Food Research 8 (5) : 13 - 23 (October 2024)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

Enhancement of polyphenols and haematological profile of rats fed with traditional African soups

[1,2]Ejueyitsi, E.O., [1]George, B.O., [1]Anigboro, A.A. and [3,*]Okpoghono, J.

[1]*Department of Biochemistry, Faculty of Science, Delta State University, P.M.B. 1, Abraka, Delta State, Nigeria*

[2]*Longlife Healthcare Medical and Diagnostics Nigeria Limited, Golden Plaza, Shop D10 and D11, Shell Contractors Gate Road Warri, Delta State, Nigeria*

[3]*Department of Biochemistry, Delta State University of Science and Technology, Ozoro, Delta State, Nigeria*

Article history:
Received: 17 June 2022
Received in revised form: 25 August 2022
Accepted: 15 June 2023
Available Online: 2 September 2024

Keywords:
Flavonoids,
Haematology,
Polyphenols,
Soup,
Spices,
Total iron binding capacity

DOI:
https://doi.org/10.26656/fr.2017.8(5).321

Abstract

Nigeria is a multi-ethnic and multicultural country where several traditional soups are indigenous to the various social groups. When soups are prepared with the right spices, it can be truly healthy with multiple biochemical/nutritional benefits. The objective of the study is to determine some blood parameters, total iron binding capacity (TIBC), polyphenols and flavonoid content of rats fed with indigenous soups in Delta State, Nigeria. A total of twenty-five mature male albino Wistar rats were divided into five groups of five rats each. Group 1 rats served as control, rats in Group 2 were given *Igbagba* soup (okro pepper soup), rats in Group 3 were administered *Oghwo ameidi* (palm nut soup; soup made from African palm fruit; *Elaeis guineensis*). Group 4 and Group 5 rats were given *Offe ikere* (pepper soup) and aqueous tween 80 respectively. The rats in each group were allowed free access to clean drinking water and feed for 28 days experimental period. The results indicated that rats given soup extracts (Group 2, 3 and 4) their serum total polyphenol content was 20.75, 12.24 and 9.83 mg/g, respectively, while the control value was 2.81 mg/g. Significant increases in TIBC, total flavonoids, packed cell volume (PCV), haemoglobin (Hb), red blood cells (RBC), and white blood cells (WBC), were observed in rats given the soup extracts when compared with control. The study has shown that African indigenous soups may improve haematological indices owing to their polyphenol and flavonoids content.

1. Introduction

Soups offer a feasible means to blend a few nourishment ingredients into one wholesome meal, subsequently contributing to dietary differing qualities (Sivakumar *et al.,* 2018). Additional time, conventional cooking information has been misplaced and there's an urgent need to discover ways to maximize nutritional benefits utilizing basic recipes. The joining of these traditional nourishments into more calorie dishes such as soups can be a practical way to advance their utilization. The arrangement and choice of dietary nourishments are significant to support a healthy way of life. Urban customers frequently depend on quick foods for normal meals, and these foods are ordinarily high in sugar, fat and salt content (Vimbainashe *et al.,* 2020). Undernourishment could be a worldwide issue influencing nearly 900 million individuals around the world, and around 2 billion suffer from nutrient lack (Vimbainashe *et al*., 2020). Dietary enhancement can be a long-term methodology to combat malnutrition, through expanding utilization of nearby nourishments such as conventional vegetables.

Traditional food speaks to a critical portion of the culture, history, character and legacy of a region or nation and are key components within the dietary design (Rekha *et al.,* 2010). Poor diet choices can lead to ailing health and constant weight-related disarranges. Dietary utilization of nutrient wealthy soups is related with a lower risk of obesity (Sivakumar *et al.,* 2018). Traditional spices and verdant vegetables are good nourishment choices owing to their characteristic anti-diabetic, antifungal, antiinflammation and antioxidant properties (Ekakitie *et al.,* 2021, Ekakitie, Orororo and Okpoghono, 2021; George *et al.,* 2013; George *et al.,* 2012; Vimbainashe *et al.,* 2020).

Polyphenols are among the foremost alluring phytochemicals because of their antioxidant action (Ruiz

*Corresponding author.
Email: *okpoghono@gmail.com*

eISSN: 2550-2166 / © 2024 The Authors.
Published by Rynnye Lyan Resources

RESEARCH PAPER

-Torralba *et al.,* 2018). These components are known as auxiliary plant metabolites and possess moreover antimicrobial, antiviral and anti-inflammatory properties in conjunction with high antioxidant capacity (Ruiz-Torralba *et al.,* 2018; Onakurhefe *et al*., 2020). Research also underpins the significance of examining the antioxidant properties of the plant portion that is really consumed (Vanzani *et al.,* 2011; Okpoghono *et al*., 2021; Onakurhefe *et al.,* 2020; Omoike *et al.,* 2022), instead of centering the attention on the impacts of individual compounds. At last, in spite of the fact that a few compounds may contribute to the antioxidant properties in complex frameworks, polyphenols are regularly considered the primary source of the antioxidant movement but little information supports a precise relationship (Vanzani *et al.,* 2011; Ugwu et al., 2023; Okpoghono, Osioma, Metie *et al.,* 2023). Iron is the foremost difficult mineral to add to foods because it is difficult to guarantee satisfactory absorption. A fundamental issue is the presence of absorption inhibitors, such as phenolic compounds, within the food vehicle (Otuaga *et al.,* 2020a; Otuaga *et al.,* 2020b; Okpoghono, Omoriare, Igue *et al.,* 2023), that will decrease the bioavailability. The total iron-binding capacity (TIBC) test measures the blood's capacity to connect itself to iron and transport it around the body (Aksan *et al.,* 2020). Consequently, this study is aimed at assessing and comparing the TIBC and polyphenols content of rats fed withAfrican indigenous soups.

2. Materials and methods

2.1 Sample area

Three popularly consumed soups from different localities were selected for this study, based on the preliminary survey conducted by Ejueyitsi *et al.* (2022) on the type of food respondents eat in the different locality/senatorial districts of Delta State. The soups were as follows; pepper soup (*Offe ikere*) from Umutu in Ukwuani LGA, Okro pepper soup (*Igbabga ofofo*) from Egbokodo Itsekiri in Warri South LGA and palm nut soup (*Oghwo ameidi*) from Ekeranvwe in Ughelli North LGA., Delta State Nigeria. Soups were prepared according to descriptions from correspondents in various localities. All the experimental measures conformed to Delta State University, Abraka, ethical committee guidelines with approved number, REC/FOS/20/010 for this study.

2.2 Soups preparation

The soups and ingredients used were prepared following previous procedures outlined by Ejueyitsi *et al*. (2023).

2.2.1 Pepper soup (Offe ikere)

Water (1 L) was poured into a pot. Yam, plantain, spices and pepper were added and allowed to boil for 5 mins. This was followed by adding meat and fish, then allowed to cook for 15 mins. Thereafter, maggi and salt was added to taste. The pepper soup was served with yam and plantain.

2.2.2 Okro pepper soup (Igbagba ofofo)

A total of 1 L of water was put in a pot. Then all the spices and ingredients except okro were added in the pot on the fire, and then allowed to boil for 10 mins. This was followed by the addition of grated okro and potash then allowed to boil for about 10 mins. Salt and maggi was added to taste.

2.2.3 Palm nut soup (Oghwo ameidi)

The fresh palm nut was boiled for 30 mins in a pot, and then the kernel was pound carefully to allow quick extraction of the content with water. Extract (1.5 L) was collected in a clean pot using plastic sieve. The pot with the extract was allowed to boil until it thickened for 30 mins. Ingredients (chopped onions *(Allium cepa)*, pepper *(Capsicum frutescens)*, ground crayfish, spices (*Heinsia crinite, Aframomum sceptrum,* and *Glycyrrhiza glabra)*, maggi cube and cooked meat were added, then allowed to cook for 30 mins. Thereafter, fresh fish was added and allowed to cook for 10 mins. Salt was added to taste then simmered for 2 mins and the soup was ready.

2.3 Preparation of soup extracts

The three soups were evaporated to dryness in an electric oven (40°C), then 50 g of the dried samples was homogenized in 450 mL aqueous tween 80 (5% tween 80) to dissolve the oil present in the soups, and then each soup sample were filtered with clean muslin cloth. The concentrations of the extracts were calculated to be 0.1 g/mL and was administered to rats at a protective dose of 400 mg/kg body weight.

2.4 Treatment of animals

The rats were divided into five groups of five rats each. The rats in each group were allowed free access to feed and clean drinking water. The oral administration of the soup extracts and tween 80 was done daily for a period of 28 days. The experimental groupings were as follows:

Group 1: control: rats were given feed and water only, soup extracts and tween 80 were not given.

Group 2: feed and okro pepper soup extract.

Group 3: feed and palm nut soup extract.

 © 2024 The Authors. Published by Rynnye Lyan Resources

Group 4: feed and pepper soup extract.

Group 5: feed and tween 80 (rats were given 1mL/kg body weight of 5% tween 80).

2.5 Blood collection and preparation of tissue homogenate

The rats were sacrificed on the 29th day. The blood was collected by cardiac puncture using hypodermic syringe and needle and then transferred to an anticoagulant-free test tube. Afterwards, the clotted blood was centrifuged at 2,500×*g* for 15 mins to separate the serum. Approximately 1 g of various tissues (liver and kidney) was homogenized in 9 mL of normal saline and centrifuged at 2,500 rpm for 15 mins to obtain the supernatant which was stored in the refrigerator for further biochemical analysis.

2.6 Biochemical analysis

2.6.1 Determination of polyphenols

Assessment of polyphenols was carried out using high-performance liquid chromatography (HPLC) analyses method described by Seal (2016) The mobile phase contains 1% aqueous acetic acid solution (solvent A) and acetonitrile (solvent B), and 2 mL/min was adjusted as the flow rate. The column was controlled at 28°C and the sample injection volume was 5 µL. The HPLC chromatograms were detected using a photodiode array UV detector at three different wavelengths: 272, 280 and 310 nm according to compounds analysed. The compound was identified based on its retention time and by spiking with the standards under the same conditions. Sample quantification was done by measuring the integrated peak area and the content was calculated.

$$\text{Concentration of polyphenols (mg/g)} = \frac{\text{Peak area}}{\text{Standard peak area}} \times \text{Standard concentration}$$

Total polyphenol standard concentration = 29.81 mg/g, total polyphenol standard peak area 21513.8440 mg/g, individual polyphenol standard concentration = 1.99 mg/g, individual polyphenol standard peak area 1434.2563 mg/g.

2.6.2 Proanthocyanidin

Proanthocyanidins (condensed tannins) were determined according to the method of Sun *et al.* (1998). To 50 µL of diluted sample, 3 mL of 4% vanillin solution in methanol and 1.5 mL of concentrated HCl were added. The mixture was allowed to stand for 15 mins, and absorption was measured at 500 nm against methanol as a blank. catechin solution was used for the preparation of the calibration curve. The amount of total condensed tannins is expressed as mg (+)- catechin /g DW.

2.6.3 Flavanones

Quantitative determination of flavanones was accomplished using the 2,4-Dinitrophenylhydrazine (DNP) method proposed by Nagy and Grancai (1996). Sample (40 µL) was dissolved in 80 µL of DNP solution (For 5 mL: 50 mg of DNP in 100 µL of 96% sulphuric acid and 4850 µL of methanol), mixed and incubated at 50°C for 50 mins in a water bath, then cooled at room temperature and 280 µL of 10% potassium hydroxide (KOH) was added. Finally, the absorbance was measured at 486 nm. The total content of flavanones was determined using a calibration curve based on pinocembrin (0.5, 1, 2, 3, 4, and 5 mg/mL) with the absorbance of 0.123, 0.243, 0.323, 0.477, 0.524 and 0.832 respectively. Flavanones were expressed as µg of pinocembrin equivalent per milligram of sample extract (µg PNE/mg).

2.6.4 Flavanonols (Dihydroquercetin) and flavones (Luteolin)

Dihydroquercetin and flavones (Luteolin) were determined according to the method of Struchkov *et al.* (2018). Zero point three millilitres (0.3 mL) of 5% $NaNO_2$ aqueous solution and 5 mL of water were added to 0.1 mL of the sample. After 5 mins, 5 µL of acetic acid and 0.2 mL of 10% $AlCl_3$ were added followed by 1.5 mL of 1 M NaOH. The UV spectrum was set at the range of 300–600 nm against the blank.

Dihydroquercetin or luteolin was calculated according to the following formula:

$$\text{X (mg/mL)} = \frac{10 \times V \times A}{p \times E}$$

Where X: total content of dihydroquercetin or luteolin, V = 8 mL, A: absorption at 517 nm for luteolin, and 507 nm for dihydroquercetin, p: sample volume (mL), E: dihydroquercetin specific absorbance = 134.5 at 507 nm or luteolin specific absorbance = 63.9 at 517 nm.

2.6.5 Anthocyanins

The determination of total anthocyanins was based on the method described by Iland *et al.* (1996). The sample (200 µL) was mixed with 3.8 mL of 1 M HCl and incubated at room temperature for 3 hrs. The absorbance (A) of the acidified diluted extract was read at 520 nm against 1 M HCl as a blank solution.

Anthocyanins were calculated using the absorbance (B) of 1% w/v malvidin-3-glucoside solution as follows:

$$\text{Anthocyanins (mg/mL)} = \text{A} \times \text{Dilution factor} \times 1000/\text{ B}$$

https://doi.org/10.26656/fr.2017.8(5).321

© 2024 The Authors. Published by Rynnye Lyan Resources

RESEARCH PAPER

2.7 Haematological indices

Haematological indices such as haematocrit, erythrocyte counts, and haemoglobin and white blood cell counts were determined using the blood of the experimental rats.

2.7.1 Haematocrit

The method Thrall and Weiser (2002) was used in the determination of haematocrit. Blood was collected in capillary tubes by capillary action and then sealed at one end. Thereafter the test tubes were centrifuged at 2,500 rpm in 5 mins to separate the blood cells from the plasma. The tubes were taken from the centrifuge and read using the haematocrit reader card.

2.7.2 Haemoglobin

The cyanmethemoglobin method described by Tietz (1976), was adopted for estimation of haemoglobin. Approximately 2 mL of haemoglobin reagent were dispensed in test tubes labelled blank, control and test. Ten microliters of sample were placed into respective test tubes except for the blank and allowed to stand at room temperature for 3 mins. Approximately 2 mL of standard reagent were placed in the tube labelled standard. The spectrophotometer was set at 500 nm and then zero with the blank. The absorbance of all tubes was recorded.

2.7.3 White blood count

White blood count was determined as described by Thrall and Weiser (2002).

The dilution factor of 1:20 mL was prepared by adding 0.38 mL of the WBC reagent (Turks fluid) into 20 µL of the blood sample. Turk's fluid was added to slip before viewing using the microscope for counting with the chamber.

2.7.4 Red blood count

Red blood count was determined according to Thrall and Weiser (2002). The solution of formolcitrate was prepared by adding 10 mL formalin (40% formaldehyde) into one litre of 31.3 g/L trisodium citrate. The blood was diluted by washing one microliter (1 µL) of blood into 4.0 mL of diluents, to give a final dilution of 1 in 20. The diluted samples were mixed and then loaded in the haemocytometer. The cell number was counted after the suspension had settled.

2.7.5 Non-heme iron content

Non-heme iron content in serum, liver and kidney were measured according to the method described by Ahn *et al.* (1999). Briefly, 0.5 mL of sample was added to 3 mL of 0.1 M phosphate buffer (pH 5.5) and 1 mL of 2% ascorbic acid in 0.2 M HCl and was left to stand at room temperature for 15 mins before adding 2 mL of 11.3% trichloroacetic acid and then was centrifuged at 3000 rpm for 10 mins. The supernatant (2 mL), 10% ammonium acetate (0.8 mL) and Ferrozine reagent (0.2 mL) were mixed in a test tube and the absorbance was measured at 562 nm. A standard curve was prepared from $FeCl_3$ solution (1000 mg/L stock) which was adjusted to 10, 25, 50 and 100 mg/L. The curve was prepared by plotting the absorbance against the several concentrations of $FeCl_3$ standard solutions.

2.8 Total iron binding capacity

The total iron binding capacity in the serum was measured according to the method described by Re *et al.* (1999). Sample (0.30 mL), Tris-buffer (2.2 mL) and 0.1 mL (100 µL) iron colour reagent were added to each test tube then mixed well and incubated for 10 mins at 37°C. The absorbance of the sample against the reagent blank at 560 nm was read within 30 mins.

2.9 Statistical analysis

All the results were expressed as mean bars and means ± standard deviation (SD). Analysis of variance (ANOVA) was used to analyse the experimental data. The significant difference between means was determined at a 5% ($p<0.05$) confidence level using Duncan's Multiple Range Test. The dependence between parameters was analysed using Pearson correlation (r).

3. Results and discussion

3.1 Polyphenols content in the serum of rats given soup extract

The polyphenol content in the serum of rats given soup extract is presented in Table 1. The serum polyphenols content of the control (Group 1) rats was as follows; chlorogenic acid > kaempferol > ellagic acid > quercetin > copaene. Group 2 polyphenols content was given as; quercetin > kaempferol > chlorogenic acid > ellagic acid > skimmianine > inulin > butyrolactone > catechin > naringenin. Group 3 polyphenols content were presented as; quercetin > chlorogenic acid > kaempferol > ellagic acid > glycyrrhizin > capsaicin > glycerretol > cinnamic acid. Group 4 had the following polyphenols content order; quercetin > chlorogenic acid > kaempferol > ellagic acid > humulene > copaene> estragole > citral. The serum total polyphenols content of rats fed soup extracts *in vivo* and *in vitro* are presented in Figure 1. The total polyphenol content in the *in vitro* study, okro pepper soup, palm nut soup and pepper soup had values of 33.17, 32.37 and 29.14 mg/g, respectively. The *in vivo study* indicated that the rats given okro

 © 2024 The Authors. Published by Rynnye Lyan Resources

Table 1. Serum polyphenols content in rats.

	Polyphenols (mg/g)			
	Group 1	Group 2	Group 3	Group 4
Ellagic acid	0.208	1.520	0.640	0.490
Chlorogenic acid	0.963	3.130	1.630	1.380
Humulene	ND	ND	ND	0.250
Capsaicin	ND	ND	0.290	ND
Cinnamic acid	ND	ND	0.120	ND
Inulin	ND	0.590	ND	ND
Catechin	ND	0.130	0.120	ND
Quercetin	0.181	11.370	7.510	6.380
Kaempferol	0.330	3.020	1.230	0.620
Glycyrrhizin	ND	ND	0.590	ND
Glycerretol	ND	ND	0.230	ND
Skimmianine	ND	0.710	ND	ND
Butyrolactone	ND	0.190	ND	ND
Naringenin	ND	0.090	ND	ND
Copaene	0.127	ND	ND	0.390
Estragole	ND	ND	ND	0.200
Citral	ND	ND	ND	0.110
Total	2.810	20.750	12.240	9.830

ND: Not detected, Group 1: Control, Group 2: Feed + Okro pepper soup extract, Group 3: Feed + Palm nut soup extract, Group 4: Feed + Pepper soup extract.

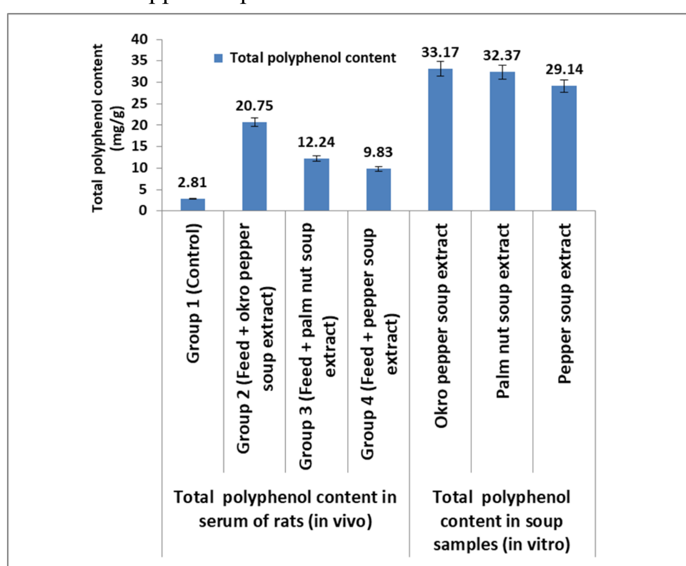

Figure 1. Total polyphenols content *in vivo* and *in vitro*.

pepper soup, palm nut soup and pepper soup extract had serum total polyphenol content of 20.75, 12.24 and 9.83 mg/g respectively while the control value was 2.81 mg/g. Polyphenols are major plant constituents and have been known to possess several biological activities such as anti-inflammatory, antioxidant and anti-carcinogenic activities (Liu *et al.,* 2017; Maseko *et al.,* 2019). The increased polyphenols contents observed in the rats fed the soups extract when compared with the control could be an indication that when the soups are frequently consumed, it may contribute substantial amounts of polyphenol compounds to the animals. Large intake of polyphenolic compounds has been associated with benefits which includes protection against stroke, cardiovascular diseases and cancer activities (Liu *et al.,* 2017; Maseko *et al.,* 2019).

3.2 Flavonoids content of rats administered different soup extracts

The total flavonoids, flavanones, flavones and flavanonol content of rats fed different soup extracts are shown in Tables 2 and 3. Group 2, 3 and 4 had significant increase in flavonoids in the serum, liver and kidney when compared to Group 1 and 5. However, no significant differences were observed in Group 1 compared to Group 5. Group 2, 3 and 4 had significant increase in flavanones, flavones and flavanonol in the serum, liver and kidney in comparison to Group 1 and 5. Anthocyanins and proanthocyanidins content of rats given soup extracts are shown in Table 4. A significant increase in anthocyanins and proanthocyanidins was observed in serum and tissues of Groups 2 and 3 in comparison to Groups 1, 4 and 5. However, no significant difference was shown in anthocyanins and proanthocyanidin in the serum and tissues of Group 1 as compared to Group 5. The significantly lower polyphenol in the serum of rats given pepper soup (group 4) (with ingredients: dry pepper, yam, plantain, cow meat, fresh catfish, *Xylopia aethiopica*, *Monodora myristica*, *Ocimum gratissimum*, salt and maggi) when compared to palm nut soup (group 3) (with ingredients:

 © 2024 The Authors. Published by Rynnye Lyan Resources

RESEARCH PAPER

Table 2. Total flavonoids and flavanonol content of rats administered different soup extracts.

Group	Serum Total Flavonoids **	Liver Total Flavonoids*	Kidney Total Flavonoids*	Serum Flavanonol**	Liver Flavanonol*	Kidney Flavanonol*
1: Control	8.14±2.49[a]	10.31±2.34[a]	7.85±1.83[a]	0.21±0.07[a]	0.21±0.06[a]	0.22±0.07[a]
2: Feed + Okro pepper soup extract	40.14±2.95[b]	73.45±4.69[a]	50.32±6.50[b]	3.32±1.68[b]	6.39±1.60[b]	4.26±1.38[b]
3: Feed + Palm nut soup extract	30.46±1.60[c]	53.63±8.29[b]	45.17±3.81[c]	2.28±0.58[ab]	4.22±1.47[ab]	3.26±0.71[ab]
4: Feed + Pepper soup extract	20.60±3.91[d]	43.56±5.12[c]	28.96±3.36[d]	1.16±0.65[a]	2.14±0.21[a]	2.03±0.44[ab]
5: Feed + Tween 80 extract	9.23±1.58[a]	12.22±3.18[a]	8.25±1.53[a]	0.30±0.08[a]	0.24±0.51[a]	0.10±0.02[a]

Values are presented as mean±SD, n = 5. Values with different superscripts within the same column are statistically significantly different ($p<0.05$).
*Units/g wet tissue, **Units/mL

Table 3. Flavanones and flavones contents in the serum, liver and kidney of rats administered different soup extracts.

Group	Serum Flavonones**	Liver Flavonones*	Kidney Flavonones*	Serum Flavones**	Liver Flavones*	Kidney Flavones*
1: Control	0.71±0.14[a]	3.46±1.68[a]	2.30±0.92[a]	0.11±0.4[b]	0.20±0.68[a]	0.12±0.02[a]
2: Feed + Okro pepper soup extract	5.95±1.05[b]	15.59±3.61[b]	8.32±1.79[b]	2.00±0.70[a]	5.27±1.86[b]	3.34±0.24[b]
3: Feed + Palm nut soup extract	4.41±1.36[b]	10.53±1.35[c]	5.44±1.54[ab]	1.02±0.41[a]	3.05±0.45[ab]	2.04±0.99[b]
4: Feed + Pepper soup extract	2.21±0.64[bc]	7.34±1.67[c]	3.28±0.78[a]	0.40±0.25[ab]	1.91±0.27[a]	0.80±0.28[ab]
5: Feed + Tween 80 extract	0.50±0.19[a]	3.17±0.14[a]	2.20±0.75[a]	0.10±0.02[b]	0.15±0.58[a]	0.26±0.35[a]

Values are presented as mean±SD, n = 5. Values with different superscripts within the same column are statistically significantly different ($p<0.05$).
*Units/g wet tissue, **Units/mL

palm oil fruit (Elaeis guineensis), fresh catfish, cow meat, *Aframomum sceptrum, Heinsia crinite*, *Allium cepa*, *Vernonia amygdalina, Glycyrrhiza glabra*, *Capsicum frutescens*, ground crayfish, salt and maggi) and okro pepper soup (group 2) (with ingredients: *Aframomum sceptrum*, *Monodora myristica*, *Heinsia crinite*, *Abelmoschus esculentus*, dry ground pepper, *Tetrapleura tetraptera*, *Chrysobalanus icaco*, *Parinari excels*, bush meat, cow meat, dry fish, sea crab and lobster) could be due to the poor absorption of polyphenol as a result of few spicy ingredients in preparation of the soup. Although powerful effects from polyphenol administration have been observed *in vivo,* corresponding clinical benefits have been much more difficult to achieve. One major motive for these divergent findings stems from the fact that deprived flavonoids absorption from the gastrointestinal tract typically leads to low plasma flavonoid concentrations (Dryden *et al*., 2006). However, spices are food adjuncts (George *et al.,* 2012, 2015, 2019; Okonta *et al.,* 2021), used as flavouring, colouring and seasoning agent with protective properties (George and Okpoghono, 2017; Okpoghono *et al.,* 2018a, 2018b, Okpoghono, George and Achuba, 2018). There are varieties of traditional soups in Nigeria, as there are tribes. The difference between the quality and taste of one soup from another depends on the spices used in their preparation (Tchokouaha *et al.,* 2015).

3.3 Changes in haematological parameters, non-heme iron content and total iron binding capacity of rats fed soup extracts

The changes in haematological parameters, non-heme iron content and TIBC of rats fed with soup extracts are shown in Table 5. There was a significant increase in PCV, Hb, WBC and TIBC of rats in groups 2, 3 and 4 compared to control. However, PCV, Hb, WBC and RBC levels of rats in group 5 had no significant differences when compared to control. On the other hand, a significant decrease was recorded in the RBC level of rats in Groups 2, 3 and 4 compared to the control. A significant decrease was observed in PCV of rats in groups 3 and 4 compared to Group 2. There was no significant difference in Hb WBC and RBC levels of rats in groups 3 and 4 compared to group 2. Also, no significant differences were observed in non-heme iron content in the serum, liver and kidney of all the experimental groups. A significant increase was observed in serum TIBC of rats in groups 2, 3 and 4 compared with control. Also, a significant increase was observed in serum TIBC of rats in Group 2 in comparison with groups 3 and 4. The assessments of haematological indices are of importance in determining the extent of deleterious effect of the soup extracts on blood of the rats. Mishra and Tandon (2012) reported, that decrease in RBC, Hb and PCV is an indication of destruction of RBC or decreased production, which may result in anaemia. On the contrary, an increase in the count of RBC, Hb and PCV is suggestive of positive erythropoiesis and polycythaemia (Mansi and Lahham,

https://doi.org/10.26656/fr.2017.8(5).321 © 2024 The Authors. Published by Rynnye Lyan Resources

Table 4. Anthocyanins and proathocyanidin content in the serum, liver and kidney of rats administered different soup extracts.

Group	Serum Anthocyanins**	Liver Anthocyanins*	Kidney Anthocyanins*	Serum Proathocyanidin**	Liver Proathocyanidin*	Kidney Proathocyanidin*
1: Control	0.12±0.04[a]	0.15±0.03[a]	0.06±0.02 [a]	2.61±0.17[a]	2.58±0.47[a]	1.65±0.35[a]
2: Feed + Okro pepper soup extract	1.37±0.24[b]	2.34±0.95[b]	1.44±0.19[b]	8.27±1.62[b]	10.53±1.60[b]	9.36±1.62[c]
3: Feed + Palm nut soup extract	0.74±0.15[ab]	1.03±0.40[ab]	0.85±0.22[ab]	6.17±2.90[b]	7.35±1.41[b]	5.30±1.61[abc]
4: Feed + Pepper soup extract	0.41±1.44[a]	0.51±0.15[a]	0.34±0.04[a]	4.43±1.45[ab]	6.38±2.22[ab]	2.64±1.00[a]
5: Feed + Tween 80 extract	0.14±0.04[a]	0.27±0.37[a]	0.16±0.08[a]	2.27±0.21[a]	2.53±0.50[a]	2.41±0.16[a]

Values are presented as mean±SD, n = 5. Values with different superscripts within the same column are statistically significantly different ($p<0.05$).
*Units/g wet tissue, **Units/mL

Table 5. Haematology, heme iron content, and TIBC of rats given different soup extracts.

Groups	PCV (%)	Hb (g/dL)	WBC ($\times 10^9$/L)	RBC ($\times 10^{12}$/L)	Serum non-heme iron content **	Liver non-heme iron content*	Kidney non-heme iron content*	Serum TIBC (µg/dL)
1: Control	30.00±1.58[a]	21.27±6.64[a]	12.18±2.80[a]	13.19±6.00[a]	17.16±5.90[a]	18.34±3.64[a]	17.72±3.29[a]	200.17±46.93[a]
2: Feed + Okro pepper soup extract	48.00±1.58[b]	31.32±5.35[b]	24.29±2.85[b]	22.03±1.57[b]	15.40±1.47[a]	15.32±3.58[a]	14.40±5.15[a]	232.29±7.44[b]
3: Feed +Palm nut soup extract	42.20±1.48[c]	28.48±6.59[b]	20.23±3.15[b]	16.19±1.96[ac]	13.28±2.06[a]	17.46±5.53[a]	15.12±3.77[a]	224.12±17.21[c]
4: Feed + Pepper soup extract	37.00±1.58[d]	25.19±3.63[ab]	16.22±4.29[ab]	18.18±1.58[c]	14.12±3.08[a]	17.20±1.07[a]	15.62±4.65[a]	212.45±8.64[d]
5: Feed + Tween 80 extract	32.00±2.54[a]	21.25±2.28[a]	13.28±4.80[a]	12.16±5.14[a]	17.40±3.32[a]	18.60±2.22[a]	17.62±1.61[a]	205.49±16.69[a]

Values are presented as mean±SD, n = 5. Values with different superscripts within the same column are statistically significantly different ($p<0.05$).
*Units/g wet tissue, **Units/mL

https://doi.org/10.26656/fr.2017.8(5).321
© 2024 The Authors. Published by Rynnye Lyan Resources

RESEARCH PAPER

2008). Hence, a significant increase in RBC (Table 6) with no alteration in Hb and PCV in rats fed the soups indicates that the soup extracts cause no toxic effect on RBC. Studies have shown that an increased count of WBC is supposed to be helpful in boosting the immune system, a decreased count of WBC shows the suppression of leucocytes and their production from the bone marrow (Odesanmi *et al.,* 2010). Therefore, an increased count of WBC in rats fed soup extracts, as observed in the present study, suggests that the soups might be having a good potentiality to boost the immune system. The present study indicated that okro pepper soup, palm nut soup and pepper soup significantly improve haematological indices of rats 28 days after treatment. This could be due to abundant polyphenols and reducing sugars. A positive correlation of $r^2 = 0.777$ was observed between serum polyphenol (proanthocyanidin) and PCV. The correlation between Hb and serum polyphenol was positive ($r^2 = 0.391$). The correlation between serum proanthocyandin and WBC was positive ($r^2 = 0.738$). Also, a positive correlation of $r^2 = 0.922$ was observed between serum polyphenol (anthocyanins) and PCV. The correlation between Hb and serum anthocyanins was positive ($r^2 = 0.610$). Also, the correlation between serum anthocyanins and WBC was positive ($r^2 = 0.741$). Interestingly, a positive correlation ($r^2 = 0.147$) was indicated between serum non -heme iron and Hb. Additionally, it was found that experimental rats given okro pepper soup, palm nut soup, and pepper soup had significantly higher serum TIBC concentrations than the control group (Table 5). The chelating potential of the soup extracts may be related to a number of possible mechanisms. Experimental studies have shown that phytochemical content and antioxidant activity is associated with iron chelating properties, also flavonoids in plant extracts can interact with iron in live organisms (Mira *et al.,* 2002). It may be suggested that the chelating property of the soup extracts might be due to the presence of polyphenols and flavonoid substances. Quercetin, chlorogenic acid, kaempferol, skimmianine and catechin in the soups may have a chelating effect on iron, thereby decreasing non-heme iron that the body cannot get a benefit of through haemoglobin synthesis. The findings of this study agree with the report of Hallberg and Hulthén (2000) who recommended that the structure of quercetin, catechol, and catechin were found to be the principal, common structure in polyphenols which bind iron. This binding occurs possibly by direct chemical binding, particularly of ferric iron, and maybe through chelating. Studies have shown that TIBC values increase in absolute iron deficiency but a decrease in iron overload (Antai *et al*., 2009). In this study, the increase in TIBC implies that the soup extracts may have inhibited iron absorption from the gastrointestinal tract. A negative correlation was found between serum non-heme iron and TIBC ($r^2 = -0.199$). Therefore, the findings of this study suggest that the soups may have protective effects in iron overload disorders. The iron chelating ability could be involved in the management of oxidative stress-stimulated deteriorative diseases like diabetes (Adefegha *et al*., 2015).

4. Conclusion

The established HPLC assay showed a good separation of the polyphenol compounds. Therefore, the method is suitable for the estimation of polyphenols in the serum of rats given soup extracts. Polyphenols such as quercetin, chlorogenic acid, kaempferol, ellagic acid, copaene, chlorogenic acid, skimmianine, inulin, butyrolactone, catechin, naringenin, glycyrrhizin, capsaicin, glycerretol, cinnamic acid, humulene, estragole and citral were found in the serum of rats given soup extracts. However, quercetin, kaempferol and chlorogenic acid were found to be more predominant in the serum. The varied polyphenol quantities may be due to the spices used in cooking the soups. The study has shown that indigenous soups popularly consumed in Delta State, may improve haematological indices owing to their polyphenol and flavonoids content. Nevertheless, rats fed with okro pepper soup had more effect, compared to palm nut soup and pepper soup. However, the intake of African indigenous soups popularly consumed in Delta State, especially okro pepper soup should be encouraged in improvement of human nutrition. Ingredients needed in preparation of the soups are available in Africa local markets.

References

Adefegha, S.A., Oyeleye, S.I. and Oboh, G. (2015). Distribution of phenolic contents, antidiabetic potentials, antihypertensive properties, and antioxidative effects of soursop (*Annona muricata* L.) fruit parts *in vitro. Biochemistry Research International,* 2015, 347673. https://doi.org/10.1155/2015/347673

Ahn, D., Wolfe, F. and Sim, J. (1993). Three methods for determining non-heme iron in turkey meat. *Journal of Food Science*, 58(2), 288–291. https://doi.org/10.1111/j.1365-2621.1993.tb04257.x

Aksan, A., Wohlrath, M., Iqbal, T.H., Dignass, A. and Stein, J. (2020). Inflammation, but not the underlying disease or its location, predicts oral iron absorption capacity in patients with inflammatory bowel disease. *Journal of Crohn's and Colitis,* 14(3), 316–322. https://doi.org/10.1093/ecco-jcc/jjz149

Antai, A.B., Ofem, O.E., Ikpi, D.E., Ukafia, S. and Agiang, E.A. (2009). Phytochemistry and some

https://doi.org/10.26656/fr.2017.8(5).321 © 2024 The Authors. Published by Rynnye Lyan Resources

haematological changes following oral administration of ethanolic root extract of *Gonglonema latifolium* in rats. *Nigerian Journal of Physiological Sciences,* 24(1), 79–83. https://doi.org/10.4314/njps.v24i1.46388

Dryden, G.W., Song, M. and McClain, C. (2006). Polyphenols and gastrointestinal diseases. *Current Opinion in Gastroenterology,* 22(2), 165–170. https://doi.org/10.1097/01.mog.0000208463.69266.8c

Ejueyitsi, O.E., George, B.O., Anigboro, A.A. and Okpoghono, J. (2022). Assessment of Frequency and Consumption Pattern of Some Indigenous Soups in Delta State. *Tropical Journal of Natural Product Research,* 6(4), 592-598.

Ejueyitsi, O.E., George, B.O., Anigboro, A.A. and Okpoghono, J. (2023). Antioxidant and polyphenol indices of popularly consumed indigenous soups in delta state, Nigeria. *Food and Humanity*, 1, 297-307. https://doi.org/10.1016/j.foohum.2023.06.

Ekakitie, L.I., Okpoghono, J., Orororo, O.C. and Ekakitie, O.A. (2021). Ameliorative prowess of bee honey in the tissues of rats administered aluminium nitrate. *Scientific African*, 12, e00782. https://doi.org/10.1016/j.sciaf.2021.e00782

Ekakitie, L.I., Orororo, O.C. and Okpoghono, J., (2021). Changes in Haematological Parameters of Aluminium-Exposed Rats Treated with Natural Bee Honey." *IOSR Journal of Environmental Science, Toxicology and Food* Technology, 14(5), 22-25.

George, B.O, Okpoghono, J., Osioma, E. and Aina, O.O. (2012). Changes in oxidative indices in *Plasemodium berghei* mice treated with aqueous extract of *Aframomum sceptrum*. *Frontiers in Science,* 2(1), 6-9. https://doi.org/10.5923/j.fs.20120201.02

George, B.O, Osioma, E. and Okpoghono, J. (2013) Effect of methanolic extract of Aframomum sceptrum on brain lipid profile and non-enzymatic antioxidant level in Streptozotocin Induced Diabetic Rats. *Nigerian Journal of Nutritional Sciences* 34(1), 13-16.

George, B.O. and Okpoghono, J. (2017). Antioxidant activity of water, ethanol and diethyl ether extracts of *Monodora myristica and Syzygium aromaticum*. *Direct Research Journal of Public Health and Environmental Technology,* 2(4), 30-35.

George, B.O., Atalor, B. and Okpoghono, J. (2019). Role of vitamin E, vitamin A and aqueous extract of *Aframomum sceptrum* in mice, infected with malaria parasites. *Nigerian Journal of Nutritional Sciences,* 40(1), 113-117. https://doi.org/10.5923/j.fs.20120201.02

George, B.O., Osioma, E. and Okpoghono, J. (2015). Effects of Aqueous Extract of *Xylopia aethiopica* and Vitamin E on Hepatic and Oxidative Enzyme Markers in Rats Exposed to Cyanide Toxicity. *International Journal of Advanced Research,* 3(11), 392-397.

Hallberg, L. and Hulthén, L. (2000). Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron. The *American Journal of Clinical Nutrition,* 71, 1147-1160. https://doi.org/10.1093/ajcn/71.5.1147

Iland, P.G., Cynkar, W., Francis, I.L., Williams, P.J. and Coombe, B.G. (1996). Optimization of methods for the determination of total and red free glycosyl-glucose in black grape berries of *Vitis vinifera. Australian Journal of Grape and Wine Research,* 2 (3), 171−178. https://doi.org/10.1111/j.1755-0238.1996.tb00105.x

Liu, K., Xiao, X., Wang, J., Chen, C.Y.O. and Hu, H. (2017). Polyphenolic composition and antioxidant, antiproliferative, and antimicrobial activities of mushroom *Inonotus sanghuang. Food Science and Technology,* 82, 154-161. https://doi.org/10.1016/j.lwt.2017.04.041

Mansi, K. and Lahham, J. (2008). Effects of *Artemisia sieberi* Besser (A.herba-alba) on heart rate and some hematological values in normal and alloxan induced diabetic rats. *Australian Journal of Basic and Applied Science,* 4(2), 57-62.

Maseko, T., Mabhaudhi, B., Ncube, S., Tesfay, H., Araya, M., Fessehazion, V., Chimonyo, A. and Ndhlala, C. (2019). Du Plooy Postharvest drying maintains phenolic, flavonoid and gallotannin content of some cultivated African leafy vegetables. *Scientia Horticulture,* 255, 70-76. https://doi.org/10.1016/j.scienta.2019.05.019

Mira, L, Fernandez, M.T, Santos M., Rocha, R., Florencio, M.H. and Jennings, K.R. (2002). Interactions of flavonoids with iron and copper ions: A mechanism for their antioxidant activity. *Free Radical Research,* 36(11), 1199–1208. https://doi.org/10.1080/1071576021000016463

Mishra, N. and Tandon, V.L. (2012). Haematological effects of aqueous extract of Ornamental plants in male Swiss albino mice. *Veterinary World,* 5(1), 19-23. https://doi.org/10.5455/vetworld.2012.19-23

Nagy, M. and Grancai, D. (1996). Colorimetric determination of flavanones in propolis. *Pharmazie*, 51, 100–111.

Odesanmi, S.O., Lawal, R.A. and Ojokuku, S.A. (2010). Haematological effects of ethanolic fruit extract of

 © 2024 The Authors. Published by Rynnye Lyan Resources

RESEARCH PAPER

Tetrapleura tetraptera in male Dutch white rabbits. *Research Journal of Medicinal Plant,* 4(4), 213- 217. https://doi.org/10.3923/rjmp.2010.213.217

Okonta, C.I., Okpoghono, J., George, B.O., Joseph, O. and Obiejogo, J. (2021). Antihyperlipidemic and Antioxidant Activity of *Syzygium aromaticum* Extract in Rats Fed Cycas Diet. *Tropical Journal of Natural Product Research,* 5(5), 959-962. https://doi.org/10.26538/tjnpr/v5i5.26

Okpoghono, J., George, B.O., Achuba, F.I., Igue, U.B., Okom, S.U., Seigha, A.A., Ugwuka, L.C. and Ayogoi, KI. (2021). Impact of Crude Petroleum Oil and *Monodora myristica* on Membrane bound ATPases and Erythrocyte osmotic fragility in-vivo. *Uniport Journal of Engineering and Scientific Research*, 6(1), 65-77.

Okpoghono, J., Achuba, F.I. and George, B.O. (2018a). Influence of *Monodora myristica* extracts on lipid profile of rats fed crude petroleum oil contaminated catfish diet. *Sokoto Journal of Medical Laboratory Science,* 3(1), 19 – 27. https://doi.org/10.1016/j.ijvsm.2018.03.006

Okpoghono, J., Achuba, F.I. and George, B.O. (2018b). Protective effect of *Monodora myristica* extracts on crude petroleum oil-contaminated catfish (*Clarias gariepinus*) diet in rats. *International Journal of Veterinary Science and Medicine*, 6, 117–122.

Okpoghono, J., George, B.O. and Achuba, F.I. (2018). Assessment of antioxidant indices after incorporating crude oil contaminated catfish and African nutmeg (*Monodora myristica*) extract into rats diet. *Journal of Applied Sciences and Environmental Management,* 22(2), 197-202. https://doi.org/10.4314/jasem.v22i2.7

Okpoghono, J., Omoriare, U.J., Igue, U.B. and Seigha, A.A. (2023). Evaluation of liver and kidney function markers of mature albino male rats fed with maize-plantain pudding delicacy cooked in metallic plates and edible plant leaves. *Journal of Applied Sciences and Environmental Management,* 27 (4), 759-766. https://doi.org/10.4314/jasem.v27i4.17

Okpoghono, J., Osioma, E., Metie, S.O., Igue, U.B., Isoje, E.F. and Okom, S.U. (2023). *Xylopiaa ethiopica* HPLC-DAD polyphenol profiling and antioxidant status from South-South region of Nigeria. *Ovidius University Annals of Chemistry,* 34 (2),104 – 111.

Omoike, G.O., George, B.O., Ugwu, E.O. and Okpoghono, J. (2022). Changes in the antioxidant properties of *Ocimum africanum* leaves aqueous extract from the south eastern region of Nigeria after in vitro gastrointestinal digestion, *International Journal of Food Properties,* 25(1), 2000-2008.

Onakurhefe, P., Achuba, F.I., George, B.O. and Okpoghono, J. (2020). Effect of *Elaeis guineensis* (Jacq) leaf extracts on crude oil-induced genotoxicity in Wistar albino rats. *Scientific African,* 7, e00280. doi:10.1016/j.sciaf.2020.e00280

Otuaga, E.J., Okpoghono, J. and George, B.O. (2020a). Non-heme iron content and antioxidant activities of Wistar rats fed aqueous extract of *Elaeis guineensis* (banga soup) cooked using different utensils. *Journal of Applied Sciences and Environmental Management,* 24(6), 1071-1077. https://doi.org/10.4314/jasem.v24i6.20

Otuaga, E.J., Okpoghono, J. and George, B.O. (2020b). Proximate Composition, Phytochemicals and Antioxidant Status of Banga Soup (*Elaeis guineensis* extract). *FUPRE Journal of Scientific and Industrial Research,* 4(2), 64-74.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A, Yang, M. and Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine,* 26, 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Rekha, M.N., Yadav, A.R., Dharmesh, S., Chauhan, A.S. and Ramteke, R.S. (2008). Evaluation of antioxidant properties of dry soup mix extracts containing Dill (*Anethum sowa* L.) Leaf. *Food and Bioprocess Technology*, 3(3), 441–449. https://doi.org/10.1007/s11947-008-0123-5

Ruiz-Torralba, A., Guerra-Hernández, E.J. and García-Villanova, B. (2018). Antioxidant capacity, polyphenol content and contribution to dietary intake of 52 fruits sold in Spain. *CyTA - Journal of Food,* 16(1), 1131–1138. https://doi.org/10.1080/19476337.2018.1517828

Seal, T. (2016). Quantitative HPLC analysis of phenolic acids, flavonoids and ascorbic acid in four different solvent extracts of two wild edible leaves, *Sonchus arvensis* and *Oenanthe linearis* of North-Eastern region in India. *Applied Pharmaceutical Science*, 6 (2), 157-166. https://doi.org/10.7324/JAPS.2016.60225

Sivakumar, D., Chen, L. and Sultanbawa, Y. (2018). A Comprehensive Review on Beneficial Dietary Phytochemicals in common traditional southern African leafy vegetables. *Food Science and Nutrition,* 6(4), 714–727. https://doi.org/10.1002/fsn3.643

Struchkov, P., Beloborodov V., Kolkhir V., Voskoboynikova I. and Savvateev, A. (2018). Comparison of spectrophotometric methods of total flavonoid assay based on complex formation with aluminium chloride as applied to multicomponent

© 2024 The Authors. Published by Rynnye Lyan Resources

herbal drug angionorm. *Journal of Pharmaceutical Negative Results,* 9, JPNR_22_17. https://doi.org/10.4103/jpnr.JPNR_22_17

Sun, B., Richardo-Da-Silvia, J.M. and Spranger, I. (1998). Critical factors of vanillin assay for catechins and proanthocyanidins. *Journal of Agricultural and Food Chemistry,* 46, 4267– 4274. https://doi.org/10.1021/jf980366j

Tchokouaha, L.A.R. Tchamgoue, A.D., Ann, O.M., Ogochukwu, O., Daniel, F., Ifeanyi, O., Ifunanya, O., Priscilla, N. and Agbor, G.A. (2015). Comparative Antioxidant Capacity of the Spices of Some Traditional Soups in Nigeria. *Scholars Academic Journal of Biosciences*, 3(1B),79-84.

Thrall, M.A. and Weiser, M.G. (2002). Haematology. In Hendrix, C.M. (Ed.) Laboratory Procedures for Veterinary Technicians. 4th ed., p. 29 -74. Missouri, USA: Mosby Incorporated.

Tietz, N.W. (1976). Fundamentals of clinical chemistry, p. 723. Philadelphia, USA: W B Saunders Co.

Ugwu, E.O., George, B.O., Omoike, G.O. and Okpoghono, J. (2021). Bioaccessibility of polyphenols and flavonoids in *Ocimum africanum* leaves extract. *Uniport Journal of Engineering and Scientific Research*, 6(1), 78-84.

Vanzani, P., Rossetto, M., De Marco, V., Sacchetti, L.E., Paoletti, M.G. and Rigo, A. (2011). Wild Mediterranean plants as traditional food: a valuable source of antioxidants. *Journal of Food Science,* 76 (1), 46–51. https://doi.org/10.1111/j.1750-3841.2010.01949.x

Vimbainashe, E., Sultanbawa, M.Y. and Sivakumar, D. (2020). Enhancement of the phytonutrient content of a gluten-free soup using a composite of vegetables. *International Journal of Food Properties,* 23(1), 1051-1065. https://doi.org/10.1080/10942912.2020.1778028

 © 2024 The Authors. Published by Rynnye Lyan Resources