CHAPTER VI

DISCUSSION

6.1 Fructose Determination

The general formula for determining fructose content, as described in 2.5, can be validly used only if the correction factor (f) is constant. It is therefore necessary to determine experimentally whether f is constant.

The experimental relationship between f value and G/F ratio at constant content of glucose, shown in Table 5-1, was neither constant nor simply correlated.

The experimental relationship between f value and G/F ratio at constant total content of sugars, shown in Table 5-2, was rather constant. It appeared that, at constant total content of sugars, the general formula may be applicable. Never the less f value was truly dependent on the total content of sugars, calculated f value in Table 5-1 should decrease or increase when the total content of sugars was varied in one direction. Such results may be the consequence of false assumptions underlying the formulation.

The calculated f values at constant content of glucose and constant total content of sugars at different G/F ratios were presented in Table 5-3.

Glucose isomerase specifically isomerizes glucose to fructose. Fructose content of isomerized glucose syrup can be calculated as the difference of apparent glucose contents before and after isomerization. Validity of such consideration was demonstrated by experimental results shown in Table 5-4.

6.2 Composition of Tapioca Flour

Except for the starch content, the analytical composition of the tapioca flour used in this investigation as shown in Table 5-5, conformed with standard characteristics for grade three tapioca flour (see Table 3-2).

6.3 Production of Glucose Syrup

6.3.1 Effect of special heat treatment and inactivation of Termamyl on the yield of glucose

Special heat treatment was suggested for better gelatinization (1,18). Inactivation of alpha-amylase before amyloglucosidase addition was suggested in the enzyme-enzyme conversion process (16). However, experimental results shown in Table 5-6 demonstrated that special heat treatment and inactivation of Termamyl did not affect yield of glucose produced from tapioca flour. Moreover, severe heating condition of these treatments produced intensely browning discoloration (36,37) which could only be removed with a large dosage of activated carbon. Extent of the discoloration carried out according to the procedure in 4.3.4.2 was shown in Table 5-7.

6.3.2 Effect of deionized and tap water on the yield of glucose

Experimental results shown in Table 5-8 demonstrated that either deionized or tap water could be used as solvent in producing glucose syrup. Termamyl produced from Bacillus licheniformis requires trace of calcium to maintain its activity and stability (1,17). Glucose syrup then could be produced with deionized water without subsequent cation exchange treatment prior to isomerization because deionized water still contained a small amount of calcium which could fulfill the trace calcium requirement of Termamyl.

The higher ICUMSA color index of syrup produced with tap water was probably due to more insoluble and soluble impurities in water according to the results shown in Table 5-9.

6.4 Pre-treatment of Glucose Syrup

6.4.1 Effect of pre-treatment on the yield of fructose

According to the results shown in Table 5-10, pre-treatment of glucose syrup before isomerization had an effect on the yield of fructose. Sample 1,2 and 3 yielded insignificantly different amounts of fructose because they were all prepared with deionized water which contained low calcium content at the beginning. Color by-product in sample 4 might inhibit Sweetzyme type A then cause the reduction of fructose yield. These results conform with the curve shown in Figure 3-29 on the influence of syrup purity (37).

Glucose syrup before isomerization had varying ICUMSA color index as shown in Table 5-11. The difference in color might be due to its different pre-treatment characteristics. The increase of color index after isomerization could be the result of heating effect during the isomerization process.

6.4.2 Effect of cation exchange on the yield of fructose

Glucose syrup with and without pre-treatment of cation exchange gave slightly different yields of fructose as shown by the results in Table 5-12. Although calcium acted as inhibitor to Sweetzyme (35), Sweetzyme might exhibit high activity initially until magnesium in the enzyme had been replaced by calcium.

6.5 Isomerization of Glucose Syrup to Fructose Syrup

6.5.1 Effect of cobalt chloride on the yield of fructose

Table 5-13 showed that, addition of cobalt chloride to glucose syrup gave a significantly increased amount of fructose. These results confirm the importance of cobalt in activating and retaining the activity of Sweetzyme type A during batch isomerization (28).

The higher ICUMSA color index observed in fructose syrup produced with addition of cobalt chloride was probably due to the presence of higher fructose content in the final product shown in Table 5-14. Fructose is more susceptible to browning than glucose (16).

6.5.2 Effect of Mg/Ca ratio on the yield of fructose

Glucose syrup prepared with different Mg/Ca ratios gave insignificantly different amounts of fructose according to the results shown in Table 5-15. Similar yield of fructose was obtained in sample 1,2 and 3 which confirms with the results shown in Table 3-5 on the influence of magnesium (32). Sample 2 and 4 which had the same Mg/Ca ratio but different total magnesium and calcium content, did not give significantly different amounts of total fructose yield after three isomerizations. However, for sample 4, rapidly decreasing fructose yields were observed in subsequent isomerizations.

6.5.3 Effect of polyphosphate and EDTA addition on the yield of fructose

Glucose syrup prepared according to 4.3.6.4 gave different levels of total fructose yield as shown in Table 5-16. Polyphosphate and EDTA are chelating agents which can combine with calcium, magnesium and cobalt (38). If glucose syrup is prepared with tap water, the added polyphosphate or EDTA may bind with calcium and make it unnecessary to be treated with cation exchange before isomerization. However, polyphosphate and EDTA can bind with cobalt stronger than calcium (38). These chelating agents can then be used only if the interaction between cobalt and Sweetzyme is irreversible. Similarly total yield of fructose was observed in sample 2 and 3 after three isomerizations. However, they were much lower than the others. The affinity between cobalt and polyphosphate might be so high that little or no cobalt was available for Sweetzyme type A. The difference in yields of fructose between sample 4 and 5 was possibly due to the binding effect of cobalt with Sweetzyme type A or EDTA depending on which was initially present.

The binding characteristics between chelating agents such as polyphosphate and EDTA and atoms such as calcium, magnesium and cobalt could be demonstrated by using eriochrome black T indicator. Experimental results shown in Table 5-17 indicated that eriochrome black T indicator reacted with calcium and magnesium giving wine-red solution as seen in sample 2. If there was no calcium or magnesium in the solution such as in the case of deionized water (sample 1), the indicator would give blue solution. Polyphosphate or EDTA added to tap water before and after reacting with indicator, could bind with calcium and magnesium giving blue solution as seen in sample 2,3,8 and 9. These two chelating agents were stable on heating at 60^{o}C for 20 hours and they still retained their ion-binding properties as indicated by the results in sample 4 and 9. EDTA dosages used in sample 8, 10 and 12-16 were insufficient to bind with calcium, magnesium and cobalt. On the other hand, the amount of EDTA in sample 9 and 11 was far too high to be recommended. The result of sample 7 demonstrated that polyphosphate specifically bound with cobalt and liberated calcium and magnesium into bulk solution. Observation on sample 10 showed that EDTA could be bound with calcium and magnesium in the presence of cobalt.

6.5.4 Effect of pure glucose and prepared glucose syrup on the yield of fructose

Experimental results on production of fructose syrup from pure glucose and prepared glucose syrup using different dosages of Sweetzyme type A were shown in Table 5-18. The results demonstrated that the amount of impurities that might be present in the prepared glucose syrup was not sufficient to affect yield of fructose. Dosage of 1.6 gm Sweetzyme type A per 1,000 gm of glucose was too low and required very long time for isomerization.

6.5.5 Determination of the activity of Sweetzyme type A

The determined activity of Sweetzyme type A was 426.6 $\pm$ 5.3 GINU/gm instead of the claimed activity of 508 GINU/gm. It was thus necessary to find the residual activity of the enzyme before use to ensure that the enzyme dosage was appropriate.

6.5.6 Determination of Sweetzyme type A dosage in the first isomerization

As indicated in Table 5-19, the appropriate dosage of Sweetzyme type A (activity 426.6 GINU/gm) for the first isomerization was found to be 33.8 gm per 1,000 gm of glucose.

6.5.7 Effect of magnesium reduction on the yield of fructose

According to the experimental results shown in Table 5-20, reduction of magnesium content in the glucose syrup did not lower the yield of fructose. The residual activities of the enzyme were comparable to those working in glucose syrup with high content of magnesium. Reasons for the unexpectedly low % residual activities in comparison to Figure 3-30 (37) were probably due to the significant pH drop during isomerization and the presence of oxygen in glucose syrup.

6.6 Post-treatment of Fructose Syrup

6.6.1 Color determination of fructose syrup before and after activated carbon treatment

The results in Table 5-21 showed that the high color index just after the first isomerization carried out according to the procedure in 4.3.6.1 might be due to the leaching of color from the enzyme. However, the color index of fructose syrup after activated carbon treatment was still high as shown by the results presented in Table 5-22. The color formation might be the result of heating fructose at high temperature (16).

6.6.2 Cobalt determination of fructose syrup before and after cation exchange

Cobalt must be removed because of its toxicity which can cause symptoms such as cutaneous flushing, chest pains, dermatitis, tinnitus, vomiting and nerve deafness. From Table 5-23, no cobalt was detectable in fructose syrup indicating that cobalt could be effectively removed after the isomerization process by cation exchange.