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PRODUCTION AND COMPARATIVE ASSESSMENT OF ALCOHOLIC DRINKS PRODUCED FROM CASSAVA, MAIZE AND PLANTAIN FLOUR USING LOCALLY PRODUCED AND IMPORTED ENZYMES

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Abstract: *Alcoholic drinks produced from cassava, maize and plantain flour using local hydrolyzed enzyme and imported enzymes were evaluated and compared in this study. About 2 kg of each flour substrate was divided into two portions; a portion was hydrolyzed using imported enzymes (α-amylase and amylo-glucosidase) while the other portion was processed with local hydrolyzed enzyme (malt) for production of alcoholic drink. Physicochemical, color and sensory attributes of the alcoholic drink samples were examined. Results showed that there were significant differences (p<0.05) in the physicochemical and color profile of the samples. Alcoholic drink produced from plantain with the minimum degree Brix liberated the highest alcohol content, followed by cassava and maize. Generally, low lightness, negative a* (greenness) and b* (blueness) confers alcoholic drinks with grey color appearance. Sensory test of the alcoholic drinks were all acceptable by the panelists. Thus, cassava, maize and plantain could serve as a good substrate for the production of alcoholic drinks.*

Keywords: Colour profile, sensory evaluation, physicochemical properties, alcoholic drink

1. Introduction

Cassava (*Manihot esculenta* Crantz) is one of the leading food and feed plants in the world [1]. It is the most important source of calories in the tropics after rice and corn, providing energy nourishment for more than half a billion people worldwide [2]. Maize (*Zea mays* L.) is an important cereal grain providing nutrients for humans and animals and serving as a basic raw-material for the production of starch, oil and protein, alcoholic beverages and food sweeteners [3]. Maize compares well with other cereals as energy source; it is rich in vitamin B and minerals such as phosphorus, magnesium, manganese, zinc, iron and small amounts of potassium [4]. Plantain (*Musa paradisiaca*) have a high carbohydrate content and low fat content and is traditionally grown in West Africa for food and used as part of local staple diets or processed into flour that can be stored for later use [5]. Alcoholic beverages are integral to daily life; alcohol has been consumed since prehistoric periods. Various types of local, traditional and indigenous alcoholic beverages are produced from local food stuffs using traditional and indigenous techniques [6]. In Nigeria, there has been local production of ethanol from cereal and other starch substrate. Fermented products like *burukutu* from maize and *pito* from millet or guinea-corn (percentage alcohol varies from 3-6% Alcohol by volume (ABV); palm wine, tapped from raffia and oil palm trees (3-4% Alcohol by volume ABV) and gin like distillates *ogogoro, kinkana, kai*

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kai and *apetesi* (ranging from 40-80% Alcohol by volume ABV) have been prepared an drunk in Nigeria [7].

In developing countries including Nigeria, indigenous food processing techniques such as fermentation and traditional distillation of alcoholic drinks from starch materials has continuously been practiced by vast ethnic groups with locally available equipments, utensils, substrates and poor processing condition which pose challenges for commercialization[8]. The consumption pattern of the alcoholic drinks is still at household in the rural settings [6]. Also of concern are health problems like food poisoning and food intoxication owing to lack of regulated processing standards and poor acceptable hygienic and content specifications [9]. Harnessing laboratory pilot scale production of alcoholic drinks from starch substrates is still limited due to non-readily available hydrolyzing enzymes. Hence, malting of local cereals could as well provide an alternative means of obtaining hydrolyzing enzymes. The knowledge of physicochemical properties, color profile and sensory characteristics of alcoholic drinks could give health wise insight of behavioural pattern of compositing compounds, improved final product quality and safety as well as positioning alcoholic drinks for potential commercialization. Considering the highlighted possibilities, an investigation was therefore geared towards the evaluation and comparative assessment of alcoholic drinks produced from cassava, maize and plantain flour using locally sourced and imported enzyme.

2. Materials and methods

Materials: Matured cassava roots, wholesome maize, and unripe plantain fruits were purchased from Kuto market (7.5 ^{o}N, 4.5 ^{o}E), Abeokuta, Nigeria. Hydrolyzing enzymes (α-amylase and amylo-glucosidase) were sourced at Enzymology laboratory of the Department of Biotechnology, FIIRO, Nigeria. Brewer's yeast was purchased from a commercial dealer at Ojota, Lagos, Nigeria. All analytical chemical used were of analytical grade.

Preparation of cassava flour

The fresh cassava roots were processed into flour using the method described by [10]. 2.5 kg of fresh cassava roots were peeled manually with a sharp knife, washed and grated in a locally fabricated mechanical grater. It was thereafter packed into hessian sack and dewatered by pressing in a mechanical press to dewater the mesh. The dewatered lump was pulverized with hands and sifted on a local raffia made sieve of mesh (0.3cm x 0.3cm) mounted on a rectangular wooden frame 40 cm^2 to remove the fibres. The sifted cassava meal obtained was allowed to dry in oven dryer at 60 ^{o}C for 24 h. The dried meal was milled and packaged in high density polyethylene film and kept under refrigerated storage until ready for further analysis.

Preparation of maize flour

The method of [11] was used for the preparation of maize flour. 2.5 kg of wholesome maize kernels were sorted, cleaned by winnowing and thereafter passed through discs of hammer mill to crush grains, and coarse maize flour was obtained.

Preparation of plantain flour

The preparation of plantain flour was carried using the method of [12] with modification. 2.5 kg of unripe plantain fruits were washed with distilled water, peeled and sliced to about 5 mm using a stainless steel knife. The slices were subsequently steamed for 5 min to inactivate enzymes. Subsequently, the pulp was drained and dried in an oven drier at 60 °C for 24 h, after which the dried plantain slices were milled into flour using attrition mill, sieved and packed in high density polythene bags.

Preparation of malt for local hydrolyzed enzyme

The preparation of sorghum malt was done using the modified method described by [13]. 1.5 kg of grains was steeped in 3 L of distilled water after prior sterilization with sodium hypochlorite solution (containing 1% available chlorine for 20 min). Steeping lasted for 24 h. Drained grains were thereafter germinated for 96 h in germination trays in an atmosphere of near water saturation. Grains were turned every 12 h and watered by spraying with 60 ml distilled water at 24 h intervals, germinating grains were withdrawn and immediately kiln-dried. Kilning was done for 24 h at 40 °C in a forced draught oven. The malt obtained was ground using a laboratory blender and thereafter packaged.

Preparation of alcoholic drinks using imported enzyme and local hydrolyzed enzyme

The method of [10, 14,] were employed for the preparation of alcoholic drinks from cassava, maize and plantain flour using imported enzyme and local hydrolyzed enzyme with modification, as presented in Figure 1. 1 kg of each flour substrate was stirred in 5000 ml of water to form slurry. The mixture was gelatinized at 70 ^oC for 20 min until smooth gel was formed and thereafter 4 ml of α-amylase was added to the gelatinized starch and incubated at 70 ^oC for 60 min. 4 ml of aqueous solution of amylo-glucosidase was then added to the liquefied sample at 50 ^oC and incubated at 70 ^oC for 60 min. While for local hydrolyzed enzyme; 250 g of malt was added to the gelatinized mash, stirred and the mixture was incubated at 60 ^oC for 5 h. The mixture (for both imported and local hydrolyzed enzyme) was sieved using a sieving cloth after the hydrolysis. The sieved sweet hydrolysate wort was boiled at 70 ^oC for 5 min to arrest further enzyme action.

Fermentation and distillation process of hydrolysate wort

The fermentation and distillation process of hydrolysate wort obtained using imported and local hydrolyzed enzymes were carried out according to the method of [10]. Fermentation yeast inoculum (100 ml of 15% yeast inoculum (15 g of dry brewer's yeast rehydrated in 100 ml of distilled water at 37 °C for 10 min) was added to the hydrolysate wort (at 30 ^oC) before anaerobic incubation for 5 days at 28-30 °C. The crude ethanol obtained was filtrated and distilled at temperatures between 78-80 ^oC using distillation apparatus (setup). The resulting product was an alcoholic drink from different flour substrates (Figure 1).

Physicochemical properties of alcoholic drinks

Determination of total titratable acidity

The total titratable acidity of the samples was determined according to the method illustrated by [15]. 250 ml beaker was filled with 100 ml distilled water and a few drops of phenolphthalein indicator were added and mixed. The mixture was titrated with 0.1N NaOH to a pale pink color. 10 ml of alcoholic drink sample was measured into the mixture and titrated against 0.1 NaOH to a pale pink color which persisted for at least 30 s.

$$Total\ titratable\ acidity (A) = 75 \times N \times \frac{T}{S}$$

Where; N = normality, T = titre value, S = sample volume and 75 = constant

Determination of pH

The method of [15] was used for determination of pH. HANNA Digital bench top pH meter equipped with an electrode probe was used for the determination. The electrode probe was calibrated and then placed into a beaker containing the alcoholic drink sample to obtain the pH readings.

Determination of total soluble solids

Total soluble solids of the alcoholic drink samples were determined according to the method of [15]. A hand held refractometer (Atago's master series, Japan) was used. The refractometer was standardized by placing a drop of distilled water on the prism and placed such that it allowed entry of sunlight into the prism. The eye-piece was used to observe the standardization after adjusting the coarse and fine adjustment knob properly. A drop of the sample was placed on the prism of refractometer and the lid was closed. The readings were taken by the graduated mark which indicated the total soluble solid values of the samples and was recorded in ^{o}Brix.

Determination of density

The method described by [16] was used for the determination of density. An empty graduated cylinder was weighed and the mass recorded. 100ml of each sample was poured into the cylinder and the mass was also recorded. The volume of the sample was gotten by subtracting the mass of the sample from the mass of the cylinder. Hence, the density was calculated as:

Density = mass/volume (g/cm^3)

Determination of alcohol content

The alcohol content of the drink samples was determined using the method of [17]. This was established by combining the results of two simple test measurements, that of a refractometer (to record sugar content) and a hydrometer (to determinate the specific gravity). The alcohol content (%) was calculated according to the equation below;

$$Alcohol\ content = R - [(SG - 1) \times 1000]$$

where R = refractometer reading, S.G = specific gravity

Colour profile of the alcoholic drinks

The colour profile of indigenous alcohol drinks were obtained using the method described by[18] with the use of Adobe Photoshop 6.0 software, normalized to L*-Lightness, a*- redness/greenness and b* - yellowness/blueness according to following Equations (3-5).

$$L* = \frac{L}{255} \times 100$$

$$a* = a \times \frac{240}{255} - 120$$

$$b* = b \times \frac{240}{255} - 120$$

The colour difference between the indigenous alcohol drinks was determined by taking the Euclidean distance between them, according to [19] as shown in Equation (6):

$$\Delta E* = \sqrt{(L_o - L*)^2 + (a_o - a*)^2 + (b_o - b^*)^2}$$

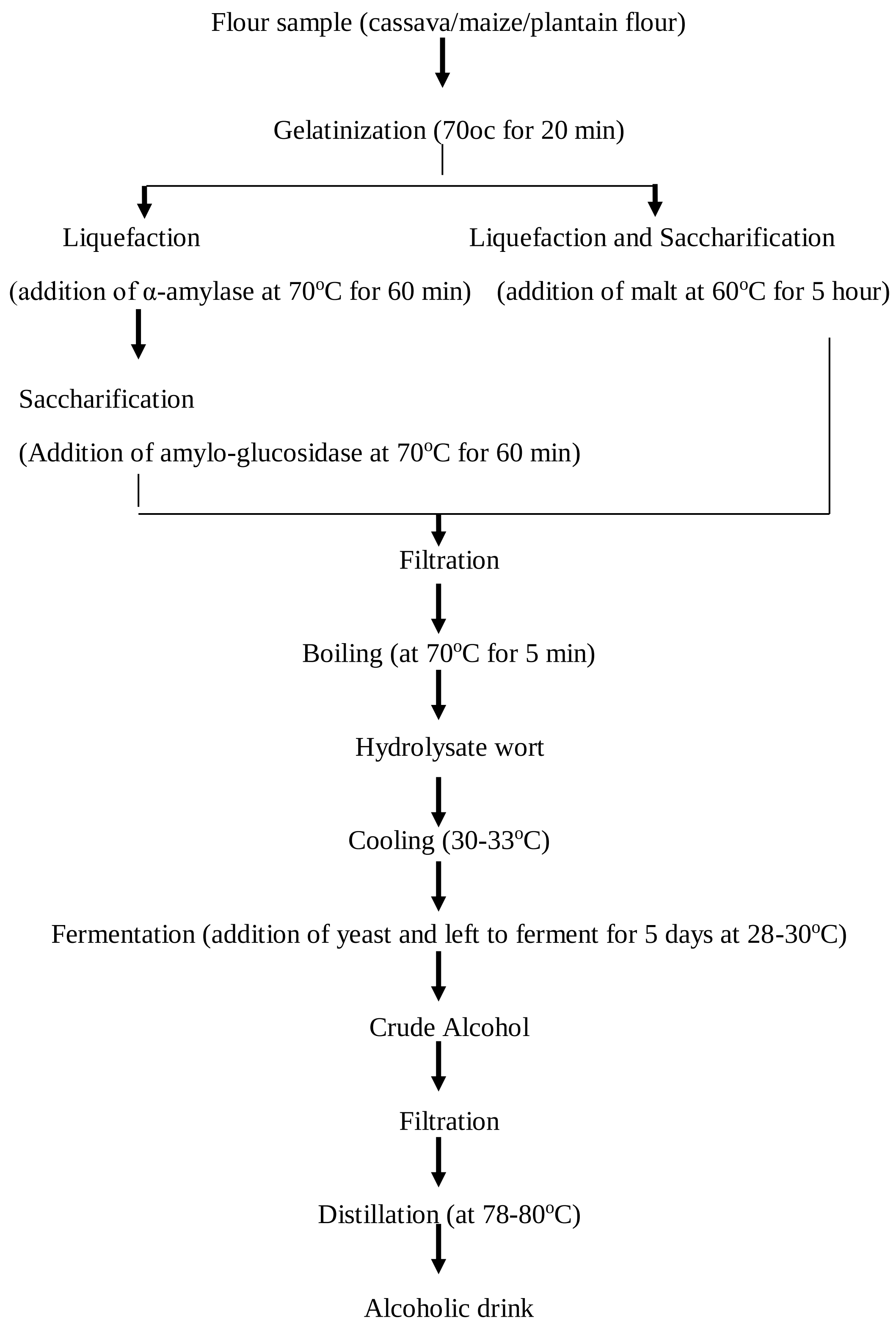

Fig.1. Flowchart for the production of alcoholic drink from cassava, maize and plantain flour
Source: IITA (2005) and Ocloo and Ayernor [10]

Sensory evaluation of the alcoholic drinks

Sensory evaluation was conducted using the modified method of [15]. 50 semi trained panelists made up of student in Department of Food Technology, Moshood Abiola Polytechnic was used. 25 ml each of the alcoholic drinks samples were presented to the panelists and evaluated based on attributes such as color, aroma, appearance, sensation and overall acceptability on a nine (9) point hedonic scale where (1)- dislike extremely and (9)- like extremely. Panelists were instructed to chew cracker biscuits and water between testing of samples. Each panelist was provided with enough privacy to avoid biased assessment.

Statistical analysis

All the experimental data were analyzed in triplicate and subjected to analysis of variance (ANOVA) to determine significant differences ($p<0.05$) using SPSS 22 software (IBM, USA), while means were separated using Duncan multiple Range test [20].

3. Results and Discussion

Physicochemical properties of alcoholic drinks produced from plantain, cassava and maize flour

The physicochemical properties of alcoholic drinks produced from plantain, cassava and maize flour showed significant differences ($p < 0.05$) with the exception of density (Table 1). Earlier study reported that total titratable acidity and pH values are critical parameters to determine the flavor and shelf life of indigenous fermented beverages [21].

Table 1

Physicochemical properties of alcoholic drinks produced from plantain, cassava and maize flour

Sample	Total titratable acidity (%)	pH	Total soluble solid (°Brix)	Density (ml/m^3)	Alcohol content (%)
A	3.00^{f} (0.06)	2.30^{a} (0.00)	0.20^{ab} (0.14)	0.99^{a} (0.00)	5.03^{b} (1.05)
B	2.08^{d} (0.03)	2.45^{ab} (0.07)	0.10^{a} (0.00)	0.99^{a} (0.00)	4.93^{b} (0.00)
C	0.42^{a} (0.05)	2.75^{c} (0.07)	0.15^{a} (0.07)	1.00^{a} (0.00)	3.28^{a} (0.90)
D	1.32^{c} (0.05)	2.60^{bc} (0.14)	0.25^{ab} (0.07)	0.99^{a} (0.00)	4.61^{ab} (0.46)
E	2.82^{e} (0.11)	2.45^{ab} (0.07)	0.40^{b} (0.14)	0.99^{a} (0.00)	4.06^{ab} (0.10)
F	0.96^{b} (0.08)	2.55^{bc} (0.07)	0.20^{ab} (0.00)	0.99^{a} (0.00)	4.24^{ab} (0.16)

Mean values with different subscripts within a column are significantly different ($p < 0.05$). A – Alcoholic drink produced from plantain flour using imported enzyme, B - Alcoholic drink produced from plantain flour using local hydrolyzed enzyme, C - Alcoholic drink produced from cassava flour using imported enzyme, D- Alcoholic drink produced from cassava flour using local hydrolyzed enzyme, E - Alcoholic drink produced from maize flour using imported enzyme, F- Alcoholic drink produced from maize flour using local hydrolyzed enzyme

The alcoholic drinks produced in this study confirms inverse relationship between total titratable acidity and pH value, i.e. increase in total titratable acidity was relative to decrease in pH value and vice versa. This observation is in agreement with studies conducted on some indigenous fermented beverage such as distilled local liquor [21, 22] and *Tchapalo* [23].

In addition, lower percentage of total titratable acidity was estimated in alcoholic drinks produced from cassava flour while that from plantain flour indicated a higher percentage. Fermentable sugar naturally present in plantain flour might have favoured rapid metabolism processes to give rise to higher lactic acid [17]. On the other hand, the pH of the alcoholic drinks from plantain and maize flour using local hydrolyzed enzyme demonstrated higher values (less acidic) whereas the use of imported enzyme gave least pH (more acidic). In comparison with previous research studies, higher pH conferring less acidic medium and total titratable acidity values have been detailed on traditional beverages from cassava drink (pH 3.7), maize drink (pH 4.1), plantain drink (pH 3.3) [6] and other liquors [24,25]. In respect to the type of enzyme used, imported enzyme influenced higher total titratable acidity than its' local hydrolyzed substitute enzyme except for the alcoholic drink produced from cassava. The reduced enzymatic activity of the malt could be attributed to higher substrate concentrations of the flour samples for enzymatic hydrolysis [26, 27]. Total soluble solids reflect the available sugar content in a medium and forms part of the important indexes during fermentation process to influence the percentage of alcohol content in alcoholic beverages [23]. The total soluble solid of the alcoholic drinks revealed maize flour (using imported enzyme) gave the maximum ^{o}Brix level while the minimum degree Brix was obtained in plantain drink (using local hydrolyzed enzyme). As with total titratable acidity, imported enzyme influenced higher total soluble solid that the local hydrolyzed enzyme except for the alcoholic drink produced from cassava which shows an inverse relationship. The maximum degree Brix observed possibly showed that not all dissolved solids were available for metabolism during fermentation [23]. Several authors demonstrated significant correlation between total soluble solid and alcohol content and thereafter suggested that the quantity of alcohol liberated in a fermenting medium depends on the utilization of available soluble solids [15, 23]. These premises are in agreement with the observation of this study, alcoholic drink produced from plantain with the minimum degree Brix liberated the highest alcohol content, followed by cassava and maize. Similar practical example was established in indigenous distilled liquor (*Areki*), where the observed least total dissolved solids liquor was proportional to higher alcohol content [23]. Nonetheless, the light alcohol contents (3.28-5.03%) observed for the drinks produced was in conformity with other local alcoholic beverages reported by several authors; *tella*: 2-6% [28], maize and banana extract: 3.32-4.63% [17], banana alcohol: < 8% v/v [29], cassava spirits: 7.8-26.3% [6] and *Kachasu* from maize meal: 9-41% v/v [30]. The density of a liquid sample is defined as mass of the liquid per volume [31]. Irrespective of the fermenting substrate source (plantain, cassava and maize flours) and type of hydrolyzing enzymes (local and imported) used, the density showed similar values with no significant differences ($p < 0.05$). In essence, the constant values suggested that the alcoholic drink density was not influenced by and with type of hydrolyzing enzymes. Yohannes *et al.*[31] deduced that density is not an accurate parameter to measure ethanol concentration. However, the ≤ 1 ml/m^3 observed for the alcoholic drinks is comparable to similar range previously reported by [16,32] for alcoholic fruit beverages.

Color profile of alcoholic spirit drinks produced from plantain, cassava and maize flour

The color profile of alcoholic drinks produced from plantain, cassava and maize flour is presented in Table 2. Reports on local drinks have shown that color test is one of the most preferred quality attribute of beverages [33]. Alcoholic drink produced from cassava flour using imported enzyme gave the maximum lightness. Similar trend was observed for plantain flour while for alcoholic drink produced from maize flour, local hydrolyzed enzyme gave increased lightness. These variations in lightness could be due to concentration of white mucilage present in the alcoholic drinks after distillation process [34]. The negative a* (greenness) was observed in all the produced alcoholic drinks. Similar greenness (-4.30-2.22) was reported for an alcoholic fermented plum beverage [35]. The imported enzyme might have influenced higher greenness than substrate sources as depicted by the values. In respect to negative b* (blueness), the substrate sources had greater effect, wherein, plantain flour alcoholic drink was observed with the highest blueness, followed by cassava and maize flour. The negative values of a^* and b^* indicated that all the samples were more red and yellow but the yellow parameters showed a higher values which is also event in the values of ΔE which are very close. According to [34], generally low red/green axis and yellow/blue axis on the color plane confers fermented product appearance tending towards grey color.

Table 2

Color profile of alcoholic drinks produced from plantain, cassava and maize flour

Sample	L*	a*	b*	ΔE
A	7.51^b (0.02)	-0.32^c (0.01)	-0.96^a (0.01)	169.71^b (0.00)
B	7.39^a (0.04)	-0.44^b (0.02)	-0.95^a (0.02)	169.70^a (0.00)
C	8.84^f (0.02)	-0.45^{ab} (0.00)	-0.88^b (0.01)	169.73^c (0.00)
D	8.32^e (0.03)	-0.43^b (0.01)	-0.67^c (0.00)	169.73^c (0.00)
E	7.66^c (0.01)	-0.31^c (0.01)	-0.46^d (0.00)	169.73^c (0.01)
F	7.89^d (0.00)	-0.48^a (0.02)	-0.44^d (0.02)	169.73^c (0.00)

Mean values with different subscripts within a column are significantly different ($p < 0.05$). L* - Lightness, a* - redness/greenness, b* - yellowness/blueness, ΔE - color difference. A – Alcoholic drink produced from plantain flour using imported enzyme, B - Alcoholic drink produced from plantain flour using local hydrolyzed enzyme, C - Alcoholic drink produced from cassava flour using imported enzyme, D- Alcoholic drink produced from cassava flour using local hydrolyzed enzyme, E - Alcoholic drink produced from maize flour using imported enzyme, F- Alcoholic drink produced from maize flour using local hydrolyzed enzyme

The color difference within the alcoholic drink showed that cassava and maize flour drinks had same differences but the plantain flour gave the least difference. The total colour change (ΔE*) was parameter considered for the overall colour difference evaluation among the alcoholic drinks produced. It could be suggested that the color difference was affected by the substrate source rather than the hydrolyzing enzymes.

Sensory evaluation of alcoholic drinks

Table 3 indicates the sensory evaluation of alcoholic drinks produced from plantain, cassava and maize flour. The multi-sensory experiences including appearance, color, aroma, taste etc. just before or during food/beverage intake depicts the product sensorial attributes [36]. The sensory evaluation of the alcoholic drinks revealed slight significant differences ($p<0.05$). The appearance of alcoholic drink from plantain flour using imported enzyme had the highest value while cassava flour produced with local hydrolyzed enzyme gave the least value. For aroma, highest score was adjudged to alcoholic drink produced from cassava drink using imported enzyme while plantain flour with same processed enzyme has least score. Based on overall acceptability, alcohol drinks produced using imported enzyme was rated the most acceptable whereas the least rating was scored by same substrate using malt.

Table 3

Sensory evaluation of alcoholic drinks produced from plantain, cassava and maize flour

Sample	Appearance	Color	Aroma	Taste	Overall acceptability
A	8.00^{b}	7.60^{ab}	6.90^{a}	7.10^{ab}	7.65^{ab}
B	7.75^{b}	7.85^{ab}	7.10^{a}	6.95^{ab}	7.35^{a}
C	7.80^{b}	8.05^{b}	8.15^{b}	7.75^{b}	8.15^{b}
D	6.85^{a}	7.00^{a}	7.15^{a}	6.55^{a}	7.10^{a}
E	7.20^{ab}	7.30^{ab}	7.35^{ab}	7.00^{ab}	7.40^{a}
F	7.50^{ab}	7.75^{ab}	7.05^{a}	6.85^{ab}	7.25^{a}

Mean values with different subscripts within a column are significantly different ($p < 0.05$). A – Alcoholic drink produced from plantain flour using imported enzyme, B-Alcoholic drink produced from plantain flour using local hydrolyzed enzyme, C - Alcoholic drink produced from cassava flour using imported enzyme, D-Alcoholic drink produced from cassava flour using local hydrolyzed enzyme, E-Alcoholic drink produced from maize flour using imported enzyme, F- Alcoholic drink produced from maize flour using local hydrolyzed enzyme

4. Conclusion

The drinks produced from plantain, cassava and maize flour revealed closely related low acidity, soluble solid, density and alcohol content which imply good substrate for the production of alcoholic drinks. Inverse trends relationship between total titratable acidity and pH value was confirmed. Higher percentage of acidity in plantain drink informed better alcoholic flavor than the other alcoholic drinks. Significant differences ($p < 0.05$) in color profile of the alcoholic drinks produced from cassava, maize and plantain flour depend on substrate source; the low lightness, negative a* (greenness) and b* (blueness) confers alcoholic drinks with grey color appearance. However consumer preference differs when choosing the most preferred alcoholic drink. Alcoholic drink produced from cassava using imported enzyme was highly rated in color, aroma, taste and overall acceptability compared to that produced from maize and plantain flour. Further studies should investigate the volatile components of the alcoholic drinks in order to enumerate possible toxic elements or health benefiting compounds that might have liberated during processing.

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6. Disclosure statement

No potential conflict of interest was reported by the authors.

7. Informed consent

Written informed consent was obtained from all study participants.

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