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Original Research Article

Effect of Water Cooking on the Biochemical Characteristics of Leafy Vegetables (*Ficus exasperata*) Consumed in Northern Côte d'Ivoire

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Abstract: Leafy vegetables play an important role in the diets of all populations around the world, particularly in Africa, Asia and Oceania, where they provide an essential part of the nutritional and medicinal needs. *Ficus exasperata* leaves could improve the nutrition and health of consuming populations. the leaves of *Ficus exasperata* are little known in terms of food for the Ivorian population. The objective of this study is to study the effect of cooking in water on some biochemical parameters of leafy or gratory vegetables (*Ficus exasperata*) used in the preparation of traditional dishes in the North of Côte d'Ivoire. *Ficus exasperata* was cooked for 20 to 40 min and the biochemical analyzes were carried out at different times from 20 to 40 min. The results revealed significant differences at the 5% level. The results showed that fiber and lipid levels increased after 20 minutes of cooking. On the other hand, cooking in water after 40 min resulted in losses of 14.41% of ashes, 55% of proteins, 96% of phytochemicals, 81.74% of phytates and 84.94% of oxalates. The recommended cooking time is 20 minutes because most of the nutrients are barely denatured. It would then be recommended to cook these leafy vegetables for 20 minutes to minimize nutrient losses.

Keyword: Leafy vegetables, biochemical characteristics, water cooking, nutrients, food safety.

INTRODUCTION

In most developing countries, malnutrition, particularly undernutrition, is one of the main causes of mortality and morbidity among young children (WHO/UNICEF, 2006; Lartey, 2008). In fact, 800 million inhabitants of these countries suffer from chronic undernourishment with 200 million children under the age of 5 suffering from protein-energy malnutrition (FAO, 1996). Many millions more suffer from diseases caused by dietary deficiencies such as deficiencies in proteins, vitamins and minerals essential for health and physical development (Black, 2008). However, recent evidence has shown that the number of undernourished people has increased in recent years. Moreover, the increase in the world population which could reach 9 billion inhabitants by 2050, the effects of climate change, urban planning and land degradation, means that access to future food products will be difficult, which will accentuate malnutrition. Indeed, the use of traditional leafy vegetables would be useful and beneficial to prevent and fight against both malnutrition and diseases linked to oxidative stress. In Ivory Coast, most leafy vegetables which have interesting nutritional values are often grown in urban and peri-urban areas. As a result, gratory leaves (Ficus exasperata) generally used in the preparation of traditional dishes in the north of Côte d'Ivoire represent an important source of carbohydrates, minerals and polyphenols (Koné F. *et al*., 2023). However, the young leaves of *F. exaperata* are prescribed as a common anti-ulcer remedy. Some studies have revealed that traditional leafy vegetables contain a significant amount of hydrocyanic acid, oxalic acid, alkaloids and saponins which may pose health risks to consumers (Orech F *et al*., 2005). This is why it is recommended to cook them before consumption. It should be noted that pre-cooking at temperatures at short times followed by elimination of the cooking water partially or completely rids leafy vegetables of their harmful substances (Sorensen J *et al*., 1994). The consumption of many leafy vegetables requires cooking to avoid their irritating or toxic effects (Richard, 2007). Cooking vegetables makes it possible to improve their

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digestibility by modifying the structure of dietary fibers, but it also leads to a more or less marked reduction in nutritional value, either by destruction of heat-labile and/or oxidizable substances, or by diffusion of water-soluble constituents. in the cooking water. Cooking modifies the texture of the food and destroys the microorganisms and enzymes responsible for degradation (Sharma and Le Maguer, 1996). It also modifies the nutritional value of vegetables. However, it could also lead to undesirable consequences such as the degradation of nutrients (vitamins) and the formation of molecules. It should, however, be noted that in all these previous works, most of which were carried out more than 10 years ago, the effect of cooking in water on the biochemical characteristics of leafy vegetables has never been specified. (*Ficus exasperata*). Thus, in order to make our contribution to improving the nutritional and health status of the Ivorian populations, this present study set out to evaluate the effect of the cooking method on the biochemical characteristics of leafy vegetables (*Ficus exasperata*) consumed in the north of Côte d'Ivoire.

MATERIALS AND METHODS

Plant material

The plant material consists of leafy vegetables in the adult stage which are *Ficus exasperata Vahl* (Moraceae) (Figure 1).

Methods

Sampling

The leafy vegetables were harvested in November 2020 in a field located in the town of Divo (south-west of Côte d'Ivoire). These leaves were put in bags and then transported to the Biocatalysis and Bioprocesses Laboratory at Nangui Abrogoua University (Abidjan) for analyses. Once received, the leaves are stored at room temperature in a ventilated room within the laboratory, so as to protect them from any factors likely to alter them. The identification and authentication of these plant species were carried out at the National Floristic Center of the Felix.

Figure 1: Leaves of *Ficus exasperata* or gratoire (Koné F *et al*., 2023)

Houphouët Boigny University in Abidjan. The leaves of *Ficus exasperata* were selected for this study after a survey for the preparation of dishes in the North.

Production of cooked leaf powder

The production of the powder from the cooked leaves was done in several stages. The leaves are first sorted and washed in tap water. Then 250 g of fresh leaves were immersed in a stainless steel container, containing 1.5 L of boiling demineralized water (100°C) for 20, 30 and 40 min. After cooking, the leaves were cooled and allowed to drain at room temperature for 5 min, before being dried in an oven (Memmert) at 45°C for 72 h. Then, the dried leaves were crushed using an electric blender (Nasco) into more or less fine particles. Finally, sieving was carried out using a sieve with a mesh size of 250 µm in order to obtain finer particles while removing large particles and certain indigestible fibers. The powders obtained were stored in a jar at room temperature and constituted the samples analyzed.

Biochemical analysis of leaf powders

The analysis of some biochemical parameters of the samples was carried out in triplicate according to the standard methods of the AOAC (2000). The moisture content was obtained by drying the samples in an oven at 105 °C to constant weight. The ash content was determined by incinerating the dried samples in a muffle furnace at 550 °C for 4 hours. The crude fiber content was estimated by weighing the insoluble residue

obtained with acid (H2SO4, 0.25 M) and alkali (NaOH, 0.3 M). Crude protein content was calculated by multiplying the estimated nitrogen content by 6.25, using the Kjeldahl method. The crude fat level was carried out using the Soxhlet extraction method. Carbohydrate content was calculated using the weight difference method. All approximates have been expressed as a percentage based on dry matter.

Vitamin C content

The vitamin C content of the samples was determined according to the method described by Pongracz (1971). A sample of 10 g of ground samples in 20 mL of metaphosphoric acid (3%) / acetic acid (8%) solution. The ground material was centrifuged at 4000 rpm for 20 min. The supernatant was then collected in a volumetric flask. The supernatant (1 mL) was titrated with 2,6-dichlorophenol indophenol. The appearance of a persistent champagne pink color for 15 seconds marked the end of the dosage. One (1) mL of a standard solution of pure ascorbic acid (1 mg/mL) was also titrated with 2,6-dichlorophenol indophenol (DCPIP).

Extraction of total phenolic compounds

Total phenolic compounds of the leafy vegetable samples were extracted with methanol according to the method of Singleton *et al*., (1999). One (1) g of leafy vegetable was dissolved in 10 mL of methanol (70%; v/v). The resulting mixture was homogenized by manual stirring for 2 min at room temperature, then centrifuged at 4200 rpm for 5 min in a centrifuge (TGL-16M). The pellet was collected in 10 mL of methanol (70%; v/v) and centrifuged again. A third extraction was carried out under the same conditions. The 3 supernatants were combined in a 50 mL flask and the volume was adjusted with distilled water to the mark. This mixture constituted the total phenolic extract.

Polyphenol content

The polyphenol content was determined according to the method described by Singleton V *et al*., (1999). A sample of 1 mL of the methanolic extract was introduced into a test tube and added with 1 mL of Folin-ciocalteu reaction diluted 1/10 (v/v). The tube was left to stand for 3 min and then 1 mL of 20% (w/v) sodium carbonate solution was added. The contents of the tube were made up to 10 mL with distilled water and the whole was placed in the dark for 30 min. The Optical Density (OD) reading was carried out at 765 nm using a BK_UV 1000 brand spectrophotometer against a blank. The quantity of polyphenols was determined using a standard curve established from a stock solution of gallic acid (1 mg/mL).

Flavonoid content

The method for determining the flavonoid content is that described by Meda M *et al*., (2005). A sample of 0.5 mL of the methanolic extract was introduced into a test tube. To the contents of the tube were added successively 0.5 mL of distilled water, 0.5 mL of aluminum chloride (10% w/v), 0.5 mL of potassium acetate (1M) and 2 mL of 'distilled water. The tube was left to stand for 30 min in the dark and the OD was read at 415 nm using a spectrophotometer against a blank. The flavonoid content of the samples was determined using a standard range established from a stock solution of quercetin (0.1 mg/mL).

Tannin content

The tannin content was determined according to the method described by Bainbridge Z *et al*., (1996). A sample of 1 mL of the methanolic extract was introduced into a test tube. To the contents of the tube was added a volume of 5 mL of vanillin reagent. The tube was left to stand for 30 min in the dark and the OD was read at 500 nm using a spectrophotometer against a blank. The tannin content of the samples was determined using a standard range established from a stock solution of tannic acid (2 mg/mL) under the same conditions as the test.

Determination of the antinutritional properties of the leaves Oxalate content

The oxalate content of the leaves was determined according to the method described by Day and Underwood (1986). One (1) gram of the finely ground sample was homogenized in 75 mL of 3M sulfuric acid (H2SO4) with magnetic stirring for 1 hour. The mixture was filtered with Whatman filter paper, then 25 mL of the filtrate was taken and hot titrated with permanganate (KMnO4) solution until it turned pink for 30 seconds.

Phytate content

Phytates were determined according to the method of Latta and Eskin (1980) using Wade's reagent. One gram of dried and ground sample was homogenized in 20 mL of 0.65 N HCl. The mixture obtained was stirred for 12 h at room temperature (28 °C). Everything was centrifuged at 3000 rpm for 40 min. To 0.5 mL of supernatant were added 3 mL of Wade's reagent. Then the tube was left to rest for 20 min in the dark and the absorbance reading was taken with a spectrophotometer at 490 nm against a blank. Finally, a calibration curve was produced using a range of phytic acid concentrations ranging from 0 to 10 mg/mL. The results were expressed in mg phytic acid equivalent (EAP)/100g dry matter (DM).

Statistical analyzes

The results obtained were subjected to analysis of variance (ANOVA) with the STATISTICA 7.1 software. In the event of a significant difference between the parameters studied, the classification of the means was done according to the Duncan test. The significance level is $\alpha = 0.05$. Statistical differences with a probability value less than 0.05 ($P < 0.05$) are considered significant. When the probability is greater than 0.05 ($P > 0.05$) the statistical differences are not significant.

RESULTS

Effect of cooking on the Physico-chemical characteristics of *F. exasperate*.

The biochemical properties of *Ficus exasperata* at different cooking times are presented in Table I. The analysis shows that the ash and protein contents of *F. exasperata* decrease during cooking respectively from 16.79 ± 0.17 to 14, 37 ± 0.00% and from 12.60 ± 0.01 to 5.66 ± 0.01%. On the other hand, cooking *F. exasperata* leaves in water leads to an increase in lipid content (from 3.62 ± 0.02 to 4.92 ± 0.01%) and fiber content (from 15 .52 ± 0.02 to 22.41 ± 0.01%). However, the carbohydrate content of *F. exasperata* leaves appears to increase and ranges from 51.47 ± 0.20 to 54.12 ± 0.03%. These observed contents are statistically different at the 5% threshold.

Table I: Physico-chemical characteristics of *F. exasperata* during cooking

Parameters	Cooking time (min)			
	0	**20**	**30**	**40**
Ashes (%)	16.79 ± 0.17^{c}	15.18 ± 0.04^{b}	15.05 ± 0.03^{b}	14.37 ± 0.00^{a}
Fats (%)	3.62 ± 0.02^{a}	3.95 ± 0.02^{b}	$4.32 \pm 0{,}02^{c}$	4.92 ± 0.01^{d}
Carbohydrates (%)	51.47 ± 0.20^{a}	54.12 ± 0.03^{d}	$53{,}18 \pm 0{,}03^{c}$	52.65 ± 0.02^{b}
Fibers (%)	15.52 ± 0.02^{a}	16.85 ± 0.02^{b}	19.34 ± 0.01^{c}	22.41 ± 0.01^{d}
Proteins (%)	1.60 ± 0.01^{d}	9.90 ± 0.00^{c}	8.310 ± 0.01^{b}	5.66 ± 0.01^{a}
EV(Kcal/100g)	288.88 ± 0.64^{c}	291.60 ± 0.14^{d}	284.04 ± 0.14^{b}	277.48 ± 0.12^{a}

The values obtained are means ± standard deviations determined in 3 tests. On the lines of each parameter, the means assigned different letters are significantly different at P < 0.05 according to the Duncan test. VE=Energy value

Effect of cooking on the phytochemical characteristics of *F. exasperata*

The polyphenol content of *F. exasperata* leaves boiled in water is presented in Figure 2. The results show that boiling in water leads to a significant decrease (P < 0.05) in all determined polyphenols. Indeed, a loss rate of 96.47% is observed in the total polyphenol content after 40 min water cooking.

As for tannins (Fig 2), a decrease from 50.38 ± 2.28 (T0) to 1.70 ± 0.29 mg/100 g of DM (T40) was observed, i.e. a loss rate of 96 .47%. Concerning flavonoids, cooking resulted in a decrease from 116.54 ± 0.77 (T0) to 3.12 ± 0.74 g/100g of DM (T40 = time at 40 min), which corresponds to a loss rate of 97.32%.

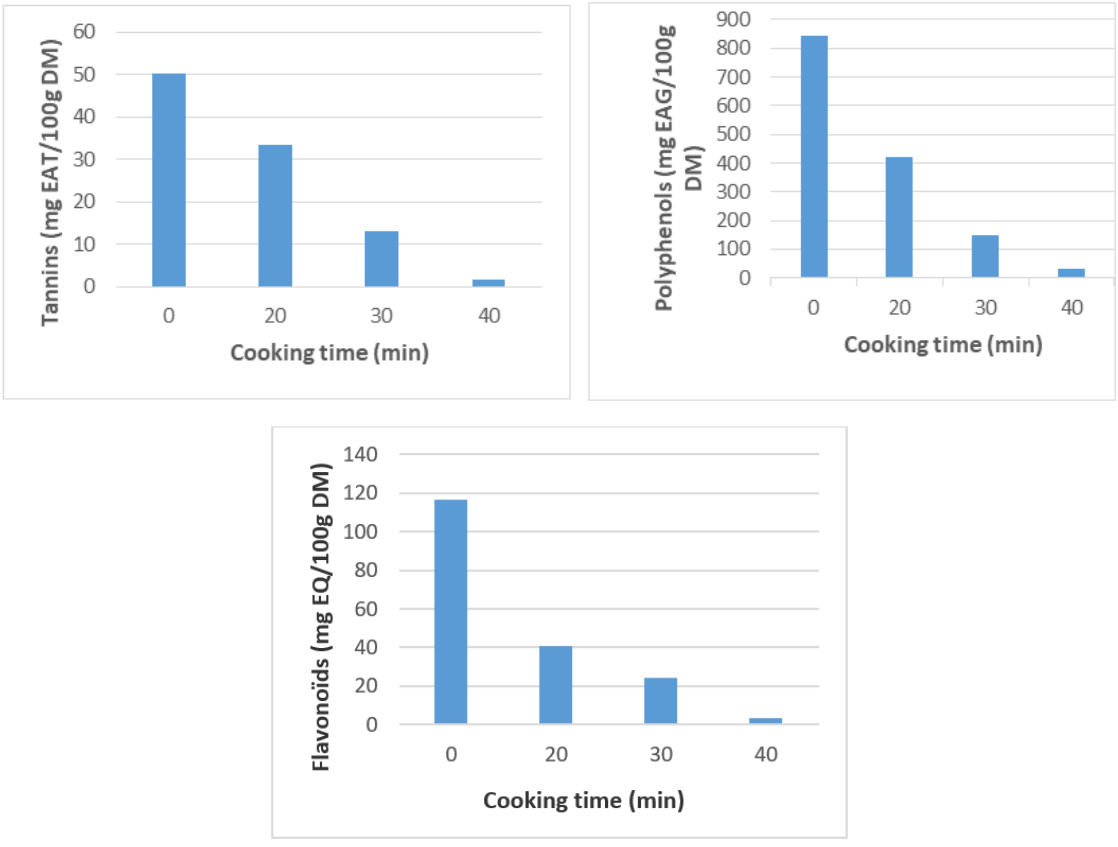

Figure 2: Phytochemical characteristics of *F. exasperata* during cooking

Effect of cooking on the contents of anti-nutritional compounds in *F. exasperata*

Figure 3 shows the variation in the content of anti-nutritional compounds in *F. exasperata* during boiling. Phytate content in leaves fresh *F. exasperata*, which is 7.45 ± 0.24 mg/100 g of DM, decreases significantly ($P < 0.05$) during the different cooking times to reach the value of 1.36 ± 0, mg/100 g of DM after 40 min of cooking. The same observation is noted with the oxalate content which decreases significantly ($P < 0.05$) from 36.33 ± 3.18 (T0) to 5.47 ± 0.18 mg/100 g of DM (T40).

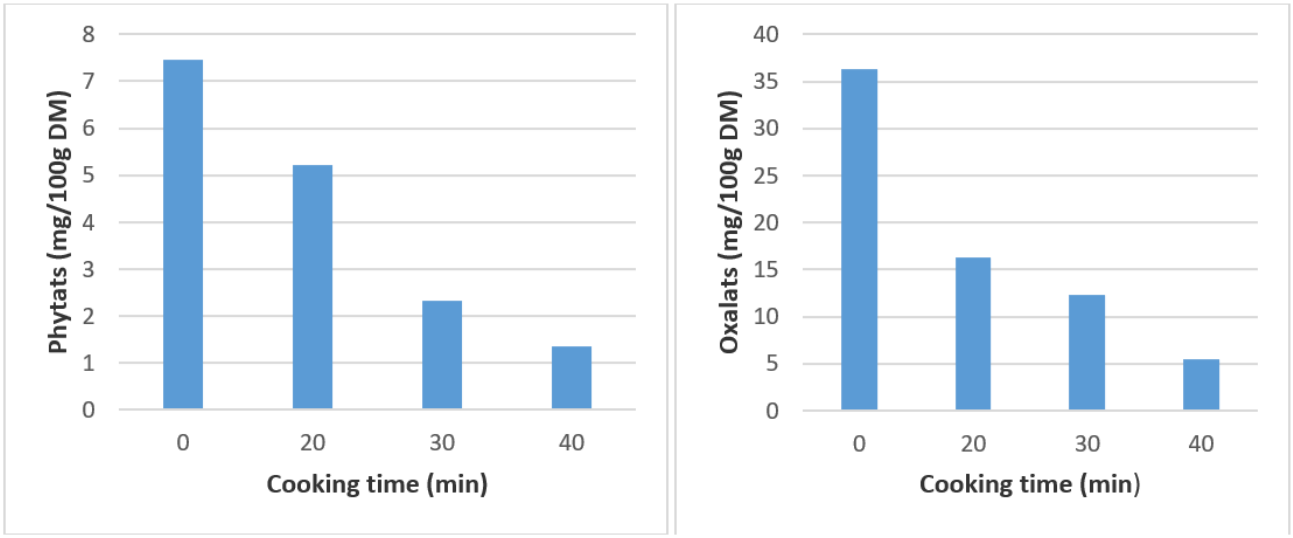

Figure 3: Contents of anti-nutritional compounds in *F. exasperata* during cooking

DISCUSSION

During cooking in water for 20 to 40 minutes of the leafy vegetables studied, a drop in the ash rate was recorded. Indeed, this loss of ash is explained by the leaching of mineral elements in the cooking water (Oboh, 2005). According to the work of Zoro (2016), the ash rate in potato leaves (I. batatas) and M. arboreus leaves decreases from 10.77 ± 0.06% to 6.53 ± 0.2%. Thus, the leaves of Ficus exasperata could be considered a good source of minerals when the cooking time in water does not exceed 20 min.

After 40 min of cooking in water, the *F. exasperata* leaves studied lost approximately 55% of protein. This phenomenon would essentially be attributed to their solubilization and diffusion in the cooking water due to the heat (Lund, 1997). Low protein in cooked leafy vegetables would require supplementation with animal proteins or legumes in order to reduce protein-energy malnutrition in populations (Uusiku N *et al*., 2010). So, cooking F. exasperata leaves for 20 minutes would be recommended because the loss rate reaches 9.90%, hence the use of animal proteins.

From 20 min to 40 min of cooking in water, the fat content increases from 3.95 ± 0.02% to 4.92 ± 0.01%. These low lipid values obtained show that leafy vegetables contain little lipids and therefore have a low energy value (Sobowale et al., 2011). However, a plant-based diet based on leafy vegetables provides the body with a quantity of essential fatty acids. Thus, cooking led to an increase in the lipids of F. exasperata due to the softening of the texture of the cell walls (Vodouhe S *et al*., 2012). Indeed, lipids play an essential role in the constitution of cell membranes (FNB, 2001; Bedigian, 2004).

Cooking causes a reduction in the polyphenol content because they are soluble in water during boiling. The loss rate estimated at 50.28% after 20 min shows that the loss of tannins is not very high. Leaching or thermolability of specific flavonoids could be the basis for the decrease in the content of phenolic compounds (Yamaguchi T *et al*., 2001). The effect of cooking on the content of polyphenolic compounds could affect the medicinal and dietary potential of leafy vegetables highlighted by Wong S *et al*., (2006). Polyphenols are antioxidants that fight against free radicals responsible for disorganization in the body, causing cancer, degeneration diseases and cellular aging (Vauzour D *et al*., 2010).

During cooking in water, tannins decrease (33.55 ± 2.75 mg/100g DM to 1.70 ± 0.29 mg/100g DM). Tannins are heat labile and exhibit antioxidant activity (Hagerman *et al*., 1998; Gülçin I *et al*., 2003; Hung and Nhi, 2012).

However, the decrease of oxalates and phytates in *F. exasperata* leaves during cooking indicated 84.94 and 81.74%, respectively. From this point of view, cooking leafy vegetables appears to be a detoxification

process allowing the partial or total elimination of these anti-nutritional factors (Ekop et Eddy, 2005).

CONCLUSION

The objective of this study is to evaluate the effect of the cooking method on the biochemical characteristics of leafy vegetables (Ficus exasperata) consumed in the north of Côte d'Ivoire. Cooking the leaves of Ficus exasperata in water generally resulted in nutrient losses of 20 to 40 min. However, the fiber and lipid levels have increased and the recommended cooking time is 20 minutes because most of the nutrients are little denatured. It is in this sense that it would be interesting to reduce the cooking time of leafy vegetables in water even further compared to that usually done by households (around 35 to 45 min). It would be desirable to integrate *Ficus exasperata* into the eating habits of the Ivorian population to ensure nutritional balance.

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