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Effect of Selected Processing Methods on Nutrient, Phytochemical, and Carotenoid Profiles of Two Cultivars of *Pterocarpus santalinoides*

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Abstract: The aim of this research was to compare the nutrient and potential medicinal properties of two cultivars of *Pterocarpus santalinoides* leaves. Two cultivars of *Pterocarpus santalinoides* (Nturukpa) leaves **(**light green Avuo and dark green Oseleukwu) were investigated for their nutrient composition, phytochemical content, and carotenoid profiles. The effects of oven-drying at 55 °C for 1 hour and 20 minutes, sautéing in palm oil for 3 minutes, and blanching in boiling water for 5 minutes on the nutrients and phytochemicals were evaluated. The leaves were rich in protein (14.0%) and vitamin B_6 (73 mg/100 g). The leaves were also moderate sources of iron (6.2-7.4%), magnesium (42-44%), zinc (1.2%), and potassium (45.0-55.0%). Raw light green Avuo contained the highest amount of folate (vitamin B_9). The phenolic contents, cyanogenic glycosides, anthocyanins, and phytates of the two *P. santalinoides* cultivars were statistically similar ($p < 0.05$). Steroids, oxalate, tannins, and alkaloids (7.7%) were higher in the light-coloured cultivar (Avuo), while saponins and flavonoids (1.1%) were higher (p <0.5) in the dark green cultivar (Oseleukwu). The α-carotene, trans-β-carotene, and total β-carotene contents of the two *P. santalinoides* cultivars were statistically similar ($p < 0.05$). The most predominant peak in the chromatogram of the *P. santalinoides* was trans-beta-carotene. The nutrient and phytochemical composition of the two cultivars of *P. santalinoides* indicates that they are good sources of proteins and micronutrients, and their phytochemical content suggests that they possess medicinal value.

Keywords: *Pterocarpus santalinoides* cultivars; nturukpa; nutrients; phytochemicals; carotenoid profile; effects of processing.

1. Introduction

Pterocarpus santalinoides L'Herit. ex DC is a deciduous tree native to West Africa, belonging to the family Papilio*noideae* [1]. It is also found in the South. America - Paraguay, Brazil, Bolivia, Peru, Ecuador, Colombia, Venezuela, the Guyanas; and the Caribbean [2]. The young leaves are used as a culinary vegetable .in Nigeria. The raw seed was found to be toxic, but can be eaten when roasted. Its common names include nturukpa (Igbo), gbengbe (Yoruba), gunduru (Hausa), ikyarakwa or kereke (Tiv), and uturukpa. *P. santalinoides* (Igede). was found to possess significant antioxidant and antidiabetic activities [3]. Further studies are required to establish the importance of *P. santalinoides* in the treatment of diseases associated with oxidative stress and hyperlipidaemia. In traditional settings in

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rural Nigeria, the bark, roots, and leaves of *P. santalinoides* are used in medicinal preparations [4]. They are used to treat bronchial complaints, amoebic dysentery, stomach-ache, and sleeping sickness; to prevent abortion and ease childbirth, and as a tonic. The leaf is used for the treatment of stomach-aches, diarrhoea, and diabetes mellitus, as well as to enhance wound healing [5]. *Pterocarpus santalinoides* is used in Cameroonian traditional medicine to treat cardiovascular diseases, including hypertension [6]. Previous research has revealed the presence of various bioactive compounds, including flavonoids, tannins, saponins, steroids, alkaloids, and triterpenoids, which have determined the ethnobotanical uses of Pterocarpus santalinoides [7-9]. The role of underutilised green leafy vegetables in combating hidden hunger (micronutrient deficiencies) and promoting food security cannot be overemphasised [10]. This underutilised food source, which offers additional health benefits, requires further research to justify and scientifically validate its utilisation. The present research addresses the need for in-depth compositional analysis of the nutrient, phytochemical, and carotenoid profile of two cultivars of *P. santalinoides* leaves. It provides scientific information about them and their potential for use as food and as medicine. The effect of selected processing methods on the nutrients was also investigated.

2. Materials and Methods

2.1 Procurement of samples

The fresh tender leaves of two cultivars of *Pterocarpus santalinoides* (*Nturukpa*) were obtained from Eke Market in Afikpo North Local Government of Ebonyi State and bagged separately in polythene bags (Figures 1a and 1b).

2.2 Sample preparation

At the laboratory, the two cultivars of *P. santalinoides* twigs were destalked, and the tender leaves selected, washed in potable water, drained, and sliced into 1.5 m pieces. The sliced leaves were divided into four 500g portions, and each portion was subjected to a different processing treatment (oven drying at 50 °C for 1 hour and 20 minutes; sautéing with 20 mL of palm oil for 3 minutes; blanching at 100 °C in boiling water). The raw samples were used as the controls.

Figure 1a. *Pterocarpus santalinoide*s, (*Avuo*)

Figure 1b. Dark green *Pterocarpus santalinoides* (*Oselukwu*)

2.3. Determination of nutrient content

The moisture content, ash, protein, fat, crude fibre, and carbohydrate contents were determined according to the methods of the Association of Official Analytical Chemists [11]. The values obtained for protein, fat, and carbohydrate were used to calculate the energy content of the samples. The calcium and magnesium contents of the sample extract were determined using the Versanate EDTA complexometric titration method, as described by AOAC [11]. The atomic absorption spectrophotometer (AAS) was used to determine the concentration of trace elements as described by

AOAC [11]. The iodine value of the samples was determined by the Wijs method as described by Samantha *et al.* [12]. Vitamins B_1, B_2, B_3, B_6, B_9, and vitamin E were determined according to the method described by AOAC [11]. The vitamin C content of the samples was determined using the 2,4-dinitrophenylhydrazine (DNPH) method, as described by AOAC [11].

2.4. Determination of Phytochemicals

Preliminary qualitative phytochemical screening of the extract was carried out to determine the presence of secondary metabolites using the method described by Harborne [13]. Alkaloids were qualitatively detected by Dragendroff reagent (potassium bismuth iodide), flavonoids by Benedict's solutions, saponins by frothing test, tannins by Wohler's test, and phenols by Ferric chloride solution as described by Ajuru *et al*. [14]. Saponins, total phenols, and tannins in the leaves of Pterocarpus santalinoides were determined spectrophotometrically by the Folin-Ciocalteau method [16]. The method described by AOAC [11] was used for determining phytic acid. Alkaloid content was determined using the alkaline precipitation gravimetric method [11]. The Folin-Dennis colourimetric method, as described by Kirk and Sawyer [15], was used to determine tannin content in the sample. The flavonoid content was determined according to the AOAC method [11]. Oxalate was determined by the method described by Iwuoha and Kalu [16]. The alkaline titration method of AOAC [11] was used for the determination of cyanogenic glycosides in the sample

2.5. Protocol for carotenoid analysis of vegetables

Only the raw and sautéed leafy green vegetables were used to determine the carotenoid profile. Chromatographic peaks were identified based on the standards available.

2.5.1. Sample Extraction

A portion of about 10 g of homogenous sample was weighed into a mortar, and about 3 g of Hyflosupercel (celite) was added. The mixture was ground with 50 mL of cold acetone. After proper maceration in the mortar, the mixture was filtered with suction using a Buchner funnel with filter paper. The mortar, pestle, funnel, and residue were washed with small amounts of acetone, and the washings were received in the suction flask through the filter paper. Extraction was repeated 3-4 times until the final residue washed with acetone became devoid of colour.

The extract was *trans*ferred into a chromatographic column, which was previously packed with Alumina and made wet with petroleum ether (to remove chlorophylls and other esters that could interfere with the analysis). The collected fraction was then *trans*ferred to 500 ml separating funnel with Teflon stop cock. Twenty (20) mL of petroleum ether (PE) was added, followed by the addition of 300 mL of distilled water (slowly along the walls of the funnel) without shaking to avoid the formation of an emulsion. The two phases were allowed to separate, and the aqueous lower phase was discarded.

About 200 mL of distilled water was added about 4 times to wash and remove any residual acetone. During the last washing, it was ensured that the lower phase was completely discarded while the upper phase was retained. The petroleum ether phase was collected in a 25 mL volumetric flask (by making the solution pass through a small funnel containing anhydrous Sodium sulphate (about 15 g) to remove residual water). The funnel was washed with petroleum ether, and the washings were collected into the volumetric flask. The volume was made up to the mark using Petroleum ether, and the total carotenoids were determined spectrophotometrically. The total carotenoids (TC) content was calculated using the formula:

$$TC\ (\mu g/g) = A \times \text{volume (mL)} \times 10^4 / A^{1\%}\,1\text{cm} \times \text{sample weight (g)}$$

Where A = absorbance

Volume = total volume of extract: 25mL

$A_{1\%cm}$ = absorption coefficient of carotene in PE (2592)

2.5.2. Identification of the Carotenoids

The high-performance Liquid chromatography (HPLC) technique was used. Separation of carotenoids in samples was carried out using Waters e2695 HPLC systems equipped with a photodiode Array (PDA) Detector. The Petroleum ether extract in the extraction steps above was concentrated and

dried under nitrogen gas. It was reconstituted in 1 mL of a 50:50 (v/v) mixture of dichloromethane and methanol and filtered through a 0.22 µm PTFE syringe filter (Millipore) directly into injection vials. Ten microliters were then injected into the system. High-performance Liquid chromatography (HPLC) conditions were as follows.

Mobile phase: 50 % Methyl-tert-butyl ether (MTBE): 50 % Methanol; Column: Polymeric YMC $C_{30,}$ 5µm, 4.6 × 250mm; Isocratic elution for 10 minutes; Flow rate: 1 mL/minute; Equilibration: 10 minutes; Injection volume: 20µL.

Identification and Quantification of chromatograms were generated at 450 nm, and the identification of Lutein, α-carotene, and β-carotene (*cis* and *trans* isomers) was performed using external standards based on the calibration curve, along with verification of the absorption spectrum and co-elution with available authentic standards.

2.6. Statistical Analysis

Data were analysed using Statistical Package for Social Sciences (SPSS) version 23 (IBM SPSS Inc., Chicago, IL, USA). One-way analysis of Variance (ANOVA) was used to analyse the results. Means of duplicate/triplicate values were obtained, and separation of means was carried out using the Duncan Multiple Range test. Means were separated at a 95% confidence level.

3. Results and Discussion

Two cultivars of *Pterocarpus santalinoides (Nturukpa)* leaves (light green cultivar locally known as *Avuo* and dark green cultivar with a harder texture, locally known as *Oselukwu* were used for this research work. They are both forest trees.

3.1 Effect of Processing Methods on the Proximate Composition of Two Cultivars of P. santalinoides

The effect of processing methods on the proximate composition of two cultivars of *Pterocarpus santalinoides* (Light green Avuo and Dark green Oseleukwu) is shown in Table 1. All the proximate parameters of the leaves were significantly affected by processing. Oven-dried samples had higher fibre, ash, and protein contents. Sautéed samples had higher fat content.

Table 1. Effect of processing on the proximate composition of two cultivars of *Pterocarpus santalinoides*

Cultivars	Processing methods	Moisture content (%)	Crude Protein (%)	Crude Fat (%)	Crude Fibre (%)	Ash (%)	Carbohydrate (%)	Energy value (kcal)
Avuo **(Light green)**	**Raw**	63.05^{c}±0.07	14.44^{f}±0.01	1.96^{d}±0.01	2.11^{c}±0.01	3.73^{c}±0.02	14.69^{f} ±0.03	134.16^{f}±0.04
	Oven drying	44.68^{g} ±0.01	16.27^{b}±0.01	0.81^{f}±0.01	3.72^{a}±0.01	4.07^{b}±0.02	30.45^{b} ±0.01	194.17^{d}±0.03
	Sautéing	46.35^{e} ±0.03	15.88^{d}±0.02	3.42^{b}±0.01	3.60^{b}±0.01	4.01^{c}±0.01	26.75^{d} ±0.01	201.28^{b}±0.01
	Blanching	76.66^{a} ±0.01	12.95^{g}±0.01	0.18^{h}±0.01	1.61^{h}±0.01	1.66^{g}±0.02	6.95^{h} ±0.00	81.18^{h} ±0.00
Oseleukwu **(Dark green)**	**Raw**	57.79^{d} ±0.02	14.75^{e}±0.02	2.76^{c}±0.01	2.34^{d}±0.01	3.88^{d}±0.01	18.48^{e}±0.01	157.76^{e}±0.03
	Oven drying	45.15^{f} ±0.01	16.42^{a}±0.01	1.13^{e}±0.01	3.01^{c}±0.01	4.15^{a}±0.01	30.14^{c} ±0.03	196.41^{c}±0.01
	Sautéing	36.84^{h} ±0.01	16.01^{c}±0.01	3.98^{a}±0.01	2.02^{f}±0.01	4.07^{b}±0.02	37.09^{a} ±0.02	248.20^{a}±0.01
	Blanching	71.82^{b} 0.01	12.68^{h}±0.02	0.27^{g}±0.01	1.95^{g}±0.02	1.84^{f}±0.02	11.44^{g} ±0.03	98.91^{g} ±0.01

Results are the means of duplicate determinations. Means in the same column with the same superscript are not significantly different at (p<0.05). Means in the same column with different superscripts are significantly different at (p<0.05). *Avuo* =Light green *P. santalinoides* cultivar; *Oselukwu* = Dark green *P. santalinoides* cultivar

Pterocarpus santalinoides leaves contain high amounts of crude protein (14.44-14.75%), which is enhanced by sautéing and oven drying (15.88-16.01% and 16.27-16.42%, respectively). These processing methods also increased the ash, crude fibre, carbohydrate, and energy contents. Blanching resulted in the highest reduction in all nutrients. The fat content ranged from 1.96% to 2.76% in the leaf samples, while the ash content varied from 3.73% to 3.88%. However, these values are relatively high when compared with the data obtained by Agiang et al. for fresh *P. santalinoides* [17].

3.2 Effect of Processing Methods on the Vitamin Content of Two Cultivars of P. santalinoides.

The effect of processing methods on the vitamin content of two cultivars of *P. santalinoides* is shown in Table 2. Pro-vitamin A and vitamin E were improved by sautéing; vitamin B_6 was improved by blanching in the light green cultivar. Additionally, oven drying resulted in moderate retention of certain vitamins, such as vitamin B6 and vitamin C, in the leaves of *Pterocarpus santalinoides* (Nturukpa) cultivars.

Table 2. Effect of processing methods on the vitamin content of two cultivars of *Pterocarpus santalinoides*

Cultivar	Processing methods	Vitamin B_1 (mg/100)	Vitamin B_2 (mg/100)	Vitamin B_3 (mg/100)	Vitamin B_6 (mg/100g)	Vitamin B_9 (mg/100)	Pro-vitamin A µg/100g	Vitamin C (mg/100g)	Vitamin E (mg/100)
Avuo (Light green)	Raw	2.89^{e}± 0.01	0.22^{b} ±0.01	0.78^{b} ±0.03	73.05^{cd}±0.01	3.69^{a}±0.01	28.09^{e}±1.93	18.86^{b}±0.01	1.86^{c}±0.00
	Oven drying	2.43^{f}±0.04	0.15^{b} ±0.03	0.59^{b} ±0.01	68.03^{cde}±0.02	2.88^{d}±0.05	23.14^{cd}±1.15	15.84^{d}±0.02	1.28^{g}±0.00
	Sauteing	2.34^{g}±0.02	0.17^{b} ±0.03	0.65^{b}±0.01	56.22^{e}±0.01	2.79^{e}±0.00	243.72^{a}±11.02	14.84^{e}±0.01	3.59^{b}±0.00
	Blanching	2.48^{e}±0.00	0.23^{b} ±0.04	0.74^{b}±0.02	94.17^{a} ±0.01	3.19^{b}±0.09	15.82^{de}±2.71	13.42^{f}±0.01	1.33^{e}±0.00
Oselukwu (Dark green)	Raw	3.16^{a}±0.04	0.33^{ab}±0.02	0.91^{b} ±0.00	87.35^{ab}±0.01	2.99^{c} ±0.01	19.08^{cde}±0.58	20.39^{a}±0.01	1.55^{d}±0.00
	Oven drying	2.76^{d}±0.00	0.24^{b} ±0.02	1.82^{a}±0.00	79.67^{bc}±0.00	2.91^{cd}±0.01	14.19^{de}±1.91	17.02^{c}±0.02	1.31^{f}±0.00
	Sauteing	2.86^{c}± 0.02	0.28^{ab}±0.03	0.78^{b}±0.08	62.73^{de}±0.02	2.45^{f} ±0.01	225.25^{b}±7.52	15.82^{d}±0.03	4.30^{a}±0.00
	Blanching	3.06^{b}±0.01	0.55^{a} ±0.35	0.46^{b}±0.53	41.07^{f}±14.16	2.91^{cd}±0.00	8.95^{e}±0.35	14.85^{c}±0.02	0.69^{h}±0.00

Results are the means of duplicate determinations. Means in the same column with the same superscript are not significantly different at (p<0.05). Means in the same column with different superscripts are significantly different at (p<0.05). *Avuo* =Light green *P. santalinoides* cultivar; *Oselukwu* = Dark green *P. santalinoides* cultivar

There were no significant variations ($p < 0.05$) in the provitamin A content of *P. santalinoides* between the raw and other processed samples, except for sautéing, which was statistically different ($p < 0.05$). α-Carotene, *trans-*β-carotene, and total carotene contents of the leaves were statistically similar.

Pro-vitamin A acts in the body as an antioxidant, regulates metabolic reactions, maintains ocular health and immune function [18]. The vitamin has an integral role in regulating total metabolic reactions in the body. The highest concentration of provitamin A (243.72 µg) in this study is lower than the RDA required for pregnancy and lactation, according to the WHO [19], which is 800 µg. Raw Avuo contained the highest amount of folate (3.69mg/100g), while raw dark green Oseleukwu contained the highest amount of vitamin B_2 (0.33mg/100g) and vitamin B_3 (87.35mg/100g).

The vitamin C content of *P. santalinoides* was significantly lower (p<0.05) than *Gnetum africanum* (36.22 mg/100g), *Talinium triangulare* (65.34 mg/100g) and *Telfairia occidentalis* (86.20 mg/100g) reported by Oladejo [20]. Vitamin C is an antioxidant which also regenerates the active antioxidant form of vitamin E and enhances non-haem iron absorption [21]. It helps with cell adhesion, promotes the quick healing of wounds and cuts, and also fights mouth infections.

The highest concentration of vitamin C (20.39 mg) in this study is lower than the required daily allowance (RDA) during pregnancy and lactation, as per the WHO [19], which is 55.0 mg/100g. The highest concentration of vitamin E (4.30 mg) in the study is also lower than the Recommended Dietary Allowance (RDA) during pregnancy and lactation, as reported by Kominiarek et al. [22], which is 15.0 mg. However, Pterocarpus santalinoides (Nturukpa leaves) is a good source, providing at least 25% of the daily requirement of provitamin A, vitamin B6, vitamin C, Vitamin B1, and vitamin E.

3.3 Effect of Processing Methods on Mineral Content of Two Cultivars of P. santalinoides

The effect of processing methods on the mineral content of two cultivars of *P. santalinoides* is shown in Table 3. Some minerals were improved by processing (e.g., calcium retention in oven drying and sautéing, potassium retention in sautéing); additionally, the magnesium content was improved in oven-dried Avuo. Therefore, sautéing is considered a better processing method for retaining minerals, followed by oven drying.

The raw vegetables contained the highest amount of zinc (1.22-1.26 mg/100g), which was enhanced by oven-drying and sautéing. Raw Oseleukwu contained the highest amount of iodine (63.3 mg/100 g). The highest concentration of iron (7.75 mg/100g) in this study is lower than the Recommended Dietary Allowance (RDA) required during pregnancy and lactation, according to the

World Health Organisation (WHO) [19], which is 27.0 mg/100g. The highest concentration of zinc was found to be 1.29 mg/100g. Marginal zinc deficiency can reduce immunity. Those deficient in zinc, particularly children, are prone to increased diarrheal and respiratory problems.

Table 3. Effect of processing methods on mineral content of two cultivars of *Pterocarpus santalinoides* (mg/100g)

Cultivars	Processing Methods	Iron (Fe)	Magnesium (Mg)	Zinc (Zn)	Calcium (Ca)	Selenium (Se)	Potassium (K)	Iodine (I)
Avuo (Light green)	Raw	6.20^{c} ±0.28	42.12^{d}±0.04	1.22^{a}±0.06	3.99^{b}±0.03	0.26^{cd}±0.02	45.00^{bc}±4.24	50.88^{c}±0.03
	Oven Drying	6.95^{abc}±0.21	42.19^{d}±0.04	1.26^{a} ±0.05	4.08^{a}±0.04	0.34^{ab}±0.01	47.00^{b}±2.83	45.99^{f}±0.04
	Sauteing	6.70^{bc}±0.28	42.16^{d}±0.06	1.29^{a} ±0.03	4.09^{a} ±0.03	0.38^{a} ±0.03	55.00^{a}±2.83	48.84^{d}±0.01
	Blanching	7.05^{abc}±0.21	41.90^{e}±0.04	1.10^{b}±0.03	3.88^{c}±0.03	0.17^{ef}±0.04	40.00^{c}±1.41	50.39^{c}±0.07
Oselukwu (Dark green)	Raw	7.40^{ab}±0.28	44.77^{b}±0.04	1.27^{a}±0.04	3.67^{c}±0.04	0.19^{e}±0.01	55.00^{a}±2.83	63.33^{a} ±0.03
	Oven Drying	7.50^{ab}±0.57	44.90^{a}±0.03	1.26^{a} ±0.01	3.71^{de}±0.04	0.21^{de}±0.03	59.50^{a}±2.12	48.44^{de}±0.02
	Sauteing	7.35^{ab}±0.64	44.81^{ab}±0.05	1.28^{a} ±0.02	3.77^{d}±0.03	0.10^{bc}±0.02	57.00^{a}±2.83	48.12^{e}±0.78
	Blanching	7.75^{a}±0.35	43.32^{c}±0.08	1.24^{a} ±0.02	3.50^{f}±0.03	0.12^{f}±0.02	48.00^{b}±2.83	59.71^{b}±0.09

Results are the means of duplicate determinations. Means in the same column with the same superscript are not significantly different at (p<0.05). Means in the same column with different superscripts are significantly different at (p<0.05). *Avuo* =Light green *P. santalinoides* cultivar; *Oselukwu* = Dark green *P. santalinoides* cultivar

3.4 Effect of Processing on the Phytochemical Composition of P. santalinoides

Table 4. Effects of processing on phytochemical content of two cultivars of *P. santalinoides* leaves (mg/100g)

Cultivars	Processing methods	Steroid	Phenol	Saponin	Oxalate	Cyanogenic Glycoside	Anthocyanin	Tannin	Phytate	Alkaloid	Flavonoid
Avuo (Light green)	Raw	0.26^{a} ±0.03	0.17^{ab} ±0.01	0.34^{cd} ±0.03	0.39^{a} ±0.02	1.04^{a} ±0.73	0.21^{ab} ±0.02	1.36^{a} ±0.01	0.49^{a} ±0.02	7.73^{a} ±0.04	0.81^{b} ±0.01
	Oven Drying	0.24^{ab} ±0.02	0.14^{bc} ±0.01	0.32^{cd} ±0.02	0.36^{ab} ±0.03	1.48^{a} ±0.03	0.15^{cd} ±0.02	1.28^{b} ±0.02	0.41^{d} ±0.02	4.62^{c} ±0.03	0.67^{c} ±0.01
	Sauteing	0.26^{a} ±0.02	0.13^{bc} ±0.02	0.33^{cd} ±0.03	0.38^{ab} ±0.02	1.49^{a} ±0.02	0.16^{bcd} ±0.02	1.29^{b} ±0.01	0.43^{bcd} ±0.01	4.52^{cd} ±0.02	0.62^{d} ±0.02
	Blanching	0.22^{abc} ±0.02	0.11^{c} ±0.01	0.28^{d} ±0.03	0.29^{c} ±0.02	1.42^{a} ±0.02	0.12^{d} ±0.02	1.12^{c} ±0.03	0.48^{ab} ±0.02	3.38^{f} ±0.11	0.71^{c} ±0.01
Oselukwu (Dark green)	Raw	0.22^{bcd} ±0.01	0.22^{a} ±0.04	0.43^{a} ±0.04	0.33^{bc} ±0.02	1.46^{a} ±0.02	0.25^{a} ±0.02	1.11^{c} ±0.01	0.51^{a} ±0.01	7.23^{b} ±0.04	1.09^{a} ±0.01
	Oven Drying	0.18^{cd} ±0.02	0.21^{a} ±0.01	0.41^{ab} ±0.02	0.24^{d} ±0.02	1.38^{a} ±0.04	0.19^{abc} ±0.02	0.84^{e} ±0.00	0.47^{abc} ±0.04	4.42^{d} ±0.03	0.62^{d} ±0.03
	Sauteing	0.19^{bcd} ±0.02	0.19^{a} ±0.02	0.36^{bc} ±0.02	0.28^{cd} ±0.02	1.40^{a} ±0.03	0.22^{a} ±0.03	0.78^{f} ±0.01	0.42^{cd} ±0.02	3.68^{e} ±0.03	0.69^{c} ±0.01
	Blanching	0.14^{d} ±0.02	0.15^{bc} ±0.02	0.3^{cd} ±0.02	0.24^{d} ±0.02	1.31^{a} ±0.03	0.17^{bcd} ±0.02	1.04^{d} ±0.02	0.49^{a} ±0.02	3.79^{e} ±0.02	0.59^{d} ±0.02

Results are the means of duplicate determinations. Means in the same column with the same superscript are not significantly different at (p<0.05). Means in the same column with different superscripts are significantly different at (p<0.05). *Avuo* =Light green *P. santalinoides* cultivar; *Oselukwu* = Dark green *P. santalinoides* cultivar

Calcium concentration in the leaves was found to be in appreciable amounts. The highest concentration of calcium was 4.09 mg/100g. There was a higher potassium concentration in Oseleukwu (55 mg/100g) compared to the Avuo cultivar (45.00 mg/100g). From the results, the different processing methods used in this study had no significant effect on the iodine concentration. Iodine is an essential component of the thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3), necessary for normal growth, development, and metabolism during pregnancy, infancy, and throughout life [24]. When the physiological requirements for iodine are not met, a series of functional and developmental abnormalities occurs. Severe iodine deficiency results in hypothyroidism, endemic goiter and cretinism,

endemic mental retardation, decreased fertility, increased prenatal death, and infant mortality [24]. The iodine in the sample was in an appreciable amount. For all the minerals analysed in these leafy vegetables, magnesium, potassium, and iodine were the predominant elements; iron and calcium were moderately available. At the same time, zinc and selenium (trace elements) were not present in appreciable amounts. The highest concentration of iodine (63.33 mg/100g) in this study is lower than the RDA during pregnancy and lactation according to WHO [19], which is in the range of 150.250 µg.

The effect of processing methods on the phytochemical content of two *P. santalinoides* cultivars is presented in Table 4. There were no statistically significant variations ($p>0.05$) in the phenolic content, cyanogenic glycosides, anthocyanins, and phytates in the two *P. santanoloides* cultivars. Sterol, oxalate, tannin and alkaloids were higher in the light green cultivar, while saponins and flavonoids were higher in the dark green cultivar.

P. santalinoides leaves are edible, and their phytochemical contents were less than 5g or above, which has been reported as an indication of phytochemical toxicity [24]. The low tannin content (sautéed Osw and oven-dried Osw) in this study suggests that the leaves have little to no astringent properties [25]. Tannins are water-soluble phenolic compounds that precipitate proteins from aqueous solution. They occur in all vascular plants. Tannins bind to proteins, making them biologically unavailable. They protect the kidney and have antiviral, antibacterial, and anti-parasitic properties [25]. Leaves with tannins are used for the treatment of intestinal disorders, such as diarrhoea and dysentery [26]. Tannins hasten the healing of wounds and inflamed mucous membranes [25]. *P. santalinoides* has been found to be rich in phytochemicals [27].

3.5 Effect of Processing on the Carotenoid Profile of Two Cultivars of P. santalinoides

The effect of processing methods on the carotenoid profile content of two cultivars of *P. santalinoides* is shown in Table 5. Carotenoid content was maximally improved by sautéing, irrespective of the cultivar. Therefore, sautéing is considered a more effective processing method for retaining carotenoids. Oven drying resulted in minimal retention of carotenoids in the leaves of *P. santalinoides* (nturukpa) cultivars.

Table 5. Effect of processing on carotenoid profile of two cultivars of *P. santalinoides* (µg/g).

Cultivars	Processing methods	α-Carotene	13-*cis* β-Carotene	*trans* β-Carotene	9-*cis* β Carotene	Total β Carotene
Avuo (Light green)	Raw	$1.66^{b}\pm 0.01$	$6.01^{c}\pm 0.98$	$16.80^{b}\pm 3.56$	$4.45^{c}\pm 0.66$	$27.26^{b}\pm 1.92$
	Oven Drying	$2.22^{b}\pm 0.50$	$5.09^{cd}\pm 0.16$	$13.64^{bc}\pm 1.35$	$3.30^{cd}\pm 0.21$	$22.03^{bc}\pm 1.41$
	Sauteing	$23.83^{a}\pm 0.74$	$86.31^{a}\pm 0.52$	$108.15^{a}\pm 6.88$	$38.01^{a}\pm 0.68$	$232.46^{a}\pm 8.08$
	Blanching	$1.51^{b}\pm 0.32$	$3.58^{fg}\pm 0.25$	$9.26^{bc}\pm 2.35$	$2.23^{de}\pm 0.26$	$15.07^{bcd}\pm 2.86$
Oselukwu (Dark green)	Raw	$2.42^{b}\pm 0.33$	$4.77^{de}\pm 0.29$	$10.57^{bc}\pm 0.92$	$2.54^{de}\pm 0.21$	$17.87^{bcd}\pm 0.41$
	Oven Drying	$1.71^{b}\pm 0.86$	$3.80^{ef}\pm 0.45$	$7.26^{bc}\pm 1.15$	$2.02^{de}\pm 0.24$	$13.08^{cd}\pm 1.83$
	Sauteing	$22.53^{a}\pm 1.12$	$76.71^{b}\pm 0.01$	$102.75^{a}\pm 9.39$	$33.89^{b}\pm 1.27$	$225.23^{a}\pm 11.02$
	Blanching	$1.74^{b}\pm 0.15$	$2.58^{g}\pm 0.57$	$3.97^{c}\pm 0.33$	$1.53^{e}\pm 0.02$	$8.08^{d}\pm 0.42$

Results are the means of duplicate determinations. Means in the same column with the same superscript are not significantly different at ($p<0.05$). Means in the same column with different superscripts are significantly different at ($p<0.05$). *Avuo* =Light green *P. santalinoides* cultivar; *Oselukwu* = Dark green *P. santalinoides* cultivar

The increase in carotenoid concentration in the sautéed samples may be attributed to the dehydration of the cellular matrix and the improved extractability of carotenoids from the vegetables. This process involved the addition of minimal or no water in the sautéed samples [28].

Carotenoids are lipophilic, and they tend to be more bioavailable in fats. Moderate levels of α-carotene were found in both cultivars of *Pterocarpus santalinoides* vegetables, which may be related to the "channelled" conversion of α-carotene to lutein in the biosynthetic pathway through hydroxylase enzymes [29]. The increase in carotenoid concentration in the sautéed samples may be attributed to the dehydration of the cellular matrix and the improved extractability of carotenoids from the vegetables. This process involved the addition of minimal or no water [28].

Raw light green Avuo contained statistically higher 13-cis-β-carotene (6.01 µg/g) than the raw Osw (4.77 µg/g). Sautéed Avuo (86.31 µg/g) was statistically higher than sautéed Osw (76.71 µg/g). The 13-cis β-carotene data in raw and blanched samples in the study are moderately lower than raw *Telferia occidentalis* (26.47 µg/g) and cooked *Telferia occidentalis* (50.78 µg/g) reported by Okpalanma *et al.* [30].

Available literature suggests that the consequences of *trans*- and *cis*-isomerisation are changes in bioavailability and physiological activity [31]. Trans β-carotene is also an isomer of **β**-carotene Okpalanma *et al.* [30]. Sautéing may have caused the denaturation of carotene binding proteins, releasing the carotenoids so that they can be extracted more easily [28].

Many geometric isomers of carotene, including *trans*, 9-*cis*, 13-*cis*, and 15-*cis* isomeric forms, exist in food and human tissues [31]. The major carotene isomers in human blood circulation are trans-carotene, with small amounts of 13-*cis*- and 9-*cis*-carotene. However, circulating levels of the cis-isomers of β-carotene are not responsive to increased consumption of their isomers [31]. Besides, literature data suggest that each carotenoid shows an individual pattern of absorption, plasma transport, and metabolism [31]. The levels of *cis* isomers of carotene are much higher in leafy vegetables. The consequences of *trans-cis* isomerisation are changes in bioavailability and physiological activity [30]. The 9-cis-β-carotene level was significantly higher in sautéed light green cultivar (38.01µg/g) and sautéed dark green cultivar (33.89 µg/g). (Okpalanma *et al.* [31] reported that 9-cis-β-carotene of raw *Pterocarpus mildbraedii* was 12.30 µg/g and was significantly higher than the result obtained in this study (4.45 µg/g, 2.54 µg/g) but the sautéed samples of the two cultivars in this study (38.01 µg/g, and 33.89 µg/g) were higher than the cooked sample of *Pterocarpus mildbraedii* (27.25 µg/g) reported by Okpalanma *et al.* [31]. The 9-cis-β carotene content observed in this study was lower than that in raw and cooked *Talinum triangulare* (8.53 µg/g and 44.29 µg/g) reported by Okpalanma and Ojimelukwe [32] and *Solanum melongena* (eggplant) (1.48 µg/g) reported by Djuikwo *et al.* [29]. Increase in the carotenoid concentration in the sautéed samples may be due to dehydration of the cellular matrix and improved extractability of carotenoids from the vegetables [28].

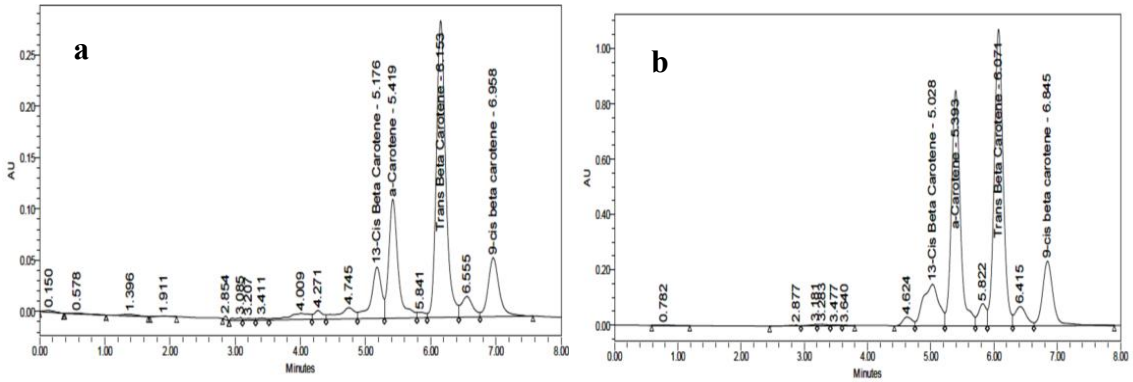

Figure 2a. Carotenoid profiles of raw (**a**) and sautéed (**b**) *P. santalinoides* (Light green cultivar).

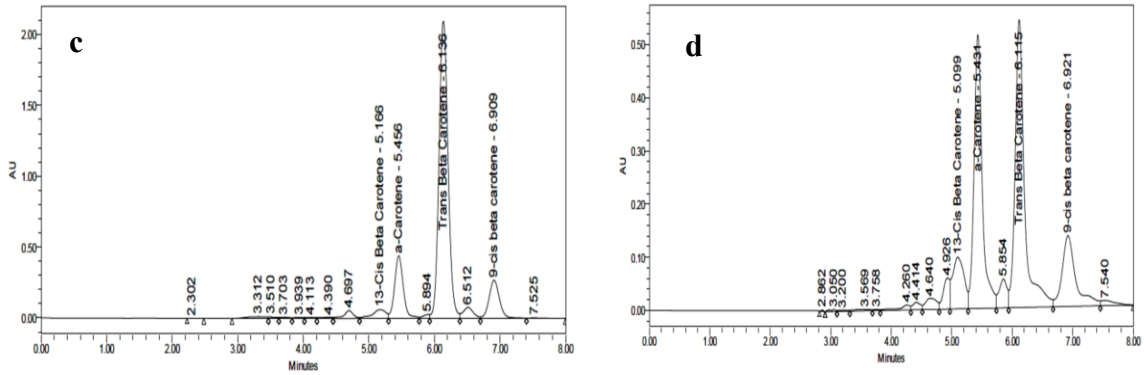

Figure 2b. Carotenoid profiles of raw (**c**) and sautéed (**d**) *P. santalinoides* (Dark green cultivar).

3.5.1 Total β-Carotene

Sautéing significantly improved ($p < 0.05$) the total *β*-carotene concentrations of the green vegetables compared with other processing methods. The total *β*-carotene (Tβ-c) content was significantly higher in sautéed light green leaves (232.46 µg/g,) and sautéed dark green Osw-225.23µg/g) than in raw light green leaf Avuo (27.26 µg/g), and raw dark green Osw (17.87µg/g), because denaturation of carotene binding proteins releases the carotenoids so that they can be extracted more easily [28]. Cooking methods are known to affect the retention of carotenoids. Total β-carotene isomerises into 13- and 9-*cis*-*β*-carotenes; to quantify *β*-carotene, it is necessary to add all the isomers. Therefore, the Avuo cultivar of *P. santalinoides* is a better source of carotenoids. The *β*-carotene content of vegetables varies widely [33].

3.5.2 Carotenoid Profile of the Raw Light Green P. santalinoides (Avuo cultivar)

The carotenoid profile of the raw light green *P. santalinoides* is shown in Figure 2a. The chromatogram shows up to four major peaks and several minor peaks. 13-cis-β-carotene eluted at about 5.2 minutes; *α*-carotene eluted at 5.4 minutes. *trans*-*β*-carotene eluted at 6.15 minutes (and was the highest peak), while 9-*cis*-*β*-carotene eluted after 7 minutes. The other peaks were not identified due to a lack of standards (see Figure 2a).

3.5.3 Carotenoid Profile of the Sauteed Light Green P. santalinoides

The carotenoid profile of the sautéed sample is shown in Figure 2a. Up to 7 peaks with broader peak areas than the raw samples were observed. While 13-cis-β-carotene eluted within 5 minutes, β-carotene eluted within 5.4 minutes. *trans*–*β*-carotene eluted within 6 minutes while 9-*cis*-*β*-carotene eluted after 6.8 minutes. There were about four other small but broad peaks which were not identified due to a lack of calibration standards. The peaks are more than those observed for the dark green cultivar.

3.5.4 Carotenoid Profile of the Raw Dark Green P. santalinoides

The Carotenoid profile of the raw dark green *P. santalinoides* (Oseleukwu cultivar) is shown in Figure 2b. The chromatogram shows four major peaks and several minor peaks. 13-cis-β-carotene eluted at about 5.2 minutes; *β*-carotene eluted at 5.5 minutes. *trans*-*β*-carotene eluted at 6.14 minutes (and was the highest peak) while 9-*cis*-*β*-carotene eluted after 6.91 minutes.

3.5.5 Carotenoid Profile of the Sautéed Dark Green P. santalinoides

The carotenoid profile of the raw *P. santalinoides* (dark green cultivar) is shown in Figure 2b. The chromatogram contains four major peaks and several minor peaks. 13-cis-β-carotene eluted at about 5.1 minutes; *α*-carotene eluted at 5.4 minutes. *trans*-*β*-carotene eluted at 6.12 minutes (and was the highest peak) while 9-cis-β-carotene eluted after 6.92 minutes.

3.5.6 Carotenoid Profiles and Their Nutritional Benefits

Sautéed *P. santalinoides* could be useful in alleviating vitamin A deficiency in developing countries because of its high concentrations of 13-cis β-carotene [29]. Out of the several different geometric isomers of *β*-carotene that exist in food and human tissues, the major *β*-carotene isomers in human circulation are *trans*-*β*-carotene, with a small amount of 13-*cis*- and 9-*cis*-*β*-carotene [30]. The results showed that the identification of the carotenoids according to the standard protocol was precise. Two classes of carotenoids, namely xanthophylls and carotenes, were identified and quantified under the HPLC conditions used. Only the carotenoids with major peak areas were further identified. β-carotene is the most abundant carotenoid and exhibits numerous pharmaceutical properties, including antioxidant, anti-obesity, anti-cancer, anti-ageing, anti-atherosclerotic and anti-sunburn properties. It also possesses hepatoprotective and neuroprotective properties. It improves vision and prevents night blindness [34]. Leaves from the genus Amaranthus contain higher amounts of carotenoids than *P. santalinoides* leaves (*Amaranthus. viridis* L. (2538 µg/g DW): *A. gangeticus* L. (789 µg/g DW), and *A.*

tristis L. (675 µg/g DW). [**36].** *P. brachycarpa* and *T. mongolicum* are also good sources of carotenoids [37, 38]. *P. santalinoides* leaves contain appreciable amounts of blood-forming elements and will therefore go a long way in preventing anaemia [35]. The nutrient composition and carotenoid content of these two *P. santalinoides* cultivars are expected to promote a healthy immune system in humans.

3.5.7 Comparison of Nutrients in the Two Cultivars of P. leaves

The results of this study revealed that *Pterocarpus santalinoides* leaves are a good source of macro- and micronutrients, phytochemicals, and carotenoids. The dark green *P. santalinoides* contained more protein, fat, crude fibre, ash, and carbohydrate than the light green cultivar. The light green cultivar contained more vitamin B9, while the dark green cultivar had more vitamins B1 and vitamin C. The dark green cultivar had more iron, magnesium, potassium, and iodine, while the light green cultivar had more selenium. The light green *P. santalinoides* cultivar contained more steroids, oxalate, tannin, total carotenoids, and alkaloids, while the dark green cultivar contained more saponin and flavonoids. The iodine and zinc content of these underutilised vegetables exceeds the World Health Organisation's recommended daily allowances for pregnant and lactating women. They are also moderate sources of iron. Of the three processing methods investigated, sautéing and oven drying at 50°C resulted in better retention of nutrients than blanching. Most of the carotenoids were better retained in light green Avuo leaf compared to dark green Oselukwu leaf; hence, Avuo was a better source of carotenoids. Compared to many other green leafy vegetables, *Pterocarpus santalioides* leaves should be sautéed to maximize carotenoid retention.

4. Conclusion

The two cultivars of *Pterocarpus santalinoides* analysed in this study are very good sources of nutrients and phytochemicals. While the dark green-coloured cultivar was found to provide more nutrients, the light green-coloured cultivar contained more phytochemicals. They are both very good sources of zinc and iodine. Further studies should be conducted to establish the medicinal value of *P. santalinoides* for the prevention of anaemia and the management of non-communicable diseases.

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