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Enzymatic production of glucose syrup from Sudanese sorghum and millet

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Abstract
Glucose syrup produce from two sorghum (*Sorghum bicolor* L. Moench) cultivars and two millet (*Pennisetum glaucum*) cultivars starches were investigated. The starch extracted by two deferent methods, laboratory wet milling (control) and enzymatic wet milling (BE) then hydrolyzed by alpha amylase, glucoamylase and pullulanase enzymes. TSS, reducing sugar, moisture and solid matter of glucose syrup were varied between 39- 41, 81.73%-93.47% , 50.9%-59.79% and 40.21%-49.10% respectively, for the laboratory wet milling and enzymatic wet milling of sorghum and millet cultivars. Dextrose equivalent results of glucose syrup revealed 99.63%, 86.88%, 95.80% and 96.33% for laboratory wet milling (control) and 92.86%, 89.24%, 92.92% and 88.58% for enzymatic wet milling for Tabat, Wed baco, Ashana and Demby, respectively. The color of glucose syrup varied between the medium yellow for Tabat pepsin treated sample and light straw color for Tabat, Wad baco and Demby control samples.

Keywords: glucose syrup, sorghum, millet, alpha amylase, glucoamylase, pullulanase

Introduction

Glucose syrup is a concentrated aqueous solution of various sugars like glucose, maltose and other nutritive saccharides obtained from the hydrolysis of edible starch. Glucose ($C_6H_{12}O_6$) contains six carbon atoms, one of which is part of an aldehyde group known as an aldohexose, commonly presents in a form of white substance or as a solid crystal. Glucose also known as confectioners' syrup and can be dissolved in water as an aqueous solution (Vandamme *et al.*, 2002) [21]. Glucose is important in food industries as sweetener, as well as in the production of antibiotics (Vandamme *et al*., 2002 and Nigam and Singh, 1995) [21, 13]. In addition, organic acids like lactic, citric and ascorbic (Vitamin C) acids are also being produced from glucose (Vandamme *et al*., 2002; Madihah *et al*., 2001) [21, 11]. In UK, glucose syrup and high maltose syrup are used in brewing as fermentable carbohydrate. Liquid glucose is used as substrate for the production of stabilizer xanthan gum from *Xanthomonas campestris* and for the growth of mycoprotein from *Fusarium graminearum* (Vandamme *et al*., 2002) [21]. The production of glucose, maltose and dextrin from starch of maize (Sutherland *et al*., 1986) [19], banana (Igoe, 1989; Bello-Perez *et al*., 2002) [8, 2] and sweet potato (Omemu *et al*., 2004) [14] has been well documented in many parts of the world. The production of these important products of starch hydrolysis is not found in Sudan. The production of glucose syrup from starch has two main procedures; hydrolysis by acid or by enzymes. Enzymatic production of glucose syrup from starch is a multistage process involving: liquefaction, saccharification, purification and concentration. Generally, the key features of the liquefaction enzymes are, 1- High dextrose yields with minimal by products formation, 2- Fast viscosity reduction-enabling high dry substance levels. 3- Low color formation and reducing the refinery costs (Pontoh and Low, 1995) [16]. The saccharification is done by using an exo-acting glucoamylase, which is specialized in cleaving α-1, 4 glucosidic bonds and slowly hydrolyzes α-1, 6 glucosidic bonds present in maltodextrins. Therefore, recently pullulanase, which efficiently used to hydrolyze α-1, 6 glucosidic bonds (Hebeda, 1992; Van der Maarel *et al*., 2002; Ezeji and Bahl, 2006 and Brienzo *et al*., 2008) [7, 20, 6, 4]. As general enzymatic hydrolysis is more preferable as it gives better yield, less byproducts and easy to maintain.

In the liquefaction step, gelatinization increased the rate of hydrolysis while saccharification is a step to further hydrolyze the liquefied starch. Yield of hydrolysis depends on substrate concentration, type of starch, enzyme dose, time taken, Speed of agitation, granule size and viscosity of the raw starch. Lesser substrate concentrations are preferable so as to avoid substrate inhibition. Greater hydrolysis time and high enzyme dose showed the highest increase in glucose yield percentage as temperature rises (Alias, 2009) [1].

DE is a measure of the total reducing sugars calculated as D-glucose on dry basis. Starch hydrolysates with dextrose equivalent (DE) below 20 are referred to as maltodextrins, while those with DE above 20 are referred to as maltose syrup or glucose syrup (White *et al*., 2003) [23]. Starch hydrolysates with the same DE can have different properties and molecular compositions depending on the starch and how it is digested (Marchal *et al*., 1999) [12]. The color of starch-derived sweeteners is often referred to as 'water white,' but it is more meaningful to express color of syrup in terms of absorbance (optical density) dextrose syrup color, is expressed in absorbance measured against a reference standard of water at 450 nm and 600 nm. Several reactions can cause color development in starch-derived sweeteners, because they contain reducing sugars, they will react with proteins and amino acids via the non-enzymatic browning reaction between sugars and primary or secondary amines (Milliard reaction) to form what are referred to as 'color bodies. (Waller and Feather, 1983) [22]. Color development that occurs in the absence of nitrogenous compounds with the

application of heat or acids is the result of caramelization. Excessive heating of starch-derived sweeteners will result in partial caramelization and development of undesirable flavors.

Materials and Methods

Materials

Starch obtained from two cultivars of sorghum and millet grown in Sudan, sorghum, Tabat obtained from Alfao Research Station, Wad baco from Gadarif local market and the two millet cultivars were obtained from Al obied Research Station.

Extracted by two deferent wet milling procedures**:**

- Laboratory wet milling was conducted according to Steinke and Johnson (1991) steeping for 24hours (control A).
- Enzymatic wet-milling was done using the two stage modified procedure described by Johnston and Singh (2001) steeping for 7hours (BE).

Chemicals and enzymes

Chemicals used in this study were of analytical grade obtained from Lab Line Company and some of the enzymes and standard reagents were obtained from outside the country (China and Saudi Arabia).

Methods

Glucose syrup production procedure

Gelatinization step

This was done as described by Bello- Perez *et al*., (2002) [3]. A 30 % of each starch was gelatinized by cooking in boiling water bath for 8 minutes.

Liquefaction step

Liquefaction is the hydrolysis of the starch to oligosaccharides: glucose polymers of up to ten glucose residues. This was done by adding stable alpha amylase enzyme (EC 3.2.1.1) (1000 units for gram starch) for 2h at pH 6.0 - 6.5 (1000 units for gram starch). During the liquefaction step the starch hydrates and is broken down by the action of the enzyme:

Starch + H_2O (α amylase) → oligosaccharides

Saccharification Step

After liquefaction, the pH was lowered to between 4 and 5 and the liquid was cooled to around 55C°. This inactivates the liquefaction enzyme and creates conditions suitable for the sacchrification enzymes, Glucoamylase (EC 3.2.1.3) 100 units per gram of starch and Pullulanase (EC 3.2.1.41) 0.5 units per gram of starch added for 3 h. After sacchrification time was completed, the syrup was heated for 10 minutes in boiling water bath to inactivate the enzymes, and then concentrated by heating to achieve 40% total soluble solids (TSS). The glucose syrup was stored in deep freezer for further analysis.

Reducing sugars determination:

Reducing sugars in starch hydrolysate were estimated by Lane and Eynon's method as reported by Ranganna (1986) [17].

Moisture content of glucose syrup

Moisture content was determined according to A.O.A.C (1990) methods. In an oven provided with a fan at 70C°under vacuum for 4 to 6 hours.

Solid matters

Solid matters were calculated by deference.

Solid matters = 100 – Moisture content

Dextrose equivalent

Dextrose equivalent was calculated using the following formula:

$$\text{Dextrose equivalent (\%)} = \frac{y \times 100}{100 - M}$$

Where

Y: reducing sugar

M: moisture content

Color of glucose syrup

The color of glucose syrup was measured using spectrophotometer by the method described by Askar and Treptow, (1993) with some modifications. Ten grams of sample were extracted with 100 ml ethanol (60%) then filtrated throw (Whatman No. 1 filter paper) .The absorbance of the extract was measured with a spectrophotometer (model:Jenway 6305 spectrophotometer)at 420 nm with 60% ethanol as a blank.

Statistical Analysis

Data was assessed by analysis of variance (ANOVA) using CRD with three replicates. Means were compared using Duncan (1955) [5] Multiple- Range Test (DMRT) with a probability $P<0.05$.

Results and Discussion

The result of total soluble solids (TSS), reducing sugars (RS), moisture content, solid matters (SM) and dextrose equivalent (DE) of the glucose syrup from the sorghum starches, extracted by BE (Enzymatic wet milling) and the control A (Laboratory wet milling) are shown in Table (1).

The Total soluble solids (TSS) of the Tabat glucose syrup were found to be 40.00 and 39.00 for BE and the control sample respectively, while Wad- baco gave 41.00 for BE and the control samples.

The reducing sugars (RS) content of the Tabat glucose syrup were found to be 85.05 and 93.47% for the BE and the control sample, while Wad-baco reducing sugars gave 81.73 and 82.45% for the BE and the control sample respectively. Significant ($P\leq0.05$) difference between the enzymatic treated samples and the control samples were observed, on the other hand the reducing sugars were significantly ($P\leq0.05$) different between the two cultivars.

The moisture content of Tabat glucose syrup were found to be 59.79 and 56.58% for the BE and the control samples respectively. While the moisture content of Wad-baco glucose syrup was found to be 53.15 and 50.90%for enzymatic treated sample and the control sample respectively.

The solid matters (SM) of Tabat glucose syrup were found to be 40.21 and 43.42% for BE and the control sample, which were lower significantly ($P\leq0.05$) compared to 46.85 and 49.10% for the enzymatic treated sample and the control of Wad-baco respectively. The dextrose equivalent (DE) of Tabat glucose syrup was found to be 92.86 and 99.63% for

the BE and the control samples, while the BE and the control of Wad-baco cultivar gave dextrose equivalent of 89.24 and 86.88 respectively. Statistical analysis of data showed significant ($P \leq 0.05$) difference between the dextrose equivalent of the enzymatic treated samples and the control samples. Also, the dextrose equivalent varied significantly ($P \leq 0.05$) in respect to the cultivars. The dextrose equivalent which is the reducing power of the syrup revealed that the control of Tabat had the highest mean value compared to enzymatic treated samples and the control of Wad-baco. However, these results were higher than 78.28% for sorghum syrup and 73.50% for yellow maize syrup produced by using amyloglucosidase only, reported by Zainab *et al.*, (2011) [24], that may enhance the positive effect or the advantage of using pullulanase together with glucoamylase .Jensen and Norman, 1984 and Poliakoff and Licence, 2007, they mentioned that the use of a debranching enzyme would increase the rate of overall saccharification process and reduce the total amount of glucoamylase that is required for complete conversion process.

Color of the sorghum glucose syrup

The color of the glucose syrup from the sorghum starches, extracted by BE and the control A (Enzymatic wet milling) and the control A (Laboratory wet milling) are presented in Table (2).

One of the most important quality criteria in glucose syrup its color since it has negative effect not only on sensorial characteristics (flavor, appearance) but also on the nutritional value. The color of glucose syrup were measured using spectrophotometer at wave length 420nm, the results were expressed in terms of absorbance (optical density). The results were found to be 0.167 and 0.121 for Tabat and Wed baco treated sample, while the control samples were 0.051 and 0.054 for Tabat and Wed baco respectively. The results of the control samples were lower significantly ($P \leq 0.05$) compared to enzyme treated sample. There were no significant ($P \leq 0.05$) differences between the two control samples. Pancoast (1980) [15] reported visual color related to specific optical densities, Tabat and Wed baco treated samples were found to be medium yellow to yellow and light yellow respectively. The control samples gave light straw color for the two investigated cultivars, which were better than the treated sample. The standard color of glucose syrup when offered for sale is usually described as 'water white' or colorless (Kearsly and Dziedzic, 1995) [10].

Properties of millet glucose syrup

The total soluble solids (TSS), reducing sugars (RS) , moisture content, solid matters (SM) and dextrose equivalent (DE) of glucose syrup from the millet starches , extracted by BE (Enzymatic wet milling) and the control A (Laboratory wet milling) are given in Table (3). The total soluble solids (TSS) of glucose syrup for Ashana were found to be 39.00 and 40.00 for BE and the control sample, also, 39.00 and 40.50% for BE and the control sample of Demby cultivar respectively. Reducing sugars (RS) of Ashana glucose syrup were found to be 85.64 and 89.27%for the BE and the control sample, while Demby glucose syrup gave a reducing sugars of 81.36 and 90.29 for BE and the control samples respectively. Significant ($P \leq 0.05$) differences of reducing sugars between Ashana and Demby glucose syrup treated and control samples were observed. Demby control sample gave the best reducing sugar which led to the highest dextrose equivalent.

The moisture content of glucose syrup was found to be 58.60, 57.50, 55.93 and57.59% for Ashana BE, Ashana A, Demby BE, and Demby A sample. The moisture content showed significant ($P \leq 0.05$) difference between enzymatic treatment and the control samples.

The solid matters (SM) from the glucose syrup were found to be 41.20 and 42.50% for the BE treatment and the control sample of Ashana cultivar, for Demby cultivar SM was 44.07 and 42.41% for BE and control samples respectively. These values of SM were higher than 37.2% for glucose syrup from millet starch reported by Zainab *et al.*, (2011) [24]. Statistical analysis showed significant variation at level $P \leq 0.05$ between the two treatments and the two investigated cultivars.

The dextrose equivalent (DE) of Ashana glucose syrup was found to be 92.92 and 95.80% for BE and the control samples, while Demby glucose syrup gave the values of 88.58 and 96.33% for BE and the control samples respectively. Statistical analysis showed significant ($P \leq 0.05$) difference between the enzymatic treatment and the control sample, also there is significant ($P \leq 0.05$) variation between the two millet cultivars. However, Zainab *et al.*, (2011) [24] who used one hydrolysis enzyme (amyloglucosidase) reported 65.66% DE for millet syrup.

Color of millet glucose syrup

The color of the glucose syrup from the millet starches, extracted by BE (Enzymatic wet milling) and the control A (Laboratory wet milling) are presented in Table (2).

The results expressed in terms of absorbance (optical density), were found to be 0.073 and 0.062 for Ashana and Demby enzymatic treated sample, while the control samples were 0.129 and 0.050 for Ashana and Demby respectively. Statistical analysis showed significant ($P \leq 0.05$) differences between the control and the treated sample also between the two investigated millet cultivars.

The enzymatic treated samples gave straw and light straw color, while the control gave light yellow and light straw color for Ashana and Demby respectively as described in the Handbook of Sugars by Pancoast, (1980) [15]. Demby glucose syrup gave better color than Ashana glucose syrup for the treated and control sample. This result may be due to the primary color of the millet grains.

Conclusions

The glucose syrup prepared from the starches obtained by the enzymatic wet milling gave low dextrose equivalent compared to the control; the enzymatic wet milling gave higher starch yield. Glucose syrup from Tabat and Wad baco control samples gave better color than the treated sample, Demby glucose syrup gave better color than Ashana glucose syrup for the treated and control sample.

Table 1: Total soluble solids (TSS), reducing sugars (RS), moisture content, solid matters (SM) and dextrose equivalent (DE) % of sorghum glucose syrup:

Treatment	TSS		RS		Moisture content		SM		DE	
	Tabat	Wad-baco	Tabat	Wad-baco	Tabat	Wad-baco	Tabat	Wad-baco	Tabat	Wad-baco
BE	$40.00^{b} \pm 0.00$	$41.00^{a} \pm 0.00$	$85.05^{b} \pm 0.00$	$81.73^{d} \pm 0.17$	$59.79^{a} \pm 0.12$	$53.15^{c} \pm 0.17$	$40.21^{d} \pm 0.12$	$46.85^{b} \pm 0.17$	$92.86^{b} \pm 0.00$	$89.24^{c} \pm 0.18$

A (control)	$39.00^{c} \pm 0.00$	$41.00^{a} \pm 0.00$	$93.47^{a} \pm 0.00$	$82.45^{c} \pm 0.00$	$56.58^{b} \pm 0.07$	$50.90^{d} \pm 0.12$	$43.42^{c} \pm 0.07$	$49.10^{a} \pm 0.12$	$99.63^{a} \pm 0.00$	$86.88^{d} \pm 0.00$
$Lsd_{0.05}$	0.0005954*		0.1575*		0.2382*		0.2382*		0.1786*	
SE±	0.0001826		0.0483		0.07303		0.07303		0.05477	

Values are mean±SD
*= significant
**= highly significant
Mean value(s) having different superscript(s) in columns and rows are significantly (P≤0.05) different according to DMRT
A (control): steeping for 24h with 0.2% SO_2
BE: steeping for 7h with 3h in distilled 4h in 0.1M sodium acetate buffer pH (4-4.5) + 250mg pepsin enzyme

Table 2: Color of sorghum and millet glucose syrup (optical density- absorbance):

Sample	Sorghum		Millet	
	Tabat	Wad-baco	Ashana	Dembi
BE	0.167^{b}±0.02	0.121^{a}±0.002	0.073^{b}±0.01	0.062^{c}±0.003
A	0.051^{c} ±0.04	0.054^{c}±0.03	0.129^{a}±0.01	0.050^{d}±0.004
$Lsd_{0.05}$	0.045*		0.011*	
SE±	0.018		0.0076	

Values are mean ± SD
Mean value(s) having different superscript(s) in a column are significantly (P≤0.05) different according to DMRT

Table 3: Total soluble solids (TSS), reducing sugars (RS), moisture content, solid matters (SM) and dextrose equivalent (DE) % of millet glucose syrup:

Treatment	TSS		RS		Moisture content		SM		DE	
	Ashana	Demby	Ashana	Demby	Ashana	Demby	Ashana	Demby	Ashana	Demby
BE	$39.00^{c} \pm 0.00$	$39.00^{c} \pm 0.00$	$85.64^{c} \pm 0.27$	$81.36^{d} \pm 0.11$	$58.60^{a} \pm 0.21$	$55.93^{c} \pm 0.14$	$41.20^{c} \pm 0.19$	$44.07^{a} \pm 0.14$	$92.92^{b} \pm 0.29$	88.58^{c} ±0.12
A (control)	$40.00^{b} \pm 0.00$	$40.50^{a} \pm 0.00$	$89.27^{b} \pm 0.29$	$90.29^{a} \pm 0.16$	$57.50^{b} \pm 0.18$	$57.59^{b} \pm 0.09$	$42.50^{b} \pm 0.18$	$42.41^{b} \pm 0.09$	$95.80^{a} \pm 1.58$	96.33^{a} ±0.17
$Lsd_{0.05}$	0.0005954*		0.421*		0.3094*		0.3036*		1.513*	
SE±	0.0001826		0.1291		0.09487		0.09309		0.464	

Values are mean ± SD
*= significant
**= highly significant
Mean value(s) having different superscript(s) in columns and rows are significantly (P≤0.05) different according to DMRT
A (control): steeping for 24h with 0.2% SO_2
BE: steeping for 7h with 4h in 0.1 M sodium acetate buffer pH (4-4.5) + 250 mg pepsin enzyme

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