

Thesis Title            Immobilized Brewer's Yeast Containing Invertase for  
                         Continuous Inversion of Sucrose

Name                    Miss Sivalee Boonkunlaya

Thesis Advisor        Assitant Professor Surapong Navankasattusas, Ph.D.

Department            Food Technology

Academic Year        1986

#### ABSTRACT

Consumption of high fructose corn syrup in developed countries such as the United State of America, Japan and Canada is currently gaining popularity. Invert sugar can be used in food product like high fructose corn syrup. There are three basic processes of invert sugar production from sucrose namely acid hydrolysis, ion-exchange resins and enzymatic conversion (invertase). The advantages of enzymatic conversion are low ash, less color and flavor development, and less costly than acid hydrolysis. However free enzyme can be used only once and some of the enzyme may be contaminated in the product. This research was directed to develop immobilized brewer's yeast containing invertase. Optimal activity pH and optimal temperature, half life, kinetics of immobilized invertase in a batch and continuous process, and optimal condition for continuous inversion of sucrose were determined. The results of varying contents of yeast cell and albumin, following a  $4^2$  factorial experimental plan indicated that the content of yeast, the content of albumin and their interactive effects affected physical stability and activity at 99% significant

level. The optimal composition for immobilization consisted of 5% yeast (weight by volume of liquid) and 4% albumin (weight by volume of liquid). Substitution of albumin by gelatine at 75% or higher may cause difficulty in bead forming. Immobilization of the yeast with gelatin instead of albumin at different content of sodium alginate, gelatine and glutaraldehyde according to a  $2^3$  factorial experiments showed that sodium alginate, interactive effects of gelatine and glutaraldehyde, three factors interaction between sodium alginate, gelatine and glutaraldehyde would reduce the physical stability, but glutaraldehyde would increase the physical stability. Two factors interaction of sodium alginate and glutaraldehyde, gelatine and glutaraldehyde would increase activity. The immobilized yeast with gelatine could not be used in continuous process of sucrose inversion because of its swelling. Increasing albumin in the use of gelatine and albumin in the immobilization of yeast would decrease activity. The immobilized yeast with high ratio of gelatine to albumin would swell more proportionally. Increasing the soaking time of beads in calcium chloride with 1% glutaraldehyde (volume by volume of liquid) would decrease activity. Washing the beads with phosphate buffer pH 6 for over 12 hours would soften and deform the beads. Beads washed with phosphate buffer pH 6 were vacuum dried. Both optimal activity pH and stability pH of immobilized invertase were 4-6. The optimal temperature for inversion of sucrose was 60°C. Thermal stabilities of immobilized invertase over the temperature range from 30-70°C in 50% sucrose solution (weight by weight) pH 5 were indifferent. The dried immobilized invertase could be kept at 4°C with an estimated half life of 520 days.

Batch hydrolysis of 15% sucrose solution (weight by weight) pH 5 with immobilized invertase had a half life of 8 days at 60°C. The activation energies for deactivation of immobilized invertase was 16309.58 cal/g mol. Immobilized invertase showed characteristics of substrate inhibition. The  $K_m(\text{app})$  was 220.70 mM,  $K_s$  was 5660.50 mM and  $V_{\text{max}}(\text{app})$  was 33647.60 units/g immobilized invertase respectively. Continuous hydrolysis of sucrose by immobilized invertase in packed-bed reactor had residence time distribution between plug-flow and perfect mixing. The optimal condition for production of invert sugar with 3 g of immobilized yeast in packed-bed reactor (1 cm inside diameter and 30 cm length) of 18 ml capacity was that 15% sucrose solution (weight by weight) pH 5 was fed to the packed-bed reactor with space time of 18 minutes at 60°C. The immobilized invertase had an estimated half life of 10 days at 60°C. The production of invert sugar could be scaled up to 4-fold while conversion was slightly different. The kinetics of continuous hydrolysis of sucrose by immobilized invertase in steady state had  $K_m(\text{app})$  of 87.60 mM,  $K_s$  of 1363.70 mM and  $V_{\text{max}}(\text{app})$  of 9800 units/g immobilized invertase respectively.

Invert sugar with 90% conversion consisted of fructose, glucose and sucrose could be kept for over 4 months without crystallization.