BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY DURING FOOD PROCESSING OF BEET STALKS AND LEAVES (*BETA VULGARIS L.*)

BEETROOT ANTIOXIDANT AND PHENOLIC CONTENT

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We assessed the effect of cooking methods (baking and steaming) on the phytochemicals and antioxidant activity of aqueous and ethanolic extracts of beet (*Beta vulgaris* L.) stalks and leaves using FRAP and DPPH assays. Steaming for 45 minutes increased ($p < 0.05$) the concentration of phenolic compounds compared to raw beet stalks and leaves. In contrast, we observed a reduction of these compounds ($p < 0.05$) when beet leaves and stalks were baked. The type of solvent used to prepare the extracts also influenced the concentration of phytochemicals and antioxidant activity. The ethanolic extract was more efficient in extracting phenolic compounds from the steamed samples and yielded the highest antioxidant values measured by the DPPH assay ($p < 0.05$).. The results suggest that beet leaves and stalks should be consumed after steaming. This vegetable can contribute to phenolic compounds in a balanced diet, contributing to the reduction of food waste.

KEYWORDS: BEETROOT, ANTIOXIDANT, PHENOLIC COMPOUNDS, OXIDATIVE STRESS, COOKING, WASTE PRODUCTS

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INTRODUCTION

Beetroot (*Beta vulgaris* L.) is an example of a vegetable with antioxidant capacity mainly attributed to nitrogen compounds called betalains[1]. The antioxidant activity of this compound is greater than that of the catequins or even alfa-tocopherols[2]. Also, the intake of these compounds could contribute to the treatment of oxidative-stress related disorders[2]. They are used as a dye in a number of food products, such as ice cream, wine, jams, marmalade, dry mixes and yogurt[3].

Beetroot and its juice are a rich source of nitrate and has recently gained popularity due to the effect of nitrate consumption on increasing the bioavailability of nitric oxide (NO) in the organism, which can enhance performance during exercises and reduce the risk of coronary diseases[4, 5]. However, despite the popularity of beetroot, unconventional parts usually determined as food waste such as stalks and leaves are less investigated.

The use of unconventional foods parts such as beet stalks and leaves may reduce food waste, which increased 50% in the world since 1973[6]. Also, focusing on these parts could increase the access to high nutritional foods at a very low cost, since these parts are not commercially valuable. Hence, investigating the nutritional value of these components could increase the market price for beetroot[7]. Moreover, few studies measured the bioactive compounds and antioxidant properties of unconventional foods in the last years[8].

Vegetables are usually consumed after being submitted to a cooking method to improve the taste, flavor, texture, color and microbiological safety. However, cooking methods could modify the concentration of bioactive compounds and consequently its antioxidant properties[9]. Also, there is no consensus about the best cooking methods and its impacts on bioactive compounds. The eminent thermal processing of cooking can lead to oxidation and loss of water-soluble compounds by steaming/leaching[10]; however, these increased temperatures can also inhibit pro-oxidant enzymes, thus promoting a higher antioxidant profile on foods[11]. Therefore, it is important to know how different cooking processes can affect antioxidant activity and phenolic compounds in different food[12]. Moreover, there is no specific information about beet stalks and leaves.

On this basis, the aim of the present study was to investigate the effects of two different cooking techniques (baking and steaming) on the antioxidant properties and bioactive concentrations of beetroot stalks and leaves (*Beta vulgaris* L.).

MATERIALS AND METHODS

Beet leaf and stalk samples

Fresh and organic beet leaves and stalks were obtained from street markets located in Limeira, SP, Brazil. The samples were cleaned and stored in sealed plastic bags at 4°C until the time for analysis.

Chemical composition

Moisture, protein and ash concentrations were determined by the methods of the Association of Official Analytical Chemists (AOAC, 1990)[13]. Protein content was determined by the micro-Kjeldahl method (Factor of 6.25). Lipid content was determined by the Soxhlet method using ethyl ether, and carbohydrate content was calculated as the difference. All analyses were conducted on raw stalks and leaves. The water gain or loss during the baking and steam cooking processes was analyzed using the moisture assay after the cooking process[13].

Cooking methods

a) *Baking*: After cleaning, the raw beet leaves and stalks were dried in an oven (Clarice - Queen 4B, Pinhalzinho SC, Brazil) using four combinations of time and temperature (180°C for 30

minutes; 180°C for 45 minutes; 210°C for 30 minutes, and 210°C for 45 minutes) and stored in sealed plastic bags at - 80°C until the time for analysis. b) *Steaming*: After cleaning, the raw beetroot stalks and leaves were subjected to moist heat and placed in stainless steel pans for steaming (100°C) for two time periods (30 and 45 minutes). The samples obtained were stored in sealed plastic bags at - 80°C until the time for analysis.

Beet leaf and stalk extracts

Raw, baked and steamed beet leaves and stalks were weighed (2.5 ± 0.1 g) and 20 mL of solvent (distilled water or ethanol p.a.) were added and the preparation was stirred in shaker tubes (Phoenix Luferco AP56) for 5 minutes. The samples were then centrifuged (Centrifuge 5810 R -. Germany) at 4000 rpm for 15 minutes at room temperature (± 25° C). After centrifugation, the supernatant was filtered (Whatman number 3), 20 ml of the same solvent was added to the residue and the stirring, centrifugation and filtration procedure was repeated under the same conditions. The extracts obtained in both steps were homogenized and the volume was completed to 50 mL with water or ethanol. The extracts were then placed in glass vials wrapped with aluminum foil and stored at -80°C until the time for analysis.

Total phenolic concentration

The Folin-Ciocalteu method[14] was used to quantify the concentration of total phenolic compounds in the extracts. The analyses were conducted on microplates and the samples were analyzed in concentrated form. Reading was performed at 750 nm (Multi-Detection Microplate reader Synergy-BIOTEK, Winooski, VT) and the analyses were performed in triplicate. The results are expressed as mg gallic acid equivalent/100 g raw sample (mg GAE/100 g raw sample) after a standard curve was constructed using solutions with gallic acid (50 to 300 $\mu L.mL^{-1}$). The equation of the curve used to calculate the concentration of phenolic compounds was: Concentration ($\mu g.mL^{-1}$) = 0.0037x Abs (750 nm) - 0.1144 (R^2 = 0.999)

Total flavonoid concentration

The flavonoid concentration of the extracts was measured by the method of Ghasemi[15]. The readings were performed at 510 nm (Multi-Detection Microplate reader Synergy-BIOTEK, Winooski, VT). For analysis of the results a calibration curve was constructed under the same conditions using solutions with increasing concentrations of Quercetin (6.25 to 200 $\mu L.mL^{-1}$). The experiments were performed in triplicates and the results are expressed as mg quercetin/g dry $sample^{-1}$ (mg QE/100 g raw sample). The curve equation used to calculate flavonoid concentration in the samples was: concentration (QE $\mu g.mL^{-1}$) = 3.0246 x Abs - 0.0015 (R^2 = 0.997).

Determination of antioxidant activity by the DPPH method (2,2-diphenyl-1-picrylhydrazyl)

Antioxidant activity was measured by the DPPH method as described by Brand-Williams[16]. The absorbance was measured at 515 nm using a microplate reader (Multi-Detection Microplate reader Synergy-BIOTEK, Winooski, VT). All analyses were performed in triplicate. The percentage of the remaining amount of DPPH was calculated as follows: % DPPHr = 100 – [(white abs – abs sample x 100) ÷ white abs].

Ferric reducing antioxidant power (FRAP assay)

The FRAP assay was carried out by the method described by Benzie and Strain[17]. The absorbance was read at 593 nm (Multi-Detection Microplate Reader Synergy-BIOTEK, Winooski, VT). All samples were analyzed in triplicate. Antioxidant activity was calculated based on the change in absorbance reading for 6 minutes (ΔA593nm) using default Fe values (standard $FeSO_4$ curve) obtained from the following formula: Abs 0-6 min (ΔA593 nm) test sample = Abs 0-6 min (ΔA593 nm) standard x [Fe^{2+}] (mmol / L).

Statistical analysis

Data is reported as mean ± standard deviation (SD) of three measurements (n = 3) and were analyzed by two-way ANOVA multiple comparisons analysis at the 95% confidence level. The Pearson correlation test was used to further identify the correlation between phenolic concentration and antioxidant activity measured by FRAP assay. GraphPad Prism software version 5.00 (Trial) was used to analyze the results.

RESULTS

Chemical composition

Organic beet leaves and stalks presented 92.0 ± 0.1 g/100 g of moisture and 3.1 ± 0.1 g/100 g of ashes, 1.8 ± 0.2 g/100 g of total proteins, 0.2 ± 0.1 g/100 g of total lipids, and 2.9 g/100g of carbohydrates. After cooking, we observed differences in the final moisture concentration. While raw beetroot stalks and leaves presented 92% moisture, baking promoted almost 38% dehydration independently of time or temperature. In contrast, the moist heat process preserved the initial moisture and did not promote dehydration.

Cooking yield

After the cooking process, the total yield varied according to the method and time of cooking. Baked samples showed the following yields: ~20% at 180/30°C, ~17% at 180/45°C, ~11% at 210/30°C, and ~8% at 210/45°C, while samples steamed for 30 and 45 minutes showed yields of 80% and 75%, respectively.

CONCENTRATIONS OF BIOACTIVE COMPOUNDS AND ANTIOXIDANT PROPERTIES

Phenolic compounds

The type of solvent employed to prepare the extracts affected the bioactive concentration and the antioxidant properties of the samples. As shown in Table 1, ethanol was more efficient in extracting the phenolic compounds from the raw material and after applying the steam cooking method ($p<0.05$). In contrast, the solvent applied after the baking process did not influence the phenolic concentration ($p>0.05$). The baking process reduced the phenolic concentration by about 75% ($p<0.05$), while the steam cooking process was more effective in preserving the phenolic compounds of beetroot stalks and leaves.

Flavonoids content

Ethanol was more efficient than water in extracting flavonoids ($p<0.05$) and had an expressive action on raw and steamed samples ($p<0.05$) (Table 2). When beet leaves and stalks were submitted to the steam cooking process, the ethanolic extract yielded a twice fold flavonoid content compared to the raw material, although the difference was not statistically significant ($p>0.05$). Baking reduced the flavonoid content ($p<0.05$) independently of time or temperature.

Antioxidant properties

Antioxidant properties were significantly reduced ($p<0.05$) when beet leaves and stalks were subjected to different cooking methods (Table 3). FRAP results were influenced by the type of solvent used for extraction. The antioxidant properties determined by the FRAP assay were significantly higher ($p<0.05$) when ethanol was used to prepare the raw sample extract. These results were inversely related to DPPH, with the antioxidant properties determined by the DPPH

method being significantly higher ($p<0.05$) when water was used to prepare the raw sample extract. However, when samples were steamed or submitted to temperatures higher than 180°C, the aqueous extracts showed higher ($p<0.05$) antioxidant properties by the FRAP assay regardless of processing time. The inverse tendency was observed in the DPPH results. Analysis of the aqueous samples submitted to either cooking process showed lower ($p<0.05$) antioxidant properties by the DPPH method regardless of cooking time.

Correlation assay

Figure 1 illustrates the correlation between phenolic concentration and FRAP assay ($p<0.05$). This correlation was higher for the aqueous extract ($r = 0.92$) than the ethanolic extract ($r = 0.89$). FRAP correlation with phenolic compounds agrees with the FRAP assay results that demonstrated higher antioxidant properties of the aqueous extracts (Table 3).

DISCUSSION

In summary, the bioactive compounds decreased when beet stems and leaves were submitted to the baking process ($p<0.05$). However, after cooking, the type of solvent used to prepare the extracts influenced the antioxidant activity results. The aqueous extract showed higher FRAP values ($p<0.05$) while the ethanolic extract showed better results for the DPPH assay ($p<0.05$). This difference can be attributed to the solubility of the main components of the molecule with antioxidant activity, such as number of phenolic rings or catecholic structure[18].

The total yield of beet leaves and stalks after a cooking process was directly related to water gain or loss. Usually, the dry cooking process (baking) results in water losses, which increase with high temperature and long periods of time. In contrast, the steaming process causes less water loss than baking and may have influenced the other results. We did not use a steaming cooking process because it is not common to consume boiled leaves. Furthermore, the use of boiling water results in a high loss of soluble antioxidants by leaching and diffusion into the cooking medium, as shown in a recent meta-analysis[19, 20].

The extraction of bioactive compounds is expected to increase when the sample has lower moisture content due the larger contact surface. However, in the present study the results were expressed on a dry basis (g.100 g^{-1} dry sample), showing the effect of the solvent on the efficiency of extraction of bioactive compounds. The results showed a higher efficiency for ethanol when the food matrix had a high moisture concentration (steaming). However, there was no difference when different solvents (ethanol or water) were applied to extract bioactive compounds from baked samples.

Many factors contribute to the efficiency of extraction from a food matrix, the most important being type of solvent, temperature, solvent volume, pH, use of ultrasound, and material state[21]. Therefore, further studies are necessary to analyze the best conditions for the extraction of bioactive compounds and other factors that can contribute to the efficiency of extraction on beets leaves and stalks.

The steam cooking method seems to be better than the consumption of raw beets stems and leaves (Table 1). Similar to the present study, Bernaert[22] observed that steaming was a better method for maintaining phenolic compounds in leeks. Other studies also demonstrated that steaming increases total phenolic content in different kinds of vegetables[23, 24]. The steaming method seems to be a valuable cooking process to increase phenolic bioavaibility[22]. Thus, it is important to evaluate the best thermal process to preserve the ability of vegetables to deliver their maximum health benefits for consumption[12, 25].t

Nevertheless, although baking could have various effects on bioactive compounds depending on the food matrix and other variables, we observed reduced antioxidant activity measured by the FRAP and DPPH assays (Table 3). In this case, thermal processing by baking reduced phenolic

compounds, but maintained other compounds with redox potential such as vitamins that could be extracted from the matrix. Moreover, thermal treatments can break down the glucosides of flavonoids to form other compounds or derivatives with higher antioxidant properties and different biological mechanisms of action[26].

Cooking processes can induce numerous chemical reactions such as Maillard reaction, Strecker degradation, and the hydrolysis of esters and glycosides due to the applied heating. Thus, this process could lead to the generation of new antioxidant compounds[27, 28]. The extent to which these phytochemicals change during thermal processing depends on the sensitivity of the compound to modification or degradation, the processing method and the length of exposure to a processing technique[22].

Recently, Tian[29] observed that the air-frying method produced a notable increase in DPPH radical-scavenging activity in potatoes when compared to other cooking methods, while baking had the most detrimental effect. The authors suggested that temperature and time used in the cooking methods were conducive to the generation of new antioxidants with high DPPH radical-scavenging activity due to the number of hydroxyl/imino-groups and the position of the hydroxyl[29].

The steaming process increased the FRAP results when compared to raw samples in this study. Steaming probably induces the breakdown of compounds, which could lead to a variety of phenolics compounds with varying compositions[30] and its action is more expressive when water is used as a solvent. Steaming also increased the antioxidant activity measured by FRAP assay in carrot, broccoli and courgette with aqueous extracts when compared to raw foods[31]. However, further investigations should identify and quantify these compounds in order to explain this phenomenon.

Several studies have demonstrated the health benefits of beet consumption due to the betalains, nitrate and phenolic compounds[5, 32, 33]. However, although leafy vegetables are sources of minerals, vitamins, antioxidants and pigments, few studies have evaluated the functional properties of beet stalks and leaves. Thus, the pioneering results of the present study will improve the understanding of the antioxidant properties of beet stalks and leaves. These results could have major influence in the beetroot market price due to improved application and cooking methods specifications[7]. Hence, it is important to identify and quantify the principal phenolic compounds and their changes as a function of the cooking method used in order to recommend the best form of consumption.

CONCLUSIONS

The results of the present study demonstrated that the bioactive compounds and antioxidant properties of beet leaves and stalks were affected by different cooking methods (baking and steaming). Steaming was the method that retained the greatest amounts of phenolic and flavonoid compounds and maintained the ferric reducing antioxidant power (FRAP assay). Thus, from a health-promotion point of view, this method was preferable for cooking beetroot stems and leaves. Moreover, the type of solvent used to prepare the extracts influenced the bioactive compounds and antioxidant capacity of beet stems and leaves, indicating that further studies should be conducted to optimize the extraction conditions. These results lead us to conclude that beet stems and leaves should also be considered as part of the usual diet due to their nutritional value, thereby improving the full use of food.

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