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Quality Evaluation of Glucose Syrup Produced from Saccharification of Cassava Starch by Amyloglucosidase

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ABSTRACT

The production of glucose syrup is a complex biochemical process that involved the breakdown of glycosidic bond of ά-1, 4 and ά-1, 6 of amylose and amylopectin of the starch granules, respectively. The quality attributes of glucose syrup produced from the saccharification of cassava starch by amyloglucosidase was evaluated in this study. The results showed that sample dry weight of the glucose syrup samples ranged from 0.066 - 0.083 g; reducing sugar, 46.13 – 74.23%; dextrose equivalent, 57.75 – 96.41 DE; colour intensity, 0.162 – 0.208; titratable acidity, 0.10 – 0.30. Besides, the glucose syrup samples contained low amount of copper, arsenic and lead in the ranges of 0.015- 0.023, 0.0001 - 0.002 and 0.01 – 0.012 mg/kg, respectively. The sensory attributes of the glucose syrup samples revealed that they possessed good taste (5.04 – 5.96), appearance (5.08 – 5.78), texture (5.10 – 5.81), aroma (5.14 – 5.86), fluidity (5.03 – 5.60) and overall consumers' acceptability (5.10 – 5.78). The glucose syrup produced at pH 4.5 and 55 ^{0}C had the optimal taste and appearance, while those produced at pH 5 and 60 ^{0}C had the optimal texture, aroma and fluidity. However, the glucose syrup produced at pH 4.5 and 55 ^{0}C had the highest reducing sugar, dextrose equivalent, while those produced at pH 5 and 60 ^{0}C had the least sample dry aroma and fluidity. Notably, the glucose syrup produced at pH 4.5 and 60 ^{0}C had the lowest amount of copper and arsenic, while glucose syrup produced at pH 4 and 60 ^{0}C had the lowest amount of lead.

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Introduction

The industrial significance of high-quality glucose syrup is particularly paramount to the food and pharmaceutical sectors. For instance, the glucose, a major product of starch hydrolysis, is widely used as a bulk sweetener in the food production, pharmaceutical formulations, and confectionery industry. The generation of glucose, maltose, and dextrins from starch sources such as maize, plantain, banana, cassava, and sweet potato has been globally well-documented. In Nigeria, however, starch hydrolysis products were primarily derived from cassava, a tuber cultivated extensively in the southern regions. Factually, the starch conversion into various sweeteners could be accomplished through either chemical (acid) or enzymatic processes, with enzymes offering several advantages over acid hydrolysis. Acid hydrolysis often resulted in undesirable by-products, including unwanted color and flavor compounds. It is also less effective due to its inability to address α-1,6-glucosidic linkages, while the means of controlling the process could be challenging. In contrast, enzymatic hydrolysis is more efficient and controllable, thereby yielding higher quality sweeteners. Furthermore, research has identified enzymes in local agricultural products such as rice, barley, and sorghum, which could be activated during malting to enhance starch breakdown [1-13].

Cassava (Manihot esculenta Crantz) is a vital crop in tropical regions. It is environmentally friendly, as its cultivation did not contribute to air pollution or exacerbate greenhouse gas emissions. Cassava starch served as a versatile raw material for producing a variety of sweeteners, including maltose, glucose syrup, glucose, and fructose, using either acid, enzymatic processes, or a combination of both. As a perennial woody shrub, cassava's root is a valuable source of glucose production. It is a cost-effective and readily available substrate in tropical countries. Cassava could be cultivated at altitudes ranging from sea level to 250 m, requiring a minimum annual rainfall of 1,000. Its high tolerance to drought enabled it to survive dry seasons with low soil moisture and high humidity. Additionally, cassava thrived more in poor-quality soils, hence making it more resilient than many other major food crops. A key role of cassava roots is storing energy for the plant's growth, primarily in the form of starch, which constituted the largest component of dry matter in fresh roots. Cassava's advantages as an energy crop for fuel production included its potential for high alcohol yields per hectare and its superior resistance to drought and plant diseases compared to sugarcane. Nutritionally, cassava is rich in calcium and ascorbic acid (vitamin C) and contained significant amounts of thiamine, riboflavin, and niacin. The carbohydrate fraction of cassava roots is predominantly starch, comprising 20-35% of the fresh tuber. Other carbohydrate components such as fructose, dextrose, and dextrin might also be present, though it is unclear if these compounds were found in the live root or formed post-harvest as starch and sucrose breakdown products. Therefore, this study aimed to evaluate the quality attributes of glucose syrup produced from the saccharification of cassava starch through the enzymatic activities of amyloglucosidase [14-20].

Materials and Methods

Methods

Maltodextrin, with optimal Reducing Sugar content (17.84%) and Dextrose Equivalent (DE) (14.74) was produced during a previous liquefaction process of cassava starch using alpha-amylase at pH 6.5, 70 °C, and 60 min. The pure culture of thermostable amyloglucosidase (derived from Aspergillus niger, optimized for pH 4.5 and 60 °C) was sourced from the Federal Institute of Industrial Research, Oshodi (FIIRO), Nigeria. Rochelle salt and Dinitrosalicylic acid (DNS) were procured from Pascal Store, Akure, Nigeria [21].

Description of Fermentor

A prototype fermentor was designed and fabricated to operate in conjunction with a thermostatic water bath (DK-600 SANFA Electrical Thermostatic Water Bath Boiler) for the liquefaction and saccharification processes, as depicted in Figure 1. The fermentor is equipped with a variable motor gear (specifications: Type (TIPO): Var 10/0, and Code (Condice): AC3999) sourced from GIFA Transmission Bologna, Italy.

Production of Substrate

The substrate was prepared by making a 10% (w/v) cassava starch suspension with distilled water, resulting in a 10% slurry. Typically, 10 g of starch was mixed with 100 ml of distilled water to create the slurry. A 40 ppm Ca^{2+} solution was added to stabilize the enzyme. The pH of the slurry was adjusted to 6.0, 6.5, and 7.0 using a citrate-phosphate buffer, respectively according to the modification of the methods previously described by Pele et al. Gelatinization was performed by heating the mixture to 97 °C and maintaining this temperature for 10 min. The gelatinized starch was then cooled to 65, 70, and 75 °C, respectively, for subsequent processing. Liquefaction was carried out by adding 2% (w/v) alpha-amylase and incubating the mixture at the respective temperatures for 40, 50, and 60 min. The fermentor was connected to a thermostatic water bath, maintaining agitation at 50 rpm. Samples were withdrawn at regular intervals to monitor the reaction kinetics. The mixture was heated to 97 °C for 15 to 20 min to terminate enzyme activity, then centrifuged (80-2 Centrifuge, Med-Lab Scientific Company, England) at 2500 rpm for 10 min to obtain the supernatant. A standard glucose curve was prepared to determine the optimal conditions for cassava starch liquefaction, which was then used as the substrate for the saccharification process.

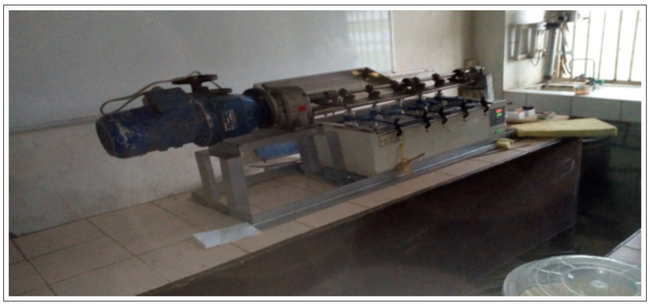

Figure 1: The Fermentor used for Liquefaction and Saccharification

Note: (Motor) Kw: 0.75; Poles: 4; Rpm min–rpm max: 350–1750; Type: mas 20P; Code: 29602117; Mount POS: 2.5.4. Bonfiglioli Riduttori, Italy.

Characterization of Amyloglucosidase

The optimum conditions for cassava starch hydrolysis were determined using a pure culture of thermostable amyloglucosidase during saccharification. Enzyme activity was assessed across different pH levels, temperatures, and time intervals. A completely randomized 3 × 3 × 12 experimental design was employed, consisting of three pH values (4.0, 4.5, and 5.0), three temperatures (50, 55 and 60 °C), and twelve-time intervals (6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, and 72 h).

Determination of Enzyme Activity in Amyloglucosidase

The optimum samples from the liquefaction process were cooled to temperatures of 50, 55, and 60 °C, while the pH was adjusted to 4.0, 4.5, and 5.0 using diluted hydrochloric acid. Saccharification was initiated by adding 2% (w/v) of amyloglucosidase, with reaction periods set at 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, and 72 h at the specified temperatures and pH values. The fermentor was clamped to the thermostatic water bath, maintaining a speed of 50 rpm. Samples were withdrawn at regular intervals to monitor the process. Enzyme activity was halted by heating the mixture to 97 °C for 20 min, followed by centrifugation at 2500 rpm to collect the supernatant for further analysis. A standard curve for glucose production was generated to determine the optimum conditions for the saccharification of cassava starch.

Determination of Physicochemical Properties of Glucose Syrup

The dry weight of the sample was assessed by measuring moisture loss on a wet weight basis. The pH was measured using a pH 200 HM Digital meter (CE 0112309), while the degree Brix was determined with an Antago Hand refractometer. Specific gravity was assessed using gravity bottles, and the refractive index was measured with an Abbe refractometer. Viscosity was evaluated using an Ostwald viscometer. The reducing sugar content was determined using the dinitrosalicylic acid method as outlined by Miller, and the dextrose equivalent (DE) was calculated following the method described by Betiku et al. Titratable acidity of the glucose syrups was assessed according to the procedure established by James, and color intensity was measured using the method specified by FAO [22-26].

Determination of Selective Heavy Metals of Glucose Samples

The copper, arsenic, and lead concentrations were measured using atomic absorption spectrophotometric methods, as outlined by James. For each sample, 2 g were precisely weighed using an analytical balance and placed into a pre-dried and cooled crucible. The samples were charred on a Bunsen burner within a fume cupboard for 30 min to remove most of the smoke. Following this, they were transferred into a pre-heated muffle furnace set at 550 °C using a laboratory spatula. The samples were allowed to remain in the furnace for 3 h until they converted to a white or light grey ash. If any samples retained a black or dark coloration after this duration, they were moistened with a small amount of water to dissolve salts, dried in an oven, and the ashing process was repeated. After ashing, the metal ions were quantified using a flame atomic absorption spectrophotometer [25].

Sensory Evaluation of Glucose Samples

A sensory evaluation of the glucose syrups was conducted with a semi-trained panel of 30 members who were familiar with sweeteners. The overall acceptability of the glucose syrups was assessed using a 7-point hedonic scale, where 1 indicated “dislike extremely,” 2 represented “dislike moderately,” 3 signified “dislike,” 4 indicated “neither like nor dislike,” 5 represented “like,” 6 indicated “like moderately,” and 7 signified “like extremely” [26].

Statistical Analyses

All procedures were conducted in triplicates, data obtained from the experiment were analyzed using a completely randomized experimental design and statistical methods. The analysis was conducted using Microsoft Excel (version 2010), SPSS (version 20), and Minitab (version 17). Significant differences were obtained at 95% level of siginificance ($p<0.05$).

Results and Discussion

Physicochemical Properties of Glucose Syrup Produced from Cassava Starch by Pure Amyloglucosidase

The results of the physicochemical properties of glucose syrup produced from cassava starch by pure amyloglucosidase are shown in Table 1. Reducing sugar content is a direct measure of the glucose produced from the enzymatic breakdown of starch. The results showed that the reducing sugar content increased with higher temperatures and optimal pH conditions, with the highest value at pH 4.5, 55 °C (74.23%) and pH 4, 60 °C (73.87%). This trend is consistent with previous findings [26] that higher efficiency in starch hydrolysis by amyloglucosidase under mildly acidic conditions (pH 4.5) and at elevated temperatures (55–60 °C) achieves higher efficiency in starch hydrolysis. Moreso, higher temperatures enhanced the kinetic energy of molecules, thereby increasing the rate of enzyme-substrate interactions, which accelerated starch breakdown into reducing sugars. Similarly, Ali & Ahmed found that cassava starch hydrolysis was significantly improved at 55 °C, with a 60% increase in reducing sugar content. However, at lower temperatures and higher pH (such as pH 5, 50 °C, with 46.13% reducing sugar), enzyme efficiency decreased, thus reflecting suboptimal hydrolysis conditions. This finding agreed with pass study that observed reduced amyloglucosidase activity at pH values above 5.0 due to decreased enzyme-substrate binding affinity. The Dextrose Equivalent (DE), which indicated the percentage of reducing sugars in the syrup, provided insight into the extent of starch hydrolysis. However, the Table 1 further revealed that the DE is highest at pH 4.5, 55 °C (96.41) and pH 4.5, 60 °C (94.90), respectively. The current high DE aligned with high reducing sugar content and suggested nearly complete hydrolysis of cassava starch, as amyloglucosidase effectively converted starch into glucose under these conditions. The high DE values (above 90) were desirable in industries such as confectionery and sweetened beverages, as they produced sweeter syrups with a cleaner taste. Moreso, Patel & Singh confirmed that glucose syrups with DE values >90 were preferable in high-sweetness applications, as these syrups exhibited better functional and sensory properties. The results (Table 1) for DE values under other conditions, such as pH 4, 50 °C (60.57), indicated partial starch hydrolysis, in line with previous research showing that DE tends to decrease at lower temperatures [26-29].

Table 1: Physicochemical Properties of Glucose Syrup Produced from Cassava by Pure Amyloglucosidase

Sample	Sample Dry weight (g)	Percentage Reducing Sugar (%)	Dextrose Equivalent (DE)	Colour Intensity	Titratable Acidity (%)	pH	Brix (o)	Specific Gravity	Refractive Index	Vicosity (dpa.s)
pH 4,50ºC	$0.083^{a}\pm0.00$	$50.27^{g}\pm0.09$	$60.57^{h}\pm0.06$	$0.201^{c}\pm0.00$	$0.30^{a}\pm0.02$	$5.10^{i}\pm0.03$	$9.70^{g}\pm 0.02$	$1.03^{c}\pm0.00$	$1.3425^{g}\pm0.00$	$1.0412^{h}\pm0.00$
pH 4, 55 ºC	$0.082^{a}\pm0.00$	$61.44^{e}\pm0.04$	$74.92^{f}\pm0.08$	$0.205^{b}\pm0.00$	$0.25^{b}\pm0.01$	$5.25^{g}\pm0.01$	$11.40^{e}\pm0.01$	$1.04^{b}\pm0.00$	$1.3430^{f}\pm0.00$	$1.0415^{g}\pm0.00$
pH 4, 60 ºC	$0.08^{b}\pm0.00$	$73.87^{b}\pm0.06$	$92.34^{c}\pm0.04$	$0.208^{a}\pm0.00$	$0.20^{c}\pm0.01$	$5.30^{f}\pm0.01$	$14.80^{b}\pm0.01$	$1.06^{a}\pm0.00$	$1.3463^{c}\pm0.00$	$1.0420^{f}\pm0.00$
pH 4.5, 50 ºC	$0.081^{ab}\pm0.00$	$50.09^{h}\pm0.09$	$61.83^{g}\pm0.09$	$0.170^{f}\pm0.00$	$0.25^{b}\pm0.01$	$5.15^{h}\pm0.03$	$9.10^{h}\pm0.02$	$1.03^{c}\pm0.00$	$1.3422^{h}\pm0.00$	$1.0511^{e}\pm0.00$
pH 4.5, 55 ºC	$0.077^{c}\pm0.00$	$74.23^{a}\pm0.05$	$96.41^{a}\pm0.08$	$0.174^{e}\pm0.00$	$0.20^{c}\pm0.02$	$5.60^{e}\pm0.02$	$15.20^{a}\pm0.03$	$1.06^{a}\pm0.00$	$1.3465^{a}\pm0.00$	$1.0588^{a}\pm0.00$
pH 4.5, 60 ºC	$0.075^{d}\pm0.00$	$71.17^{c}\pm0.07$	$94.90^{b}\pm0.07$	$0.177^{d}\pm0.00$	$0.15^{d}\pm0.02$	$5.80^{c}\pm0.02$	$14.30^{c}\pm0.02$	$1.06^{a}\pm0.00$	$1.3460^{b}\pm0.00$	$1.0580^{b}\pm0.00$
pH 5, 50 ºC	$0.08^{b}\pm0.00$	$46.13^{i}\pm0.06$	$57.75^{i}\pm0.04$	$0.162^{h}\pm0.00$	$0.20^{c}\pm0.03$	$5.70^{d}\pm0.01$	$7.10^{i}\pm0.02$	$1.03^{c}\pm0.00$	$1.3420^{i}\pm0.00$	$1.0335^{i}\pm0.00$
pH 5, 55 ºC	$0.075^{d}\pm0.00$	$61.80^{d}\pm0.08$	$82.40^{e}\pm0.04$	$0.166^{g}\pm0.00$	$0.15^{d}\pm0.01$	$6.65^{b}\pm0.01$	$11.50^{d}\pm0.03$	$1.04^{b}\pm0.00$	$1.3436^{d}\pm0.00$	$1.0528^{d}\pm0.00$
pH 5, 60 ºC	$0.066^{e}\pm0.00$	$60.00^{f}\pm0.05$	$90.90^{d}\pm0.05$	$0.169^{f}\pm0.01$	$0.10^{e}\pm0.02$	$6.80^{a}\pm0.01$	$11.20^{f}\pm0.01$	$1.04^{b}\pm0.00$	$1.3434^{e}\pm0.00$	$1.0540^{c}\pm0.00$

Values are means of triplicates
Values along the same column followed by different superscripts are significantly different ($p<0.05$)

Color intensity is an important parameter in determining the quality of glucose syrup, particularly in applications requiring high transparency. The highest (0.208) and lowest (0.162) color intensities were obtained at pH 4, 60 °C and pH 5, 50 °C, respectively, suggesting that higher temperatures might lead to mild browning reactions such as Maillard reactions or caramelization. For instance, past work noted that the slight increase in color intensity at higher temperatures is typical due to the thermal degradation of sugars, which caused darkening in glucose syrups. This is crucial for food industries where syrup color could affect product appearance, especially in clear beverages and transparent confectioneries. Moreso, the titratable acidity and pH were critical factors for ensuring enzyme stability during hydrolysis and for the overall stability of glucose syrup. In this study, titratable acidity was highest at pH 4, 50 °C (0.30%), while the pH values ranged from pH 5.10 to pH 6.80, depending on the conditions. The maintenance of an acidic environment (around pH 4.5) is essential for optimal amyloglucosidase activity and minimal by-product formation during hydrolysis. The observed decrease in titratable acidity at higher pH (such as 0.10% at pH 5, 60 °C) aligned with past findings, that reported the effect of lower acidity on the sweetness and overall stability of glucose syrup over time [30-32].

Brix and specific gravity were measures of the total soluble solids and the density of the syrup, respectively. Therefore, the highest Brix values (Table 1) for glucose syrup samples from pH 4.5, 55 °C (15.20 °Brix) and pH 4, 60 °C (14.80 °Brix), reflected the high concentrations of sugars in these syrups. High Brix values were desirable in syrup production, as they ensure a thick consistency and long shelf life. Specific gravity also followed the same trend, with values increasing at higher sugar concentrations. For instance, pH 4.5, 55 °C yielded a specific gravity of 1.06, which is a reflection of high sugar content. This result is consistent with past, that demonstrated the correlation of higher specific gravity with more concentrated syrups, which were particularly valuable for applications in confectioneries where texture and thickness were key factors of production [32,33].

The refractive index and viscosity were critical points for understanding the clarity and flow properties of the syrup. The higher refractive index values in the present study (1.3465 at pH 4.5, 55 °C) is associated with higher sugar content and better clarity, as which is similar to the past reported observation demonstrated. Viscosity also observably increased with higher sugar content and pH, reaching a peak at pH 4.5, 55 °C (1.0588 dPa.s), indicating a thicker and more concentrated syrup. Notably, a high viscous syrups were preferred for applications in the food industry where texture played a significant role, such as in sauces, candies, and certain beverages. This is supported by the observations of another study that reported increase in viscosity with higher DE, sugar content, and Brix, thereby providing a product with richer texture that is suitable for various industrial uses [31,34].

Undesirable (Heavy) Elements in Glucose Syrup Produced from Cassava by Pure Amyloglucosidase

The results of undesirable elements (copper, arsenic, and lead) in glucose syrup produced from cassava starch saccharification by amyloglucosidase at various pH and temperature conditions are shown in Table 2. The results provided important insights into the safety and quality of the syrup. The presence of heavy metals, even in trace amounts, could possibly affect the syrup's safety for consumption, and it is essential to ensure that these levels remained within permissible limits as set by the food safety regulations. The copper content in the glucose syrup samples ranged from 0.015 to 0.023 mg/kg with the highest (0.023 mg/kg) and lowest (0.015 mg/kg) from samples at pH 4, 50 °C and pH 4.5, 60 °C, respectively. Although copper is an essential trace element necessary for various biological functions, but its excessive levels is prone to toxicity. However, the copper levels in this study were well within the World Health Organization (WHO) permissible limits (<2 mg/kg) for food products. The decrease in copper content at higher pH values and temperatures may be attributed to the reduction in metal ion solubility or interactions with organic acids formed during the hydrolysis process, which could precipitate copper out of the solution [35,36].

Table 2: Undesirable (Heavy) Elements in Glucose Syrup Produced from Cassava by Pure Amyloglucosidase

Sample	Copper (mg/kg)	Arsenic (mg/kg)	Lead (mg/kg)
pH 4, 50 ºC	0.023^{a}	0.002^{a}	0.011^{e}
pH 4, 55 ºC	0.023^{b}	0.002b	0.01^{f}
pH 4, 60 ºC	0.023^{c}	0.0011^{c}	0.01^{g}
pH 4.5, 50 ºC	0.018^{f}	0.0002^{e}	0.012^{a}
pH 4.5, 55 ºC	0.016^{g}	0.0002^{e}	0.011^{b}
pH 4.5, 60 ºC	0.015^{h}	0.0001^{e}	0.011^{d}
pH 5, 50 ºC	0.023^{c}	0.0014^{b}	0.011^{c}
pH 5, 55 ºC	0.022^{d}	0.001^{c}	0.011^{e}
pH 5, 60 ºC	0.021^{e}	0.001^{d}	0.010^{f}

Values are means of triplicates.
Values along the same column followed by different superscripts are significantly different ($p<0.05$)

The arsenic content ranged from 0.0001 to 0.002 mg/kg, with the lowest (0.0001 mg/kg), and the highest (0.002 mg/kg) levels recorded from pH 4.5, 60 °C and pH 4, 50 °C glucose syrup, respectively. Arsenic is a toxic element, and its presence in food, even in trace amounts, posed health risks. Interestingly, the arsenic levels found in this study were well below the maximum Codex Alimentarius Commission's permissible limit (0.1 mg/kg) for food products. The lower arsenic content at higher pH levels, particularly at pH 4.5, suggests that the enzymatic conditions favored the minimal contamination of the syrup with arsenic. This could be due to the better control of processing conditions, which minimized its dissolution or release during the hydrolysis process [37].

Lead content in the glucose syrup samples ranged from 0.010 to 0.012 mg/kg. The highest lead concentration (0.012 mg/kg) was observed at pH 4.5, 50 °C, while the lowest (0.010 mg/kg) was recorded at pH 4, 60 °C. Lead is a highly toxic heavy metal, and its accumulation in the human body could cause severe health problems, particularly in children. Notably, the lead levels in this study are within the maximum allowable limit of 0.1 mg/kg specified by the European Commission for food products. The variations in lead content at different pH and temperature conditions might be due to the interaction of lead ions with starch or enzymatic hydrolysis products, which could reduce its solubility at higher temperatures. The lower lead concentration at pH 4, 60 °C indicated that optimal enzymatic conditions not only improve the syrup's quality but also helped in minimizing contamination with toxic metals [38,39].

The heavy metal content observed in this study is comparable to findings in previous research on glucose syrup production from cassava. For instance, a past study reported similarly low levels of copper, arsenic, and lead in cassava glucose syrup, with the conclusion that controlled enzymatic processing minimized the risk of contaminations. Another study also highlighted the importance of monitoring metal contaminants during starch hydrolysis, particularly in food products intended for human consumption. Besides, Ibrahim & Salawu emphasized that the safety of glucose syrups could be improved through the use of pure enzymes like amyloglucosidase, which reduced the likelihood of contamination with undesirable elements. These findings aligned with the results of this study, showing that careful control of pH and temperature conditions during enzymatic hydrolysis significantly reduced the presence of heavy metals in the final syrup product [39,36,31].

Sensory Evaluation of Glucose Syrup Produced from Cassava by Pure Amyloglucosidase

The results of the sensory evaluation of glucose syrup produced from the saccharification of cassava starch by amyloglucosidase at various pH and temperature conditions are shown in Table 3. The results provided valuable insights into the consumer acceptability and overall quality of glucose syrup produced from cassava starch saccharification by amyloglucosidase under various pH and temperature conditions. Each sensory attribute: taste, appearance, texture, aroma, fluidity, and general acceptability played a critical role in determining the suitability of the syrup for industrial applications, particularly in the food and beverage sectors. The taste scores ranged from 5.04 to 5.96, with the highest score observed at pH 4.5, 55 °C (5.96) and the lowest at pH 5, 50 °C (5.04). This suggested that the glucose syrup produced under mildly acidic conditions and moderate temperatures resulted in a more favorable taste. Moreso, the higher temperatures enhanced the enzymatic breakdown of starch into glucose, which increased the sweetness of the syrup, as evidenced by the higher Dextrose Equivalent (DE) values presented in Table 1. Adebiyi & Abiodun noted that sweeter glucose syrups with higher DE values, especially those produced under optimal pH and temperature conditions, were more palatable and preferred in taste by consumers. Similarly, a study found that amyloglucosidase performed best at around pH 4.5 to 5.0, resulting in syrups with better taste profiles due to more efficient starch hydrolysis. Appearance scores ranged from 5.08 to 5.78, with the highest score recorded at pH 4.5, 55 °C (5.78) and the lowest at pH 4.5, 50 °C (5.08). The clear appearance of glucose syrup is critical for applications in beverages and transparent products, and these scores suggested that syrups produced at higher temperatures and mildly acidic pH values (around pH 4.5 and 55 °C) offered superior visual qualities. The appearance of the syrup also correlated with the color intensity presented in Table 1, as higher color intensities were noted at higher temperatures. The present observation is similar to the past work that reported a glucose syrups produced under moderate heat show clearer to have more desirable appearances, through avoidance of excessive browning (due to Maillard reactions) and minimum sugar crystallization [31,39].

Table 3: Sensory Attributes of Glucose Syrup Produced from Cassava Starch by pure Amyloglucosidase

Sample	Taste	Appearance	Texture	Aroma	Fluidity	General Acceptability
pH 4, 50 °C	5.26^{g}	5.21^{g}	5.13^{h}	5.14^{i}	5.03^{h}	5.20^{f}
pH 4, 55 °C	5.34^{e}	5.56^{d}	5.67^{c}	5.26^{g}	5.15^{e}	5.25^{e}
pH 4, 60 °C	5.88^{b}	5.75^{b}	5.51^{e}	5.67^{d}	5.40^{c}	5.76^{b}
pH 4.5, 50 °C	5.16^{h}	5.08^{i}	5.10^{i}	5.22$^{\underline{h}}$	5.07^{g}	5.14^{g}
pH 4.5, 55 °C	5.96^{a}	5.78^{a}	5.62^{d}	5.76^{b}	5.15^{e}	5.78^{a}
pH 4.5, 60 °C	5.75^{c}	5.70^{c}	5.78^{b}	5.71^{c}	5.45^{b}	5.61^{c}
pH 5, 50 °C	5.04^{i}	5.11^{h}	5.16^{g}	5.30^{f}	5.11^{f}	5.10^{h}
pH 5, 55 °C	5.38^{d}	5.25^{f}	5.42f	5.41^{e}	5.21^{d}	5.51^{d}
pH 5, 60 °C	5.30^{f}	5.48^{e}	5.81a	5.86^{a}	5.60^{a}	5.78^{a}

Texture scores ranged from 5.10 to 5.81, with the highest score at pH 5, 60 °C (5.81) and the lowest at pH 4.5, 50 °C (5.10). This indicated that higher temperatures improve the syrup's consistency, likely due to increased sugar concentration and viscosity (Table 1). A thicker and more uniform texture is typically preferred in syrups used for confectionery, sauces, and other products where texture played a vital role in consumer satisfaction. For instance, previous findings found that higher temperatures during glucose syrup production enhanced its viscosity and texture, providing a smoother mouthfeel, especially at higher sugar concentrations, which was observed at pH 5, 60 °C [40].

Aroma scores ranged from 5.14 to 5.86, with the highest score recorded at pH 5, 60 °C (5.86) and the lowest at pH 4, 50 °C (5.14). The aroma of glucose syrup is often mild, but subtle differences may arise from the caramelization or Maillard reactions occurring at higher temperatures. Therefore, syrups produced at higher temperatures tend to have a more pleasant aroma, as similar reports showed that mild browning reactions enhanced the sensory profile of syrups through introduction of desirable aromas [30].

In the same vein, the fluidity scores ranged from 5.03 to 5.60, with the highest score at pH 5, 60 °C (5.60) and the lowest at pH 4, 50 °C (5.03). Fluidity, or the syrup's flow properties, is a critical parameter in applications like beverages and confectionery coatings, where ease of pouring and spreading is important. The higher temperatures appeared to enhance the fluidity of the syrup, likely due to the reduced viscosity at these conditions. Fluidity is influenced by both the sugar concentration (Brix) and viscosity as previously reported when higher Brix values in syrups produced at higher temperatures, contributed to its better fluidity. Moreso, another work reported that the maintenance of an optimal balance between viscosity and fluidity is a key factor for syrup quality [31,39].

The general acceptability scores ranged from 5.10 to 5.78, with the highest scores observed at pH 4.5, 55 °C (5.78) and pH 5, 60 °C (5.78), indicating that these conditions produced the most favorable syrups in terms of overall quality. The various attributes (taste, appearance, texture, etc.) of the syrup sample provided the best balance between sugar concentration, clarity, and consistency, which resulted in a product that is well-received by the consumers.

The sensory results of this study are consistent with previous findings that noted syrups produced under optimal enzymatic conditions (around pH 4.5 to 5.0 and temperatures of 55–60 °C) to be more acceptable to consumers, with favorable taste and appearance. Ibrahim & Salawu also found that syrups with higher DE and Brix values tended to score higher in taste and texture, as through its sweeter flavor and smoother consistency. However, the processing temperatures >55 °C enhance the sensory attributes of syrups, including aroma and texture, while minimizing undesirable browning reactions. The sensory evaluation of glucose syrup produced from cassava starch saccharification by amyloglucosidase revealed that the most favorable product is obtained at pH 4.5, 55 °C and pH 5, 60 °C, where syrups scored highest in general acceptability, taste, appearance, and texture [30,31,36,41].

Conclusion

The present study evaluated the physicochemical properties, safety and sensory attributes of glucose syrup produced from the saccharification of cassava starch by pure amyloglucosidase under varying pH and temperature conditions. The results demonstrated that optimal enzymatic hydrolysis conditions, particularly pH 4.5 and 55 °C produced glucose syrup with high reducing sugar content, Dextrose Equivalent (DE), Brix, and specific gravity, making it suitable for various industrial applications, including food, beverages, and pharmaceuticals. The highest DE values, correlating with a near-complete hydrolysis of cassava starch, were observed at pH 4.5, 55 °C, and pH 4, 60 °C, thereby yielding syrups that possessed the desirable qualities of high sweetness and clarity. The safety analysis of the levels of undesirable elements such as copper, arsenic, and lead reflected that the syrup samples were well below permissible limits, affirming that controlled enzymatic processing minimized contamination risks. Furthermore, sensory evaluation revealed that syrups produced under optimal conditions scored highest in terms of taste, appearance, texture, and general acceptability, indicating consumer preference for syrups produced at mildly acidic pH levels and moderate temperatures. The findings of this study were consistent with existing literature on enzymatic starch hydrolysis, underscoring the importance of carefully optimizing pH and temperature to maximize enzyme efficiency while ensuring the production of high-quality, safe, and consumer-preferred glucose syrup. Future research could explore further improvements in enzyme formulation and process control to enhance syrup quality, particularly for specialized industrial applications.

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