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Utilization of Sorghum (*Feterita*) Starch in Production of Fructose Syrup

Elamin A. Elkhalifa*, Nadia K. A. Abdalla, Sarah, A. M. Abdelkareem

Department of Food Engineering and Technology, Faculty of Engineering and Technology, University of Gezira, Wad Medani, Sudan

Abstract The experiments of this research were conducted for the isolation of starch from sorghum grains (Feterita) by wet milling process and subsequent use for production of fructose syrup by enzyme hydrolysis. Chemical composition and percentage of starch, amylose, water soluble amylose, total soluble sugars and reducing sugars were determined. Isolated starch was cooked by heating and liquefied by α–amylase and saccharified by amyloglucosidase. The glucose syrup produced was treated with isomerase to produce fructose syrup. The percentages of moisture, protein, fat, ash, crude fiber and total carbohydrate of Feterita were 4.9, 12.8, 2.5, 1.7, 1.8 and 76.3, respectively. Feterita grains contained 49% starch. Amylose and water soluble amylose were 32.2% and 5.6, respectively. Total soluble sugars were 2.9%. In hydrolysate reducing sugars, total soluble solids (TSS) and glucose contents increased successively during hydrolysis of starch. The glucose content was increased from 38.14 mmole/L to 360.35 mmole/L whereas glucose conversion to fructose reached 50%.

Keywords Sorghum, *Feterita*, Starch, Hydrolysate, α-amylase, Amyloglucosidase, Isomerase

1. Introduction

In the Sudan, starch is available from varieties of cheap sources (sorghum, maize, millet, cassava, sweet potato and potato) and the cheapest source is *Feterita*. The enzyme technology of glucoamylase is applied to the starch conversion process, giving added economic benefits to produce dextrose and dextrose syrups. In this process high starch concentrations can be used which requires less steam energy in subsequent evaporation steps. The conversion of glucose to fructose via-the action of enzyme glucose isomerase remains one of the most important transformations used in industry [1].

The chemical isomerization of glucose to fructose is possible especially under condition of high temperature and alkaline pH [2-4]. However, chemical isomerization is not employed commercially because of the usual production of non-metabolizable materials such as psicose, formate and colored materials that are costly to remove [5]. The technique for the production of high fructose syrup (HFS) was first developed in Japan and later improved in United States [6]. In the U.S. corn starch is treated with the enzymes α–amylase and amyloglucosidease to produce glucose. The latter is then treated further with glucose isomerase to produce significant fructose content and hence greater sweetening capacity [5]. In the United States ten million tons of HFS are produced annually and used to replace sucrose in the majority of its uses [1]. Since 2000, production of high fructose corn syrup (HFCS) has declined by about 10 percent, with 2015 production totaling 8.5 million tons [7]. Fructose play an important role in the diet of the diabetics as it is only slowly absorbed by the stomach and intestinal tract and hence dose not influence the blood glucose level [8].

In modern developments of starch industry, the starch can be converted easily to glucose and then further to HFS using immobilized enzymes process that can be recovered for reuse. Considering this, successful HFS industry can be developed in the Sudan adding economic value to the high production of sorghum crop in the country.

The objectives of this study to utilize enzymes of α–amylase, amyloglucosidase and glucose isomerase to convert the *Feterita* starch to fructose syrup.

2. Materials and Methods

2.1. Materials

Sorghum grains (Feterita) were purchased from Wad Medani local market. α–amylase, amyloglucosidase and isomerase enzymes were obtained from NOVO Nordisk A/S Denmark. The standard glucose kits were obtained from MDSS GmbH, Hannover, Germany.

2.2. Proximate Analyses

The percentages of moisture, ash and fiber were determined using A.O.A.C. methods [9], while the protein content was determined by A.A.C.C. methods [10] and the

* Corresponding author:
benkhalifa_99@yahoo.com (Elamin A. Elkhalifa)
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fat content was determined by A.O.C.S. methods [11]. Total carbohydrates were obtained by difference between the sum of the other major compositions, namely moisture, protein, fat, ash and fiber from 100 percent.

2.3. Starch Isolation

The starch isolated from *Feterita* grains by steeping first in an anti-bacterial mercuric chloride solution overnight and then by wet grinding in sodium chloride solution according to the procedure of Badenhuizen [12]. The isolated starch percentage was calculated using the following formula:

$$\frac{\text{Yield of starch fraction (g) X100}}{\text{Weight of grains sample (g)}}$$

2.3.1. Amylose

Amylose content in Feterita starch was released by treatment with diluted alkali according to the procedure of Williams, *et al.* [13]. The extracted amylose content was determined against amylose standard with iodine reagent at wavelength 600 nm.

2.3.2. Water-Soluble Amylose

Water-soluble amylose content in Feterita starch using hot water was determined against amylose standard with iodine reagent at wavelength 600 nm according to the procedure of Juliano *et al.* [14].

2.4. Total Soluble Sugars

Total soluble sugars content in defatted *Feterita* flour was extracted with aqueous ethyl alcohol, followed by treatment with phenol- sulphuric acid to produce golden yellow color. The absorbance was measured at 490 nm against glucose standard with different concentrations [15]. The percentage of total soluble sugars was calculated using the following formula:

$$\frac{Cstd}{Astd} \times A_{extract} \times \frac{1gm}{1.000.000} \times \frac{DF}{0.1gm\ sample} \times 100$$

Where:
Cstd = Conc. of standard (μg)
Astd = Absorbance of standard
$A_{extract}$ = Absorbance of 1ml sample extract
DF = Dilution factor (100 ml)

2.5. Conversion of Sorghum Starch to Fructose

Glucose was produced by enzymatic hydrolysis and then converted to fructose syrup, under optimum temperature, pH and incubation period required for the activity of the three enzymes as described by the method of Cheetham [16].

$$\text{Starch} \xrightarrow{\alpha\text{-amylase}} \text{Dextrin + Oligosaccharides} \xrightarrow{\text{amyloglucosidase}} \text{glucose units} \xrightarrow{\text{isomerase}} \text{Fructose + glucose}$$

2.5.1. Determination of pH

Electrometric method employing pH-meter with glass electrode (assembly) was used for pH measurements. The pH-meter was adjusted with standard buffer solutions. The pH of the hydrolysate after each enzyme treatment was recorded.

2.5.2. Determination of total Soluble Solids (TSS)

The Abbe refractometer was adjusted at 20°C to give zero reading using distilled water. 2-3 drops of starch slurry was transferred by a glass rod to the instrument. The reading was recorded in Brix to represent TSS [17]. The procedure was also used for determining TSS in hydrolysate sample.

2.5.3. Determination of Reducing Sugars

Determination of reducing sugar using Lane and Eynon method. This method was used for determination of reducing sugars and other substances [18]. The percentage of reducing sugars was calculated using the following equation:

$$\text{Reducing sugars \%} = \frac{100\ Ff}{VC}$$

Where:
Ff = the correction factor;
V = the volume (ml) of the test solution used in the titration;
C = the concentration (g/100 ml) of the sample in the test solution.

2.5.4. Determination of Glucose Content

The glucose content in starch mixture and hydrolsate sample was determined according to Tinder [19]. GOD-PAP enzymatic colorimetric method sold as a Kit was adopted for determination of glucose (MDSS GmbH, Hannover, Germany). The red Quinoneimine formed is proportional to the amount of glucose present in the sample as presented in the following reaction:

$$\text{Glucose} + 2\ H_2O + O_2 \xrightarrow{GOD} \text{Gluconic acid} + H_2O_2$$

$$2\ H_2O_2 + \text{Phenol} + \text{4-amino-antipyrine} \xrightarrow{PAP} 4H_2O + \text{Quinoneimine}$$

The amount of glucose was calculated as follows:

$$\text{Glucose concentration (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{conc. standard}$$

2.5.5. Determination of Fructose Content

Fructose content was determined by amount of glucose which was converted to fructose by isomerase. Fructose content was calculated by subtracting the unconverted glucose in the enzymic reaction from the total glucose in the sample.

3. Results and Discussion

3.1. Chemical Composition of Sorghum (*Feterita*)

The results obtained for the study of sorghum analysis are presented in Table 1.

Table (1). Proximate analysis of sorghum grains (*Feterita*)

Moisture	Protein	Fat	Ash	Crude fiber	Carbohydrate
			%		
4.9	12.8	2.5	1.7	1.8	76.3

The moisture content reported in this investigation was found to be 4.9%. The estimated value of protein content was 12.8% which was lower than the value of 13.4 reported by Eggum *et al.* [20]. This variation may be related to differences in genetically and environmental conditions, sorghum has low fat content, the fat content of studied Feterita was found 2.5%, which was less than Eggum *et al.* [20] who reported that fat content of Feterita was 4.1%. These differences may be related to genetic and climate variation. The ash content of food stuffs represent the inorganic residue remaining after the organic matter has been burned. The ash content of Feterita was found 1.7%, which was less than Eggum *et al.* [20] who reported that ash content of Feterita 2.07%.

The fiber content of studied Feterita was found to be 1.8% which was less than Eggum *et al.* [20] who found that fiber content of Feterita was 2.1%. This difference may be also to environmental and genetic variations. The carbohydrate of Feterita was 76.3% which was within the range reported by other workers [20-22].

Table (2). The percentages of starch amylose, water soluble amylose and total soluble sugars in sorghum (*Feterita*)

Sample	Components %			
	Starch	Amylose	Water soluble amylose	Total soluble sugar
Feterita grains	49	32.2	5.6	2.9

3.2. The Isolated Starch

The percentage of starch was found 49%. This result was higher than the result of Abd Elnour [23] who reported that the percentage of starch in *Ferterita* was found to be 44.2% in grains without decortication then increased to 57.05% in decorticated seeds once, and increased to 61.8% when *Feterita* was decorticated twice. Amylose and water soluble amylose content were found to be 32.2% and 5.6%, respectively.

Buddair [24] reported that *Feterita* starch had the highest amylose content than other sorghum varieties (*Dabar* and *Tetron*). Rooney and Saldivar [25] reported that the amylose content in starch was 30%. This variation in result may be due to the genetic makeup of the sorghum varieties. The percentage of total soluble sugars was found 2.9% as reported in Table 2.

3.3. Starch Hydrolysate

All polysaccharides can be hydrolyzed with acids or enzymes to yield monosaccharides. The results of pH, total soluble solids (TSS) and reducing sugars in starch slurry and hydrolysate samples are presented in Table 3.

Table (3). pH, total soluble solids and reducing sugars in *Feterita* starch hydrolysate

Treatment	Steps of hydrolysis	pH	TSS Bx	Reducing sugars %
Starch slurry 30% solids	-	6.80	0.0	0.0
Starch slurry (α–amylase)	Liquefaction	5.60	25.5	10.2
Starch hydrolysate (amyloglucosidase)	Saccharification	4.41	28.8	22.2
Starch hydrolysate (isomerase)	Isomerization	7.50	36.0	42.2

The result showed that in starch slurry the TSS and reducing sugars were not reported. The pH of starch slurry was found 6.8.

In liquefaction step the pH was adjusted at pH 5.6. After hydrolysis of starch slurry with α–amylase for 2 hours, the TSS and reducing sugars were found 25.5°Bx and 10.2%, respectively.

In Saccharification step using amyloglucosidase hydrolysis for 48 hours, the pH was adjusted at 4.41 and temperature set at 60°C. The TSS was increased to 28.8°Bx and a double increase in reducing sugars to 22.2% compared to liquefaction step was obtained. Hence, reducing sugars and total soluble solids increased due to hydrolysis of starch by amyloglucosidase enzyme.

In the isomerization step the incubation conditions for isomerase were set at pH 7.5 and temperature at 60°C for 2 hours. The total soluble solids was increased to 36.0°Bx and reducing sugars content in the hydrolysate was substantially increased to 42.2% compared to the previous Saccharification step. In this stage of hydrolysis part of glucose is converted to fructose which has greater reducing power toward Fehling's reagent Shallenberger and Birch [26].

Glucose content in starch slurry was found 0.0938 mmole/l (Table 4). When α–amylase was added to the starch slurry, starch was broken down to soluble dextrin and oligosaccharide and glucose content increased to 38.14 mmole/l. While the hydrolysis continued by the addition of

amyloglucosidase enzyme, the glucose concentration in the hydrolysate was substantially increased to 360.35 mmole/l due to hydrolysis of dextrin to glucose by amyloglucosidase enzyme. In the isomerization step the incubation conditions for isomerase enzyme was set at pH 7.5 and 60°C for 2 hours, more than half of glucose content was converted into fructose. Accordingly glucose content was reduced to 172.75 mmole/l (Table 4). The isomerization of glucose gives fructose, the most sweet natural sugar. Fructose syrup competes with sucrose of cane sugar in many food applications.

Table (4). Glucose and fructose contents of sorghum (*Feterita*) starch hydrolysate

Treatment	Steps of hydrolysis	Glucose content mmole/l	Fructose content mmole/l
Starch slurry 30% solids	-	0.0938	-
Starch slurry (α–amylase)	Liquefaction	38.14	-
Starch hydrolysate (amyloglucosidase)	Saccharification	360.35	-
Starch hydrolysate (isomerase)	Isomerization	172.75	187.6

4. Conclusions

Feterita grain is relatively rich in protein and carbohydrates compared to other sorghum varieties. The starch isolated from *Feterita* grains had high amylose and water soluble amylose contents. Addition of isomerase enzyme converted the starch hydrolysate to a mixture of glucose and fructose whereas glucose conversion to fructose reached 50%. *Feterita* starch can be processed through enzymatic conversion to produce fructose syrup.

Further research is needed to isolate the starch through dry milling process. Also further investigation is needed to study the physical and chemical properties of fructose syrup produced from *Feterita* starch.

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REFERENCES

[1] Housler, H. and Stutz, A.E. (2001). D-xylose (D-glucose isomerase and related enzymes in carbohydrate synthesis). *Topics current chemistry, (215 L): 77-114.*

[2] Poulsen, P; Borge, R. and Zittan, I.E. (1977). Process of isomerazing glucose. (Novo Industry A/S Denmark). *United States Patents 4, 025, 289.*

[3] Scallet, B.L; Shieh, K; Ethernthal, I. and Slapshak, L. (1974). Studies in the isomerization of D-glucose. *Die Starke, (26): 405-408.*

[4] Barker, S.A; Somers, P.J. and Hatt, B.W. (1975). Methods in high fructose syrup manufacture (Novo Industry A/S Denmark). *United States Patent 3, 875, 140.*

[5] Blanchard, P.H. and Geiger, E.O. (1984). Production of high fructose corn syrup in the USA. *Sugar Technology Reviews, Elsevier Science Publishers, Amsterdam, 1-94.*

[6] White, P.J. and Pollak, L.M. (1995). Corn as a food source in the United States: Part II. Processes, products, composition and nutritive value. *Cereal Foods World 40 (10): 756-762.*

[7] United States Department of Agriculture, USDA. (2017). U.S. Sugar Production. *Economic Research Service*.

[8] Davis, E.A. (1995). Functionality of sugars: Physiological interactions in foods. *American Journal of Clinical Nutrition 62 (1): 170-177*.

[9] A.O.A.C. (1984). *Official Methods of Analysis 14th edition*. Association of Official Analytical Chemists, Washington, D.C., USA.

[10] A.A.C.C. (1983). *Approved methods of analysis.* American Association of Cereal Chemists, St. Paul, USA.

[11] A.O.C.S. (1981). *Official Tentative Methods of Analysis 3rd edition*, Association Oil Chemists, Society, Champaign, IL 61920, USA.

[12] Badenhuizen, N.P. (1964). *General Method for Starch Isolation pp. 14-15*. In: Methods in Carbohydrate Chemistry, vol. V (R.L. Whistler, R.J. Smith, J.N. Bemiller, and M.L. Wokform (eds.). Academic Press, New York, USA.

[13] Williams, V.R; WU, W.T; Tsai, H.Y. and Bates, H.G. (1958). Varietal differences in amylose content of rice starch. *J. Agric. Food Chem. 6: 47-48.*

[14] Juliano, B.O; Contano, A.V. and Vidal, A.J. (1968). Note on a limitation of the starch-iodine blue test for milled rice amylose. *Cereal Chem. (45) : 63-65.*

[15] Dubois, M; Gilles, K.A; Hamilton, J.K; Rebers, P.A. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem. 28: 350-356.*

[16] Cheetham, P.S.J. (1987). The Application of Enzymes in Industry, pp 274-379. In: A. Wiseman (ed.). *Handbook of Enzyme Biotechnology.* Eilis Horwood Limited, Chichester, England.

[17] A.O.A.C. (2000). *Official Methods of Analysis 17th edition*. Total soluble solids and titratable acidity of fruits and fruits products. Association of Official Analytical Chemists, Washington, D.C., USA.

[18] International Commission Uniform Methods of Sugar Analysis (ICUMSA). (1998). Determination of reducing sugars by Lane Eynon constant volume procedure *Official Method Book with first supplement, ICUMSA Publications Department C/O British Sugar Technical Center*. Norwich Research Park, Coney, Norwich NR4, 7UB, England.

[19] Tinder, P. (1969). Glucose-GOD-PAP-enzymatic colorimetric method. *Ann. Clin Biochem. 6: 24.*

[20] Eggum, B.D; Monawar, L; Bach Knudesn, K.E; Munk, L. and

Axleel, J. (1983). Nutritional quality of sorghum and sorghum foods, from Sudan. *Journal of Cereal Science (10): 127-137.*

[21] Reichert, R.D. (1982). Sorghum dry milling. In: Sorghum in the eighties. *Proceeding of the International Symposium on Sorghum. ICRISAT, Patancheru, A.P. India.*

[22] Elkhalifa, E.A. (2000). Sorghum-based Traditional Foods of Sudan. Preparation and Quality Aspects. *Proceeding of the 1997 International Conference on Traditional Foods. p p 117- 127, CFTRI, Mysore. India.*

[23] Abd Elnour, M.K. (2001). The Effect of Decortications on Wet-milling and Starch Quality of Sorghum and Millet Grains. *M.Sc. Thesis, University of Khartoum, Khartoum, Sudan.*

[24] Buddair, A.A. (1977). Chemical Studies on Sorghum Grains and their Products. *M.Sc. Thesis, University of Khartoum, Sudan.*

[25] Rooney, L.W. and S.O. Saldivar. (1991). *Sorghum Hand Book of Cereal and Technology*. (K.J. Iorenz and K. Kalp (eds.)), Marcel Dekkar, New York, USA.

[26] Shallenberger, R.S. and Brich, G.G. (1975). *Sugar Chemistry*. The AVI Publishing Company, Inc. West Port, Connecticut, U.S.A.