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Evaluation of the Physicochemical and Antioxidants Properties of Freeze-Dried Beetroot Colourants Extract Using Different Levels of Citric Acid

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Research Article

Abstract

This research investigated freeze-dried beetroot colourants extracted using citric acid as green organic solvent at 1-5% citric acid concentration and water as the control. The physicochemical and antioxidants activities were determined using standard methods of analysis. Mineral investigation showed that phosphorus and potassium were predominant, though with a significant decrease from 150.98 mg/100g at 0 % citric acid to 101.28 mg/100g at 5 % citric acid for phosphorous. However, the potassium content ranged from 131.78 mg/100g at 0 % to 152.76 mg/100g at 3 %, the citric acid extracted beetroot colourants had higher potassium content. Also, as the concentration of citric acid increased, there was a significant ($p<0.05$) decrease in the pH, with increase in titratable acidity and lightness of the samples. The antioxidant properties showed a noteworthy radical scavenging ability with a strong IC_{50} values of DPPH (2,2-diphenyl-1-picrylhydrazyl), and ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity ranging between 48.60 to 85.63 µg/mL and 48.87 – 85.76 µg/mL respectively. This study reveals promising potentials in the freeze-dried extracts that could be utilized in the food product development and pharmaceutical industries to enhance life quality as well as the impact of different citric acid concentration in beetroot colourant extraction and its relevance as solvent in beetroots colourants extraction.

Keywords: Antioxidants, Freeze-dried, Colourant, Minerals, Beetroots.
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1. Introduction

Colours are visual feature affecting recognitions, desire and subsequent selection and consumption of food. The colour of food are appearance enhancers and it is an important organoleptic parameter that could indicate the freshness, safety and acceptability of the food in modern consumers expectation [1]. In addition, colour is associated with certain flavours and colour of food which largely influences its acceptance [2,3].

Basically, food colours are classified as synthetic or natural. Synthetic colorants are being chemically synthesized from petrochemical sources though these colorants are increasingly being replaced by natural colorants. The natural food colours are obtained from nature and these includes plants materials (such as the roots, leaves, stems, bark, wood shavings, flowers, fruits, rinds, hulls, husks), insects and microorganisms such as algae and fungi. [4,5]. Several advantages of the use of natural colorants aside being safer, they are easily obtained from renewable sources, eco-friendly, lustrous and attractive to the human eye [6] compared to synthetic. The synthetic colours are petrochemicals coal tar derivatives, and most contain an azo-group that has been linked to food intoxication [7], health complications such as allergies, resulting in hyperactivity in children, and carcinogenic pathologies, nausea and breathing complications and oxidative stress (8-11). These problems have instigated the recent ban on some red dyes by the United State Food and Drugs Administration [12]. Hence, increased growing interest in natural colours as consumers are avoiding foods containing synthetic colorants [13, 9] culminating into the need for replacement of these synthetic colorants by natural pigments made from renewable sources for use in the food industries.

Cultivars of Beetroot (*Beta vulgaris*) are biennial, herbaceous plant grown throughout Europe and North America and in some part of West Africa. In Nigeria, Beetroots are grown mainly in Jos, Plateau State of Northern Nigeria [14]. Beetroots are generally used as food for humans. It is a valuable source of dietary fibres, antioxidants, minerals, and vitamins [15,16]. Dietary intake of beetroots antioxidants has been proven to prevent against various chronic disorders, such as cancer, diabetes, coronary disease and neurodegenerative diseases such as Alzheimer disease and Parkinson disease [17]. Beetroots (red and yellow beetroot *Beta vulgaris* L. ssp. *vulgaris)* impart a red-violet and yellow colour respectively, influenced by the betalains (betanins or betacyanins) and betaxanthin pigments [18].

The production of colour from beetroots could reduce the increasing cost of dyes importation in Nigeria, where about 95 per cent of the locally used dyes are imported at an average cost of N40 billion in 2022 [19]. This could lead to the reduction of foreign exchange expenditure, accessing of its health enhancing ingredients, boost exports, and promote environmental conservation. Currently, the extraction of these colours components in beetroot is carried out using conventional technologies which involves thermal extraction (use of high temperature) and toxic solvents which is found to be deleterious to health [20].

The use of green mild acid solvent such as citric acid has been recommended in extractions of plants materials and freeze-drying method of colour extraction has been proven to give a high colour yield and lower degradation of some colour constituents [21]. Colorants from plants are characterized by mineral and with the high content of several secondary metabolites which are

active biological compounds of antioxidant properties [22]. It is however pertinent to determine the minerals, physical and the antiradicals' ability of the freeze-dried beetroot colour extract using some of antioxidant methods commonly used such as DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) to predict antioxidant capacity. The data will establish the efficiency of the acidic medium of extraction and other health beneficial roles this extract could play aside from being a food colorant.

2.0 Materials and Methods

2.1 Sample collection

Freshly harvested red beetroot of 10 kg was purchased from a farmland in Jos Plateau state, Nigeria and pre-processing operation which involves sorting, peeling and cleaning was carried out within 24 h of purchase.

2.2 Preparation of beetroot colour extraction

The beetroot colorant extraction was carried out using the slightly modified method described by Aykın-Dinçer *et al*. [23] and following the preliminary study carried out. In the present study, cold centrifugation was utilized instead of water bath in the extraction as described in the original method. This is to improve the efficiency and yield of the extraction process without the application of heat. Essentially, beetroot extract was produced using aqueous extraction of distilled 0.30M Citric acid at 0, 1, 2, 3, 4, 5 % of 0.3M of citric acid. The peeled beetroot corms were shredded using a grater and blended in the prepared solvent (3 g:100 ml extraction solvent) and the mixture was allowed to stay for 4 h and stirred intermittently, sieved using a muslin cloth followed by filtering using a Whatman (No.1) filter paper. Thereafter, the mixture was centrifuged at 4 ^{o}C, 10000 rpm for 15 minutes. The supernatant was collected and the mixture centrifuged for the second time at 4 ^{o}C, 12000 rpm for 10 minutes in order to ensure that there is no more sediment or precipitate present in the solution. Finally, the supernatant was collected poured in the drying trays and placed in freeze dryer (CRC FD-10N-50 model) for 16 h, 80 Pa and -40 ^{o}C (cold trap temperature) to obtain the freeze-dried beetroots food colourant samples.

2.3 Analytical Methods

2.3.1 Determination of minerals contents

The mineral (potassium, zinc, phosphorus, magnesium, calcium, iron, and selenium) content of the freeze-dried beetroot colorant extract was determined using the method described by AOAC [24].

2.3.2 Determination of physicochemical properties

pH values

The pH value of the freeze-dried beetroot sample extracts was measured with a glass-electrode digital pH meter at room temperature. Prior to analysis, the pH meter was standardized using phosphate buffer solutions of pH values 4.0 and 7.0 [24].

Total titratable acidity (TTA)

The TTA was determined by following the procedure adopted by Khalil *et al*. [25]. Five grams (5 g) of the freeze-dried beetroot extract was weighed and homogenized in a conical flask with 25 ml of distilled water and followed by filtration using Whatman No.1 filter paper. Thereafter, 0.1 M NaOH solution was titrated against the extract filtrate using three drops of phenolphthalein as an indicator until a pink colour endpoint and the TTA was calculated using the formular;

$$\% \text{ TTA} = \frac{abcd}{w}\ x\ 100 \quad (1)$$

Where, a = Titre value; b = Molarity of NaOH used, c = volume made up, d = ml equivalent weight of citric acid =0.06404; f = weight sample used

Colour

The colour characteristics of the freeze-dried beetroot colourant extracts were measured using a colourimeter (Chroma-Meter, Tokyo, Japan) and the colour parameters, L*, a* and b* which indicates the lightness, redness, and yellowness of the samples were determined.

2.4 Determination of Antioxidants activities

2.4.1 Determination of 1,1-diphenyl-2-picryl-hydrazil (DPPH) radical scavenging activity

The DPPH of the samples was determined by following method described by Silva *et al*. [26]. The anti-radical activity of each sample was determined using a dilution series, in order to obtain a large spectrum of sample concentrations. The colour sample (200 µL) was mixed with 2800 µl of 80 µM methanol solution of DPPH, allowed to stand for 30 min in the dark at room temperature and the absorbance measured at 517 nm using a spectrophotometer (INESA SPECTRO.125N ENGLAND) against the blank containing 1 ml methanol in place of the extract.

The IC_{50} values were calculated using the formular;

$$\% \text{ inhibition} = \frac{Ao - A1}{A0}\ x\ 100 \quad (2)$$

A_1 is the extract/standard absorbance and A_0 is the control absorbance. The inhibition curves were drawn and IC_{50} values computed.

2.4.2 Determination of ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) free radical scavenging assay

The ABTS radical scavenging activity was determined using ABTS stock solution of 7 mM and 2.45 mM potassium peroxydisulfate (v/v = 2:1) was used to prepare the ABTS solution in dark at room temperature overnight. The ABTS working solution was then diluted from ABTS solution by ethanol to adjust the absorbance to 0.700 at the wavelength of 734 nm. A 100 µl of the beetroot colour extract was then mixed with 3.9 ml ABTS working solution and this was thoroughly vortex and allowed

to react for 6 minutes. The absorbance was then read at 734 nm and Trolox solution (50, 75, 100, 125 and 150 mg/L) was used as standard to generate the straight-line equation [27].

2.5 Statistical Analysis

Analysis was carried out in triplicate and the data were subjected to one-way analysis of variance (ANOVA) as described by Iwe (28) using the version 20 of SPSS statistical software The means were compared using Duncan multiple range test at $p \leq 0.05$ probability level.

3.0 Results and Discussion

3.1 Mineral composition of freeze-dried beetroot extract

The mineral composition is as presented in Table 1 Minerals are essential inorganic elements required for structural and functional roles in the human body [29]. Amongst the major minerals, potassium, calcium and magnesium ranged between 131.73 – 152.76 mg/100g, at 0 and 3 % citric acid, 28,49- 40.81 mg/100g at 0 and 5 % citric acid, 40.13 to 59.65 mg/100g, at 0 and 4 % citric acid respectively

Table 1. Mineral content (mg/100g) **of freeze-dried beetroot colorant extract**

	% Citric acid					
	0	1	2	3	4	5
Potassium	$131.73^{e}\pm0.40$	$141.22^{d}\pm0.25$	$150.68^{b}\pm0.06$	$152.76^{a}\pm0.01$	$149.79^{c}\pm0.30$	$150.48^{b}\pm0.33$
Calcium	$28.49^{f}\pm0.00$	$30.75^{e}\pm0.00$	$38.74^{d}\pm0.03$	$40.24^{c}\pm0.01$	$40.81^{a}\pm0.01$	$40.43^{b}\pm0.00$
Magnesium	40.13 ± 0.00^{f}	47.27 ± 0.00^{c}	$47.02^{d}\pm0.00$	$44.08^{e}\pm0.00$	$59.65^{a}\pm0.00$	$58.99^{b}\pm0.00$
Phosphorus	$150.89^{a}\pm0.02$	$136.08^{d}\pm0.25$	$120.74^{e}\pm0.24$	$141.02^{c}\pm0.18$	$148.98^{b}\pm0.12$	$101.28^{f}\pm0.58$
Zinc	$4.07^{f}\pm0.00$	$4.10^{e}\pm0.00$	$5.15^{c}\pm0.00$	$4.17^{d}\pm0.00$	$5.91^{a}\pm0.00$	$5.89^{b}\pm0.00$
Selenium	$2.72^{e}\pm0.00$	$3.28^{a}\pm0.00$	$2.95^{c}\pm0.00$	$2.99^{b}\pm0.00$	$2.78^{d}\pm0.00$	$2.46^{f}\pm0.00$
Iron	$5.01^{c}\pm0.00$	$5.54^{b}\pm0.01$	$6.45^{a}\pm0.00$	$4.80^{e}\pm0.00$	$4.94^{d}\pm0.00$	$4.84^{e}\pm0.04$

Means with the same superscript within a row are not significantly different from each other at $p< 0.05$ %

The increase in magnesium content with higher citric acid concentrations is consistent with its role as a divalent cation that can be effectively chelated by organic acids such as citric acid aiding its extraction from plant tissues [30]. For phosphorous, the highest value was recorded at 0 % (150.98 mg/100g) and 5 % citric acid (101.28 mg/100g). The inverse relationship between citric acid concentration and phosphorus content is notable which could be as a result of possible interference in solubility and stability due to pH alteration.

Zinc, selenium and iron content of the freeze-dried beetroot colour extracts samples ranged between 4.07 – 5.91 mg/100g at 0 and 4 % citric acid, 2.72 – 3.28 mg/100g and 4.80 - 6.45 mg/100g at 3 and 4 % respectively. The selenium and iron content shows a distinct fluctuating trend in this study and could be attributed to the complex redox chemistry and its interaction with organic acids [31]. In addition, chelation of iron by citric acid has been reported [32]. The overall trend suggests citric acid aids in the release of these minerals [33] and this has been attributed to the breaking down of cell walls and chelating metal ions resulting in enhancement of the extraction of minerals from plant matrices [34,35]. The decrease in mineral content at higher citric acid concentrations might be due to it reaching a saturation point for optimum extraction [34]. These values are higher than the mineral content reported in *Sambucus wightiana* extract using methanol as extraction solvent [36]. This result implies a strong interaction of the minerals with the extracting medium, revealing the ease of penetration of the acid to a nutritional advantage evidenced by the high amount of phosphorus, magnesium and potassium as observed in this research work. These minerals are important for energy metabolism and signal cell signalling muscle contraction, functioning of nerve cells, phosphorus is utilised complex physiological responses and as cofactors in some enzymatic reactions [37] and potassium regulates osmotic pressure, nerve impulses conduction and maintains acid-base balance.

3.2 Physicochemical parameters of the freeze-dried beetroot colour extracts

The results of the physicochemical properties of the freeze-dried beetroot colourant are as presented in Table 2. According to the results, the least L* colour value was observed in the water extracted beetroot colourant at 16.00, lightness increased in the citric acid extracted colourants. According to Borjan *et a*l. [38], water components rather than organic compounds enable pigments release by stretching the plant tissue. Higher values of L* at 26.60-28.21 was reported by [25] for methanolic beetroot extracted colorant. The *a** value measures the level of red to green where positive and negative value indicates redness and greenness respectively. The a* values was highest at 1 % citric acid extracted beetroot colorant. Though there was no significant ($p<0.05$) difference amongst the samples. These variations in the colour values of the beetroot colour extracts could be due to the differences in the extraction of inherent colouring compounds [39]. The positive *b** value measures the level of yellow while the negative value measures the level blueness of the samples. This result shows that the water extracted colourant has the lowest, though there is no significant ($p<0.05$) difference at 0 and 1 % beetroot colourants.

The mean pH values of the samples ranged between 5.05 to 6.09. As expected, there was an increase in acidity of the freeze-dried extract as the percentage citric acid increases. This is an important parameter in the stability of betalain pigments reported to be stable in acid medium range of 4-6 [39]. The total amount of acid neutralized by a strong base is referred to as titratable acidity (TTA). The TTA determined in the samples ranged between 0.33 to 0.53 %. The concentration of organic acids is an important parameter in its use in food products as it may affects the flavour and subsequent application in foods [40].

Table 2. Physical properties of freeze-dried beetroot colorants extracts

	% Citric acid					
Parameters	0	1	2	3	4	5
L*	16.00^{b}±0.89	16.06^{b}±0.95	16.75^{ab}±0.32	17.14^{ab}±0.29	17.22^{ab}±0.94	17.57^{a}±0.55
a*	6.00^{a}±0.17	6.31^{a}±0.16	5.80^{a}±0.22	4.54^{b}±0.23	4.87^{b}±0.70	2.73^{c}±0.34
b*	-1.47^{c}±1.74	-1.39^{c}±0.13	0.54^{b}±0.09	0.57^{a}±0.11	0.48^{b}±0.05	0.43^{b}±0.05
pH	6.00^{a}±0.02	5.90^{b}±0.01	5.64^{c}±0.00	5.29^{d}±0.01	5.10^{e}±0.03	5.05^{f}±0.01
TTA	0.37^{f}±0.00	0.38^{e}±0.00	0.41^{d}±0.00	0.43c±0.00	0.44^{b}±0.00	0.53^{a}±0.01

Means with the same superscript within a row are not significantly different from each other at p< 0.05 %.
L*= lightness, a*= redness, and b*= yellowness

3.3 Antioxidants activities of the freeze beetroots colorant samples

Table 3 and 4 shows the ABTS and DPPH radical scavenging activity of the free-dried beetroots colour extract sample at different concentrations and the corresponding IC_{50} values. There was a significant ($p<0.05$) difference among the samples with respect to the ABTS and DPPH radical scavenging activities. All the beetroot colour extracts have the propensity to reduce free radicals, as indicated by the dose-dependent increase in percentage inhibition. However, the control (100 % water) extract samples showed the least value at each concentration in both the DPPH and ABTS results. The higher activity in the citric acid solvents extracted colorants could suggest that organic acid is able to extract more bioactive compounds with higher antioxidants activities. Citric acid is also an antioxidant [41] and this could also have contributed to the anti-radical activities of the colour samples. This study shows that inhibition of the freeze-dried beetroots colorants samples was concentration dependent also use of citric acid in extraction led to the increase in percentage inhibition of the extracts. This is however a common trend observed in antioxidant activity assays of plant extracts, as a higher concentration of bioactive compounds responsible for antioxidant activity becomes available to scavenge free radicals [42].

Table 3. ABTS free radical scavenging activities and IC_{50} of the beetroot colourant (%)

	% Citric acid					
Concentration (µg/mL)	0	1	2	3	4	5
50	47.55^{e}±0.14	47.54^{e}±0.07	48.42^{d}±0.14	48.62^{c}±0.00	49.96^{a} ±.07	49.58^{b}±0.14
75	49.04^{e}±0.07	49.91^{d} ±0.07	51.91^{b}±0.07	49.87^{d}±0.00	51.46^{c}±0.14	52.55^{a}±0.16
100	49.79^{f}±0.07	52.12^{e} ±0.00	55.40±0.07^{a}	53.40^{d}±0.14	54.40^{c}±0.0	54.93^{b}±0.12
125	52.95^{d}±0.01	55.69^{c} ±0.07	59.51±0.14^{a}	56.31^{b}±0.05	56.39^{b}±0.13	56.52^{b}±0.14
150	57.48^{e}±0.00	58.52^{cd}±0.14	61.30±0.14^{a}	58.97^{b}±0.00	58.56^{c}±0.14	58.30^{d}±0.18
IC50	85.63^{a}±0.23	74.90 ^{b}±0.38	70.71±2.68^{c}	60.65^{d}±0.05	53.09^{e}±0.03	48.60^{f}±0.70

Means with the same superscript are not significantly different from each other at p< 0.05 %

Table 4. DPPH radical scavenging activities and IC50 of the beetroot colourant extracts

	% Citric acid					
Concentration (µg/mL)	0	1	2	3	4	5
50	47.55±0.15^{e}	47.58±0.06^{e}	48.41±0.14^{d}	48.62±0.02^{c}	49.97±0.08^{a}	49.60±0.13^{b}
75	49.12±0.11^{e}	49.97±0.08^{d}	51.89±0.10^{b}	49.73±0.25^{d}	51.43±0.17^{c}	52.51±0.13^{a}
100	49.64±0.29^{f}	52.08±0.07^{d}	55.38±0.10^{a}	53.40±0.14^{c}	54.39±0.09^{b}	54.68±0.31^{b}
125	52.88±0.10^{d}	55.70±0.69^{c}	59.45±0.13^{a}	56.34±0.11^{b}	56.30±0.15^{b}	56.43±0.15^{b}
150	57.50±0.03^{d}	58.52±0.15c^{d}	61.34±0.13^{a}	58.88±0.17^{b}	58.58±0.13^{b}	58.30±0.18^{c}
IC_{50}	85.76±0.23^{a}	74.91±0.40^{b}	60.35±0.37^{d}	71.03±2.56^{c}	53.08±0.02^{e}	48.87±0.62^{f}

Means with the same superscript are not significantly different from each other at p< 0.05 %

This shows that a higher concentration of the beetroot extract leads to a greater ability to neutralize ABTS radicals. The IC_{50} values, signifies the beetroot extract concentration needed to inhibit 50% of the ABTS radicals ranged from 48.60 to 85.63 µg/mL. A Low IC_{50} value is indicative of good inhibition of the enzyme and based on the classification by Seeta et al. [43], IC_{50} is divided into very strong (IC_{50} < 50 µg/mL), strong (IC_{50}: 50–100 µg/mL), moderate (IC_{50}: 101–150 µg/mL), and weak (IC_{50}: 250–500 µg/mL). This implies that the IC_{50} antioxidant activity of these extracts at 0- 4 % citric extract can be classified as strong while the beetroot extract at 5 % exhibited a stronger antioxidant capacity. This result is higher compared to the report of IC_{50} values of 39.74 and 13.42 µg/mL for DPPH and ABTS ethanol extract of *Juniperus phonecia* reported by Guaname *et al*. [44] and on the other hand within the range (58.70-91.72 µg/mL) reported for *Artemisia campestris* using different organic extracts [45]. Higher IC_{50} (lowest antioxidant activity) is observed for the colourant derived from the 0 % citric acid extraction solvent. The lower IC_{50} value in the freeze-dried citric acid beetroot colourant extracts had the best inhibition of enzyme and higher antioxidant potency. This also implies that a smaller amount of the extract is needed to achieve a substantial level of radical scavenging of Reactive oxygen species (ROS) generated in the system. This makes these colour extracts potentially valuable source of natural antioxidants and as such an added benefit in food applications [46]. The free radical scavenging ability and high antioxidant activity of betanin are linked to the presence of phenolic hydroxy groups in the structure and the unsaturated bonds on the benzene ring [47].

The presence of ROS at low or appropriate concentrations plays positive role in body cells. However, at high concentration of ROS, it is referred to as oxidative stress which may contribute to cellular malfunctioning, damaging proteins, nucleic acids and lipids and it has been proven to be a facilitating factor of degenerative diseases such as diabetes, renal failure amongst others [48]. This implies that the use of these extracts can contribute to the neutralizing of the free radicals thereby offering cell protection and therapeutics uses.

Conclusion

Freeze-dried beetroots colourants extracts were successfully produced at varying (0- 5 %) concentration of citric acid. The study revealed a significant increase in potassium, calcium, magnesium, zinc, selenium and iron, decrease in pH, increase in total titratable acidity as the percentage citric acid concentration increases. A deeper L* colour was obtained amongst the citric acid extract at 1 %. Generally, a strong radical scavenging ability for the ABTS and DPPH of the extracts as the citric acid concentration increased with a more pronounced radical scavenging activity at 5 % citric acid in this study. The utilization of citric acid in its extraction could confer a nutritional and health advantage beyond its use as colourant which can be explored in food product development.

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Conflict of interest

The authors declared that there is no conflict of interest

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