CHAPTER V

EXPERIMENTAL RESULTS

5.1 Analysis for Glucose and Fructose

5.1.1 Relationship between factor, f and G/F ratio

Relationship between factor, f and G/F ratio was studied in 2 cases according to 4.3.1.3.1 and 4.3.1.3.2.

Table 5-1 Relationship between f value and G/F ratio at constant content of glucose

25 ml of sample solution				Calculated f value	G/F ratio
True G, mg	True F, mg	G_t, mg	G_I, mg		
65.0	4.3	69.6	66.7	3.1	15.1
65.0	6.5	72.8	66.7	16.2	10.0
65.0	13.0	78.7	66.7	13.0	5.0
65.0	32.5	97.9	66.7	25.0	2.0
65.0	65.0	128.6	67.6	16.2	1.0

Table 5-2 Relationship between f value and G/F ratio at constant total content of sugars

25 ml of sample solution				Calculated f value	G/F ratio
True G, mg	True F, mg	G_t, mg	G_I, mg		
60.9	4.1	66.2	62.8	5.9	14.9
59.1	5.9	66.1	61.2	5.9	10.0
54.2	10.8	65.4	56.0	7.7	5.0
43.3	21.7	62.9	46.5	4.1	2.0
32.5	32.5	61.9	35.1	5.7	1.0

Table 5-3 Relationship between f value and G/F ratio at constant content of glucose and at constant total content of sugars

G/F ratio	f value	
	Constant content of glucose	Constant total content of sugars
14.9-15.1	3.1	5.9
10.0	16.2	5.9
5.0	13.0	7.7
2.0	25.0	4.1
1.0	16.2	5.7

5.1.2 Fructose determination by Iodometric method.

Fructose content was determined by Iodometric method according to 4.3.1.4

Table 5-4 Fructose determination at different G/F ratio

25 ml of sample solution					G/F ratio
True G, mg	True F, mg	True M, mg	G_I, mg	Calculated F, mg	
75.0	-	-	75.6 ± 0.0	-	∞
65.0	10.0	-	65.9 ± 0.4	9.7 ± 0.4	6.5
50.0	25.0	-	52.0 ± 0.0	23.6 ± 0.0	2.0
42.0	33.0	-	44.7 ± 0.0	30.9 ± 0.0	1.3
38.0	37.0	-	41.3 ± 0.2	34.3 ± 0.2	1.0
75.0	-	5.0	79.5 ± 0.4	-	∞
65.0	10.0	5.0	70.1 ± 0.0	9.4 ± 0.4	6.5
50.0	25.0	5.0	55.9 ± 0.4	23.6 ± 0.8	2.0
42.0	33.0	5.0	49.1 ± 0.4	30.4 ± 0.8	1.3
38.0	37.0	5.0	45.4 ± 0.3	34.1 ± 0.7	1.0

5.2 Composition of Tapioca Flour

The composition of tapioca flour was determined according to 4.3.2.1 - 4.3.2.8 .

Table 5-5 Composition of tapioca flour

Characteristics	Sample no.	
	1	2
% Moisture content	9.7 ± 0.7	12.4 ± 0.3
% Ash (on dry basis)	0.40 ± 0.09	0.40 ± 0.01
% Acid insoluble ash (on dry basis)	0.10 ± 0.01	0.08 ± 0.00
% Protein (on dry basis)	0.37 ± 0.05	0.00 ± 0.00
% Starch (on dry basis)	88.6 ± 1.0	89.3 ± 0.8
Pulp, ml/50 gm of flour max	0.1	0.1
pH of aqueous extract	5.95	5.6
% Fineness max	1.0	1.0

5.3 Production of Glucose Syrup

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

Four samples of starch slurry were prepared according to 4.3.4.2.

Table 5-6 Effect of special heat treatment and inactivation of Termamyl on the yield of glucose

Sample no.	Treatment		% yield of glucose
	Special heat treatment	Inactivation of Termamyl	
1	+	+	101.7 ± 0.4
2	+	-	100.9 ± 0.6
3	-	+	101.3 ± 0.5
4	-	-	100.4 ± 0.9

Note + = Treatment applied

\- = Treatment not applied

Table 5-7 ICUMSA color index of glucose syrup produced according to 4.3.4.2

Sample no.	Treatment		ICUMSA color index
	Special heat treatment	Inactivation of Termamyl	
1	+	+	736.5
2	+	-	631.4
3	-	+	722.0
4	-	-	623.0

Note + = Treatment applied

\- = Treatment not applied

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

Two samples of starch slurry were prepared according to 4.3.4.3 .

Table 5-8 Effect of deionized and tap water on the yield of glucose

Sample no.	Types of water used	Calcium content ppm	% yield of glucose
1	Deionized water	1	100.4 ± 0.9
2	Tap water	16	100.3 ± 0.4

Table 5-9 ICUMSA color index of glucose syrup produced according to 4.3.4.3

Sample no.	Types of water used	ICUMSA color index
1	Deionized water	623.0
2	Tap water	751.0

5.4 Pre-treatment of Glucose Syrup

5.4.1 Effect of pre-treatment on the yield of fructose

Four samples of glucose syrup (30° Brix) were pre-treated according to 4.3.5.2 and isomerized according to 4.3.6.1.

Table 5-10 Effect of pre-treatment on the yield of fructose

Sample no.	Pre-treatment			% yield of fructose
	Activated carbon treatment	Cation exchange	Anion exchange	
1	+	+	+	14.3 ± 0.6
2	+	+	-	15.2 ± 0.8
3	+	-	-	15.1 ± 1.1
4	-	-	-	11.0 ± 0.4

Note + = Pre-treatment applied

\- = Pre-treatment not applied

Table 5-11 ICUMSA color index of syrup pre-treated according to 4.3.5.2 and isomerized according to 4.3.6.1

Sample no.	Pre-treatment			ICUMSA color index	
	Activated carbon treatment	Cation exchange	Anion exchange	Before isomerization	After isomerization
1	+	+	+	143.7	224.9
2	+	+	-	171.7	274.8
3	+	-	-	199.8	277.9
4	-	-	-	723.3	780.3

Note + = Pre-treatment applied

\- = Pre-treatment not applied

5.4.2 Effect of cation exchange on the yield of fructose

Two samples of glucose syrup (30° Brix) were pre-treated according to 4.3.5.3 and isomerized according to 4.3.6.1.

Table 5-12 Effect of cation exchange on the yield of fructose

Sample no.	Pre-treatment			Calcium Content ppm	% yield of fructose
	Activated carbon treatment	Cation exchange	Anion exchange		
1	-	+	-	2	11.1 ± 0.4
2	-	-	-	20	9.4 ± 0.5

Note + = Pre-treatment applied

\- = Pre-treatment not applied

5.5 Isomerization of Glucose Syrup to Fructose Syrup

5.5.1 Effect of cobalt chloride on the yield of fructose

Two samples of glucose syrup (30° Brix) were pre-treated according to 4.3.6.2 and isomerized according to 4.3.6.1 .

Table 5-13 Effect of cobalt chloride on the yield of fructose

Sample no.	Sample characteristics	% yield of fructose
1	Addition of cobalt chloride	15.1 ± 1.1
2	No addition of cobalt chloride	7.2 ± 0.4

Table 5-14 ICUMSA color index of syrup pre-treated according to 4.3.6.2 and isomerized according to 4.3.6.1

Sample no.	Sample characteristics	ICUMSA color index	
		Before isomerization	After isomerization
1	Addition of cobalt chloride	199.8	277.9
2	No addition of cobalt chloride	199.8	263.8

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

Four samples of pure glucose syrup (28 °Brix) were prepared according to 4.3.6.3 and isomerized according to 4.3.6.1 .

Table 5-15 Effect of Mg/Ca ratio on the yield of fructose

Sample no.	Mg/Ca ratio	% yield of fructose			Total yield
		1st isomerization	2nd isomerization	3rd isomerization	
1	10	6.5 ± 0.3	3.7 ± 0.2	2.8 ± 0.2	13.0 ± 0.7
2	20*	6.5 ± 0.1	3.7 ± 0.2	2.8 ± 0.5	13.0 ± 0.8
3	32	6.5 ± 0.1	3.7 ± 0.5	2.8 ± 0.3	13.0 ± 0.9
4	20**	7.7 ± 0.3	3.3 ± 0.5	2.2 ± 0.01	13.2 ± 0.8

Note * Sample 2 was prepared with deionized water and added with magnesium sulfate to get Mg/Ca ratio = 20

** Sample 4 was prepared with tap water and added with magnesium sulfate to get Mg/Ca ratio = 20

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

Five samples of pure glucose syrup (28°Brix) were prepared according to 4.3.6.4 and isomerized according to 4.3.6.1.

Table 5-16 Effect of polyphosphate and EDTA addition on the yield of fructose

Sample no.	% yield of fructose			
	1st isomerization	2nd isomerization	3rd isomerization	Total yield
1	6.6 ± 0.04	3.7 ± 0.3	2.5 ± 0.1	12.8 ± 0.4
2	2.3 ± 0.1	1.8 ± 0.1	1.3 ± 0.2	5.4 ± 0.4
3	2.3 ± 0.3	1.9 ± 0.2	1.4 ± 0.1	5.6 ± 0.6
4	3.9 ± 0.1	3.4 ± 0.08	2.6 ± 0.04	9.9 ± 0.2
5	5.1 ± 0.2	4.0 ± 0.03	3.0 ± 0.2	12.1 ± 0.4

Note 1 = Sample prepared with deionized water and adjusted Mg/Ca to 10

2 = Sample prepared by mixing polyphosphate, cobalt chloride and Sweetzyme type A together

3 = Sample prepared by mixing cobalt chloride and Sweetzyme type A for 1 hour before polyphosphate addition

Table 5-16 (cont.) Effect of polyphosphate and EDTA addition on the yield of fructose

Note 4 = Sample prepared by mixing EDTA, cobalt chloride and Sweetzyme type A together

5 = Sample prepared by mixing cobalt chloride and Sweetzyme type A for 1 hour before EDTA addition

Table 5-17 Color of solutions with eriochrome black T indicator prepared according to 4.3.3.1.2 and 4.3.6.4

Sample no.	Sample Preparation	Color with eriochrome black T indicator			
		Before heating at 60°C, 20 hr	After heating at 60°C, 20 hr	PO_4 addition (3gm/l)	EDTA addition (0.275gm/l)
1	Deionized water	Blue			
2	Tap water	Wine-red		Violet blue	
3	Tap water/PO_4 (3 gm/l)	Violet-blue			
4	Tap water/PO_4 (3 gm/l)	Violet-blue	Blue-violet		
5	Deionized water/ $CoCl_2$	Blue			
6	Tap water/$CoCl_2$/ PO_4 (3 gm/l)	Pink-red	Pale wine-red		
7	Tap water/$CoCl_2$/ PO_4 (3 gm/l)	Pink-red		Pale Pink red	
8	Tap water/EDTA (0.225 gm/l)	Pink-red			Blue
9	Tap water/EDTA (0.5 gm/l)	Blue	Blue		

Table 5-17 (cont.) Color of solutions with eriochrome black T indicator prepared according to 4.3.3.1.2 and 4.3.6.4

Sample no.	Sample Preparation	Color with eriochrome black T indicator			
		Before heating at 60°C, 20 hr	After heating at 60°C, 20 hr	PO_4 addition (3gm/l)	EDTA addition (0.275gm/l)
10	Tap water/$CoCl_2$/ EDTA (0.225 gm/1)	Wine-red			Violet blue
11	Tap water/$CoCl_2$/ EDTA (0.5 gm/1)	Violet-blue	Blue-violet		
12-16	Syrup from 4.3.6.4		Wine-red		

Note Sample 1-11 prepared according to 4.3.3.1.2

Sample 12-16 prepared according to 4.3.6.4

5,5,4 Effect of pure glucose and prepared glucose syrup on the yield of fructose

Four samples of syrup (40 °Brix) were prepared according to 4,3,6,5 and isomerized according to 4.3.6.1 .

Table 5-18 Effect of pure glucose and prepared glucose syrup on the yield of fructose

Sample no.	Syrup characteristics		Sweetzyme type A dosage		% yield of fructose
	Pure glucose	Prepared glucose	1.6 gm/ 1,000 gm G	33.8 gm/ 1,000 gm G	
1	+	-	+	-	6.4 ± 0.1
2	-	+	+	-	6.9 ± 0.3
3	+	-	-	+	44.7 ± 0.4
4	-	+	-	+	46.1 ± 0.0

Note + = Addition

- = No addition

5.5.5 Activity of Sweetzyme type A

The activity of Sweetzyme type A determined according to 4.3.6.6 was 426.6 ± 5.3 GINU/gm enzyme.

5.5.6 Determination of Sweetayme type A dosage in the first isomerization

Three samples of pure glucose syrup (40^{o} Brix) with different amounts of Sweetzyme type A were isomerzed according to 4.3.6.7.

Table 5-19 Sweetzyme type A dosage in the first isomerization

Sample no.	Sweetzyme type A dosage, gm/1,000 gm glucose	% yield of fructose
1	25.0	37.8 ± 0.3
2	30.0	40.5 ± 0.6
3	33.8	44.7 ± 0.4

5.5.7 Effect of magnesium reduction on the yield of fructose

Two samples of pure glucose syrup (40 °Brix) were prepared according to 4.3.6.8 and isomerized according to 4.3.6.1.

Table 5-20 Effect of magnesium reduction on the yield of fructose

Sample no.	$MgSO_4$ addition gm/l syrup	% residual activity / % yield of fructose					
		1st isomerization	2nd isomerization	3rd isomerization	4th isomerization	5th isomerization	6th isomerization
1	2.0	100.0 / 45.0±0.1	75.4 / 36.0±0.3	63.6 / 14.8±0.2	66.3 / 5.3±0.3	59.2 / 3.4±0.4	59.2 / 1.7
2	0.1	100.0 / 45.1±0.2	72.8 / 35.9±0.1	56.7 / 20.5±0.1	55.9 / 10.8±0.2	49.1 / 6.4±0.1	51.1 / 4.0

Table 5-21 ICUMSA color index of fructose syrup prepared according to 4.3.6.8 and isomerized according to 4.3.6.1

Sample no.	$MgSO_4$ addition gm/l syrup	ICUMSA color index after isomerization					
		1st isomerizat^{n}	2nd isomerizat^{n}	3rd isomerizat^{n}	4th isomerizat^{n}	5th isomerizat^{n}	6th isomerizat^{n}
1	2.0	385.3	129.9	93.4	91.5	91.5	101.7
2	0.1	365.5	138.1	99.6	99.7	99.8	101.8

5.6 Post-treatment of Fructose Syrup

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

The color of fructose syrup before and after activated carbon treatment was determined according to 4.3.7.2 .

Table 5-22 ICUMSA color index of fructose syrup before and after activated carbon treatment

Sample	ICUMSA color index	
	Before activated carbon treatment	After activated carbon treatment
Fructose syrup	385.3	167.6

5.6.2 Cobalt determination of fructose syrup before and after cation exchange

The cobalt content of fructose syrup before and after cation exchange was determined according to 4.3.7.3.

Table 5-23 Cobalt content of fructose syrup before and after cation exchange

Sample	Cobalt content, ppm	
	Before cation exchange	After cation exchange
Fructose syrup	20	0