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Effect of Steam Blanching on Carotenoids, Phenolic Compounds Content and Antioxidant Activity of Dried Pumpkin's Pulp (*Cucurbita moschata*) Farmed with Three Biological Fertilizers

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Authors' contributions

This work was carried out in collaboration among all authors. Author BGV, realized the field work and wrote the first draft of the manuscript. Authors DAW, DDFF, LEG, CB, NS and Glo managed the analysis of the study and performed the statistical analysis. Authors TIA and GI designed the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aims to assess the effect of steam blanching on the phytochemical composition and the antioxidant properties of *Cucurbita moschata* pulp, obtained with the use of three biological fertilizers.

Study Design: The study was done on five samples of pumpkin pulp grown in different conditions. All the samples were evaluated before and after steam blanching treatment followed by hot air drying. The experimental design was a randomized complete block design with three replications.

Place and Duration of Study: Laboratory of Biochemistry-University of Douala, Cameroon. The duration of study was seven months, from March to September 2018.

Methodology: Pumpkin were grown in May-September 2018 using the following fertilizers: ash at 10kg/25m²; bovine compost at 62.5 kg/25m²; ash + bovine compost (1:1). Besides negative control without fertilizers and positive control represented by NPK (20-10-10) at 2 kg/25m² have been done. After harvesting, carotenoids, phenolic compounds, flavonoids contents, and antioxidant activities were determined using standard methods before and after a steam blanching treatment followed by hot air drying process.

Results: Pumpkin pulp fertilized with ash, after a steam blanching treatment had the highest contents of total carotenoids, β -carotene, lycopene respectively of 696.03 ± 7.57; 584.86 ± 15.50 and 115.00 ± 1.25 mg/100g of edible portion and the lowest percentages of loss of these bioactive compounds. Pumpkin pulp fertilized with bovine compost and ash showed the lowest rates of loss of total phenolic compounds, respectively 0.62% and 4.25%, while those fertilized with the positive control (NPK) showed the highest rate of loss of total phenolic compounds (60%). Steam blanching treatment had a significant impact on phytonutrient contents and total phenolic compounds extracted from dried pumpkin pulp exert greater antioxidant activity.

Conclusion: Pumpkin pulp fertilized with ash provides the highest carotenoids content after steam blanching treatment and this treatment increases the antioxidant activity and probably the shelf life of dried pumpkin pulp.

Keywords: Carotenoids; phenolic compound; Cucurbita moschata; fertilizers; drying.

1. INTRODUCTION

Pumpkin is a good source of natural bioactive compounds and antioxidants such as flavonoids, β-carotene and lycopene, according to research [1]. Several works showed the property of phenolic compounds such as natural antioxidants found in functional food to guarantee the cell constituents protection against oxidative damage [2,3]. The biological potential of phenolic compound have also been demonstrated in the fight against plant pathogens and plant parasitic nematodes [4]. In the other hands, carotenoids are well known for their nutritional properties and health promoting effects and represent attractive ingredients to develop innovative functional foods. nutraceutical and pharmaceutical However, preparations [5]. many factors influence the quality of these compounds in foods, including plant development stage, environmental factors, and cultural practices [6]. Some studies has shown the influence of some fertilizers on crop production [7]. Concerning pumpkin, previous research has shown the effect of some fertilizers on the phytochemical and

antioxidant potential of fresh Cucurbita moschata pulp [8]. However, fruits and vegetables more often undergo technological processing of culinary transformation with a view to their consumption by the populations. Several studies have therefore shown the influence of these treatments on the nutritional and phytochemical composition of some fruits [9]. Concerning pumpkin, some treatments such as steam blanching and frving have been used to improve the shelf life of pumpkin products and enhance their consumption [10]. Concerning these treatments, some works showed that steaming is the most used by consumers [11]. Moreover, this treatment do not guarantee medium and long term preservation of the pumpkin pulp, but preserves carotenoid content and few nutrients [9]. Some works showing the effects of fertilizers on phytochemical composition of pumpkins [8], and according to the fact that, foods more often undergo technological processing of culinary transformation, such as steaming for improve their consumption [11], this study aims to assess the effect of hard treatment such as steaming blanching on phytochemical composition and the antioxidant properties of pumpkin pulp (*Cucurbita moschata*), obtained with the use of tree biological fertilizers.

2. MATERIALS AND METHODS

2.1 Site Description and Experimental Layout

The study was carried out in the agro-ecological zone of the western highlands, in the Koung-khi sub-division and Baa-Bayangam's locality. The climate is the "Cameroonian altitude" type, marked by two seasons of unequal lengths: a dry season, which runs from mid-November to mid-March, and a rainy season which lasts from mid-March to mid-November. Average temperatures are low (19°C), and heavy rain (1500-2000 mm) falls in a single mode configuration. The previous crop at this site is leafy vegetables. For the experiment the treatment consisted of:

(i) Negative control (T0);

(ii) Ash alone applied at 10 kg/25m² (T1);

(iii) Bovine compost alone applied at 62.5 kg/25m² (T2);

(iv) 50% of ash applied at 10 kg/25m² with 50% of bovine compost applied at 62.5 kg/25m² (T3);
(v) NPK (20-10-10) fertilizer of 2 kg/25m², positive control (T4).

The experimental design was a randomized complete block design with three replications. The area of each experimental block was 25 m². Plots were separated by a margin of 1 m apart while blocks were 4 m separated from each other. The experiment was performed in the same location in 2018. Before the start of the experiment in 2018, chemical parameters were first analyzed. Thus, 2 kg of soil samples were randomly collected (0-20 cm depth) from ten points in the study area using steel coring tubes. They were mixed together, air dried at 35°C for 24 hours. The samples were then sieved and analyzed for particle size, pH, organic carbon, organic matter, total nitrogen (N), phosphorous (P), potassium (K), natrum (Na), calcium (Ca) and magnesium (Mg) undefined.

The soil pH was measured using a CG822 type pH meter fitted with a combined pH electrode. Two types of pH were measured: pH-KCl and pH-H₂O. The actual acidity (pH-H₂O) was measured in a 1: 2.5 soil-water suspension (10g of soil in 25 ml of distilled water) at least 16 hours after preparation.

The determination of the organic carbon and organic matter was carried out by the method of Walkley and Black as described by [12].

2.2 Biological Material

Pumpkin seeds were purchased from a local producer, coming from *Cucurbita moschata* specie and Butternut variety. The fertilizers used for the study are traditional fertilizers particularly, ash, bovine compost, ash-bovine compost mixture and chemical NPK fertilizer purchased from a local producer. Ash has been obtained as follows: The untreated eucalyptus branches and stems devoid of any plastic waste were burned by combustion in an oven at 550°C for 4 hours. After cooling, the raw ash obtained was sieved using a 200 micron diameter sieve. The fine ash obtained was then stored in sealed plastic bags for future use.

Bovine compost was obtained as following: This compost was prepared essentially from urine and feces of cattle mixed with wood chips, water and shredded plant residues in a ratio of 1:4. The materials were piled up in successive layers starting with them. Stems of plants are cut into pieces, and followed by the excrement of urine and bovine feces, water is added to the optimum. When forming the pile, a few bamboo stems were pushed in to facilitate ventilation. When the pile is ready, it is surrounded by a layer of mud 3 cm thick. The bamboo stems were removed on the second day of composting, leaving holes that allow aeration. After five days, the temperature rises to 70°C and the holes are closed in turn. The first turnaround was done after three weeks. The humidity of the pile was adjusted with water and cattle dung, and the overturned pile was again sealed from the air with mud. The compost was used after three months when growing the squash. Before the start of the experiment, fertilizers samples were analyzed for organic carbon, organic, N, P, K, Ca, Mg and pH following the methods described above.

2.3 Crop and Application of Fertilizer

The fields were plowed during the period from March to April 2018. Two weeks before sowing, the biological fertilizers were applied to the soil as background manure, then in maintenance manure 30, 60 and 90 days after sowing. The spreading was done by burying 10 to 20 cm of the fertilizer. The pumpkin seeds were sown 2 cm deep in May 2018. For each hole, 02 pumpkin seeds were sown with the tip facing down. A spacing of 1.60 m between the rows and 70 cm on the rows, for a density of one plant per square meter has been achieved. Hand weeding was done on a monthly basis during the period of the experiment. The plants were not pruned during cultivation. Irrigation was carried out in order to keep the soil fresh. Maintenance of the crop was carried out until harvest.

2.4 Harvest and Steam Blanching Treatment of Fruits

The harvest took place five months later in September when the fruits reached maturity. The fruits were considered mature when the peduncle became dry and brittle. After harvesting, the fruits were weighed and the moisture, dry matter contents were determined by standard methods [13]. The fresh pulp was obtained after removing pericarp using a stainless knife. The fresh pulp were then dried in oven. The method used for drying pumpkin samples is described by [10]. Peeled slices of 5 cm thick were steamed at 85°C for 30 min. The steamed slices were then cooled for 5 min and sliced again to 5 mm thick, which were then dried in an oven at 80°C for 4 hours and cooled in a desiccator for 15 min. The dried pulp samples were then finely ground and a test portion of 2.5 g per sample was weighed.

2.5 Evaluation of the Effect of Fertilizers on the Phytochemical Composition and Antioxidant Potential

The quantification of carotenoids, phenolic compounds and evaluation of antioxidant potential of the samples have been evaluated.

Total carotenoids were extracted by ultrasonication in an ultrasonic generator with 300 W power and 40 kHz frequency, according to the modified method of [14] using the mixture of hexane-acetone solvent (3:1). In a 20 ml test tube, 2.5 g of pumpkin pulp were introduced and 10 ml of solvent were added. The whole was brought to ultra-sonication for 40 min at 20°C. The crude extract obtained was recovered and then centrifuged for 15 min at 6500 rpm and at 10°C. The supernatant was concentrated in vacuum using a vacuum concentrator. The dry extracts obtained were subsequently used to determine the content of total carotenoids, ßcarotene and lycopene in each sample of pumpkin pulp. Carotenoid extraction was carried out until the pumpkin pulp became discolored. After their extraction, carotenoids were quantified by spectrophotometry using the method described by [15].

The total phenolic compounds were extracted by ultra-sonication using the ethanol-water solvent mixture (70:30). In a 10 ml test tube, 1g of

pumpkin pulp was introduced and 5 ml of solvent were added. The whole was brought to ultrasonication for 40 min at 20°C. The crude extract obtained was recovered and then centrifuged for 15 min at 6500 rpm and at a temperature of 10°C. The supernatant was concentrated in vacum using a vacuum concentrator. The dry extracts obtained were subsequently used to determine total phenolic compounds and flavonoids contents in each sample of pumpkin pulp. Total phenolic compounds and flavonoids contents were subsequently quantified by spectrophotometry using the method described by [16].

The evaluation of the antioxidant activities was done by trapping the free radical DPPH and ABTS and was carried out according to the modified method of [17].

2.6 Statistical Analysis

Microsoft excel and graphpad prism software were used for data processing. Mean, standard deviations, ordered two-dimensional analysis of variance and weighted percentages were compared between different groups using the Bonferroni and Kruska Wallis test at the P≤0.05 significance level [9].

3. RESULTS AND DISCUSSION

3.1 Chemical Composition of Fertilizers

Table 1 showed the results of the analysis of ash, bovine compost and NPK fertilizer used for soil fertilization. Chemical characterization studies showed variability in the composition of ash, bovine compost and NPK used for soil fertilization. The results showed that ash was rich in mineral elements including potassium (65%), phosphorus (3.2%), calcium (3.1%) and magnesium (2.8%). Iron and manganese accumulated a percentage of 3%. The other elements such as chlorine, sodium and sulfur was present in trace amounts respectively in proportions of 0.002%, 0.03% and 0.033%. Bovine compost had a high organic matter (35.45%) and organic carbon (12.9%) content. The mineralization of the nitrogen contained in the bovine compost used was illustrated by the C/N ratio of a value of 15. The pH of bovine compost and ash was 7.05 and 10.1, respectively. NPK was essentially rich in mineral elements including nitrogen (20%), phosphorus (10%) and potassium (10%).

3.2 Effect of Fertilizers on Water Content

Table 2 showed the evolution of mass and water contents of pumpkin according to the fertilizers. Pumpkin fertilized with bovine compost (T2) produced the fruits with the largest masses (1.99 \pm 0.068 kg) compared to those fertilized with ash (T1) (1.198 \pm 0.068 kg). The pumpkins fertilized with T1 (87.57 \pm 0.30%) produced fruits with the lowest water content, while those fertilized with T2 (93.26 \pm 0.38%) produced fruits with the highest water content.

3.3 Effect of Fertilizers on Phytochemical and Antioxidant Properties

The results of the evaluation of the influence of fertilizers before and after steam blanching of pumpkin pulp on their carotenoids, *B*-carotene, lycopene, total phenolic compounds and flavonoids contents are presented in the figure 1. It emerged that fresh pumpkin pulp fertilized with treatment T3 (ash + bovine compost mixture), showed the highest carotenoids, ß-carotene and lycopene contents compared to other fertilizers. This result was higher than those obtained by [13] and could be explained by the extraction methods. In this study, the Ultrasonic Assisted Extraction (UAE) was the extraction method used and some research showed that UAE and MAE (microwave assisted extraction) are increasingly used in the extraction of natural products and also could increase the extraction yield of phytonutrients [18]. Fig. 1 also showed the effect of steam blanching on the levels of carotenoids, B-carotene and lycopene contents according to different fertilizers used. Pumpkins pulp fertilized with ash (T1) showed the lowest rate and highest contents of total carotenoids, β-carotene and lycopene respectively of 696.03 ± 7.57; 584.86 ± 15.50 and 115.00 ± 1.25 mg/100g of DM. This could be explained by the water contents in these different pumpkins pulp. Indeed, the pumpkin pulp fertilized with mineral ash (T1) had the lowest water content, reducing the drying time in the oven and, as a result, the exposure time of the carotenoids to heat, which contributed to a lower percentage of loss of these phytonutrients. Because of their high water content, which contributed to increasing their time of exposure to heat and thus their percentage of loss, those fertilized with compost had the highest percentages of loss of total carotenoids, *B*-carotene and lycopene; as a result, they had the lowest total carotenoids, ßcarotene, and lycopene contents of 484.69 ± 21.95; 420.40 ± 0.001 and 97.98 ± 2.23 mg/100g

of DM. This result is in agreement with those of [19] who showed that in tomato juice, the degradation and isomerization of all trans lutein, β-carotene and lycopene, increases during storage in light, and when the temperature rises. The heat treatment of the matrices improved the partial degradation of the carotenoids by isomerization from trans to cis form. This was due to the breakdown of carotenoid-protein complexes [20]. However pumpkin pulp fertilized with bovine compost, showed the lowest rate and highest contents of total phenolic and flavonoid contents. This could be due to the fibers texture of pumpkin pulp obtained with compost fertilizers which could contribute to reduce the losses of phenolic compound by their linkage to the fibers. However this hypothesis have to be explored in next studies.

Table 3 showed that the percentages of losses of total phenolic compounds are significantly lower than those of total carotenoids. This could be explained by the stability of phenolic compounds to heat. This could also be explained by the sensitivity of carotenoids to heat and oxygen. Indeed, carotenoids are a family of thermolabile molecules, sensitive to temperature and oxygen. Their unsaturated chemical structures make them unstable and very reactive. Several authors had shown the effect of temperature on the stability of carotenoids. Thus, [21,22], and [23] investigated the kinetics of isomerization of all trans-carotene and lycopene in cis-isomer forms in mango puree and carrot subjected to combined high pressure-temperature treatments in depth. The polyene chain of carotenoids is subject to isomerization of trans-carotenoids to cis-carotenoids. Their oxidation, temperature, exposure to light, presence of acid, and adsorption to active surfaces could facilitate isomerization.

The results presented in Figs. 2 and 3 showed that the phenolic compounds extracted from pumpkins pulp presented a greater antioxidant potential than the carotenoids extracted whatever the test used, DPPH or ABTS. These results could be explained firstly by the low percentage loss of phenolic compounds during drying and secondly by the transformation of these phenolic compounds into other phenols with high antioxidant potential under the effect of the temperature, which is an activator of the PAL enzyme involved in the biosynthesis of these phenolic compounds. Indeed, the temperature can modify the total phenolic content in the fruits after harvest; heat stress caused by high temperatures leads to an increase in PAL and CHS activities which results in the new synthesis of phenolic compounds [24]. On the other hand, there was a significant decrease in the antioxidant potential of carotenoids extracts and a significant increase of antioxidant potential of phenolic compounds extracts in dried pumpkins pulp, compared to those of fresh pumpkins pulp. This decrease of the inhibition potential of free radicals DPPH and ABTS was mainly linked to the decreases in the levels of carotenoids which was destroyed by heat during drying. These results also showed that dried pumpkin pulp fertilized with bovine compost was those which exhibited the highest scavenging power for free radicals DPPH and ABTS. This could be explained by their high β -carotene and flavonoids contents. Thus, the antioxidant power of dried pumpkins pulp was attributed in this case to phenolic compounds, in particular flavonoids. The percentage inhibition of free radicals DPPH and ABTS significantly increased after drying to reach values above 80%. These values are higher than those obtained by [25], who evaluated the scavenging power of the DPPH radical by fractions of phenolic compounds and carotenoids extracted from ripe pumpkin and obtained reduction rates of this radical which are around 50%. This difference could be explained by growing conditions, environment, soil type, pumpkins species and variety. This antioxidant potential was strongly correlated with β-carotene and flavonoids contents with a Pearson correlation coefficient of 0.9. Showing the carotenoids and phenolic compound contents [26] of fresh and dried pumpkin pulp, and according to their biological potential, these results also suggest that pumpkin pulp could be used in formulating with other medicinal plants as biological crop protection agent [27].



(a)

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(b)

(c)



(e)







Fig. 2. % inhibition of free radical DPPH from steam blanching pumpkin pulp Means with the same letter in each diagram are not significantly different at $P \le .05$ level of probability



Fig. 3. % inhibition of free radical ABTS from steam blanching pumpkin pulp Means with the same letter in each diagram are not significantly different at $P \le .05$ level of probability

Parameters	Fertilizers			
	Ash	Bovine compost	NPK	
SiO ₂ (%)	0.29	-	-	
P (%)	3.20	0.65	10	
K (%)	65.00	1.40	10	
Ca (%)	3.10	-	-	
Mg (%)	2.80	-	-	
Na (%)	0.03	-	-	
S (%)	0.033	-	-	
Fe (%)	1.53	-	-	
Mn (%)	1.45	-	-	
Cl (%)	0.002	-	-	
N (%)	-	0.86	20	
Organic carbon (%)	-	12.90	-	
Organic matter (%)	-	35.45	-	
C/N Ratio	-	15.00	-	
рН	10.2	7.05	-	

Table 1. Chemical composition of fertilizers

Table 2. Evolution of the masses and water contents of pumpkins according to fertilizers used.

	Treatment							
	T4	T2	T1	Т3	Т0	Р		
Mass (kg)	2.38 ^a ± 0.36	1.99 ^b ± 0.22	1.2 ^c ± 0.07	1.56 ^d ± 0.12	1.08 ^e ± 0.08	0.0001		
Water content (%)	85.55 ^a ± 0.30	$93.26^{b} \pm 0.38$	87.57 ^c ± 0.30	88.06 ^c ± 0.28	88.73 ^c ± 0.64	0.01		

Means with the same letter in each row are not significantly different at P ≤ .05 level of probability. To (Negative control); T1 (Ash); T2 (Bovine compost); T3 (Ash + Bovine compost); T4 (NPK positive control)

Paramètres		Losses (%)					
	ТО	T1	T2	Т3	T4	P-value	
Total carotenoids	77.51 ^a ± 1.00	40.26 ^b ± 2.53	76.71 ^ª ± 1.96	72.40 ^c ± 0.62	31.63 ^ª ±0.40	0.001	
β-carotene	77.80 ^a ± 0.80	38.20 ^b ± 2.89	76.59 ^ª ± 0.34	73.91 ^a ± 0.58	26.72 ^c ±8.38	0.009	
Lycopene	49.25 ^a ± 0.87	19.32 ^b ± 3.18	55.00 ^ª ± 1.56	48.04 ^a ± 1.41	12.62 ^b ± 6.60	0.001	
Total phenolics	14.85 ^a ± 2.92	10.03 ^a ± 1.09	5.46 ^b ± 1.53	12.72 ^a ± 2.45	62.90 ^c ±1.47	0.001	
Flavonoïds	$6.32^{a} \pm 3.95$	4.05 ^b ± 1.18	$2.50^{\circ} \pm 0.22$	6.80 ^a ± 1.55	$3.70^{d} \pm 0.13$	0.001	

Table 3. Losses (%) of bioactive compounds after steam blanching

Means with the same letter in each row are not significantly different at P ≤.05 level of probability. To (Negative control); T1 (Ash); T2 (Bovine compost); T3 (Ash + Bovine compost); T4 (NPK positive control)

4. CONCLUSION

This study revealed that, the pumpkin pulp fertilized with ash had the lowest rate of losses and the highest contents of total carotenoids, β -carotene, and lycopene after steam blanching. In the other hand, pumpkin fertilized with bovine compost showed the lowest rate of loss of total phenolic compounds and flavonoids compared to those fertilized with the positive control which exhibited the highest rate of loss of total phenolic compounds. The phenolic compounds extracted from these dried fruits exerted greater antioxidant potential than fresh fruits. Hence, steam blanching provides higher antioxidant potential and could also increases the shelf life of dried pumpkin pulp.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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