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Effect of cooking on the chemical composition of five leafy vegetables consumed in Cameroun

Effet de la cuisson sur la composition chimique de cinq légumes feuilles consommés au Cameroun

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Abstract
Tropical leafy vegetables are an interesting source of nutrients however, they contain components called anti-nutrients which interfere with nutriments thus preventing their biodisponibilities. The various elements contained in the vegetables sheets can be influenced by several factors. The aim of this study is to evaluate the effect of cooking method on the nutritional composition of five leafy vegetables consumed in Cameroon. *Cucurma maxima, Hibiscus cannabinus, Moringa oleifera, Solanum nigrum, Vernonia calvoana* were collected from home gardens. After harvesting, each vegetable was divided into three batches. A raw control batch (C), a batch cooked without kanwa (SN) and another batch cooked with kanwa (AN). The determination of total protein, total lipid, total and reducing sugar contents was carried out. The mineral content of the vegetables was determined by absorption spectrophotometry, while the oxalate content was determined by titration. Total carotenoids were also quantified. The results show that cooking with or without kanwa resulted in significant losses in protein, total carotenoids, total and soluble sugars for all vegetables. Cooking without kanwa resulted in an increase in lipid content in the *M. oleifera*, *H. cannabinu*, *V. calvoana* and *C. maxima* samples. The mineral content decreased with cooking without kanwa but increased after cooking with kanwa. cooking caused a reduction in oxalate content in all leafy vegetables.
Key words: Leafy vegetables, cooking, kanwa, nutrients, anti-nutrients.

Résumé
Les légumes feuilles tropicaux sont une source intéressante des nutriments, cependant, ils renferment des composantes appelées antinutriments qui interfères avec des nutriments empêchant ainsi leurs biodisponibilités. Les différents éléments contenus dans les légumes feuilles peuvent être influencés par plusieurs facteurs. Le but de cette étude est d'évaluer l'effet de la méthode de cuisson sur la composition nutritionnelle de cinq légumes feuilles consommés au Cameroun. *Cucurma maxima, Hibiscus cannabinus, Moringa oleifera, Solanum nigrum, Vernonia calvoana* ont été collectés dans des jardins familiaux. Après la récolte, chaque légume a été divisé en trois lots. Un lot témoin cru (C), un lot ayant subi une cuisson sans kanwa (SN), un autre lot ayant subi une cuisson avec kanwa (AN). La détermination des teneurs en protéines totales, lipides totaux, les sucres totaux et réducteurs a été effectuée. Celle des teneurs en minéraux des légumes a été effectuée par spectrophotométrie d'absorption, alors que les teneurs en oxalates ont été déterminées par titration. Les caroténoïdes totaux ont également été quantifiés. Des résultats, il ressort que la cuisson avec ou sans kanwa a occasionné des pertes significatives des teneurs en protéines, caroténoïdes totaux, sucres totaux et solubles quel que soit le légume considéré. La cuisson sans kanwa a plutôt permis l'augmentation des teneurs en lipides dans les échantillons de *M. oleifera*, *H. cannabinu*, *V. calvoana* et *C. maxima*. Les teneurs en minéraux quant à elles baissent avec la cuisson sans kanwa mais augmentent après cuisson avec kanwa. Le blanchiment a occasionné la réduction des teneurs en oxalates dans tous les légumes feuilles.
Mots clés : Légumes feuilles, cuisson, kanwa, nutriments, antinutriments.

I. Introduction

Most leafy vegetables are not consumed in their raw state. Therefore, they require pre-treatment (Yadang *et al.,* 2009) and cooking to avoid irritating or toxic effects (Richard, 2007). These treatments include washing, cutting, blanching, incorporation of food additives. The way in which foods are processed and cooked changes their nutritional value. Indeed, it is known that these operations change the chemical composition of agricultural products (Burotti *et al.,* 2020).

Generally, traditional leafy vegetables are consumed cooked in the preparation of many regional dishes, such as ndolè and foléré in Cameroon. The bitterness of *V. calvoana* (ndolè) and the acidity of *H. sabdariffa* (foléré) require several culinary operations such as washing with rubbing, blanching with or without kanwa. These treatments, although necessary, are not without drawbacks. Indeed, the cooking water carries with it other elements than bitterness or acidity, in particular mineral salts and vitamins (Tarek, 2002; Onyeike *et al.,* 2003; Ejoh *et al.,* 2005a). Processing also has its advantages. Blanching and cooking in water reduce the levels of anti-nutritional factors in vegetables which is favorable for the biodisponibility of their nutrients contents (Farinde *et al.,* 2018).

Despite the efforts of researchers in recent years to increase the level of knowledge of the nutritional potential of wild leafy vegetables, there is still an information gap to be filled, especially on the effect of processing methods on several of them (Adjatin, 2006, Burotti *et al.,* 2020). This study therefore aims to evaluate the influence of cooking on some nutritional parameters of leafy vegetables consumed in Cameroon.

II. Materials and methods

II.1- Sampling

C. maxima, H. cannabinus, M. oleifera, S. nigrum, V. calvoana were collected from home gardens. After harvesting, each species was rinsed with running water, trimmed and cut before being subjected to various treatments. cooking without kanwa (batch 1) consisted of soaking 450g of vegetables in a covered pot containing 1L of boiling water. After 10 to 20 minutes, depending on the species, the leaves were softened and transferred to a sieve for cooling. For cooking with kanwa (Batch 2), each species was boiled with 10 g of kanwa. After boiling 1L of water, the kanwa was added and the mixture homogenised until it dissolved. The leaves were then introduced into this boiling water. This operation is stopped as soon as the leaves have wilted. The third batch was made up of vegetables that had not been treated at all.

Each batch was spread out in the *RIVIERA α BAR* ventilated dryer for 24 hours at 45°C and then reduced to powder using a *CULATTI* type MFC electric grinder and sieved with a 2 mm sieve. The powder obtained was stored in hermetically sealed glass jars and used for the various analyses.

Kanwa

Limestone (Kanwa) is a mineral condiment that contains a mixture of salts composed of other minerals but is very rich in sodium carbonate, (NaCO3, NaHCO3, 2H2O) and its content can be as high as 99% depending on the deposits (Britton, 1995). In cooking, Cameroonian women use kanwa to soften foods that are difficult to cook and to reduce flatulence (beans, cowpeas), and to wash and preserve the green colour of vegetables (Britton, 1995; Kegah *et al.,* 2020).

II.2- Analysis of the chemical composition of different leafy vegetables

II.2.1- Determination of crude protein

The total protein content was determined by the method of Lowry *et al.* (1951)**.** This is the result of two reactions: The Biuret reaction between the peptide bonds of the proteins with the cupric bonds in an alkaline medium and the reduction of phosphomolybdenum-phosphotungsten by tyrosine and tryptophan present in the treated protein. The stain is a blue violet with an absorption maximum at 650 nm.

II.2.2- Determination of some mineral composition

Minerals (calcium, iron, zinc) were determined spectrophotometrically. Sodium contents were determined by flame photometry (Benton and Vernon, 1990).

II.2.3- Determination of crude fat

Hexane soluble material in food was extracted from dried sample using a Soxhlet Extraction apparatus. The hexane was evaporated and residue was weighed (Bourely, 1982**).**

II.2.4- Determination of total sugars

Total sugars were extracted and determined spectrophotometrically according to the method of Dubois et *al.* (1956) quoted by Karamoko *et al.* (2016). In the presence of 1.5N sulphuric acid and under heat, the available carbohydrates are hydrolysed to simple sugars. The 3,5-dinitrosalicylic acid (DNS) reacts with the simple sugars under heat to give a red-brown complex (3-amino 5-nitrosalicylic acid) which shows an absorption maximum at 540 nm.

II.2.5- Determination of Total oxalate contents

Total oxalate contents were determined by hot titration of an aliquot of the sample extract with 0.01 M KMnO solution (AOAC, 1970**).** Approximately 0.75 g of dry, ground sample was added to a 100 mL flask containing 76 mL of distilled water and 4 mL of 6 N HCl. The mixture was heated in a boiling water bath for 1 hour, cooled in an ice bath and the volume adjusted to the mark with distilled water. After a first filtration, 2 aliquots of 40 mL were introduced into 2 beakers which received 20 mL of 6 N HCl each. The mixture was evaporated to half the initial volume and filtered. The precipitate retained on the filter paper was washed several times with hot water until a volume of about 60 mL was obtained. The filtrate obtained was then treated with 3 drops of methyl red and 25% ammonia until a faint yellow colour was obtained. The solution was then boiled in a water bath, cooled in an ice tray and filtered to remove the precipitate of ferrous ions. The filtrate obtained was boiled, then 5 mL of 5% CaCl2 was added under constant stirring and left to stand overnight. It was then filtered and the calcium oxalate precipitate retained on the paper was transferred to a beaker with distilled water and 25% sulphuric acid until completely dissolved. To precipitate the heavy metals, 5 mL of tungstophosphonate reagent was added to the acidified extracts and the mixture was centrifuged at 5000 rpm for 15 min. The supernatant was then titrated under constant stirring with 0.05M potassium permanganate solution. A control was carried out in parallel and calibration was carried out using an oxalic acid solution.

II.2.6- Determination of carotenoid content

The determination of the carotenoid content of the leafy vegetables was done by the method of Wolff, 1968. One gram (01g) of sample was triturated in 10ml of hexane. The solution was collected in a 20ml flask and made up to the mark with hexane. The absorbance was determined between 420 and 490 nm by spectrophotometer. The control vessel was filled with hexane.

II.2.6- Statistical analysis

The results were processed by analysis of variances at a threshold of 5% with SPSS 20.0 software and by Duncan's multiple comparison test.

III. Results and discussion

III.1- The influence of cooking on some nutrients

Proteins contents

The crude proteins contents vary with the type of vegetable (Figure1). *M. oleifera*, with 16.99±0.5g/100g DM has the highest content and *C. maxima* (5.62±0.65g/100g DM) the lowest value. The protein content of *M. oleifera* although high, is lower than that found by Ibok *et al.,* 2008 for the same species (27.51g/100 DM). Nevertheless, all the five leafy vegetables studied have higher protein contents than those reported for spinach (3.1g/100g DM) and for *A. triangularis* leaves 2.9g/100g DM by Salazar *et al.*, 2005**.** These results show that *M. oleifera* could be a potential source of protein that is less expensive and available to consumers.

Cooking with or without kanwa resulted in significant losses in protein content for all vegetables. Indeed, a 66.07% reduction was obtained for *M. oleifera* cooked with kanwa and 60.07% for that cooked without kanwa.

The reduction observed for the samples cooked with kanwa could be explained by the fact that the proteins would have been solubilised in the water following their denaturation during cooking. Heat is capable of destroying the nutritional component of perishables including vegetables because many of the nutritional factors found in vegetables are liable to heat (Olayinka *et al.,* 2015).

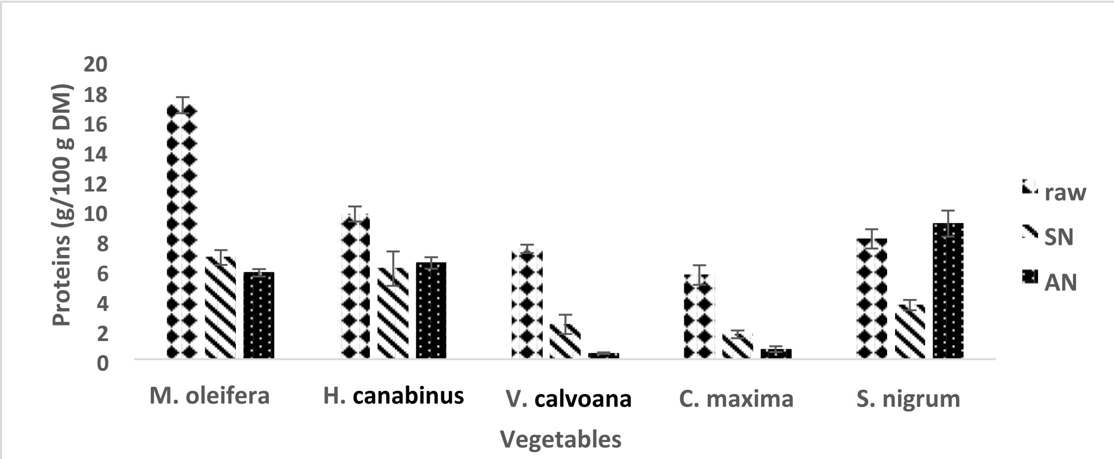

Figure 1: Proteins contents of leafy vegetables according to cooking method.

C= Raw SN =Without kanwa AN =With kanwa

Fats contents

M. oleifera gave the highest fat content (10.816±0.26g/100g DM) followed by *S. nigrum* (10.28±0.345g/100g DM), *H. canabinus* (10.14±0.173g/100g DM) and *C. maxima* (7.01±0.158g/100 DM) (Figure 2).

The fats contents of all the vegetables studied are higher compared to those of *H. sabdariffa* leaves (4.75g/100g DM) and *V. unguiculata* leaves (4.23g/100g DM) obtained by Oulai *et al*. (2014). *V. calvoana* and *C. maxima* have similar contents to *G. latifolium* (6.07g/100g DM) and *T. indica* (7.20g/100g DM) reported by Afolabi 2008; Ajayi *et al.* 2005**.** Lipids also act as a carrier for fat-soluble vitamins (A, D, E and K), ensuring their transport within the body and are precursors to molecules essential to the body (Murray *et al.*, 2013). However, the dietary importance of these fats depends on their fatty acid composition.

Cooking without kanwa resulted in an increase in fat content in *M. oleifera* (93.89%), *H. cannabinus* (13.01%), *V. calvoana* (14.98%) and *C. maxima* (72.82%). Similarly, after cooking with kanwa, there was an increase of 12.02% for *Moringa*, 82.87% for *V. calvoana* and 94.70% for *C. maxima.* The softening of the cells by cooking would have made the lipids more available hence the increase (Vodouhe *et al.*, 2012).

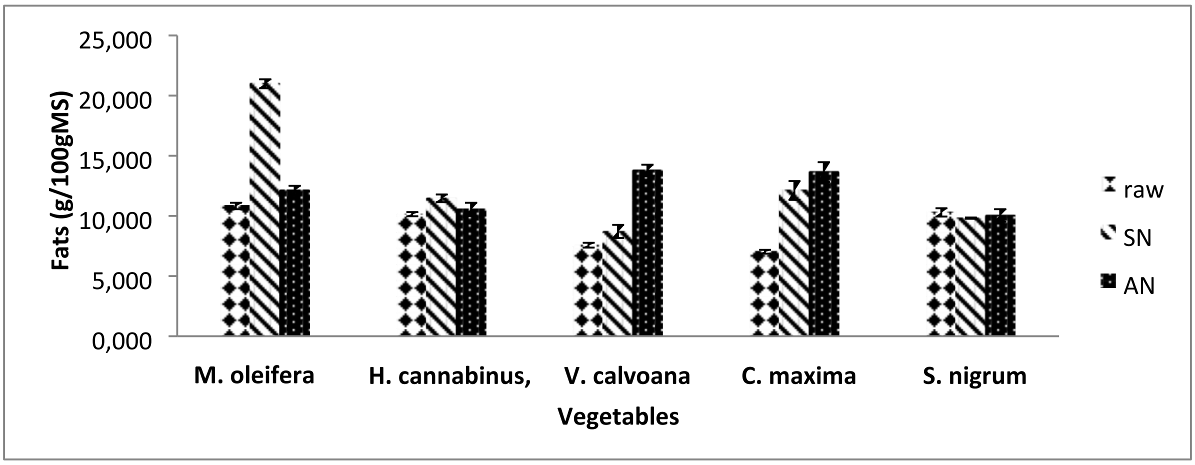

Figure 2: Fats contents of leafy vegetables according to cooking method.
C= Raw SN = Without kanwa AN =With kanwa

Total and reducing sugars contents

The results of the analyses reveal that the total sugar contents of *M. oleifera* (20.50±0.91g/100g DM, *S. nigrum* (18.52±3.34g/100g DM) and *C. maxima* (17.48±0.56g/100g DM) between which there is no significant difference are the highest (Figure 3). Concerning free sugars, the highest content is given by *M. oleifera* (17.63g/100g MS) and the lowest by *C. maxima* (5.82±0.67g/100g MS) (Figure 4).

Figures 3 and 4 show that cooking with or without kanwa reduces the sugar content of the vegetables. A reduction of (38.44%) was observed in the leaves of *M. oleifera for* total sugars and 94.99% for *V. calvoana* for reducing sugars. Bill, 2003 reported that sugars undergo irreversible chemical modifications due to heat and the hydration.

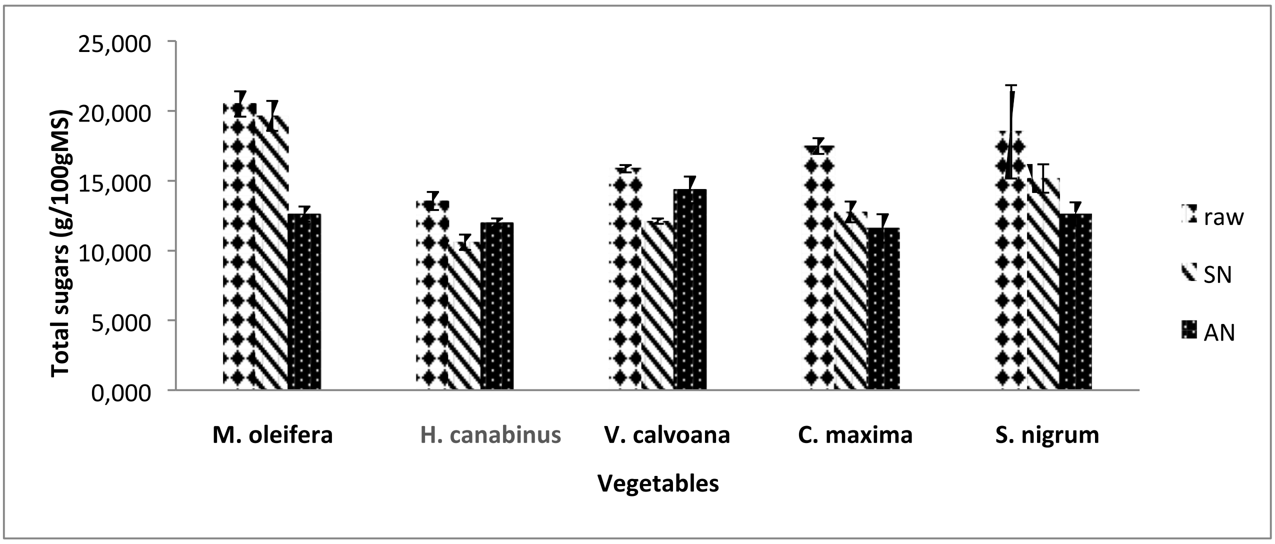

Figure 3: Total sugars contents of leafy vegetables according to cooking method.

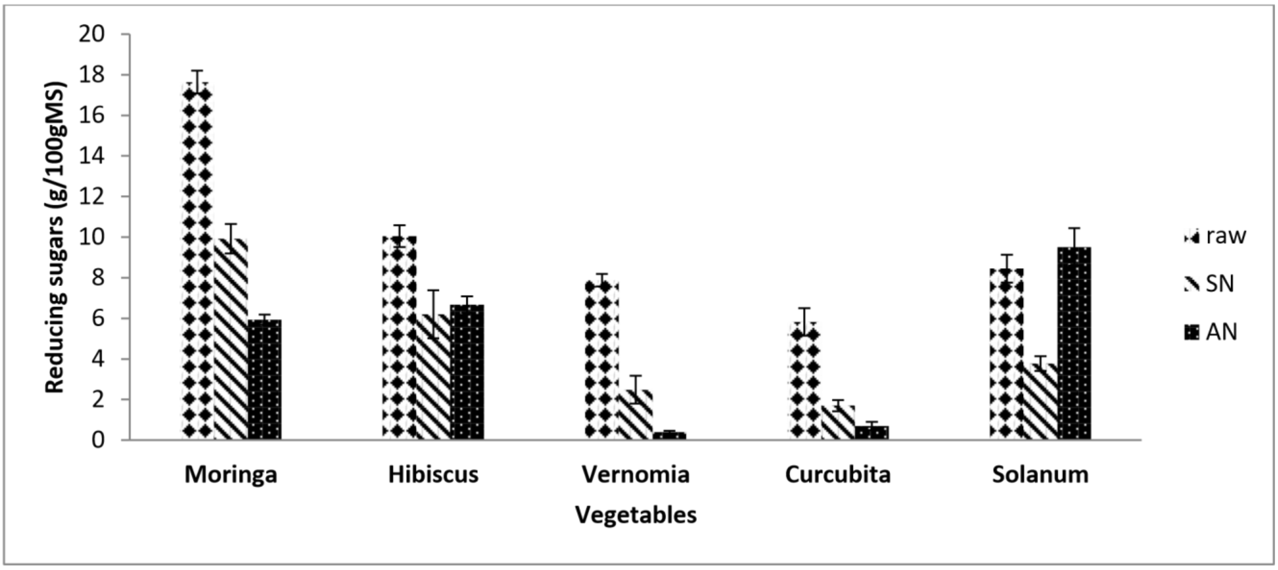

Figure 4: Reducing sugars contents of leafy vegetables according to cooking method.

Total carotenoids contents

Cooking with or without kanwa significantly ($P < 0.05$) affects the carotenoid levels concentrations for all vegetables (Figure 5). Their rate of loss varied from one vegetable to another. The highest reduction was observed in the *H. cannabinus* sample (23.6%) after cooking with kanwa and 15.12% in *V; calvoana* after cooking without kanwa. These losses are the result of cooking and leaching (Food, 2020). Heat causes the photooxydation of the carotenoids cis and trans and led to the epoxydation and cleavage before fragmentations in series of composed of small masses, thus losing their biological activity (Aman *et al*., 2005).

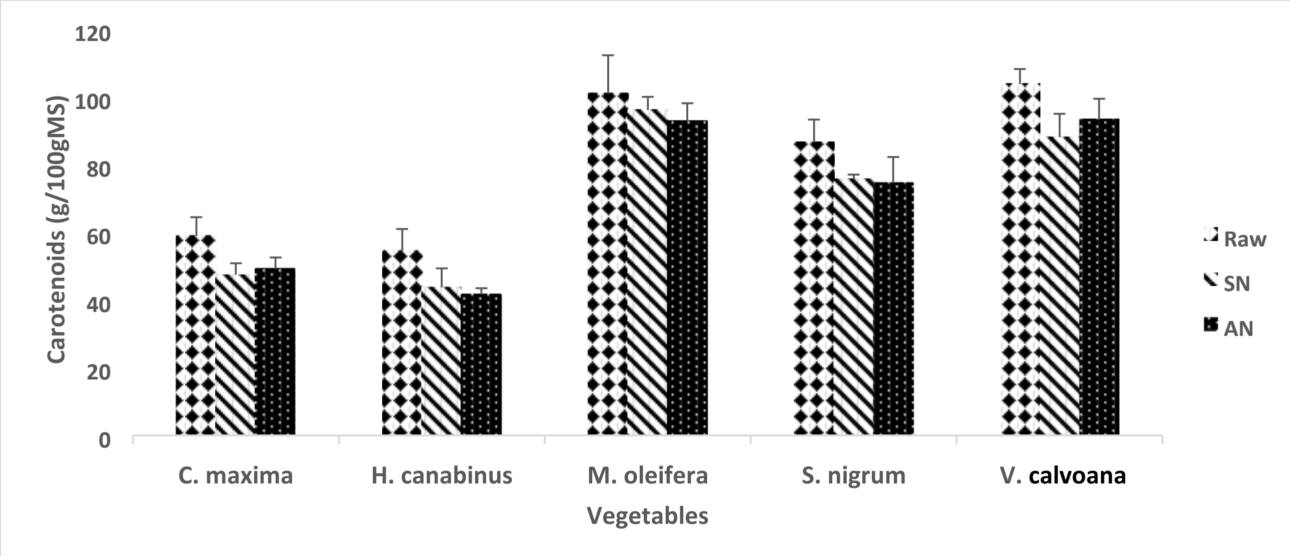

Figure 5: Carotenoids contents of leafy vegetables according to the cooking method.

III.2- Influence of cooking on some minerals

Calcium contents

The results presented in Figure 6 show that cooking without kanwa reduced calcium levels. With *S. nigrum*, for example, a reduction of 34.91% was observed. This loss in calcium is due to leaching and the solubility of calcium in water. In the case of bleaching with kanwa, an increase of 51.04%, 50.11% and 17.58% was observed for *C. maxima*, *V. calvoana*, and *H. canabinus* respectively. This increase being greater, is understandable when one knows that the kanwa used has a significant calcium content (812.38 ± 38.73mg/100gMS). Calcium is involved in the regulation of nerve and muscle function, blood clotting and the activation of many enzymes. The recommended dietary allowance (RDA) varies from 500 to 1200mg of calcium per day, depending on age and physiological state (ANSES, 2016). Therefore, the consumption of our vegetables can meet an individual's calcium requirements.

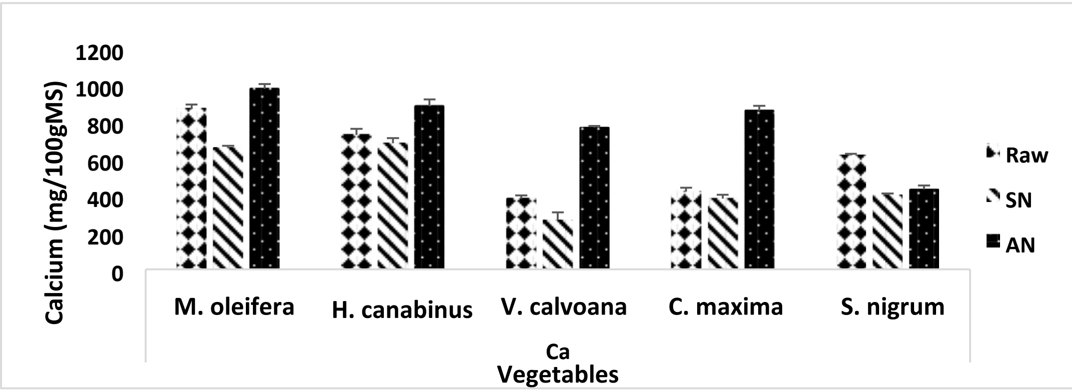

Figure 6: Calcium contents of leafy vegetables according to cooking method.

C= Raw SN =Without kanwa AN =With kanwa

Sodium contents

Figure 7 shows that, the sodium content of cooked samples without kanwa decreased significantly compared to raw samples. The greatest reduction is noted in *V. calvoana* with 57.69%, followed by *S. nigrum*, *H. canabinus*, *C. maxima* and *M. oleifera* with 45.23%, 44.55%, 38.23% and 7.97% respectively. The solubility of sodium in water is well known. The sodium present in the vegetable diffused into the cooking water, thus reducing its content in the samples. Bamidele *et al.,* 2017 had the same observation after blanching of some leafy vegetables consumed in Nigeria. The low sodium content after blanching of the leafy vegetables qualifies them as good for healthy people, for hypertensive people (Adepoju and Oyewole, 2008).

All the samples cooked with kanwa had a significant increase in sodium content ranging from 59.15% for *H. canabinus* to 94.44% for *S. nigrum*. Considering the very high sodium content (3578.83±10.2%MS) of the kanwa used for bleaching, it could be said that kanwa contributed significantly to this increase in the different leafy vegetables. While kanwa promotes a colour and texture that is appreciated by consumers (Ejoh *et al.,* 2005b), it significantly increases the sodium content in the vegetables. Excessive consumption of sodium in food promotes high blood pressure or oedema in some people.

Na is needed to maintain the osmotic balance of body fluids, the body pH, to control glucose absorption and to enhance normal protein retention during growth (NRC, 1989). Its presence in an individual's diet is therefore desirable. The RDA of Na for an adult male is 2500mg (NRC, 1980).

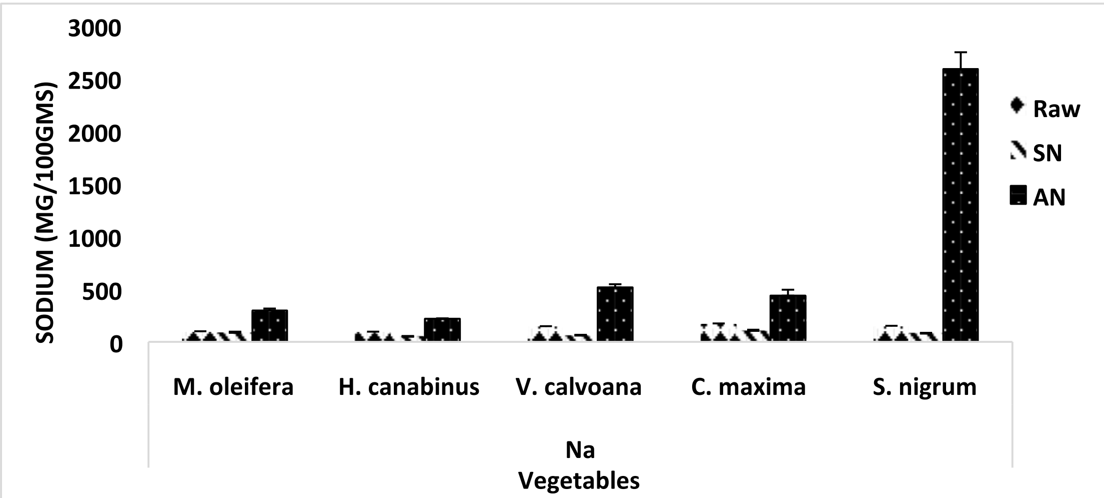

Figure 7: Sodium contents of leafy vegetables according to cooking method.

C= Raw SN =Without kanwa AN =With kanwa

Iron contents

The iron contents of the leafy vegetables are expressed according to the treatments applied (Figure 8). The results show that *C. maxima* has the highest iron content (167±9.94mg/100gMS) followed by *H. canabinus* (27.60±3.46mg/100gMS) and *S. nigrum* (26.85±2.01mg/100gMS). *V. calvoana* and *M. oleifera* have the lowest iron contents 19.01±2.07mg/100gMS and 14.99±2.00mg/100gMS respectively. After cooking, it was found that cooking without kanwa favoured a reduction of 30.93% in *V. calvoana* samples. In contrast, in the other samples, iron levels tended to be stable. The significant decrease in the specific case of *V. calvoana* cooked without kanwa can be explained by the fact that, in order to reduce the bitterness of the vegetable after blanching, it was washed with rubbing, which favoured the leaching of iron. The increase in iron content after blanching with kanwa of the samples is 57.75% in *V. calvoana*, 39.06% in *S. nigrum.* This increase is justified by the addition of kanwa.

Indeed, the kanwa used in this study was particularly rich in iron (173.46±1.88mg/100gMS) (Ejoh, 2017). Iron is an essential trace element for the synthesis of haemoglobin and myoglobin (Idris, 2011).

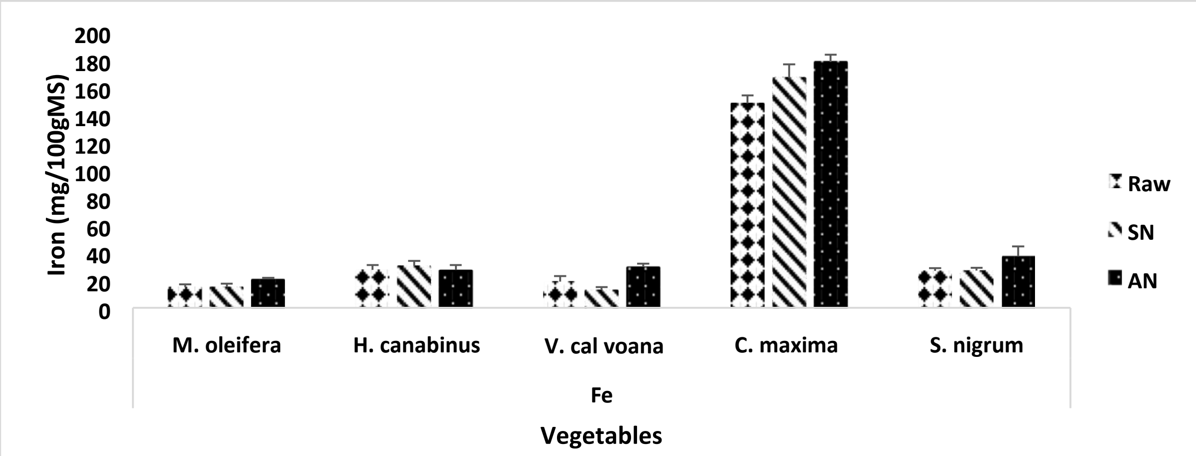

Figure 8: Iron contents of leafy vegetables according to cooking method.

C= Raw SN =Without kanwa AN =With kanwa

Zinc contents

The zinc contents of the leafy vegetables studied varied from 9±0.9mg/100g DM for *C. maxima* to 19.7 ± 2.4mg/100g DM for *M. oleifera*. The results show no significant difference between the bleaches applied to *H. canabinus*. In contrast, the zinc contents of *C. maxima* showed losses of 35.55% and 33.33% when the bleaches with and without kanwa were applied. This reduction in zinc in the treated samples is due to its hydrolysis and dissipation in the cooking water. These results are similar to those reported by Onyeike *et al.*, 2004 who showed that zinc levels decreased in cooked vegetables.

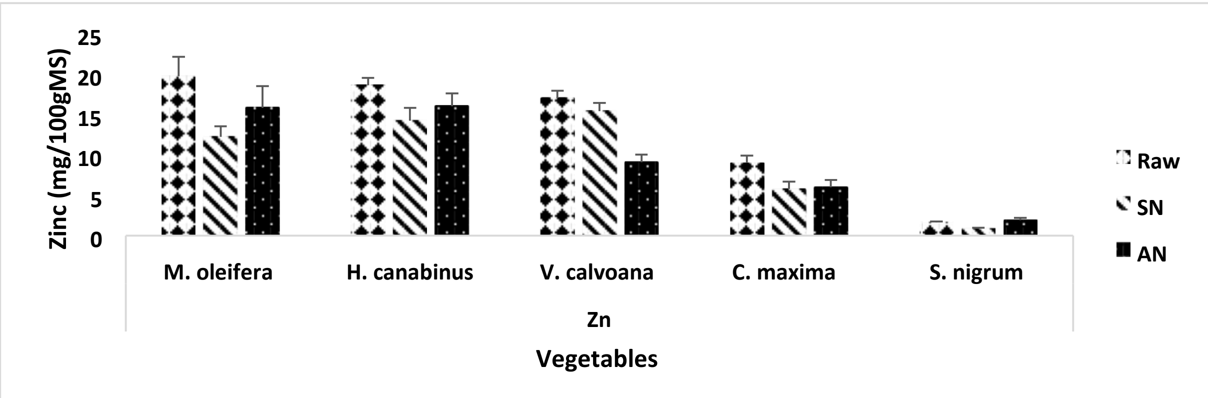

Figure 9: Zinc contents of leafy vegetables according to cooking method.

C= Raw SN =Without kanwa AN =With kanwa

III.3- Influence of cooking on total oxalate contents

The results in Figure 10 show that *M. oleifera* has the highest value (0.561±0.03g/100g DM) followed by *S. nigrum* (0.431±0.02g/100g DM). Cooking resulted in the reduction of oxalate contents although the rate of reduction depends on the leafy vegetable. With *Solanum*, cooking with or without kanwa resulted in a reduction of 76.7% and 74.4% respectively. For *Vernonia*, 15.4% reduction was observed when cooked without kanwa and 25.6% after cooking with kanwa.

Shashi and Salil (1999) found similar results after bleaching in water of vegetables such as *A. tricolor*, *C. album,* and *S. oleracia.* The high percentage of oxalate reduction during cooking is believed to be due to its solubility in water. Oxalates in foods contain a soluble and an insoluble fraction (Bhandari and Kawabata, 2004). During cooking, there is interaction between the leaves and hot water, which leads to considerable cell disruption and facilitates the leaching of soluble oxalates (Duru *et al.*, 2020). Oxalates by forming complexes with some nutrients such as calcium and proteins reduce their bioavailability (Sefa-Dedeh *et al.*, 2004). Therefore, reducing the level of oxalates in cooked samples would improve the bioavailability of calcium and reduce the risk of kidney stones in humans.

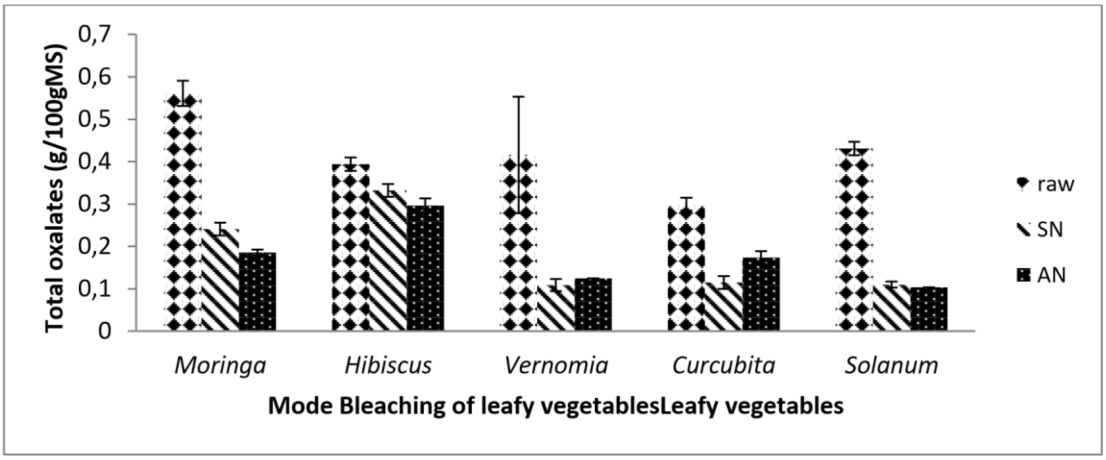

Figure 10: Total oxalate contents of leafy vegetables according to cooking method

C= Raw SN =Without kanwa AN =With kanwa

IV. Molar ratio of anti-nutrients to minerals

To predict the bioavailability of calcium, oxalate/calcium ratios were calculated. If these values are higher than the critical value (2.5), calcium is complexed by the oxalate. The results show that this critical value is higher than the calculated values for all the samples studied. Compared to raw vegetables, all ratio values are much smaller when the samples are cooked.

In general, it can be observed that cooking with or without kanwa contributes to reducing the oxalate/calcium ratio values. Indeed, the reduction of oxalate levels would have a beneficial effect on the bioavailability of calcium **(**Shashi and Salil, 1999**).**

Table: Oxalate/Calcium ratios of leafy vegetables according to treatments.

Echantillons	*C. maxima*	*S. nigrum*	*M. oleifera*	*V. calvoana*	*H. canabinus*
C	0,7	0,7	0,6	1,1	0,5
SN	0,1	0,3	0,2	0,2	0,4
AN	0,2	0,2	0,3	0,2	0,3

C= Raw SN =Without kanwa AN =With kanwa

V. Conclusion

This study showed that cooking significantly affected the nutritional composition of the leafy vegetables. Cooking with or without kanwa reduced the protein, sugar, total carotenoids and zinc contents. The lipid content increased instead. While cooking without kanwa reduced calcium, sodium and iron contents, cooking with kanwa increased them. The total oxalate content was reduced for all leafy vegetables when cooked with or without kanwa, thus improving calcium bioavailability.

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