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Effect of Processing on Chemical Composition of *Struchium sparganophora* (Linn.) Ktze.

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Abstract: The effect of processing on the antioxidant properties and proximate composition of *Struchium sparganophora* leafy vegetable was evaluated. The processing included: abrasion with cold water (WH); abrasion and blanching with boiled water (WHH); abrasion with 10g of common salt (WS) and unprocessed vegetable (NP). Standard methods were used to analyze the processed and unprocessed vegetables for antioxidant activities, antioxidant phenolic compounds and the proximate. The results of the study showed that Protein content of unprocessed *S. sparganophora* was 3.64g/100g and was reduced to 1.94-2.66g/100g by processing. Fat and ash contents were likewise reduced by processing while fibre and moisture contents were not reduced by abrasion with cold water. *S. sparganophora* had antioxidant activity of 93.9%. Abrasion with cold water did not affect this value significantly (P=0.05). Neither did it affect the concentrations of anthocyanin and proanthocyanidin significantly (P=0.05). However, total phenol and flavonoid concentrations were significantly affected by processing. *Struchium sparganophora* is best consumed unprocessed, however, abrasion with cold water might be an alternative if the nutrients and the antioxidants in *Struchium sparganophora* will not be compromised
Keywords: *Struchium sparganophora*, common salt, Protein content, antioxidant.

INTRODUCTION

Struchium sparganophora (Linn.) Ktze. commonly called water bitter leaf and locally called "ewuro odo" in the south western Nigeria belongs to the Asteraceae Family. *S. sparganophora* is a water plant consumed as a leafy vegetable in Nigeria.

Vegetables play significant role in human nutrition, especially as a source of vitamins, minerals and dietary fibre [1]. Vegetables contain compounds that are valuable antioxidants and protectants. The main protective action of vegetables has been attributed to the presence of antioxidants, especially antioxidants vitamins and phenolic compounds such as flavonoids[2-3].

In Nigeria, green leafy vegetables are usually subjected to various post-harvest treatments such as blanching, soaking, abrasion with or without salt, in order to improve their palatability and to remove the bitter taste and some of the acids present in the vegetables [2]. The various processing techniques had been reported to alter both the nutrient, antinutrient and antioxidant property of some commonly consumed plant foods in Nigeria [4, 2].

This study therefore evaluated the effects of commonly practiced vegetable processing methods on the chemical compositions of *S. sparganophora*.

MATERIALS AND METHODS

Samples of *S. sparganophora* used in this study were collected at the fish pond of Department of Agriculture, Ife Central local Government, Ile-Ife, Osun-state, Nigeria in 2010. Prior to analysis, the plant leaves were destalked and washed with distilled water. The vegetable was then separated to 4 parts (about 500 g each). A portion was left unprocessed (NP) while other 3 parts were processed. A part was abraded with cold water (WH); the 2nd part was abraded with hot water (WHH) while the 3rd part was abraded with salt (WS). The processed and the unprocessed vegetables were oven dried at 45^0C for 24 hours. The dried leaves were milled and stored in the refrigerator for analysis.

Antioxidant Assay

The hydrogen donating or radical scavenging of the extract was determined using the stable radical DPPH (2, 2-diphenyl-2-picrylhydrazyl hydrate) according to the method described by Brand-Williams [5]. DPPH reacts with an antioxidant compound which can donate hydrogen, it is reduced. The change in colour from deep violet to light yellow was measured spectrophotometrically at 517 nm. 1 mg/ml of extract

solution was prepared. 1 ml of methanol was then pipetted into the cuvette followed by addition of 1 ml DPPH reagent in the spectrophotometer and kept in the dark for 30 minutes. The absorbance was read at 517 nm. This served as control. The absorptions of sample test containing 1ml of extract solution and 1ml of DPPH was prepared, kept in the dark for 30 minutes and read at 517 nm.

Determination of Total Phenols Content

Total phenol content was determined by the method of Singleton and Rossi [6] using the Folin – Ciocalteau reagent in alkaline medium. To 0.1 ml of extract, 0.9 ml of distilled water was added. 0.2 ml of Folin's regent was subsequently added and vortexed. After 5 minutes, 1 ml of 7% Na_2CO_3 solution was added to the mixture. The solution was diluted to 2.5 ml and incubated for 90 minutes at room temperature. Then, absorbance was read at 750 nm against reagent blank. For the standard solution, a stock solution of gallic acid 1 mg/ml was prepared. Then aliquots of 0.2, 0.4, 0.6, 0.8 and 1 ml were taken and each was made up to a total of 2 ml. From this range of concentrations, 0.1 ml was taken into different test tubes. The standard curve was prepared using 100, 200, 300, 400, 500 µg/ml solutions of gallic acid in methanol:water (70:30, v/v). Total phenol values was expressed in terms of gallic acid equivalent (mg/g of sample), which is a common reference compound.

Determination of Total Flavonoid Content

Total flavonoid content was determined using $AlCl_3$ method as described by Lamaison and Carnet[7]. To 0.1 ml of extract, 0.4 ml of distilled water was added, followed by 0.1 ml of 5% $NaNO_2$. After 5 minutes, 0.1 ml of 10% $AlCl_3$ was added, and then 0.2 ml of 1M NaOH and the volume was made up to 2.5 ml with distilled water. Absorbance was measured against reagent blank at 510 nm. For the standard solution, a stock solution of quercetin 1 mg/ml was prepared. Then aliquots of 0.2, 0.4, 0.6, 0.8 and 1 ml were taken and made up to a total of 2 ml. From this range of concentrations, 0.1 ml was taken into different test tubes. The standard curve was prepared using 100, 200, 300, 400, 500 µg/ml solutions of quercetin acid in methanol:water (70:30, v/v). Total flavonoid values was expressed in terms of quercetin equivalent (mg/g of sample), which is a common reference compound.

Determination of Total Proanthocyanidin Content

The proanthocyanidin content was determined using a modified method of Porter *et al*. [8] using the AlCl / Butan – 1-0l assay method. Extracts were diluted to provide spectrophotometric readings between 0.10 and 0.80 absorbance units. A 1.0 ml sample aliquot of adequately diluted extract was added to 9.0 ml of concentrated hydrochloric acid in n-butanol (10/90, v/v) in a screw top vial. The resulting solution was mixed by vortexing at a mid-range setting (5 to 6) for 10 to 15 seconds. Samples were heated for 90 minutes in an 85ºC water bath (Haake W19; Haake, Berlin, Germany) and then cooled to 15 to 25ºC in an ice bath. The absorbance at 550 nm was measured on a UV-visible spectrophotometer. A control solution of each extract was prepared to account for background absorbance due to pigments in the extracts. The control solution consisted of the dilute extract prepared in the hydrochloric acid/n – butanol solvent without heating.

Determination of Total Anthocyanin Content

The total anthocyanin content of the test samples was determined using the pH differential method of Fuleki and Francis [9] as described by Guisti and Wrolstad [10]. A pH 1.0 buffer solution was prepared by mixing 125 ml of 0.2 N KCl with 385 ml of 0.2 N HCl and 490 ml distilled water. The pH of the buffer was adjusted to pH 1.0 with 0.2 N HCl. A pH 4.5 buffer solution was prepared by mixing 440 ml 1.0 M sodium acetate with 200 ml 1.0 M HCl and 360 ml distilled water. The pH of the solution was measured and adjusted to pH 4.5 with 1.0 M HCl. 2 ml of anthocyanin extract was diluted to 50 ml in each of the pH 1.0 and 4.5 buffers and was allowed to equilibrate in the dark for 2 hours. The absorbance of the samples at 512 (A512 nm) and 700 nm (A700 nm) was measured on a Shimadzu 265 UV – visible spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD).

Proximate composition analysis

Crude protein, Carbohydrate, Ash, Crude fibre, Ether extract and Moisture contents were determined using the routine chemical analytical methods of Association of Official Analytical Chemists (AOAC)[11].

Moisture content:

About 2 g samples of the extracted pulp were measured out in triplicate and oven-dried at 90^0C for 72 hours when constant weight constituted the moisture content. The difference expressed as a percentage of the initial weight was the percent moisture content.

Crude fibre content:

To about 2 g homogenized pulp was added 1.25% hot tetra-oxo-sulphate (VI) acid in a 500 ml beaker. The content was covered and refluxed for 20 minutes. Filtration was done using vacuum pump and trap. The residue was washed thrice with hot water to remove the acid traces. The filtrate was then returned to the beaker and 200 ml hot 1.25% sodium hydroxide was added and refluxed for 30 minutes. Further filtration was done and the residue was washed, first with hot water followed with butan-1-ol and finally with hot tetra-oxo-sulphate VI acid. The residue was collected into a crucible, oven dried at 100ºC for 24 hours, cooled in a dessicator and weighed to obtain the weight of the crucible plus fibrous residue. The crucible was then transferred into a furnace set at 660 ºC for three hours for ashing. The content was thereafter cooled, and weighed to the obtain weight of

crucible plus ash. Thereafter, fibre content and % crude fibre content were calculated

Crude protein:

About 0.5 g of the homogenized pulp was weighed into Kjedahl flask. To the sample was added a 1: 2 mixture of perchloric acid and H_2SO_4. The sample was left to settle for 20 minutes and then transferred to a digester for one hour till sample turned colourless. The sample was then left to cool under fume-cupboard. The digest was distilled in a 500 ml flask using 100 ml distilled H_2O, 50 ml NaOH and selenium tablet. The flask was immediately covered and placed in a condenser. Attached to the condenser was a conical flask containing 50 ml boric acid which changed from red to green and volume was made up to 100 ml. The solution was then titrated using phenophtalein indicator against standardized sulphuric acid till the colour turned to red. From the entire procedure, the % Nitrogen was calculated.

Ether Extract/Fat content

About 2 g of sample was transferred into a fat free extraction thimble. The soxhlet extractor with a reflux condenser and a small flask was oven-dried, weighed and fitted up into a complete soxhlet apparatus. The fat free extraction thimble containing the sample was plugged lightly with cotton wool, petroleum ether was added and the thimble was placed in the soxhlet extractor. Additional petroleum ether was added until the barrel of the 300 ml extractor was half full. The condenser was replaced tightly and then placed on hot plate. The content was allowed to boil for 2 hours ensuring that the ether siphon until the siphoning was no longer noticed. The flask was detached and the contents of the extractor barrel was siphoned into ether stock bottle. The material was well drained. The content of the extractor thimble is removed and dried. The condenser and flask are replaced and then dried until the flask is practically dried. The flask is detached, cleaned and dried to constant weight. The difference between the initial flask weight and the final weight was ether extract (fat) content. The percent ether extract was then calculated.

Ash:

About 2 g of the sample was weighed into a crucible (Platinum), transfer into a murffle furnace. Ignite at 600ºC for 3 hours. It was removed and allowed to cool in a desiccator and final weight was taken, using analytical weighing balance.

Statistical analysis

Mean values of each of the analysed nutrients for each of the processing methods were generated from the three replicates and standard errors were calculated to reveal the degree of spread of the data.

RESULTS

The result of the proximate composition of *Struchium sparganophora* leafy vegetable as shown in Table 1, revealed that the leaf has 3.64g/100g protein. The food processing technique cause a significant reduction (P=0.05) in the protein (1.94-2.66g/100g) content. The trend was similar for ether extract (fat) and ash content. The ash content is an index of the mineral contents present in crops. However, there was no significant difference in the fibre contents of *S. sparganophora* that was not processed (4.35g/100g) and the vegetable that was abraded with cold water (4.08g/100g). Similarly, moisture content did not differ significantly (P=0.05) in the processed vegetable, the vegetable that was abraded with cold water and the one that was abraded with hot/boiled water. The carbohydrate content by difference was highest in *S. sparganophora* that was abraded with hot/boiled water (89.71g/100g) and lowest in the unprocessed vegetable (83.82g/100g). There was no significant difference (P=0.05) in the carbohydrate content of the vegetable that was abraded with cold water (87.94g/100g) and the one that was abraded with salt (87.90g/100g). (NaCl).

The result of the antioxidant properties of *Struchium sparganophora* leafy vegetable is presented on Table 2. The antioxidant activity of *S. sparganophora* is 93.6%. This value is not significantly different (P=0.05) from the vegetable that was abraded with cold water (92.9%). However abrasion of the vegetable with hot water and common salt significantly reduced the values. The values of the antioxidant activities of the vegetable processed with hot water and common salt did not differ significantly (P=0.05). The total phenol (mg/100g) concentration of *S. sparganophora* is 8628. The food processing techniques cause a significant decrease (P=0.05) in the total phenol concentration (2256-5822). The same trend was observed in the flavonoid concentration (6186mg/100g) of *S. sparganophora*. The values decreased significantly (P=0.05) a crossed the processing techniques (1635-4642mg/100g). However, abrasion with cold water did not significantly (P=0.05) affect the concentrations of anthocyanin and proanthocyanidin in *S. sparganophora*.

TABLE 1: Proximate Composition of *Struchium sparganophora* as affected by processing (Dry weight basis)

Processing methods	Crude protein g/100g	Crude fibre	Ether extract	Ash	Moisture	Carbohydrate
NP	3.64±0.12a	4.35±0.02ab	1.30±0.02a	6.43±0.11a	0.46±0.02a	83.82±0.04c
WH	2.66±0.03b	4.08±0.04a	0.89±0.02b	4.00±0.08c	0.43±0.02a	87.94±0.12b
WHH	1.94±0.05c	3.52±0.06c	0.59±0.13b	3.82±0.07c	0.42±0.12a	89.71±0.05a
WS	2.34±0.02bc	3.78±0.03bc	0.79±0.02b	4.88±0.12b	0.31±0.11b	87.90±0.04b

TABLE 2: Antioxidant properties of *Struchium sparganophora* as affected by processing (Dry weight basis)

	Antioxidant activities (%)	Phenol (mg/100g)	Flavonoid (mg/100g)	Anthocyanin (mg/100g)	Proanthocyanidin (mg/100g)
NP	93.2±0.12a	8628±0.13a	6186±0.08a	3.52±0.15a	1.01±0.20a
WH	92.9±0.03a	5822±0.11b	4642±0.12b	3.25±0.09a	0.99±0.13a
WHH	90.7±0.13b	2256±0.10d	1635±0.08d	1.00±0.13c	0.05±0.09c
WS	90.4±0.13b	5232±0.09c	4404±0.02c	1.84±0.15b	0.17±0.13b

Means with the same letter in each column are not significantly different at 5% level of Probability using Duncan's multiple range test.

DISCUSSION

Fruits and vegetables have been reported to b e very good sources of antioxidants especially vitamin C and phenols. In Nigeria, most green leafy vegetables are usually processed (blanched or abraded) before consumption. Processing is usually done in order to reduce the bitter taste or make it more palatable [2]. Nutritional factors are widely considered to be critical for human health. Overwhelming evidence from epidemiological studies indicate that diet rich in fruit and vegetables are associated with a lower risk of several degenerative diseases, however, the health-promoting capacity of fruit and vegetables strictly depends on their processing history [12].

In this study, blanching with hot water and abrasion with common salt were found to be more detrimental to the proximate composition and antioxidant properties of *Struchium sparganophora* leafy vegetable. However, abrasion with cold water did not significantly affect the chemical composition especially the protein, fibre, antioxidant activity and the anthocyanin contents of this vegetable. According to Oboh *et al.* [2], abrasion with salt in *Cnidoscolus acontifolus* brought about the highest decrease in the protein content, the decrease in the nutrient content caused by the processing most especially abrasion with salt could be attributed to the fact that some nutrients were leached off by water when they were being mechanically squeezed. The protein values for both the processed and unprocessed *Struchium sparganophora* leaf vegetable were comparable to the reported values for *Solanum meloneana, Solanum aethiopicum* (4.8%), *Solanum lycospersicum* (2.8%) *and Solanum nigrum* (3.2%), [13] and *Solanum macrocarpon* [2]. The antioxidant activity of *Struchium sparganophora* was higher than those reported for *Biden pilosa* (91.8%), *Celosia trigyna* (81.7%), *Crassocephalum crepidioides* (91.7%), *Launaea taraxacifolia* (67.2%) and *Solanum nigrum* (91.4%) [14].

Phenols have antioxidant capacities that are much stronger than those of Vitamin C and E. Flavonols and Flavonones are Flavonoids of particular importance because they have been found to posses' antioxidant and free radical scavenging activity in vegetables[3]. Some evidence has shown that flavonoids could protect membrane lipids from oxidation and a major source of flavonoids is vegetables and fruits [3]. The total phenol content of *Struchium sparganophora* is 8.6%, this value is much higher than what Oboh [2] reported for some commonly consumed green leafy vegetables in Nigeria namely: *Amaranthus cruentus, Ocimum gratissimum, Talfairia occidentalis, Baselia alba, Solanum macrocarpon, Corchorus olitorus,* which is 0.3%, while *Struchim sparganophora and Vernonia amygdalina* had 0.1% and 0.2% respectively.

CONCLUSION

Struchium sparganophora leafy vegetable is best consumed unprocessed, however, if processing of this vegetable is necessary, abrasion with cold water rather than with boiled water and/or salt is recommended.

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