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Vegetables and their by-products: total phenolic compound content, total flavonoids, and antioxidant activity

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ABSTRACT

There is an underutilization of bioactive compounds, given the losses and daily waste of vegetables throughout the entire production chain. Accordingly, the objective of this study was to quantify the content of phenolic compounds and antioxidant capacity in different parts of vegetables. Analyses were performed on five vegetables (zucchini, eggplant, broccoli, green cabbage and green beans) from conventional cultivation. The levels of total phenolics, total flavonoids, and antioxidant capacity were obtained by spectrophotometric methods. Except broccoli, the analyzed by-products had levels of bioactive compounds and antioxidant capacity equal to or greater than the traditional parts of vegetable. Broccoli florets stood out with the highest levels of total phenolics (141.22 mg GAE/100 g), total flavonoids (137.38 mg CE/100 g) and antioxidant capacity (588.3 μ mol TEAC/100 g). In contrast, the lowest levels were observed in zucchini pulp (25.44 mg GAE/100 g; 13.03 mg CE/100 g; 69.78 μ mol TEAC/100 g). Therefore, the importance of full consumption of vegetables is reiterated. By-products can also be used to enrich preparations.

Keywords: vegetables, food waste, bioactive compounds.

RESUMO

Hortalças e seus subprodutos: teor de compostos fenólicos totais, flavonoides totais e capacidade antioxidante

Verifica-se um subaproveitamento dos compostos bioativos diante das perdas e desperdício diário dos vegetais ao longo da cadeia produtiva. Diante do exposto, objetivou-se no estudo quantificar o teor de compostos fenólicos e a capacidade antioxidante em diferentes partes de hortalças. As análises foram realizadas em cinco hortalças (abobrinha, berinjela, brócolis, repolho verde e vagem) do cultivo convencional (Goiânia-GO). Os teores de fenólicos totais, flavonoides totais e capacidade antioxidante foram quantificados por espectrofotometria. Exceto brócolis, verificou-se que os subprodutos analisados apresentaram teores de compostos bioativos e capacidade antioxidante igual ou superior às partes tradicionais das hortalças. O florete do brócolis se destacou com os maiores teores de fenólicos totais (141,22 mg GAE/100g), flavonoides totais (137,38 mg CE/100g) e capacidade antioxidante (588,3 μ mol TEAC/100g). Por outro lado, os menores teores foram observados na polpa da abobrinha (25,44 mg GAE/100g; 13,03 mg CE/100g; 69,78 μ mol TEAC/100g). Portanto, reitera-se a importância do consumo integral das hortalças ou o uso dos subprodutos para o enriquecimento de preparações.

Palavras-chave: verduras, resíduos de alimento, compostos bioativos.

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A critical global focus today is to improve diet quality through a healthy diet based on fruits, vegetables, cereals, seeds, and legumes while reducing environmental impacts (Conrad *et al.*, 2018). These foods are essential for human nutrition as they provide substantial amounts of water, vitamins, minerals, dietary fiber and bioactive compounds in the diet. The high content of substances with biological activities in these foods is of interest to researchers and consumers because these substances can modulate metabolic processes,

resulting in benefits for plants, as well as better health conditions for their regular consumers (Shirahigue & Ceccato-Antonini, 2020).

Of these substances, phenolic compounds are among the most abundant and widely distributed secondary metabolites in nature, being associated with numerous health benefits. Due to their ability to neutralize oxidation processes by capturing free radicals and reactive oxygen species (ROS), they can help in the prevention of respiratory diseases, cancer, and neurological and cardiovascular diseases (Maina *et al.*,

2021; Grande-Tovar *et al.*, 2021).

However, despite these benefits, the use of these compounds is estimated to be suboptimal due to daily losses and waste of food resources throughout the production chain. Unfortunately, half of the fruits and vegetables produced worldwide end up as waste, with a significant production of skin, seeds, peel, stalks, and bagasse as by-products of fruits and vegetables (Coman *et al.*, 2020; Tlais *et al.*, 2020).

While this waste leads to a series of economic, social and environmental consequences, the full utilization/

reuse of these by-products could remedy the deficiencies observed in the dietary intake of nutrients by the population (Tlais *et al.*, 2020; Gupta *et al.*, 2021). Accordingly, assessment of the content of bioactive compounds and antioxidant capacity of these by-products is of paramount importance to raise awareness concerning the best use of vegetables. Therefore, this study aimed to quantify the content of phenolic compounds and antioxidant capacity in different parts of vegetables.

MATERIAL AND METHODS

Place and period of the study

The analyses were performed at the Laboratory of Nutrition and Food Analysis (LANAL) of the Faculty of Nutrition (FANUT) of the Federal University of Goiás (UFG), from September 2021 to August 2022.

Raw materials

Vegetables (zucchini, eggplant, broccoli, green cabbage, and green beans) were obtained from the conventional cultivation system and purchased at local retailers in Goiânia (GO). The analyzed foods came from two different batches, each batch analyzed in triplicate, and the results were obtained as mean values of the six replicates.

After the vegetables were purchased, the same were sanitized under running water and divided into conventional consumption (zucchini and eggplant pulp; broccoli florets; cabbage leaves; green beans) and by-products (zucchini and eggplant peelings; broccoli stalk; cabbage heart; green bean pods). Subsequently, the samples were cut and ground using a stainless steel knife, and then the quartering technique was performed so that the analytical samples were representative of the analyzed foods. Afterward, the samples were macerated with a mortar and used for the preparation of extracts.

Quantification of bioactive compounds

Preparation of extracts

A procedure adapted from Rufino *et al.* (2007) was used to obtain extracts. 1 g of the foods (weight adjusted for each

vegetable) were placed in a Falcon tube containing 10 mL methanol. The tubes, in the absence of light, remained for 1 h in an ultrasonic bath. Subsequently, the extracts were centrifuged (4,000 rpm/15 min) in a refrigerated benchtop centrifuge (model 350-R; MPW brand) and filtered using filter paper.

Determination of total phenolic compounds

The total phenolic content was determined by spectrophotometry using Folin Ciocalteu's reagent (Singleton & Rossi, 1965; Genovese *et al.*, 2008). For such, 0.25 mL of the extracts were transferred to test tubes wrapped with aluminum foil. Then, 2.75 mL of a 3% Folin Ciocalteu solution was added to the tubes, which were vortexed for 10 seconds and left to stand for 5 min. Subsequently, 0.25 mL of 10% sodium carbonate solution was added to the tubes, which were then vortexed for 10 s and left to rest for 60 min in the absence of light and at room temperature.

The concentration of total phenolics was determined from optical absorbance values obtained ($\lambda = 765$ nm) by interpolating to the values of a previously constructed gallic acid standard curve. The results were expressed as mg GAE (gallic acid equivalent)/100 g of food.

Determination of total flavonoids

The determination of total flavonoids was performed by a spectrophotometric method described by Kim *et al.* (2003). 1 mL of the extracts was transferred to test tubes wrapped with aluminum foil containing 4 mL distilled water. Subsequently, at time 0, 0.3 mL of 5% sodium nitrite was added to the tubes and vortexed for 10 s. After 5 min, 0.3 mL of 10% aluminum chloride was added and then vortexed for 10 s and left to rest for 6 min. Subsequently, 2 mL of 1 M sodium hydroxide was added to the mixture and the tubes were vortexed for 10 s. Finally, immediately after, the mixture was diluted by adding 2.4 mL of distilled water and again homogenized in a vortex for 10 s.

The concentration of total flavonoids was determined from optical absorbance values obtained ($\lambda = 510$ nm), by interpolating to the values of a previously

constructed catechin standard curve. The results were expressed in mg CE (catechin equivalent)/100 g food.

Determination of antioxidant capacity *in vitro*

Antioxidant capacity was determined by spectrophotometric methods using the DPPH radical (2,2-diphenyl-1-picryl-hydrazyl), described by Brand-Williams *et al.* (1995) and Sanchez-Moreno *et al.* (1998). Briefly, 0.1 mL of the extracts was transferred to test tubes covered with aluminum foil. Then, 3.9 mL of the DPPH reagent solution (60 $\mu\text{mol/L}$) was added to the tubes. Subsequently, the tubes were vortexed for 10 s and then kept for 120 min in the dark at room temperature.

This method is based on the capture of the DPPH radical by antioxidants, thus producing a decrease in optical absorbance at a wavelength of 517 nm. A standard curve was constructed using solutions of trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and the results expressed in $\mu\text{mol TEAC}$ (Trolox-equivalent antioxidant capacity)/100 g food.

Statistical analysis

Statistical analyses were performed using SYSTAT software (v.12, SYSTAT Corporation). Data were analyzed by one-way analysis of variance (ANOVA). In the first analysis, each food was compared independently. Each food was compared by the part where the samples were collected (pulp, bark, floret, stalk, leaf, heart, seed and pods), specific for each food, these being the independent variables. The dependent variables were the amount of total phenolics, total flavonoids and antioxidant capacity. For subsequent analyses, all foods and their parts were compared together for the three dependent variables above. The assumptions of independence and normality for ANOVA analysis were evaluated by scatter plots and histograms of the residuals, respectively.

RESULTS AND DISCUSSION

There were significant differences in total phenolic contents for three foods compared to the parts in which the samples were collected ($p < 0.05$). For zucchini, the peel (69.87 mg GAE/100

g) showed significantly higher levels of total phenolics compared to pulp (25.44 mg GAE/100 g) (Table 1). Similar results were also reported by Serquiz *et al.* (2018) and Cangussu *et al.* (2021) in their studies with atemoya (*Annona x atemoya*) and umbu (*Spondias tuberosa*). According to those authors, these results may be associated with the defensive role played by phenolic compounds against external aggressors, thus leading to higher reserves of phenolic compounds in the outermost parts of the food.

For broccoli, the florets (141.22 mg GAE/100 g) showed higher contents of total phenolics compared to the stalk (62.42 mg GAE/100 g). For green cabbage, significant differences were also found, with samples taken from the heart (63.88 mg GAE/100 g) showing a higher concentration of total phenolics compared to leaves (45.11 mg GAE/100 g) (Table 1).

In a study performed with leeks comparing the content of phenolic compounds in the bulb and leaf of the foodstuff, a higher content of total phenolics was observed in the leaves (4.99 mg GAE/100 g for cultivar A and 5.55 mg GAE/100 g for cultivar B) compared to the bulb (3.55 mg GAE/100 g for cultivar A and 4.34 mg GAE/100 g for cultivar B). Thus, the authors highlighted the possibility of using the leek leaf in different culinary preparations, since it is usually discarded during the pre-preparation stages (Damin *et al.*, 2020).

For the other foods (eggplant and green beans), no significant differences were found between the amount of total phenolics in the different parts where the samples were collected ($p > 0.05$; Table 1). Thus, it can be emphasized that the use of the edible parts of these vegetables or their by-products does not interfere in the intake of total phenolic compounds. Whole vegetable consumption is recommended in order to promote an increase in the intake of bioactive compounds.

Regarding total flavonoid contents, as well as total phenolic results, zucchini, broccoli and green cabbage vegetables showed statistically

significant differences in their different parts ($p < 0.05$) (Table 1).

For zucchini, the peel (59.06 mg CE/100 g) showed significantly higher levels of total flavonoids compared to the pulp (13.03 mg CE/100 g). For broccoli, the florets (137.38 mg CE/100 g) showed higher values of total flavonoids compared to the stalk (37.78 mg CE/100 g). Suleria *et al.* (2020) compared the concentration of total flavonoids in peels and pulps of different fruit species and also determined higher concentrations in the peels. According to the authors, exposure of the external part of the food to sunlight leads to abundant and diverse synthesis of flavonoids.

The samples taken from the heart (22.44 mg CE/100 g) displayed a higher content of flavonoids compared to the leaves (14.76 mg CE/100 g) (Table 1). Masood *et al.* (2023) also verified different contents of total flavonoids in different parts of the food. Analyzing onion (*Allium cepa*) extracts, these authors found a higher concentration of total flavonoids in the peel (40.33 - 72.33 mg QE/g) in comparison to the bulb (15.67 - 26.33 mg QE/g) of onions ($p < 0.01$).

For the other foods (eggplant and green beans), no differences were found between the levels of total flavonoids in the different parts from which the samples were collected ($p > 0.05$; Table 1). Thus, in the absence of the conventional edible parts, the by-products are ideal for use.

Regarding antioxidant capacity, the spectrophotometric method using the DPPH radical measures the ability to quench the radical by decreasing optical absorbance due to electron donation from the extracts to the radical (Grande-Tovar *et al.*, 2021). As a result, significant differences were observed for three foods compared to the parts in which the samples were collected ($p < 0.05$).

In zucchini, peel (159.13 $\mu\text{mol TEAC}/100\text{ g}$) showed significantly higher levels of antioxidant capacity compared to pulp (69.78 $\mu\text{mol TEAC}/100\text{ g}$). For broccoli, florets (588.3 $\mu\text{mol TEAC}/100\text{ g}$) exhibited higher levels of antioxidant capacity

compared to stalk (223.10 $\mu\text{mol TEAC}/100\text{ g}$). For green beans, a statistically significant difference was also verified, where samples removed from the pods (168.53 $\mu\text{mol TEAC}/100\text{ g}$) showed greater antioxidant capacity compared to seeds (124.37 $\mu\text{mol TEAC}/100\text{ g}$) (Table 1). For the other foods (eggplant and cabbage), no significant differences were found between antioxidant capacity in different parts of the foods ($p > 0.05$; Table 1). According to Láscaris *et al.* (2020), Suleria *et al.* (2020) and Pazinato *et al.* (2021), besides such differences in food parts, these values vary according to factors to which the plants are exposed, such as humidity, soil type, climate, maturation, genetic factors, and storage forms. In addition, sample preparation methods, the preparation of extracts, extraction time, and use of different solvents (analysis variables) can interfere with the results of *in vitro* antioxidant capacity.

Regarding the analysis of the foods as a whole, significant differences were found among the foods and their parts respectively to total phenolic content ($p < 0.001$).

Broccoli florets presented the highest total phenolic content (141.22 mg GAE/100 g), followed by zucchini peel (69.87 mg GAE/100 g), cabbage heart (63.88 mg GAE/100 g) and broccoli stalks (62.42 mg GAE/100 g) with similar values of total phenolics. Samples of cabbage leaf (45.11 mg GAE/100 g), green beans (37.53 mg GAE/100 g), eggplant peel (36.30 mg GAE/100 g), green bean pods (33.92 mg GAE/100 g) and eggplant pulp (30.24 mg GAE/100 g) showed similar and significantly lower values of total phenolics compared to the previously mentioned foods. Zucchini pulp presented the lowest content of total phenolics (25.44 mg GAE/100 g) (Table 1).

Vegetable by-products, such as zucchini peel, cabbage hearts and broccoli stalks, had higher levels of total phenolic compounds than traditional parts used for vegetable consumption, such as cabbage leaves, eggplant, and zucchini pulp (Table 1). Damin *et al.*

Table 1. Content of bioactive compounds and antioxidant capacity of different parts of vegetables. Goiânia, UFG, 2022.

Vegetables	Total phenolics (mgGAE*/100 g) (mean \pm SD****)	Total flavonoids (mg CE**/100 g) (mean \pm SD)	Antioxidant capacity (μ mol TEAC***/100 g) (mean \pm SD)
Zucchini			
Pulp	25.44 \pm 2.93 ^{bd}	13.03 \pm 1.74 ^{bd}	69.78 \pm 14.57 ^{bc}
Peel	69.87 \pm 7.91 ^{ab}	59.06 \pm 2.53 ^{ab}	159.13 \pm 11.1 ^{ab}
Eggplant			
Pulp	30.24 \pm 13.83 ^{ac}	23.51 \pm 15.06 ^{ac}	204.58 \pm 110.54 ^{ab}
Peel	36.3 \pm 2.54 ^{ac}	32.08 \pm 3.63 ^{ac}	250.94 \pm 65.93 ^{ab}
Broccoli			
Floret	141.22 \pm 13.44 ^{aA}	137.38 \pm 15.39 ^{aA}	588.3 \pm 116.58 ^{aA}
Stalk	62.42 \pm 7.41 ^{bb}	37.78 \pm 2.46 ^{bc}	223.10 \pm 18.67 ^{bb}
Cabbage			
Leaf	45.11 \pm 3.77 ^{bc}	14.76 \pm 4.22 ^{bd}	164.13 \pm 24.82 ^{ab}
Heart	63.88 \pm 0.68 ^{ab}	22.44 \pm 1.99 ^{ac}	198.87 \pm 53.66 ^{ab}
Green beans			
Seed	37.53 \pm 6.24 ^{ac}	30.29 \pm 3.08 ^{ac}	124.37 \pm 3.1 ^{bc}
Pod	33.92 \pm 7.99 ^{ac}	31.14 \pm 9.39 ^{ac}	168.53 \pm 43.57 ^{ab}

Equal superscript letters in different parts of the same food indicate no statistically significant difference ($p > 0.05$); Equal superscript capital letters in the same column indicate no statistically significant difference ($p > 0.001$); *GAE= Gallic acid equivalent; **CE= Catechin equivalent; ***TEAC= Trolox-equivalent antioxidant capacity; ****SD= Standard deviation.

(2020) found similar results as in the present study, observing that leek leaf contains higher contents of minerals, total phenolics and antioxidant capacity than the bulb. Therefore, these by-products should not be wasted, but included in the diet, contemplating the nutritional, economic and sustainable objectives of the Dietetic Technique.

Significant differences were found between foods and their parts with respect to total flavonoids ($p < 0.001$). Broccoli florets was the sample with the highest content of total flavonoids (137.38 mg CE/100 g), followed by zucchini peel (59.06 mg CE/100 g).

Broccoli stalk (37.78 mg CE/100 g), eggplant skin (32.08 mg CE/100 g), green bean pods (31.14 mg CE/100 g) and green beans (30.29 mg CE/100 g), eggplant pulp (23.51 mg CE/100 g) and green cabbage heart (22.44 mg CE/100 g) had similar and significantly lower values of total flavonoids compared to those mentioned above. The samples with the lowest levels of total flavonoids were cabbage leaf (14.76 mg CE/100 g) and zucchini pulp (13.03 mg CE/100 g) (Table 1).

Identical to the results of total phenolic compounds, zucchini peel is a by-product that stands out because it had a significant content of total phenolics and total flavonoids. Zucchini peel had a four-fold higher total flavonoid content than pulp (Table 1). Accordingly, Serquiz *et al.* (2018) highlighted the functional potential that can be lost by the population when using only the conventional parts of vegetables.

Significant differences were observed between the foods and their parts about antioxidant capacity ($p < 0.001$). The food with the highest antioxidant capacity was broccoli florets (588.3 μ mol TEAC/100 g).

Samples of eggplant peel (250.94 μ mol TEAC/100 g), broccoli stalk (223.10 μ mol TEAC/100 g), eggplant pulp (204.58 μ mol TEAC/100 g), green cabbage heart (198.87 μ mol TEAC/100 g), green bean pods (168.53 μ mol TEAC/100 g), cabbage leaf (164.13 μ mol TEAC/100 g) and zucchini peel (159.13 μ mol TEAC/100 g) had similar and significantly lower values in relation to antioxidant capacity compared to the foodstuffs cited above. The foods with

the lowest antioxidant capacity were green beans (124.37 μ mol TEAC/100 g) and zucchini pulp (69.78 μ mol TEAC/100 g) (Table 1).

Broccoli florets stood out in the three analyses performed. According to Bashmil *et al.* (2021), this result is expected because several studies have indicated a causal correlation between the concentration of phenolic compounds and antioxidant capacity *in vitro*. In addition, it should be noted that the broccoli stalk had three times higher antioxidant capacity than zucchini pulp. Therefore, in order to increase bioactive compounds in food, zucchini pulp could easily be replaced by unused parts of vegetables, such as zucchini peel, broccoli stalk, cabbage heart and eggplant peel.

Except broccoli, it can be concluded that the by-products analyzed had equal or higher levels of bioactive compounds and antioxidant capacity than the more traditional parts used for vegetable consumption. Among the analyzed samples, broccoli florets stood out with the highest contents of total phenolics, total flavonoids, and

antioxidant capacity. On the other hand, the lowest levels were observed on zucchini pulp.

Given the above, we reiterate the importance of the consumption of whole foods from the plant kingdom, because the by-products are important sources of bioactive compounds that help reduce the risk to develop non-transmissible chronic diseases, in addition to contributing to the nutritional, economic and sustainable objectives of food.

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