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Proceeding Paper

Bioactive Compounds and Antioxidant Activity of Selected Pumpkin Cultivars: Impact of Cooking Treatments †

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Abstract: Pumpkin (*Cucurbita moschata*) undergoes several cooking processes before consumption, leading to alterations in both its physical attributes and chemical composition. Therefore, the objective of this work is to evaluate the effect of different cooking treatments (convection oven, steaming, microwaving, and boiling) on bioactive compounds and antioxidant activity in different pumpkin cultivars: Cuyano INTA, Dorado INTA, Paquito INTA, and Cokena INTA. The results showed high variability in the concentration of bioactive compounds and antioxidant activity between the cultivars ($p < 0.001$). The stability of bioactive compounds and antioxidant activity after cooking was found to be genotype × cooking treatment interaction dependent ($p < 0.001$). Nevertheless, the free radical scavenging activity exhibited by cooked pumpkins was found to be high, in the range of 69.93 to 256.44 μM Trolox g^{-1} fw.

Keywords: *Cucurbita moschata*; cooking methods; phytochemicals; biological properties

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1. Introduction

Fruits and vegetables are known to contain significant amounts of bioactive compounds denominated phytochemicals with biological activities. These compounds have the potential to provide health benefits beyond fundamental nutrition, diminishing the susceptibility to degenerative illnesses, such as cancer and cardiovascular disease [1]. For this reason, the interest of the public and health professionals in functional foods in the prevention of diseases is gaining ground. In light of this, pumpkin (*Cucurbita moschata*) has attracted the attention of researchers due to its nutritional profile and health-promoting properties. Pumpkin flesh is a source of micronutrients, and its seeds possess significant amounts of proteins, minerals, phytosterols, and essential fatty acids. Furthermore, pumpkin flesh is rich in bioactive compounds, especially carotenoids, polyphenols, amino acids, vitamins, and minerals. It is an excellent source of trace elements such as potassium, phosphate, and magnesium [2]. It must be emphasized that the genetic diversity of the germplasms of pumpkin leads to great variation in the shape, size, flavor, color, and nutritional content, and its nutrient composition also differs depending on the origin and cultivation environment [3].

Pumpkin is consumed directly or as byproducts, including pumpkin puree, soup, jams, pastries, and baked goods [4]. Cooking processes lead to several changes in physical characteristics and chemical composition caused by thermal degradation, dilution, and the solubilization of constituents that leak into the water used for treatment [5]. Boiling and steaming, involving cooking the vegetable by immersing it in boiling water or cooking it in the steam produced from boiling water for several minutes, can generally lead to the loss of nutritional compounds and flavor [6]. In addition, food processing procedures can modify the bioaccessibility and bioavailability of bioactive compounds as well as antioxidant levels and activities [7].

The aim of the present work is to evaluate the effect of different cooking treatments on the bioactive compounds and antioxidant activity of selected pumpkin cultivars.

2. Materials and Methods

2.1. Plant Material

Four pumpkin cultivars (*Cucurbita moschata*), Cuyano INTA, Dorado INTA, Paquito INTA, and Cokena INTA, were grown during 2021–2022 using a randomized complete block design at INTA's experimental field located in La Consulta, Mendoza, Argentina. Biological replicates (five plants per replication) were randomly separated into four groups to evaluate the effect of the cooking method. During the cultivation, all of the agronomic practices—irrigation and weeding—were performed according to standard procedures. The fresh fruits were harvested at commercial maturity. After harvest, the pumpkins were washed in fresh water, and the parts with phytopathologies and seeds were removed. Then, about 200 g of each species were collected using a corer for each treatment. Cooking was performed by convection oven, steaming, microwaving, and boiling (Table S1). Samples were then frozen at −20 °C, freeze-dried, and ground before analysis.

2.2. Bioactive Compounds and Antioxidant Activity

Total carotenoids content (TC) was determined by spectrophotometry at 450 nm following the methodology proposed by Pacheco et al. [8]; total phenolic content (TPC) was measured with the Folin–Ciocalteu method [9], individual phenolic compounds were analyzed according to Rinaldi et al. [7], and the enantiomeric of tyrosine (Tyr) and tryptophane (Trp) analysis was performed following the method described by Botella et al. [10].

The antioxidant activity was based on the evaluation of free radical scavenging activity (DPPH assay) [11]. Trolox was used as standard at the concentration of 50–500 µM. The antioxidant activity of the sample was expressed as µM Trolox equivalent antioxidant capacity (TEAC) per g fresh weight.

2.3. Statistical Analysis

The values reported are the means of three replicates along with their corresponding standard deviation. The Tukey test was applied for pairwise comparisons, with statistical significance determined for p values < 0.05. The percent contribution of the variance of each factor and interactions was calculated from the sum of squares of the effects. The data were statistically analyzed with InfoStat-Statistical Software (version 2015l, 2020).

3. Results and Discussion

3.1. Effect of Cooking Methods on Total Carotenoids and Total Phenolic Content

Carotenoids and phenolic compounds are a significant group of bioactive compounds that are associated with a diverse range of health-promoting effects. Table 1 shows the content of carotenoids and total phenolic compounds in raw and cooked samples of the pumpkin cultivars under study. The highest content of TC was found in the Cokena INTA cultivar, while Paquito INTA exhibited the highest TPC levels. There was significant variation in the TC and TPC of the samples obtained through different cooking methods.

Table 1. Total carotenoids and total phenolic content of fresh and cooked samples of different pumpkin cultivars.

Cultivar	Cooking Treatment	TC [1]	TPC [2]
Cuyano INTA	Raw	20.53 ± 0.08 [3] k	154.57 ± 3.12 jk
	Boiling	7.42 ± 0.62 c	23.68 ± 3.24 a
	Baking	3.87 ± 0.07 a	105.08 ± 0.16 efg
	Steaming	9.94 ± 0.15 d	121.84 ± 5.32 fghi
	Microwaving	14.49 ± 0.31 g	75.21 ± 1.10 d
Cokena INTA	Raw	31.51 ± 0.19 n	103.90 ± 0.42 ef
	Boiling	20.82 ± 0.51 k	39.88 ± 0.30 ab
	Baking	12.37 ± 0.36 f	105.99 ± 4.82 efg
	Steaming	31.14 ± 0.11 n	115.69 ± 1.80 efg
	Microwaving	18.64 ± 0.57 i	84.22 ± 2.36 de
Dorado INTA	Raw	25.59 ± 0.25 m	126.81 ± 0.56 ghi
	Boiling	4.31 ± 0.14 a	50.58 ± 25.85 bc
	Baking	6.11 ± 0.04 b	137.99 ± 3.31 ij
	Steaming	15.60 ± 0.06 h	128.98 ± 5.09 hi
	Microwaving	11.45 ± 0.13 e	152.30 ± 10.10 ij
Paquito INTA	Raw	23.05 ± 0.07 l	195.48 ± 7.91 l
	Boiling	15.67 ± 0.24 h	68.87 ± 4.61 c
	Baking	7.49 ± 0.22 c	162.95 ± 1.23 k
	Steaming	19.57 ± 0.15 j	207.81 ± 5.18 l
	Microwaving	11.41 ± 0.30 e	196.51 ± 2.75 l

[1] TC: total carotenoids content expressed as µg g^{-1} fw. [2] TPC: total phenolic content expressed as µg GAE g^{-1} fw. [3] Means and standard deviations of triplicate analyses. Values followed by at least one different superscript letter in the same column are significantly different ($p \leq 0.05$) according to Tukey's test.

The ANOVA analysis showed significant differences for levels of both bioactive compounds among cultivars and cooking treatments. The cultivar × cooking treatments interaction was significant at $p < 0.05$. However, ANOVA analysis revealed that cooking methods accounted for 57% and 52% of the variation in TC and TPC concentration, respectively. Meanwhile, the interaction between cultivar and cooking treatment played a comparatively minor role. Among the studied cooking methods, baking was the most aggressive, leading to significant losses of TC content ranging from 61% in Cokena INTA to 81% in Cuyano INTA as compared with raw samples. However, boiling pumpkins also resulted in a significant reduction in the levels of TC in Cuyano INTA and Dorado INTA cultivars; in fact, one-third and one-sixth of TC were observed compared to non-treated samples. In contrast, steaming and microwaving preserved about 48% to 100% and 45% to 70% of TC, respectively. In particular, Cokena INTA retained about 100% of TC after steaming, and Cuyano INTA retained about 70% of TC after microwaving. The effect of the thermal treatment on TPC was significantly different than in the case of TC levels. As expected, boiling pumpkins resulted in significant losses of TPC, ranging from 62% in Cokena INTA to 85% in Cuyano INTA. These compounds exhibit high solubility in water; therefore, it is reasonable to anticipate greater losses during cooking methods involving immersion [10]. Nevertheless, baking tended to reduce TPC only in Cuyano INTA and Paquito INTA, whereas after steaming and microwaving, the TPC only fell in Cuyano INTA to about 21% and 51%, respectively, of that reported in raw pumpkins. The thermal degradation of TC observed in the current research agrees with previously reported results [12]. Tomás-Barberán et al. [12] reported the thermal instability of carotenoids and their susceptibility to degradation, and noted that light, heat, and oxygen induced isomerization during thermal processing. On the other hand, the reduction in TPC may be due to the breakdown of phenolics during cooking. In steaming, the lower temperature (compared to the boiling point and microwaving) may explain the increased preservation of phenolic compounds.

3.2. Effect of Cooking Methods on Individual Phenolic and Enantiomers Amino Acid Content

The effects of the treatments on the overall changes in the phenols and enantiomeric amino acids in the pumpkin cultivars are reported in Tables S1–S5. With regard to phenolic compounds, the pumpkin flesh of Paquito INTA presented significant levels of catechin, naringenin, and luteolin. Meanwhile, Cuyano INTA showed high levels of sinapic and chlorogenic acids. It was observed that in all investigated pumpkin cultivars, after thermal treatment the catechin, apigenin, and luteolin content decreased in comparison with the reference sample (raw pumpkin). Meanwhile, other phenolic compounds, such as flavonols, hydrocinnamic acids, and phenyl alcohol, increased. The cooking process can improve the extractability of these phenols either by the inactivation of enzymes related to the degradation of such compounds [11] or the alteration of the matrix in which phenols are embedded, such as the breakdown of the cell wall structure and partial hydrolysis [13].

Concerning Tyr and Trp, Table S5 shows that L-Tyr was the most abundant of the AAs, while D-Tyr was not detected in any of the cultivars studied. Moreover, both L enantiomers (Tyr and Trp) were present at a ratio of 80–90%; meanwhile, D-Trp constituted approximately 2% of the total AAs level (Figure 1). The ANOVA analysis showed significant differences for the levels of enantiomers studied between cultivars and cooking methods. The cultivar × cooking treatments interaction was significant at $p < 0.05$ (Table S6). When boiling pumpkin, there were significant losses in most amino acids (L-AAs), except for L-Tyr in the Paquito INTA variety, which increased; nevertheless, D-Trp levels exhibited a rise in all cases except for the Dorado INTA cultivar, likely due to the water-based cooking process [14,15]. Steaming generally increased L-AAs levels, except for L-Tyr and L-Trp in Dorado INTA and Paquito INTA, respectively, with significant losses in D-Trp in some varieties. Microwave cooking retained or increased the amino acid content, except in the Cuyano INTA variety, aligning with findings from Ito et al. [16] (Figure 1). In a complex matrix like food, pinpointing the specific cause for alterations in individual amino acids becomes challenging [17]. The genotype influences the expression of genes associated with the biosynthesis of compounds, while cooking methods impact the extractability and stability of compounds [18].

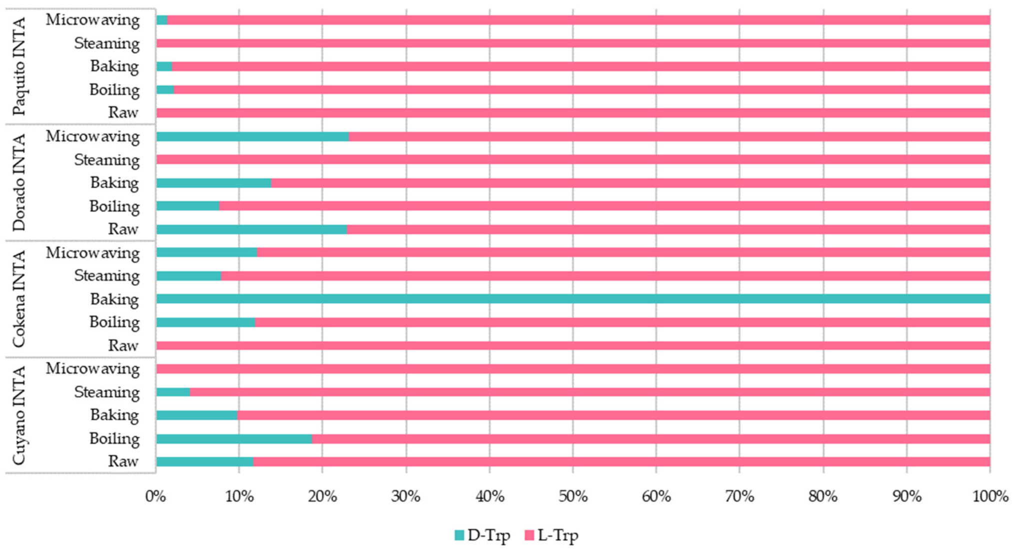

Figure 1. A distribution pattern bar diagram illustrating the levels of D- and L-Trp in pumpkin samples from various cultivars subjected to different cooking methods.

3.3. Effect of Cooking Methods on Antioxidant Activity

In terms of nutritional levels, the antioxidant activity of fruits and vegetables represents a very important characteristic of quality. Antioxidant activity was assessed by the evaluation of the free radical scavenging ability (DPPH test). As shown in Figure 2, all of the four pumpkin cultivars exhibited appreciable scavenging properties against

DPPH radicals. The antioxidant activity of fresh pumpkin ranged from 149.59 ± 4.10 to 213.20 ± 5.59 µM Trolox g^{-1} fw in Cokena INTA and Cuyano INTA, respectively. The free radical scavenging activity exhibited by cooked pumpkins was found to be in the range of 69.93 to 256.44 µM Trolox g^{-1} fw. It was evident that the steamed pumpkin flesh was the most powerful antioxidant, as its radical scavenging activities were significantly different from those of any of the other kinds of cooked pumpkin flesh. Meanwhile, the boiled samples exhibited the lowest radical scavenging activity; this could be attributed to the heat-degraded bioactive compounds having leached into the cooking medium.

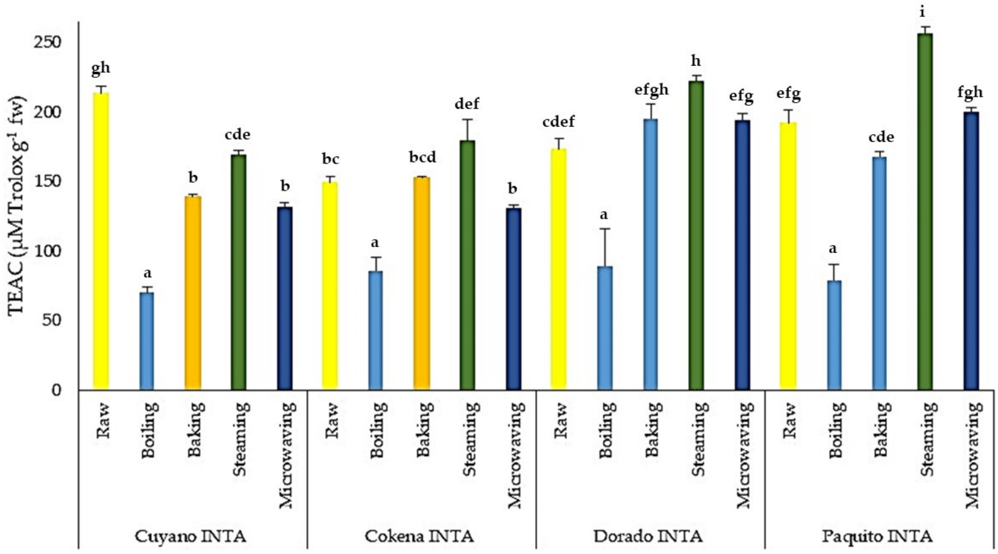

Figure 2. Antioxidant activity by DPPH test in pumpkin cultivars. Bars indicate mean values of three replicates, expressed as µM Trolox g^{-1} fw ± SE. Mean values with a common letter are not significantly different at $p < 0.05$ (Tukey test).

4. Conclusions

This research showed that traditional cooking treatments have an impact on the levels of bioactive compounds and antioxidant activities of selected pumpkin cultivars. Additionally, the results obtained show that *Cucurbita moschata* is a great source of phenolic compounds, particularly catechin, and amino acids such as L-Trp, thereby making it a desirable ingredient to a well-rounded diet. Furthermore, the presence of D-Trp in select cultivars underscores the existence of naturally occurring D-amino acids in unprocessed foods. Moreover, the free radical scavenging activity exhibited by cooked pumpkins was found to be high, in the range of 69.93 to 256.44 µM Trolox g^{-1} fw. These findings hold promise for utilization in pumpkin breeding programs, offering the potential to create novel pumpkin cultivars chosen for their exceptional nutritional qualities.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/Foods2023-14969/s1, Table S1: Temperature and time for each cooking treatment; Table S2: Phenolic compounds: flavanol, flavanones, flavonols, and flavonones levels in samples of pumpkin exposed to different cooking treatments; Table S3: Phenolic compounds: hydrocinnamic acid levels in samples of pumpkin exposed to different cooking treatments; Table S4: Phenolic compounds: hydroxybenzoic and phenyl alcohol levels in samples of pumpkin exposed to different cooking treatments; Table S5: Enantiomers D and L (Trp and Tyr) levels in samples of pumpkin exposed to different cooking treatments; Table S6: ANOVA table of Try and Trp variation due to cultivar, cooking treatment, and their interaction.

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