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# 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 x 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

Plant-based foods are known to contain significant amounts of bioactive compounds. 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 that reason, the interest of the public and health professionals in functional foods in the prevention of diseases is gaining ground. In this context, pumpkin (*Cucurbita moschata*), has attracted the attention of researchers due to its nutritional profile and health-promoting properties. Pumpkin flesh is rich in micronutrients, and its seeds have relatively high levels 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 us potassium, phosphate, and magnesium [2]. It must be emphasized that the shape, size, flavor, color, and nutritional content of pumpkin have genetic variation, and its nutrient composition differs depending on the origin and cultivation environment [3].

Pumpkin is consumed directly or processed into products such as pumpkin puree, soup, jams, pastries, and baked goods [4]. Cooking processes cause several changes in physical characteristics and chemical composition due to thermal degradation, dilution, and leaching into the water used for treatment [5]. Boiling and steaming, which involve immersing the vegetable in boiling water or exposing it to steam for several minutes, can generally lead to 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 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 at INTA's experimental field located in La Consulta, Mendoza, Argentina during 2021-2022 using a randomized complete block design. Each pumpkin cultivar was cultivated with four biological replicates with five plants per replication. Pumpkin fruits from each biological replicate were randomly separated in four groups to evaluate the effect of the cooking method. During the cultivation, standard agrotechnical and care procedures - irrigation and weeding - were performed. The fresh fruits were harvested at the 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 a 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 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*

All the results were expressed as mean ± standard deviation. The statistical analysis was conducted as a factorial design of two factors with three biological replicates. Pair-wise limit multiple comparisons and mean separations were performed by using Tukey test (*p* values < 0.05 were significant). The percent contribution of variance of each factor and interactions was calculated from the sum of squares of the effects. Statistical analyses were performed using InfoStat-Statistical Software (2020).

## 3. Results and discussion

### *3.1. Effect of cooking methods on total carotenoids and total phenolic content*

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

**Table 1.** Total carotenoids and total phenolic content of fresh and cooked 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 ± 3.31ij |
| | 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 revealed significant differences for levels of both bioactive compounds among cultivars and cooking treatments. The cultivar x cooking treatments interaction was significant at $p < 0.05$. Nevertheless, ANOVA analysis showed that cooking methods contributed in 57% and 52% of variation of the levels of TC and TPC, respectively; and cultivar x cooking treatment interaction played a minor role. Among the studied cooking methods, baking cooking was the most aggressive, leading to significant losses of TC content from 61% in Cokena INTA to 81% in Cuyano INTA as compared with raw samples. However, boiled pumpkins also resulted in significant reduction in the levels of TC in Cuyano INTA and Dorado INTA cultivars, in fact a one third and one sixth of TC compared to non-treated samples were observed. On the other hand, steaming and microwave cooking of pumpkins retained about 48% to 100% and 45% to 70% of TC, respectively. In particular, Cokena INTA retained about 100% of TC after steaming cooking and Cuyano INTA about 70% of TC after microwave cooking. The effect of the thermal treatment on TPC was significantly different than in the case of TC levels. As expected, boiling cooking in pumpkins resulted in significant losses of TPC ranging to 62% in Cokena INTA to 85% in Cuyano INTA. These compounds are very soluble in water so it should be expected that their losses will be higher in cooked methods carried out in immersion cooking [10]. Nevertheless, baking tended to reduce TPC only in Cuyano INTA and Paquito INTA, whereas after steaming and microwave, the TPC only fell in Cuyano INTA to about 21% and 51% of that reported in raw pumpkins. Thermal degradation of TC observed in the current research agrees with those results previously reported [12]. The authors reported thermal instability of carotenoids and susceptible to degradation and 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 effect of the treatments applied on the overall changes of phenols and enantiomeric amino acids in pumpkin cultivars are reported in Table S1 to S5. Whit 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, others phenolic compounds like flavonols, hydrocinnamic acids and phenyl alcohol increased. The increase in the content of these phenols after the cooking process can be associated with an increase in the extractability of these compounds either by inactivation of enzymes related to the degradation of such compounds [11] or alteration of the matrix in which phenols are embedded, such as gelatinization of proteins and other components [13].

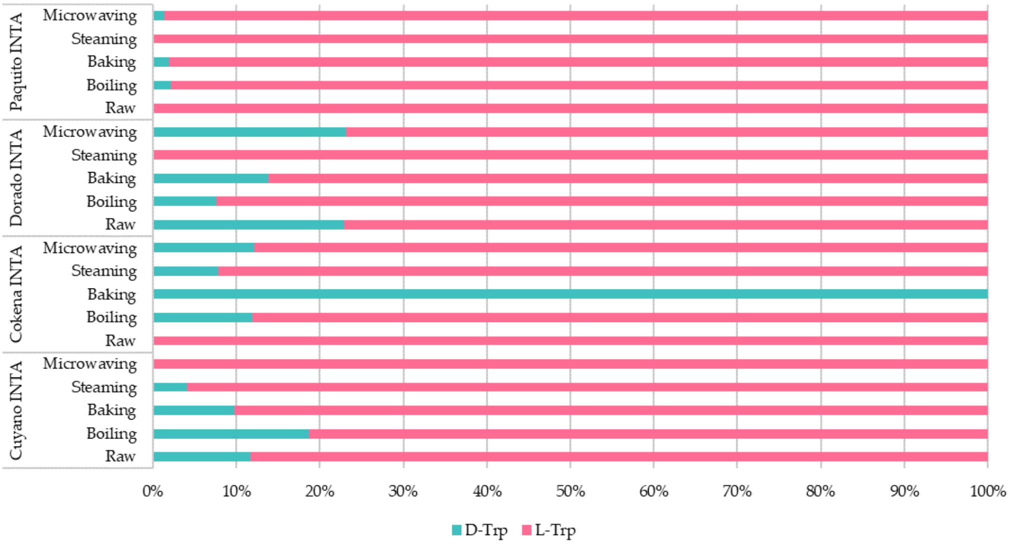

**Figure 1.** A distribution pattern bar diagram illustrating the levels of D- and L-Trp in pumpkin samples from various cultivars subjected to different cooking methods.

Concerning Tyr and Trp, Table S5 shows that D-Tyr levels were below the limit of detection in all cultivars analyzed, while its enantiomer L-Tyr was the most abundant. Moreover, both L enantiomers (Tyr and Trp), were found at a ratio of 80–90%, meanwhile D-Trp was found around the 2% with regard to total AAs content. The ANOVA analysis revealed significant differences for levels of AAs among cultivars and cooking treatments. The cultivar x 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; however, D-Trp levels increased in all cases except for the Dorando 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 amino acid content, except in the Cuyano INTA variety, aligning with findings from Ito et al. [16], Figure 2. In a complex matrix like food where it is difficult to determine the specific reason for changes in individual AAs [17]. The genotype affects expression of genes related to the biosynthesis of compounds, while cooking methods influence the extractability and stability of compounds because of thermal extract ability [18].

### *3.2. Effect of cooking methods on antioxidant activity*

Antioxidant activity of fruit and vegetables is very important quality characteristics of nutritional levels. Antioxidant activity was measured by the evaluation of the free radical scavenging ability (DPPH test). As shown in Figure 2, all 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 high, in the range of 69.93 to 256.44 µM Trolox $g^{-1}$ fw. It was evident that the steaming pumpkin flesh was proven to be the most powerful antioxidant, as their radical-scavenging activities were significantly different from those of any other kinds of cooked pumpkin flesh. Meanwhile, the boiled samples exhibited the lowest radical-scavenging activity, and this could be attributed to the heat-degraded bioactive compounds having leached into the cooking medium.

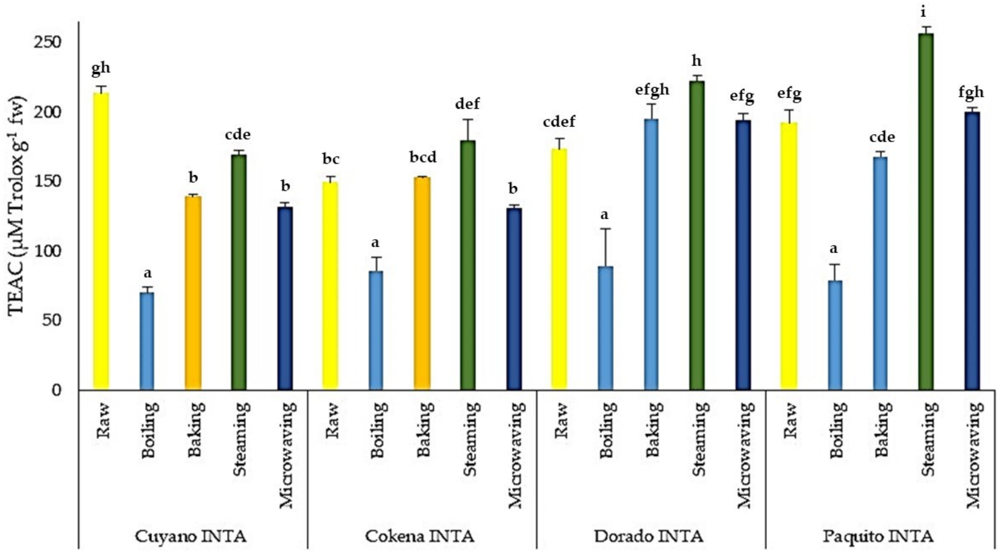

**Figure 2.** Antioxidant activity by DPPH test in pumpkin cultivars. Bars indicate mean values of three replicates, expressed as µM Trolox $g^{-1}$ fw ± SE. Mean values with a common letter are not significantly different at $p < 0.05$ (Tukey test).

## 4. Conclusions

This research showed that traditional cooking treatments have an incidence on the levels of the bioactive compounds and antioxidant activity of selected pumpkin cultivars. In addition, the data obtained reveal that *Cucurbita moschata* is a good source of phenolic compounds, particularly catechin, and amino acids as L-Trp, make 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: www.mdpi.com/xxx/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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