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Impact of Spices on Micronutrients, Phenolic Compounds, in Vitro and in Vivo Antioxidant Potentialities in Eggplant Leaves Cooked with Water

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Abstract: Eggplant leaves (*Solanum macrocarpon*) are rich in nutrients which are unfortunately lost during cooking. To reduce micronutrients losses and enhance antioxidant potentialities, they were cooked in water for 30 minutes with onion, ginger and Guinea pepper. The analyzes focused on the determination of vitamin C, β-carotene, phenolic compounds, free radical-scavenging and lipid peroxidation inhibitory activities. In Wistar rats, thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD) and glutathione peroxidase activities were also evaluated during 1 month. The results indicated that vitamin C remained low in eggplant leaves cooked with onion and spices. Cooked eggplant market leaves β-carotene content (187.11 µg/100 FM) increase from 447.43 µg/100 FM (with onion) to 905.44 µg/100 FM (with Guinea pepper). Ginger, Guinea pepper and onion improved eggplant field leaves free radical scavenging activities, which previous IC_{50} value (12.00 µg/ml) became 0.67, 0.83 and 3.08 µg/ml respectively. TBARS rate increased only for rats which received eggplant market leaves cooked with onion from 0.02 to 0.03 nmol/ml. SOD activities increased during the firsts 2 weeks of experiment. Glutathione peroxidase activities is high in serum of rats which received eggplant field leaves cooked with onion (18.70 nmol/min/ml). Onion, ginger and Guinea pepper enhance eggplant antioxidant potentialities during cooking.

Keywords: Eggplant Leaves, Onion, Spices, Micronutrients, Antioxidant Activities, Cooking, Wistar Rats

1. Introduction

Eggplant leaves (*Solanum macrocarpon*) are recommended to human well-being as they contain high level of nutrients, fibers and phenolic compounds [1]. They contribute to overcome anemia and other nutrients deficiencies [2] and have antioxidant, hypoglycemic, hypolipidemic and weight reduction effects on human organism [3]. Eggplant leaves are usually cooked with other vegetables (onions, tomatoes) and spices (pepper) to improve their taste and their flavor. In addition, spices contain phenolic compounds which give them a potential antioxidant activity. These antioxidants have the capacity to scavenge the free radicals and prevent the damage caused by them in human organism [4, 5].

One of the challenge is that eggplant leaves are consumed after cooking processes in order to increase their palatability, their digestibility and eliminate anti-nutritional factors [6]. But, cooking occurred micronutrients and phenolic compounds' losses in leafy vegetables which increased with cooking time and conditions [7]. However, in spices, heating treatments increase antioxidant activities [8]. So, such properties could be used to prevent micronutrients losses and increase cooked food antioxidant capacities to overcome oxidative stress due to free radicals [9].

Some studies have been conducted in this field of research. One of them has indicated that cooking vegetables with acidulate and antioxidant spices (tamarind, turmeric and onion) increase the retention of β-carotene in leafy vegetables [10].

Another one has revealed that spices (turmeric, nutmeg, Guinea pepper and cloves) can reduced micronutrients losses and increase antioxidant activities during steaming [11]. As the most cooking process used is cooking with water, there is a need to evaluate the impact of spices on eggplant leaves during this process. The required spices were onion, ginger and Guinea pepper. Their impact on vitamin C and β-carotene preservation during cooking with water has been studied. Moreover, there in vitro and in vivo impact on boiled eggplant leaves has been evaluated in Wistar rats.

2. Materials and Methods

2.1. Materials

Eggplant leaves were collected in field and in market. The experimental field was made in Bingerville, a town localized at 16.20 km of Abidjan (5°21'20" of North latitude and 3°53'07" of West longitude). A nursery was made and 5 weeks after sowing, plants were transplanting as follow: 1 m between line and 0.5 m between plants on line. Watering was done in morning and in evening for two weeks after transplanting and then, when necessary. Leaves harvest begging 6 weeks after transplanting and took place over 6 weeks. For this study, only leaves harvest at the 9th week after transplanting were taken into account because, according to Kouamé-Osnou et al. [12], at this stage, nutrients are in greatest quantity in leaves.

Eggplant leaves were also collected in « Gouro Market » a leafy vegetables wholesale trade in Abidjan, Côte d'Ivoire. Violet onions and yellow ginger were also collected in the same market and Guinea pepper was harvested in a field in Dabou, a town localized at 38.76 km of Abidjan (5°19'32" of North latitude and 4°22'36" of West longitude).

Animal material consisted of Wistar rats (male and female) to assess in vivo antioxidant activities. They were collected and treat according to the Vivarium of Felix Houphouët-Boigny University ethical code (Côte d'Ivoire). Thus, 44 rats aged 2 months and weighing between 75 and 170 g at the start of the study were used and divided into 11 batches of 4 rats each. Males and females were separated. The rats were acclimatized for one week to the laboratory rearing conditions.

2.2. Methods

2.2.1. Sampling

On field, eggplant leaves were harvested early in the morning at the 9th week after transplanting. At market, Eggplant leaves were collected from 3 sellers chosen randomly, then, mixed. All samples were transported to the laboratory for the analysis. The leaves were destalked, cleaned, washed under running water. Guinea pepper were burned before dried under the sun for 1 week. Ginger was pelled, cut into fine slices and dried in an air-conditioned room at 16°C for 72 hours. Guinea pepper and ginger were ground with a grinder (Moulinex Lm 241025). Onions were cut into fine slices and used in fresh form as made by women at home.

The batches of rats were distributed as follows: 1 control batch (receiving nothing), 1 batch receiving vitamin C, 4 batches receiving respectively onion, ginger, Guinea pepper and the Ginger-Guinea pepper mixture and 5 batches receiving each a leafy vegetable cooked with or without spices. The experiment was conducted for 1 month.

2.2.2. Cooking Method

Eggplant leaves were cooked with water for 30 minutes with and without onions and spices. Ginger and Guinea pepper were mixed in the proportion 1:1. For 100 g of eggplant leaves, 1 g of onions or spices was added. A part for vitamin C and β-carotene analysis which was made on fresh leaves sample, the other analysis recurred dried samples. Samples in these cases were dried at 16°C during 72h.

2.2.3. Vitamin C and β-carotene Analysis

Vitamin C was extracted in 10 g of eggplant leaves cooked with or without onions or spices and stabilized with 10ml of a mixture of metaphosphoric acid (0.3M) and acetic acid (1.4M) solution. The extract was centrifuged at 100 rpm for 15 min then filtered on Wathman n°4 paper. The dosage was made with 2,6-dichlorophenol-indophenol [13]. β-carotene were extracted according to Tee et al. method [14]. A quantity of 10 g of sample were mixed with 40 ml of ethanol during 3 min. and put in a separating funnel with 50 ml of hexane. The detection was made at 450 nm by colorimetric method with a spectrophotometer (Rayleigh UV-1800). A β-carotene solution (1 µl.ml^{-1}) was used as standard.

2.2.4. Total Phenolic and Flavonoid Extraction and Determination

Phenolic compounds were extracted according to Bala et al. method [15]. Total phenolic compounds were determined by Folin-Ciocalteu method at 765 nm and expressed as gallic acid equivalents (GAE) in milligrams per gram DM using the standard curve of gallic acid [16]. Total flavonoids were determined at 415 nm and expressed at quercetin equivalents (QE) in milligrams per gram DM using the standard curve of quercetin [17].

2.2.5. Free Radical Scavenging Activities and Anti-Radical Power Determination

Free radical scavenging activity of the extracts was measured with the DPPH method [18]. This test consists in evaluate the capacity of extract to fixed DPPH free radical by the measurement of color diminution at 517 nm. Vitamin C (100 µg/ml) was used as standard. The sample concentration which can inhibit 50% of DPPH (IC_{50}) was determined on graphic and expressed the antiradical activity. It allowed to calculate the antiradical power [19] as follow:

$$ARP = \frac{\text{DPPH solution concentration (µmol of reduced DPPH)}}{IC_{50} \text{ x } 10^{-3} \text{ (µg /ml)}} \quad (1)$$

ARP: antiradical power
DPPH: 2,2-diphenyl-1-picrylhydrazyl
IC_{50}: Sample concentration which inhibit 50% of DPPH

2.2.6. Lipid Peroxidation Inhibitory Activity Determination

Lipid peroxidation inhibitory activity was determined

according to ammonium thiocyanate test with some slight modifications [20]. A quantity of 0.5 ml of samples extracts at graduate concentrations (0.2 to 6 mg/ml) was mixed to 0.2 ml of linoleic acid (20 mg/ml in ethanol 99%) and 0.4 ml of phosphate buffer (50 mM; pH 7.4). The mixture was heated in a water bath at 40°C for 15 min. Then, 0.1 ml of mixture was added to the reaction mixture (3 ml of ethanol (70%), 0.1 ml of ammonium thiocyanate (30 mg/ml) and 0.05 ml of $FeSO_4$ (2.45 mg/ml in HCl 3.5% (v/v)). The absorbance was determined at 500 nm after incubation at ambient temperature during 3 min. Gallic acid (100 µg/ml) was used as standard.

2.2.7. Rat Gavage and Blood Sampling

The rats were force-fed (with onion and spices and eggplant leaves cooked with or without onion and spices) for 1 month using a probe. A quantity of 10 g of onion, spices or eggplant leaves powder was soaked in 100 ml of methanol and homogenized using a rotary shaker for 24 h. The extract was filtered through Whatman paper and oven dried [15]. The amount of residue to be administered to a rat was determined according to BURGEAP method [21]. Thus, 20 mg of each residue was mixed with 23.81 ml of virgin olive oil and stored in jars for the gavage of rats according to their weight. For the gavage, 0.5 ml of extract was administered for a 100 g weight rat.

A blood sample was taken from the tails of the rats at the beginning, after 2 weeks and at the end of the month. About 4 ml of blood was collected in red tubes. Blood was centrifuged at 900 rpm for 5 min at 4°C. Serum was collected and stored at -20°C for determination of antioxidant enzymes. The rats were sacrificed at the end of the study.

2.2.8. TBARS

TBARS (Thiobarbituric Acid Reactive Substances) essay was carried out according to Satoh method [22]. To precipitate serum proteins, 2.5 ml of 20% (m/V) TCA were added to 0.5 ml of rat serum and the mixture was then centrifuged at 1500 g for 10 min. Then, 2.5 ml of sulfuric acid and 2 ml TBA (0.2%) were added to the sediment, shaken, and incubated for 30 min in a boiling bath. After addition of 4 ml of n-butanol the solution was centrifuged, cooled and the absorbance was read at 532 nm. The calibration curve obtained using different concentrations of 1,1,3,3-tetramethoxypropane as a standard was used to determine the concentration of TBA-MDA adducts in the samples.

2.2.9. Superoxide Dismutase (SOD) and Glutathione Peroxidase

The SOD assay was carried out according to protocol 574601 of the "Superoxide Dismutase Assay" kit from Calbiochem (Merk). A quantity of 10 µl of SOD standard or serum was added to 200 µl of radical detector. The reaction was initiated by adding 20 µl of diluted xanthine oxidase. The mixture was shaken and incubated for 20 min at room temperature. Absorbance was read at 450 nm.

Glutathione peroxidase assay was carried out according to protocol 353919 of the "Glutathione Peroxidase Assay" kit from Calbiochem (Merk). In non-enzymatic wells, 120 µl of buffer was added to 50 µl of co-substrate mix. In the enzyme wells, 100 µl of buffer, 50 µl of co-substrate mixture and 20 µl of bovine enterocyte glutathione peroxidase were mixed. Serum (20 µl) was incorporated into 100 µl of buffer and 50 µl of co-substrate mixture. The reaction was initiated by adding 20 µl of cumene hydroperoxide. The mixture was well stirred, and the absorbance was read at 340 nm every minute for 5 minutes.

2.2.10. Statistical Analysis

Data analysis and graphic representations were made with Graph Pad Prism 5.0 (Microsoft U.S.A). Results made in triplicate were expressed as means with standard deviation. A one-way ANOVA was performed, and means were separated using Tukey test or Dunnett test ($p \leq 0.05$).

3. Results

3.1. Vitamin C, β-carotene, Total Phenolic and Flavonoid Compound Content in Onion, Spices and Eggplant Leaves Cooked with and Without Onions and Spices

Table 1 present vitamin C, β-carotene, total phenolic and flavonoid content of onion and spices. Onion' vitamin C level (24.78 mg/100g FM) was higher than that of spices and differed significantly to them. Ginger had the highest β-carotene content (545.49 µg/100 g FM) and Guinea pepper the lowest one (103.60 µg/100 g FM). Ginger total phenolic and flavonoids content (185.17 mg GAE/g DM and 108.58 QE/g DM respectively) were significantly higher than in onion and the other spices. In spice mixture (Ginger-Guinea pepper) total phenolic compound and flavonoids rate were not improved. There were about 69.25 mg GAE/g DM and 13.5 QE/g DM respectively.

Table 1. *Vitamin C, β-carotene, total phenolic and flavonoid compounds content in onion and spices (alone and mixed).*

Spices	Vitamin C (mg/100g FM)	β-carotene (µg/100g FM)	Total phenolic (mg GAE/g DM)	Flavonoid (mg QE/g DM)
Onion	24.78 ± 0.01^{b}	246.63 ± 8.95^{c}	7.75 ± 0.2^{a}	2.42 ± 0.12^{a}
Ginger	3.53 ± 0.01^{a}	545.49 ± 2.19^{d}	185.17 ± 2.43^{c}	108.58 ± 1.85^{c}
Guinea pepper	3.53 ± 0.01^{a}	103.6 ± 2.99^{a}	69.58 ± 11.37^{b}	47.58 ± 7.17^{b}
Ginger-Guinea pepper	3.53 ± 0.01^{a}	136.54 ± 0.79^{b}	69.25 ± 1.06^{b}	13.5 ± 0.54^{a}

In row, values with different letter differed significantly (Tukey test, $p \leq 0.05$).

Vitamin C content is low in cooked eggplant leaves with or without onion and spices (Table 2). Cooked eggplant market leaves β-carotene content (187.11 µg/100 g FM) increase with onion and spices from 447.43 µg/100 g FM (with onion) to 905.44 µg/100 g FM (with Guinea pepper). However, onion and spices didn't improve cooked eggplant field leaves

β-carotene content. Cooked eggplant market leaves total phenolic content (86.33 mg GAE/g DM) decrease significantly while adding ginger (9.27 mg GAE/g DM) and Guinea pepper (12.18 mg GAE/g DM) and ginger-Guinea pepper mixture (65.25 mg GAE/g DM). But, there was an increase of total phenolic compound rate while cooked with onion (91.17 mg GAE/g DM) However total phenolic compound of eggplant field leaves cooked with onion (134.75 mg GAE/g DM) and ginger-Guinea pepper mixture (171.00 mg GAE/g DM) content were important. Cooked eggplant market leaves flavonoid rate (4.08 mg QE/g DM) was only improved by onion incorporation (39.17 mg QE/g DM). For eggplant field leaves flavonoid content (101.67 mg QE/g DM) there was a decrease while adding onion and spices.

Table 2. *Vitamin C, β-carotene, total phenolic and flavonoid compounds content in eggplant leaves cooked with and without onion and spices.*

Eggplant leaves	Vitamin C (mg/100g FM)	β-carotene (µg/100g FM)	Total phenolic (mg GAE/g DM)	Flavonoid (mg QE/g DM)
Cooked market leaves	7.08±0.01^{b}	187.11±2.59^{c}	86.83±3.90^{d}	4.08±1.36^{a}
Market leaves onion	7.08±0.01^{b}	447.43±0.88^{e}	91.17±0.51^{d}	39.17±0.59^{d}
Market leaves ginger	3.53±0.01^{a}	842.13±1.03^{f}	9.27±0.15^{a}	4.32±0.60^{a}
Market leaves pepper	0±0^{a}	905.44±5.93^{g}	12.18±0.22^{a}	8.03±0.20^{a}
Market leaves ginger-pepper	3.53±0.01^{a}	187.29±1.88^{c}	65.25±0.89^{b}	6.07±0.31^{a}
Cooked field leaves	3.53±0.01^{a}	286.15±1.52^{d}	248.00±0.35^{g}	101.67±0.3^{g}
Field leaves onion	3.53±0.01^{a}	98.10±1.87^{a}	134.75±0.74^{e}	43.50±0.20^{e}
Field leaves ginger	3.53±0.01^{a}	111.91±1.31^{b}	81.83±0.62^{d}	35.00±0.71^{c}
Field leaves pepper	3.53±0.01^{a}	161.17±0.52^{c}	79.50±0.20^{c}	18.42±0.85^{b}
Field leaves ginger-pepper	3.53±0.01^{a}	186.65±1.04^{c}	171.00±0.35^{f}	73.83±0.72^{f}

In row, values with different letter differed significantly (Tukey test, $p \leq 0.05$)

3.2. In Vitro Antioxidant Activities in Onion, Spices and Eggplant Leaves Cooked with and Without Onion and Spices

3.2.1. Free Radical Scavenging Activities and Antiradical Power of Onion, Spices and Eggplant Leaves Cooked with and Without Onion and Spices

DPPH free radical scavenging activities of vitamin C, onion, spices and eggplant leaves cooked with and without onion and spices increased with concentrations (Figure 1). Vitamin C inhibitory concentration of 50% DPPH (IC_{50}) was about 1.75 µg/ml. Among spices, ginger, and Guinea pepper IC_{50} values (1.17 and 1.58 µg/ml respectively) were better than that of vitamin C, indicating a good antiradical capacity and therefore an important antiradical power (85.71 and 63.16 µmol.ml/µg for ginger and Guinea pepper respectively) (Table 3). These improved eggplant field leaves free radical scavenging activities. Indeed it IC_{50} value which was 12.00 µg/ml became 0.67 µg/ml while cooked with ginger and 0.83 µg/ml while cooked with Guinea pepper. So, the antiradical power of eggplant field leaves cooked with ginger and Guinea pepper became higher than that of vitamin C (166.67 µmol.ml/µg and 133.33 µmol.ml/µg respectively). In eggplant market leaves, spices have not improved free radical scavenging activities. Onion have a less important IC_{50} value (35.42 µg/ml). But, when added to eggplant leaves, it improved their free radical scavenging activities with a IC_{50} value of 3.83 µg/ml (in eggplant market leaves) and 3.08 µg/ml (in eggplant field leaves).

Table 3. *Onion, spices and eggplant leaves cooked with and without onion and spices IC_{50} and antiradical power.*

Spices and eggplant leaves	IC_{50} (µg/ml)	ARP (µmol.ml/µg)
Vitamin C	1.75±0.20^{a}	57.93±6.83^{d}
Onion	35.42±0.20^{c}	2.82±0.02^{a}
Ginger	1.17±0.37^{a}	85.71±0.12^{d}
Pepper	1.58±0.51^{a}	63.16±0.23^{d}
Ginger-pepper	6.92±0.42^{a}	14.46±0.40^{b}
Cooked market leaves	72.77±0.52^{d}	1.37±0.01^{a}
Market leaves onion	3.83±0.24^{a}	26.19±1.68^{c}
Market leaves ginger	16.00±1.78^{b}	6.33±0.76^{a}
Market leaves pepper	15.93±2.52^{b}	6.45±1.12^{a}
Market leaves ginger-pepper	20.60±1.35^{c}	4.87±0.31^{a}
Cooked field leaves	12.00±0.82^{b}	8.37±0.57^{a}
Field leaves onion	3.08±0.72^{a}	34.26±7.96^{c}
Field leaves ginger	0.67±0.24^{a}	166.67±47.14^{d}
Field leaves pepper	0.83±0.24^{a}	133.33±47.14^{d}
Field leaves ginger-pepper	8.83±0.24^{b}	11.33±0.31^{b}

In row, value with different letter differed significantly (Dunnett test, $p \leq 0.05$).

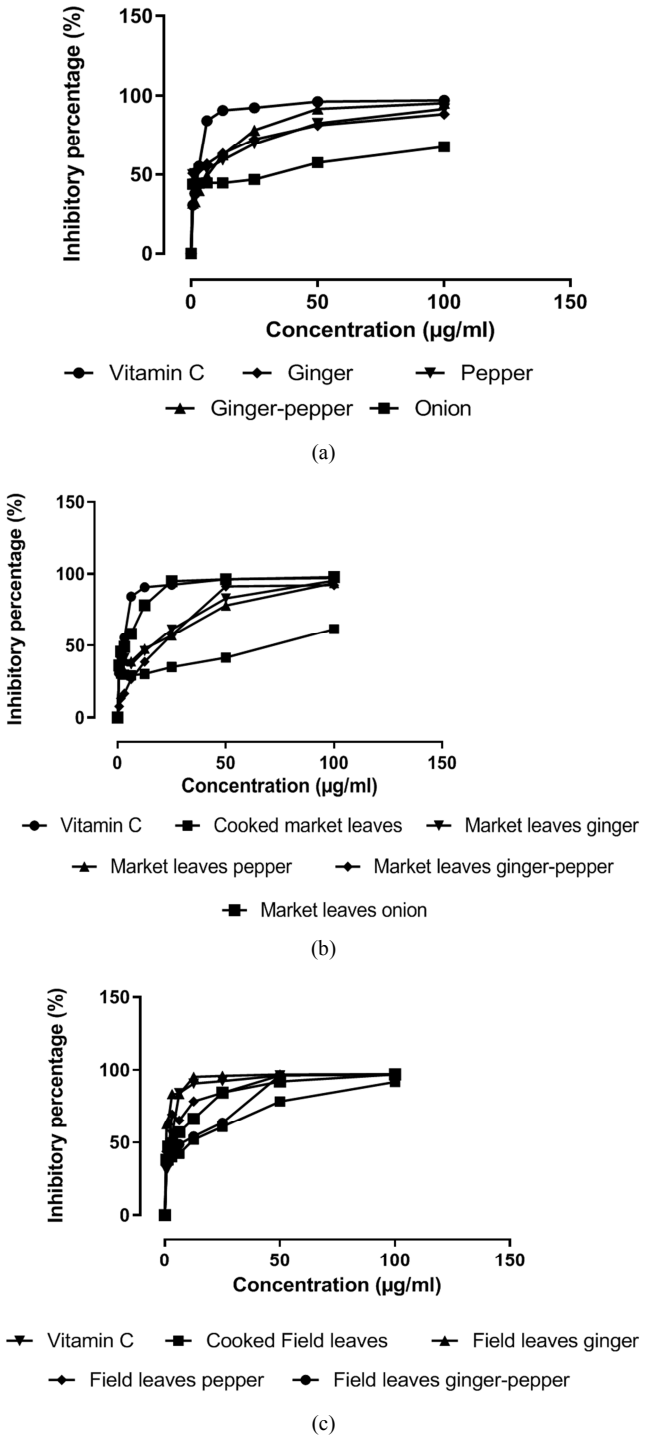


Figure 1. *Evolution of antiradical activities of onion and spices (a), eggplant market leaves cooked with and without onion and spices (b) and eggplant field leaves cooked with and without onion and spices (c).*

3.2.2. Lipid Peroxidation Inhibitory Activities of Onion, Spices and Eggplant Leaves Cooked with and Without Spices

Lipid peroxidation inhibitory activity of onion, spices and eggplant leaves cooked with and without spices were upper and differed significantly to that of standard (gallic acid) and increased with concentration (Figure 2). Indeed, gallic acid lipid peroxidation activity varied from 65.32 to 74.69% with an IC_{50} value of 156.33 µg/ml. Ginger, Guinea pepper and onion lipid peroxidation IC_{50} value (115.00, 116.67 and 122.00 µg/ml respectively) are higher and differ significantly to that of gallic acid. Only eggplant market leaves cooked with ginger and Guinea pepper lipid peroxidation inhibitory activity didn't differed of that of gallic acid.

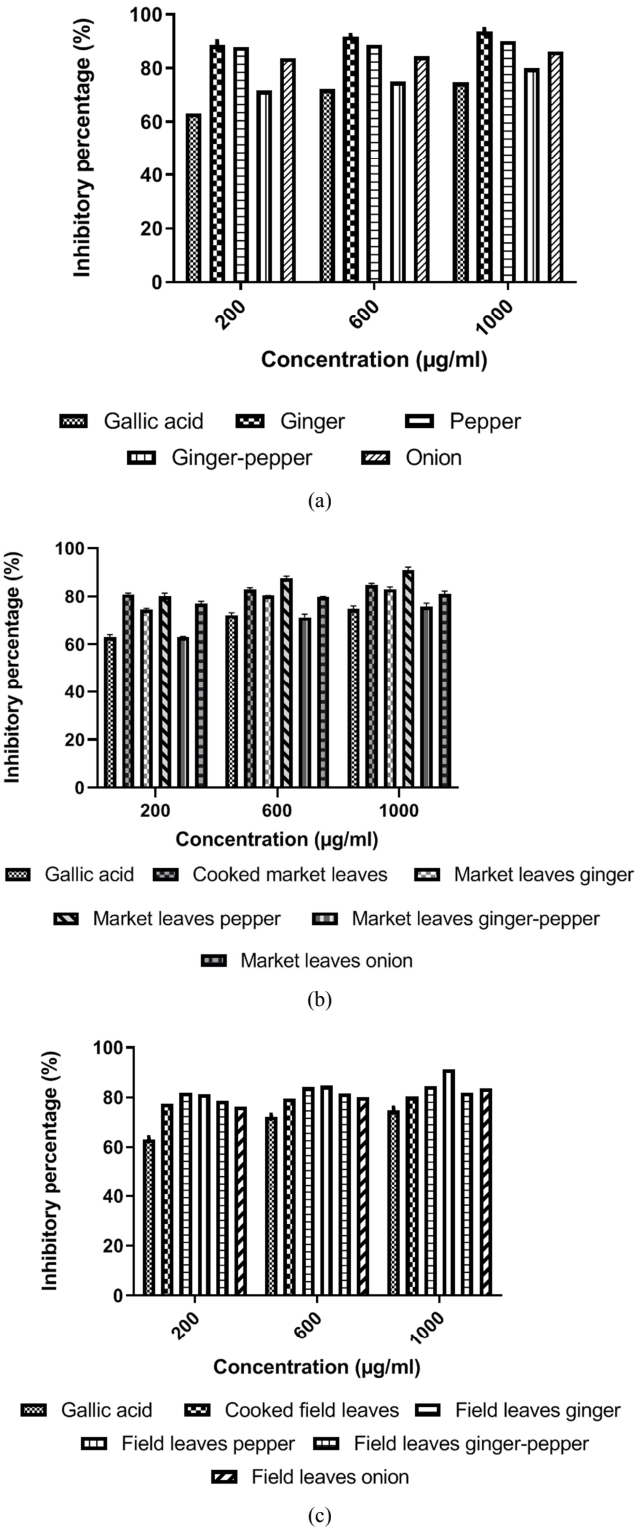
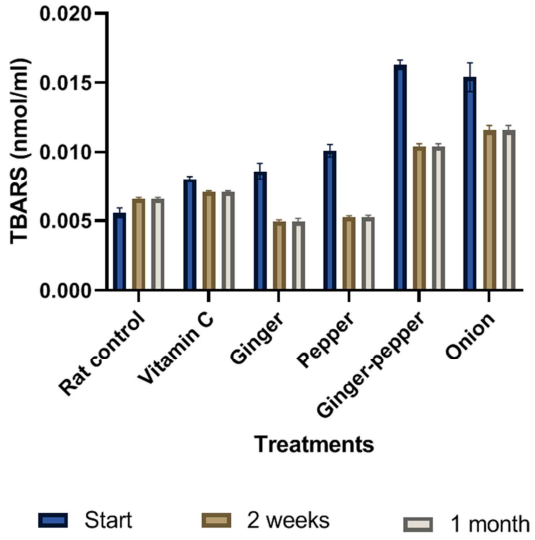


Figure 2. *Evolution of lipid peroxidation inhibitory of onion and spices (a), eggplant market leaves cooked with and without onion and spices (b) and eggplant field leaves cooked with and without onion and spices (c).*

3.3. In Vivo Antioxidant Activities of Onion, Spices and Eggplant Leaves Cooked with or Without Spices

3.3.1. TBARS Activities

The determination of substances resulting from lipoperoxidation and reacting with thiobarbituric acid indicates the presence of free radicals in the serum of rats. Free radicals rate in rats' serum decrease from the beginning to the end of the experiment for all rats' batches except for rats which received eggplant market leaves cooked with onion. Indeed, free radicals in this batch increase from 0.02 to 0.03 nmol/ml (Figure 3).



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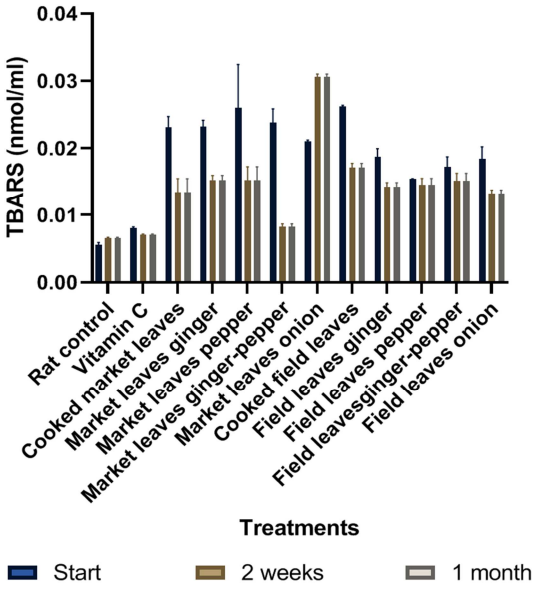
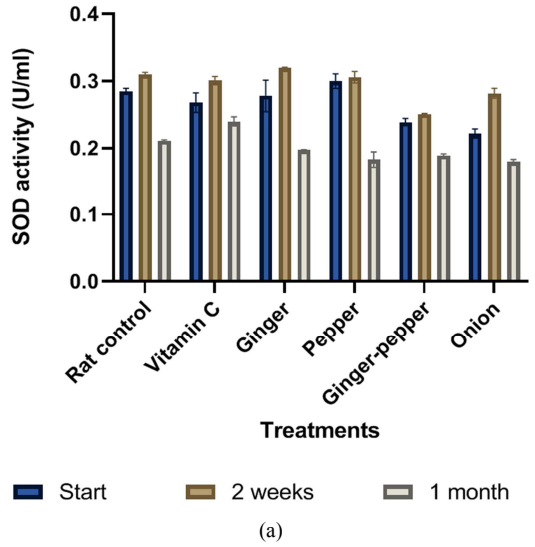


(b)

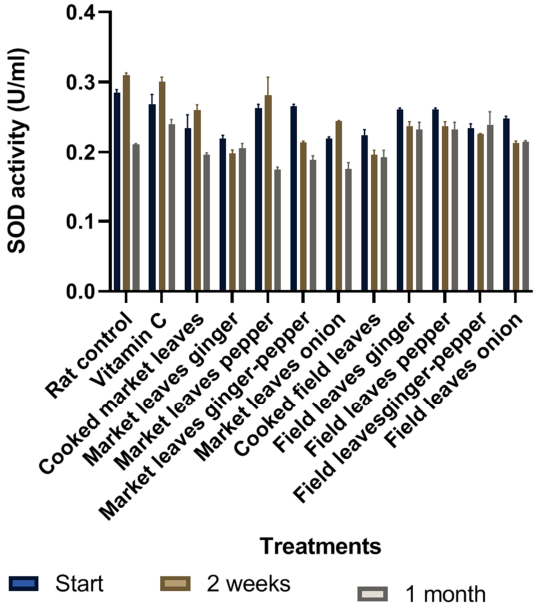
Figure 3. *TBARS activity evolution in serum of rats receiving onion, spices (a) and eggplant market and field leaves cooked with and without onion and spices (b).*

3.3.2. Superoxyde Dismutase (SOD) Activities

SOD activities increase during the firsts 2 weeks of rats' gavage then it decreases after the last 2 weeks for onion, spices and eggplant market leaves. But, for eggplant field leaves cooked with and without onion and spices, SOD activities decreased. At 2 weeks, there was no significant difference between SOD activity of the control (0.31 U/ml) and that of ginger (0.31 U/ml) and guinea pepper-cloves (0.31 U/ml). But it slightly differed to that of onion (0.28 U/ml) (Figure 4).



(a)



(b)

Figure 4. *SOD activity evolution in serum of rats receiving onion, spices (a) and eggplant market and field leaves cooked with and without onion and spices (b).*

3.3.3. Glutathione Peroxidase Activity

Glutathione peroxidase activities also decrease during the experiment for most treatments. But at 2 weeks of the experience, it is high in serum of rats control (29.90 nmol/min ml) and in that of rats which received eggplant field leaves cooked with onion (18.70 nmol/min/ml). For rats which received eggplant field leaves with ginger or Guinea pepper, glutathione peroxidase activity was around 11.50 nmol/min/ml (Figure 5).

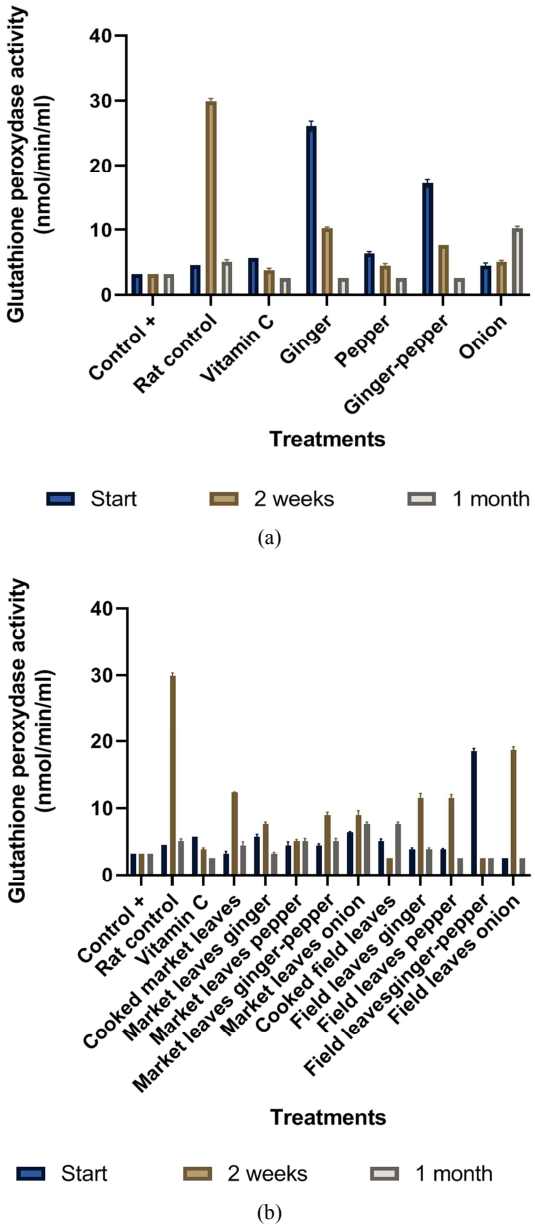


Figure 5. *Glutathione peroxydase activity evolution in serum of rats receiving onion and spices (a) and eggplant market and field leaves cooked with and without onion and spices (b).*

4. Discussion

The effect of onion, ginger and Guinea pepper on cooked eggplant leaves vitamin C, β-carotene, in vitro and in vivo antioxidant potentialities has been evaluated in this study. Previously, onion and spices vitamin C, β-carotene, phenolic and flavonoid compounds have been determined. Onion vitamin C rate is more important than in ginger and Guinea pepper. This is due to the fact that onion has been used in fresh form while the spices have been dried before utilization. Indeed, vitamin C is a water soluble and thermolabil vitamin as revealed in Ejoh et al. study [23]. So, during drying process this vitamin was evaporated, favoring a low content in spices.

Ginger had an important β-carotene content probably due to his flesh yellow color. Moreover, this β-carotene rate is higher than 90 mg/100g FM as indicated by Kandlakunta et al. [24] in their study. Ginger had also the most important total phenolic and flavonoids content. This suggest an important antioxidant activity because phenolic compounds are implicated in oxidative stress inhibition by donating a single electron or hydrogen atom for reduction [25]. Total phenolic and flavonoid compound rate was very low in onion. This was probably due to the part of onion used. In fact, Prakash et al. [26] has indicated that total phenolic compound rate differed according to onion layers. So, for violet onion, total phenolic compounds were about 43.50 mg GAE/g DM in outer layers, 14.90 mg GAE/g DM in middle layers and 4.60 mg GAE/g DM in inner layers. Total phenolic compound and flavonoids rate weren't improved in mixed spices. This suggest any synergistic effect of ginger over Guinea pepper.

Vitamin C content is low in all cooked eggplant leaves. This is due to boiling process and to the fact that vitamin C did not resist to high temperature [23]. Onion and spices improved eggplant market leaves β-carotene content. This was due to their own β-carotene rate and to their antioxidant potentialities. Indeed, Gayathri et al. [10] have revealed that turmeric and onion increased β-carotene retention in amaranth leaves during boiling. However, there was a decrease of β-carotene rate in eggplant field leaves, in comparison to eggplant market leaves, while cooking with onion and spices. This could be due to cultivar and also to an eventual oxidation and isomerization of β-carotene [27].

Eggplant market leaves total phenolic and flavonoids compounds rate increased with onion to eggplant market leaves. This is due to onion antioxidant potentialities. The increase of total phenolic compounds in eggplant field leaves cooked with ginger-Guinea pepper mixture suggests a positive synergistic effect of ginger and Guinea pepper. It could be due to the collapsed cells, inter-tissue cracks, and thin intracellular bonds around the middle area of eggplant leaves [28].

The increase of DPPH free radical scavenging activities with concentration, observed in Guinea pepper, was due to their total phenolic content and had a positive effect on eggplant leaves free radicals scavenging activities and antiradical power. Moreover, ginger and Guinea pepper IC_{50} value are upper than that indicated by Shobana and Akhilender [4] in their study (7.50 µg/ml and 5.50 µg/ml respectively). Onion IC_{50} value is lower than that revealed by Prakash et al. [26] which varied between 0.70 mg/ml (in outer layers) and 12.7 mg/ml (in inner layers). But it improved eggplant leaves free radical scavenging activities while added during cooking. This could be due to its own antioxidant potentialities but also to the fact that heating treatment increase antioxidant activities [4, 8].

Lipid peroxidation inhibitory activity of onion, spices and eggplant leaves boiled with and without onion and spices is upper than that of gallic acid and increased with concentration. This indicates their potentiality to inhibit linoleic acid lipid peroxidation due to their flavonoids content which have the capacity to reduce peroxyl radicals by electrons transfer thanks to their low redox potential [29]. The improvement of lipid peroxidation inhibition in eggplant leaves cooked with

onion and spices is due to their flavonoids content and to boiling process as indicated by Shobana and Akhilender [4].

Rats which received eggplant leaves boiled with or without onion and spices have high free radicals in their blood serum than rats' control. The free radical detection in rats' serum revealed a lipoperoxidation activity and suggest a high in vivo antioxidant potentiality [30]. SOD activities increase during the firsts 2 weeks of rats' gavage regardless of the treatments. This suggests food capacity to catalyze the conversion of the superoxide O_2^- to H_2O_2 and O_2 and to provide a defense system under oxidation conditions, in which O_2^- appears to play an important role [31]. The SOD activities in serum of rats receiving eggplant field leaves cooked with onion and spices were higher than that receiving eggplant market leaves cooked with onion and spices. This is due to varieties and onion and spices high antioxidant capacities. Moreover, Jung et al. [32] have revealed an SOD-like activity of 0.58 U/ml in *Solanum melongena* leaves. Glutathione peroxidase activities decreased during the experiment for most treatments but were more important for rats which received eggplant field leaves cooked without onion and spices. This suggests the formation of oxidative glutathione (GSSG) by reduced glutathione GSH and potential antioxidant capacity due to eggplant leaves consumption.

5. Conclusion

This study has been conducted to evaluate the impact of onion, ginger and Guinea pepper on vitamin C and β-carotene losses reduction and antioxidant potentialities enhancing during eggplant leaves cooking. Onion and spices did not influence vitamin C losses. However, they improved β-carotene and antioxidant activities by increasing eggplant leaves scavenging power and improving their ability to inhibit lipid peroxidation. The detection of free radical, SOD and glutathione peroxidase activities in rats' serum suggest the benefit to add onion and spices to eggplant leaves during cooking. This is great for human well-being and will be better by reducing cooking time.

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References

[1] Oboh, G.; Ekperigin, M. M.; Kazeem, M. I., (2005). Nutritional and haemolytic properties of eggplants (*Solanum macrocarpon*) leaves. *J. Food Compos Anal*, 18 (2-3), 153–160. https://doi.org/10.1007/s00217-006-0410-1.

[2] Vyas, S.; Collin, S.; Bertin, E.; Davys, G.; Mathur, B. Leaf concentrate ace adolescent year alternate toiron and folic acid supplements for anemic girls: randomized controlled trial in India. *Pub Health Nutr*, (2009); pp. 1-6. https://doi.org/10.1017/S1368980009991224.

[3] Famuwagun, A. A.; Taiwo K. A.; Gbadamosi, S. O.; Oyedele, D. J.; Aluko, R. E.; Adebooye, O. C., (2017). Extraction Optimization and Antioxidant Properties of African Eggplant (*Solanum macrocarpon*) Leaf Polyphenols. *Hindawi J Food Quality*, pp. 14. DOI: 10.1155/2017/2159183.

[4] Shobana, S.; Akhilender, N. K., (2000). Antioxidant activity of selected Indian spices. *Prostaglandins Leukot Essent Fatty Acids*, 62 (2), 107–110. https://doi.org/10.1054/plef.1999.0128. PubMed.

[5] Subhasree, B.; Baskar, R.; Laxmi, R. K.; Lijina R. S.; Rajasekaran, P., (2009). Evaluation of antioxidant potential in selected green leafy vegetables. *Food Chem*, 115: 1213-1220.

[6] Richard, R. P., (2007). Contribution à la compréhension de la cuisson domestique sous pression de vapeur: Etude expérimentale et modélisation des transferts, de l'évolution de la texture des légumes et du fonctionnement d'un autocuiseur. Thèse. Ecole doctorale, ABIES, AgroParis Tech, France, 289 p.

[7] Agbo, A. E.; Gbogouri, A. G.; N'Zi, J. C.; Kouassi, K.; Fondio, L.; Kouamé, C., (2019). Evaluation des pertes en micronutriments et en oxalates au cours de la cuisson à l'eau et à la vapeur des feuilles d'épinard Malabar (*Basella alba*) et de célosie (*Celosia argentea*). *Agro. Afr.*, 31 (2), 100-110.

[8] Benkeblia N., (2005). Free-Radical Scavenging Capacity and Antioxidant Properties of Some Selected Onions (*Allium cepa* L.) and Garlic (*Allium sativum* L.) Extracts. *Braz Arch Biol Technol*, 48 (5), 753-759.

[9] García-Alonso, M.; Pascual-Teresa, S.; Santos-Buelga, C.; Rivas-Gonzalo, J. C., (2004). Evaluation of the antioxidant properties of fruits. *Food Chem,* 84, 13-18. https://doi.org/10.1016/S0308-8146(03)00160-2

[10] Gayathri, G. N.; Platel, K.; Prakash, J.; Srinivasan, K., (2004). Influence of antioxidant spices on the retention of β-carotene in vegetables during domestic cooking processes. *Food Chemistry*, 84, 35–43. https://doi.org/10.1016/S0308-8146(03)00164-X.

[11] Agbo, A. E.; Méité, S.; Gbogouri, G. A.; Gouekou, A. D.; Kouamé, C. E.; Kouassi, K.; Brou, K., (2020). Impact of antioxidant spices on vitamin B_9 and β-carotene in steamed sweet potato leaves. *Acta Hort*, *1292*, 383-392. DOI: 10.17660/ActaHortic.2020.1292.51.

[12] Kouamé-Osnou, C. E. A.; Agbo A. E. C.; Traoré S.; Gbogouri G. A., (2020). Evolution of Nutrient Content of Eggplant Leaves (Solanum Macrocarpon) "Gboma" Variety during Plant Growth. *Int JAgric Biol Sci,* pp. 59-68.

[13] Abdulnabi, A. A.; Emhemed, A. H.; Hussein, G. d.; Biacs, P. A., (1997). Determination of antioxidant vitamins in tomatoes. *Food Chem*, 60: 207-212.

[14] Tee, E. S.; Kudalasevan, R.; Young, S. I.; Khor, S. C.; Zakiyah, O., (1996). *Laboratory Procedures in Nutrient Analysis of Foods*. Kuala Lumpur, Malaysia: Division of Human Nutrition, Institute of Medical Research, pp. 55.

[15] Bala, I. M.; Manigundan, K.; Usha, J.; Shanmuga P. A., (2014). Antioxidant and Anti-proliferative activity of methanolic leaf extract of *Eupatorium glandulosum*. *L Int J Adv Res,* 2 (7), 717-723.

[16] Mc Donald, S.; Prenzler, P. D.; Autolovich, M.; Robards, K., (2001). Phenolic content and antioxidant activity of olive extracts. *Food Chem*, 73, 73-84. https://doi.org/10.1016/S0308-8146 (00)00288-0.

[17] Chang, C.; Yang, M.; Wen, H.; Chern, J., (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anals*, 10, 178-182.

[18] Parejo, I.; Codina, C.; Petrakis, C.; Kefalas, P., (2000). Evaluation of scavenging activity assessed by Co (II)/EDTA-induced luminol chemilunescence and DDPH (2,2-diphenyl-1-pycryl-hydrazyl) free radical assay. *J Pharmacol Toxicol Methods*, 44, 507-512. https://doi.org/10.1016/S1056-8719 (01)00110-1. PubMed.

[19] Kroyer, G. T., (2004). Red clover extract as antioxidant active and functional food ingredient. *Innov Food Sci Emerg Technol*. 5, 101–105. https://doi.org/10.1016/S1466-8564 (03)00040-7.

[20] Lee, C. Y.; Sharma, A.; Cheong, J. E.; Nelson, J. L., (2009). Synthesis and antioxidant properties of dendritic polyphenols. *Bioorg Med Chem Lett*, 19, 6326-6330. https://doi.org/10.1016/j.bmcl.2009.09.088. PubMed.

[21] BURGEAP, (2008). Paramètres d'exposition pour le calcul des doses. Rapport, France, pp. 1-15.

[22] Satoh, K., (1978). Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clin Chim Acta*, 90 (1), 37-43.

[23] Ejoh, A. R.; Tanya, A. N.; Djuikwo, N. V.; Mbofung, C. M., (2005). Effect of processing and preservation methods on vitamin C and total carotenoid levels of some *Vernonia* (bitter leaf) species. *Afri. J. Food Nut. Sc.* 5 (2), 12 p.

[24] Kandlakunta, B.; Rajendran, A.; Thingnganing, L., (2008). Carotene content of some common (cereals, pulses, vegetables, spices and condiments) and unconventional sources of plant origin, *Food Chem*, 106, 85–89.

[25] Gramza, A.; Khokhar, S.; Yoko, S.; Gliszczynska-Swiglo, A.; Hes, M.; Korczak J., (2006). Antioxidant activity of tea extracts in lipids and correlation with polyphenol content. *Eur J Lip Sci Technol,* 108 (4), 351-362. https://doi.org/10.1002/ejlt.200500330.

[26] Prakash, D.; Singh, B. N.; Upadhyay, G., (2007). Antioxidant and free radical scavenging activities of phenols from onion (*Allium cepa*). *Food Chem,* 102: 1389-1393.

[27] Oulai, P. D.; Zoué, L. T.; Niamké, S., (2015). Evaluation of nutritive and antioxidant properties of blanched leafy vegetables consumed in northern Côte d'Ivoire. *Pol. J. Food Nutr. Sci*. 65 (1), 31–38. https://doi.org/10.1515/pjfns-2015-0003.

[28] Salamatullah, A. M.; Alkaltham, M. S.; Hayat, K..; Ahmed, M. A.; Arzoo, S.; Husain, F. M.; Alzahrani, A., (2021). Bioactive and Antimicrobial Properties of Eggplant (*Solanum melongena* L.) under Microwave Cooking. *Sustainability*, 13, 1-12. https://doi.org/10.3390/su13031519.

[29] N'khili, E. Z., (2009). Polyphénols de l'alimentation: extraction, interaction avec les ions du fer et du cuivre, oxydation et pouvoir antioxydant. Thèse de doctorat, Montpellier, France, pp. 306.

[30] Arakaki, D. G.; Candido, C. J.; da Silva, A. F.; Guimarães R. D. A.; Hiane P. A., (2016). In vitro and in vivo antioxidant activity of the pulp of Jatobá-do-cerrado. *Food Sci. Technol*, 36 (1), 166-170. DOI: 10.1590/1678-457X.0084.

[31] Nagami, H.; Umakoshi, H.; Shimanouchi, T.; Kuboi, R., (2004). Variable SOD-like activity of liposome modified with Mn(II)-porphyrin derivative complex. *Biochem. Eng. J.*, 21, 221-227.

[32] Jung, E.-J.; Bae, M.-S.; Jo, E.-K.; Jo, Y.-H.; Lee, S.-C., (2011). Antioxidant activity of different parts of eggplant. *J Med Plant Res*, 5 (18), 4610-4615.