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Effects of cooking processes on total polyphenolic contents in seeds extracts of 17 varieties of Bambara groundnut (*Vigna subterranea* (L.) verdcourt)

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ABSTRACT

The effects of three cooking modes commonly used in sub-Saharan Africa on the total polyphenolic contents of 17 varieties of groundnut from the Institute of environment and agricultural research (INERA) of Burkina Faso and the Chadian Institute of agronomic research for development (ITRAD) of Chad have been studied. These total polyphenolic contents were assessed before and after cooking using a common and simple method based on Folin-Ciocalteu's reagent. Total antioxidants (TAC) and anthocyanins (TAT) contents of raw seeds were respectively assessed using the Ferric Reducing Antioxidant Power (FRAP) and differential pH methods. TAC was ranged from 5.018 (for KVS314) to 10.727 mg of Tolox Equivalents/g (for KVS225). KVS97, M4 and KVS350 varieties were distinguished by TAT greater than 160 µg/g dry seeds. Results of the determination of total polyphenolic contents (TPC) after cooking indicated that the three cooking processes had negative effects which involved decreases in TPC in studied seeds. Indeed, there were rates of decrease about 13.53%, 31.19% and 52.06% respectively for cooking processes by roasting, at pH7 and at pH8. Among these three cooking modes, cooking by roasting allowed better retention (86.47%) of total polyphenolic contents.

KEYWORDS

Total antioxidant content;
Total polyphenolic content;
Folin-Ciocalteu's reagent;
Fabaceae;
FRAP;
Total anthocyanin content.

INTRODUCTION

Legumes are an important part of the food of people in South of the Sahara. They are also sources of important macro and micronutrients and therefore play a crucial role in human nutrition[1,2]. Polyphenolic compounds represent a large group of phytochemical micronutrients in cereals, legumes, especially in fruits and vegetables. They embrace a wide range of plant substances having in common an aromatic ring with one or more

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hydroxyl groups. The great diversity of the chemical structures of polyphenols, explains their largest expansion of secondary metabolites. Through the literature many polyphenolic compounds identified by appropriate study techniques, are divided into several classes including phenolic acids, tannins and flavonoids. They have some bioactive properties such as antioxidant activities, the ability to stimulate natural enzymatic systems of body detoxification and therefore offer some benefits to health in the prevention of diseases such as cancer, heart disease and hypertension[3,4,5].

Food preparations consist of transformations of the raw food product into finished products intended for consumption. These different modes of food processing have an impact on the sensory and nutritional quality aspects due to many chemical reactions and the physical and biological processes occurring. From this point of view, investigations on the effects (physicochemical properties) of any mode of processing become a priority in order to provide foods containing all nutritional qualities required for the welfare of consumers.

Apart from the effects of preservation, thermal transformation mode can influence the qualitative, sensory and nutritional aspects of finished products. Thermal transformation mode can take various forms, including cooking, roasting, heating in the microwave. The literature revealed that it can increase or decrease the total polyphenolic contents and antioxidant activity. Indeed, Amakura et al.[6] noted an increase in the total polyphenolic contents during thermal processing of red berries in confiture. The same observation was made by Malika et al.[7] during the steaming of vegetables. But they have a decrease of polyphenolic contents when cooking these vegetables in water. Many mechanisms have been proposed to explain the polymerization and oxidation of polyphenols, thermal degradation, depolymerization of condensed tannins (polyphenols with high molecular weight) and the generation of Maillard reactions (occurring between a carbonyl compound like a reducing sugar, and an amine, like an amino acid, peptide, or protein) products, probably responsible of the evolution of the total polyphenolic contents of during processing[8].

In this paper, effects of three traditional modes (all based on heat) of transformation including roasting of the seeds of *Vigna subterranea* on a hot plate for 15 min; cookings at pH7 and pH8 for 2 hours have been discussed. Studied varieties of *Vigna subterranea* were collected in Burkina Faso and Chad. The determination of total polyphenolic contents (TPC) before and after cooking allowed identifying cooking process which preserved more total polyphenols in finished products.

EXPERIMENTAL

Chemicals and reagents

6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Gallic acid, Fe (III)(TPTZ)2Cl3 (TPTZ = 2,4,6-tripyridyl-s-triazine), Ferric chloride were obtained from Sigma chemical Inc, USA. All other reagents and chemicals used were of analytical grade.

Plant material

The study focused on twelve (12) : KVS350, KVS314, KVS311, KVS109, KVS67, KVS288, KVS153, KVS360, KVS141, KVS225, KVS97, KVS312 and five (5): M2, D2, M3, M4, M7, varieties of *V. subterranea* respectively provided by the INERA in Burkina Faso and ITRAD in Chad.

Sampling and sample preparation

(a) Extraction of raw plant material

Seeds of different varieties of *V. subterranea* have been crushed. 3 g of powder of each variety are extracted with 15 mL of the following solvent system: acetone-water- acetic acid (70: 29.5: 0.5) in maceration for 24 hours at 4°C. Extracts were filtered and residues were extracted again twice with 10 mL of the above solvent system for twenty-four (24) hours. The filtrates are collected and stored in the refrigerator at 4°C until the measurements of TPC, TAC and TAT[9].

(b) Cooking extraction of cooked plant material

To extract polyphenolic compounds of cooked seeds, we proceeded as follows:

- 5g of the different varieties of seeds have been put into the beaker and roasted on a hot plate for 15 min. The roasted seeds are ground and 3 g of powder from each variety was extracted in the same conditions as the raw seeds.
- 5g of seeds of different varieties of *Vigna subterranea* were cooked with convenient (no

water had not to be poured after cooking) volume (110 mL) of neutral (pH7) and basic (pH8) water for 2 hours; then crushed into a paste. 3g of this paste of each variety was macerated for 24 hours at 4°C in 15mL of the solvent system acetone-water-acid acetic (70: 29.5: 0.5). After filtration, residues were extracted twice with 10 mL of the same solvent system at 4°C for 24 hours.

Determination of TPC using Folin-Ciocalteu assay

Total phenolic phytochemical contents were measured using the Folin-Ciocalteu method[10]. Briefly, 60 µL of appropriate diluted samples and a standard solution of gallic acid (3,4,5-trihydroxybenzoic acid) were mixed to 60 µL of Folin-Ciocalteu's Reagent (FCR-1: 10 dilution) and left to stand for 8 min at room temperature to allow for the FCR to react completely with the oxidible substances or phenolates. 120 µL of Na2CO3 (7.5 % in water) were added to destroy the residual reagent. Absorbances were measured at 765 nm using a 96-well (glass vials 250 µL) microplate spectrophotometer (Microplate Autoreader MP96; SAFAS Instruments) after incubation for 30 min at 37 °C against distilled water as a blank. Total phenolic contents of the samples determined from the calibration curve equation ($y = 43.57x + 0.200$, $R^2 = 0.990$) were expressed in mg of gallic acid equivalents (GAE) /gram of dry material. All measurements were performed in three replications.

Determination of TAT using differential pH assay

Total anthocyanin contents of the extracts were evaluated using the pH-differential method in which two buffer systems were used: the solution of potassium chloride, pH1.0 (0.025 M) and acetate solution, pH4.5 (0.025 M). 100 µL of the properly diluted extract were mixed with 200 µL of the corresponding buffers and the absorbance was read against a blank at 510 nm and 700 nm, 15 min later. The absorbance A was calculated as follows: $A = (A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}$. The monomer concentration of anthocyanic pigments in the extract was calculated as the cyanidin-3-glucoside $\left(mg/L = \frac{A \times PM \times DF \times 1000}{\varepsilon \times l}\right)$ [11] where A: absorbance; PM: molecular weight; (449.2); DF: dilution factor; ε: molar absorptivity (26900). Total anthocyanin contents were expressed in milligrams of cyanidin-3-glucoside/gram of dry material.

Determination of TAC using FRAP (Ferric Reducing Antioxidant Power) assay

In this method, a ferric salt, Fe (III)$(TPTZ)_2Cl_3$ (TPTZ = 2,4,6-tripyridyl-s-triazine) was used as an oxidant[12,13]. It was prepared by mixing 1 mL (1 mL, 10 mM in 40 mM) TPTZ, 10 mL of a solution of sodium acetate buffer (pH = 3.6) and 1 mL of solution of Fe (III), H_2O (13). About 30 µL of distilled water was mixed to 20 µL of appropriately diluted extract and then, 200 µL of solution FRAP were added. Absorbances of the intense blue discoloration were measured at 593 nm using a microplate (spectrophotometer SAFAS, MP96) drive about 10 minutes after. A calibration curve was established using Trolox as reference of antioxidant. Results, determined from the calibration curve equation ($y = 25.47x + 0.068$; $R^2 = 1$), were expressed in mg of Trolox Equivalent (TE)/g dry material. All measurements were performed in three replications.

Statistical analyses

All experiments were conducted in three replications. The results are expressed in mean ± SD. Analysis of variance (ANOVA) allowed to appreciate the differences between varieties for their antioxidant, polyphenolic and anthocyanin contents using the statistical software Genstat, 14th Edition. The $P < 0.05$ values were considered statistically significant[14].

RESULTS AND DISCUSSION

TABLE 1 : Establishment of the standard curves

standard curves	Standard	Equations	coefficients of correlation
RFC	Gallic Acid	y = 43.57x + 0,200	$R^2 = 0.990$
FRAP	Trolox	y = 25.47x + 0.068	$R^2 = 1$

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TABLE 2 : Total antioxidant (TAC), polyphenolic (TPC) and anthocyanins (TAT) contents in the different varieties of raw seeds of *V. subterranea*

Varieties	Phytochemicals (mg/g)		
	TAC	TPC	TAT
KVS153	7.408±0.081 efg	2.997±0.051 c	0.01±0.000a
KVS225	10.727±0.043 l	4.406±0.055 f	0.034±0.001 d
KVS311	6.027±0.184 b	2.51±0.067 b	0.011±0.000ab
M2	6.974±0.017 cd	2.381±0.002 b	0.033±0.000 d
KVS67	7.223±0.092 def	2.876±0.027 c	0.012±0.000 ab
KVS288	6.929±0.101 cd	2.964±0.014 c	0.03±0.000 d
KVS312	8.466±0.013 hi	4.536±0.089 f	0.03±0.000 d
KVS314	5.018±0.014 a	2.284±0.021 b	0.02±0.000 c
KVS97	8.44±0.010 hi	4.533±0.128 f	0.331±0.002 h
D2	7.616±0.084 fg	2.386±0.054 b	0.016±0.000 bc
KVS360	9.233±0.219 j	3.595±0.006 d	0.034±0.000d
M4	8.154±0.085 h	3.673±0.089 de	0.281±0.001 g
KVS109	6.701±0.155 c	2.828±.045 c	0.012±0.000 ab
M3	7.704±0.036 g	2.538±0.046 b	0.034±0.000 d
KVS141	8.593±0.021i	3.736±0.051 de	0.053±0.001 e
KVS350	5.101±0.090 a	1.86±0.072 a	0.161±0.004 f
M7	9.721±0.079 k	3.913±0.083 e	0.03±0.000 d

Data are expressed as means ± SE of triplicate experiments. Means in a column not having a common letter are different (P<0.05).

Studied varieties of Bambara groundnut were characterized by the color yellow-cream, black purple or variegated pericarp. Results of the measurements of total antioxidant, polyphenolic and anthocyanin contents depending on the cooking modes are recorded in TABLE 2.

Total antioxidant contents (TAC)

The analysis of TABLE 2 revealed that there were more or less significant differences between varieties of *Vigna subterranea* from the point of view of their total antioxidant contenst. Indeed, there was a relative variation for 113.77% either contents ranged from 5.018 for KVS314, KVS350 varieties (P>0.05) to 10.727 mg of TE/g of dry seeds for the KVS225 variety which had the highest TAC (TABLE 2). There was no significant difference (P>0.05) between KVS109, KVS288, and M2 varieties having an approximative TAC of 6.702 mg/g. These varieties had a greater (P<0.05) TAC than KVS314, KVS350 ones. The latter were less rich than M4, KVS97 and KVS312 varieties with a TAC of about 8.15 mg of TE/g of dry material.

This study showed that the contents of antioxidants were different in extracts of studied varieties of *Vigna subterranea*. Some varieties had practically the same (P>0.05) of antioxidants contents while others showed significant statistical differences (P<0.05).

Total polyphenolic contents (TPC)

Several previous studies showed that the antioxidant activity of the extracts was related to micronutrients as polyphenols in general and in particular anthocyanins contents. The correlation coefficient obtained (0.824) showed that antioxidant activity of extracts of the 17 varieties of *Vigna subterranea* depended on more than 80% of the total polyphenolic contents. Indeed, a variation of the total polyphenolic contents of a given variety caused a consequential amendment of the antioxidant capacity. Total polyphenolic contents varied from 1.861 for KVS350 to 4.536 mg Gallic acid equivalents (GAE)/g of dry material for KVS225, KVS97 and KVS312 (TABLE 2, Figure 1) ; there was then, a relative variation of 143.73%. KVS314, M2, D2, KVS311 and M3 varieties had approximately the same total polyphenolic contents (P>0.05) of about 2.285 mg of GAE/g of dry seeds. These above mentioned varieties were less rich in polyphenols than KV109, KVS67, KVS288 and KVS153 with an average TPC of about 2.828 mg of GAE/g. This explained once more the variability of total antioxidant contents in extracts of different varieties of *Vigna subterranea*.

Total anthocyanins contents (TAT)

In the majority of studied groundnut extracts, anthocyanins are low. Their contents were certainly linked to the color of pericarp; some being more colorful than others. Thus the three varieties that were distinguished by the purple color of pericarp had the highest total anthocyanins contents (TABLE 2 and Figure 2). Among the 17 varieties studied, there were only three varieties (KVS350, M4 and KVS97) whose TAT were respectively valuable 0.161, 0.281 and 0.331 mg/g of dry

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TABLE 3 : TPC of the different varieties of *Vigna subterranea* after cooking processes

Varieties	TPC (mg/g)			
	raw seeds	roasted seeds	seeds cooked at pH7	seeds cooked at pH8
KVS153	2.997±0,051 c	2.844±0.049 ef	1.317±0.029 de	1.579±0.021 d
KVS225	4.406±0.055 f	4.078±0.086 j	1.239±0.091 d	3.556±0.061 l
KVS311	2.51±0.067 b	2.031±0.020 b	1.55±0.041 fg	1.865±0.048 e
M2	2.381±0.002 b	2.032±0.014 b	0.661±0.087 a	1.501±0.024 cd
KVS67	2.876±0.027 c	2.39±0.020 d	1.434±0.068 ef	1.925±0.033 e
KVS288	2.964±0.014 c	2.945±0.031 f	2.236±0.074 h	2.558±0.115 i
KVS312	4.536±.089 f	3.297±0.030 g	2.366±0.071 h	2.615±0.012 i
KVS314	2.284±0.021 b	1.755±0.009 a	0.801±0.043 ab	1.286±0.114 b
KVS97	4.533±0.128 f	3.873±0.060 i	2.908±0.056 i	3.068±0.086 j
D2	2.386±0.054 b	2.099±0.013 bc	1.504±0.016 fg	1.98±0.030 ef
KVS360	3.595±0.006 d	2.711±0.044 e	0.997±0.066 c	2.666±0.019 i
M4	3.673±0.089de	3.546±0.105 h	0.96±0.028 bc	2.325±0.055 gh
KVS109	2.828±0.045 c	2.189±0.006 bc	1.622±0.044 g	2.143±0.019 fg
M3	2.538±0.046 b	2.265±0.013 cd	0.647±0.045 a	1.023±0.025 a
KVS141	3.736±0.051de	3.169±0.025 g	1.563±0.062 fg	2.49±0.031 hi
KVS350	1.86±0.072 a	1.666±0.040 a	0.961±0.073 bc	1.302±0.024 bc
M7	3.913±0.083 e	3.809±0.030 i	3.125±0.059 j	3.286±0.003 k

Total polyphenolic contents are expressed in mg/g of dry seeds (Folin-Ciocalteu method) of GAE; Data are expressed as means ± SE of triplicate experiments. Means in a column not having a common letter are different (P<0.05).

TABLE 4 : Means of degradation and retention rates of polyphenols depending on cooking process

Cooking mode	Mean of TPC (mg/g)	Rates of decrease (%)	Rates of retention (%)
Raw seeds	3.177	0	100
roasted seeds	2.747	13.53	86.47
Seeds cooked at pH8	2.186	31.19	68.81
Seeds cooked at pH7	1.523	52.06	47.94

seeds.

The analysis of TABLE 3, it was an actual decrease of polyphenolic in different varieties of seeds after culinary transformations. Indeed, in extracts of roasted seeds (Figure 3, TABLE 3), the TPC varied from grade 1.666 for KVS314, KVS350 varieties to 4.078 mg of GAE/g of dry seeds for the KVS225 variety. The mean value of TPC in these roasted extracts was 2.747 mg of GAE/g (TABLE 4). Compared to the raw seeds extracts with a mean value for total polyphenolic of 3.177 mg of GAE/g, there was a reduction of 13.53% (TABLE 4). Indeed, reductions in rates were quite high in extracts of roasted seeds of KVS311, KVS109, KVS314, KVS360 and KVS312 varieties which contained, respectively, 2.51; 2.828; 2.284; 3.595; 4.536 mg of GAE/g of dry seeds, contained respectively only 2.031; 2.189; 1.755; 2.711 and 3.297 mg of GAE/g of dry seeds of TPC after the culinary transformation. From results, moderated reduction of decrease in TPC values of KVS350 M3, D2, KVS97, M2, KVS141 and KVS67 extracts were noticed. Finally, the extracts from the roasted seeds of the varieties KVS288, M7, M4, KVS153, and KVS225 have low reduction rates approximately 3.86%. Indeed, mean value of TPC in these extracts was 3.444 mg of GAE/g of dry seeds after culinary processing against 3.590 mg of GAE/g of dry seeds for raw seeds. These decreases could be explained by the degradation of some phenolic compounds known as heat-labile (most of the polyphenols cannot support temperatures exceeding 40°C during the culinary transformation).

Nevertheless, cooking process by roasting of these

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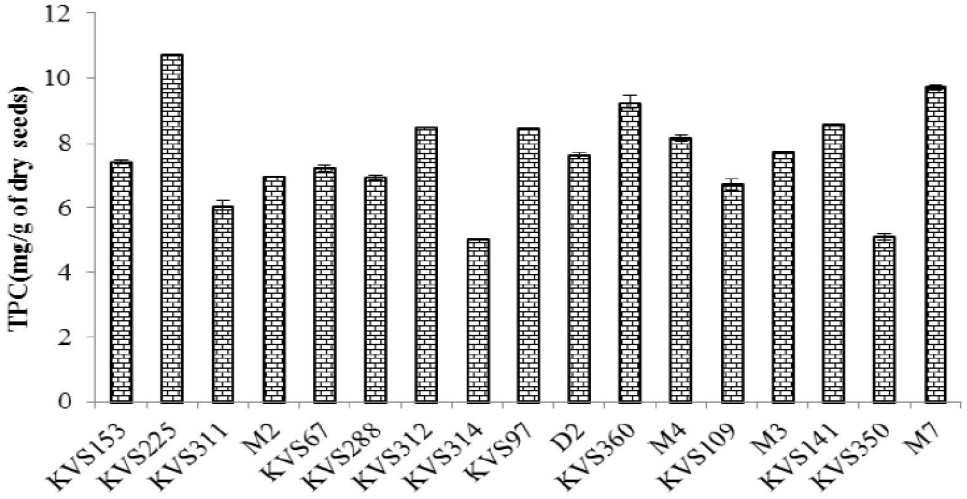

Figure 1 : Histogram of TPC values of the different varieties of seeds of *V. subterranean*

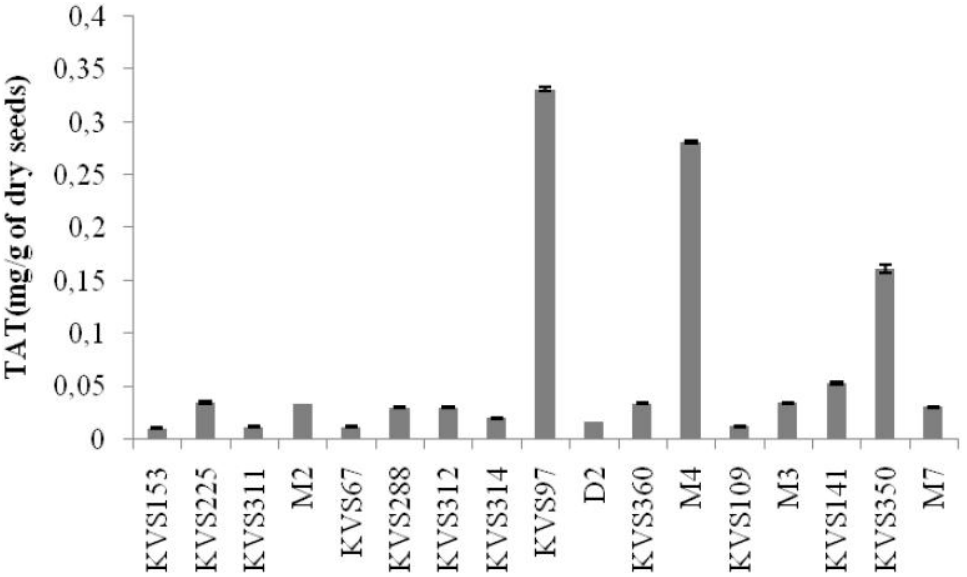

Figure 2 : Histogram of TAT values of the different varieties of seeds of *Vigna subterranea*

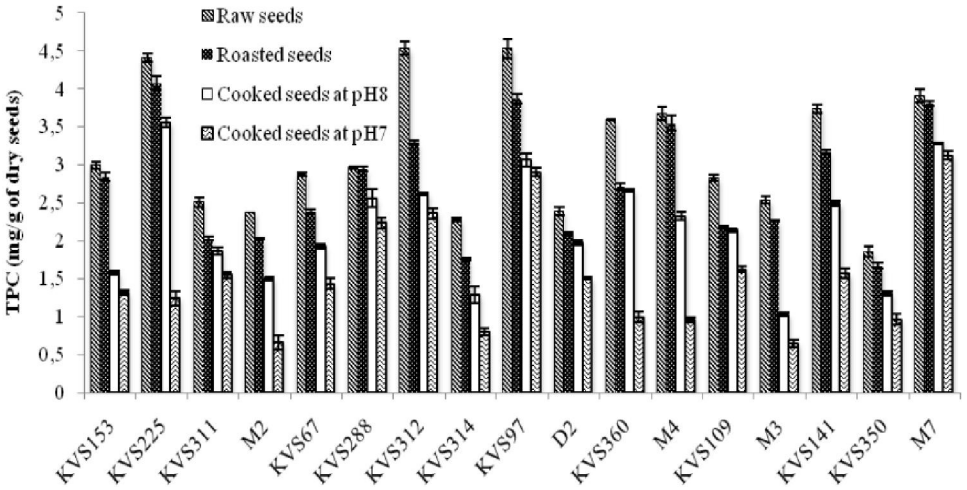

Figure 3 : Comparative histogram of TPC values of the different varieties of seeds of *Vigna Subterranea* after cooking processes

seeds of *Vigna subterranea* allowed retention of 86.47% of total polyphenols (TABLE 4).

Furthermore, results of the seeds of different varieties *Vigna subterranea* cooked at pH7 (Figure 3, TABLE 3), gave a global reduction rate of 52.06% in total polyphenolic (TABLE 4) contents ranging from 0.647 for KVS314, M2, M3 varieties to 3.125 mg of Gallic acid equivalents/g of dry seeds (based on the ratio of the mass of dry seed and that of the paste obtained after cooking) for M7 variety which had the highest TPC. The global retention rate of TPC in this case was low (47.94%). There was in this case, a strong decrease of TPC from extracts of roasted seeds. Indeed, the mean value of TPC in this case was 1.523 mg of GAE/g while 2.747 mg of GAE/g in extracts of roasted seeds (TABLE 3). This mode of cooking of the seeds in water at pH7 for 2 hours gave the highest rate of reduction in total polyphenolic contents in seeds of *Vigna subterranea* ; so a retention rate of the lowest TPC (TABLE 4) than that of roasted seeds.

Concerning, seeds of *Vigna subterranea* cooked at pH8, the total polyphenolic contents varied from 1.302 for the KVS350 variety to 3.556 mg of GAE/g of dry seeds (based on the ratio of the mass of dry seed and that of the paste obtained after cooking) for KVS225 variety (Figure 3, TABLE 3). The mean value of TPC after the culinary transformation was 2.186 mg of GAE/g of dry seeds. Compared to the raw seeds extracts, there was a global retention rate (68.81%) higher than that of extracts from seeds cooked at pH7 but lower than that of roasted seeds. Compared to cooking process at pH7, cooking at pH8, preserved polyphenols (global reduction rate of 31.19%). However, reduction rates of TPC measured respectively after cooking at pH7 and pH8 conditions remained higher than those obtained after roasting the seeds (Figure 3). Better retention of TPC in the roasted seeds could be explained by the fact that this traditional way of transformation required only 15 min versus 2 hours for the two other cooking processes.

Results obtained indicated that the thermal transformation had effects on the TPC. These effects were expressed negatively in all varieties studied seeds. The rates of decrease varied from one variety to another and could be explained by the nature and the contents of the different phenolic compounds specific to each variety.

Data on the impact of thermal processes in relation to the contents and composition in the seed polyphenols are rare. Views of researchers often differ on this plan. For some, the heat treatment of fruit resulted an increase of TPC while for others, this treatment resulted a decrease of the latter. Thus Amakura et al.[6] studied the impact of thermal processing of different types of red berries into jam, and observed a small increase in content of TPC. According to these authors this TPC increase would be due to a heating-induced release of phenolic compounds originally associated with the cell walls, and therefore related to the degradation of these walls. Malika B et al.[7] have studied the total polyphenolic content in six raw and cooked following vegetables: lettuce, green cabbage, spinach, fennel, green beans, and green celery. A global loss of 30.82% of total polyphenolic contents in relation to raw was observed after cooking in distilled water for about 11 min. Cooking in water, pears, Renard[15] found a limited loss procyanidins, associated with an important liberation of hydroxycinnamic acids in the cooking water. More recently Colin-Harold[16] studied the impact of the transformation of apple to compote, step by step. Thus, when cooking the fruit at 80°C for 15 min, an increase of 50% of total polyphenolic contents was observed. The author hypothesized that cooking helped to facilitate the extraction of compounds. Pasteurization (90°C for 5 min) this product pre-cooked trained then a loss of 30% of total polyphenols. Van der Sluis et al.[17] studied the effect of storage for 4 days at 85°C on the stability of polyphenols from apple juice and identified the different behaviors depending on the family of compounds. Temperature-sensitive are flavonoids (average loss of 75% depending on the type of molecules), and phloridzin and chlorogenic acid were the most stable (15% loss). Authors attributed the loss of flavonoids to mechanisms of acidic and basic hydrolyses. Indeed, considering that natural environment was slightly acid where polyphenols such as anthocyanins are more stable, we could understand that degradations by acid hydrolysis were more pronounced during cooking at pH7 than basic hydrolysis altering just organic acids of polyphenolic glycoside links.

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