Trop J Nat Prod Res, June 2021; 5(6):1094-1100
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Effect of Different Blanching Treatments on the Nutritional Composition, Phytochemical Contents and Antioxidant Activity of Dried *Moringa oleifera* Lam. Leaf Flour

Abimbola T. Ola-Adedoyin*, Samuel O. Etatuvie, Ifreke M. Luke, Kayode O. Olaniyan

Products Development & Quality Assurance Department, Nigeria Natural Medicine Development Agency, Lagos, Nigeria

ARTICLE INFO

Article history:
Received 15 December 2020
Revised 25 May 2021
Accepted 23 June 2021
Published online 01 July 2021

ABSTRACT

Moringa oleifera leaf is an exceptional vegetable with excellent nutritional and medicinal properties. Blanching improves nutrients availability in the leaf. This study aimed to investigate the effect of different blanching treatments on the nutritional, phytochemicals, and antioxidant properties of dried *M. oleifera* leaf. Freshly harvested *M. oleifera* leaf was divided into five parts. Four parts were blanched using different treatments while the fifth was unblanched. Blanching was done at 100°C for 3 minutes. Each part was dried using an electric dryer at 50°C for 7 hours and analyzed for nutritional content by proximate analysis using Standard Official Methods. HPLC was used to determine Vitamin A and C, Gallic acid for Phenol and flavonoid while antioxidant activities were evaluated with four in-vitro scavenging assays, using ascorbic acid as a standard. There was significant difference between the results for both the blanched and unblanched dried *M. oleifera* leaf: Moisture content (5.83-8.21%; 5.76%), protein (23.02-26.43%; 23.37%), carbohydrate (32.32-39.22%; 38.37%), crude fat (0.75-2.37%; 1.67%), crude fibre (17.74-29.31%; 23.87%), Vitamin-C (69.08-234.32mg/100g; 78.65mg/100g), Vitamin-A (17.10-25.99IU; 41.96IU), phenol (27.93-59.25mgGAE/g; 59.64mgGAE/g), flavonoid (27.98-51.77mgRE/g; 56.93mgRE/g), total antioxidant (12.00-71.56%; 27.36%), DPPH (18.06-87.52; 11.98-67.72%), FRAP (0.09-0.595%; 0.092-0.556%), and nitric oxide (5.12-82.16%; 19.74-68.79%) respectively ($p<0.05$). Blanching of *M. oleifera* leaf before drying should be encouraged to improve its nutritional value and for use as a functional food because of its abundant natural antioxidants. All blanching treatments exhibited loss of Vitamin A and minimal loss of phenol and flavonoid. Steam blanching at 100°C for 3 minutes should be encouraged.

Keywords: Blanching treatment, Antioxidant activities, Nutritional, Phytochemicals content, *Moringa oleifera*

Introduction

The world is increasingly faced with health challenges in which the abandonment of plant foods with medicinal properties is one of the main causative factors. Plants had been used for decades as food and medicine to enhance natural health. Plant foods have the ability to reduce the development of chronic diseases due to their different potent phytochemicals and numerous antioxidants. [1,2]

Moringa oleifera Lam. commonly called *Moringa* is one of the vegetables of the *Brassicales* order and belongs to the family *Moringaceae* and is a fast-growing plant with high drought tolerance.[3,4] *Moringa* is an exceptional vegetable of high valued nutritional properties, diverse phytochemicals and natural antioxidants.[5] *Moringa* leaf has moderate carbohydrate, low fat, high fibre and high-quality protein with all its essential amino acids in good proportions. The leaf is rich in minerals such as iron, calcium, phosphorous, copper, and vitamins such as B, C, D, E and betacarotene; a precursor for vitamin A.[6,7]

*Corresponding author. E mail: abimbolagrace05@gmail.com
Tel: +234 8096087186

Citation: Ola- Adedoyin AT, Etatuvie SO, Luke IM, Olaniyan KO. Effect of Different Blanching Treatments on the Nutritional Composition, Phytochemical Contents and Antioxidant Activity of Dried *Moringa Oleifera* Lam.. Leaf Flour. Trop J Nat Prod Res. 2021; 5(6):1094-1100. doi.org/10.26538/tjnpr/v5i6.19

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

The leaf also has low calorie which makes it a good supplement for the obese. In nursing mothers, *Moringa* leaf richness in phytosterols and its compounds leads to increase in estrogen production.

This production induces the openings of mammary glands ducts to produce more milk. It is also a good source of iron and calcium for pregnant women because of its richness in minerals.[8] Diverse phytochemicals are present in *Moringa* leaf such as tannins, sterols, terpenoids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugar along with anti-cancerous agents like glucosinolates, isothiocyanates, glucoside compounds and glycerol-1-9-octadecanoate. *Moringa* leaf has abundant natural antioxidants. These natural antioxidants improve the antioxidants system in the body and scavenge free radicals which causes chronic diseases like diabetes, cancer, inflammatory and cardiovascular disease, thereby maintaining and preserving good health. [8,9] Bioactive compounds like quercetin, kaempferol, zeatin, campesterol, sitosterol contributes to the medicinal properties of *Moringa* leaf.[10] *Moringa* leaf also contains therapeutically active compounds which have been isolated scientifically. They are various glycosides of thiocarbonate and isocyanide class e.g. pterygosperm moringyne, niaziridin, 4-(α-L-rhamnopyranosyloxyl benzyl isothiocyanate, 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate.[11] Moringa leaf has the capability to be applied in medicine and functional food preparation.[4] It can be eaten fresh, cooked or stored as dried powder for months without refrigeration.[12]

Blanching is a preliminary treatment in processing of vegetables. Blanching is commonly done at household level for the softening and removal of undesirable colour and taste from vegetables. In food processing industries blanching is an important unit operation mainly

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

used to inactivate enzymes that causes quality loss in stored vegetables. It helps to remove pesticides, reduce microbial load, and increase the bioavailability of nutrients. Blanching process should be done in a short time in order to maintain the chemical and the physical quality of the food product. Blanching is usually done prior to drying of vegetables.[13,14] Drying is a general method done for the preservation of vegetables. It brings the moisture content of the food product to a safe level where micro-organisms will not be able to survive.[15] Some studies have investigated the effect of few blanching treatments on nutritional, phytochemical and antioxidant activities of *Moringa oleifera* leaf.[5,16] However, commonly used household blanching treatments and its effect on the nutritional, phytochemical and antioxidant properties of *Moringa oleifera* leaf have not yet been reported. As a result of the gap in the above studies, there is a need to determine the effect of these different blanching treatments on its nutritional composition, phytochemical contents and antioxidant activity. Therefore, the objective of this study was to investigate the nutritional composition, phytochemical contents and antioxidant activities of blanched and unblanched dried *Moringa oleifera* leaf powder using different blanching treatments (boil water, boil water with sodium chloride, steam, and boil water with sodium bicarbonate).

Materials and Methods

Samples preparation

Fresh *Moringa oleifera* leaf was harvested from the botanical garden of Nigerian Natural Medicine Development Agency, Victoria Island, Lagos in February 2020 and deposited at the herbarium with voucher number MPNH/2020/1252. It was thereafter transferred to the laboratory department. About 1.5 kg of the leaf was harvested and divided into 5 parts of 300 g each. The leaf was sorted, all visibly deceased and discoloured leaf was removed. The leaf was separated from the petioles, washed properly with running water and allowed to drain. The blanching process was done at 100°C for 3mins. After blanching, the leaf was immediately cooled with running water to stop the blanching process.

1st part: Boil water blanching
2nd part: Boil water + sodium chloride blanching
3rd part: Steam blanching
4th part: Boil water + sodium bicarbonate blanching
5th part: Unblanched

Each part was dried separately at 50°C for 7 hours using the electric dryer. After drying, it was milled separately. Each leaf part was subjected to analysis for nutritional composition (proximate analysis and vitamins determination), phytochemical content and antioxidant activities.

Determination of Nutritional Composition

Proximate analysis was determined by standard methods of Association of Official Analytical Chemist.[17] Moisture content was determined until a constant weight was gotten. Ash content was determined by incineration in a muffle furnace at 550^{0}C. Crude fat was determined by Soxhlet extraction using the standard method. Protein determination was determined by the kjeldhal method as described by the standard method and conversion factor of 6.25 was used to calculate the protein content. Crude fibre was determined by digestion of the sample using the standard method. Carbohydrate was determined by difference as follows: 100 - (ash% + protein% + fat% + fibre% + moisture%).

Determination of Vitamin A

Vitamin A was determined using HPLC (High Performance Liquid Chromagraphy). 1 g of sample was weighed into a beaker and 10 ml of HPLC grade methanol was used to titrate the sample in a mortar. The homogenate with the residue was poured into a universal bottle and a further 10ml of methanol was used to wash the remnant of the sample into the bottle. This was left to stand for about 10 hours.

The sample was filtered using 0.45 µm cartridge filter, further cleanup was done before the sample was injected into HPLC.

Determination of Vitamin C (Estimation of Ascorbic Acid)

Ascorbic acid content was estimated by the method of Roe and Keuther[18] with slight modification. Ascorbate was extracted into 4% TCA by homogenizing 1 g of sample in it and the volume was made up to 10 mL with 4% TCA. The supernatant obtained after centrifugation at 2000 rpm for 10 minutes was treated with a pinch of activated charcoal, shaken well and kept for 10 minutes. Centrifugation was done one more time to eliminate the charcoal remnant. The quantity of the clear supernatants obtained was noted. Two aliquots different from the supernatant were taken for the assay (0.5 mL and 1.0 mL). The assay volumes were made up to 2.0 mL with 4% TCA (Trichloroacetic acid) 0.2 to 1.0 mL of the working standard solution containing 20-100 g of ascorbate respectively were pipetted into clean dry test tubes, the volumes of which were also made up to 2.0 ml with 4% trichloroacetic acid. 2,4-Dinitrophenylhydrazine (DNPH) reagent (0.5 mL) was added to all the tubes, followed by two drops of 10% thiourea solution. The osazones formed after incubation at 37°C for 3 hours, were dissolved in 2.5 mL of 85% H_2SO4, in cold, with no appreciable rise in temperature. To the blank alone, DNPH reagent and thiourea were added after the addition of H_2SO4. After incubation for 30 minutes at room temperature, the samples were read at 540 nm and the levels of ascorbic acid in the samples were determined using the standard graph was plotted on an electronic calculator to get the linear regression mode and written as mg ascorbate/g of leaf.

Determination of phytochemical contents

Estimation of total phenolic compound**:** 0.5 g sample of extract dissolve in 50 mL of water. 0.5 mL was added to 0.1 mL of Folin-Ciocalteu reagent (0.5 N) mixed and incubated at room temperature for 15 minutes. Later, 2.5 mL of sodium carbonate solution (7.5% w/v) was added and incubated for another 30 minutes at room temperature. The absorbance was measured at a wavelength of 760 nm. The total phenol concentration was written as Gallic Acid equivalent (GAE) (mg/g of dry mass) and is the mostly used reference value.

Total flavonoid content estimation**:** 1 mL of sample solution (100 µg/mL) was mixed with 3 mL of methanol, 0.2 mL of 10% Aluminum chloride, 0.2 mL of 1 M potassium acetate and 5.6 mL of distilled water. The mixture was incubated at room temperature for 30 minutes and the absorbance was measured at 415 nm. The calibration curve was done by using quercetin solutions at different concentrations in methanol.

Total antioxidant capacity determination: solution of the sample extract (1 mL) at a concentration of 100 mg/mL was mixed with 3 mL of reagent solution (0.6M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95°C for 90 minutes. Later the samples were cooled to room temperature, and the absorbance of each aqueous solution was measured at 695 nm. The total antioxidant capacity was measured as equivalent of ascorbic acid.

Determination of antioxidant activity

1,1 diphenyl-2-picryl hydrayl(DPPH) radical scavenging activity assay: An aliquot of 0.5 mL of extract in ethanol (95%) at different concentrations (25, 50, 75, 100 µg/mL) was mixed with 2.0 mL of DPPH solution (0.004 g in 100 mL methanol). The control was made of only DPPH solution in place of the sample and methanol was used as the blank. The mixture was strongly shaken and left in an upright position at room temperature. After 30 minutes the decrease in absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm. The percentage (%) inhibition activity was gotten from the below equation:

$$\text{DDPH radical scavenging activity (\%): } \frac{[(A_0 - A_1)]}{A_0} \times 100.$$

Where, A_0 is the absorbance of the Control and A_1 is the absorbance of the extract or standard.

Nitric oxide scavenging activity assay: 4 mL sample of plant extract and standard solution of different concentrations (25, 50, 75, 100 µg/mL) were taken in different test tubes and 1 mL of Sodium

nitroprusside (5mm in phosphate buffered saline) solution was added into the test tubes. They were incubated for 2 hours at 30°C to complete the reaction. 2 mL sample was withdrawn from the mixture and mixed with 1.2 mL of Griess reagent (1% Sulphanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2% H_3PO_4). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthylethylene diamine was measured at 550 nm.[19] Ascorbic acid was used as a standard.

Reducing power scavenging activity assay: Various concentrations of the extracts (25 to 100 µg/mL) in 1.0 mL of deionized water were mixed with phosphate buffer (2.5 mL) and potassium ferricyanide (2.5 mL). The mixture was maturated at 50°C for 20 min. Aliquot of trichloroacetic acid (2.5 mL) were added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper surface of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly made ferric chloride solution (0.5 mL). The absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations was used as standard.

Statistical analysis

Data was presented as mean±standard deviation (SD). All samples were run in duplicate. The difference between groups was analyzed by one-way analysis of variance (ANOVA) was compared with Duncan's Multiple Range Test (DMRT) and was used to determine the mean differences at 95% confidence interval. Mean difference at $P<0.05$ were considered significantly different. The result was used was analysed using IBM-SPSS version 25.0.

Results and Discussion

Table 1 shows the nutritional contents of the blanched and unblanched dried Moringa leaf. Proximate and vitamin analysis plays an important role in the determination of the nutritional composition of dried *Moringa* leaf. The carbohydrate content of the blanched samples ranges between (32.32 to 39.22%) and unblanched (UB) sample was (38.37%). The carbohydrate content of Boil Water Blanched (BWB) was significantly higher than the UB sample ($p<0.05$). The BWB sample had the highest carbohydrate content (39.22%) followed by the unblanched (UB) sample (38.37%) while the steam blanched (SB) sample and boil water + sodium bicarbonate blanched (BWSB) sample had lowest carbohydrate content of (32.85 and 32.32%) respectively. The BWB sample carbohydrate content increases significantly than all other samples($p<0.05$). The protein content of the blanched leaf ranges between (23.02 to 26.43%) and UB was (23.37%). Significantly BWB sample had the highest protein content of (26.43%) than the UB sample ($p<0.05$). BWB increases significantly than every other samples ($p<0.05$). UB sample yielded higher protein content (23.37%) than boil water + sodium chloride (BWSC) sample of (23.02%) but lower than BWSB sample (24.67%) ($p<0.05$). However, no significant difference was observed between SB and UB ($p>0.05$).

The crude fat of the blanched samples ranges between (0.75 to 2.37%) and UB sample at (1.67%) BWB sample had the highest crude fat value (2.37%) and significantly higher than the UB sample (1.67%)($p<0.05$). BWB increases significantly higher than and all other blanched samples ($p<0.05$). The UB sample had the highest crude fat as compared BWB, SB, and BWSB ($p<0.05$).

The moisture content of the blanched samples ranges between (5.83 to 8.21%) and unblanched sample was (5.76%) BWSB sample (8.21%) was significantly higher than sample BW, BWSC, SB, and UB ($p<0.05$) No significant difference between moisture contents of group SB and control ($p>0.05$). The ash value of blanched sample ranges between (6.87 to10.66%) and UB sample was (5.98%). BWSB sample was significantly higher than every other sample ($p<0.05$). The crude fibre of the blanched sample ranges between 17.74 to 29.31% and the UB, 23.87%. Crude fiber content (23.40%) of sample BWSB was significantly lower than the values for sample SB (29.31%) and BWSC (24.51%) ($p<0.05$) but significantly higher than group BWB (17.74%). No significant difference between crude fibre contents of BWSB and UB ($p>0.05$).

The vitamin C content of the blanched samples ranges between (69.08 to 234.32 mg/100 g) and unblanched sample was (78.65 mg/100 g). Vitamin C content was significantly higher in SB sample (234.32 mg/100 g) than all other samples ($p<0.05$), followed by BWSC sample (103.84mg/100mg) while the BWSB sample had a significantly lower vitamin C content of (69.08 mg/100 g). No statistically significant difference between samples BWB and UB ($p>0.05$). The vitamin A content of the blanched samples ranges between (17.10 to 25.99 IU) and the UB sample (41.96 IU). The UB sample had the highest vitamin A content (41.96 IU) as compared to other samples ($p<0.05$) except BWB (25.99 IU) sample which had the highest retention of vitamin A amongst the blanched samples. No significant difference was observed between SB, BWSB and BWB and SB ($p>0.05$)

BWB sample had highest carbohydrate content when compared to the blanched sample. The carbohydrate content of BWB sample was significantly higher than all other blanched samples and unblanched sample ($p<0.05$). Both blanched and unblanched Moringa leaves are good sources of energy. As reported by Meher *et al.*[6] that *Moringa* leaf is a good source of carbohydrate. The protein content of BWB sample was significantly higher than the UB sample ($p<0.05$). The protein value was higher than the results gotten by Mutiara *et al.*[20] and Isitua *et al.*[11] of (24.59 and 24.31%) protein content respectively but lower than results gotten by Alves *et al.*[21] of decolorized *Moringa* leaf flour. The high protein in BWB sample may be due to impact, reduction of particle size on the leaf which may lead to cell separation and this leads to extraction of more protein from the sample. Moringa leaf is a good source of cheap and abundantly available source of quality protein. There was significance difference in the crude fat content of BWB sample ($p<0.05$) when compared with UB sample. BWB sample had the highest fat content amongst all the samples. The high crude fat content of BWB can be due to high temperature of blanching which the leaf was subjected to, this causes the extraction of more fat content from the leaf. The values are lower than the findings of Isitua *et al.*[11] Moringa leaf is low in fat content as reported by Meher *et al.* [6.] Moisture content of all samples was less than 10; this inhibits microbial growth and makes storage easy for a long period of time at room temperature. The UB sample had the lowest moisture content amongst all the samples. The moisture content of the UB sample was significantly different from all blanched samples ($p<0.05$). Blanching causes disorderliness of the cells and makes it easy for moisture to escape. But the altering of the blanching process with cool water increases the moisture content of the disordered cells, which leads to increase in moisture content of the blanched leaf. Since both blanched and unblanched leaf was dried at the same temperature, this makes all blanched samples have the highest moisture content than the unblanched sample. There was significance increase in the ash content of BWSB sample ($p<0.05$) when compared with UB sample. BWSB had the highest ash content when compared to other blanching treatments, this may be due to addition of sodium biocarbonate to the water that was used for its blanching treatment. This was similar to the findings of Alves *et al.*[21] where undecolorized Moringa leaf has lower ash content compared to the decolorized leaf but lower to the value of Alves *et al.*[21] and Isitua *et al.*[11] Ash content of a food substance is the amount of minerals present in it. The (10.66%) ash content of BWSB shows that *Moringa* leaf is rich in minerals. There was significance increase in SB and UB sample fibre content ($p<0.05$). SB had the highest crude fibre content than the UB sample and all other blanched samples. Steam blanching prior drying serves as a protector to fibre content of Moringa leaf, other blanching treatment loss was due to leaching of nutrients into water. The findings were similar in Alves *et al.*[21], where the crude fibre of decolorized *Moringa* leaf was higher than the undecolourized leaf.

There was significant increase in vitamin C retention of SB than the UB ($p<0.05$). Blanching before drying offers a protective effect on *Moringa* leaf. This was similar to the report made by Nobosse *et al.*[5.] There was loss in vitamin C content of unblanched sample because of its easy liability to heat and oxidation on exposure heat. The great loss of vitamin C by other blanching treatments was due to the solubility of vitamin C in water, as a water-soluble vitamin and its instability to high temperature.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

UB sample had the highest retention of vitamin A when compared to other samples. Vitamin A content of UB sample increases significantly than all blanched samples ($p<0.05$) All blanching treatments had a negative effect on vitamin A. This might be due to washing away of the green colour during blanching which may signifies the presence of beta-carotene in the leaf. This was similar to the findings of Alves *et al.*[21] where non-decolorized *Moringa* leaf has higher antioxidant activity and vitamin A been an antioxidant agent. BWB with high vitamin A content compared to other blanched samples was due to the impact of boiled water on the leaf which causes the extraction of more vitamin A and since it is not a water-soluble vitamin it was not destroyed by blanching. But other blanched samples BWSC and BWSB had the lowest vitamin A content this may be due to the addition of sodium chloride and sodium bicarbonate to the blanching water.

Table 2 shows the phytochemical content of the blanched and unblanched dried leaf samples of *Moringa* flour. The phenolic content of the blanched samples ranges between (27.93 to 59.25 mg/100 g), UB (59.64 mg/100 g) and SB sample of (59.25 mg/100 g) had the highest phenolic content amongst all blanched samples. The UB and SB samples were not statistically different ($p>0.05$) but significantly higher than samples BWB, BWSC and BWSB ($p<0.05$). Sample BWB (52.30 mg/100 g) was significantly higher than sample BWSC (44.54 mg/100 g) and sample BWSB (27.98 mg/100 g) ($p<0.05$).

The Flavonoid contents of the blanched samples ranges between (27.98 to 51.77 mg/100 g) and UB was (56.93 mg/100 g**)**. The UB, SB, BWB and BWSC was not statistically different from each other ($p>0.05$) but was significantly different from BWSB (27.98 mg/100 g), ($p<0.05$). The total antioxidant capacity (TAC) of the blanched samples ranges between (12.00 to 71.56 mg/100 g) and unblanched sample (27.36 mg/100 g). BWSC sample (71.56 mg/100 g) was significantly higher than the UB sample ($p<0.05$). BWSC (71.56 mg/100 g) and BWB samples (71.20 mg/100 g) were not statistically different from each other ($p>0.05$) but was significantly higher than BWSB (12.0 mg/100 g) and UB (27.36 mg/100 g) ($p<0.05$).

In this study, phenol and flavonoid are of high content. Gallic Acid Equivalent (GAE) and Rutein Equivalent (RE) were used as standard for phenol and flavonoid respectively. UB sample had the highest antioxidants but not significantly different from steam blanched ($p>0.05$). Steam blanched had the highest phenolic content when compared with other blanching treatments. Phenols are known as secondary metabolites and non-nutritive compound but when consumed it has many health advantages. They are considered as the main compound that is responsible for oxidation.[15] UB sample had the highest flavonoid content compared to all the blanched samples but was not significantly different from SB sample ($p>0.05$). SB had the highest flavonoid amongst all blanched samples. The results of the above two phytochemical contents were similar to the results of Nobosse *et al.*[5] and Wickramasinghe *et al.*[15], in the study phenol and flavonoid content was higher in unblanched *Moringa* leaf. Antioxidant activity in plant extract is generally attributed to the presence of phenol compounds and other antioxidant components.[5] The high value of Phenol and flavonoid compounds shows that *Moringa* leaf is embedded with high antioxidant potential, this implies that *Moringa* leaf can be used as a functional food to improve the nutritional and health potential of the consumer. Both have health benefits such as antioxidant, anti-tumor, hypoglycemic, antiviral, anti-bacteria and anti-inflammatory.[11,15] Total Antioxidant Capacity (TAC). The TAC of BWSC sample increase significantly than the UB sample ($p<0.05$). BWSC had the highest antioxidant content than all other blanching treatments. The high value in BWSC may be due to activation of enzymes which lead to increase in antioxidant capacity.

Table 3 shows the DPPH % inhibition of the blanched and unblanched *Moringa oleifera* leaf samples. Percentage inhibition of all groups increased with increase in concentration with a minimum at 25µg/ml and maximum at 100 µg/mL. At a concentration of 25µg/ml, the percentage inhibition of all blanched samples ranges between (18.06 to 31.95%) all blanched samples had higher inhibition than the unblanched (11.98%). SB sample (31.95%) was significantly higher than the UB sample($p<0.05$). All blanched and UB samples were significantly lower than the F (ascorbic acid) (45.05%), ($p<0.05$). SB (31.95%)>BWB (24.88%) > BWSC (20.05%).

Table 1: Nutritional Composition of blanched and unblanched dried *Moringa oleifera* leaf flour

Parameters	BWB	BWSC	SB	BWSB	UB
Carbohydrate (%)	39.22^{a}±0.15	37.88^{b}±0.19	32.86^{c}±0.11	32.32^{d}±0.28	38.37^{e}±0.11
Protein (%)	26.43^{a}±0.25	23.02^{b}±0.12	23.54^{c}±0.13	24.67^{d}±0.25	23.37^{bc}±0.12
Crude Fat (%)	2.37^{a}±0.06	0.98^{b}±0.08	0.61^{c}±0.28	0.75^{d}±0.02	1.67^{e}±0.04
Moisture (%)	7.40^{b}±0.06	6.31^{a}±0.08	5.83^{c}±0.08	8.21^{d}±0.11	5.76^{ce}±0.08
Ash (%)	6.87^{a}±0.50	7.32^{b}±0.11	7.85^{c}±0.04	10.66^{d}±0.26	5.98^{e}±0.10
Crude Fibre (%)	17.74^{a}±0.45	24.51^{b}±0.43	29.31^{c}±0.08	23.40^{d}±0.38	23.87^{bd}±0.09
VITAMIN C (mg/100g)	83.17^{a}±0.45	103.84^{b}±0.45	234.32^{c}±4.01	69.08^{d}±0.80	78.65^{ae}±0.62
Vitamin A (IU)	25.99^{ab}±4.36	17.10^{b}±2.52	19.69^{bc}±2.40	18.96^{b}±2.73	41.96^{ad}±15.62

BWB: Boil water Blanching, BWSC: Boil water + sodium chloride Blanching, SB: Steam Blanching; BWSB: Boil water + sodium bicarbonate Blanching; UB: Unblanched. n = 2 mean ± SD. Values in a row followed by different letters in superscript are significantly different ($p < 0.05$).

Table 2: Phytochemical Content of Blanched and Unblanched *Moringa oleifera* Dried Leaf

Phytochemical content	BWB	BWSC	SB	BWSB	UB
Phenol (mg/100)	52.30^{a}±2.21	44.54^{b}±1.16	59.25^{c}±0.06	27.93^{d}±0.30	59.64^{ce}±0.11
Flavonoid (mg/100)	51.73^{a}±0.13	51.48^{a}±2.41	51.77^{a}±0.38	27.98^{b}±0.57	56.93^{a}±7.04
Total Antioxidant Capacity (mg/100)	71.20^{a}±0.86	**71.56^{a}±0.12**	70.03^{b}±0.35	12.00^{d}±0.23	27.36^{c}±0.17

BWB: Boil water blanching; BWSC: Boil water + sodium chloride blanching; SB: Steam blanching; BWSB: Boil water + sodium bicarbonate blanching; UB: Unblanching. n=2 mean± SD. Values in a row followed by different letters in superscript are significantly different ($p < 0.05$).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

At 50 µg/mL concentration, all the samples were significantly lower than the sample F (56.55%) ($p<0.05$). UB sample (29.37%) was significantly lower than all blanched samples ($p<0.05$) but no significant difference between samples BWB and SB, BWB and BWSB ($p>0.05$).

At 75 µg/mL concentration, Sample BWB (72.71%) was significantly higher than all other samples except the standard-F (79.92%) ($p<0.05$). Sample BWB (72.71%) was significantly than UB sample (48.50%). Samples BWSC and SB were not statistically different from each other($p>0.05$) but significantly different from samples BWSB and UB ($p<0.05$).

At 100 µg/mL concentration, blanched samples range between (79.37-87.52%). BWB and SB samples were not statistically different ($p>0.05$) but significantly higher than BWSC, BWSB, and UB ($p<0.05$).

DPPH (2,2-diphenyl-1-picrylhydrazyl) Antioxidant activities of *Moringa oleifera* leaf was measured using DPPH radical scavenging assay. There was continuous increase in DPPH absorbance with increase in the concentration of the sample and this implies increase in antioxidant activity. Phenols are considered as important compounds that exhibit antioxidants activities in plants and its increase leads to increase in antioxidant activity. The highest DPPH scavenging activity was found in SB at (25-50 µg/mL), BWSC at (75 µg/mL) and SB at (100 µg/mL) than the UB sample. SB sample increases significantly than the UB sample ($p<0.05$). This could be due to inactivation of polyphenol enzymes during blanching, which leads to increase in phenolic content and further increase in antioxidant activity of the leaf. The standard used ascorbic acid had the highest antioxidant scavenging activity than blanched and all unblanched samples. This was similar to the results of Unigbe *et al*.,[22] Omede [23] and Pujimulyani *et al*.[14] The result was different from Nobosse *et al*.[5,] This variation may be influenced by age of leaf, geographical location of the plant or method of extraction.

As shown in Table 4, the reducing activity of sample F which is the standard was significantly higher than all other groups at various concentrations (25 to 100 µg/mL). At 25 µg/mL, sample BWSC (0.13%) increases significantly ($p<0.05$) than the UB sample (0.09%). Samples BWB (0.09%) and UB (0.09%) were not significantly different and had significantly lower than the other samples ($p<0.05$). Group BWSC (0.13%) was significantly higher than SB (0.12%).

At 50µg/ml, BWSC sample (0.24%) was significantly higher than all other samples ($p<0.05$) except sample F (0.24%) ($p<0.05$). Samples BWB and UB, and SB and BWSB were not significantly different ($p>0.05$).

At 75 µg/mL, the percentage inhibition of blanched samples ranges between (0.31 to 0.39%) and UB (0.33%). BWSC sample (0.39%) was significantly higher than UB sample and BWB, SB, BWSB samples but lower than sample F (0.38%), ($p<0.05$). BWB (0.31%) and BWSB (0.33%) samples were not significantly different ($p>0.05$) but lower than SB (0.40%) and UB samples (0.33%), ($p<0.05$).

At 100 µg/mL concentration, BWSB sample (0.39%) also exhibited significantly higher reducing power than other samples ($p<0.05$) except for sample F. Samples BWB and BWSB were not significantly different ($p>0.05$).

There are few studies conducted on reducing power activity of *Moringa* leaf. In this study, the antioxidant effect of the reducing power activity increases with increase in the concentration of the assay used, this was similar to the findings of Saranya *et al*. [25] BWSC sample had the highest antioxidant activities when compared with all the blanched samples in all concentration. BWSC sample significantly increases ($p<0.05$) than the unblanched sample. The antioxidant reducing power activity of the standard sample F (ascorbic acid) was higher than all the samples.

Table 5 shows the % inhibition of the blanched and unblanched samples with nitric oxide. The standard recorded significantly higher values than all the groups at various concentrations of Nitric oxide. BWB increases significantly ($p<0.05$) than all other samples except at 75 µg/mL where group BWB (70.78%) and F (76.07%) were not statistically different ($p>0.05$). Percentage inhibition increases with increase in the concentration of Nitric oxide (down the column from 25 µg/mL to 100 µg/mL) across all groups with group BWB recording the highest values after the standard.

At 25 µg/ml, samples BWSC (8.26%) and SB (5.12%) recorded the least % inhibition as compared with UB (19.74%) and other samples ($p<0.05$). No significant difference between BWSB (17.84%) and UB (19.74%), ($p>0.05$) but they were significantly higher than other sample except sample F ($p<0.05$).

At 50 µg/ml,BWB sample(42.70%) had highest % inhibition than UB sample(37.8%). the BWB increases significantly than UB sample ($p<0.05$). All the samples had higher significantly % inhibition than sample BWSB (30.93%) except SB sample (24.96) ($p<0.05$)

At 100 µg/ml Nitric oxide, BWB (82.16%) and BWSC (74.48%) were significantly higher than SB (69.07%), BWSB (68.50%) and UB (68.79%) ($P<0.05$). However, SB and UB were not significantly higher than control ($p>0.05$).

Nitric Oxide Scavenging Activity**:** The antioxidant scavenging activity increases with increase in concentration of assay in all samples. Sample F, which is the standard had the highest nitric oxide scavenging activity in all concentrations. BWB sample had the highest scavenging activity of Nitric oxide amongst all the samples and at various concentrations. BWB sample increases significantly ($p<0.05$) than the UB sample. The result was similar to Saranya *et al*.[25] who studied using three vegetables (*Alternanthera sessilis*, *Cardisbspermum helicalabum* and *Celosia*). Nitric oxide in medicinal plants prevents pathogenesis and progression of cardiovascular system. It also helps to prevent pathogenesis of endothelial damage in diabetes patient.[26]

Moringa oleifera leaf, a valuable and affordable source of vitamin, macro-nutrient and phytochemical compounds. As a result of its important nutritional composition, it serves as another food to combat malnutrition, its numerous natural antioxidants can help to stop ageing, scavenge free radicals and alter oxidation process. Presence of its phenolic compounds which is the reason behind its therapeutic potential, fights against various diseases such as cancer, diabetes, cardiovascular disease. It also displayed free radical scavenging activity over DPPH, ferric reducing power and nitric oxide. Therefore, from the results of this study, the consumption of dried Moringa leaf is recommended to access its nutritional composition alongside its health benefits to maintain good health status and prevent chronic disease. The use of Moringa dried leaf is also recommended as a supplement and functional food to achieve its nutritional and therapeutic properties.

Precisely, it is not yet clear where the concentration of the DPPH starts diminishing because the higher the concentration the higher the percentage of the scavenging activity. Therefore, it is necessary to conduct further studies to know the point of declination of the efficacy of the scavenging activities.

Table 3: DPPH Scavenging Activity (%) of blanched and unblanched *Moringa oleifera* dried leaves

Concentration (µg/mL)	BWB	BWSC	SB	BWSB	UB	F
25 µg/mL	24.88^{a}±1.05	20.05^{b}±1.99	31.95^{c}±0.71	18.06^{b}±1.29	11.98^{d}±0.24	45.05^{e}±0.68
50 µg/mL	48.51^{a}±0.59	40.02^{b}±1.06	50.17^{ac}±0.35	47.34^{da}±0.35	29.37^{e}±0.82	56.55^{f}±1.36
75 µg/mL	72.71^{a}±0.71	69.80^{b}±1.06	69.47^{b}±0.12	64.98^{c}±1.76	48.50^{d}±1.77	76.92^{e}±0.76
100 µg/mL	87.11^{a}±0.12	83.78^{b}±0.35	87.52^{ac}±1.17	79.37^{d}±0.50	67.72^{e}±0.24	89.82^{f}±0.50

BWB: Boil water blanching; BWSC: Boil water + sodium chloride blanching; SB: Steam blanching; BWSB: Boil water + sodium bicarbonate

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 4: Reducing Power Activity (%) of blanched and unblanched dried *Moringa oleifera* leaf flour

Concentration (µg/mL)	BWB	BWSC	SB	BWSB	UB	F
25 µg/mL	0.09^{a}±0.02	0.13^{b}±0.03	0.12^{c}±0.01	0.11^{d}±0.01	0.09^{ae}±0.00	0.24^{f}±0.06
50 µg/mL	0.19^{a}±0.03	0.24^{b}±0.05	0.23^{c}±0.05	0.22^{cd}±0.04	0.20^{ae}±0.04	0.38^{f}±0.02
75 µg/mL	0.31^{a}±0.09	0.39^{b}±0.12	0.40^{c}±0.04	0.33^{ad}±0.09	0.33^{d}±0.08	0.48^{e}±0.03
100 µg/mL	0.54^{a}±0.04	0.60^{b}±0.04	0.58^{c}±0.06	0.54^{ad}±0.07	0.56^{ce}±0.05	0.63^{f}±0.01

BWB: Boil water Blanching; BWSC: Boil water + sodium chloride Blanching; SB: Steam Blanching; BWSB: Boil water + sodium bicarbonate Blanching; UB: Unblanched; F: Ascorbic acid. n=2 mean± SD. Values in a row followed by different letters in superscript are significantly different ($p < 0.05$)

Table 5: Nitric Oxide Scavenging Activity (%) of blanched and unblanched *Moringa oleifera* leaf flour

Concentration (µg/mL)	BWB	BWSC	SB	BWSB	UB	F
25 µg/mL	26.95^{a}±1.07	8.26^{b}±0.67	5.12^{c}±0.27	17.84^{d}±1.34	19.74^{de}±1.34	47.89^{f}±0.34
50 µg/mL	42.70^{a}±0.80	36.24^{b}±0.81	24.96^{c}±0.13	30.93^{d}±0.81	37.86^{e}±0.13	63.09^{f}±0.59
75 µg/mL	70.78^{a}±1.07	58.82^{b}±0.54	48.49^{c}±1.21	60.72^{d}±1.61	53.89^{e}±0.27	76.07^{af}±116
100 µg/mL	82.16^{a}±0.81	74.48^{b}±0.94	69.07^{c}±1.34	68.50^{cd}±1.34	68.79^{cd}±0.67	84.91^{e}±0.75

BWB: Boil water Blanching; BWSC: Boil water + sodium chloride Blanching; SB: Steam Blanching; BWSB: Boil water + sodium bicarbonate Blanching; UB: Unblanch; F: Ascorbic acid. n=2 mean± SD. Values in a row followed by different letters in superscript are significantly different ($p < 0.05$)

Conclusion

In this study, blanching prior to drying has been found to be an important process in the preservation of *Moringa oleifera* leaf which is seasonal and highly perishable. *Moringa* leaf which is easily accessible and in abundance during its season is an affordable source of important nutrient and valuable medicinal properties. It can serve as another food to fight malnutrition in developing countries and can also be used as a functional food because of its additional health benefits. Blanching has improved the carbohydrate, protein, vitamin C, fibre, ash while unblanched prior to drying retained more vitamin A and phytochemical contents of *Moringa* leaf. There was vitamin A loss and minimal loss of phytochemical contents in steam blanched leaf. Steam blanching at 100^{o}C for 3 minutes should be encouraged.

Conflict of interest

Authors declare no Conflict of Interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgement

We would like to appreciate the research and non-research staff of Nigerian Natural Medicine Development Agency, for the provision of the sample used, for the accommodating environment and supply of experimental facilities for this research work.

References

1. Jhansi D, Manjula K. Functional and nutraceutical properties of herbals and its application in food. Int J Sci Res. 2016; 5(7):196-198.
2. Indriasari Y, Wignyanto W, Kumalaningsih S. Effect of blanching on Saponins and Nutritional Content of Moringa Leaves Extract. J Food Res. 2016; 5(3):55-60
3. Ahmad Faitzat AR, Muhammad DI, Saie BK. Health Benefits of *Moringa oleifera*. Arab Pac J. Pl .2014; 15(20):8571-8576
4. Valdez-Solana MA, Mejia-Gar VY, Tellez -Valencia A, Garcia-Arenas G, Salas-Pacheco J, Alba-Romeo JJ, Sierra-Campos E. Nutritional Content and Elemental and Phytochemical Analyses of *Moringa oleifera* Grown in Mexico. J Chem. 2015:1-9
5. Nobosse P, Fombany EN, Mbofung CF. Effect of blanching methods on the ant phytochemical and antioxidant activities of *Moringa oleifera* leaves. Am J Food Sci Technol. 2017; 5(2):53-60
6. Meher J, Kannan R, Meher R. Effects of Blanching Media on the Yield Stress of Green Leafy Vegetable Puree. Intern. J Rec Technol Eng. 2019; 4:855-860
7. Rajput H, Prasad SGM, Srivastav P, Singh N, Suraj L, Chandra.R. Chemical and Phytochemical Properties of Fresh and Dried *Moringa oleifera* (PKM-1) leaf powder. Chem Sci Rev Lett. 2017; 6(22):1004-1009
8. Gopalakrishnan L, Doriya K, Santhoshkuma D. Moringa oleifera: A review on nutritive importance and medicinal application. Food Sci Hum Well. 2016; 5(2): 49-56
9. Fitriana WD, Ersam T, Shimizu K, Fatmawati S. Antioxidant activity of *Moringa oleifera* extracts. Indo J Chem. 2016; 16(3): 297-301.
10. Das PK, Siddika MA, Asha SY, Aktar S, Islam F, Khanam, JA, Rakib, MA. Investigation of phytochemicals and antioxidant activities in the leaves methanolic extract from *Moringa oleifera* plants grown in Bangladesh. J Pharmacogn Phytochem. 2019; 8(4):2502-2508
11. Isitua CC, Sanchez-Muros, Lozano MJ, Jaramillo CJ, DutanF. Phytochemical and nutritional properties of dried leaf powder of *Moringa oleifera* Lam. From Machala EL Oro Province of Ecuador. Asian J Plant Sci Res. 2015; 5(2):8-16
12. Oyeyinka AT, Oyeyinka SA. Moringa oleifera a Food Fortificant: Recent Trends and Prospects. J Saudi Soc Agric Sci .2018; 17(2) :127-136
13. Xiao H, Pan Z, Deng L, El-Mashad M, Yang X, Mujumdar AS, Gao Z, Zhang Q. Recent developments and trends in thermal blanching- A comprehensive review. Inf Proc Agric. 2017;4(2): 101-127

14. Pujimulyani D, Raharjo S, Marsono Y, Santos U. The effects of blanching treatment on the radical scavenging activity of white saffon. Int Food Res J. 2010; 17:615-624
15. Wickramasinghe YW, Wickramasinghe I, Wijesekara I. 'Effect of Steam Blanching, Dehydration Temperature &Time, on the Sensory and Nutritional Properties of a Herbal Tea Developed from *Moringa oleifera* Leaves. Int J Food Sci. 2020; 2020:1-11
16. Indriasari Y, Wignyanto W, Kumalaningsih S. Effect of blanching on Saponins and Nutritional Content of *Moringa* Leaves Extract. J Food Res. 2016; 5(3):55-60
17. AOAC Association Official methods of Analysis, AOAC International, Gaithersburg, MD, USA, (18th edition). 2000. 320-325 p.
18. Roe JH, Keuther CA. The determination of ascorbic acid in whole blood and urine through the 2, 4-dinitrophenylhydrazine. J Bio Chem. 1943; 147:399-407.
19. Ellis DJ, Yuan Z, Seto E. Determination of protein, lysine deacetylation. Curr Protoc Protein Sci. 2008; 14:12
20. Mutiara T, Harinjo, Estioasih T, Sriwahyuni E. Nutrient Content of Kelor (*Moringa oleifera* Lam.) leaves Powder under different blanching methods. Food Pub Health. 2012; 2(6): 296-300.
21. Alves AS, Teixeira EM, Oliveira GC, Arantes-Pereira L, Oliveira CC, Costa LL. Physicochemical characterization and antioxidant activity of decolorized *Moringa oleifera* Lam. Leaf flour. Nutr. 2017; 42:31.
22. Unuigbe CA, Okeri HA, Erharuruyi O, Oghenero EE, Obamed DA. Phytochemical and antioxidant evaluation of *Moringa oleifera* (Moringaceae) leaf and seed. J Pharm Bio. 2014; 11(2):51-57
23. Omede A. Total Polyphenolic Content and Antioxidant Properties of *Moringa oleifera* Leaf extracts. Anim Res Int. 2016; 13(2): 2454-2462
24. Inbathamizh L, Padmini E. Effect of geographical properties on the phytochemical composition and antioxidant potential of *Moringa oleifera* flower. Bio Med Rx. 2013; 1(3): 239-247
25. Saranya R, Deanesan G, Ramesh S, Gopi R. Quality and Antioxidant Potential of Leafy Vegetables. J Food Proc Technol. 2017; 8(9):1-6
26. Sierra-Campos E, Valdez-Solana MA, Perez-Velazquez JR, Garcia-Arenas G, Tellez- Valencia A, Avitia-Dominguez C. Moringa oleifera leaves extract regulate the activity of nitric oxide synthases and paraoxonase 1 in diabetic rat. MOJ Biorg Org Chem. 2018; 2(5):236-241