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Effect of Household Cooking Methods on Polyphenols and Antioxidant Capacity of Endogenous Leafy Vegetables

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Abstract – Leafy vegetables are often suggested to pretreatments prior to use. One of the usual practice is the cooking methods which presumably affect biochemical and physicochemical compounds. This study aims to evaluate various cooking methods of leafy vegetables, namely precooking without additive, cooking with additive (traditional potash called Kanmu or ash filtrate) or cooking without additive on total polyphenols content and antioxidant activity of three traditional leafy vegetables (Justicia tenella, Acmella uliginosa and Sesamum radiatum). Total polyphenol contents (TPC) ranged between 0.36 – 0.60 g GAE/100g DW. Traditional cooking methods affected biochemical and physicochemical compounds depending on species. For instance, precooking and cooking with potash decreased significantly ($p<0.05$) TPC in J. tenella leaves while no significant effect was evidenceed in the case of cooking with ash filtrate and cooking without additive. Regarding antioxidant capacity, precooking and cooking without additive significantly increased it [raw 39.01 – 63.9%), P and CWA (85.58 – 90.89%)] whilst cooking with additive decreased it (CP, CF; 10.77-44.44%).

Keywords – Justicia tenella, Acmella uliginosa, Sesamum radiatum, bioactives coumpounds.

I. Introduction

Leafy vegetables are usually consumed after some culinary treatments like squeeze washing, pre-cooking or cooking [1], [2] and these treatments provoke contrasting physico-chemical changes, depending on the species. [3] reported losses in antioxidant components, particularly low antioxidant activity of brocolli (*Brassica oleracea*, Brassicaeae) while other studies mentionned that cooking increased antioxidant components of leafy vegetables [4], [5].

In Benin, processing techniques of traditional leafy vegetables before consumption were documented [2]. Irrespective of culinary treatments [6], [1], [2], some additives such as ash filtrate and alkaline rock named 'kanmu' or 'kanwa' was usually used [2]. Details regarding the nutritional impacts, essentially biochemical and physicochemical changes of theses practices are not fully documented. Some interactions between nutritional contituents and additive probaly impact bioavailable of nutritients. As already pointed out, the cooking of *Vernonia amygdalina* leaves with kanwa decreased significantly phenolics compounds, saponin and oxalic acid [1]. Due to the mineral potential of additives used (ash filtrate, 'kanmu'), pretraitements associating these additives can presumably impact nutritional and bioactive constituents of leafy vegetables.

This study aims to evaluate the effect of household cooking methods on total phenolics coumpounds and antioxydante capacity of leafy vegetables: *Sesamum radiatum* L. (Black benniseed, Pedaliaceae), *Acmella uliginosa* (L.) Jansen (Toothache plant, Asteraceae) and *Justicia tenella* (Nees) T. Anderson (Acanthaceae).

II. Material And Methods

2.1- Sample collection

Samples of traditional leafy vegetables were collected in markets, farms and traditional garden of villages in the Department of Donga in North-West Benin (in Natitingou, Pam-pam, Perma, Tiélé). After collection, edible part was immediately separated from branches, put in sealed polyethylene bag, and in cooler with ice before returning in laboratory. Alkaline rock (kanmu or kanwa) was bought from Natitingou market and ash filtrate was obtained by filtration of sorghum stem ash collected in three households.

2.2- Processing treatments and materials preparation for analysis

For each species, sample of 100 g of leaf was cleaned with distillate water before being submitted to different treatments. The quantities of water and softening ingredients used were indicated by housewives in the sampling areas. Water temperature before leaves addition was set at 90°C ± 3°C. Processing methods of all leafy vegetables were summarized on figures 1 and 2 base on household techniques. Spinaches (*A. uliginosa* and *J. tenella*) were cooked following three scenarii : (i) cooking with potash (alkali rock: CP) or ash filtrate (CF); (ii) cooking without additive (CWA) and (iii) precooking without additive (P). Regarding *S. radiatum*, only scenarii 1 and 2 (CP/CF, CWA) were applied.

After cooking, samples and raw leaves were freeze-dried (-55°C), then milled in Moulinex (Waring, 32B780) and stored in airtight containers for analysis.

2.3- Physicochemical analyses

Total phenolic content

Total phenolic content (TPC) was determined followed the modified method of [7] by [8] and [9] to determined TPC in leafy vegetables flour fruitfully. Extraction was realized twice by centrifugation using HCl/methanol (1%v/v). Gallic acid (Aldrich Chemical Company Steinheim, Germany) was used as standard and the optical density was measured at 760 mm with a spectrophotometer (Jenway, UV 6715). Data was expressed in Gallic Acid Equivalent (GAE)/ 100g Dry Weight (DW).

Antioxidant capacity

DPPH (2.2-diphenyl-1-picrylhydrazyl acid) method was used to assess antioxidant capacity of raw and cooked leafy vegetables following the modified method of [10] used by [3]. 25 mg of freeze-dried flour of leafy was extracted with 1.125 ml of aqueous methanol (80%) during 2 h under continuous stirring at room temperature twice. The supernatant was filtrated with Whatman N°1 paper and diluted with 5% aqueous methanol.

Statistical analysis

One-way Analysis of Variance was performed using STATISTICA (v6) program to evaluate effect of cooking methods on total phenolics and antioxidant capacity. The effect of traditional cooking methods was compared to raw leafy using the test of Newman and Keuls at 5% to structure mean values.

III. Results

3.1- Kknowledge on the processing of studied leafy vegetable

A result of survey to know cooking parameters for studied leafy vegetables was indicated in table 1. It was noticed that, cooking parameters were different according to species and household's methods.

Table 1: Cooking conditions of fresh leaves using different cooking methods (100 g)

Species	Cooking modes	Cooking time (min)	Quantity of water (ml)	Quantity of potash/ ash filtrate (g)
Sesamum radiatum	CP/CF	9	915	5.9/26.7
	CWA	9	915	-
Justicia tenella	CP/CF	9	610	5.50/7.3
	CWA	9	610	-
	P	3	1200	-
Acmella uliginosa	CP/CF	5	713	3.3/23.7
	CWA	5	713	-
	P	7	1170	-

CP= Cooking with potash, CF= cooking with ash filtrate, CWA= cooking without additive, P= precooking

3.2- Total Polyphenolic content (TPC)

TPC of uncooked leafy was presented in Table 2. Precooking (P) and cooking with potash (CP) significantly decreased the TPC ($p<0.05$) for *J. tenella*, while no significant effect was observed for cooking without additive (CWA) and cooking with ash filtrate (CF). In contrast, significant increase of TPC was observed after precooking for *A. uliginosa* but no significant effect was observed for other cooking methods (CP, CWA, and CF) for this species. As far as *S. radiatum* is concerned, no significant effect for all cooking method was observed on TPC.

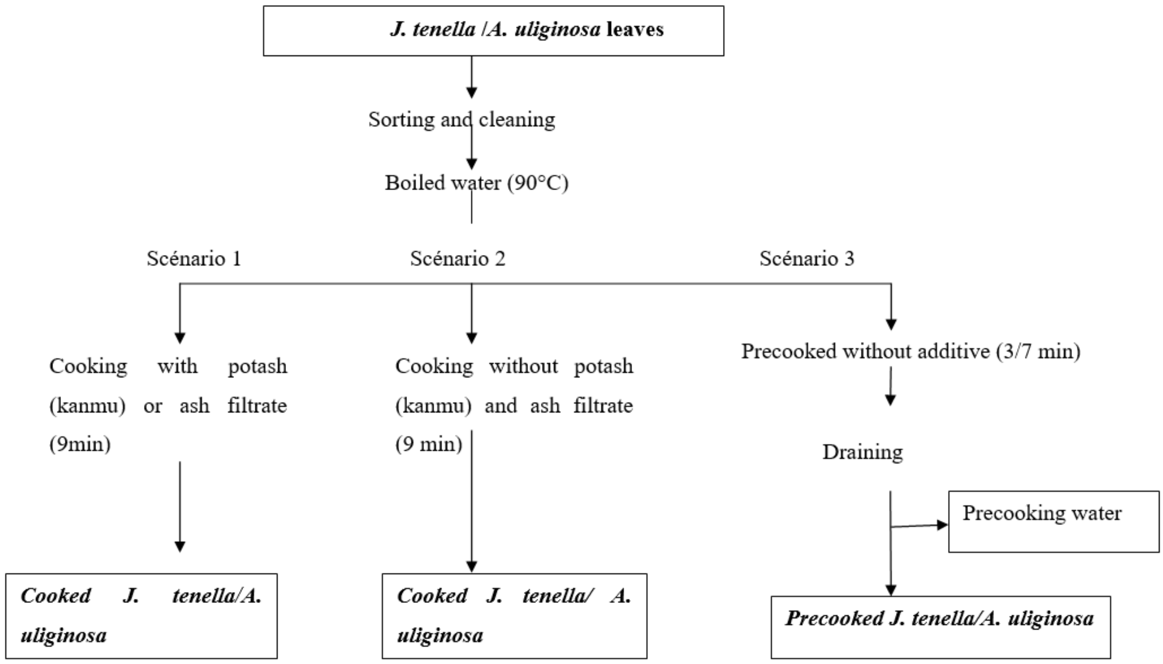

Figure 1: Processing methods applied for *Justicia tenella* and *Acmella uliginosa.*

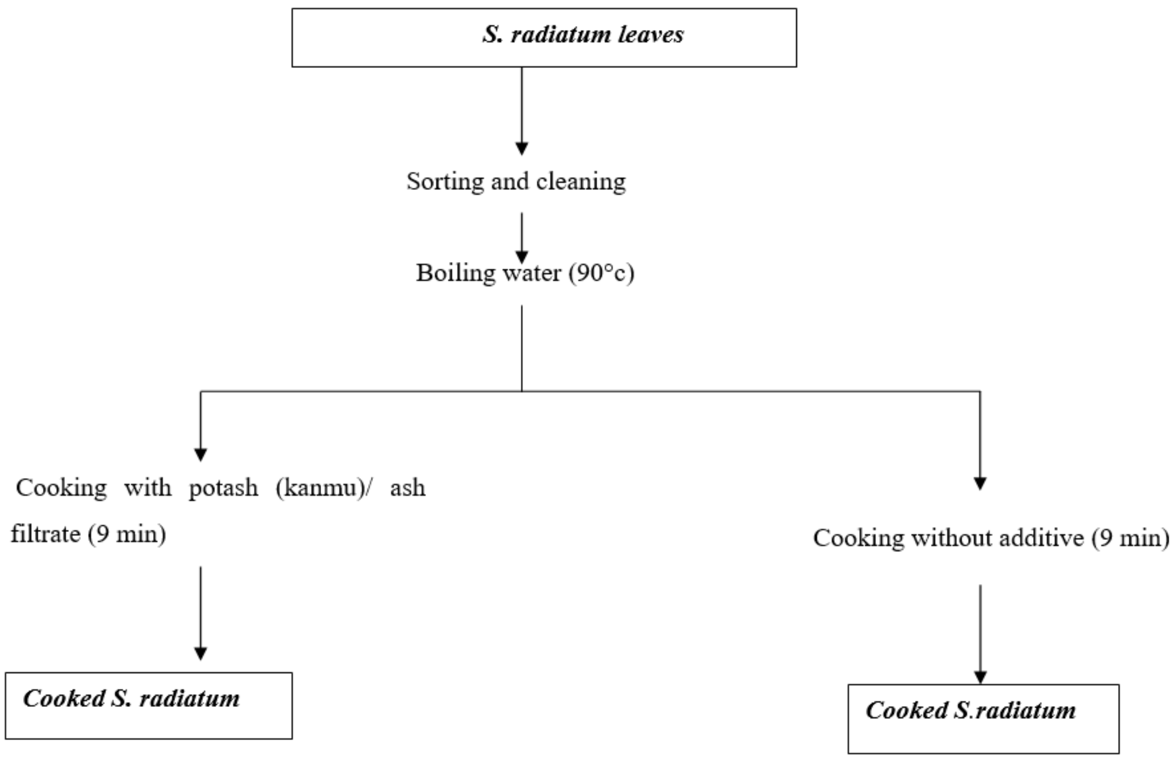

Figure 2: Processing methods applied for *Sesamum radiatum*

Table 2: Total phenolic compounds in leafy vegetables after different treatments

Parameters	Species	R	P	CWA	CP	CF
TPC (g GAE/100g DW)	*Justicia tenella*	0.56±0.12a	0.38±0.01b	0.59±0.12a	0.44±0.07bc	0.53±0.12ac
	Acmella uliginosa	0.36±0.05a	0.62±0.11b	0.43 ±0.04a	0.37±0.07a	0.43±0.08a
	Sesamum radiatum	0.60±0.15a	-.	0.58±0.03a	0.53±0.20a	0.55±0.11a

Means ±SD, values with different letters on the same line are significantly different ($p<0.05$), GAE: Gallic Acid Equivalent, R= raw, P= precooking, CWA= cooking without additive, CP= cooking with potash, CF= cooking with ash filtrate

Table 3: Antioxidant capacity of leafy vegetables during cooking

Antioxidant capacity	Species	C	P	CSA	CP	CF
DPPH (%)	*Justicia tenella*	74.08±7.62b	63.22±18.1ab	52.95±16.63a	3.78±2.08c	6.25±22.32d
	Acmella uliginosa	39.01±6.51c	85.58±4b	88.26±2.09b	15.84±14.16a	22.16±8.52a
	Sesamum radiatum	63.9±34 a	.-	90.89±0.3c	0.77±7.16b	44.44±25.6a

Results of ANOVA + Newman and Keuls test: Means ±SD, values with different letters on the same line that have different letters are significantly different ($p<0.05$), R= raw, P= precooking, CWA= cooking without additive, CP= cooking with potash, CF= cooking with ash filtrate

3.3- Effect of cooking methods on antioxidant capacity

Inhibition percent of DPPH radical varied between 3.78 - 74.08%, 15.84 - 39.01% and 10.77 - 90.89% for *J. tenella, A. uliginosa* and *S. radiatum,* respectively (Table 4). A significant decrease of antioxidant activity was recorded during treatments for *J. tenella* ($p<0.05$). Only precooking significantly increased antioxidant activity of *A. uliginosa*, while only cooking without additive significantly increased it in the case of *A. uliginosa* and *S. radiatum.*

IV. Discussion

The high variability of TPC was associated to leafy species and it showed two antagonist behaviors depending on the treatments. An increase of TPC in *A. uliginosa* (72.2%) and *J. tenella* (5.35%) during precooking was reported during cooking of *Spinacia oleracea*, *Petroselinum Crispum*, *Anethum graveolens* and other traditional leafy vegetables such as *Ocimum gratissimum, Talinum triangulare, Amaranthus* hybridus*, Brassica juncea*, *Brassica rapa* and *Solanum retroflexum* Dum [4], [5], [11], [12], [13]. This increase might be rooted in the breakdown of cell wall and liberation of TPC that is more quantified [14]. The raise of free flavonols content subsequent to cooking could also explain this increase [15]. Conversely, a significant decrease of TPC was noticed during heat treatments of fruits, leafy vegetables, sorghum and legumes [16], [17], [18], [19], [4], [20], [8], [21], [22]. A decrease induced by precooking method could be explained in one hand by leakage of some TPC or number of flavonoids affected by heat treatment in boiling water [23], [24] or by breakdown of molecule link during cooking [25]. In other hand, TPC decrease could be due to the formation of hydroxyl groups during cooking and formation of insoluble complex with minerals or proteins; in this form, identification of reactive hydroxyl groups could decrease [26].

In general, tenderizer or additives induce a downward trend for TPC, probably due to their linkage with polyphenol's hydroxyl groups [27]. Actually, traditional tenderizer had variable mineral composition such as Ca, Fe, Zn and Na [27]. The Kanmu used in this study contains 94, 82 and 0.054 mg/ 100g DW of calcium, iron and zinc, respectively.

The DPPH percent inhibition ranged between 39.0 and 74.1% and lower than that of BHA (93.6%), one synthetic antioxidant. Culinary effect varied within species. Antioxidant activity decreased in the case of *J. tenella* or increased after precooking of *A. uliginosa* or cooking with tenderizer. Wachtel–Galor *et al.* [28] in the case of Brassicaceae also mentioned this variability of antioxidant activity as a result of cooking methods. Antioxidant activity decreased from 14.65 to 94.95% and, this is in line with [3] and [11] who reported the decrease of antioxidant activity after cooking during 2-3 and 6 min. Amin *et al*. [29] also mentioned that blanching reduced *Amaranthus* leaves antioxidant activity. The reduction of scavenging activity after cooking with potash or ash filtrate could be affected by samples pH. Actually, the pH of leaves obtained with ash filtrate ranged between 9.02 and 9.91 while those obtained with potash varied from 10.15 to 10.30. [30] found that antioxidant activity decreased significantly when the pH is 10 and it is low at pH=10 than pH= 9. As far as the increase in scavenging activity (during *A. uliginosa* precooking, *A. uliginosa* and *S. radiatum* cooking without additive) is concerned, we are agreement with other research works [31]. Turkmen *et al.* [4] obtained the same trend after cooking of pepper and green beans. Boiling of plantain, pumpkin, mushrooms and leafy vegetables [32], [5], [33], [34] increased their antioxidant activity. According to [35], this rise could be due to breakage of the cell wall and cellular compartments that release more antioxidant components or due to chemical reactions with production of antioxidants compounds. The increase of antioxidant activity in cooked leaves could be also due to inactivation of oxidative enzymes by heating [36]. According to [37], enhance of scavenging activity after tomato cooking could be caused by increase of discharge of phyto-chemical substances while [31] attributed the increase of activity in cooked leaves to formation of novel products with antioxidant activity.

V. CONCLUSION

Beside a large variation nutritional composition of studied species, different cooking processes had contrasting physicochemical changes depending on species. Actually, precooking affects significantly TPC content of some of leafy vegetables. Cooking with potash increase significantly TPC content of *J. tenella* and nitrate in all studied leafy vegetables., ash filtrate does not influence significantly TPC, in all species. In contrast, cooking with additive decrease significantly antioxidant capacity of all studied species. Antioxidant activity of all species is reduced during cooking with tenderizer. The effect of traditional cooking methods on vitamins content could be explored to understand their contribution in reduction antioxidant capacity. It will be important to identify the appropriate cooking methods that decrease antinutritional content and preserve vitamins and mineral.

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