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EFFECT OF TEMPERATURE-TIME PAIR ON THE NUTRITIONAL VALUE AND ANTIOXIDANT ACTIVITY OF *TETRAPLEURA TETRAPTERA* HEATED PULP POWDER

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ABSTRACT

This study was carried out to determine the effects of heating time and temperature on the nutritional composition, phenolic compounds and antioxidant activity of *T. tetraptera* fruit pulp. The pulp were extracted from mature dried fruits, grinded into powder and baking at different temperatures (75, 90 and 100°C) and times (10, 20 and 30 min). The heat-treated samples were then analysed using standard methods. The results revealed that when both time and temperature of heating increased, the moisture (10.88 to 9.33 %), ash (5.42 to 3.84 %) and protein (7.01 to 4.00 %) rates in samples decreased while carbohydrates (74.05 to 77.53 %), fat (1.18 to 2.25 %) and fibres (11.77 to 15.78 %) rates increased. In addition, the increase in temperature and heating time has led to significant decreases in the levels of calcium, magnesium, potassium, phosphorus, iron and copper, reducing considerably the spice's contribution of these minerals to a diet. Furthermore, it was observed that total phenols and flavonoids contents, and DPPH free radical scavenging activity increased significantly with increase in heating time from 10 to 30 minutes at the tested temperatures. In contrast, total tannins (55.11 to 32.26 mg /100g), phytates (9.99 to 5.38 mg/100g) and oxalates (300.62 to 171.53 mg/100g) contents in samples decreased significantly with increase in heating temperature and time in comparison to unheated samples. It is therefore suggested that cooking of this spice should be done within the shortest possible temperature and time to retain most of its nutrients or around 100°C for an optimum time in order to obtain significant antioxidant effect.

KEYWORDS: *Tetrapleura tetraptera;* heating; antioxidant activity; nutritional value; phenolic contents.

1. INTRODUCTION

Since their discovery between the 6th and 7th century BC, spices have been an essential component of human nutrition across continents and over time, and continue to play a prominent role in the cultures of many peoples around the world (Raghavan, 2007). In Africa, Latin America, Asia and the Mediterranean, all kinds of spices and aromatic herbs are omnipresent in people's diets (Borquaye et al., 2017). The term "spices" refers to chlorophyll-free aromatic products, which come mainly from tropical countries, while the term "aromatic herbs" is used to refer to plants which fresh or dried herbaceous parts are used (Balasasirekha, 2014). They are used for their gustatory qualities but also for their therapeutic virtues such as antibacterial, antioxidant, antiseptic, analgesic, energizer, anti-inflammatory, antiemetic and antispasmodic (Nadeem and Riaz, 2012). These virtues would be due to the presence of bioactive molecules within them that are of growing interest nowadays (Romson et al., 2011).

As most others foods, spices are composed of proteins, fats, carbohydrates, and water, plus small amounts of other compounds such as minerals, vitamins, pigments, flavour and bioactive elements. It is important to understand how these components react when heated or mixed with other foods during domestic preparation.

Furthermore, thermal treatments are well known to have variable effects on chemical composition, bioactive compounds and antioxidant properties of plant samples. Effects include little or no change, significant losses or enhancement (Agbemafle et al., 2012). On the other hands, depending of their chemical composition, some spices must be added at the beginning of cooking, others must not cook otherwise they will lose all their qualities (Sophie, 2006). Thus, a proper control of when a spice is added could allow the consumer to benefit from all its

advantages.

According to Egharevba and Gamaniel (2017), the current demand for spices of all kinds is growing worldwide. Locally, the fruits of species such as *Coelocaryon oxycarpum, Xylopia aethiopica, Monodora myristica* and *Tetrapleura tetraptera* seem particularly prized by the populations for their therapeutic properties and the pleasant aroma they give off (Ouattara et al., 2016; N'zebo et al., 2018). In particular, *Tetrapleura tetraptera* fruits also called "Chêrê-chêrê" or "Chêboué" in south-eastern Côte d'Ivoire have recognized antioxidant, antibacterial, anti-inflammatory, anti-convulsant, anti-diabetic, hypotensive, etc. properties and are integrated into domestic preparations (N'zebo et al., 2018). Although the nutritional and bioactive properties of this spice are well studied, there is little information on the influence of time-temperature pair on it nutritional value and bioactive compounds found in the literature. Thus, in this study, the antioxidant and nutritional properties of *T. tetraptera* pulp powder and the effects of heat treatment were assessed.

2. MATERIAL AND METHODS

2.1. Samples collection, preparation and heat treatment

Mature dried fruits of *T. tetraptera* were randomly harvested from the plant at Awabo village (5°30'14.2"N and 4°01'30.6"W) in south eastern Côte d'Ivoire. Fruit were authenticated at the Department of Botany, Nangui Abrogoua University (Côte d'Ivoire). After sorting, the selected mature dried fruits were washed and then the pulp were extracted. The obtained pulp were first dried in an oven at 45°C for 72 hours, then crushed using a blender (Binatone BLG 550) to obtain the fresh powder (T_0). Thereafter, heat treatments were applied to the fresh powder using Dusyant's (2015**)** method with a slight modification. Five grams (5 g) of fresh pulp powder were spread into pyrex petri dishes. Finally, the dishes containing the powder samples were heated in an electric muffle furnace (Nabertherm, Germany) at various temperatures (75, 90 and 100°C) and times (10, 20 and 30 minutes). After heating, the obtained powder samples (T_0 to T_9) were kept cold (4°C) until the analyses.

2.2. Proximate composition analysis

Ash, moisture, crude fibre, crude protein, crude fat and carbohydrates contents were determined according to the standard methods of AOAC (2000). Moisture content was determined by the difference of weight before and after drying 10 g of sample in an oven (Memmert, Germany) at 105°C until constant weight at least or 72 h. Crude protein content (N x 65) was estimated by the macro-Kjeldahl nitrogen assay method using a digestion apparatus. The fat content was determined by Soxhlet extraction using hexane as a solvent. Ash fraction was determined by incineration of dried sample (5 g) in a muffle furnace (Nabertherm, Germany) at 550°C for 12 h. The percentage residue weight was expressed as ash content. Fibre estimate was obtained from the loss in weight of dried residue following the digestion for fat-free samples with 1.25 % each of H_2SO_4 and NaOH solutions.

2.3. pH and total titratable acidity (TTA)

This determination was carried out according to the method of Sadler and Murphy (2010). One gram of the crushed sample was completely dissolved in 50 mL distilled water and 5 mL of the sample with 2 drops of phenolphthalein indicator were added to a 100 mL conical flask. The mixture was titrated against 0.1 N solution of sodium hydroxide until the end-point which reached when a changed of colour was observed. The pH was measured directly using a pH-meter (Bentchop Model) as described by Sadler and Murphy (2010).

2.4. Minerals analysis

Minerals such as Ca, Fe, Mg, Zn, Cu and I were determined using AOAC (2000) method. Pulp powder was digested with a mixture of concentrated sulfuric acid (18.01mol/L), percloric acid (11.80mol/L) and nitric acid (14.44mol/L) and analysed using an atomic absorption spectrophotometer. Na and K were determined by flame emission photometer while total phosphorus was determined as orthophosphate by the ascorbic acid method after acid digestion and neutralization using phenolphthalein indicator and combined reagent (AOAC, 2000).

2.5. Phytochemical composition analysis

2.5.1. Extraction of phenolic compounds

Extraction of phenolic compounds was carried out according to Singleton et al. (1999) method. A sample (10 g) of *T. tetraptera* fruit pulp powder was extracted by stirring with 50 mL of methanol 50 % (v/v) at 25°C for 24 h and filtered through Whatman paper N°4. The residue was then extracted with 2 additional 50 mL portions of methanol. The combined methanolic extracts were evaporated at 40°C in a rotary evaporator (Heildolph, Germany) until 25 mL, prior to phenolic compound contents determination.

2.5.2. Determination of total phenolic content

Contents of total phenolic compounds were estimated according Folin-Ciocalteu method (Singleton et al., 1999). A volume of 1 mL of methanolic extract of each sample was added to 1 mL of Folin-Ciocalteu solution in a test tube. After 3 min, 1 mL of 20 % sodium carbonate solution was added to the mixture and adjusted to 10 mL with distilled water. The mixture was allowed to stand at room temperature in a dark environment for 30 min. Absorbance was measured against the blank reagent at 725 nm. Gallic acid was used for the calibration curve with a concentration range of 50-1000 µg/mL. Results were expressed as mg gallic acid equivalent (GAE) per 100g dry weight.

2.5.3. Determination of total flavonoids content

Total flavonoids content was determined according to the method of Meda et al. (2005), but slightly modified. A

volume of 0.5 mL of methanolic extract of sample was diluted in 0.5 mL of distilled water. Then, 0.5 mL of aluminium chloride 10 % (w/v) and the same volume of sodium acetate 1 M were added. Finally, 2 mL of distilled water was added and absorption reading at 415 nm was carried out after 30 min against a blank sample consisting of a 4 mL methanolic extract without aluminium chloride. Quercetin was used for the calibration curve with a concentration range of 0-100 µg/mL. Results were expressed as mg of quercetin equivalent (QE) per 100 g dry weight.

2.5.4. Determination of total tannins content

Tannins content was determined using the method described by Bainbridge et al. (1996). A volume of 1 mL of each methanolic extract was collected and mixed with 5 mL of reaction solution [vanillin 0.1 mg/mL in sulphuric acid 70 % (v/v)]. The mixture was left to stand at room temperature in a dark for 20 min. The absorbance was measured at 500 nm against a blank (without extract). Tannic acid was used for the calibration curve with a concentration range of h0-100 µg/mL. The results were expressed as mg of tannic acid equivalents (TAE) per 100 g dry weight.

2.5.5. Determination of antinutritional factors

The oxalate assay was performed according to Day and Underwood (1986) procedure using $KMnO_4$. 1 g of the ground sample was added to 75 mL of 3 N H_2SO_4. The mixture was carried under magnetic stirring for 1 h and filtered using Whatman filter paper N°1. Thus, 25 mL of the filtrate was collected and titrated against 0.05 N $KMnO_4$ solution until a faint pink colour appeared that persisted for 30 s.

Phytates content were estimated according to the method described by Latta and Eskin (1980). 1 g of sample was homogenized in 20 mL of 0.65 N HCl in a mechanical shaker for 12h at the room temperature. The extract was centrifuged at 12,000 rpm for 40 min and the supernatant was used for phytates estimation. To 0.5 mL of the supernatant, 3 mL of wade reagent (0.03 % solution of $FeCl_3.6H_2O$ and 0.3 % of sulfosalicylic acid in distilled water) was added and the mixture was allowed to stand at room temperature for 15 min. Absorption was measured at 490 nm against a blank. Sodium salt of phytic acid (0-10 µg/mL) was used as standard for construction of calibration curve. Result was expressed as mg phytic acid equivalent (PAE) per 100g dry weight.

2.5.6. Determination of molar ratio of anti-nutrients to minerals

The molar ratio of anti-nutrients to minerals was obtained by dividing the mole of anti-nutrient with the mole of minerals (Sengev et al., 2016).

2.6. Determination of antioxidant activity

2.6.1. Method of DPPH free radical scavenging

The antioxidant activity of the extracts was evaluated by the DPPH free radical scavenging method described by Hatano et al. (1988). The methanolic extract previously obtained was completely evaporated with rotavapor at 35°C until a dry extract was obtained. Then, 20 mg of dry extract was dissolved in 100 mL of methanol (70%, v/v) to obtain a 200 µg/mL stock solution. From this stock solution, different concentrations of extracts (20 to 200 µg/mL) were prepared by dilutions in the appropriate volume of methanol (70 %, v/v). For the evaluation of antioxidant activity in 2 mL extract (various concentrations), 1 mL DPPH (0.3 mM in 70 % methanol, v/v) was added and the mixture was thoroughly agitated for 5 min. A control was also performed containing 2 mL of solvent instead of the extract. The absorbance of the sample and control were measured at 517 nm with a spectrophotometer (T80+UV/VIS Spectrophotometer) after 30 min incubation in the dark at room temperature (28±2°C) against a blank for each extract concentration. Ascorbic acid was used as a positive control under the same conditions. The DPPH radical scavenging activity (%) was plotted against the extract concentration (µg/mL) to determine the concentration of extract necessary to decrease DPPH radical scavenging activity by 50 % (IC_{50}).

2.6.2. Ferric reducing antioxidant power (FRAP) assay

The ferric ion reducing power of the methanolic extract was determined according to Oyaizu (1986) method. One mL of the fruit extract (20-200 µg/mL) was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide solution (1%, w/v). The mixture was incubated at 50°C for 20 minutes. Then, 2.5 mL of TCA (10 %, w/v) was added and the mixture was centrifuged at 3000 rpm. The upper layer of the solution was mixed with 2.5 mL distilled water and $FeCl_3$ (0.5 mL, 0.1 %). The absorbance was measured at 700 nm against blank. Higher absorbance indicated a higher reducing power. Ascorbic acid was used as a positive control under the same conditions. The FRAP of the sample was expressed as ascorbic acid equivalent (µg AAE/mL extract). EC_{50} value was the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation from regression analysis.

3. Statistical Analysis

All chemical analyses and assays were performed in triplicate. Results were expressed as mean values ± standard deviation (SD). Analysis of variance (ANOVA), followed by Duncan's test, was conducted to analyse data at the 95 % confidence level, using STATISTICA software (version 12, ©StatSoft, Inc. 1984-2014).

4. RESULTS AND DISCUSSION

4.1. Effect of heating on the nutritional value of the fruit pulp

4.1.1. Proximate composition

The nutritional composition of *T. tetraptera* fruit pulp

before and after heating is recorded in Table 1. Statistical analysis revealed significant differences ($p \leq 0.05$) in moisture, ash, fibre, protein, fat and carbohydrates contents of different samples. Generally, moisture, ash and protein contents of samples heated to 75, 90 and 100 °C for 10, 20 and 30 minutes decrease significantly, while the fibre, carbohydrates and fat contents increase significantly for the same times. The decrease or increase is more pronounced at 30 minutes of heating regardless of the temperature applied.

The moisture content of the sample was found to range from 9.33 to 10.88 % (Table 1). Similar low moisture content (8 to 14 %) have been reported in some spices (Tchiegang and Mbougueng, 2005). The low moisture content is indicative of the fact that this spice can stay on the shelf for a longer period. Heating have a significant effect on the moisture content depending on temperature and time. Indeed, for heating times of 10, 20 and 30 minutes, the decrease in moisture content of pulp powder samples varies between 2.30 and 3.77 % after heating at 75°C, between 4.59 and 6.80 % after heating at 90°C and between 8.18 and 14.25 % after treatment at 100°C. These decreases in moisture content of heated samples would result from the loss of moisture by evaporation during heating. It appears that as the temperature and duration of the treatment increase, the samples are more dehydrated, so the resulting moisture loss is greater.

Protein content found in untreated powder (7.01±0.66 %) decreases significantly ($p \leq 0.05$) after heating. The greater the temperature and heating time, the greater the decrease. Protein losses estimated between 18.97 and 42.94 % for all heated samples suggest denaturation of cellular proteins during heating. Indeed, the exposure of proteins to high heat causes them to coagulate. Many of these proteins coagulate completely around 71 to 85°C. As the cooking temperature of the food increases, they dry out, harden and shrink (Gisslen, 2011). So, it would be appreciable to add the spice powder at the end of cooking in order to better benefit from the quality of the proteins.

Fat contents of *T. tetraptera* pulp powder samples (treated and untreated) were ranging from 1.18 to 2.25 %. However, the significant increase in fat content of *T. tetraptera* powder samples heated after 30 minutes (from 1.18 to 1.51 % at 75°C; from 1.18 to 2.34 % at 90°C and from 1.18 to 2.55 % at 100°C) could be explained by the moisture loss and dry matter concentration observed at the end of the treatment (Vodouhe et al., 2012). In addition, the low fat content of heated or unheated powder samples of *T. tetraptera* may partly explain their low calorific values. Moreover, the calorific values recorded after 30 minutes of heating (295.78 kcal/100g at 75°C; 298.92 kcal/100g at 90°C and 299.23 kcal/100g at 100°C) are included in the range reported for some unprocessed Ghanaian spices (243.17 to 402.77 kcal/100g) (Borquaye et al., 2017).

The fibre content of the fresh pulp of *T. tetraptera* obtained was 11.78 %. This value increase in the samples between 1.19 and 34.84 % after heat treatment. That could be due to the release of insoluble fibres from their food matrix under the effect of heat. Indeed, the increase in temperature during heating would cause the breakage of weak polysaccharide bonds and the cleavage of glycosidic bonds, which would result in an easier release of previously bound dietary fibres. Regarding the Recommended Dietary Allowance (RDA) for fibre estimated to 19-38 g/day (Trumbo et al., 2002), a portion of 100 g of *T. tetraptera* pulp powder would provide between 30 and 83 % of the fibre intake per day. Thus, *T. tetraptera* pulp powders obtained before or after heat treatment are a good source of fibre. Dietary fibres improve laxation, reduce risk of coronary heart disease and assist in maintaining normal blood glucose levels (Trumbo et al., 2002). Otherwise, the increase in carbohydrate content of the samples following the increase in temperature and processing time is probably due to the loss of moisture during oven processing, thus leading to a concentration of dry matter in the spice powder samples.

4.1.2. Mineral content

This study shows that heat treatment at temperatures of 75 to 100 °C for maximum durations of 30 minutes of *T. tetraptera* pulp powder induces a significant decrease in macroelements contents such as magnesium, calcium, potassium and phosphorus but also in trace elements like iron and zinc (Table 2). This would explain the significant decrease in ash content observed in these same samples. The rates of ash reduction observed in the various powder samples after 30 minutes of heating at 75, 90 or 100°C are less than 30 %. A priori, the treatment of the spice at these times (10 to 30 minutes) and temperatures (75 to 100 °C) would therefore not negatively impact its mineral quality.

Calcium, magnesium, sodium, phosphorus and potassium are essential macroelements. Calcium is necessary for blood coagulation and plays an essential role in the functioning of certain enzymes. It also plays a very important role in bone formation and neuromuscular functions. The RDA of calcium is 0.5 g/day for adults and 1 g/day for children (Igwe and Eleazu, 2018). Magnesium is important for muscle relaxation. Magnesium RDA is 400 mg/day and dietary intakes of magnesium below normal can represent a huge risk factor for hypertension, ischemic heart disease, stroke, congenital malformations, muscle degeneration, bleeding disorders, etc (Eleazu et al., 2012). Potassium is a mineral and electrolyte essential for heart and tissue health, skeletal contraction and gastrointestinal functions. The RDA for potassium is 3 to 4 g/day (Vasudevan et al., 2011). High levels of potassium tend to reduce the adverse effects of sodium on blood pressure and also reduce the risk of cardiovascular disease, osteoporosis and kidney stones. Phosphorus is involved with calcium in the growth and maintenance of bones, teeth and

muscles (Turan et al., 2003). The RDA for phosphorus is 800 mg/day (WHO/FAO, 2004). Sodium is associated with the regulation of acid-base balance, maintaining osmotic pressure and protecting the body from dehydration (Ujowundu et al., 2010). Iron, zinc and copper are very important trace elements for the body. Iron plays an important role in preventing anaemia, which affects more than one million people worldwide (Trowbridge and Martorell, 2002). Zinc is a cofactor of glutathione peroxidase and alcohol dehydrogenase. It is involved in the synthesis of DNA, RNA and proteins, regulates blood sugar, insulin levels and is necessary for the development of reproductive organs. Zinc deficiency in some diets is often due to their high levels of phytates leading to growth disorders in children, as weakened immunity can increase morbidity and mortality from common infections. The RDAs for iron and zinc are 8 and 6 mg/day, respectively (WHO/FAO, 2004). With reference to RDAs for calcium, magnesium, phosphorus, potassium, iron and zinc, the heat treatments applied would considerably reduce the spice's contribution of these minerals to a diet.

Table 1: Chemical composition and estimated energy value of heated powder samples of *T. tetraptera* pulp.

T° of heating	Time (min)	Sample	Moisture (%)	pH	Titratable acidity (%)	Ash (%)	Carbohydrates (%)	Proteins (%)	Fat (%)	Fibres (%)	Energy value (Kcal/100g)
Control	**0**	T_0	10.88±0.15^{g}	6.40±0.00^{d}	0.34±0.02^{a}	5.42±0.24^{d}	74.62±0.51^{ab}	7.01±0.66^{e}	1.18±0.01^{a}	11.78±0.02^{a}	293.60±0.15^{bc}
75°C	**10**	T_1	10.63±0.06^{f}	6.46±0.06^{d}	0.32±0.02^{a}	4.47±0.20^{c}	76.83±0.07^{c}	5.68±0.03^{d}	1.25±0.01^{a}	11.78±0.02^{a}	298.64±0.32^{ef}
	20	T_2	10.49±0.09^{e}	6.10±0.00^{e}	0.45±0.03^{b}	4.42±0.08^{c}	77.53±0.29^{d}	4.94±0.33^{c}	1.34±0.02^{ab}	11.77±0.01^{a}	300.13±0.03^{f}
	30	T_3	10.47±0.07^{e}	6.37±0.06^{d}	0.49±0.01^{cd}	4.20±0.01^{bc}	76.48±0.35^{c}	4.46±0.32^{b}	1.51±0.01^{b}	14.34±0.04^{c}	296.62±0.56^{d}
90°C	**10**	T_4	10.38±0.08^{e}	5.77±0.05^{b}	0.50±0.00^{d}	4.42±0.54^{c}	74.56±0.41^{ab}	5.62±0.00^{d}	1.33±0.04^{ab}	15.00±0.03^{f}	291.11±1.83^{a}
	20	T_5	10.25±0.04^{d}	5.90±0.20^{c}	0.50±0.00^{d}	4.22±0.18^{bc}	75.01±0.16^{b}	4.93±0.33^{c}	1.93±0.05^{c}	14.79±0.06^{e}	296.14±0.21^{d}
	30	T_6	10.14±0.03^{d}	6.10±0.01^{e}	0.47±0.01^{bc}	4.16±0.45^{bc}	76.44±0.17^{c}	4.50±0.33^{b}	2.24±0.02^{e}	13.61±0.04^{b}	305.17±0.23^{g}
100°C	**10**	T_7	9.99±0.10^{c}	5.30±0.00^{a}	0.53±0.01^{e}	4.29±0.01^{bc}	74.05±0.42^{a}	4.96±0.33^{c}	2.07±0.33^{c}	15.78±0.02^{g}	292.44±0.07^{ab}
	20	T_8	9.72±0.01^{b}	6.07±0.06^{e}	0.49±0.01^{cd}	4.05±0.25^{ab}	74.31±0.06^{ab}	4.75±0.00^{bc}	2.13±0.40^{cd}	15.69±0.07^{g}	294.31±0.32^{c}
	30	T_9	9.33±0.04^{a}	6.10±0.01^{e}	0.49±0.01^{cd}	3.84±0.01^{a}	76.53±0.20^{c}	4.00±0.33^{a}	2.25±0.15^{de}	14.63±0.06^{d}	297.34±0.72^{d}

Values in the same column having different superscripts are significantly different (p≤0.05).

Table 2: Minerals contents (mg/100g MS) of heated powder samples of *T. tetraptera* pulp.

T° of heating	Time (min)	Sample	Macroelement					Microelement			
			Na	Ca	Mg	P	K	Fe	Zn	I	Cu
Control	**0**	T_0	0.43±0.01^{d}	187.33±0.58^{a}	141.33±0.58^{b}	303.33±5.77^{a}	1303.67±0.57^{a}	0.83±0.00^{a}	0.23±0.00^{d}	0.10±0.01^{a}	0.27±0.00^{a}
75°C	**10**	T_1	0.40±0.01^{h}	171.67±0.58^{b}	135.33±0.58^{d}	310.00±0.00^{a}	1132.33±0.57^{e}	0.75±0.03^{c}	0.22±0.00^{e}	0.09±0.00^{b}	0.27±0.00^{a}
	20	T_2	0.41±0.00^{g}	165.33±0.58^{c}	137.00±0.00^{bcd}	299.33±5.76^{b}	1202.00±1.73^{c}	0.65±0.00^{d}	0.21±0.00^{f}	0.08±0.00^{c}	0.22±0.01^{c}
	30	T_3	0.41±0.00^{g}	155.33±0.58^{d}	128.67±0.57^{e}	293.33±5.77^{b}	1011.00±1.00^{g}	0.78±0.00^{b}	0.22±0.00^{e}	0.07±0.00^{cd}	0.17±0.00^{f}
90°C	**10**	T_4	0.44±0.01^{c}	166.00±1.00^{c}	135.67±0.58^{cd}	300.00±0.00^{ab}	1095.67±0.57^{f}	0.82±0.02^{a}	0.24±0.00^{b}	0.06±0.00^{de}	0.25±0.00^{b}
	20	T_5	0.39±0.00^{i}	143.00±1.00^{e}	141.00±8.72^{b}	303.33±5.77^{a}	1204.33±0.57^{b}	0.65±0.11^{d}	0.21±0.00^{f}	0.06±0.00^{de}	0.21±0.00^{d}
	30	T_6	0.41±0.00^{f}	129.00±1.00^{h}	138.00±1.00^{bcd}	310.00±0.00^{a}	956.67±0.58^{h}	0.56±0.00^{e}	0.21±0.00^{f}	0.06±0.00^{de}	0.15±0.00^{g}
	10	T_7	0.42±0.01^{e}	141.00±1.00^{f}	140.67±0.58^{bc}	310.00±0.00^{a}	884.33±0.58^{j}	0.58±0.00^{e}	0.23±0.00^{c}	0.06±0.00^{e}	0.21±0.01^{d}
100°C	**20**	T_8	0.45±0.00^{b}	136.33±0.58^{g}	149.33±1.15^{a}	306.67±5.77^{a}	1159.00±0.00^{d}	0.65±0.00^{d}	0.24±0.01^{b}	0.06±0.01^{e}	0.18±0.00^{e}
	30	T_9	0.48±0.00^{a}	121.33±0.58^{i}	146.67±0.58^{a}	306.67±5.77^{a}	939.33±0.58^{i}	0.65±0.00^{d}	0.25±0.00^{a}	0.01±0.00^{f}	0.15±0.01^{g}

Values in the same column having different superscripts are significantly different (p≤0.05).

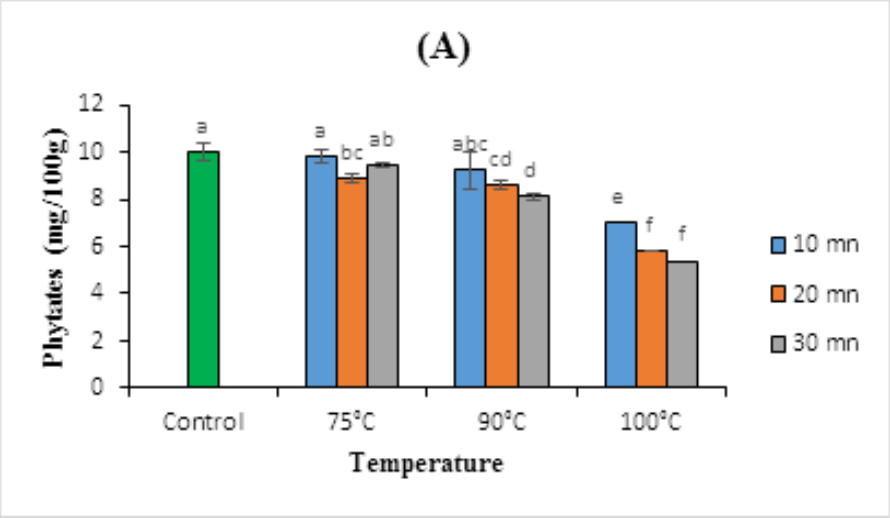
4.1.3. Antinutritional factors and mineral bioavailability

Heat treatment results also show that for temperatures between 75 and 100°C and durations of 10 to 30 minutes, the oxalate and phytates losses in various *T. tetraptera* powders samples are significant (Figure 1). They range from 300.62±11.32 to 171.53±7.19 mg/100g for oxalates and from 9.99±0.38 to 5.38±0.16 mg/100g for phytates. It appears that the longer the heating time and temperature, the greater the loss of oxalates and phytates remains. This reduction in oxalate and phytates levels during heating would be due to the heat-labile nature of these compounds, but also to the formation of insoluble complexes between these compounds and other constituents of the spice during the operation (Soetan and Oyewole, 2009). Thus, the reduction is all the more important as it could be beneficial to consumer health, as phytates and oxalates are anti-nutritional factors in food.

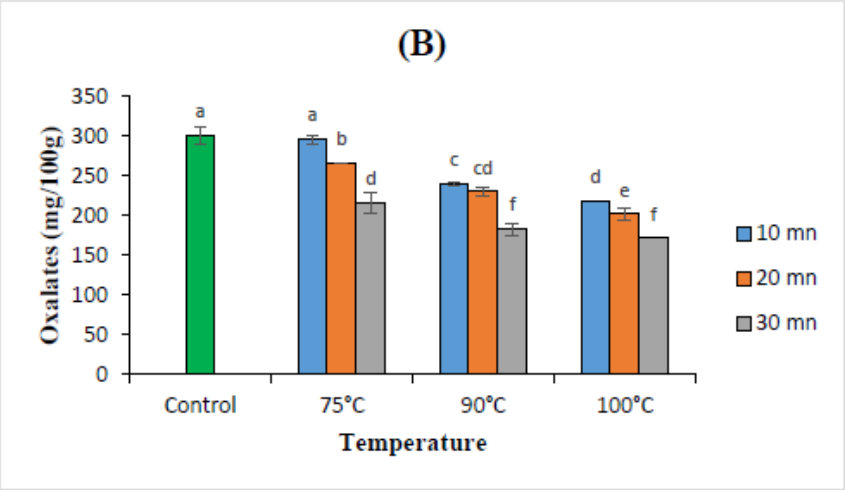
The importance of minerals to the body has justified the interest of determining the anti-nutritional factors to mineral ratios (Table 3). Indeed, oxalates and phytates are anti-nutritional compounds that chelate divalent cations such as calcium, magnesium, zinc and iron, reducing their bioavailability (Sandberg, 2002). The calculated ratios [phytates]/[Ca], [oxalates]/[Ca], [phytates]/[Zn] and [phytates]*[Ca]/[Zn] in different treated samples (T_1 to T_9) are generally below the respective critical levels of 0.24, 1.00, 15.00 and 0.50 mol/kg (Borquaye et al., 2017). This indicates that the heat treatment of the pulp of *T. tetraptera* at 75 to 100°C for 10 to 30 min does not interfere with the bioavailability of Ca and Zn. However, for the [phytates]/[Fe] ratio, it would appear that treating the powder at temperatures around 100°C for periods of 20 to 30 min would promote iron bioavailability. This can be explained by the fact that at these times and temperatures, oxalate and phytates losses are very higher.

Table 3: Anti-nutritional factors to mineral ratios of heated powder samples of *T. tetraptera.*

Samples	[Phy]/[Ca]	[Oxa]/[Ca]	[Phy]/[Zn]	[Phy]/[Fe]	[Phy]*[Ca]/[Zn]
T_0	0.003	0.729	4.335	1.026	0.203
T_1	0.003	0.783	4.355	1.107	0.187
T_2	0.003	0.731	4.062	1.160	0.168
T_3	0.004	0.634	4.226	1.030	0.164
T_4	0.003	0.657	3.794	0.955	0.157
T_5	0.004	0.732	3.924	1.128	0.140
T_6	0.004	0.643	3.688	1.226	0.119
T_7	0.003	0.706	2.918	1.025	0.103
T_8	0.002	0.673	2.390	0.760	0.081
T_9	0.003	0.642	2.159	0.702	0.065
Threshold value	**0.240**	**1.000**	**15.000**	**1.000**	**0.500**



Data are presented as means ±standard deviation. Means with different letters are significantly different, $p \leq 0.05$.



Data are presented as means ±standard deviation. Means with different letters are significantly different, $p \leq 0.05$.
Figure 1: Variation in phytates (A) and oxalates (B) contents of *T. tetraptera* powders affected by different heating times and temperatures.

4.1.4 Phenolic contents

Heat treatment theoretically leads to a reduction in the water content of food. As a result of this water reduction, the dry matter increases and consequently the contents of certain nutrients. This is what has been observed regarding the phenolic content of the spice studied (Table 4). The results show a significant increase in polyphenol and flavonoid levels after heat treatment at 75°C, 90°C and 100°C. The large increase in total phenol content could also be explained by the heat-induced degradation of cellular tissues that induces the release of phenolic compounds trapped in the fibre of food plants for easier absorption in the small intestine (Guihua et al., 2007). Another possible explanation for the increase in polyphenol content is the inhibition of enzymatic oxidation reactions of these compounds under the influence of heat (Dewanto et al., 2002). These results are in agreement with those reported by many authors in the literature. Indeed, Fanasca et al. (2009) observed a 23% increase in polyphenol content in asparagus after cooking. Similarly, Bushra et al. (2014) and Dusyant (2015) reported a significant increase in the total phenol content of ginger, saffron and cloves after 15 to 60 min of oven heat treatment.

Like polyphenols, the increase in flavonoid content in heated *T. tetraptera* powder samples suggests that heat treatment induces an increase in flavonoid extractability probably due to the destruction of the plant's cell membranes. This increase is also beneficial for the body because many flavonoids are powerful metallo-enzyme inhibitors (lipoxygenase, myeloperoxidase and NADPH oxidase) that generate free radicals. The antioxidant activity of flavonoids could therefore reduce oxidative cellular stress. It should be noted that oxidative stress has been shown to be involved in the pathogenesis of various neurodegenerative diseases such as Alzheimer's and Parkinson's diseases, as well as amyotrophic lateral sclerosis (Eleazu et al., 2012). The results obtained are in agreement with the observations of Temitope et al. (2010) and Dusyant (2015) who reported that flavonoids contained in spices such as saffron, basil, onion, garlic, cloves and ginger were resistant to thermal denaturation. These authors even observed an increase in the flavonoid content of these spices after heating at temperatures greater than or equal to 100°C for 15 to 120 minutes. Chen et al. (2011) also noted an increase in the flavonoid content of orange peel extracts (*Citrus sinensis* (L.) Osbeck) heated to temperatures above 90°C. Similarly, Ho and Lin's (2008) work on lemon peel (*Citrus reticulata*) treated at 100°C for 30 to 180 minutes also showed that the flavonoid content increases with the duration of the heat treatment.

Dry baking resulted in a significant decrease of tannin content of the *T. tetraptera* pulp powder samples. This reduction in tannin levels after heating may be mainly due to the fact that these compounds are heat-labile and degrade under heat action (Udensi et al., 2007). These results are also consistent with those of Rehman and Shah (2005) who stated that tannin levels in black, red and white beans were significantly reduced after regular domestic cooking or pressure cooking at 121°C for 20 minutes respectively.

4.2. Effect of heating on antioxidant activity of the fruit pulp

The evaluation of antioxidant activity revealed that DPPH scavenging activity of extracts from heated powders were high compared to the control (T_0), regardless to the heating time and extract concentration (Figure 2). In addition, when the heating temperature and time were increased, the DPPH scavenging activity of extracts also increases. Similarly, when the heating temperature reaches 100°C, the reducing power of heated powders seems to be higher than that of the control for heating time between 10 and 30 minutes (Figure 3). These observations could be explained by the presence

of a greater quantity of various antioxidant phenolic compounds (phenolic acids, flavonoids, tannins, anthocyanins, coumarins, etc.) in the heated samples compared to the control. Indeed, phenolic compounds responsible of plants antioxidant activity are more easily released in treated samples following heat-induced degradation of the plant cell wall (Guihua et al., 2007). Our findings are similar to those of Romson et al. (2011) who noted a higher DPPH radical inhibition activity in Thai curry paste extracts heated to 80, 90 and 100°C for 10, 20 and 30 minutes. Similarly, Khatun et al. (2006) also reported a significant increase in DPPH scavenging activity of extracts from clove and turmeric samples heated to 100°C. These authors also noted that the scavenging activity of DPPH increased with increasing heating time (from 1 to 6 hours).

The inhibitory concentration (IC_{50}) obtained with the control sample extract (untreated) was 67.75±2.26 µg/mL, while vitamin C concentration was 5.50±0.25 µg/mL (Table 5). Compared to those of extracts from various spices such as coriander, cumin, ginger, sumac, cinnamon, sweet bay leaf, etc. founded by Ereifej et al. (2016) (77 to 1260 µg/mL), it shows that *T. tetraptera* pulp powder could be a better natural antioxidant. Furthermore, an important fact to note is that for temperatures around 100°C and a heating time equal or greater than 20 minutes, the IC_{50} values of extracts (≤ 4.76±0.76 µg/mL) still lower than those of vitamin C. This observation would suggest a significant antioxidant effect of the powder after heating at 100°C because most effective scavengers of the free radical DPPH are those with the lowest IC_{50} values (Kholkhal, 2014). Likewise, considering the efficient concentration (EC_{50}) values obtained, it appears that the ferric reducing antioxidant power of heated powders becomes higher than vitamin C value (60.30±2.22 µg/mL) for heating at 100°C for 20 minutes (28.60±17.75 µg AAE/mL) and 30 minutes (17.27±5.55 µg AAE/mL) (Table 5). This indicated that the compounds present in powders heated at 100°C from 20 minutes had a much higher reducing ability than the other samples. So, it implies that heat treatment could improve both DPPH radical scavenging and FRAP activities.

Table 4: Mean values of phenolic contents of *T. tetraptera* heated powder samples.

T° of heating	Time (min)	Sample	Polyphenols (mg GAE/100g)	Flavonoids (mg QE/100g)	Tannins (mg TAE/100g)
Control	**0**	$\mathbf{T_0}$	2407.10±8.35^{h}	16.04±0.62^{e}	55.11±0.43^{a}
75°C	**10**	$\mathbf{T_1}$	2466.69±11.80^{g}	17.10±0.86^{de}	53.54±0.87^{b}
	20	$\mathbf{T_2}$	2492.16±13.32^{f}	17.40±0.67^{d}	52.28±0.43^{c}
	30	$\mathbf{T_3}$	2510.53±6.54^{e}	17.77±0.48^{d}	46.82±0.59^{e}
90°C	**10**	$\mathbf{T_4}$	2533.21±7.56^{d}	19.46±0.54^{c}	50.71±0.57^{d}
	20	$\mathbf{T_5}$	2547.96±11.82^{cd}	19.94±0.50^{c}	45.59±0.76^{f}
	30	$\mathbf{T_6}$	2562.18±2.24^{c}	22.22±0.85^{b}	44.87±0.16^{f}
	10	$\mathbf{T_7}$	2551.88±10.82^{c}	22.03±0.54^{b}	45.24±0.16^{f}
100°C	**20**	$\mathbf{T_8}$	2629.09±9.19^{b}	24.42±0.81^{a}	40.44±0.44^{g}
	30	$\mathbf{T_9}$	2693.81±7.78^{a}	25.45±0.75^{a}	32.26±0.33^{h}

Values in the same column having different superscripts are significantly different (p≤0.05).

Table 5: IC_{50} and EC_{50} values of heated samples of *T. tetraptera* pulp powder compared to ascorbic acid as reference.

T° of heating	Time (min)	Sample	DPPH IC_{50} (µg/mL)	FRAP EC_{50}(µg AAE/mL)
Control	**0**	$\mathbf{T_0}$	67.75±2.26	73.39±11.68
75°C	**10**	$\mathbf{T_1}$	56.09±4.16	109.81±16.05
	20	$\mathbf{T_2}$	38.38±3.70	73.81±7.57
	30	$\mathbf{T_3}$	31.07±6.82	107.63±4.21
90°C	**10**	$\mathbf{T_4}$	26.06±3.19	177.16±13.53
	20	$\mathbf{T_5}$	20.15±5.00	124.05±33.11
	30	$\mathbf{T_6}$	15.42±4.93	109.86±9.36
	10	$\mathbf{T_7}$	11.34±0.58	88.01±10.50
100°C	**20**	$\mathbf{T_8}$	4.76±0.76	28.60±17.75
	30	$\mathbf{T_9}$	-	17.27±5.55
Ascorbic acid (reference)			5.50±0.25	60.30±2.22

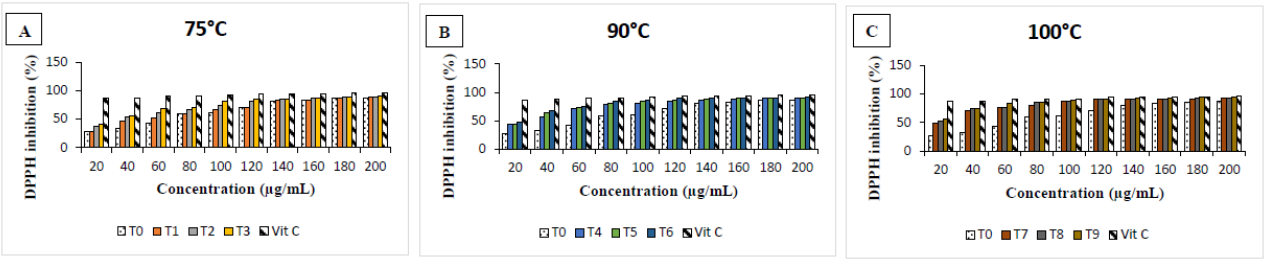


Figure 2: DPPH inhibition of extracts from heated and unheated samples of *T. tetraptera* pulp powder comparing to vitamin C.

A: samples were heated at 75°C for 10 min (T1), 20 min (T2) and 30 min (T3); B: samples were heated at 90°C for 10 min (T4), 20 min (T5) and 30 min (T6); C: samples were heated at 100°C for 10 min (T7), 20 min (T8) and 30 min (T9); T0 was the unheated sample; Vit C: vitamin C.

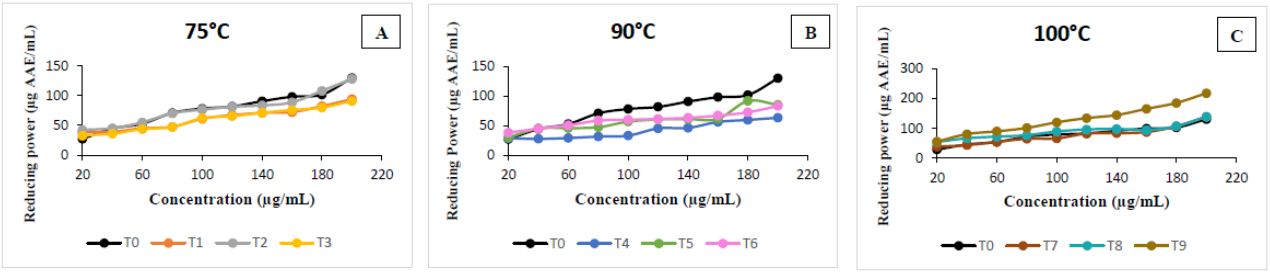


Figure 3: Reducing power assay of extracts from heated and unheated samples of *T. tetraptera* pulp powder expressed as ascorbic acid equivalent.

A: samples were heated at 75°C for 10 min (T1), 20 min (T2) and 30 min (T3); B: samples were heated at 90°C for 10 min (T4), 20 min (T5) and 30 min (T6); C: samples were heated at 100°C for 10 min (T7), 20 min (T8) and 30 min (T9); T0 was the unheated sample

5. CONCLUSION

The present study concludes that proximate composition of *T. tetraptera* powder was affected significantly both by temperature and heating time. Generally, the higher the time and temperature, the greater the effects (decrease or increase). Additionally, the study revealed that heating at the temperatures of 75, 90 and 100°C for 10 to 30 minutes could reduce significantly the mineral content of the fruit. However, heat treatment at temperature around 100°C for 20 to 30 minutes increased significantly the total phenolic contents, DPPH inhibition and ferric reducing potentials in the fruit.

Therefore, this study suggests an optimum cooking time-temperature, which could result in the highest retention of total phenolic contents, radical-scavenging and ferric reducing potentials in the pulp of *T. tetraptera* and lowest appropriate temperature to assure a higher quality spice for the maintenance of human health.

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