Effect of different atmospheric and subatmospheric cooking techniques on qualitative properties and microstructure of artichoke heads

Elsevier Editorial System(tm) for Food Research International

Manuscript Draft

Manuscript Number: FOODRES-D-20-02308R2

Title: Effect of different atmospheric and subatmospheric cooking techniques on qualitative properties and microstructure of artichoke heads

Article Type: Research Articles

Keywords: artichoke; texture; colour; microstructure; antioxidant activity; polyphenols

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Abstract: Quartered Violetto artichokes were cooked with different treatments (boiling, steaming, sous-vide and vacuum-cooking) at the same cook value at the product thermal centre. Bracts and hearts were then studied in terms of physical (moisture content, texture and colour), histological and chemical (phenolic, 5-hydroxymethylfurfural (HMF) and furans content, total antioxidant capacity) analyses. A deeply modified microstructure was observed for boiled and steamed samples, with an evident decrease of hardness both for bracts and hearts. Lightness were decreased by all the treatments on the two anatomical parts (with the exception of sous-vide bract) and the highest total colour difference was recorded in steamed while the lowest in sous-vide samples. Steamed and sous-vide artichoke exhibited the highest total phenolic content as well as total antioxidant capacity. On the other hand, sous-vide showed the highest concentration of HMF, 2-furan-methanol and 2,4-dihydroxy-2,5-dimetyl-3(2H)-furanone while byproduct 5-metylfuraldheide was detected only in the steamed product.

Cover Letter

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To: Editor-in-Chief of Food Research International

Dear Editor,

I would like submit to your attention the revised research paper entitled "*Effect of different atmospheric and subatmospheric cooking techniques on qualitative properties and microstructure of artichoke heads"* (FOODRES-S-20-02913R1) by Massimiliano Rinaldi, Paola Littardi, Saverio Santi, Antonella Cavazza, Maria Grimaldi, Margherita Rodolfi, Tommaso Ganino, Emma Chiavaro for the publication in Food Research International.

We would like to thank the Editor to have considered the manuscript for publication in this journal and the reviewers for their helpful suggestions and comments, which have been very useful for improving the quality of the manuscript. An itemized list of comments in response to the reviewers' observations has been prepared for each reviewer. Changes on manuscript were visualized in black bold character.

The total word count of the Manuscript (including tables and figure legends) is 5915, including also 34 references and 1 tables + 6 figures and it is prepared strictly according to the Journal format as provided in the instruction to authors.

I declare that all the co-authors have agreed for submission to Food Research International and the manuscript is not submitted or under consideration in any other journal. I also declare that there are any conflicts of interest.

I hope that you consider this paper. If there is anything else that you would like to know, please don't hesitate to get in touch with me.

Looking forward to hear you soon. Sincerely yours,

Parma, 27.08.2020

With my Best Regards

Prof. Massimiliano Rinaldi

***Detailed Response to Reviewers**

Reviewer #1: The authors have greatly improved their manuscript based on all reviewer comments.

Some additional minor comments:

- Introduction lines 8-15: this sentence has not yet been fixed, please revise.

Thank you. The sentence was corrected.

- Section 2.2 line 32: the sentence should start 'Quartered artichoke globes …'

Thank you. Mistake was corrected

- Section 2.3 line 27-30: should read 'pre-selected sample locations based …' and the end of the sentence needs fixing.

Thank you. Sentence was completed

- Section 2.4 line 5: What is a 'semithinLeitz' microtome?

- The Figure captions are all mixed up. Please fix.

- Figure 4 (structure of all artichoke samples): Why are there different scalebars for different treatments? This makes it difficult for both the authors and the readers to draw meaningful conclusions about the structural differences. Can some of the images be replaced so that at least all bract or heart samples are shown at the same magnification?

Thank you. Authors decided to merge different pictures in the same plate for limiting the number of Figures. Otherwise, we were obliged to add other Figures but the aim of the different magnifications was to underline only particular aspects of the tissue and not to compare all the samples at the same magnification.

Once these additional comments have been addressed, in particular the images in Figure 4, the manuscript will likely be suitable for publication.

Reviewer #2: General comments

The authors attended to most of the comments and observations made by the reviewers; however, there are still corrections that need to be addressed. In general, the manuscript could be good, only if the authors attend carefully the comments. Some of the specific comments are presented below:

Comment 1: The authors mention that they corrected the numbering of the lines; however, the error still persists in the reviewed version. Manuscripts must be typewritten, with 2 cm margins. Each page must be numbered, and lines must be consecutively numbered from the start to the end of the manuscript

Thank you. Pages were numbered as suggested as well as margins were corrected but actually consecutive line numbering is not allowed by the submission system that automatically adds line numbers.

Comment 2: in the reviewed version, authors mention that a description of the detailed procedure followed for the preparation of the extracts has been added to section 2.5.2. However, in such document,

section 2.5.2. correspond to Determination of 5-HMF and furans. The authors probably meant that "a description of the detailed procedure followed for the preparation of the extracts has been added to section 2.5.1.

Thank you. Reviewer is right, but we referred to the old paragraph number in the original submission to successfully fix the suggested comment.

Comment 3: Authors reply that values are not "exactly equal", but show the same trend, as confirmed by correlation value of 0.95, probably because the main antioxidant compounds are polyphenols as reported in many references about artichokes. However, is highly recommended to add or show how the inhibition percentage was calculated, as the reference by Lutz et al. (2011) For example…

Thank you. Inhibition % calculation was better explain as suggested

Comment 4: The numbers of the figures in the section "Captions for figures" do not correspond to the figures presented in the reviewed version. Please correct them

Thank you. Caption for figures was corrected as suggested

Highlights

- Boiling and steaming deeply modified the microstructure
- Steaming gave the best results for the nutritional quality
- Vacuum cooking maintained good colour and texture
- Sous-vide cooking presented the highest content of HMF

Effect of different atmospheric and subatmospheric cooking techniques on qualitative properties and microstructure of artichoke heads

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Abstract

Quartered Violetto artichokes were cooked with different treatments (boiling, steaming, sous vide and vacuum cooking) at the same cooking value at the thermal centre. Then, the physical (moisture content, texture and colour), histological and chemical (phenolic, 5-hydroxymethylfurfural (HMF) and furan content, total antioxidant capacity) features of bracts and hearts were assessed. A deeply modified microstructure was observed in boiled and steamed samples with an evident decrease in hardness both for bracts and hearts. Lightness of two anatomical parts was decreased by all the treatments (with the exception of sous vide bracts). The highest total colour difference was recorded for steamed samples, whereas the lowest was noted for sous vide samples. Steamed and sous vide artichoke exhibited the highest total phenolic content and total antioxidant capacity. Sous vide samples exhibited the highest concentrations of HMF, 2-furan-methanol and 2,4-dihydroxy-2,5-dimetyl-3(2H)-furanone, whereas the by-product 5-metylfuraldheide was only detected in the steamed product.

Keywords: artichoke; texture; colour; microstructure; antioxidant activity; polyphenols

1. Introduction

Based on their nutritional value, plant-based foods are currently regarded as a simple way to incorporate the concepts of health and wellness via natural products into our diet. Furthermore, among plant-based foods, the use and consumption of edible flowers, which constitute not only a garnish but also a traditional source of food in many parts of the World and particularly in Italy (Guarrera & Savo, 2013), **it is reported to be under expansion**. Among edible flowers, artichoke flowers or globe artichoke (*Cynara cardunculus* var. *Scolymus* L.) were traditionally consumed in the Mediterranean basin. In a recent review, their nutraceutical properties and potential utilization as functional ingredients was reported (Gostin & Waisundara, 2019). As confirmation of tradition, Italy is the leading world producer of artichoke followed by Egypt and Spain, with estimated annual harvests of 390 kt, 324 kt and 208 kt, respectively (FAOSTAT, 2018). In addition, a 44% increase in global artichoke consumption over the last 20 years was reported (Dosi et al., 2013), confirming consumers' interest in this edible flower.

Thus, in addition to the utilization of globe artichoke as a functional ingredient, the effects of innovative cooking techniques on this edible flower deserve attention and research due to increasing demand of healthy cooked vegetables by consumers. Ferracane et al. (2008) studied the effects of the most common cooking methods (boiling, steaming and frying) on artichokes and reported increases in total antioxidant capacity but significant variations in colour. However, several papers demonstrated that some products of the Maillard reaction, such as 5-hydroxymethylfurfural (HMF), display diverse harmful effects on human health. In fact, in addition to the beneficial activities of HMF, such as antioxidative, antiproliferative, antiallergic, anti-inflammatory, antihypoxic, antisickling, and antihyperuricemic effects (Zhao et al., 2013), the HMF and furans generated by thermal treatment are associated with carcinogenicity, neoplastic transformation, hepato- and nephrotoxicity and must be mitigated (Pérez-Burillo, Rufián-Henares, & Pastoriza, 2019). HMF is considered potentially

carcinogenic to humans or might be metabolized to potentially carcinogenic compounds in humans. HMF and acrylamide are regarded as the most important heat-induced contaminant (Capuano & Fogliano, 2011). Among proposed strategies for HMF reduction, vacuum treatment represents a potentially useful technology, but the processing parameters must be properly adjusted to minimize loss of sensory attributes (Lee et al., 2019).

Similarly, vacuum cooking techniques have also been used and represent an emerging technology in cooking also to reduce the quality losses caused by classical cooking methods mainly due to degradation at high temperatures and leaching into cooking water. An example of this technique is sous vide cooking, in which the product is vacuum-packed and cooked in a sealed plastic bag at low temperatures without contact with oxygen and water (Guillén et al., 2017). However, the application of sous vide cooking to artichoke was reported to cause a significant change in colour, producing an artichoke that is yellower and lighter compared with those cooked directly by boiling (Guillén et al., 2017) due to increased retention of chlorophyll b in the vacuum-packaged samples. Thus, vacuum cooking is a promising cooking technique as demonstrated by Iborra- Bernad et al. (2013, 2014 and 2015) and Martínez-Monzó (2013) on other vegetables. In this cooking technique, products are cooked inside a device with sub-atmospheric pressure, which causes the water to boiling below 100 °C, but without the mechanical compression of a bag as required in sous vide.

Comprehensive data on the comparison of atmospheric and vacuum cooking techniques are not available in the literature; thus, the aim of this work was to evaluate the effects of thermal treatments on the microstructural, physical (texture, colour, water content) and chemical (HMF, total antioxidant capacity, total phenol content) properties of the hearts and bracts of globe artichoke.

2. Materials and Methods

2.1 Chemicals

Water (MilliQ), 96% ethanol, 99% methanol, acetone, sodium carbonate, Folin-Ciocalteu reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl), 5-hydroxymethylfurfural, formalin, acetic acid and gallic acid analytical standard were purchased from Sigma-Aldrich.

2.2. Samples and storage

Freshly harvested artichokes (*Cynara cardunculus* var. Scolymus L.) cv. Violetto were kindly donated by the company Fiordelisi srl, Stornarella (FG), Italy. Artichokes were transported under refrigerated conditions within 24 h from harvesting and stored at 4 °C until use. First two external bracts (the leave-like structures protecting the flower) were removed from the heads of artichoke, and then the artichokes were quartered and uncooked or cooked using four different methods as reported below:

Boiling (B): **Quartered artichoke globes** were added to boiling tap water in a covered stainless-steel pot (food/water ratio, 1:5) and cooked on a moderate flame in triplicate. For each cooking trial, 10 quarters were boiled; cooked samples were drained for 5 min.

Steaming (ST): Thirty quarters were used. The treatments were performed at 100 °C under atmospheric pressure in a Combi-Steam SL oven (V-Zug, Zurich, Switzerland) following the method proposed by Rinaldi et al. (2010).

Sous vide (SV): Thirty artichoke quarters divided into three vacuum bags (OPA/PP 15/65, Orved, Musile di Piave, Italy) were placed under vacuum using a packaging machine (Lavezzini Univac, Fiorenzuola d'Arda, PC, Italy). The samples were cooked in a stirred water bath at 85 °C (JULABO Labortechnik GmbH, Seelbach, Germany).

Vacuum cooking (VC): The samples were treated using the system described in European Patent EP2671476A2. Thirty artichoke quartered globes were placed in a closed container filled with tap

water (food/water ratio, 1:5) in which the pressure was brought to 0.8 bar by using a vacuum pump (Paoluccio Schirò-Tecla srl, Verona, Italy). Subsequently, the container was inserted in a preheated ventilated oven at 130 °C.

For all cooking trials, cooked samples were cooled for 30 min on an open rack at room temperature to ensure equilibration to ambient temperature and then immediately analysed. Pictures of all samples are reported in Figure 1.

All cooking conditions were defined by means of preliminary tests to achieve the same degree of cooking at the thermal centre expressed in terms of the cook value, which corresponded to an acceptable cooking level expressed by a group of 20 untrained people who assessed samples cooked at different degrees during preliminary sensory experiments. The cook value was calculated from the integration of the heat penetration curve during preliminary tests as follows:

$$C_{Tref}^{z} = \int_0^t 10^{(T-T_{ref})/z}\, dt$$

where

t = time (min)

T_{ref} = reference temperature; set to 100 °C

z = temperature increase that induces a 10-fold increase of the reaction rate of the chemical reaction used as a reference; z was set at 33 °C, as previously reported (Vittadini et al., 2005). All the cooking trials were designed to achieve a C_0 at centre equal to a 10-min equivalent that corresponded to 12 min for B, 23 min for ST, 38 min for SV and 37 min for VC. C_0 values at the samples' surfaces were 12 min for B, 13.3 min for SV, 18 min for VC and 23 for ST.

The steam and ventilated oven air temperatures and water temperatures in the stirred bath as well as those at the samples' centre and surface were monitored using 0.9-mm wire thermocouples (K-type; Ni/Al-Ni/Cr) with an acquisition rate of 5 s.

2.3 Physical analyses of hearts and bracts

The moisture content (g/100 g) of raw and cooked artichokes was evaluated in triplicate using the gravimetric technique following the official method (AOAC, 2002).

Texture analysis was performed by Warner-Bratzler shear force analysis on both hearts (the tender fleshy centre of the immature artichoke flower) and bracts using a TA.XT2 Texture Analyzer equipped with a 25-kg load cell (Stable Micro Systems, Godalming, UK) and Texture Expert software for data analysis. Samples were placed perpendicular to the blade, and shearing was performed at a velocity of 5 mm/s. The maximum force of the peak was used as a measure of sample hardness (tenderness) and expressed in Newton (N). Ten samples were analysed for each cooking technique.

Colour was determined for ten pre-selected locations samples based on the outer surface of bracts and hearts by limiting the measurements to an area of about 1 cm x 1.5 cm around positions depicted in Figure 1. The analyses were performed using a Minolta Colorimeter (CM 2600d, Minolta Co., Osaka, Japan) equipped with a standard illuminant D65 and a 10° position of the standard observer. The instrument was calibrated before each analysis with white and black standard tiles. Lightness (*L**) was quantified on each sample using Spectramagic software (Ver. 3.6). In addition, the colorimetric parameter hue angle (H°) (colour of sample as defined by its location in a 360° axis; 0 or 360°= red, 90° = yellow, 180° = green and 270° = blue) and chroma (C*) (colour saturation increasing from 0) were also obtained. Total colour differences (ΔE) between raw and cooked samples were also calculated. Ten samples were analysed for each cooking technique.

2.4 Histological analysis on hearts and bracts

Ten samples (artichoke bracts and hearts) for each treatment were fixed in FAA solution (formalin: acetic acid: 60% ethanol solution, 2:1:17 v/v) (Ruzin, 1999). After two weeks, the samples were

dehydrated with gradual alcohol concentrations. The inclusion was made in a methacrylate resin (Technovit 7100, Heraeus Kulzer & Co., Wehrheim, Germany), and the resulting blocks were sectioned at 3 μm thickness (transversal cuts) **with a Leitz 1512 microtome** (Leitz, Wetzlar, Germany). The sections were stained with Toluidine Blue (TBO) solution (Ruzin, 1999) for the evaluation of structure variation after each treatment and with Tannins Solution (Ruzin, 1999) for a qualitative evaluation of tannin inclusions location and shape after each treatment. Sections were observed using a Leica DM 4000 optical microscope (Leica Imaging Systems Ltd., Wetzlar, Germany) equipped with a Leica DMC2900 digital camera (Leica Imaging Systems Ltd., Wetzlar, Germany).

2.5 Chemical Analyses on whole quartered artichoke

2.5.1 Total Phenolic Content (TPC) and total antioxidant capacity (TAC)

Samples were mixed with liquid nitrogen and minced. Then, three replicates were processed as follows: an amount corresponding to 0.5 g of dry weight were added to 20 mL of acetone, and the sample was submitted to extraction at room temperature for 30 minutes under stirring, covering the vials with aluminium foil to protect the content from light. Subsequently, vials were placed in a centrifuge at 6000 g and supernatant was recovered and transferred in a 100 mL flask. The extraction procedure was repeated for three consecutive times and solvent aliquots were brought to dryness under nitrogen flow. The extracts were then recovered with 15 mL of ethanol and filtered with a PTFE filter before analysis. An aliquot (50 μL) of sample extract was added to 1160 μL of water (MilliQ), 300 μl of sodium carbonate 20% w/w (to ensure the optimum pH for the formation of phenolate ions (Cicco & Lattanzio, 2011)) and 100 μL of the Folin-Ciocalteu reagent; the solution was then incubated at 40 ° C for 30 minutes. An identical preparation of the blank was performed but lacked the sample. Absorbance was measured at 760 nm. The TPC value was expressed as mg of GAE (gallic acid equivalent)/g of dry sample. The calibration curve was generated using 7.5 to 125 μg/mL gallic acid.

An aliquot (50 µL) of sample extract and 2 mL of DPPH (6 x 10^{-5} M solution in methanol, Lutz, Henrìquez& Escobar, 2011) were added to a cuvette. Absorbance was measured at 517 nm at time zero (T0) and after 16 minutes (T1). Methanol was used as the blank. **The antioxidant capacity was expressed as a percentage of inhibition of the DPPH radical (Lutz et al., 2011) as follows: I% = $[(A_{C(0)} - A_{S(t)})/A_{C(0)}]*100$, where $A_{C(0)}$: absorbance of the control at time = 0 min; and $A_{S(t)}$: absorbance of the sample at time = 16 min**. All analyses were performed in triplicate.

2.5.2 Determination of 5-HMF and furans

Gas chromatography coupled to mass spectrometry (GC-MS) was used for the quantitative determination of 5-HMF and the semi-quantitative analysis of the different furans. A Thermo 1300 equipped with an auto-sampler, thermostatic oven and Thermo TSQ 8000 mass spectrometer (Thermo Scientific) was employed in these experiments. Samples (1 µL) were injected in an Agilent HP-5MS UI 30 m, 0.250 mm x 0.25 µm column using helium as inert gas at a flow of 1.2 mL/min. The separation method involved a programmed temperature that increased from 35 to 250 °C at the rate of 5 °C/min. Acquisition was performed by full-scan MS in the mass range between 40 and 1000. Peak identification was achieved by using the NIST library and comparison with standards when available. Quantitative determination of 5-HMF was achieved by means of a calibration curve generated using between 25 and 200 µg/mL in the matrix (extract of raw artichoke) given that a significantly different slope was obtained compared to that obtained using the curve built in solvent. Limit of detection (LOD) and limit of quantification (LOQ) were calculated by following the Eurachem 2014 guidelines. The GC-MS technique was used to search for other compounds generated by thermal treatment, such as molecules belonging to the class of furans. Using the NIST library, it was possible to identify several markers, such as 2-furan-methanol (furfuryl alcohol), 2,4-dihydroxy-2,5-dimetyl-3(2H)-furanone and 5-metylfuraldheide.

2.6 Statistical analysis

Means and standard deviations calculated with SPSS (v. 26.0, SPSS Inc., Chicago, USA) statistical software were used to perform one-way analysis of variance (ANOVA) to evaluate the effect of the different cooking treatments at a significance level of 0.05 ($p<0.05$). A Tukey-Kramer post hoc test at a 95% confidence level was also applied using the same software to verify differences among groups.

3. Results and Discussion

3.1. Physical analyses

The moisture content was 82.0 ± 0.6 g/100 g in raw artichoke, which was consistent with that reported by Romani et al. (2006). As expected, boiling caused a significant increase in moisture content (83.3 ± 1.8) due to water absorption during boiling (Pellegrini et al., 2009). In contrast, ST samples exhibited a significant reduction in moisture content (79.3 ± 1.6) compared to raw samples probably due to water evaporation during the cooling stage as artichokes were not protected from water loss and their surface temperature was approximately 100 °C immediately after cooking. Finally, VC (80.0 ± 0.4) and SV (80.8 ± 1.2) samples did not present significant differences compared to raw and ST artichoke samples.

Hardness of the bracts is reported in Figure 2A. All cooking procedures caused a significant reduction of bract hardness as expected, and the greatest extent of softening was noted for ST despite the lowest moisture content. This finding was likely related to the measured surface cook values of artichoke samples. ST samples exhibited the highest value (23 min) and subsequently the highest level of damage due to cooking. Other cooking procedures did not exhibit any significant difference. Data on bract hardness are consistent with microstructural data. ST samples exhibited the most damaged structures (**Figure 4C**), whereas VC and SV exhibited the lowest damage (**Figure 4G and 4E**). Similarly, Iborra-Bernad et al. (2015) reported more damaged structures for traditionally cooked green beans and carrots and reduced wall thickness due to the higher temperatures compared to sous vide and vacuum cooking. B samples exhibited increased firmness compared to ST probably due to the longer cooking time in the latter samples (as temperature and cook value at the product centre were the same).

Heart hardness presented a different trend compared to bracts (Figure 2B). B and ST exhibited the highest softening effect as previously reported by Ferracane et al. (2008). Among cooked samples, VC exhibited the lowest reduction in hardness of artichoke hearts, which was confirmed by microstructural

analyses revealing that VC samples were most similar to R. Comparing gaps between cells, VC did not show solute lines, whereas these lines were found in SV. This finding indicates the filling of these gaps with liquid from the cytoplasm caused by mechanical compression in the bag, thereby resulting in the loss of cell turgor (Iborra- Bernad et al., 2014). However, SV hearts exhibited less damage compared to B and ST and significantly increased hardness as a consequence (Figure 2B).

Regarding colorimetric parameters, in general, L* decreased after all cooking procedures for both bracts and hearts (Table 1) with the exception of SV bract. Ihl, Monsalves and Bifani (1998) reported a reduction of L* in artichoke after blanching treatments with the greatest variation noted for steam, which is in accordance with obtained data. SV samples did not present significant differences compared to raw artichoke, which is consistent with findings by Iborra-Bernad et al. (2013) revealing increased lightness of green beans with sous vide than vacuum cooking due to the contact of the samples with water with vacuum cooking and the replacement of air with water.

The quantitative indicator of colourfulness Chroma (C) was reduced after all cooking procedures (Table 1) and especially for B, indicating substantial reduction of appearance and less intense colour, which changed from a vivid to a dull green, probably due to loss of chlorophyll in boiling (Yuan, Sun, Yuan, & Wang, 2009). Finally, a decrease in hue angle (H°) was also observed in external bracts of cooked artichoke (Table 1); H° was not significantly reduced in B but was reduced by other cooking procedures from green to yellow (in agreement with Ferracane et al., 2008). Decreased greenness is generally associated with chlorophyll degradation and pheophytin formation. However, more relevant changes were observed in ST artichoke, which exhibited the lowest H° values. This finding was potentially due to a change in the reflectance of the samples' surface during cooking and/or the formation of degradation products (Pellegrini et al., 2009; Armesto et al., 2016).

Colorimetric parameters of artichoke hearts showed different trend variations compared to bracts (Table 1). The hearts were brighter than bract, and all cooking trials significantly reduced L*and C

values with the greatest reductions noted for ST and VC. Koç et al. (2017) reported that the differences in the degradation of the colour of green peas depend on cooking temperature. Particularly, the lower cooking temperature used with sous vide offered better colour retention compared to vacuum cooking. The greatest variation of H° was recorded for VC with the highest variation towards yellow colour.

Concerning total colour differences, ΔE (Table 1), ST presented the highest variation, whereas SV exhibited the lowest. The results are related to the surface cook value. ST presented the highest value with the highest thermal damage as a consequence, whereas SV presented lower values due to the surface protection from oxygen provided by cooking bags. The visual appearance of raw and cooked samples are presented in pictures of both bracts and hearts of artichokes in Figure 1.

3.2 Histological analysis

3.2.1 Raw

Raw bract is composed of different tissues, including epidermal, mechanical, parenchymatic and conductive (vascular bundles) tissues (Figure 3A). One layer of cells with stomata comprises epidermal tissue. In the underlying structure, three layers of collenchyma cells are observed. In these layers, signs of cell dehydration are present due to the sample preparation process (FAA treatment, dehydration and inclusion) of the plant material. Most of the anatomic structure is composed of mesophyll (photosynthetic parenchyma) with immersed vascular bundles (phloem and xylem) surrounded by mechanical tissue. Observational analysis of bracts dyed with tannin solution reveal that the tannins are present in large quantities throughout the entire structure and especially in the tissues immediately under the epidermal tissues (Figure 3B).

Raw heart is mainly composed of parenchymatic tissue surrounded vascular bundles (Figure 3C). In this sample, the tannin solution revealed the presence of large quantities of tannins (Figure 3D), especially near the vascular bundles.

3.2.2 Boiling (B)

B treatment caused severe bract damage (epidermal detachment, collenchyma and mesophyll cells dehydration, cell separation and large fissures) due to temperature especially in the parenchyma tissue (Figure 4A) . The structural damage induced by B is greater than that noted in other samples (Figure 4A, 4C, 4E) probably due to the intensity of the ST treatment combined with the presence of water. In addition, artichoke hearts were strongly damaged after B treatment (Figure 4B). Generally, the effect of boiling on the structure confirms with that observed in vegetables by other authors (Paciulli et al., 2016;Lutz et al., 2011). Bracts and hearts showed no differences regarding the presence of tannin inclusions compared to raw samples (data not shown).

3.2.3 Steaming (ST)

ST treatment caused slight dehydration of bract cells; this phenomenon was more visible in the external part of the structure (epidermis and underlying cell layers) (Figure 4C). In other vegetables, Paciulli et al. (2016) observed that steam caused cell detachment probably due to the high temperatures of the treatment (100°C). These temperatures cause the bonds between the pectic substances of two adjacent cells to break. Another important aspect to consider is the increased cell wall thickening (Figure 4C) compared with raw samples. This observation is probably due to heat that induced solubilization of the intercellular cementing pectin, facilitating cell wall loosening (De Roeck et al., 2008; Waldron, 2004). De Roeck et al. (2008) observed that thermally treated carrots were characterized by increased amounts of water-soluble pectin and decreased amounts of chelator and sodium carbonate-soluble pectin, indicating substantial degradation and solubilization.

After ST, the artichoke heart appeared damaged as evidenced by cell separation and thickening. Furthermore, gaps were also observed due to heat treatment (Figure 4D). No difference in the presence of tannin inclusions was noted for ST (artichoke bracts and hearts) compared to raw samples.

3.2.4 Sous vide (SV)

In artichoke bract, SV treatment yielded cell separation, and this effect is most evident in parenchymatic tissue (Figure 4E). The external part of the bract did not appear damaged. Ortiz et al. (2016) observed that the texture reduction of apple after SV treatment was potentially related to depolymerisation of covalently bound pectins present in the cell walls. In our study, cell separation was observed (Figure 4E), and this phenomenon could simply be due to the depolymerisation of cell wall pectin. In hearts, cell separation was observed in the parenchymatic tissue after SV treatment (Figure 4F). In both SV bracts and hearts, no difference was observed regarding the presence of tannin inclusions compared to raw samples.

3.2.5 Vacuum cooking (VC)

In artichoke bracts, VC treatment did not cause severe damage. Cell separation at the mesophyll level (especially lacunar parenchyma) and slight dehydration of external tissues (especially epidermis and collenchyma) were observed (Figure 4G). A similar situation occurred in hearts where only sporadic cell separation was observed (Figure 4H). No differences in the presence of tannin inclusions were noted in samples subject to VC treatment (artichoke bracts and hearts) compared to raw samples.

3.3.1 Total phenolic content (TPC) and total antioxidant capacity (TAC)

Phenolic compounds in plants are located inside the vacuoles or conjugated to cell wall components (Kalt, 2005). TPC of artichoke is reported in Figure 5A. Raw and VC samples exhibited significantly lower amounts of phenolic compounds compared with other treatments. In general, the higher phenolic content after thermal treatment was probably due to an action on cell wall membranes, yielding a softening and disrupting effect that promoted the release of internal compounds (Lutz et al., 2011). The VC technique is considered a non-intensive cooking treatment (Iborra-Bernad et al., 2014). Therefore, minimal action on cell wall membranes and a total phenolic content similar to that observed for raw

samples are expected. In addition, the presence of water as medium can extract a percentage of the released compounds.

ST presented the highest amount of TPC compared to SV and B probably due to the absence of a cooking media and the highest surface cook value, which likely resulted in increased release of compounds. In fact, the presence of water, which is crucial for boiling, facilitates compound washing and dilution (Lutz et al., 2011). SV exhibited significant differences with respect to B, which is consistent with that reported by Baardseth et al. (2010). SV was performed without direct contact with the aqueous media, and minimal release of liquid from the vegetable was observed.

DPPH spectrophotometrical assay results are similar to those recorded for TPC and are shown in Figure 5B. Data reported by previous studies revealed a different behaviour depending on the type of vegetable considered (Kosewski et al. 2018) after sous vide cooking compared with raw samples. In carrots and cabbage, a significant increase after cooking has been ascribed to the interaction between different classes of compounds and the Maillard reaction (Pérez-Burillo et al., 2019). The great difference observed between VC and SV confirms the data reported by Guillén et al. (2017). Significant correlation was observed between TPC and DPPH data (R^2>0.95), confirming the high levels of polyphenolic compounds in artichokes which are responsible of their total antioxidant capacity (Gostin & Waisundara, 2019).

3.3.3 Analysis of 5-HMF and furans

The analysis of 5-HMF, a marker of the second step of the Maillard reaction, was performed using GC-MS and evidenced by the presence of a peak corresponding to the analyte of interest by comparison of the retention time and the associated mass spectrum with a standard solution of the pure compound. Spectrum analysis was also processed by the NIST Mass Spectral Library database, useful for the identification of the chemical compound by matching fragmentation “fingerprints” of the ions

generated with those from a rich collection of reliable reference spectra. The obtained response of matching with the 5-HMF standard was about 99%. Data reported in Figure 6 revealed a definite difference between the examined samples. As expected, HMF was not detected in raw samples given that HMF forms after thermal treatment. SV samples contained the highest levels of the marker; however, the thermal stress associated with this procedure does not significantly differ from the other processes. Therefore, the reason for the difference was not attributed the HMF formation rate but to its distribution in the media. In fact, given that HMF is water soluble, it was released into water during VC and B treatments and therefore removed from the vegetable itself. However, in SV, the presence of the plastic bag prevented HMF release and promoted accumulation. From the toxicological point of view, considering the typical levels of an adult diet, the intake of HMF consequent to artichoke consumption can be considered of limited concern. In fact, obtained values are similar to those reported for other cooked vegetables (Pérez-Burillo et al., 2019) and do not constitute a high contribution to the daily dietary intake such as that from coffee and cocoa beverages. The presence of 2-furan-methanol and 2,4-dihydroxy-2,5-dimetyl-3(2H)-furanone, which are products of the Maillard reaction, responsible for the darkening of a cooked product and its mutagenic properties (Swasti &Murkovic, 2012), has been found in all samples. Higher concentrations (data from semiquantitative analysis, based on area values corrected by means of an internal standard) of these compounds are noted in SV and B, whereas lower levels are noted in ST. Finally, 5-metylfuraldheide is a product of the thermal decomposition of HMF (Nikolov &Yaylayan, 2011) and was only detected in ST (data not shown).

4. Conclusions

Histological analysis revealed that traditional cooking procedures caused the most severe tissue damage, which is also reflected in the highest softening of B hearts and ST bracts and hearts. SV and VC samples were similar to raw samples from a structural point of view and in terms of heart texture. Colour parameters evidenced a general worsening and darkening of the appearance for all cooking methods, but especially for ST samples. On the other hand, although ST artichoke showed promising results concerning biological aspects (the highest total phenolic content and total antioxidant capacity) followed by SV, the latter also exhibited the highest concentration of HMF. Based on the obtained results, it has been assumed that steam could be a good choice for preserving the quality (antioxidant) of artichoke, whereas vacuum cooking is the most promising method for maintaining pleasant colour and texture. In addition, VC more efficiently reduces the formation of HMF and furans generated by thermal treatment, and these compounds are responsible for the darkening of a cooked product and accounted for its carcinogenic and mutagenic properties. Results of the present study could be useful for domestic or professional preparation of artichokes but also for industrial processing by proposing alternative cooking methods to traditional steam ones.

Acknowledgement

The authors dedicate this work to the memory of Paoluccio Schirò without whose help this would not have been possible.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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Captions for figures

Figure 1: Picture of quartered artichoke samples.

Figure 2: Hardness (Newton, N) of bracts (A) and hearts (B) of artichoke samples. Error bars represent +/- 1 standard deviation, (n = 10, sample size = 10 for each cooking trial). Different letters indicate significant differences ($p< 0.05$).

Figure 3: Transverse sections of bracts (A and B) and hearts (C and D) artichoke samples stained with Toluidine Blue O (A and C) and Tannins Solution (B and D).Magnification of tissue of artichoke bract of raw/uncooked samples (A and B). In (B) the dark color indicate the presence of tannins. Magnification of tissue of artichoke heart of raw/uncooked samples (C and D). In (D) the dark color indicate the presence of tannins.

Legend: e = epidermis; c = collenchymatic tissue; p = parenchymatic cells; vb = vascular bundles; t = tannins.

Figure 4: Transverse sections of bracts (A, C, E, and G) and hearts (B, D, F and H) artichoke samples stained with Toluidine Blue O. (A and B) magnification of tissue of artichoke samples boiled; (C and D) magnification of tissue of artichoke samples steamed; (E and F) magnification of tissue of sous vide artichoke samples; (G and H) magnification of tissue of vacuum cooked artichoke samples.

Legend: d =dehydrated cells; cd = cell detachment; f = fissures; ct = cell wall thickening.

Figure 5: Total phenolic content (TPC) (A) and total antioxidant capacity (TAC) (B) of artichoke samples. Error bars represent +/- 1 standard deviation, (n = 3, sample size = 3 for each cooking trial). Different letters indicate significant differences ($p< 0.05$).

Figure 6: 5-hydroximethylfurfural**(**HMF) (A) and furans (B) content of artichoke samples. Error bars represent +/- 1 standard deviation, (n = 3, sample size = 3 for each cooking trial). Different letters indicate significant differences ($p< 0.05$).

Table 1. Colorimetric parameters for bract and heart of artichoke samples.[a]

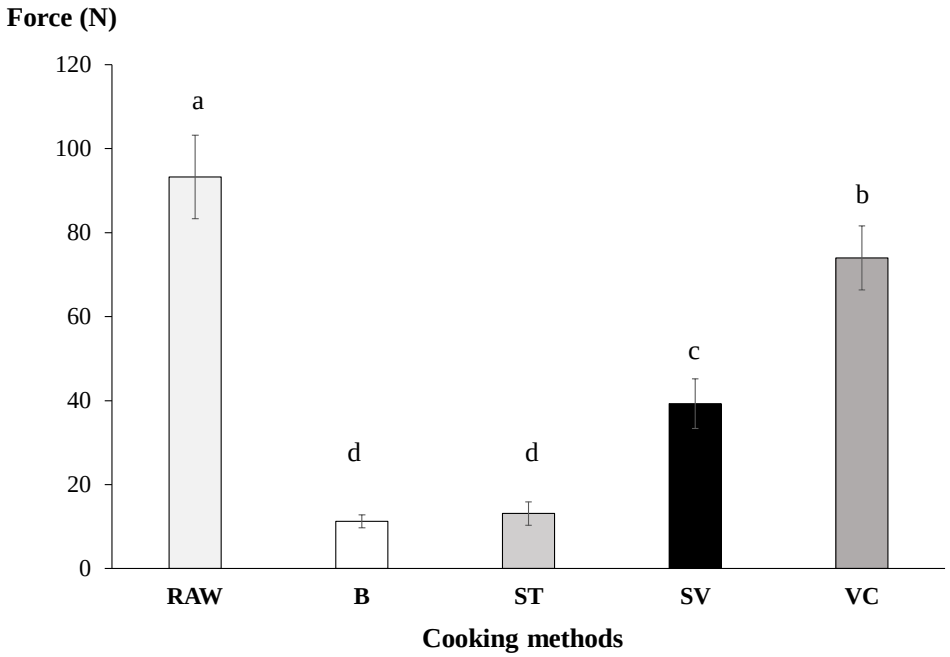
	*L**	*C*	*H°*	*ΔE*	*L**	*C*	*H°*	*ΔE*
	Bract				*Heart*			
RAW	60.4±7.8a	22.9±3.9a	98.8±9.7a	-	75.2±6.4a	22.4±2.3a	87.3±3.9b	-
B	50.1±3.0bc	15.0±2.9c	95.8±4.2a	12.4±3.3a	56.2±2.4b	16.2±2.1b	90.4±3.7a	20.2±2.3c
ST	47.6±2.8c	17.7±3.2bc	86.5±2.7c	14.2±2.5a	46.2±2.8c	13.0±2.3c	85.7±5.0b	30.6±3.0a
SV	58.8±3.3a	19.1±3.3b	86.4±2.9c	7.0±2.1b	60.5±5.2b	17.7±1.8b	84.7±3.4b	15.6±5.2d
VC	53.1±7.8b	18.4±3.8bc	90.2±6.8b	9.2±3.6b	51.3±4.8c	13.6±1.6c	74.2±4.7c	26.0±4.5b

[a]n=10. Means in column followed by different capital letters significantly differ ($p < 0.05$) among different times for the same bread.Means followed by different lowercase letters significantly differ ($p< 0.05$) among the four types of bread at the same storage time.

Figure 1.

Figure 2.

A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RAW
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SV
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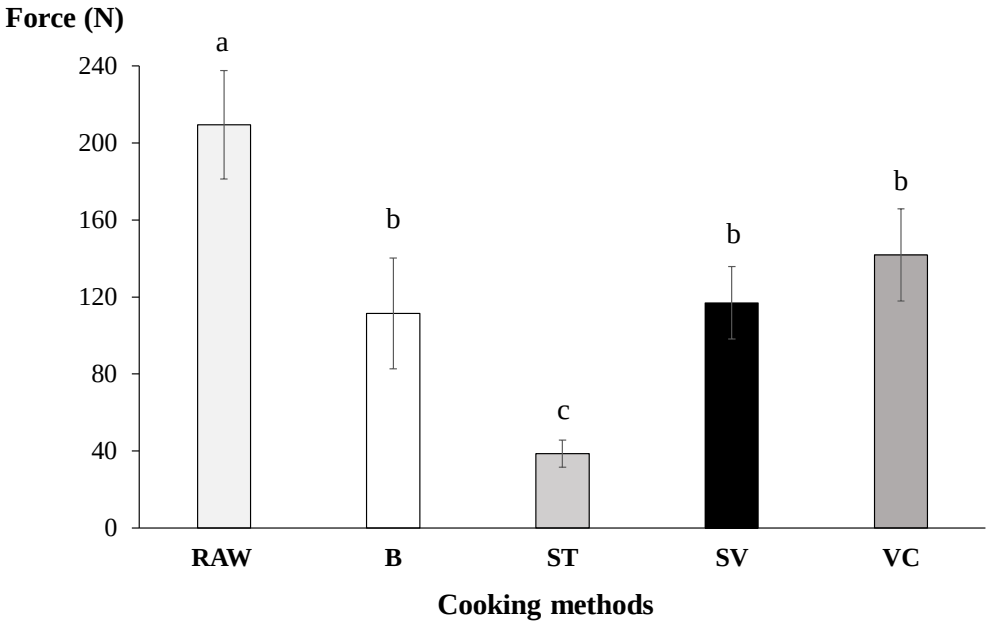
Cooking methods

B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Cooking methods

Figure 3.

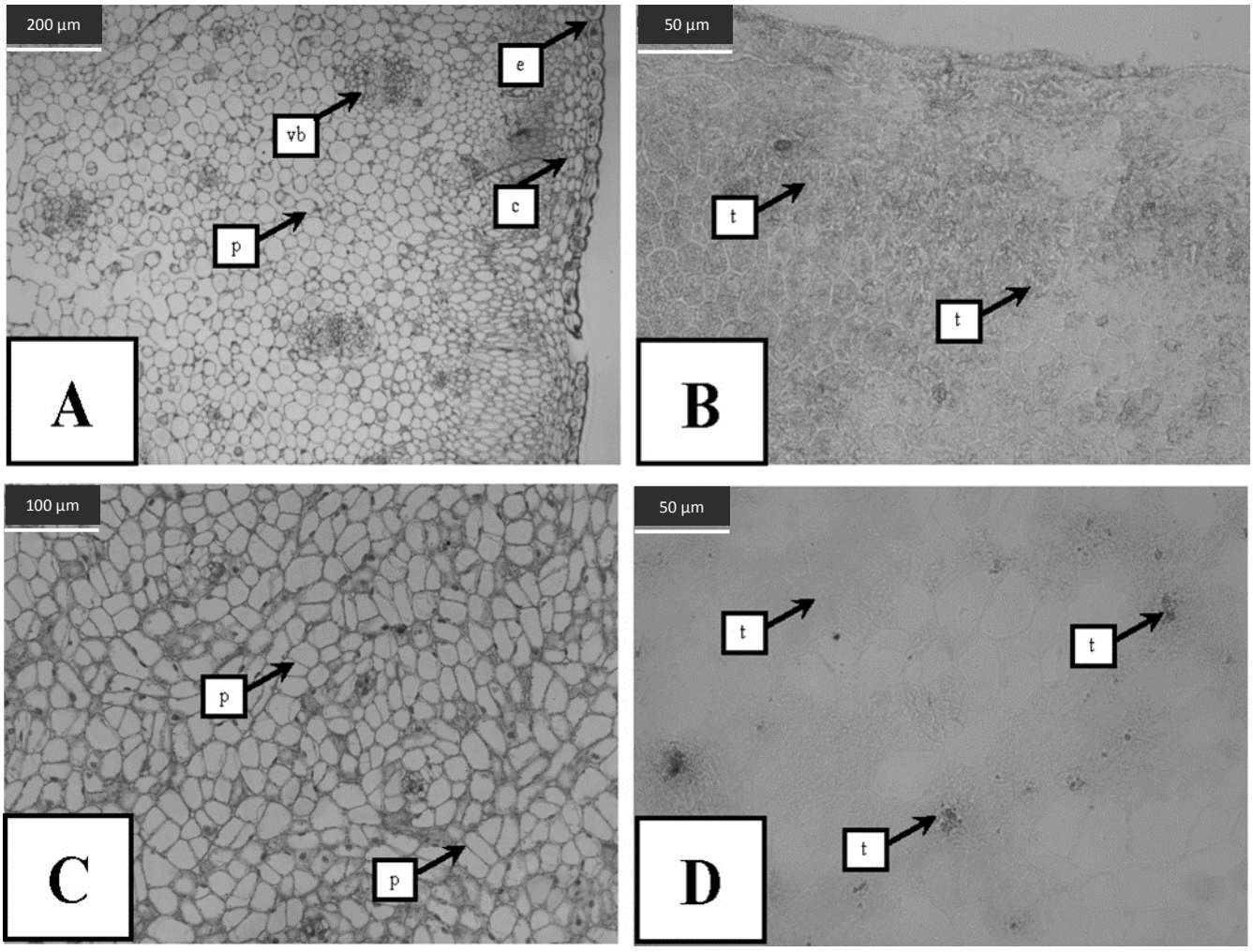

Figure 4.

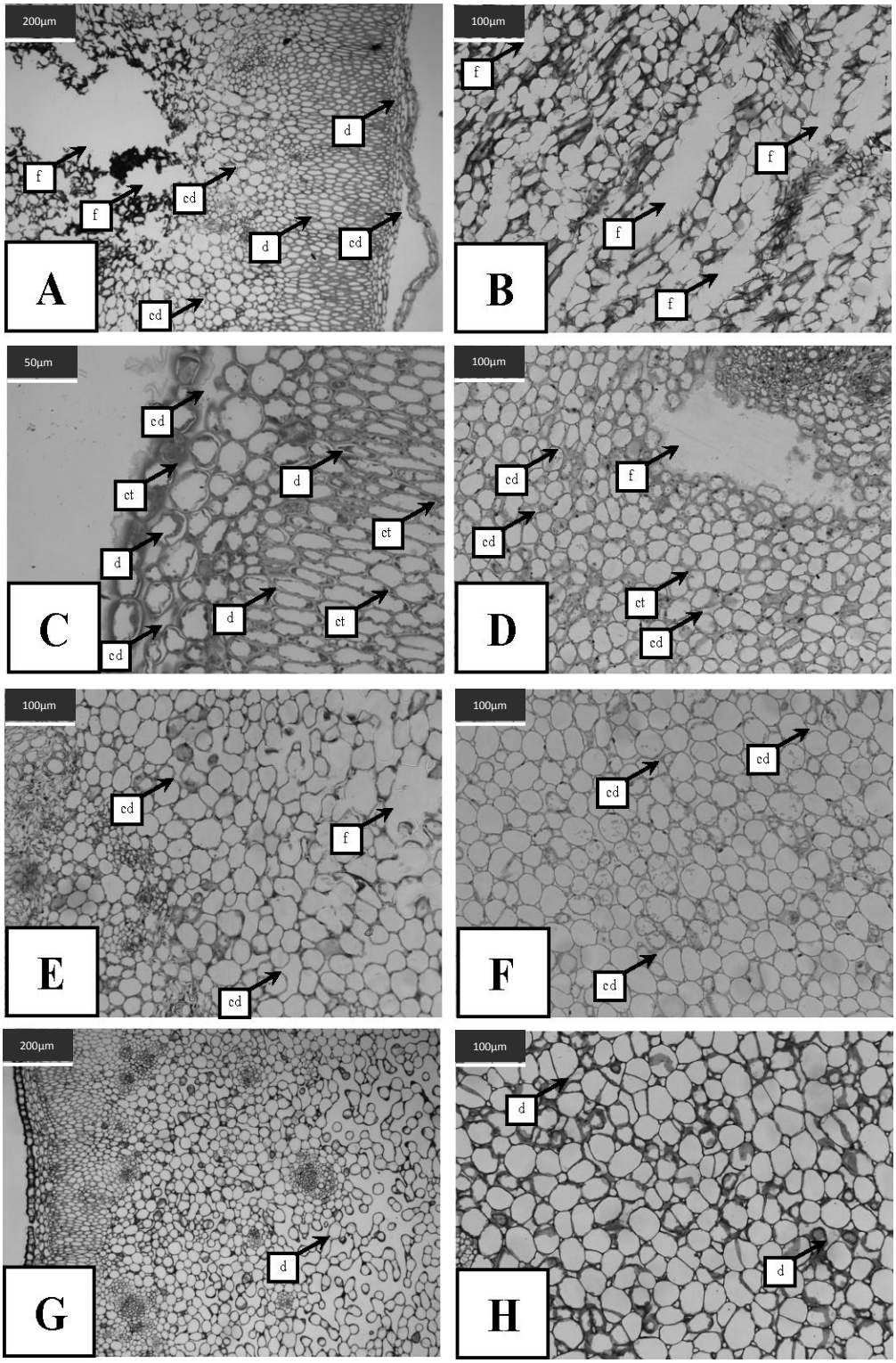
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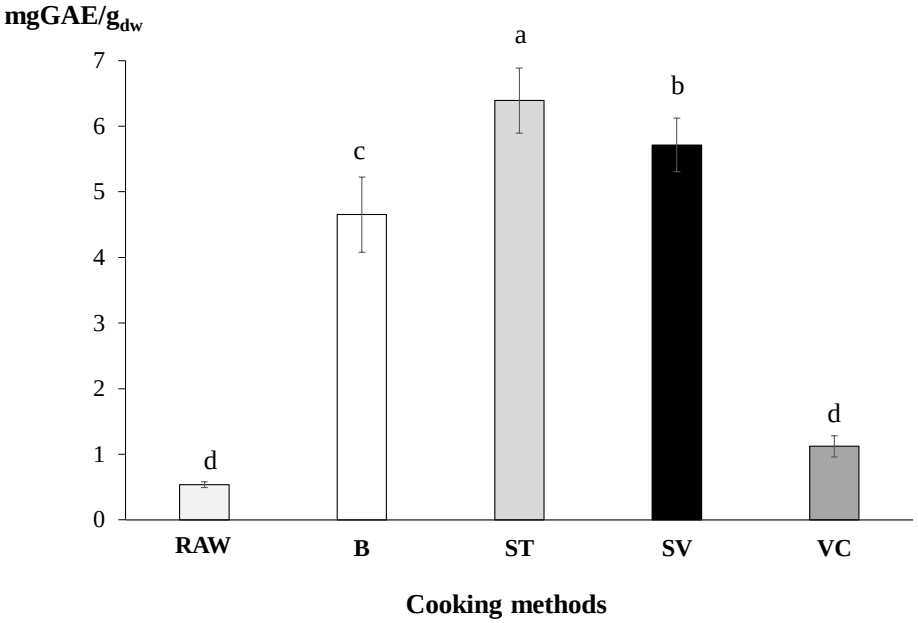
Figure 5.

A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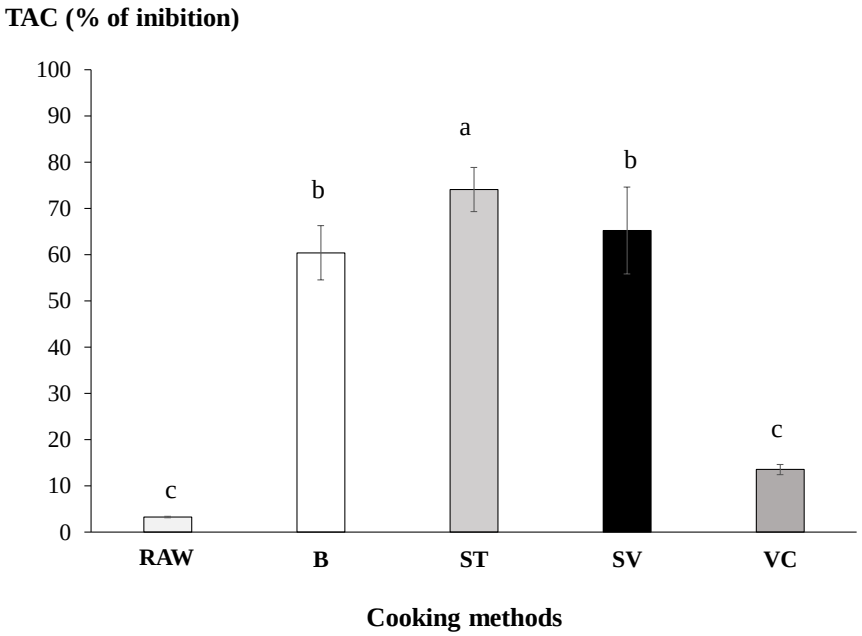
VC
Cooking methods

B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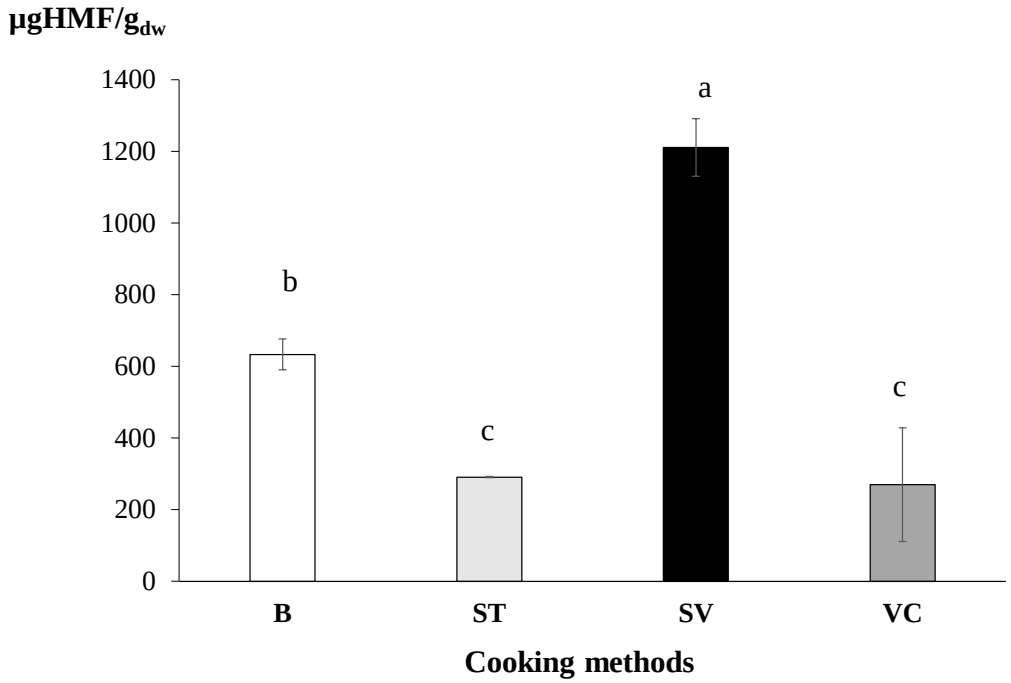
Cooking methods

Figure 6.


µgHMF/g$_{dw}$
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1200
1000
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ST
SV
VC
Cooking methods

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*Graphical Abstract

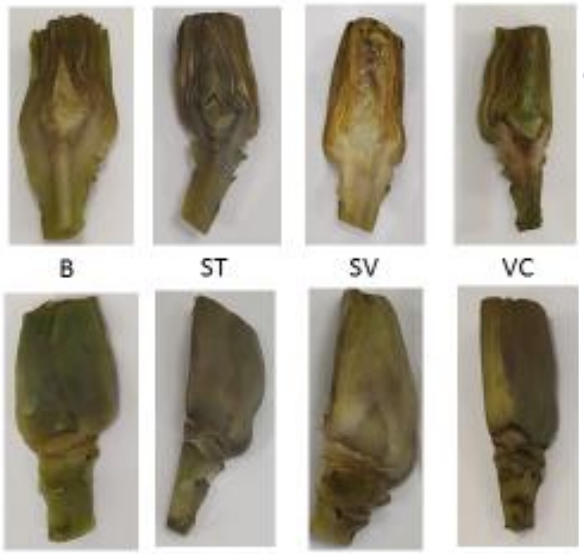
Globe artichoke Violetto

BOILING (B)

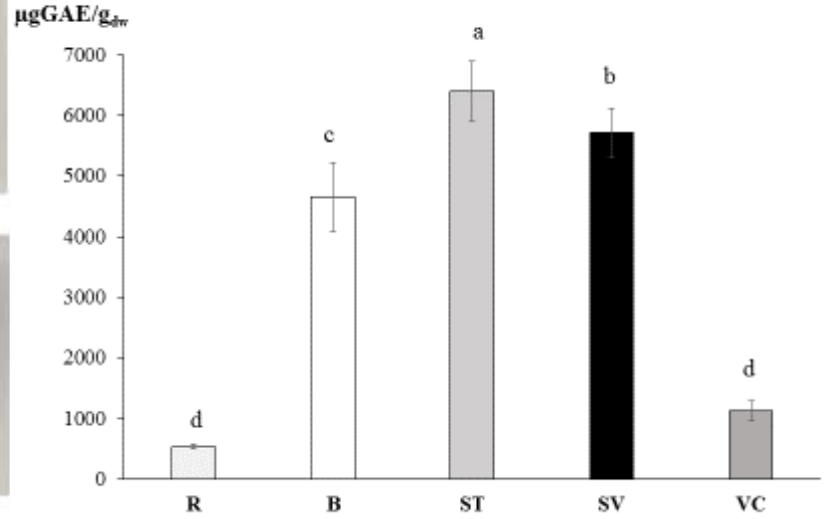
STEAMING (ST)

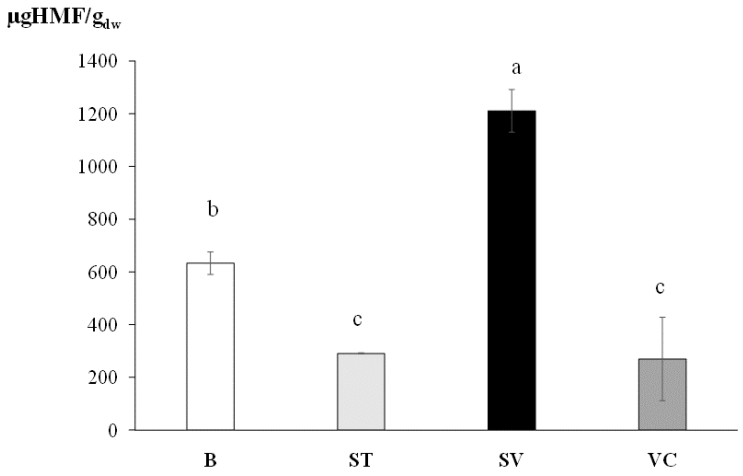
SOUS VIDE (SV)

VACUUM COOKING (VC)



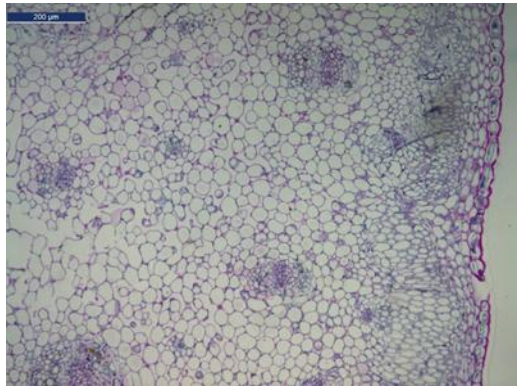
Total phenolic content





5-hydroximethylfurfural

Microstructure I



***Credit Author Statement**

Massimiliano Rinaldi: Conceptualization, Supervision; Paola Littardi: Investigation, Data Curation; Antonella Cavazza; Methodology, Writing - Original Draft; Saverio Santi: Conceptualization; Maria Grimaldi: Investigation; Margherita Rodolfi: Investigation; Tommaso Ganino: Writing - Original Draft , Conceptualization; Emma Chiavaro: Writing - Review & Editing

***Conflict of Interest form**

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: