CHAPTER IV

EXPERIMENTAL METHODS AND MATERIALS

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4.2 Materials and Chemicals

4.2.1 Analytical methods for glucose and fructose determinations

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4.2.1.3 Pure anhydrous maltose

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4.2.1.5 Fehling's solution A (copper sulfate, $CuSO_4.5H_2O$)

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4.2.1.13 0.5 N Sodium hydroxide

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4.2.2.1.2 5 N Hydrochloric acid

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4.2.2.2.3 Deionized water

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4.2.2.3.2 40% Sodium hydroxide

4.2.2.3.3 Deionized water

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4.2.2.4.1 Deionized water

4.2.2.5 pH of aqueous extract

4.2.2.5.1 Deionized water

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4.2.3.1 Color of solution with eriochrome black T indicator

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4.2.3.1.2 Eriochrome black T/sodium chloride indicator

4.2.3.1.3 Deionized water

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4.2.3.2.2 Magnesium metal, 99.99%

4.2.3.2.3 Cobalt metal, 99.99%

4.2.3.2.4 1 M Hydrochloric acid

4.2.3.2.5 5 M Hydrochloric acid

4.2.3.2.6 Deionized water

4.2.3.3 ICUMSA color index

4.2.3.3.1 Deionized water

4.2.4 Production of glucose syrup

4.2.4.1 Tapioca flour

4.2.4.2 Deionized water

4.2.4.3 Tap water

4.2.4.4 10% Sodium hydroxide

4.2.4.5 4 N Hydrochloric acid

4.2.4.6 Alpha - amylase (Termamyl 60 L, NOVO INDUSTRI)

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4.2.5 Pre - treatment of glucose syrup

4.2.5.1 Prepared glucose syrup, 35 ^{o}Brix

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4.2.5.5 Cation exchange resin (IONAC C - 240)

4.2.5.6 Anion exchange resin (IONAC A - 550)

4.2.6 Isomerization of glucose syrup to fructose syrup

4.2.6.1 Prepared glucose syrup, 40 ^{o}Brix

4.2.6.2 Pure glucose syrup, 28 ^{o}and 40 ^{o}Brix

4.2.6.3 Magnesium sulfate

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4.2.6.5 10% Sodium hydroxide

4.2.6.6 4 N Hydrochloric acid

4.2.6.7 Tris (hydroxymethyl) aminomethane (tris buffer)

4.2.6.8 Disodium dihydrogen ethylenediaminetetra - acetate dihydrate (EDTA)

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4.2.6.10 Immobilized glucose isomerase (Sweetzyme type A)

4.2.6.11 Deionized water

4.2.6.12 Tap water

4.2.7 Post - treatment of fructose syrup

4.2.7.1 Fructose syrup, 40°Brix

4.2.7.2 4 N Hydrochloric acid

4.2.7.3 Granular activated carbon (IONAC P - 50)

4.2.7.4 Cation exchange resin (IONAC C - 240)

4.2.7.5 Anion exchange resin (IONAC A - 550)

4.3 Experimental Methods and Procedures

4.3.1 Analytical methods for glucose and fructose determinations

In order to get satisfactory results of G_t and G_I, some details of Lane - Eynon method and Iodometric method will be given here based on the descriptions in the AOAC (10) and ICUMSA official methods (16).

4.3.1.1 Lane - Eynon method for G_t determination

Careful attention to the experimental details is essential if consistent results are to be obtained.

4.3.1.1.1 Preparation of reagents

4.3.1.1.1.1 Fehling's solution (Soxhlet's modification)

Fehling's solution is prepared by mixing immediately before use, equal volume of solution A and solution B which are prepared as follows:

Solution A (copper sulfate)

i) Dissolve 34.639 gm of copper sulfate pentahydrate, $CuSO_4.5H_2O$ in water and add 0.5 ml of concentrated sulfuric acid of sp. gr. 1.84.

ii) Dilute to 500 ml in a volumetric flask and filter through glass wool or filter paper if not clear.

Solution B (alkaline tartrate)

i) Dissolve 173 gm of potassium sodium tartrate, $KNaC_4H_4O_6.4H_2O$ and 50 gm of sodium hydroxide, NaOH in water and dilute to 500 ml in the volumetric flask.

ii) Allow the solution to stand for two days (at least one day) and filter off any precipitate through prepared asbestos.

Mixed Fehling's solution does not keep indefinitely and is therefore usually prepared as required by adding a volume of solution A to an exactly equal volume of solution B. It is essential that mixing be carried out in this order. Since, if solution B is added to solution A, the precipitate of cupric hydroxide initially formed may not redissolve completely.

4.3.1.1.1.2 Standard glucose solution

i) Weigh accurately about 0.8 gm of pure anhydrous glucose (4.2.1.1).

ii) Transfer quantitatively to the 250 ml volumetric flask and make up to volume. (This solution should not be used after 24 hours.)

4.3.1.1.1.3 1% methylene blue solution

Dissolve 1 gm of methylene blue in water and dilute to 100 ml.

4.3.1.1.2 Standardization of Fehling's solution

i) Pipette 10 ml of Fehling's solution (5 ml of solution A and 5 ml of solution B) into 250 ml erlenmeyer flask and fill the standard glucose solution (4.3.1.1.1.2) into a 50 ml burette.

ii) Calculate the amount of the standard glucose solution that will completely reduce all the copper in 10 ml of Fehling's solution and add all but 0,5 - 1,0 ml of it to the flask.

iii) Mix the contents of the flask in the cold by gentle swirling and gently heat the mixture to boiling for 2 minutes.

iv) At the end of 2 minutes of boiling, add, without interrupting boiling, 2 or 3 drops of the methylene blue indicator (4.3.1.1.1.3) without touching the sides of the flask.

v) Complete the titration by dropwise addition of the glucose solution till the blue color of the indicator just disappears and the boiling contents resume the reddish appearance of the cuprous oxide which was present before the indicator was added. The titration should be completed within 1 minute, so that the contents of the flask boil altogether for 3 minutes without interruption.

vi) Multiply the titre by the number of mg of anhydrous glucose in 1 ml of the standard glucose solution to obtain the "glucose factor" (F_{10}, mg of anhydrous glucose corresponding to 10 ml of Fehling's solution).

4.3.1.1.3 G_t determination

i) Fill the neutral clarified sample solution into the 50 ml burette and then proceed as in 4.3.1.1.2.

ii) Note the titre of the sample solution used in titration with 10 ml of Fehling's solution then calculate G_t (in 25 ml of sample solution) from corresponding glucose factor, F_{10} as follows:

$$G_t = \frac{F_{10} \times 25}{\text{titre of the sample solution}}$$

4.3.1.2 Iodometric method for G_I determination

4.3.1.2.1 Preparation of reagents

The reagents of Iodometric method are prepared as follows (38):

4.3.1.2.1.1 0.1 N Primary standard potassium iodate

i) Dry some A.R. potassium iodate at 120°C for 1 hour and allow it to cool in a covered vessel in a desiccator.

ii) Weigh out exactly 3.567 gm of the finely powdered potassium iodate and transfer quantitatively to a 1,000 ml volumetric flask.

iii) Add about 400 - 500 ml of water and gently rotate the flask until the salt is completely dissolved.

iv) Make up to the mark with distilled water and shake well. The solution will keep indefinitely.

4.3.1.2.1.2 0.1 N Iodine

i) Dissolve 20 gm of iodate - free potassium iodide (4.2.1.9) in 30 - 40 ml of water in a 1,000 ml volumetric flask.

ii) Weigh out about 12.7 gm of A.R. or resublimed iodine on a watch glass on a rough balance (never on an analytical balance on an account of the iodine vapor), and transfer it by means of a small dry funnel into the concentrated potassium iodide solution.

iii) Insert the glass stopper into the flask, and shake in the cold until all the iodine is dissolved.

iv) Allow the solution to acquire room temperature and make up to the mark with distilled water. The iodine solution is best preserved in the small glass - stoppered bottles. These should be filled completely and kept in a cool, dark place.

4.3.1.2.1.3 0.1 N Sodium thiosulfate

i) Weigh out 25 gm of A.R. sodium thiosulfate crystals, $Na_2S_2O_3.5H_2O$ and dissolve in boiled - out distilled water.

ii) Make up to 1,000 ml in a volumetric flask with boiled - out water. The solution should be kept in a cool, dark place.

4.3.1.2.1.4 2 N Sulfuric acid

Add 55.4 ml of concentrated sulfuric acid with sp. gr. 1.84 into water and make up to 1,000 ml.

4.3.1.2.1.5 0.5 N Sodium hydroxide

i) Weigh about 20 gm of sodium hydroxide pellets and dissolve in water.

ii) Make up to 1,000 ml with distilled water.

4.3.1.2.1.6 1% Starch

i) Make a paste of 1.0 gm of soluble starch with a little water and pour the paste, with constant stirring, into 100 ml of boiling water then boil for 1 minute.

ii) Allow the solution to cool and keep the solution in a stoppered bottle.

4.3.1.2.2 Standardization of 0.1 N sodium thiosulfate

Thiosulfate solutions prepared with ordinary distilled water usually contains an excess of carbon dioxide; this may cause a slow decomposition to take place. Moreover, decomposition may also be caused by bacterial action, particularly if the solution has been standing for some time. For these reasons, it is necessary to standardize thiosulfate solutions with 0.1 N primary standard potassium iodate every time before use.

i) Pipette 25 ml of 0.1 N potassium iodate (4.3.1.2.1.1) into a 250 ml erlenmeyer flask and add 1 gm of iodate - free potassium iodide (4.2.1.9) and 3 ml of 2 N sulfuric acid (4.3.1.2.1.4).

ii) Titrate the liberated iodine with the thiosulfate solution (4.3.1.2.1.3) with constant shaking.

iii) When the color of the liquid has become pale yellow, dilute to about 200 ml with distilled water and add 2 ml of 1% starch solution (4.3.1.2.1.6).

iv) Continue the titration until the color changes from blue to colorless.

v) Carry out a blank determination on the reagents mentioned above by using 25 ml of distilled water instead of 25 ml of 0.1 N potassium iodate.

vi) Calculate the true normality of sodium thiosulfate solution as follows:

$$\text{Normality of sodium thiosulfate} = \frac{25\ N}{(V_2 - V)}$$

When V_2 = Volume of sodium thiosulfate solution used in the determination, ml

V = Volume of sodium thiosulfate solution used in the blank determination, ml

N = Normality of 0.1 N primary standard potassium iodate, 0.1 N

4.3.1.2.3 G_I determination

Iodometric method for G_I determination are described as follows (9, 14).

i) Pipette 25 ml of the neutral clarified sample solution containing 0.05 - 0.08 gm of anhydrous glucose into a 250 ml iodine flask (4.1.1.7) and exactly add 20 ml of 0.1 N iodine (4.3.2.2.1.2) and 5 ml of 0.5 N sodium hydroxide (4.3.2.2.1.5).

ii) Insert the glass stopper into the iodine flask, shake and allow to stand in the dark for 10 minutes.

iii) Acidify with 5 ml of 2 N sulfuric acid (4.3.1.2.1.4) and immediately titrate the excess iodine with 0.1 N sodium thiosulfate (4.3.1.2.1.3) with constant shaking.

iv) When the color of the liquid has become pale yellow, dilute to about 200 ml with distilled water and add 2 ml of 1% starch solution (4.3.1.2.1.6).

v) Continue the titration until the color changes from blue to colorless.

vi) Carry out a blank determination by using 25 ml of distilled water instead of 25 ml of sample solution. The difference between the two titrations represents the amount of 0.1 N iodine required by the sample.

vii) Calculate mg of glucose in 25 ml of the sample solution determined by Iodometric method (G_I) as follows:

$$G_I = \frac{(B - S) \times N \times 180.2}{2}$$

When S = Volume of 0.1 N sodium thiosulfate used in the determination, ml

B = Volume of 0.1 N sodium thiosulfate used in the blank determination, ml

N = Normality of 0.1 N sodium thiosulfate, 0.1 N

4.3.1.3 Relationship between factor, f and G/F ratio

4.3.1.3.1 f and G/F ratio at constant content of glucose

G/F ratio in 25 ml of sample solution with constant content of glucose is shown in Table 4 - 1 and the procedure is as follows:

i) Weigh exactly 2.6 gm of pure anhydrous glucose (4.2.1.1) and dilute to 1,000 ml in volumetric flask.

Table 4-1 G/F ratio in 25 ml of sample solution with constant content of glucose

25 ml of sample solution		G/F ratio
G, mg	F, mg	
65.0	4.3	15.1
65.0	6.5	10.0
65.0	13.0	5.0
65.0	32.5	2.0
65.0	65.0	1.0

ii) Exactly add 160 ml of the glucose solution from i) into 5 beakers each contained 0.0277, 0.0416, 0.0832, 0.2080 and 0.4160 gm of pure anhydrous fructose respectively.

iii) Calculate each G/F ratio in 25 ml of sample solution then find G_t by 4.3.1.1.3 and G_I by 4.3.1.2.3.

iv) Calculate f at each G/F ratio from exact F, G_t and G_I in the formula shown in 2.5.

4.3.1.3.2 f and G/F ratio at constant total content of sugars

G/F ratio in 25 ml of sample solution with constant total content of sugars is shown in Table 4 - 2 and the procedure is as follows:

i) Weigh exactly 2.4376 gm of pure anhydrous glucose (4.2.1.1) and dilute to 1,000 ml in volumetric flask.

ii) Exactly add 160, 150, 150, 100 and 100 ml of glucose solution from i) into the 5 beakers.

iii) Add, from burette, 0, 4.7, 18.75, 40.64 and 37.5 ml of deionized water into the 5 beakers described in ii) respectively.

iv) Weigh exactly 0.026, 0.0365, 0.0731, 0.1218 and 0.2437 gm of pure anhydrous fructose into the 5 beakers described in iii) respectively.

v) Calculate each G/F ratio in 25 ml of sample solution then find G_t by 4.3.1.1.3 and G_I by 4.3.1.2.3.

vi) Calculate f at each G/F ratio from exact F, G_t and G_I in the formula shown in 2.5.

Table 4-2 G/F ratio in 25 ml of sample solution with constant total content of sugars

Composition of sample solution					25 ml of solution		G/F ratio
Solution used, ml	Water added, ml	Total volume, ml	G, mg	F, mg	G, mg	F, mg	
160.0	-	160.0	390.0	26.0	60.9	4.1	14.9
150.0	4.7	154.7	365.6	36.5	59.1	5.9	10.0
150.0	18.75	168.75	365.6	73.1	54.2	10.8	5.0
100.0	40.64	140.64	243.8	121.8	43.3	21.7	2.0
100.0	87.5	187.5	243.8	243.7	32.5	32.5	1.0

4.3.1.4 Fructose determination by Iodometric method

G/F ratio in 25 ml of sample solution with various quantities of G, F and M (maltose) is shown in Table 4 - 3 and the procedure is as follows:

i) Weigh exactly 2.5 gm of pure anhydrous glucose and dilute to 250 ml in volumetric flask.

ii) Exactly add 7.5, 6.5, 5.0, 4.2, 3.8, 7.5, 6.5, 5.0, 4.2 and 3.8 ml of glucose solution from i) into the 10 iodine flasks respectively.

iii) Add, from burette, 17.5, 18.5, 20.0, 20.8, 21.2, 17.5, 18.5, 20.0, 20.8 and 21.2 ml of deionized water into the 10 iodine flasks described in ii) respectively.

iv) Weigh exactly 0, 0.01, 0.025, 0.033, 0.037, 0, 0.01, 0.025, 0.033 and 0.037 gm of pure anhydrous fructose and put into the 10 iodine flasks described in iii) respectively.

v) Weigh exactly 0, 0, 0, 0, 0, 0.005, 0.005, 0.005, 0.005 and 0.005 gm of pure anhydrous maltose and put into the 10 iodine flasks described in iv) respectively.

vi) Calculate each G/F ratio in 25 ml of sample solution then find G_I by 4.3.1.2.3.

vii) Calculate mg of fructose at each G/F ratio by the difference between G_I containing no fructose and G_I containing various fructose contents.

4.3.2 Analysis of tapioca flour composition (20)

4.3.2.1 Moisture content

i) Dry the aluminium dish with cover (4.1.2.1.1) in the oven

Table 4-3 G/F ratio in 25 ml of sample solution with various quantities of G, F and M

Composition of sample solution						25 ml of solution			G/F ratio
Solution used, ml	Water added, ml	Total volume, ml	G, mg	F, mg	M, mg	G, my	F, mg	M, mg	
7.5	17.5	25.0	75.0	-	-	75.0	-	-	∞
6.5	18.5	25.0	65.0	10.0	-	65.0	10.0	-	6.5
5.0	20.0	25.0	50.0	25.0	-	50.0	25.0	-	2.0
4.2	20.8	25.0	42.0	33.0	-	42.0	33.0	-	1.3
3.8	21.2	25.0	38.0	37.0	-	38.0	37.0	-	1.0
7.5	17.5	25.0	75.0	-	5.0	75.0	-	5.0	∞
6.5	18.5	25.0	65.0	10.0	5.0	65.0	10.0	5.0	6.5
5.0	20.0	25.0	50.0	25.0	5.0	50.0	25.0	5.0	2.0
4.2	20.8	25.0	42.0	33.0	5.0	42.0	33.0	5.0	1.3
3.8	21.2	25.0	38.0	37.0	5.0	38.0	37.0	5.0	1.0

(4.1.2.1.2) at 105°C - 107°C for 15 minutes.

ii) Replace the lid and place the dish in the desiccator (4.1.2.1.4) to cool for 30 minutes.

iii) Weigh the dish with the cover to the fourth decimal place (4.1.2.1.5).

iv) Weigh sample (tapioca flour) plus dish with the cover to the fourth decimal place of 2 gm.

v) Transfer the dish to the oven.

vi) After 5 hours in the oven, replace the cover, and transfer the dish to the desiccator, allow to cool and weigh.

vii) Return the dish to the oven and heat again for each 30 minutes until loss of weight between successive weighings do not exceed 2 mg.

vii) Calculate the percentage of moisture content as follows:

$$\text{Percentage of moisture content} = \frac{100\ (W_1 - W_2)}{W_1 - W}$$

When W = Weight of aluminium dish with cover, gm

W_1 = Weight of aluminium dish with cover and sample before drying, gm

W_2 = Weight of aluminium dish with cover and sample after drying, gm

4.3.2.2 Ash

i) Ignite porcelain crucible (4,1.2.2.1), cool in the desiccator (4.1.2.2.4) and weigh (4.1.2.2.5).

ii) Weigh 2 gm of tapioca flour in the crucible to the fourth decimal place.

iii) Burn gently over low flame until thoroughly charred.

iv) Transfer to the muffle furnace (4.1.2.2.2) at 600 $\pm$ 2°C and burn to a white or pale grey ash (about 2 - 3 hours).

v) Transfer to the desiccator, allow to cool and weigh.

vi) Return the crucible to the muffle and ignite again for each 30 minutes until loss of weight between successive weighings do not exceed 1 mg. Preserve the crucible and contents for the determination of acid insoluble ash (4.3.2.3).

vii) Calculate the percentage of ash as follows:

$$\text{Percentage of ash (on dry basis)} = \frac{W_2 - W}{(W_1 - W)(100 - M)} \times 10^4$$

When W = Weight of porcelain crucible, gm

W_1 = Weight of porcelain crucible and sample before ignition, gm

W_2 = Weight of porcelain crucible and sample after ignition, gm

M = Moisture content, percent

4.3.2.3 Acid insoluble ash

i) Add 5 ml of concentrated hydrochloric acid (4.2.2.1.1) to the ash contained in the crucible (4.3.2.2) and heat on a boiling water bath (4.1.2.3.3) until dry.

ii) Add 25 ml of 5 N hydrochloric acid (4.2.2.1.2), cover with a watch glass (4.1.2.3.5) and heat on the boiling water bath for 15 minutes.

iii) Filter the contents of the crucible immediately through ashless filter paper (4.1.2.3.7) and wash with hot water until the washings are free from the acid.

iv) Return the filter paper and the residue to the crucible and keep it in an electric air oven (4.1.2.3.8) maintained at 105° - 110°C for about 3 hours.

v) Transfer to the muffle furnace (4.1.2.3.9) at 600 $\pm$ 2°C and burn for about 2 hours.

vi) Transfer to the desiccator (4.1.2.3.11), allow to cool and weigh.

vii) Return the crucible to the muffle furnace and ignite again for each 30 minutes until loss of weight between successive weighings do not exceed 1 mg.

viii) Calculate the percentage of acid insoluble ash as follows:

$$\text{Percentage of acid insoluble ash (on dry basis)} = \frac{W_3 - W}{(W_1 - W)(100 - M)} \times 10^4$$

When W = Weight of porcelain crucible, gm

W_1 = Weight of porcelain crucible and sample before ignition, gm

W_3 = Weight of porcelain crucible and acid insoluble ash, gm

M = Moisture content, percent

4.3.2.4 Protein

The Kjeldahl nitrogen is determined by methods of AOAC (10). Protein is calculated from the amount of nitrogen multiplied

by 6.25.

i) Weigh 2.0 to 3.0 gm of sample.

ii) Weigh 5.5 gm of catalyst (0.5 gm $CuSO_4.5H_2O$ + 5 gm K_2SO_4) (4.2.2.2.1).

iii) Mix i) and ii)in macro-Kjeldahl digestion flask (4.1.2.4.2). Add 25 ml of concentrated sulfuric acid (4.2.2.2.2) and digest until a clear solution is obtained (about 2.5 - 3.0 hours).

iv) The clear solution is diluted with 200 ml of deionized water (4.2.2.2.3). Set up the distillation apparatus (4.1.2.4.6) so that the lower end of the condenser is immersed below the liquid level of 50 ml of 4% boric acid (4.2.2,2.4).

v) Distill with 50 ml of 50% sodium hydroxide (4.2.2.2.5) until the distillate in a receiving flask attained a total volume of about 200 ml.

vi) Titrate the distillate with 0.1 N hydrochloric acid (4.2.2.2.6) using methyl red (4.2.2.2.7) as indicator.

vii) Carry out a blank determination on the reagents mentioned above but without sample.

viii) Calculate the percentage of protein as follows:

$$\text{Percentage of protein (on dry basis)} = \frac{(a - b) \times 140 \times N}{W(100 - M)} \times 6.25$$

When a = Volume of 0.1 N hydrochloric acid used in the determination with sample, ml

b = Volume of 0.1 N hydrochloric acid used in the blank determination, ml

N = Normality of hydrochloric acid, 0.1 N

W = Weight of sample, gm

M = Moisture content, percent

4.3.2.5 Starch

Two chemical methods, namely, acid hydrolysis method and diastase hydrolysis method are generally used. However, only the acid hydrolysis method is specified as it is the simplest and is fairly reliable and accurate (39).

i) Weigh accurately about 1 gm of sample and disperse in 100 ml of deionized water.

ii) Add 10 ml of concentrated sulfuric acid (4.2.2.3.1) and heat in the oven (4.1.2.5.4) at 90°C for 3 hours.

iii) Cool and neutralize with 40% sodium hydroxide (4.2.2.3.2) and transfer quantitatively to a 250 ml volumetric flask and make up to volume.

iv) Find the value of w (mg of glucose in 1 ml of the prepared solution of the material) by the Iodometric method as described in 4.3.1.2.3 and calculate the percentage of starch as follows:

$$\text{Percentage of Starch (on dry basis)} = \frac{9\,w}{W(100 - M)} \times 250$$

When w = Weight of glucose in the prepared solution of the material, mg/ml

W = Weight of sample, gm

M = Moisture content, percent

4.3.2.6 Pulp

i) Weigh 50 gm of sample and disperse in 300 ml of deionized water for 15 minutes.

ii) Filter through the sieve (4.1.2.6.4) and wash with water until the washings show substantially no starch particles.

iii) Transfer the residue to a graduated tube with conical bottom (4.1.2.6.5) and make up to volume.

iv) Allow the pulp to settle for 2 hours and measure as ml of residue.

4.3.2.7 pH of aqueous extract

i) Disperse 25 gm of sample in 50 ml of deionized water.

ii) Determine the pH of aqueous extract with the standardized electrometric pH meter (4.1.2.7.3).

4.3.2.8 Fineness

i) Weigh 100 gm of sample on the sieve (4.1.2.8.3).

ii) Sift the sample by using mechanical shaker (4.1.2.8.4).

iii) Brush the sample left on the sieve to a tared weighing dish and weigh.

4.3.3 Methods for testing the property of glucose syrup and fructose syrup

4.3.3.1 Color of solution with eriochrome black T indicator

4.3.3.1.1 Formation of color with eriochrome black T indicator (38).

i) Add 2 ml of ammonium hydroxide/ammonium chloride buffer, pH = 10 (4,2.3.1,1) into 10 ml of sample.

ii) Add a little amount of eriochrome black T/sodium chloride indicator (4.2.3.1.2) and note the color formed.

4.3.3.1.2 Effect of polyphosphate and EDTA on cobalt ion in the color formation with eriochrome black T indicator

i) Prepare 11 samples of solution and determine the color of each sample with eriochrome black T indicator (4.3.3.1.1) as follows:

- Sample 1 is prepared with deionized water and determined the color at once.

- Sample 2 is prepared with tap water and determined the color at once, then added with sodium polyphosphate (3 gm/l solution).

- Sample 3 is prepared with tap water and sodium polyphosphate (3 gm/l solution) then determined the color at once.

- Sample 4 is prepared with tap water and sodium polyphosphate (3 gm/l solution) then determined the color before and after heating at 60°C for 20 hours.

- Sample 5 is prepared with deionized water and cobalt chloride (0.1 gm/l solution) then determined the color at once.

- Sample 6 is prepared with tap water, cobalt chloride (0.1 gm/l solution) and sodium polyphosphate (3 gm/l solution) then determined the color before and after heating at 60°C for 20 hours.

- Sample 7 is prepared with tap water, cobalt chloride (0.1 gm/l solution) and sodium polyphosphate (3 gm/l solution) and determined the color at once, then added with sodium polyphosphate (3 gm/l solution).

- Sample 8 is prepared with tap water and EDTA (0.225 gm/l solution), and determined the color at once, then added with EDTA (0.275 gm/l solution).

- Sample 9 is prepared with tap water and EDTA (0.5 gm/l solution) then determined the color before and after heating at 60°C for 20 hours.

- Sample 10 is prepared with tap water, cobalt chloride (0.1 gm/l solution) and EDTA (0.225 gm/l solution) and determined the color at once, then added with EDTA (0.275 gm/l solution).

- Sample 11 is prepared with tap water, cobalt chloride (0.1 gm/l solution) and EDTA (0.5 gm/l solution) then determined the color before and after heating at 60°C for 20 hours.

ii) Note the color formed in each sample.

4.3.3.2 Ion content

Ion content in syrup is determined as follows: (40)

i) Prepare the standard curve of magnesium, calcium, and cobalt in a suitable range with an atomic absorption spectrophotometer (4.1.3.2.1).

ii) Determine the absorbance of syrup and calculate the magnesium, calcium and cobalt content from its standard curve.

4.3.3.3 ICUMSA color index

ICUMSA color index of syrup is determined as follows: (16)

i) Filter the syrup if not clear and determine °Brix of syrup with a hand refractometer (4,1.3.3.3).

ii) Find the density of syrup with a pyknometer (4.1.3.3.4).

iii) Calculate the concentration of syrup by mutiplying the °Brix value by corresponding density and dividing by 100.

iv) Transfer the syrup into a Bausch & Lomb 1 - cm cell (4.1.3.3.2) and determine the absorbance with a spectrophotometer (4.1.3.3.1) at 420 nm using deionized water as blank.

v) Calculate ICUMSA color index as follows:

$$\text{ICUMSA color index} = \frac{10^3 \times a}{b \times c}$$

When a = Absorbance of syrup at 420 nm

b = Measuring cell length, cm

c = Concentration of syrup, gm/ml

4.3.4 Production of glucose syrup

4.3.4.1 Procedure for production of glucose syrup

The production of glucose syrup is schematically shown in Figure 4 - 1 (22).

i) Prepare a starch slurry of 30% W/W dry solid and adjust pH to 7.0 with 10% sodium hydroxide (4.2.4.4).

ii) Add Termamyl (1.2 gm of Termamyl per 1,000 gm of dry solid) (4.2.4.6) and mix thoroughly.

iii) Gelatinize the sample in a steam - jacketed pan (4.1.4.11) at 100^{o}C for 15 minutes then give a special heat treatment (pressure cooking) on the starch by placing the sample in a pressure cooker (4.1.4.12) at 105^{o}C for 5 minutes,

iv) Liquefy the sample in a water bath (4.1.4.16) at 95^{o}C for 2 hours then inactivate Termamyl in the pressure cooker at 121^{o}C for 15 minutes.

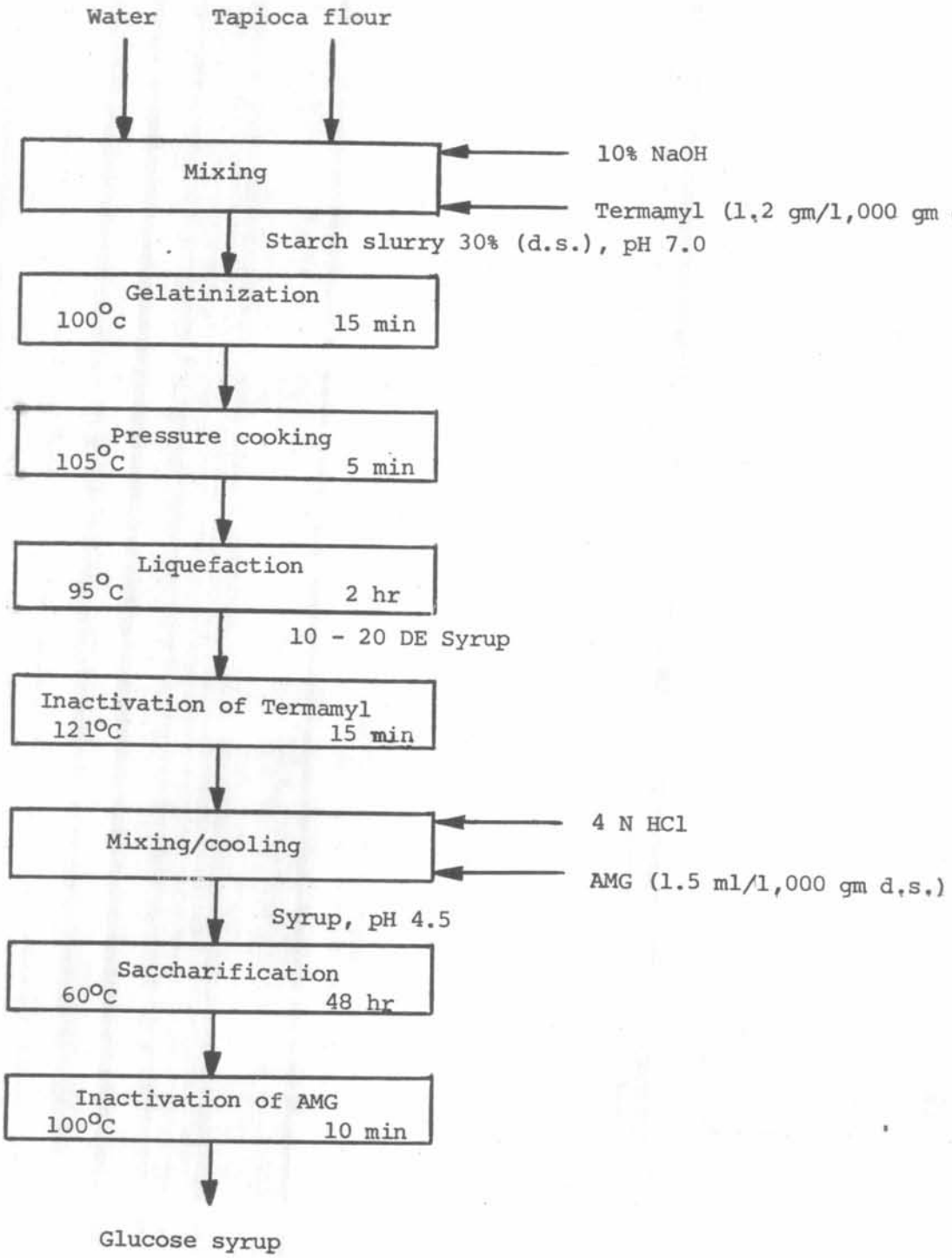

Figure 4 - 1 Production of glucose syrup (22)

v) Cool the sample to 60°C, adjust pH to 4.5 with 4 N hydrochloric acid (4.2.4.5) and add AMG (1.5 ml of AMG per 1 kg of dry solid) (4.2.4.7) then mix thoroughly.

vi) Saccharify the sample in a water bath at 60°C for 48 hours then inactivate AMG by boiling for 10 minutes.

vii) Determine G_t by 4.3.1.1.3 and find total soluble solid by hand refractometer (4.1.4.17).

viii) Find the dextrose equivalent (or % yield), which is defined as the percentage of the total reducing sugar calculated as dextrose (glucose) on a dry weight or dry substance basis, of glucose syrup.

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

i) Prepare 4 samples of starch slurry (30% W/W dry solid) with deionized water.

ii) Carry out the production of glucose syrup according to 4.3.4.1 as follows:

- Sample 1 is produced with all steps shown in Figure 4 - 1.
- Sample 2 is produced with all steps shown in Figure 4 - 1 except the inactivation of Termamyl.
- Sample 3 is produced with all steps shown in Figure 4 - 1 except the pressure cooking.
- Sample 4 is produced with all steps shown in Figure 4 - 1 except the pressure cooking and the inactivation of Termamyl.

iii) Calculate the percentage of yield of each sample and determine the color of glucose syrup by ICUMSA color index (8.3.3.3).

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

i) Prepare 2 samples of starch slurry (30% W/W dry solid) by using deionized water in the first one and tap water in the other.

ii) Carry out the production of glucose syrup according to sample 4 in 4.3.4.2.

iii) Calculate the percentage of yield of each sample and determine the color of glucose syrup by ICUMSA color index (4.3.3.3).

iv) Find the calcium content in glucose syrup by 4.3.3.2.

4.3.5 Pre - treatment of glucose syrup

4.3.5.1 Procedure for pre - treatment of glucose syrup

The pre - treatment of glucose syrup is schematically shown in Figure 4 - 2 (36).

i) Filter the prepared glucose syrup with a centrifugal filter (4.1.5.3) and a filter press (4.1.5.4).

ii) Remove the color compounds from the filtrate with a granular activated carbon column (4.2.5.4).

iii) Remove the soluble impurities and reduce the calcium content in syrup with a regenerated cation exchange (4.2.5.5) and a regenerated anion exchange column (4.2.5.6).

iv) Concentrate the purified syrup to 40 ^{o}Brix with a vacuum steam - jacketed pan (4.1.5.10).

4.3.5.2 Effect of pre - treatment on the yield of fructose

i) Carry out the production of glucose syrup with deionized water according to sample 4 in 4.3.4.2.

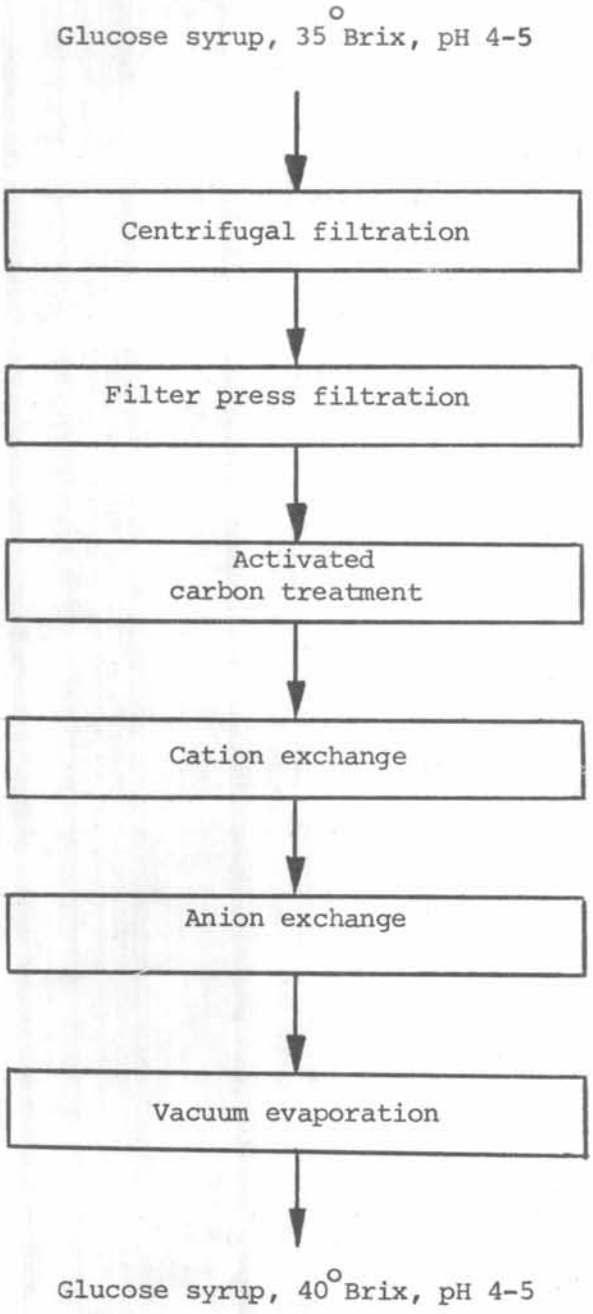

Figure 4 - 2 Pre - treatment of glucose syrup (36)

ii) Prepare 4 samples of glucose syrup (30°Brix) described in i) and pre - treat according to the pre - treatment of glucose syrup (4.3.5.1) as follows:

- Sample 1 is pre - treated with all steps shown in Figure 4 - 2 except the vacuum evaporation.

- Sample 2 is pre - treated with all steps shown in Figure 4 - 2 except the vacuum evaporation and the anion exchange.

- Sample 3 is pre - treated with all steps shown in Figrue 4 - 2 except the vacuum evaporation, the anion exchange and the cation exchange.

- Sample 4 is pre - treated with all steps shown in Figrue 4 - 2 except the vacuum evaporation, the anion exchange, the cation exchange and the activated carbon treatment.

iii) Determine the color of 4 pre - treated samples by ICUMSA color index (4.3.3.3) and carry out the isomerization of glucose syrup to fructose syrup (4.3.6.1).

iv) Calculate the % yield of fructose of each sample and determine the color of fructose syrup by ICUMSA color index (4.3.3.3).

4.3.5.3 Effect of cation exchange on the yield of fructose

i) Carry out the production of glucose syrup with tap water according to sample 4 in 4.3.4.2.

ii) Prepare 2 samples of glucose syrup (30°Brix) described in i) and pre - treat according to the pre - treatment of glucose syrup (4.3.5.1) as follows:

- Sample 1 is pre - treated with all steps shown in Figure 4 - 2 except the vacuum evaporation, the anion exchange and the activated carbon treatment.

- Sample 2 is pre - treated with all steps shown in Figure 4 - 2 except the vacuum evaporation, the anion exchange, the cation exchange and the activated carbon treatment.

iii) Isomerize glucose to fructose by the isomerization of glucose syrup to fructose syrup (4.3.6.1) and calculate the % yield of fructose of each sample.

iv) Find the calcium content in fructose syrup by 4.3.3.2.

4.3.6 Isomerization of glucose syrup to fructose syrup

4.3.6.1 Procedure for isomerization of glucose syrup to fructose syrup

The isomerization of glucose syrup to fructose syrup is schematically shown in Figure 4 - 3 (22).

i) Carry out the production of glucose syrup with deionized water according to sample 4 in 4.3.4.2.

ii) Pre - treat the glucose syrup from i) with all steps shown in Figure 4 - 2 except the cation exchange and the anion exchange,

iii) Add magnesium sulfate (2 gm/l syrup) (4.2.6.3) and cobalt chloride (0.1 gm/l syrup) (4.2.6.4) into the pre - treated glucose syrup described in ii) and adjust pH to 6.8 with 10% sodium hydroxide (4.2.6.5), then determine mg of glucose per 1 gm of total soluble solid of glucose syrup by Iodometric method (4.3.1.2.3).

iv) Add Sweetzyme type A (1.6 - 3.7 gm/1,000 gm glucose) (4.2.6.10) and mix thoroughly for 1 hour.

v) Isomerize the sample in a water bath (4.1.6.19) at 60^{o}C for 20 hours then determine mg of glucose per 1 gm of total soluble solid of fructose syrup by Iodometric method (4.3.1.2.3).

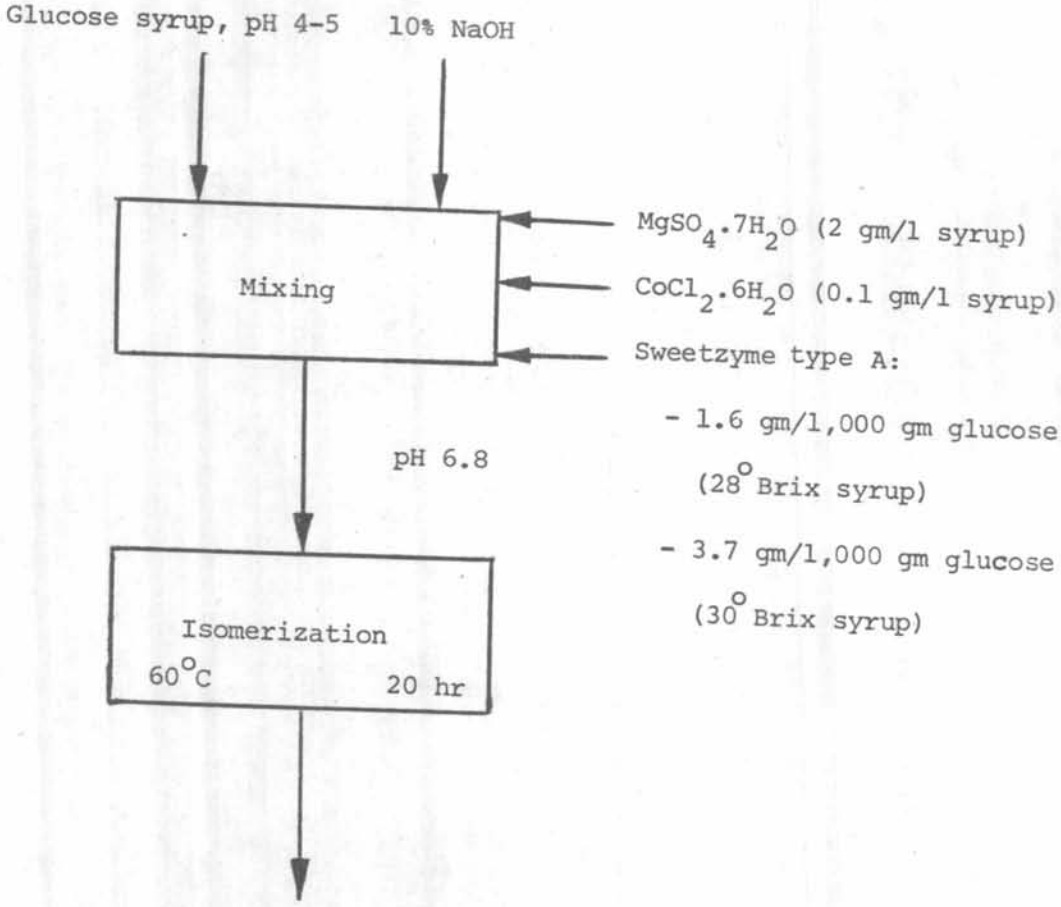

Figure 4 - 3 Isomerization of glucose syrup to fructose syrup (22)

vi) Calculate the % yield of fructose by the difference of glucose content in glucose and fructose syrup. (% yield of fructose is defined as the percentage of fructose on an initial weight of glucose basis)

4.3.6.2 Effect of cobalt chloride on the yield of fructose

i) Carry out the production of glucose syrup with deionized water according to sample 4 in 4.3.4.2.

ii) Pre - treat the glucose syrup (30^{o} Brix) described in i) with all steps shown in Figure 4.2 except the vacuum evaporation, the anion exchange and the cation exchange.

iii) Determine the color of pre - treated glucose syrup described in ii) by ICUMSA color index (4.3.3.3).

iv) Isomerize 2 samples of pre - treated glucose syrup (30^{o} Brix) according to the isomerization of glucose syrup to fructose syrup (4.3.6.1) as follows:

- Sample 1 is isomerized with all steps shown in Figure 4 - 3.
- Sample 2 is isomerized with all steps shown in Figure 4 - 3 except the addition of cobalt chloride.

v) Calculate the % yield of fructose of each sample and determine the color of fructose syrup by ICUMSA color index (4.3.3.3).

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

i) Prepare 4 samples of pure glucose syrup (28^{o} Brix) (4.2.6.2) with deionized and tap water. Calcium content in deionized water and tap water is 1 ppm and 16 ppm respectively. Each sample is prepared as follows:

- Sample 1 is prepared with deionized water and added with magnesium sulfate to get Mg/Ca ratio = 10.

- Sample 2 is prepared with deionized water and added with magnesium sulfate to get Mg/Ca ratio = 20.

- Sample 3 is prepared with deionized water and added with magnesium sulfate to get Mg/Ca ratio = 32.

- Sample 4 is prepared with tap water and added with magnesium sulfate to get Mg/Ca ratio = 20.

ii) Isomerize the 4 samples (28° Brix) with all steps shown in Figure 4 - 3 except the addition of magnesium sulfate, then separate the enzyme and re - use twice.

iii) Calculate the % total yield of fructose of each sample.

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

i) Prepare 5 samples of pure glucose syrup (28° Brix) (4.2.6.2) as follows:

- Sample 1 is prepared with deionized water then added with cobalt chloride (0.1 gm/l syrup) and magnesium sulfate to get Mg/Ca ratio = 10.

- Sample 2 is prepared with tap water then added with cobalt chloride (0.1 gm/l syrup) and sodium polyphosphate (3 gm/l syrup) (4.2.6.9).

- Sample 3 is prepared with tap water then mixed with cobalt chloride (0.1 gm/l syrup) and Sweetzyme type A (1.6 gm/1,000 gm glucose) for 1 hour before adding sodium polyphosphate (3 gm/l syrup).

- Sample 4 is prepared with tap water then added with cobalt chloride (0.1 gm/l syrup) and EDTA (0.225 gm/l syrup).

- Sample 5 is prepared with tap water then mixed with cobalt chloride (0.1 gm/l syrup) and Sweetzyme type A (1.6 gm/1,000 gm

glucose) for 1 hour before adding EDTA (0.225 gm/l syrup).

ii) Isomerize the 5 samples (28° Brix) with all steps shown in Figure 4 - 3 except the addition of magnesium sulfate, cobalt chloride in sample 1, 2, 4 and except the addition of magnesium sulfate, cobalt chloride and Sweetzyme type A in sample 3, 5. Then separate the enzyme and re - use twice.

iii) Calculate the % total yield of fructose of each sample.

iv) Determine the color of samples with eriochrome black T indicator (4.3.3.11) and compare the color with solutions according to 4.3.3.1.2.

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

i) Prepare 4 samples of glucose syrup (40° Brix) as follows:

- Sample 1 is prepared with pure glucose and deionized water, then added with magnesium sulfate to get Mg/Ca ratio = 10 and mixed thoroughly with 1.6 gm of Sweetzyme type A per 1,000 gm of glucose.

- Sample 2 is prepared with glucose syrup, which is produced according to sample 4 in 4.3.4.2 and pre- treated with all steps shown in Figure 4 - 2, then added with magnesium sulfate to get Mg/Ca ratio = 10 and mixed thoroughly with 1.6 gm of Sweetzyme type A per 1,000 gm of glucose.

- Sample 3 is prepared with pure glucose and deionized water, then added with magnesium sulfate to get Mg/Ca ratio = 10 and mixed thoroughly with 33.8 gm of Sweetzyme type A per 1,000 gm of glucose.

- Sample 4 is prepared with glucose syrup, which is produced according to sample 4 in 4.3.4.2 and pre -treated with all steps shown in

Figure 4 - 2, then added with magnesium sulfate to get Mg/Ca ratio = 10 and mixed thoroughly with 33.8 gm of Sweetzyme type A per 1,000 gm glucose.

ii) Isomerize the 4 samples (40 ^{o}Brix) with all steps shown in Figure 4 - 3 except the addition of magnesium sulfate and Sweetzyme type A.

iii) Calculate the % yield of fructose of each sample.

4.3.6.6 Determination of the activity of Sweetzyme type A

The determination of the activity of Sweetzyme type A is schematically shown in Figure 4 - 4 (31).

i) Prepare pure glucose syrup (40 ^{o}Brix) (4.2.6.2) with deionized water and add magnesium sulfate (2 gm/l syrup).

ii) Add tris buffer (12.1 gm/l syrup) (4.2.6.7) and adjust pH to 8.5 with 4 N hydrochloric acid, then determine mg of glucose per 1 gm of total soluble solid of glucose syrup by Iodometric method (4.3.1.2.3).

iii) Weigh exactly 1 gm of Sweetzyme type A into the reaction tubes (4.1.6.17) and add exactly 15 ml of pre - heated syrup to each tube.

iv) Screw the cap tightly and mix thoroughly.

v) Place the tubes on the shaker water bath (4.1.6.16) and shake at 65 ^{o}C for 1 hour.

vi) Remove the tubes and immediately place in an ice - water bath (4.1.6.18) for 5 minutes.

vii) Filter off the enzyme and determine mg of glucose per 1 gm of total soluble solid of fructose syrup by Iodometric method (4.3.1.2.3).

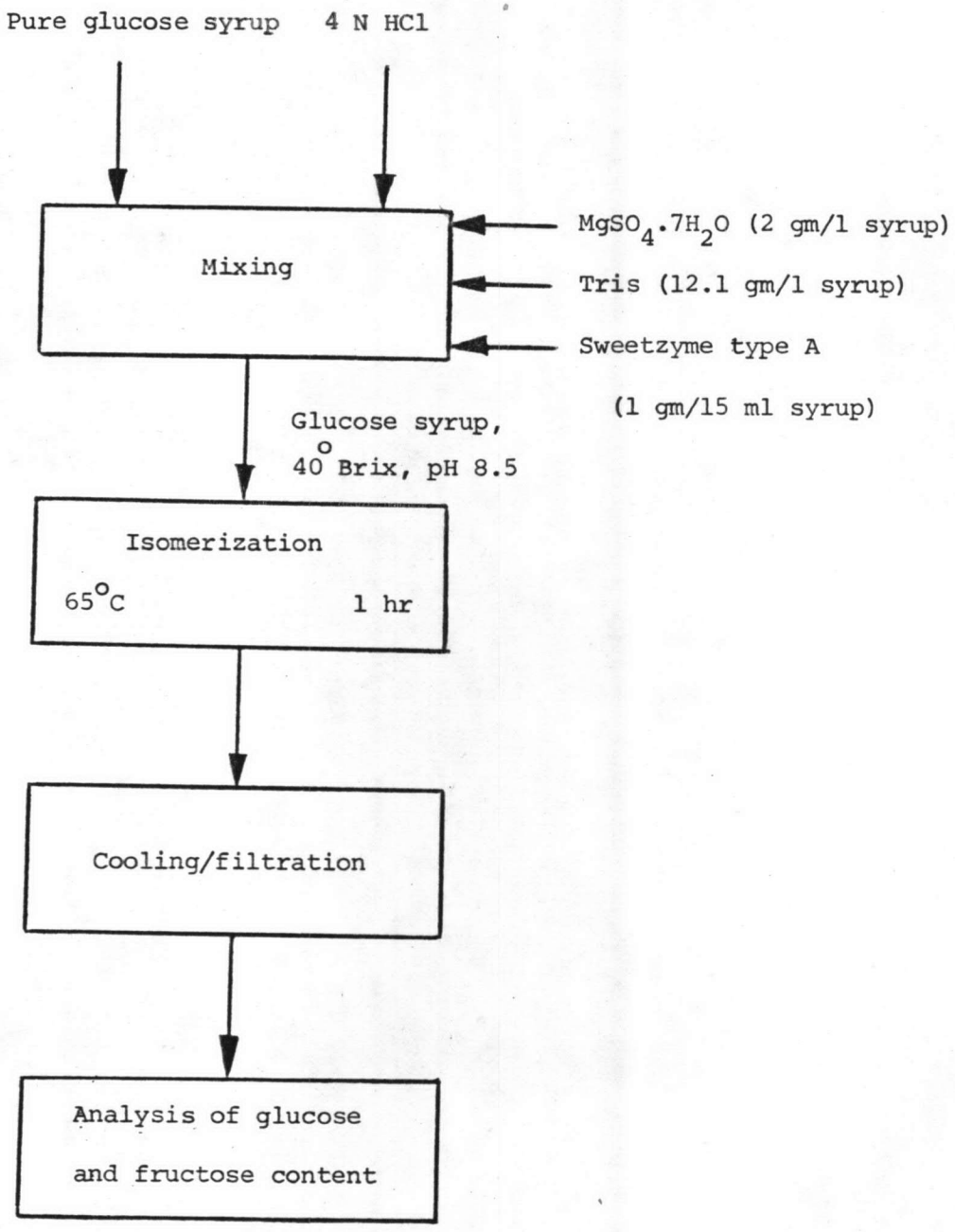

Figure 4 - 4 Determination of the activity of Sweetzyme type A (31)

viii) Calculate the conversion, X by the difference of glucose content in glucose and fructose syrup. (Conversion, X is defined as the content of fructose on an initial weight of glucose basis)

ix) Calculate the activity of Sweetzyme type A as follows (31):

$$\text{Activity} = \frac{F \times [Rf \times X - \dot{Km} \times \ln (1 - X)]}{k \times E \times \phi (X)} \quad \text{GINU/gm}$$

When F = Flow = $15 \times \rho$, gm/hour

ρ = Density of substrate = $1.1727 + \frac{Rf - 40}{200}$ $\quad 38^{\circ} < Rf < 42^{\circ}$

Rf = % W/W of glucose in substrate determined refractometrically, $^{\circ}$Brix

X = Fractional conversion

$\dot{Km}$ = Apparent Michaelis constant = 58.0

k = Rate constant for the reaction glucose $\longrightarrow$ fructose when (fructose) = 0, = 0.811

E = Amount of enzyme used, gm

$\phi(X)$ = Correction factor, the function ϕ (X) is plotted with X in Figure 4 - 5

4.3.6.7 Determination of Sweetzyme type A dosage in the first isomerization

i) Prepare 3 samples of pure glucose syrup (40° Brix) (4.2.6.2) with deionized water. Add Sweetzyme type A to each sample as follows:

- Sample 1 is mixed thoroughly with 25 gm of Sweetzyme type A per

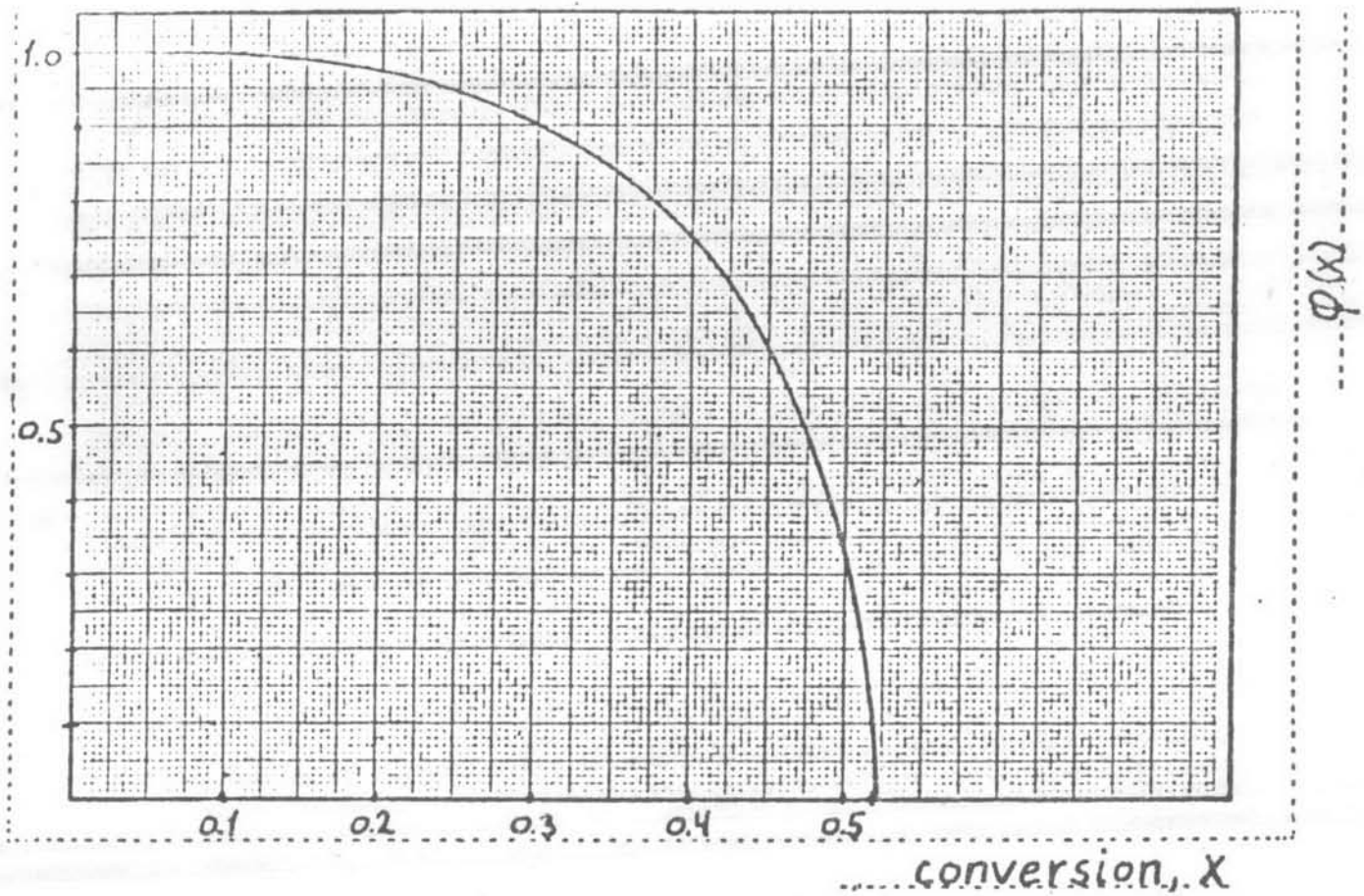

Figure 4 - 5 The relation between correction factor, Ø (X) and conversion, X (31)

1,000 gm of glucose.

- Sample 2 is mixed thoroughly with 30 gm of Sweetzyme type A per 1,000 gm of glucose.

- Sample 3 is mixed thoroughly with 33.8 gm of Sweetzyme type A per 1,000 gm of glucose.

ii) Isomerize the 3 samples (40^{o}Brix) with all steps shown in Figure 4 - 3 except the addition of Sweetzyme type A.

iii) Calculate the % yield of fructose of each sample.

4.3.6.8 Effect of Magnesium reduction on the yield of fructose

i) Prepare 2 samples of pure glucose syrup (40^{o}Brix) (4.2.6.2) with deionized water as follows:

- Sample 1 is added with 2 gm of magnesium sulfate per 1 syrup and mixed thoroughly with 33.8 gm of Sweetzyme type A per 1,000 gm of glucose.

- Sample 2 is added with 0.1 gm of magnesium sulfate per 1 syrup and mixed thoroughly with 33.8 gm of Sweetzyme type A per 1,000 gm of glucose.

ii) Isomerize the 2 samples (40^{o} Brix) with all steps shown in Figure 4 - 3 except the addition of magnesium sulfate and Sweetzyme type A. Then separate the enzyme and re - use it for 5 times.

iii) Calculate the % yield of fructose of each sample and determine the activity of Sweetzyme type A with all steps shown in Figure 4 - 4.

iv) Determine the color of fructose syrup in each sample by ICUMSA color index (4.3.3.3).

4.3.7 Post - treatment of fructose syrup

4.3.7.1 Procedure for post - treatment of fructose syrup

The post - treatment of fructose syrup is schematically shown in Figure 4 - 6 (36).

i) Immediately adjust the produced fructose syrup to pH 4 - 5 with 4 N hydrochloric acid.

ii) Remove the color compounds with a granular activated carbon column (4.2.7.3).

iii) Remove the soluble impurities and reduce the magnesium and cobalt content in syrup with a regenerated cation exchange (4.2.7.4) and a regenerated anion exchange column (4.2.7.5).

iv) Concentrate the purified syrup to 70° - 75° Brix with a vacuum steam - jacketed pan (4.1.7.6).

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

Determine the color of fructose syrup before and after activated carbon treatment by ICUMSA color index (4.3.3.3).

4.3.7.3 Cobalt determination of fructose syrup before and after cation exchange

Determine the cobalt content of fructose syrup before and after cation exchange by 4.3.3.2.

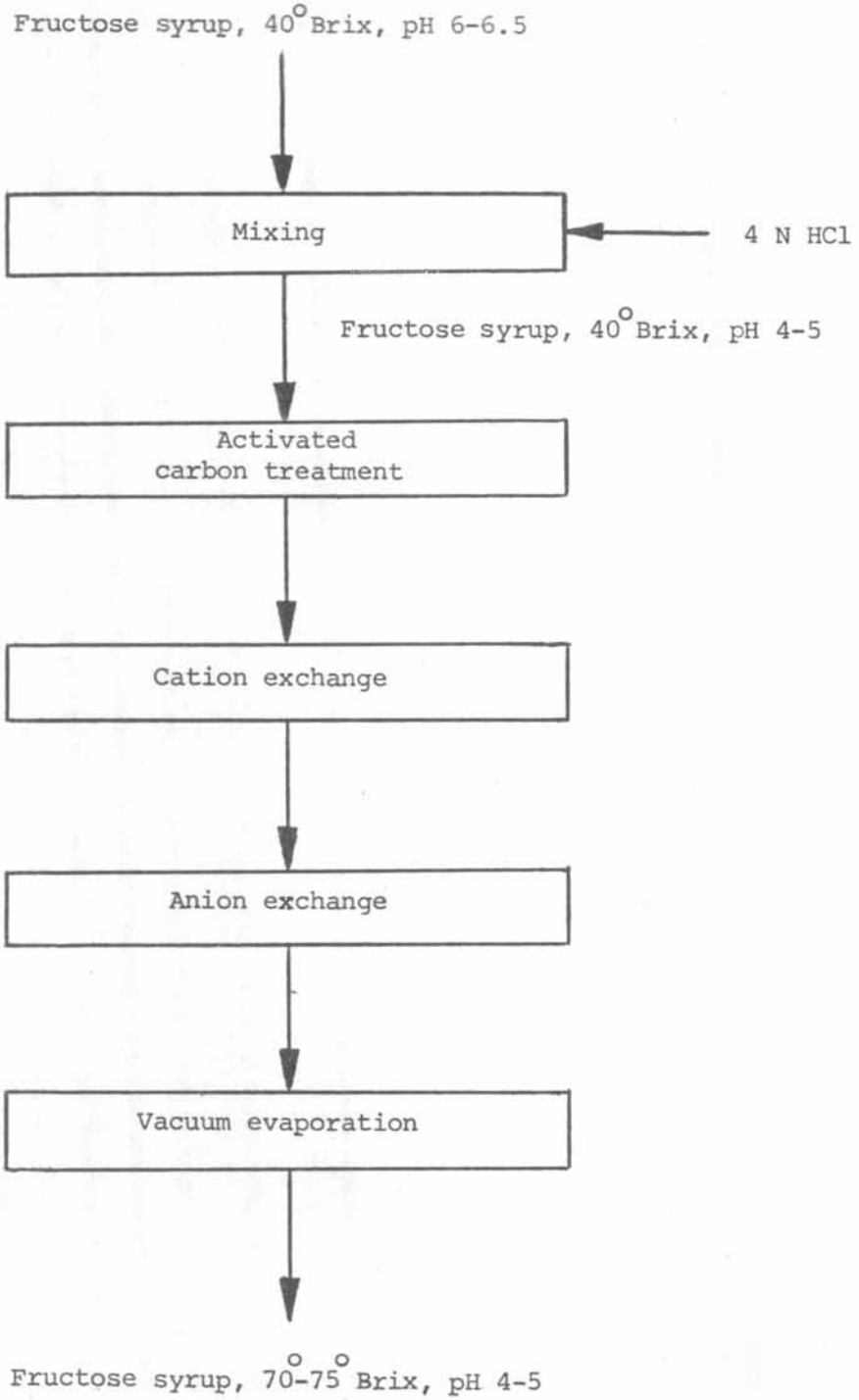

Figure 4 - 6 Post - treatment of fructose syrup (36)