydrolysis times) for industrial uses.

MATERIALS AND METHODS

Raw materials: Corn starch samples were supplied by the Yazd Mahshad Company (Yazd, Iran). Properties of the tested starch are shown in table 1. α-amylase termamyl 2-x (from *Bacillus licheniformis* with activity of 180-240 KNU/g) was supplied from Novozymes Company (Denmark). Fehling buffers, iodine solution, sodium hydroxide 1 N and hydrochloridric acid 5 N were supplied from Merck Company (Germany).

Table 1: Properties of tested starch

Properties of raw starch	
Ash contents (in d.b., %)	0.40
Moisture contents (in d.b., %)	14.00
Protein contents (in d.b., %)	0.35
Fat contents (in d.b., %)	0.20
SO_2 (ppm)	70.00
pH	4.50-6.50

Dextrose equivalent determination: For dextrose equivalent determination the modified Lane and Eynon titration (Corn Refiner Association- Method E-26), has been used. Soluble solids (ºBrix) determination has been done by refractometery method (CHD 10557 model; China).

Experimental process: Based on the results obtained in laboratory, this process was accomplished in pilot plant scale. A suspension containing 35% dry matter was liquefied to make the starch susceptible to further enzymatic breakdown by α-amylase [15]. The suspension of starch was prepared by dispersion of 1 kg of starch in 1.8 L of distilled water (35% starch suspension w/v).

According to Chronakis [3] and Slomiska *et al.* [15] pH for starch hydrolysis by α-amylase termamyl 2-x was adjusted to 6 with sodium hydroxide 1 N. Three enzyme concentrations (0.2, 0.25 and 0.3 ml) of α-amylase thermamyl 2-x per Kg of starch were added. Suspensions were put in water bath at 60, 65 and 70°C under stirring and the enzyme was allowed to react further at these temperatures for 5 hours until the required DE was obtained.

During with 300 minutes of hydrolysis with 30 minutes intervals, one fraction was removed from the water bath and its DE was determined. According to Slomiska *et al.* [15] and Placido *et al.* [14] DE and soluble solids (ºBrix) determination can express the starch hydrolysis yield.

Enzyme inactivation (pH = 4.3) was obtained by addition of hydrochloridric acid 5 N [16]. Soluble materials separated from the insoluble fibers by centrifugation (Spectrafuge16M model; England) according to Griffin and Brooks [21]. Finally, for inhibition of starch retrogradation, the obtained soluble materials were dried rapidly by spray drying (pilot plant scale GEA model; India) at 190±2°C inlet temperature and 85±2°C outlet temperature [4,6].

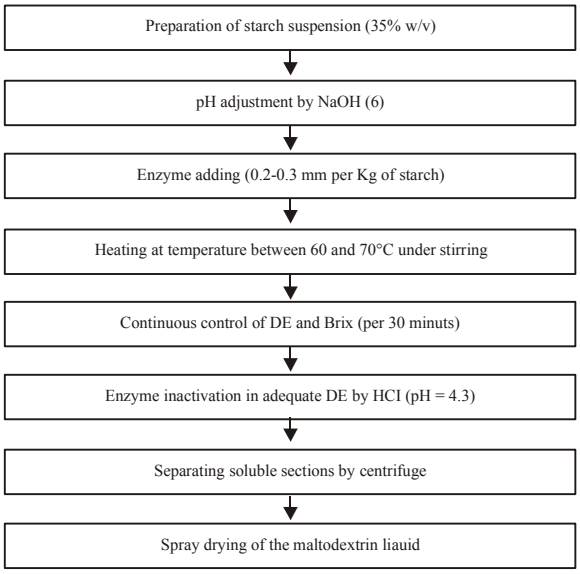


Fig. 1: Schematic flow chart of hydrolysis process for maltodextrin production by α-amylase termamyl 2-x.

The hydrolyzed starches were investigated for residual starch concentration and α-amylase activity, according to De Moraes *et al.* [22]. Figure 1, shows that schematic flow chart of hydrolysis process for maltodextrin production by α-amylase termamyl 2-x that was used in this research.

Statistical analysis: A completely randomized design with factorial arrangement and 5 replications was conducted. To study the relationship between DE and different parameters, multiple linear regression was used. Finally for approximation of DE (based on enzyme concentration, temperature and hydrolysis time) a regression model was exhibited.

RESULTS AND DISCUSSION

DE average values obtained from five replications for corn starch at each enzyme dosage, temperature and time of hydrolysis. The results from different enzyme dosages at the same temperature and time of hydrolysis do differ significantly ($p = 0.05$).

Figures 2-1 and 2-2 show the effect of temperature (at constant enzyme dosage) and enzyme dosage (at constant temperature) on DE value during 300 minutes of hydrolysis, respectively. Error bars in these figures represented the standard errors about the means of replication. Experimental results showed that after 300 minutes of hydrolysis, DE average values under 60°C hydrolysis temperature and three enzyme concentrations of α-amylase thermamyl 2-x (0.2, 0.25 and 0.3 ml per Kg of starch), respectively were 8.82, 14.87, 20.9, while hydrolysis temperature was 65°C the DE values were 10.86, 16.15, 26.73 and with 70°C hydrolysis temperature DE values were 12.07, 18.27 and 28.14.

Slomiska *et al.* [15] found that after 1 h of α-amylase activity (60-120 NU/g d.b.) on corn starch at 95°C, DE varies from 8.9 to 18.2. Using of enzyme dosage in amount of 120 NU/g d.b. gives the highest DE for com starch. DE amounts were 17.1, 23.1 and 24.1 after1, 2 and 3 hours, respectively. According to Placido *et al.* [14] 40 µL of α-amylase thermamyl 120 L (equivalent to 0.6 Kg of enzyme by ton of starch) was added to starch suspension at 100°C. Based on this report, amount of DE after 15 minutes was 17.74 and progressively increased.

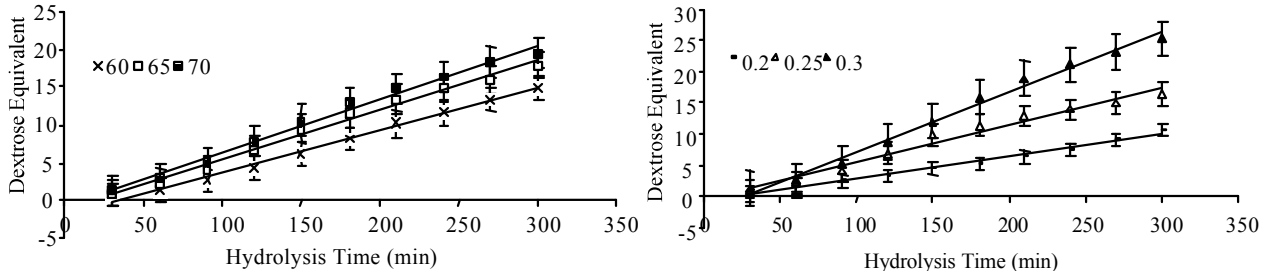
Despite the mentioned studies, the results of present study show that maltodextrin production by α-amylase termamyl 2-x is much easier and have a lot of advantages for industrial process. Benefits of this enzyme are including efficient DE development; when high DE development in a short time is a key parameter, high dextrose yield with minimal by-product formation, fast viscosity reduction, enabling high dry substance levels, low colour formation and reducing refinery costs; savings on chemicals and ion exchange costs by eliminating calcium addition [12, 23].

Correlation between variables was analyzed by multivariate regression and the best conditions for each treatment were determined. These results were checked by backward stepwise regression but all effective parameters, survived. Also equation (1), for DE estimation in applied conditions (enzyme concentrations, temperatures and hydrolysis times) was exhibited. ($R^2 = 0.875^{**}$)

DE = (82.6×Enzyme concentration) + (0.359×Hydrolysis température) + (0.0635×Hydrolysis time)-45.1 (1)

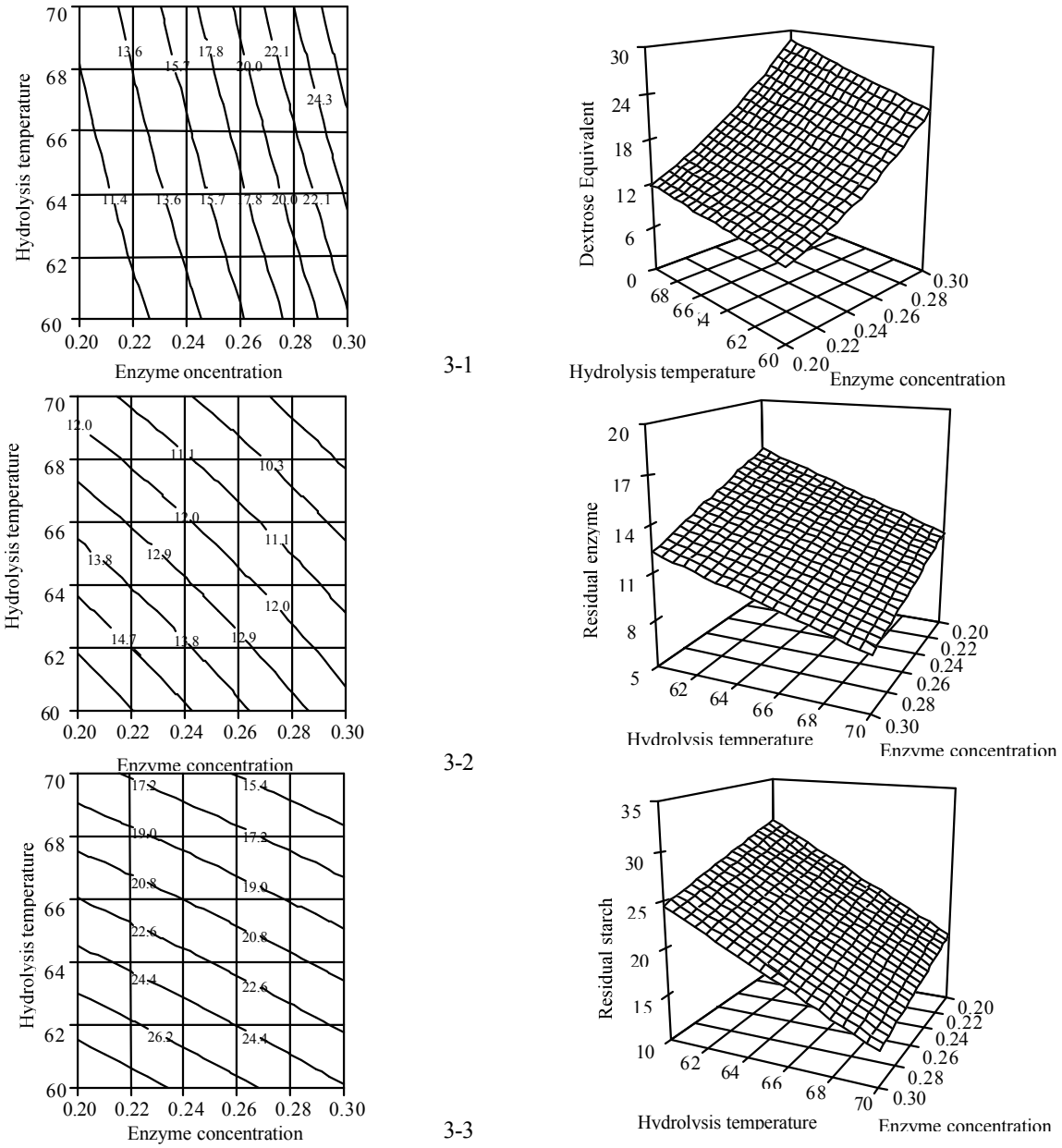
Based on the measured coefficients, the highest correlation was observed between DE and enzyme concentration while the hydrolysis time has the least relationship.

The least residual starch concentration (15%) and α-amylase activity (9%) were shown in 0.25 ml of enzyme and 70°C, meanwhile the most residual starch concentration (31%) and α-amylase activity (17%) were shown in 0.2 ml of enzyme and 60°C, after 300 minutes of hydrolysis process. Residual starch concentration had more stepper slope than residual α-amylase activity, versus enzyme dosage and hydrolysis temperature (Fig. 3).



2-1 2-2

Fig. 2: Effect of temperature at constant enzyme dosage (2-1) and enzyme dosage at constant temperature (2-2) on DE value during hydrolysis time



3-1

3-2

3-3

Fig. 3: Evaluation of DE changes (3-1), percent of residual a-amylase activity (3-2) and percent of residual starch concentration (3-3) after 300 minutes hydrolysis of corn starch by a-amylase thermamyl 2-x (enzyme concentrations between 0.2 and 0.3 ml per Kg of starch and temperature between 60 and 70ºC)

According to these results, the best enzyme concentration and hydrolysis temperature after 300 minutes of hydrolysis for maltodextrin production (with DE value between 18 and 19) were 0.25 ml of enzyme per kg of starch and 70°C, respectively.

CONCLUSION

The results of this investigation show that α-amylase thermamyl 2-x concentration, temperature and hydrolysis time have significant effects on DE value. The least residual starch concentration and residual α-amylase activity were observed in 0.25 ml of enzyme dosage and 70°C after 300 minutes. In these conditions, DE was 18.27±0.05.

The optimization of enzyme concentration, temperature and hydrolysis time will lead to maltodextrin production with specific properties. Maltodextrin that was produced in present research with DE value between 18 and 19 can be applied as anti caking agent and suitable filler in production of spray dried foods.

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