

# Influence of cooking methods on in vitro bioaccessibility of phenolics, flavonoids, and antioxidant activity of red cabbage

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## Research Article

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# Abstract

Red cabbage is rich in phytochemical compounds, and its consumption, either raw or cooked, has been linked to the prevention of several diseases. This work aimed to investigate the influence of cooking methods on *in vitro* bioaccessibility of phenolics and antioxidant activity of red cabbage. The vegetable was subjected to boiling, steaming, and microwaving for different times to evaluate color parameters, total phenolic (TPC), total flavonoid (TFC), anthocyanin content (AC), and antioxidant activity (FRAP, DPPH, and ABTS). The phytochemical bioaccessibility before and after cooking was also evaluated by *in vitro* simulated digestion. Steaming showed the most significant retention of the compounds after 20 and 25 minutes of cooking (72-86% for TPC, 72-77% for TFC, 75-79% for FRAP, 84-91% for DPPH, 70-83% for ABTS), followed by microwaving, which was more stable in 10 minutes. Microwaving decreased TFC and AC over time. Boiling did not show significant differences between the cooking times and showed more than 50% of losses of TPC, TFC, and AC and 30 to 60% of antioxidant activity. Steaming was the best cooking method, showing the most significant tendency to black coloration ( $< L^*$ ). In 10 minutes, it still showed the highest percentages of increase in TPC and the minor losses of TFC and AC in the gastric and intestinal phases. Steaming also increased the antioxidant after digestion when compared to uncooked red cabbage. These results are important to help consumers choose the most effective cooking method for red cabbage to retain its health-promoting components.

## Introduction

Vegetable consumption is often associated with several health benefits. In addition to having vitamins and minerals, vegetables are also rich in phytochemicals. Within phytochemicals is the class of flavonoids, which includes anthocyanins, a group characterized by the red, purple, violet, and blue colors present in flowers, leaves, fruits, roots, and vegetables of several plants. Vegetables that are rich in anthocyanins contribute to about 20% of the recommended daily intake of antioxidants in human food [1].

Red cabbage (*Brassica oleracea var. capitata f. rubra*) is one of the vegetables rich in anthocyanins. It pertains to the genus *Brassica*, along with cauliflower, kale, and broccoli [2]. Studies have identified some phenolic compounds that are predominant in the genus *Brassica*, such as quercetin, kaempferol, isorhamnetin, hydroxycinnamic acid, as well as vitamins C and E, and carotenoids [2–4]. These compounds function as antioxidants, decreasing the risk of chronic non-communicable diseases, helping in the reduction of oxidative stress, and assisting in the immune response [3, 5, 6].

Red cabbage consumption has been increasing due to its attractive color and health benefits [3, 7] and, although native to the Mediterranean region and South-Eastern Europe, it is currently cultivated worldwide [3]. The preparation of this vegetable varies with cultural habits and cuisines of different populations: it can be eaten raw, in salads, or cooked [4]. In Western society, for instance, boiling, steaming, and microwaving are the most common cooking methods [8]. In addition to promoting the inactivation of pathogenic microorganisms, cooking can reduce anti-nutritional factors, soften plant tissues, and also

favor the bioavailability of some nutrients [9, 10]. On the other hand, heat treatments also have adverse effects, such as the degradation of some components and the formation of toxic compounds [11].

Another condition responsible for altering the phytochemical content of vegetables is gastrointestinal digestion. This process is influenced by pH variation, enzymes, and colonic bacteria [12]. Digestion can degrade or modify the structure of bioactive compounds, leading to changes in their health-promoting properties [13]. Authors have reported a decrease in total phenolic, flavonoid, and anthocyanin contents, as well as in the antioxidant and antimicrobial activities after *in vitro* digestion of different vegetables [12–14]. Moyo et al. [14] observed that a leafy vegetable popular in Africa had its phenolic compounds, flavonoids, and antioxidant activity increased after boiling, but the digestion of the cooked vegetable was responsible for decreasing its phenolic content, and antioxidant potential.

The literature on the influence of different home-cooking methods on the physicochemical parameters, and bioaccessibility of bioactive constituents during *in vitro* digestion of red cabbage is limited and, to some extent, contradictory, presenting no consensus on the best way to prepare this vegetable. Therefore, this work aims to investigate the influence of cooking methods on *in vitro* bioaccessibility of phenolics, flavonoids, and antioxidant activity of red cabbage.

## Materials And Methods

Fresh red cabbage was purchased locally. About six units (6 kg) were cleaned, and their outer leaves were removed. Then, they were divided into four segments, and each part's center was removed. The segments were cut into *chiffonade* (ribbons) pieces using a vegetable processing machine (Britania, São Paulo, Brazil). The homogenized samples were randomly divided into 26 portions of 210 g each. Duplicate portions were used for control samples (uncooked red cabbage), and each of the tested methods (boiling, steaming, and microwaving) at their different cooking times (10 min, 15 min, 20 min, and 25 min). The tested cooking methods and times are shown in Fig. 1. For boiling, the samples were immersed in boiling water (1:6; w/w; red cabbage/water). For microwaving, the samples were placed on plates with water at room temperature (1:5; w/w) to prevent them from burning while cooking. For the steaming method, the samples were spread in a basket, which was placed inside of a pan with boiling water (1:6; w/w). Then, the steam pot with the samples inside was covered for each determined period. After the heat treatments, the samples were drained and let to cool down to room temperature. Both the uncooked and cooked cabbage were subsequently frozen in polyethylene bags at -20°C, lyophilized (Liobrás, L101, São Paulo, Brazil), and ground in a Willy knife mill (Fortinox, Distrito Federal, Brazil).

The extraction of phenolic compounds from the samples was done using a previous methodology [15] with some modifications. Done in duplicate, 1 g of each lyophilized powder sample was weighed into Falcon tubes, and 10 ml of 80% acidified methanol (0.1% HCl) was added. The tubes were vortexed, and kept in an ultrasonic bath (Unique, model USC- 2850A, Campinas, São Paulo, Brazil) for 3 min at 30°C ± 5°C. Then, they were centrifuged at 3000 rpm for 10 min at 25°C. The supernatant was removed, and the solid phase was subjected to a similar second extraction. Finally, the supernatants were combined,

filtered, and stored at  $-20^{\circ}\text{C} \pm 2^{\circ}\text{C}$  for further analysis. Total phenolic (TPC), Total flavonoid (TFC), anthocyanin content (AC) and antioxidant activity by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate), ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) and FRAP (ferric reducing antioxidant power) assays were performed, in duplicate, in 96-well microplates, and the absorbance values were measured in a Microplate Photometer (MultiSkan FC, Thermo Fisher Scientific K.K., Tokyo, Japan).

The TPC was determined using the Folin-Ciocalteu colorimetric method, described by Singleton and Rossi (1965), with some modifications. An aliquot of 10  $\mu\text{L}$  of each diluted sample was pipetted to the well with 240  $\mu\text{L}$  of distilled water, 15  $\mu\text{L}$  of Folin-Ciocalteu reagent, and 15  $\mu\text{L}$  of sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) at 20% (m/v). The absorbance was measured at 690 nm after 60 min of the solution resting in the dark. Results were expressed as mg gallic acid equivalent (GAE)/100 g cabbage powder. The TFC was determined by an aluminum chloride colorimetric assay [16]. A solution of 10  $\mu\text{L}$  of the diluted sample was mixed well with 90  $\mu\text{L}$  of sodium nitrite and left to react for 5 min. 10  $\mu\text{L}$  of a 10% aluminum chloride was added afterwards, and the solution was left to react for another 5 min. Subsequently, 90  $\mu\text{L}$  of a 1 mol/L NaOH solution was added to the well, and the absorbance was measured at 540 nm. The results were expressed as mg catechin equivalent (CE)/100 g of cabbage powder. The AC was determined by the differential pH method based on spectrophotometric readings of structural changes of anthocyanins as a function of pH, as described by Giusti and Wrolstad [17]. Two buffer solutions were prepared: potassium chloride pH 1.0 (0.025 M) and sodium acetate pH 4.5 (0.4 M). The samples were diluted in these buffer solutions and, after 30 min in the dark, the absorbances were measured in a spectrophotometer at wavelengths of 540 nm and 690 nm. The AC was expressed in mg cyanidin-3-rutinoside equivalent (C3RE)/100 g of cabbage powder, considering the molar absorptivity of 26900 L/cm.mol and the molar mass of cyanidin-3-rutinoside of 449.2 g/mol.

The DPPH radical scavenging activity of the samples was determined according to the method described by Brand-Williams et al. [18]. A DPPH solution (0.12 mmol/mL) was prepared. In the well, 10  $\mu\text{L}$  of the diluted sample was pipetted with 190  $\mu\text{L}$  of the DPPH solution. After 30 min standing in the dark, the absorbance was measured at 540 nm. The ABTS radical scavenging activity of the samples was determined as reported by Re et al. [19]. The ABTS radical was formed by reacting 5 mL of a 7 mM ABTS<sup>+</sup> solution with 88  $\mu\text{L}$  of a 140 mM potassium persulfate solution and incubated at 25°C in the absence of light for 16 h. Then, 300  $\mu\text{L}$  of ABTS and 10  $\mu\text{L}$  of each sample were placed in the microplate. The absorbance was measured at 690 nm after resting 30 min in the dark. The FRAP assay, as described by Benzie and Strain [20], was made with 100 mL of 300 mM acetate buffer at pH 3.63, 10 mL of 20 mM ferric chloride, and 10 mL of TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) previously dissolved in 40 mM hydrochloric acid. 300  $\mu\text{L}$  of FRAP, and 10  $\mu\text{L}$  of each sample were pipetted into each well. The absorbance was measured at 620 nm after 30 min of the solution resting in the dark. The DPPH, ABTS, and FRAP assays were expressed in mmol Trolox equivalent (TE)/100 g of cabbage powder.

The L\* (brightness), a\* (redness), and b\* (yellowish) color parameters of the red cabbage powder samples, both uncooked and cooked, were determined using a HunterLab MiniScan EZ colorimeter (Reston, Virginia, U.S.A.). The samples were placed in 1-cm cells and the L\*, a\*, and b\* values were

measured 6 times using Illuminant D65, at an observation angle of 10°. The hue angle parameter ( $h^\circ$ ) was calculated by  $h^\circ = \tan^{-1} (b^*/a^*) + 180^\circ$ , when  $a^* < 0$ , and  $h^\circ = \tan^{-1} (b^*/a^*)$ , when  $a^* > 0$ . The Chroma ( $C^*$ ) parameter was calculated by  $C^* = (a^{*2} + b^{*2})^{1/2}$  [21].

The cooked samples that better preserved the compounds evaluated in item 2.3, and the uncooked samples were submitted to *in vitro* gastrointestinal digestion based on the methodology described by Minekus et al. [22], with the modifications suggested by Silva et al.[15], as shown in Fig. 1.

The statistical analysis of the data was performed by the normality and homogeneity of the variances by the Shapiro-Wilk and Levene tests, respectively. The results were presented as mean  $\pm$  standard deviation. One-way analysis of variance (ANOVA) was used to determine whether there were significant differences between the red cabbage samples ( $p < 0.05$ ). For the variables that presented non-homogeneous variances ( $p < 0.05$ ), the Kruskal–Wallis test was used. Duncan's multiple range test was used to compare the means. The degree of association between the variables was assessed using Pearson's correlation coefficient.

## Results And Discussion

Phenolic acids and flavonoids are the largest groups of phenolics present in vegetables and are related to antioxidant potential since they act as reducing agents and scavengers of free radicals [15]. Therefore, the effect of cooking methods on the stability of bioactive compounds is of great interest due to the biological functions of plants, which indicate their potential as medicinal agents. The TPC, TFC, and AC of uncooked red cabbage was higher than those of the cooked samples, presenting 1604.45 mg GAE/100g, 379.49 mg CE/100g, and 1034.76 mg cy-3-glu/100g dry weight, respectively.

According to the analysis of uncooked red cabbage after the boiling, steaming, and microwaving methods (Fig. 2), it was possible to notice that exposure to heat and water significantly reduced the TPC, TFC, AC, and antioxidant activity (FRAP, DPPH and ABTS assays) ( $p < 0.05$ ). The reduction was probably due to the loss of phenolic compounds in the cooking water by leaching and thermal degradation. These results are in line with Ismail et al. [23], who confirmed that 1 min of heat treatment significantly reduced ( $p < 0.05$ ) the total phenolic content of kale, spinach, swamp cabbage, and shallots.

Comparing the three methods used, steaming showed the most significant retention during all the evaluated times: about 72–86% for TPC, 75–79% for FRAP, 84–91% for DPPH, and 70–83% for ABTS. According to Murador et al. [24] and Xu et al. [8], steaming does not involve excessive amounts of oil and avoids leaching losses. These authors still found that steaming red cabbage and kale resulted in the highest bioactive compounds and antioxidant activity compared to boiling and stir-frying methods. In the microwaving method, 54–68% of TPC, 46–68% of FRAP, 60–80% of DPPH, and 54–65% of ABTS were retained in the cooked tissue; as for the boiling method, 48–51% of TPC, 46–48% of FRAP, 72–78% of DPPH, and 44–51% of ABTS were retained in the cooked tissue. For Xu et al. [8], boiling and stir-frying were the two methods that caused significant losses of total phenolics in red cabbage. However, other

authors have reported that steaming and boiling increased the antioxidant activity of red cabbage. Murador et al. [24] and Wu et al. [25] confirmed that after boiling red cabbage for 3–4 min, the TPC was favored by as much as 21% (254 mg raw GAE/100 g to 321 mg cooked GAE/100 g).

As presented in Fig. 2, the boiling method did not show significant differences between cooking times, while the TPC, TFC, FRAP, DPPH, and ABTS were significantly more stable in 10 min of microwaving and 20 and 25 min of steaming. Depending on the cooking conditions – such as time and temperature – the compounds can be more or less affected, either increasing or decreasing their amounts. In addition, the cooking time may enhance the oxidation of the antioxidant compounds by prolonging the time that the vegetable stays in contact with heat [24].

The TFC decreased over time in the microwaving method, whereas it increased in the steaming method. 10 min of microwaving was the best method to preserve the TFC (97%), while the highest values of the steaming method (75–77%) were found after 20 and 25 min. Both methods, however, had higher values than those found in the boiling method (53%). During steaming or microwaving, the heat can promote the inactivation of polyphenol oxidase (PPO), preventing the degradation of polyphenols [8]. Microwaves have even been used in studies to inactivate enzymes and avoid nutrient leaching due to the low amount of water it requires for cooking [11, 26]. Similar results were obtained by Puupponen-Pimia et al. [27], who reported that the TFC doubled after blanching (96°C for 3 min) of cauliflower and white cabbage. All samples in the present study had decreased antioxidant activity (Fig. 2. D, E, and F) in the experimental period, similar to what Murador et al. [24] found in their ABTS assay. Our results were also similar to those obtained by Bernstein and Noreña [28], who reported that cooking, blanching, and steaming led to decreased antioxidant activity.

Compared with uncooked samples, boiling, microwaving, and steaming reduced the AC by 39–45%, 13–44%, and 9–14%, respectively. However, boiling and steaming showed no significant differences in the AC over time. Microwaving decreased the AC over time by as much as 13% for 10 min, 27% for 15 min, and 40% for 20 and 25 min. Similar to Murador et al. [24] and Xu et al. [8], the steaming method presented better stability of anthocyanins. In the study by Murador et al. [24], red cabbage boiled for 4 min had a 41% loss in its AC. Xu et al. [8] reported that stir-frying and boiling were the methods that showed the highest losses (62% and 55.5%, respectively), while steaming and microwaving had the lowest losses (46.1% and 17.5%, respectively). Likewise, for Volden et al. [29], blanching and boiling for 3 min led to losses of 59% and 41%, respectively, while steaming for 10 min resulted in a loss of 29% of AC.

Brightness values range from light to dark: 0 corresponds to black and 100 to white. Evaluating the L\* color parameter, the values ranged from 26.63 (steaming for 10, 15, 20, and 25 min) to 46.44 (boiling for 15 and 20 min, microwaving for 20 and 25 min), indicating a coloration tending to black (Table 1). Partial inactivation of enzymes, such as PPO, can be achieved in heat treatments at 85°C for 10 min. However, the enzymes can be reactivated through the renaturation process as the cooking process ends and food slowly cools down [30]. Thus, PPO can help the degradation of AC and increase the browning of the plant tissue [31]. Although the cooking time did not influence the brightness in steaming, this method was

significantly better ( $p < 0.05$ ) regarding the tendency to black coloration. Boiling and microwaving significantly increased the  $L^*$  values, indicating a slightly white color. These findings are related to the marked loss of AC after boiling since this method favors leaching in the cooking water.

Table 1

Effects of boiling, steaming, and microwaving on color parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*$ ,  $h^*$ ) of red cabbage.

Samples	Color parameters				
	$L^*$	$a^*$	$b^*$	$C^*$	$h^*$
R	33.13 ± 1.64 <sup>h</sup>	8.52 ± 0.79 <sup>h</sup>	-4.86 ± 1.68 <sup>h</sup>	9.87 ± 1.68 <sup>j</sup>	208.81 ± 6.42 <sup>g</sup>
B10	42.26 ± 1.33 <sup>h</sup>	7.22 ± 0.40 <sup>gh</sup>	-10.47 ± 0.50 <sup>gh</sup>	12.72 ± 0.50 <sup>gh</sup>	235.42 ± 0.37 <sup>cd</sup>
B15	44.85 ± 1.35 <sup>h</sup>	6.64 ± 0.10 <sup>gh</sup>	-10.38 ± 0.33 <sup>gh</sup>	12.32 ± 0.33 <sup>hi</sup>	237.39 ± 0.53 <sup>bc</sup>
B20	46.43 ± 3.22 <sup>h</sup>	6.05 ± 0.46 <sup>h</sup>	-10.03 ± 1.02 <sup>h</sup>	11.72 ± 1.02 <sup>i</sup>	238.84 ± 0.71 <sup>ab</sup>
B25	42.17 ± 1.89 <sup>h</sup>	6.44 ± 0.96 <sup>gh</sup>	-11.42 ± 1.39 <sup>h</sup>	13.11 ± 1.39 <sup>fg</sup>	240.65 ± 0.72 <sup>a</sup>
S10	28.35 ± 1.28 <sup>h</sup>	11.90 ± 0.54 <sup>h</sup>	-15.11 ± 0.51 <sup>h</sup>	19.23 ± 0.51 <sup>b</sup>	231.79 ± 0.49 <sup>e</sup>
S15	27.64 ± 0.97 <sup>h</sup>	11.67 ± 0.39 <sup>h</sup>	-14.35 ± 0.56 <sup>gh</sup>	18.51 ± 0.56 <sup>c</sup>	230.88 ± 1.67 <sup>e</sup>
S20	27.95 ± 0.87 <sup>h</sup>	10.80 ± 0.48 <sup>h</sup>	-13.88 ± 0.56 <sup>gh</sup>	17.59 ± 0.56 <sup>d</sup>	232.12 ± 0.31 <sup>e</sup>
S25	26.63 ± 0.90 <sup>h</sup>	10.66 ± 0.28 <sup>h</sup>	-13.85 ± 0.18 <sup>gh</sup>	17.48 ± 0.18 <sup>d</sup>	232.43 ± 0.56 <sup>e</sup>
M10	34.27 ± 1.42 <sup>h</sup>	13.67 ± 0.62 <sup>h</sup>	-14.96 ± 0.48 <sup>gh</sup>	20.26 ± 0.48 <sup>a</sup>	227.59 ± 1.27 <sup>f</sup>
M15	40.60 ± 2.69 <sup>h</sup>	8.23 ± 0.50 <sup>h</sup>	-12.88 ± 0.33 <sup>h</sup>	15.29 ± 0.33 <sup>e</sup>	237.42 ± 1.93 <sup>bc</sup>
M20	46.44 ± 0.94 <sup>h</sup>	7.46 ± 0.25 <sup>h</sup>	-11.29 ± 0.44 <sup>h</sup>	13.53 ± 0.44 <sup>f</sup>	236.55 ± 0.18 <sup>cd</sup>
M25	45.88 ± 1.82 <sup>h</sup>	7.12 ± 0.30 <sup>gh</sup>	-10.29 ± 0.39 <sup>gh</sup>	12.52 ± 0.39 <sup>ghi</sup>	235.32 ± 1.45 <sup>d</sup>

R: Uncooked red cabbage, B10: Boiling for 10', B15: Boiling for 15', B20: Boiling for 20', B25: Boiling for 25', S10: Steaming for 10', S15: Steaming for 15', S20: Steaming for 20', S25: Steaming for 25', M10: Microwaving for 10', M15: Microwaving for 15', M20: Microwaving for 20', M25: Microwaving for 25'. Averages followed by different letters on the same column indicate statistical difference according to Duncan's multiple range test ( $p \leq 0.05$ ).

The values of  $a^*$  and  $b^*$  represent the colors, ranging from red (+  $a^*$ ) to green (- $a^*$ ) and from yellow (+  $b^*$ ) to blue (- $b^*$ ). The  $a^*$  values of boiled (all cooking times) and microwaved (20 and 25 min) samples were smaller compared ( $p < 0.05$ ) to values of uncooked and microwaved (10 min) samples. During the steaming method,  $a^*$  values increased compared to the uncooked samples, with a tendency towards red. Parameter  $b^*$  decreased in the cooked samples (Table 1), showing a greater tendency towards blue.  $C^*$  values closer to 0 indicate more neutral colors (white/gray) while those closer to 60 indicate more intense

colors. The hue angle results indicated red color at 0 h°, yellow at 90 h°, green at 180 h°, and blue at 270 h°. The uncooked samples showed lower intensity and the C\* values of the cooked samples decreased over time (Table 1). The hue angle (h\*) indicated a color closer to blue at 208 for uncooked samples and over 230 for cooked samples. The decrease observed for parameter h\* throughout the cooking methods can be explained by the reducing number of AC present in red cabbage and by the exposure of this vegetable to oxidative conditions.

In their evaluation of the red cabbage colors, Xu et al. [8] found a decrease in a\* values in all cooking methods except for stir-frying, an increase in L\* values in samples subjected to boiling, and no significant differences in b\* values between processes. Similar results were described by Iborra-Bernad et al. [32], who demonstrated that boiling this vegetable for a longer period increased L\*, decreased a\*, and showed no significant differences in b\*.

Using Pearson's correlation coefficient, the TPC was shown to be correlated with the color measurement parameters; positive a\* (r = 0.60) and negative h\*, and L\* (r = -0.82) values indicate that higher values of TPC increase a\* but decrease h\* and L\*. Furthermore, samples with higher TPC, TFC, and AC consistently exhibited a higher antioxidant activity measured by DPPH, ABTS, and FRAP. A significant correlation was observed between all antioxidant assays (r > 0.79). In this case, steaming for 10, 15, 20, or 25 min is recommended to prevent the significant loss of phenolic compounds and the antioxidant activity of red cabbage. Under these conditions, the corresponding compound values (TPC, TFC, AC, FRAP, DPPH, and ABTS) were reduced only by 9–35%, and the color was more stable.

The antioxidant effect of bioactive compounds depends not only on the concentration present in the red cabbage, but it is also influenced by the bioaccessibility and bioavailability after digestion in the body. *In vitro* gastrointestinal digestion simulation evaluated the bioavailability of TPC, TFC, AC, and antioxidant activity of red cabbage in the three phases of digestion (oral, gastric, and intestinal) comparing uncooked samples and samples steamed for 10, 15, 20, or 25 min (Fig. 3). The TFC and AC of red cabbage decreased after the digestive process. The gastric phase showed a more significant decrease in the TFC, while the AC decreased in the intestinal phase. Phenolic compounds are easily degradable in the gastrointestinal environment, where they can be metabolized into compounds with different biological properties, thus resulting in lower bioaccessibility. The results suggest, however, that even after the entire digestive process, bioactive compounds may remain in the organism. The relatively low concentrations of polyphenols hold a significant nutritional power due to synergistic health benefits when several polyphenols are ingested [33–36].

The TPC after digestion of steamed samples decreased about 30% in the gastric phase in 15, 20 and 25 minutes. For intestinal phase the samples showed an increase of about 2–23% in relation to the uncooked sample, but there was no significant difference in the times evaluated. Regarding uncooked red cabbage, the TPC reduced 30% in the gastric phase and 17% in the intestinal phase. Steaming for 10 min showed the highest percentages of TPC increase and the lowest losses of TFC and AC in the gastric and intestinal phases after digestion. Physicochemical changes in the gastrointestinal tract (temperature, pH,



and enzymes) affect the bioavailability of antioxidant compounds. Polyphenols are sensitive to both alkaline conditions and presence of salts where a portion of the compounds can be transformed into different structural forms with other chemical properties, which would also lead to different bioavailability and biological activity [33, 34, 36, 37]. Yet an increase of some compounds may still occur since small fractions of the bioactive compounds are extracted from a food matrix and solubilized through gastric liquids; the activity of digestive and intestinal enzymes during gastrointestinal digestion, associated with the release of phenolics bound to the matrix, give place to the bioaccessible fraction [33, 34, 36, 38].

The antioxidant activity in the FRAP assay showed similar behavior to the TPC. The gastric phase showed a 15% increase in antioxidant activity in the steaming method for 10 min, and there was a significant reduction in the following cooking times. Regarding the intestinal phase of digestion, steaming showed no significant differences between the times tested but, when compared to uncooked cabbage, it increased the antioxidant potential.

For the DPPH assay, there was an increase in the antioxidant potential after the digestion of uncooked and steamed red cabbage in the following order: intestinal phase > gastric phase > control samples. Uncooked and steamed (10 min) red cabbage showed no significant differences between digested and control samples for the ABTS assay. However, in the other cooking times (15, 20, and 25 min), the intestinal phase of digestion preserved a better antioxidant activity than the gastric phase and the control sample. These results show that AC are more than bioactive compounds responsible for the antioxidant potential of red cabbage since the antioxidant activity was preserved or even increased despite the reduction of AC after digestion.

## Conclusion

When red cabbage is subjected to common home-cooking methods, such as boiling, steaming, and microwaving for 10, 15, 20, and 25 min, there is a reduction in its total phenolic content, total flavonoid content, monomeric anthocyanin content, and antioxidant activity compared to the uncooked vegetable. Furthermore, it was possible to verify that steaming promotes more stability on total phenolic compounds, anthocyanins, and color parameters, while boiling induces more significant losses. Thus, steaming can be considered the best cooking method to preserve the nutritional properties of red cabbage. This research indicated that, up to 25 min, steaming could provide higher amounts of bioaccessible antioxidants than the other methods. Given that phytochemicals and antioxidants are naturally present in food, opting for the most efficient cooking method can help people easily ingest a higher amount of antioxidants, which is beneficial for health.

## Declarations

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**Authors' Contributions** S. A. and S. Z. Conceptualization; data curation; formal analysis; investigation; methodology; writing – original draft. L. M. T. Methodology, Writing - review & editing. C. C. H. K. and S. M. R. F. supervision; resources; writing – review and editing.

**Availability of data** The authors confirm that the data supporting the findings of this study are available within the article.

**Ethics' Approval** Not applicable.

**Consent for Publication** Not applicable.

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**Conflict of interest** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Figures



Figure 1

Flowchart of cooking methods and *in vitro* digestibility of phenolics, flavonoids, and antioxidant activity of red cabbage.

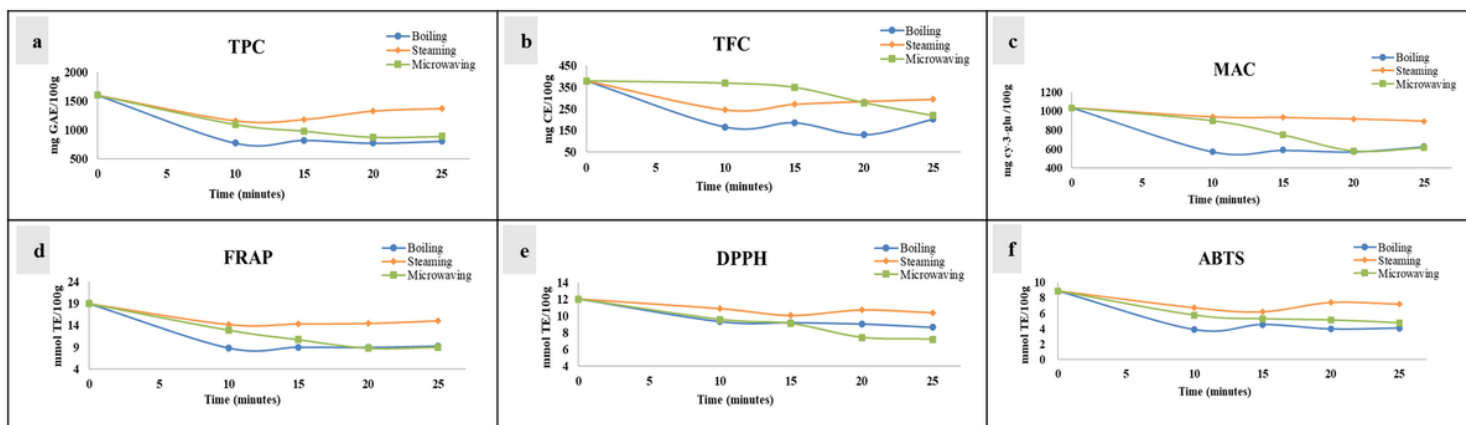


Figure 2

Total phenolic content (TPC), total flavonoid content (TFC), monomeric anthocyanin content (AC), antioxidant activity (FRAP, DPPH, and ABTS assays) of red cabbage during cooking methods and time.

### Figure 3

Total phenolic content (TPC), total flavonoid content (TFC), monomeric anthocyanin content (AC), and antioxidant activity (FRAP, DPPH, and ABTS assays) before and after gastric and intestinal phases of *in vitro* simulated digestion of red cabbage. \*The data are mean values  $\pm$  standard deviation (SD)  $n \geq 3$ . For individual bar graphs, the mean values indicated with a different uppercase letter indicates significant differences ( $p \leq 0.05$ ) between uncooked (R) samples and samples steamed for 10, 15, 20 and 25 min according to Duncan's multiple range test ( $p < 0.05$ ). The mean values indicated with a different lowercase letter indicates significant differences ( $p \leq 0.05$ ) for each part between undigested (control) and digested samples (gastric and intestinal phase).