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Effects of Pigment Removal on The Physicochemical Properties of *Hibiscus Sabdariffa* Extracts and on The Sensory Quality of Reformulated Zobo Drink

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Abstract

Various forms of drinks exist in varying sensory and organoleptic properties owing to slight modifications in their processing. Zobo drink is a formulation of extract from red fleshy calyces of *H. sabdariffa* that gained popularity in virtually all local areas in Nigeria. Red Sorrel "Zobo" has been reported to have acidic nature and blood-red colour, which give the drink its coarse texture, and hinder its acceptability in the global market. In this study, the extract of the Roselle was slightly de-pigmented with a natural adsorbent to reduce the acidity found in the plant material and to improve on the colour and sensory quality of the local drink. Physicochemical properties, pH variation over a period of five days and UV-Visible absorption of the original and slightly de-pigmented extracts were compared. There were large differences in the physical parameter values of these extracts. Pigment removal reduced the; acidity @25 ^oC with pH 2.8 to 3.5, temperature 23.2 ^oC to 22.9 ^oC, conductivity (µS/cm) 4790 to 3410; TDS (mg/L) 3114 to 2217 and turbidity (NTU) 17650 to 5050. It also reduced the; total hardness (mg/L) 2700 to 1900, total alkalinity (mg/L) 3100 to 2400, chlorides (mg/L) 1300 to 1000 except the PO_4^{2-} (mg/L) which increased from 30.9 to 39.1 and Na^+ (mg/L) 46.1 & 88.1; while K^+ (mg/L) remains constant 655. Large difference was also observed in the electromagnetic light absorption (3.501 to 2.001) at λ_{max} 500 nm. Consumer acceptability test of the drinks attested that the slightly de-pigmented drink surpassed the original local drink in all sensory qualities and consumers' acceptance. **In conclusion, t**he adsorption of sparingly soluble pigment particles on natural adsorbent altered the pH values, total dissolved solids, particle sizes, colour and other sensory properties.

Key words: Zobo drink, Pigment removal, Physicochemical properties, sensory properties.
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Introduction

Hibiscus sabdariffa is the most popular plant among the members of malvaceae family. The red sorrel is popularly called "roselle". Hibiscus has over three hundred species spread over tropical and subtropical areas of the world as they can adapt to a variety of soil in a warmer and more humid climate (Singh *et al.*, 2017). Ecologically, Roselle grows best in field conditions under the full sunlight, but can also withstand little shade and can be grown in greenhouse condition (Qi *et al.*, 2005). The origin of *Hibiscus sabdariffa* has been traced by several scholars. Cobley (1975) opined that roselle is a native plant of West Africa carried to other parts of the world such as India, Thailand, Philippines, Vietnam, Sudan, Egypt, Saudi Arabia, Malaysia, Indonesia, Mexico and China. Mat *et al.* (1985) suggested that it was originated from India, while in Abu-Tarboush *et al.* (1997) opinion it was from Saudi Arabia.

Hibiscus sabradiffa is well known for its nutritional and medicinal properties (Arvind & Alka, 2011). It is a biocompatible medicinal plant (Abbas *et al.*, 2011) that has been reported to be rich in various medically important plant chemicals called phytochemicals. The relevance of the plant in treating diverse medical problems has been thoroughly investigated by scholars in different research settings (Okereke *et al.*, 2015). Chemicals like hibiscetine, sabdaretine and gossypetine have been reported from the extracts of red calyces in addition to some flavonoids that are responsible for its antioxidant properties. The fresh calyces are also rich in nutritionally important chemicals namely; riboflavin, ascorbic acid, niacin, carotene, calcium, iron etc (Qi *et al.*, 2005).

Hibiscus sabdariffa, has a worldwide fame as a medicinal plant (Abbas *et al.*, 2011), but in Nigeria, *H. sabdariffa*, known as "Zobo" (Chau *et al.*, 2000; Kolawole and Maduenyi, 2004) is mainly cultivated to be consumed as a local drink. Some other varieties of Hibiscus (e.g. *H. altissima*) are planted for ornamental purposes and for their fibers they produce, but the main producers of *H. sabdariffa* (Egypt, Sudan, Mexico, Thailand and China) cultivate it for consumption (Naturland, 2002).

Most locally made drinks cannot be presented for global consumption probably because no attention is given to dissolution and/or distribution of particles and particle sizes in the course of processing, handling and marketing. Interestingly, Zobo gains wider acceptance in Nigeria than any other local drinks despite that there is no standard method of preparation. This may be attributed to inadequate knowledge about the effect of uneven distribution of particles, acidity level and pharmacological properties. Hence, adequate knowledge about the various methods to get rid of suspended and sparingly soluble particles in the local drinks is important in drinks that are meant to serve refreshing purposes. This research is the first ever that attempted to get rid of suspended and sparingly soluble particles in the *H. sabdariffa* extract meant for drinks formulations. "The research findings herein could have practical applications for the food and beverage industry, offering insights into potential strategies for enhancing the sensory quality and marketability of traditional beverages like Zobo while addressing health and environmental implications."

Materials and Methods

Calyxes of *Hibiscus sabdariffa* were collected from Lapai Village. The plant was identified as *Hibiscus sabdariffa* and authenticated at the Department of Biological Sciences, IBB University, Lapai, Niger State, Nigeria. A voucher specimen labeled: IBBU210511 on the plant was deposited in the herbarium of the Institution. The dry calyx of the plant was thereafter purchased at the Market of the same Village for this study.

Preparation of the slightly de-pigmented Extract

The dry calyces of *Hibiscus sabdariffa* was cleaned of extraneous matters, and pulverized into a coarse powder. The powder was macerated in distilled water (100 g of powder in 1 L of distilled water) with intermittent swirling and mixing inside a hot water bath at the temperature (70 ± 5 °C) for 15 minutes. The water soluble constituents was drained through a sieve and filtered through a muslin cloth. The filtrate was divided into two portions (A and B). A traditional method practiced in Southwestern part of Nigeria was employed and modified to adsorb pigments from Portion B as described here. Portion B was returned back inside the hot water bath for few minutes, then pigment adsorption was carried out by addition of 25 g charcoal to the warm solution and stirred for 5 mins. This was allowed to cool and filtered through a cotton wool then through a Whatmann No 1 filter paper.

The samples A and B were subjected to UV-Visible screening using a UV- spectrophotometer (MODEL: 54G-TECNO-POVA) for confirmation of the adsorption effect on the pigments contents of the plant extract.

Formulation of the Drinks from the Original and the slightly de-pigmented Extracts

The formulation of the drinks was carried out as described in 2.1 above but on a large scale production (2 Kg in 20 L of distilled water). Equal quantity of ingredients was put into each portion of the liquids, namely; cucumber, ginger, cloves, flavor (pineapples and coconut) and sugar. The solution was drained through a muslin cloth and refrigerated.

Physicochemical analysis

Physicochemical analysis was carried out in the water laboratory of the Upper Niger River Basin, Maikunkele, Minna Branch, Niger State. Parameters were determined using the methods of the American Public Health Analysis (APHA, 2005). The pH of the sample was determined electrometrically using a BLUELAB pH meter PMI-1509-0024, temperature measurements was made with a mercury-filled thermometer, conductivity with EC Meter-470 JENWAY, and turbidity with Colorimeter-900.

Consumers' Assessment of the Formulated "Zobo Drinks" from the Hibiscus Extracts

Sensory analysis

Sensory analysis of the two formulations A and B was carried out by a team of fifteen (15) pre-educated panelists. The drinks were served in 250 cm^3 transparent containers, covered and labeled as sample A and sample B. The assessors were educated on the parameters to be assessed and on the grading methods adopted for the assessment. Colour, Aroma, Taste, Texture (coarseness on the palate), and overall acceptability (i.e. Preference to available soft drinks) were analyzed and scored using the three point hedonic scale [high- most acceptable, moderate- moderately acceptable, low-fairly acceptable] as applicable to the parameters.

Results and Discussion

Colour can be considered to be the most important physical feature attracting the consumers' acceptance of food since it gives the first impression of food quality (Salih *et al.,* 2020). *Hibiscus sabdariffa* (Zobo) is a tropical plant of considerable economic potential (Duangmal *et al*., 2008). According to Shruthi *et al.* (2016), the calyces of *H. sadariffa* are rich in anthocyanins, ascorbic acid and hibiscus acid. Anthocyanins are amongst the most important classes of water soluble pigments visible to the human eye. They are responsible for many of the attractive colours including the brilliant and admiring red colour of the Zobo extract that gives sour and acidic taste that supports digestion. In this research, the pigment adsorption was an attempt to remove the sparingly soluble pigment particles that may be responsible for the sourness and/or coarseness impacted on the consumers' palates.

Result of UV- Visible Absorption

Table 1 presents the result of visible light absorption of the pigments present in the original plant extract (sample A) and in the slightly de-pigmented extract (sample B), while Figure 1 shows the light absorption bands at wavelengths range 400 - 700 nm.

Table 1: UV-VISIBLE Absorption variation between the ordinary and de-pigmented extracts

Wavelength (λ)	400	450	500	550	600	650	700

Ordinary sample	1.948	3.137	20.010	20.000	0.469	0.118	0.074
De-pigmented Sample	2.730	2.250	3.501	2.740	0.562	0.208	0.121

The maximum absorption was observed in the region at around 500 - 550 nm (λmax); 20.010 - 20.000 for sample A and 3.501 - 2.740 for sample B due probably to the aglycone of the pigment glycoside, followed by a smaller band in the range of 400 - 450 nm; 1.948 - 3.137 nm, and 2.730 - 2.250 nm attributable to the sugar moiety respectively. These results are in agreement with previous reports for the absorption of anthocyanins in the visible region. According to Enaru *et al.* (2021), the absorption maxima (λ_{max}) of anthocyanins in the visible region is commonly recorded at around 510–520 nm along with a hump in the range of 400 - 450 nm attributed to the sugar moiety attached to the anthocyanidin moiety, and its size depends on the number of sugar moieties. In both absorption bands (Figure 1), the original extract exhibited higher absorbance intensity compared to the slightly de-pigmented Zobo extract, indicating that the adsorption process has either removed some sparingly soluble pigment particles or destroyed of some pigment molecules. Anthocyanins are the major colour pigments reported for *H. sabdariffa*. Anthocyanins are kinds of pigments that have a strong absorption in the UV-visible region of the electromagnetic spectrum. Structurally, anthocyanins are found in the form of glycosides composed of an aglycone called anthocyanidin and a carbohydrate residue that may be glucose, galactose, xylose, arabinose, rutinose or rhamnose. These carbohydrate residues are usually connected to the anthocyanidin skeleton via the C3 hydroxyl group in ring C (Oancea and Draghici, 2013; Diaconeasa *et al.*, 2017).

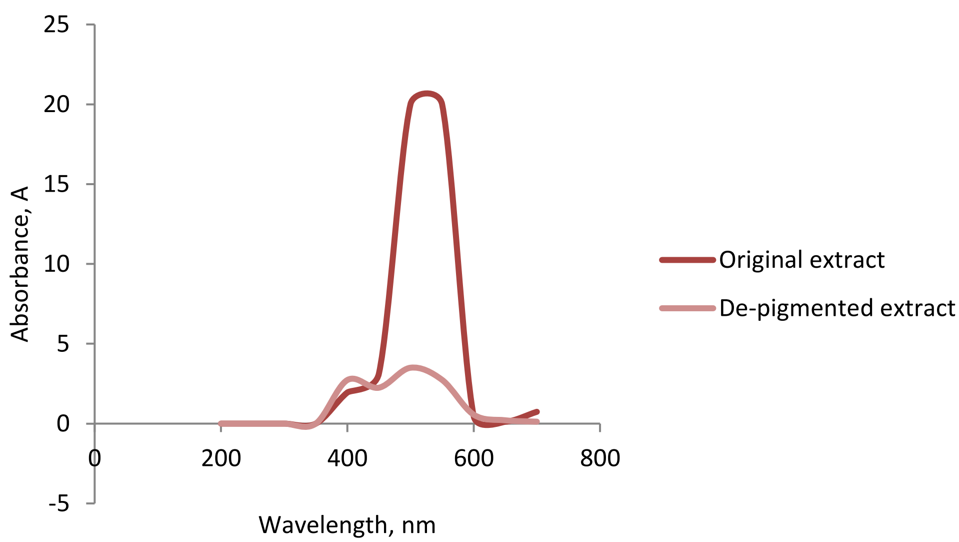

Figure 1: UV-Visible Absorptions of the original and the slightly de-pigmented extracts

pH variation

Table 2: pH variation over a period of five days

	Day 1	Day 2	Day 3	Day 4	Day 5
Ordinary sample	2.8	2.7	2.6	2.4	2.9
De-pigmented sample	3.5	3.3	3.2	3.4	3.7

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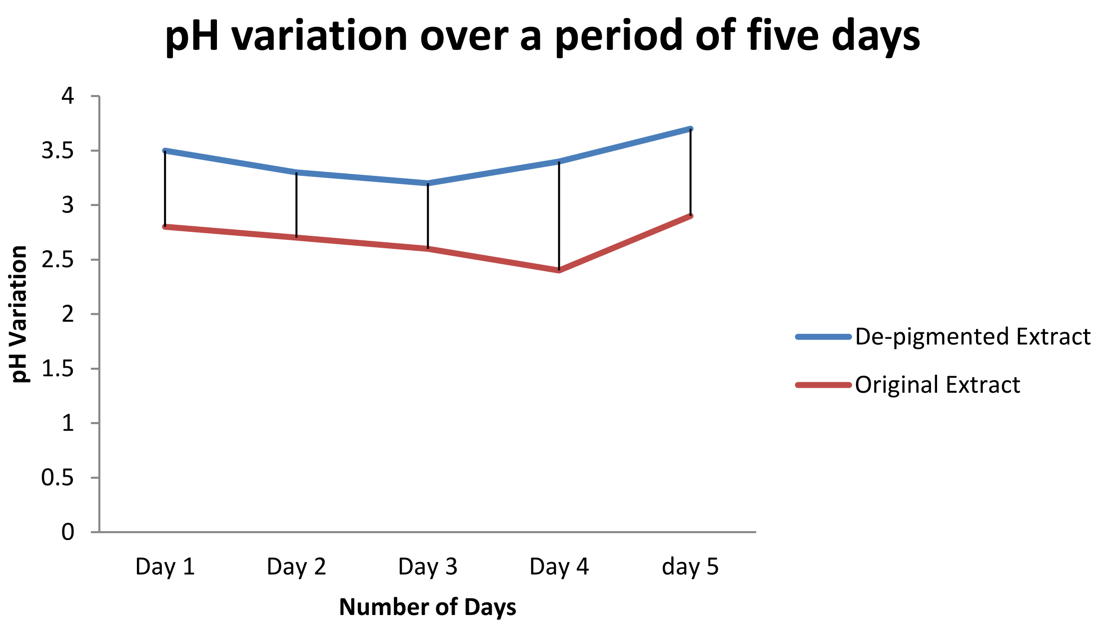

Fig 2: pH variation of the ordinary Zobo extract and the slightly de-pigmented sample over a period of five days

pH Values

The pH value is a measure of the level of acidity and/or alkalinity of a drink and other consumables. *Hibiscus sabdariffa* has been found to be acidic in nature. According to a study conducted by Falade *et al.* (2005) the dry fermented calyces of *H. sabdariffa* exhibited a very low pH value (Da-Costa-Rocha, et al., 2014). The pH values reported in the Table 2 also corroborated previous reports. The pH levels of the extract slightly de-pigmented using a natural adsorbent tends toward alkaline solution due to the removal of sparingly soluble adsorb-able pigment(s) from the original extract. The graph of the pH trends over a period of five days (Figure 2) showed that both extracts increased in their acidity (3.5 - 3.2) for the first two days, after which the de-pigmented extract reversed its course and tends towards alkaline (3.2 - 3.7). It takes the original extract additional 24 hr to effect this change, which supported our claim that colour pigment might be responsible for the level of acidity of *H sabdariffa*. The acidity of *H sabdariffa* extract could also be due to the ascorbic and glycolic acids reported to be present in the plant (Qi *et al.,* 2005, Falade *et al.,* 2005), and to citric acid and its derivatives; (2S, 3R) hydroxycitric and hibiscic acid as the dominant acid in red sorrel calyx (Da-Costa-Rocha *et al*., 2014).

Principally the source of acidity in the plant may be traceable to the condition of growth of *H. sabdariffa*. According to Ismail *et al.* (2008), the plant needs more than 12 hours of sunlight during the first months of growth to avoid premature flowering and throughout the growing and fruiting periods it can withstand relatively high temperature. It grows in a warmer and more humid climate with night time temperature not below 21°C and is most susceptible to damage from frost and fog. The plant has also been found to thrive satisfactorily on relatively infertile soils (Shruthi *et al.*, 2016).

Physicochemical parameters

Table 3 compares the results of physicochemical parameters of the original plant extract (sample A) and the slightly de-pigmented extract (sample B) as a function of particles dissolution.

Particle Size (PS) and its dissolution can have a high influence on the sensory, electrical, thermal, physical, and chemical characteristics of a finished food product (Etti *et al.*, 2020; Jillavenkatesa *et al.*, 2001; Christopher *et al.*, 2022). Wrong analyses of PS may reduce the quality of liquid products leading to high rates of rejection, and to economic losses (Christopher *et al.*, 2022). Particulate sizes and its dissolution also influence the bulk density, and flow ability of a food product (Barbosa-C´anovas *et al.*, 2005; Christopher *et al.*, 2022) especially refreshing drinks.

Table 3: Physicochemical parameters of the *H. sabdariffa* original and De-pigmented extracts

	Parameter	Method	Original Extract	De-pigmented Extract
Physical parameters	pH @ 25.0 °C	APHA 4500H B	2.8	3.5
	Temperature (°C)		23.2	22.9
	Total dissolved solids (mg/L)	APHA 2510B	3114	2217
	Turbidity (NTU)	APHA 42130B	17650	5050
	Conductivity (µS/cm)	APHA 2510B	4790	3410
Chemical parameters	Total hardness (mg/L)	APHA 2340C	2700	1900
	Total alkalinity (mg$CaCO_3$/L)	APHA 2320B	3100	2400
	Chlorides (mg/L)	APHA 4500Cl B	1300	1000
	Phosphate (mg/L)	APHA 4500-PD	30.9	39.1
	Sodium (mg/L)	APHA 3500 Na-D	46.2	88.1
	Potassium (mg/L)	APHA 3500 K-D	655	655

Result of Total Dissolved Solids (TDS)

The result of TDS showed that dissolved solids in the original sample is higher in quantity than in the slightly de-pigmented sample by 897 mg/L based on the values 3114 mg/L and 2217 mg/L respectively. This is corroborated by the higher absorption of electromagnetic light by the original sample (3.501) observed in the Visible range of the electromagnetic spectrum (Figure 1) at λ_{max} 500 nm, over the de-pigmented sample (2.001).

This shows that on addition of the adsorbent to the hot solution, the solution was saturated, some sparingly soluble solids precipitated while cooling and adsorbed on the adsorbent. This affected the values of all physical parameters including the acidity. **TDS** impacts a peculiar taste to solutions (Gloria and Token, 2015)**,** and deepens the colour.

Turbidity

Turbidity is the amount of solid suspension in a solution. The turbidity method is based upon a comparison of the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by the standard reference suspensions, the higher the intensity of scattered light, the higher the turbidity readings. In this research, the turbidity value (Table 3) was significantly high in original extract than the slightly de-pigmented extract. The large difference in the turbidity between these two extracts is an indication that the adsorbent effectively removed suspended particles in the zobo extract. Such suspended particles do have scattering effect on light. This is evident in the UV-Visible analysis results (Figure 1) at λ_{max} 500 nm, 3.501 and 2.001.

Electrical Conductivity

Conductivity of a solution refers to its capability to pass electrical flow. This ability is directly related to the concentration of ions in the solution. A liquid that is free of ions such as a pure water does not conduct electricity, while distilled water's conductivity has been measured to range from 0.5 to 3 µS/cm. Conductivity of drinking water increases from 200 µS/cm to 800 µcg/cm due to the presence of soluble ions. Conductivity is therefore a measure of TDS. Table 3 revealed that pigment removal reduced conductivity of *H. sabdariffa* extract from 4790 and 3410 µS/cm. From the result (Table 3), the relationship between electrical conductivity to the total dissolved solids (3114 mg/L and 2217 mg/L respectively) is proportional and numerically equals to 1.538 in the two samples. Evidently, the precipitated components are electrically active, polar and acidic substances.

Important electrolytes

Sodium ion (Na^+) and potassium ion (K^+) were estimated quantitatively. These cations are important electrolytes that play vital roles in maintaining proper body functioning (Mussarat *et al.*, 2022). Table 3 revealed that pigment removal increased the availability of sodium ion from 46.1 (mg/L) in the original extract to 88.1(mg/L) in the de-pigmented; while K^+ remained constant 655 (mg/L). The chlorides (1300 mg/L) are precipitated leaving 1000 (mg/L) in the solution while the PO^{4+} are becoming more available. From the result (Table 3), the relationship between anions (Chlorides (mg/L) 1300 to 1000; Phosphate (mg/L) 30.9 and 39.1) and cations shows proportional increase in Na^+ (46.2 to 88.1) and in PO^{4-} (30.9 to 39.1) in the two samples. Evidently, pigment removal allowed availability of sodium and phosphorous in form of phosphate as the process precipitated components that are chlorides inform of electrically active, polar and acidic chlorides. Sodium is important for regulating blood volume and pressure, regulating body pH, and balancing osmotic pressure, potassium is important for regulating water balance (Mussarat *et al.*, 2022); while phosphorous is important for availability of calcium for strong teeth and bone.

According to the WHO, 2000 mg (2000 ppm) of Na per day is considered safe for proper body functions and K normal intake should not increase from 4700 mg per day (Mussarat *et al.*, 2022). Phosphorus is one of the most abundant minerals in the human body. It represents approximately 1 % of total body weight and functions as a structural component of bones and teeth (Calvo, and Lamberg-Allardt, 2015). Other vital roles of phosphorus in the body metabolism are, its involvement in critical pathways to produce and store energy in phosphate bonds (ATP), regulate gene transcription, activate enzyme catalysis, buffer blood, and enable signal transduction of regulatory pathways affecting a variety of organ functions ranging from renal excretion to immune response (O'Brien, Kerstetter and Insonga, 2012). Less than 1 % of unbound inorganic phosphate (PO^{4-}) in the extracellular space is metabolically active and is now considered tightly maintained within a narrow serum concentration range (2.5–4.5 mg/dL) in adults. Phosphorus deficiency is uncommon in the midst of healthy people this is attributed to the availability of phosphorus in most of their foods (Calvo, and Lamberg-Allardt, 2015). The amount of phosphate found in this plant is evident, while the availability due to pigment removal is a plus to this research. According to Calvo *et al.* (2014), organic phosphorus is slowly and less efficiently (40–60%) absorbed, whereas inorganic phosphorus salts added to food in processing are rapidly and efficiently (80–100%) absorbed. If pigment removal could facilitate the availability of phosphorous, it should enhance the rate and efficiency of absorption. This therefore, rules out the need to add inorganic phosphorus salts as practiced in food processing. Therefore, drinks formulated by this method will be recommendable for the young women and teenagers who have the highest phosphorus requirements, presumably due to the increased need during rapid bone growth.

Alkalinity

Alkalinity is the ability of water to resist acidification. It involves measuring 25 -50 ml of water
sample. It was observed in this research that the potential of the experimental plant extracts showed acidic (pH-value) property (Table 1) that could enhance the antimicrobial activity as curative measures for some diseases. In this pH range, plant extracts may exhibit sensitivity against some organisms and could contain antimicrobial property.

Results of Consumers Assessment analysis

Table 4 compares the results of consumers' perceptions on the drinks formulated from the original (sample A) and de-pigmented (sample B) extracts of *H. sabdariffa* based on parameters as colour, aroma, taste, turbidity and acceptability rated as Low, moderate or high as applicable to the parameters.

Table 4: Sensory quality of zobo drinks prepared with two different methods as assessed by 15 respondents

S/N	Sample / Method	Colour	Aroma	Taste	Texture	Acceptability
1	A	2.07 ± 0.59	2.27 ± 0.70	2.13 ± 0.52	1.93 ± 0.26	1.27 ± 0.59
2	B	2.43 ± 0.76	2.47 ± 0.63	2.53 ± 0.52	2.13 ± 0.74	2.60 ± 0.63

Results are expressed as mean ± standard deviation. Scale: 1 = dislike, 2 = Like Moderately, 3 = Like Extremely.

Our findings showed that majority accepted the sample B over sample A. Evidently, slight modification in the preparation makes a drink serve a different purpose. By this method *H. sabariffa* extract may turn suitable for refreshment purposes rather than herbal consumption in the name of local drinks. Table 4 showed that consumers attested to the acceptability of a formulation of Zobo drink after the sparingly soluble particles are removed than the ordinary extract formulation. This research is in trend with various other researches on drinks formulation focusing on how to improve on the textures of the drinks as a way of enhancing its market-value. Salih *et al.* (2020) evaluated the sensory effects of yoghurt products by adding mixture of gum Arabic and Guar gum, and concluded that sensory characteristics (texture, colour and overall acceptability) were significantly improved except flavor that was not affected by the type and concentrations of stabilizers.

According to Yadav *et al.* (2018), tea with very fine particle fractions gives the maximum infusion profile. Particle size of a powder of a tea plays an important role in tea production process (Christopher *et al.*, 2022). Removal of sparingly soluble pigment from Zobo extract with charcoal may therefore, enhance the tea infusion profile. The de-pigmented sample showed a light pinkish-red colour which looks attractive and presentable, such appearance that could give wider acceptability to the local drink if retained after formulation.

Conclusively, the UV spectra strongly suggested that de-pigmentation exerts a significant impact on zobo colour, manifesting as a large reduction in absorbance intensity at the wavelength of the absorption maxima. The adsorption process on Zobo extract using charcoal allowed some sparingly soluble solids precipitated and adsorbed on the adsorbent. In effect, this may enhance efficient absorption of the resultant solution into the intestine if consumed as a tea and allow dissolution of additives if compounded as a soft drink.

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