Influence of microwave bag vs. conventional microwave cooking on phytochemicals of industrially and domestically processed broccoli

Erika Paulsen[1*], Diego A. Moreno[2,3], Paula M. Periago[3,4], Patricia Lema[1]

[1] Instituto de Ingeniería Química, Facultad de Ingeniería, Universidad de la República. Julio Herrera y Reissig 565, Montevideo, Uruguay

[2] Phytochemistry and Healthy Foods Laboratory, Department of Food Science and Technology, CEBAS-CSIC, Campus Universitario de Espinardo-Edificio 25, E-30100 Espinardo, Murcia, Spain

[3] Associated Unit of R&D and Innovation CEBAS-CSIC+UPCT on "Quality and Risk Assessment of Foods", CEBAS-CSIC, Campus Espinardo - 25, E-30100 Murcia, Spain

[4] Departamento de Ingeniería Agronómica (ETSIA), Instituto de Biotecnología Vegetal, Universidad Politécnica de Cartagena (UPCT). Paseo Alfonso XIII, 48, 30203 Cartagena, Murcia, Spain

*Corresponding author: erikap@fing.edu.uy. Fax: +59827107437

Abstract

Cooking vegetables in microwave bags is becoming a popular domestic cooking method, being relevant to know how this cooking method affects health-promoting phytochemicals of staples such as broccoli.

The aim of this work was to study the effect of microwave bag cooking versus conventional microwaving on bioactive compound content (glucosinolates and hydroxycinnamic acid derivatives) and other quality parameters (such as antioxidant capacity, mineral content and microbial load) of broccoli florets. The influence of cooking time on bioactive compounds content was also evaluated. The study was carried out in two independent experiments; using intact broccoli and broccoli preprocessed in industry.

~~Since ready-to-cook broccoli packaged in microwave bags is becoming more common than using the intact broccoli in retail markets, the effect of microwaving methods on bioactive compounds was evaluated in two independent experiments; using intact broccoli and broccoli preprocessed in industry.~~

Microwave bag cooked broccoli for 5 min (following label recommendation) showed higher glucosinolate content retention compared to conventional microwaving. Results suggest that volatilization could be an important phenomenon in reduction of glucosinolates during microwave cooking of broccoli florets. ~~Results suggest that besides thermal degradation, other hydrolyzation processes could also reduce glucosinolate content in conventionally cooked broccoli florets.~~ Glucosinolate profile did not change after cooking, regardless of cooking method applied. Furthermore, microwave bag cooked broccoli presented higher antioxidant capacity (by DPPH assay) than conventional microwaved broccoli. Hydroxycinnamic acid derivatives content was reduced in microwave cooking, regardless of ~~applied~~ method applied. Altogether, the use of microwave bags for microwaving is a novel method that retains main bioactive components of

broccoli. This option is a fast, easy and considerably clean cooking option to fulfill modern consumer needs.

Keywords: broccoli; industrial processing; domestic processing; microwave cooking, microwave bag; glucosinolates

1. Introduction

Modern consumers are increasingly aware of health benefits provided by the consumption of fruits and vegetables. However, current lifestyle limits the time available for home preparation of this type of products. These factors have driven the current rise in production and demand of minimally processed fruit and vegetables (Collado et al., 2019). In the case of vegetables that need to be cooked before consumption, ready-to-cook vegetables packaged in microwave bags have been developed. This type of products satisfies the demand of modern consumers, since they are fresh and easy to cook. However, it is well known that cooking processes affect food components. Therefore, it is important to know the effect of emerging cooking methods (such as microwave bag cooking) on bioactive compounds, in order to determine the effect on the health potential of the product.

Broccoli (*Brassica oleracea* var. *Italica*) is a vegetable highly valued by modern consumers due to its health-promoting properties. These properties are attributed to its high content of bioactive compounds, especially glucosinolates. Glucosinolates (GSL[1]) are characteristic bioactive compounds of *Brassica* vegetables. These compounds, and their breakdown products, have gained a great interest since their consumption has been related to reduction of risk of major chronic and degenerative diseases (Baenas, Marhuenda, García-Viguera, Zafrilla, & Moreno, 2019). Since broccoli is generally consumed cooked, many studies have been performed to investigate the effect of different cooking methods on its bioactive compounds including glucosinolates, flavonoids, flavonols, chlorophylls and carotenoids (Barakat & Rohn, 2014; López-Berenguer, C.; Carvajal, M.; Moreno, D.A.; García-Viguera, 2007; Pellegrini et al., 2010; Soares, Carrascosa, & Raposo, 2017; Tabart, Pincemail, Kevers, Defraigne, & Dommes, 2018). Microwave cooking has shown conflicting results regarding the effects on glucosinolate and polyphenol content of broccoli. Some works reported significant losses (Jones, Frisina, Winkler, Imsic, & Tomkins, 2010; Vallejo, F.; Tomás-Barberán, F.A.; García-Viguera, 2002; Yuan, Sun, Yuan, & Wang, 2009), while others

[1] GSL is the abbreviation of glucosinolates

showed retention or increase of these compounds (Barakat & Rohn, 2014; López-Berenguer, C.; Carvajal, M.; Moreno, D.A.; García-Viguera, 2007; Lu, Pang, & Yang, 2020; Soares et al., 2017; Wu, Zhao, Haytowitz, Chen, & Pehrsson, 2019). These different results could be explained due to numerous conditions applied in microwave cooking (time, power, product size, cooking with or without water, amount of water, etc.) (Tabart et al., 2018).

Cooking vegetables in microwave bags has become a popular trend for consumption, however, information about the effect of this emerging cooking method on nutritional parameters is limited (Zhong et al., 2017; Zhong, Dolan, & Almenar, 2015). How this method affects GSL content of broccoli has not been reported before. Therefore, one of the aims of this work was to study the effect of microwave bag cooking compared to conventional microwaving on GSL content, hydroxycinnamic acids and other quality parameters of broccoli (such as antioxidant capacity, mineral content and microbial loads).

Since ready-to-cook broccoli packaged in microwave bags is becoming more common than using the intact broccoli, the effect of microwaving methods on bioactive compounds was evaluated in two independent experiments; using intact broccoli and broccoli preprocessed in industry.

2. Material and methods

2.1.Plant material and experimental design

Two separate studies were carried out; I) using domestically processed broccoli (intact broccoli from grocery store); II) using broccoli preprocessed in industry (minimally processed broccoli from industry).

I. Microwave cooking using domestically processed broccoli

The aim of this experiment was to evaluate the effect of microwave bag cooking compared to conventional microwaving on phytochemical compounds content and microbiological quality of

broccoli florets, simulating a domestic processing. Effect of cooking time and refrigerated storage of the cooked product (2 d) on quality parameters was also evaluated.

Broccoli heads (*Brassica oleracea* var. *Italica* cv. Parthenon) were obtained in a local supermarket, transported to laboratory and processed immediately. Broccoli heads were cut into florets and washed with tap water. Approximately 200 g were placed into microwave bags and cooked in a domestic microwave oven (MW 213 INOX, TEKA) for 3 and 5 min at 800 W. Product cooked under the same conditions but without bag was used as control (conventional microwaving). After cooking, florets were packaged in polypropylene bags and stored in a refrigerator at 4 °C for 2 d in order to simulate domestic use of cooked food. All treatments were performed in triplicate. Figure 1 summarizes treatments performed and codes used to refer to each one. Broccoli samples were taken before and after each cooking treatment and after 2 d of storage of cooked product. Fresh and cooked samples were taken immediately for microbiological analysis. The rest of the samples were frozen in liquid N_2 and stored at −80 °C. Prior to analyses all samples were freeze dried.

II. Microwave cooking using broccoli preprocessed in industry

The effect of microwave bag cooking versus conventional microwaving on phytochemical content, antioxidant capacity and minerals of broccoli preprocessed in industry were studied, as well as the impact of cooking time. Preprocessed broccoli (cut into florets, sanitized and packaged) was sent from industry to the lab under refrigerated conditions. Broccoli florets (approximately 200 g) were cooked inside their packaging (microwave bags) using domestic microwave oven (LG MG3924-V, 1000V) for 3 and 5 min. Product cooked under the same conditions but without bag was used as control (conventional microwaving). Figure 1 summarizes treatments performed and codes used to refer to each one. All treatments were performed in triplicate. Broccoli samples were taken before and after cooking, frozen in liquid N_2 and stored at −80°C. Prior to analyses all samples were freeze dried.

2.2.Weight loss and product temperature

Each broccoli sample was weighted before and after being cooked. Weight loss was expressed as percentage (%) of initial weight.

Product temperature was always recorded at the same point, on the floret surface, immediately after cooking, using a thermocouple (Ahlborn, ALMEMO®, Germany).

*2.3.Glucosinolate (*GSL*) and hydroxycinnamic acids (HCAs[2]) content*

2.3.1. Sample extraction

Sample extraction was carried out according to (Baenas, Villaño, García-viguera, & Moreno, 2016) with minor modifications. Freeze-dried samples (50 mg) were extracted with 1.5 mL of methanol (70% v/v), heated at 70 °C for 30 min and agitated every 5 min in a vortex stirrer. After heating, samples were centrifuged (15000 × g, 15 min, 4 °C). Supernatants were collected and methanol was completely removed using a rotary evaporator under vacuum at 37 ◦C. Dry material obtained was re-dissolved in ultrapure water and filtered through a 0.22 μmØ Millex-HV13 filter (Millipore, Billerica, MA, USA).

2.3.2. HPLC-DAD-ESI–MSn qualitative and quantitative analysis of GSL and HCAs

Qualitative and quantitative analysis of GSL was performed according to Baenas et al., (2016). Briefly, for the identification of GSL, MS fragmentation patterns [M−H, MS2, and MS3] in HPLC-DAD-ESI-MSn (Agilent Technologies HPLC 1200, Waldbronn, Germany; coupled to an UltraHCT Bruker Ion Trap, Bremen, Germany), were analyzed. For quantitation of GSL and HCAs, chromatograms were registered at 227 and 330 nm respectively. Intact GSL and the HCAs were identified following UV spectra and order of elution according to retention times, based on the

[2] HCAs is the abbreviation of hydroxycinnamic acids

fragmentation patterns and data from previous analyses in the group under analogous conditions. GSL were quantified using sinigrin and glucobrassicin (Phytoplan, Germany) as external standards of aliphatic and indole glucosinolates, respectively. HCAs were quantified using chlorogenic and sinapinic acid as external standards. Results were expressed as μmol per gram of dry weight (μmol g^{-1}).

2.4.Antioxidant capacity (AOC[3])

2.4.1. Sample extraction

Sample extraction was carried out according to Baenas, Moreno, & García-Viguera (2012) with some modifications. Aliquots of 100 mg of freeze-dried fine powdered samples were extracted with 1.5 mL of methanol (70% v/v) for 60 min in an ultrasonic bath (8891 model, Cole-Parmer, USA) agitating every 20 min in a vortex stirrer. Extracts were stored in darkness and refrigerated for approximately 16 hours. After storage, they were sonicated for 1 hour and centrifuged at 10500 × g (3–16KL model, Sigma, Germany) during 5 min at room temperature. Supernatant was decanted for ORAC and DPPH analyses.

2.4.2. ORAC assay

ORAC assay was performed according to Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer (2002) using 96-well micro plates (Nunc, Roskilde, Denmark) and an Infinite® M200 micro plate reader (Tecan, Grödig, Austria). Analyses were conducted in phosphate buffer pH 7.4 at 37 °C. Peroxyl radical was generated using 2, 2′-azobis (2-amidino-propane) dihydrochloride. Fluorescein was used as substrate. Fluorescence conditions were as follows: excitation at 485 nm and emission at 520 nm. Standard curve was linear between 10 and 200 μM Trolox. Results were expressed as

[3] AOC is the abbreviation of antioxidant capacity

μmol Trolox Equivalents (TE[4]) per gram of dry weight (μmol g^{-1}). Samples corresponding to each treatment were analyzed in triplicate.

2.4.3. DPPH assay

The antioxidant capacity was determined using the free radical DPPH• according to Brand-Williams et al. (1995) with modifications according Mena et al. (2011). Changes in absorbance at 515 nm after 50 min of reaction were measured by using 96-well micro plates (Nunc, Roskilde, Denmark) and Infinite® M200 micro plate reader (Tecan, Grödig, Austria). All reactions started by adding 2 μL of the corresponding diluted extract to the well containing the DPPH stock solution (250 μL). The final volume of the assay was 252 μL. A Trolox (Sigma-Adrich, Germany) calibration curve was prepared for a concentration range of 0–200 μM. Results were expressed as μmol TE per gram of dry weight (μmol g^{-1}).

2.5. Microbiological analysis

Ten grams of each sample were aseptically placed into a sterile stomacher bag with 90 mL of Buffered Peptone Water (PW) (Scharlab, Barcelona) and homogenized in a Stomacher. Samples were analyzed for aerobic mesophilic bacteria, aerobic psychrotrophic bacteria and moulds and yeasts. Plate Count Agar (PCA) (Scharlab, Barcelona) was used for mesophilic and psychrotrophic bacteria analysis and incubated for 24–48 h at 30 °C and for 7 d at 5 °C, respectively. Rose Bengal Chloramphenicol Agar (RB) (Scharlab, Barcelona) was used for moulds and yeasts and incubated for 5 d at 25 °C. Results were expressed as colony-forming units per gram of fresh weight (cfu/g).

2.6.Minerals

[4] TE is the abbreviation of trolox equivalents

Concentration of boron (B) calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), phosphorus (P) and sulphur (S), were analyzed in samples (ca. 100 mg DW) of the freeze-dried and finely ground plant material. Samples were digested in a microwave oven (CEM Mars Xpress, North Carolina, USA) by HNO3 – HClO4 (2:1) acid digestion. Mineral determination was carried out using a Perkin–Elmer (Waltham, MA) 5500 model ICP emission spectrophotometer, at 589 nm, using a conductivity detector and quantifying by comparison with authentic standards (Servicio Ionómica, CEBAS-CSIC, Murcia, Spain). Mineral contents were expressed in g/100g and mg/kg DW, depending on the mineral.

2.7. Statistical analysis

Data are presented as the mean (n = 3) ± standard deviation (SD). For experiment using domestically processed broccoli, three-way ANOVA considering all effects and interactions (cooking method, cooking time and storage time) was performed. Significant differences were calculated using Tukey's test ($p < 0.05$). One-way ANOVA followed by a Dunnett's test ($\alpha = 0.05$) was performed to compare each treatment against fresh (uncooked) broccoli samples. The same statistical analysis was applied for experiment using broccoli preprocessed in industry, but two-way ANOVA (cooking method and cooking time) was applied. For all statistical analysis XLSTAT (Statistical and data analysis solution, USA) software was used.

~~A one-way ANOVA was performed for all studied variables. When the effects of treatments were significant, mean ratings were calculated. Significant differences were calculated using Tukey's test. Differences were considered significant when $p < 0.05$. For these analyses, the InfoStat (Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina) software was used.~~

3. Results and discussion

3.1. Microwave cooking using domestically processed broccoli

3.1.1. Weight loss (WL[5]) and product temperature after cooking

Significant differences were observed in WL and product temperature after cooking (Table 1). It has been reported that water loss in food during microwaving is higher than in other cooking methods (Xu, Chen, Cao, Xia, & Jiang, 2016). Dos Reis et al. (2015) evaluated steaming, boiling, microwaving and *sous vide*, reporting that microwaved broccoli presented the lowest moisture content. This was explained by the development of a pressure gradient inside food, generated due to heating characteristics of microwaving. This creates an outward flux of rapidly escaping vapor (Chandrasekaran et al., 2013). In this study, WL was higher in conventional microwaved broccoli than broccoli cooked inside microwave bag. This could be attributed to the fact that the bag works as a barrier to water vapor diffusion, increasing humidity and pressure around the vegetable. Consequently, humidity and pressure gradients decrease, reducing water loss during the process. Since water retention in vegetable matrices is an important factor in order to avoid thermal damages and preserve bioactive compounds (Chandrasekaran, Ramanathan, & Basak, 2013; Soares et al., 2017), the use of microwave bag could be a good alternative to preserve broccoli florets' health potential. Broccolis cooked for 5 min showed greater WL compared to those cooked for 3 min, in all conditions. This agrees with the fact that longer microwaving times cause a greater WL in food products.

The effect of cooking method on product temperature was different depending on cooking time. For 3 min cooking, product temperature was significantly higher in microwave bag cooking compared to conventional microwaving. In the case of 5 min, although broccoli cooked in microwave bag showed a higher temperature, the difference with conventional method was not significant. Higher product temperature observed in microwave bag cooking could be due to less evaporation (lower WL) shown by this cooking method. When water evaporation is lower, product temperature is higher than when there is greater evaporation. Conventional microwaving presented significant

[5] WL is the abbreviation of weight loss

differences in product temperature depending on cooking time: 5 min showed a higher product temperature compared to 3 min. Microwave bag cooking did not show significant differences in product temperature with cooking time. This could be attributed to the difference in WL between cooking times within each method.

3.1.2. Total glucosinolate (GSL) content

Figure 2-A shows the effect of cooking method, cooking time and 2 d of storage (cooked product) on total GSL content. Microwaved broccoli for 3 min showed no significant losses of total GSL content, regardless of cooking method. Significant differences between cooking methods were found for a cooking time of 5 min. Microwave bag cooked broccoli (MWB5) showed higher total GSL content (32.3 ± 2.6 µmol/g) than conventional microwaved broccoli (MW5) (26.4 ± 1.3 µmol/g). Therefore, use of microwave bag retained these bioactive compounds for longer cooking periods (5 min). This best preservation of GSL may be due to the reduction in evaporation losses. Several studies show that microwaving is suitable for the retention of various compounds of nutritional interest (Guo, Sun, Cheng, & Han, 2017; Soares et al., 2017; Xu, Chen, Cao, Xia, & Jiang, 2016; Tabart et al., 2018; Pellegrini et al., 2010). However, it has also been reported that the cell lysis and high evaporation rate in microwaving could cause losses of several compounds including GSL (Soares et al., 2017). Therefore, the lower evaporation rate (less WL) observed in microwave bag cooking could explain the greater retention of GSL.

According to the results, conventional microwaving for 5 min (MW5) significantly reduced total GSL content, so it should be avoided. Microwave bag cooking (for 3 and 5 min) and conventional microwaving for 3 min (MW3) would be recommended in order to retain total GSL content.

GSL content in cooked florets showed no variation after 2 d of storage under refrigerated conditions compared to freshly cooked product, except for MWB5 where GSL content dropped during storage

(from 32.7 ± 3.1 to 25.1 ± 1.0 µmol/g). Therefore, reduction of GSL content of cooked broccoli during storage appears as an aspect to take into account in future works.

3.1.3. Individual glucosinolate (GSL) content

Molecular ion [M−H] − (m/z) of GSL, their fragmentation ion patterns, and their retention times allowed identification of seven different compounds in all samples analyzed. Mass spectral information of intact GSL identified (Table S1), individual GSL content of all samples analyzed (Table S2) and an example of HPLC-DAD chromatogram obtained in the analysis of fresh broccoli (Figure S1) are shown in Supplementary Material.

GSL profiles did not shown differences between fresh and cooked samples. This is an interesting result, since it implies that microwaving did not significantly affect the profile of these bioactive compounds, regardless of the time and method applied. A greater loss of indolic GSL compared to aliphatic ones has been described for cooking methods such as boiling, steaming and stir-frying (Soares et al., 2017; Yuan et al., 2009). However, it has been reported that this behavior is not observed in microwaving (Yuan et al., 2009). Studies with different varieties of *Brassica* have reported that the relative distribution of GSL did not change after cooking *(*Francisco, Velasco, Moreno, García-Viguera, & Cartea, 2010; Pellegrini et al., 2010). This is in agreement with results obtained in the present study. Individual GSL content either remained constant or decreased depending on the cooking method and time, but no specific behavior pattern was found.

3.1.4. Hydroxycinnamic acids (HCAs)

In recent years, study of HCAs in foods has been increasing due to their potential beneficial properties for human health. Although they have not been extensively studied, there are some reports where antidiabetic effects and inhibitory activity against breast and hematologic cancers are

attributed, in addition to their antioxidant properties (Ruiz et al., 2018). Therefore, knowing how cooking process affects this type of compounds is of interest.

The main HCAs identified in the samples were synaptic acids and their derivatives. HACs content declined by 40% (on average) compared to fresh broccoli, in all conditions (Figure 3-A). HACs content remained unchanged after 2 d of refrigerated storage. HACs are within the group of phenolic compounds, whose low stability at high temperatures is well known. For that reason, losses could be attributed to thermal degradation. Conditions used in this study presented a higher retention of HACs level compared to other works. Vallejo, Tomás-Barberán, & García-Viguera (2003) cooked broccoli florets in microwave (1000 W, 5 min) obtaining HACs decrease of 74%. They used water for cooking, thus HACs losses were attributed to leaching phenomena. Pellegrini et al. (2010) microwaved broccoli florets (300 W, 30 min) without using water, reporting significant HCAs losses compared to fresh broccoli (72%). This greater loss of HACs was explained by the excessive cooking time applied (Guo et al., 2017). Thermal degradation seems to be the main factor that affects HCAs stability, since despite not using water for cooking (leaching effect reduced) the losses are significant.

3.1.5. Microbial analysis

The effect of cooking method, cooking time and 2 d of refrigerated storage on microbiological quality was evaluated (Figure 4).

Prior to cooking, broccoli florets had a yeast and mold count of 5.2 x 10^2 cfu/g. After cooking, the counts were <10^2 cfu/g, independently of cooking method and time applied. After 2 d of storage no significant increase in mold and yeast counts was observed.

Mesophilic bacteria counts showed significant differences between cooking time and method applied. Longer cooking times presented lower counts. Microwave bag cooking showed higher

reduction of mesophilic bacteria than conventional microwaving. This may be due to the higher product temperatures reached with microwave bag cooking. Mesophilic bacteria count did not increase in cooked product after 2 d of storage.

For 3 min cooking, there were not significant differences in psychrophilic bacteria counts between cooking method (MW3 and MWB3). However, broccoli cooked in microwave bag (MWB5) showed lower psychrophilic bacteria counts compared to conventionally microwaved broccoli for 5 min cooking (MW5). Two days storage did not show psychrophilic bacteria growth, except for MW5 samples.

To conclude, microwave bag cooking produces a greater reduction of microbial load in broccoli florets. This could be due to the fact that product reaches higher temperatures in microwave bag cooking. Longer cooking times showed a greater reduction, this could be related to higher product temperatures and longer process time. Overall, no microbial growth was observed after 2 d of refrigerated storage.

3.2. Microwave cooking using broccoli preprocessed in industry

3.2.1. Weight loss (WL) and product temperature after cooking

Results obtained using broccoli preprocessed in industry were in agreement with those obtained using domestically processed broccoli (Table 1). Cooking times of 5 min showed higher WL compared to cooking for 3 min (in all conditions). Broccoli cooked in microwave bags showed lower WL than conventional microwaved broccoli, for both 3 and 5 min. Thus, this second experiment confirms that microwave bag cooking reduced WL of broccoli florets.

As observed in domestically processed broccoli, product temperature after cooking was significantly higher in microwave bag cooking compared to conventional microwaving. Again, it was observed that cooking time had a significant effect in product temperature after conventional

microwaving. This was not so in the microwave bag method, where the product temperature reached the same value regardless of cooking time.

3.2.2. Total glucosinolate (GSL) content

Statistical differences in total GSL content were found according to cooking method and time applied (Figure 2-B). Conventionally microwaved broccoli for 3 min (IMW3) kept total GSL content, while same cooking method for 5 min (IMW5) showed a significant GSL loss compared to uncooked broccoli. Microwave bag cooking kept total GSL content compared to uncooked product, and no significant differences were observed between 3 and 5 min cooking. These results are in agreement with those obtained in domestically processed broccoli. It could be concluded that microwave bag cooking reduces total GSL loss due to a reduction on evaporation. Another result to highlight is that volatilization losses of total GSL predominated over thermal degradation losses, since broccoli cooked in microwave bag presented higher product temperature but also higher retention of GSL than conventional microwaved broccoli. Thus, volatilization phenomenon could be the predominant cause of losses of total GSL during microwaving. Several studies reported that the main causes of GSL loss during cooking process are: leaching, enzymatic and thermal degradation, but there is little mention of the volatilization phenomenon (Armesto, Gómez-Limia, Carballo, & Martínez, 2019; Campos et al., 2019; Guo et al., 2017; Pellegrini et al., 2010; Tabart et al., 2018; Vallejo, F.; Tomás-Barberán, F.A.; García-Viguera, 2002; Wu et al., 2019; Zhao et al., 2019). As a new contribution, this work proved that the use of a microwave bag can further reduce losses of total GSL compared to conventional microwaving, probably due to evaporation reduction.

It is important to consider the effect of cooking on structure of plant tissue, which can affect the extraction of compounds. Zhong et al. (2015) reported that microwave bag cooking can soften broccoli faster compared to traditional microwaving. Therefore, it is necessary to conduct further

studies in order to determine the effect of possible interactions between water loss and tissue softening on total GSL content during cooking.

3.2.3. Individual glucosinolate (GSL) content

Results were similar to those obtained using domestically processed broccoli (first experiment). GSL profile did not shown differences between fresh and cooked samples, regardless of cooking method and time applied. Individual GSL content is shown in Table S2 within Supplementary Material.

3.2.4. Hydroxycinnamic acids (HCAs)

Results obtained using broccoli preprocessed in industry were slightly different to those obtained using domestically processed broccoli. Results are shown in Figure 3-B. For 3 min cooking, conventionally microwaved broccoli kept HCAs content, while broccoli cooked in bag showed a decreased of these compounds compared to fresh broccoli. A cooking time of 5 min significantly reduced HCAs content, regardless of cooking method applied. Losses were 50% compared to fresh broccoli. It is interesting to note that intact broccoli from the first experiment (using domestically processed broccoli) had significantly higher HACs content than broccoli preprocessed in industry ($p = 0.0006$). HCAs losses with respect to uncooked broccoli were similar in both experiments.

HCAs losses were observed in samples that reached higher temperatures after cooking (IMW5, IMWB3 and IMWB5), so it could be attributed to thermal degradation. Therefore, microwave bag cooking increases HCAs losses compared to conventional microwaving, because cooking in a bag increases the product temperature. Low stability of phenols at high temperatures has been reported, where degradation or transformation has been indicated as phenomena responsible for HCAs losses (Ruiz et al., 2018).

3.2.5. Antioxidant capacity (AOC)

Data obtained from ORAC assay showed no significant differences between cooking methods and times applied. AOC remained at (92.0 ± 13.0) μmol g^{-1} (on average) in both broccoli uncooked and cooked under different conditions. These results are in agreement with other studies where microwaved broccoli florets did not show losses in AOC (dos Reis et al., 2015; Zhong et al., 2015). Zhong et al. (2015) found that AOC of broccoli florets cooked by conventional and bag microwaving are preserved or increased, respectively (AOC measured by ORAC assay). These results are explained by a balance between compound losses due to heating process and AOC increase due to release of antioxidant compounds from the vegetable matrix and formation of new antioxidant compounds (Zhong et al., 2015).

Significant differences in AOC between cooking methods were found by DPPH assay (Figure 5). In conventionally cooked broccoli, AOC was reduced by 41% and 75% for 3 and 5 min cooking respectively. In broccoli cooked in bag, AOC declined by 50%, regardless of cooking time applied. Therefore, cooking in microwave bags allowed retaining AOC to a greater extent than conventional microwaving for cooking times of 5 min. These results are in agreement with Zhong et al., (2015), who reported that broccoli cooked in microwave bag presented higher AOC compared to traditional microwaving. However, our findings show a significant decrease in AOC during cooking, while an increase was observed by Zhong et al. (2015). Our results are in agreement with those reported by Pellegrini et al. (2010), who concluded that microwaving had a detrimental effect in AOC. Discrepancies between studies could be due to various factors such as assay used, extraction efficiency, cooking conditions, cutting size and vegetable physical structure. AOC loss after cooking was mainly attributed to cell lysis diffusion and thermal degradation of compounds with AOC (Soares et al., 2017). The best retention of AOC in microwave bag cooking (for cooking time of 5 min) could be linked to retention of compounds by reducing the evaporation rate. However,

other studies should be carried out to identify what is the effect of microwave bag on these types of compounds.

Different conclusions were obtained depending on AOC determination method applied. This is mainly due to the diversity of compounds that contribute to the AOC and the complexity of this type of food matrix (Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002; Floegel, Kim, Chung, Koo, & Chun, 2011). Our work confirms the idea that AOC of complex matrices such as vegetables should be analyzed by more than one method, and the interpretation of results should be done with caution.

3.2.6. Minerals

No significant differences were found in mineral content regardless of cooking methods and time applied. Mineral content of samples is shown in Supplementary Material (Table S3). Contradictory results are found in literature regarding changes in mineral content of vegetables during microwaving. On one hand, some studies have reported mineral loss after microwaving different vegetable matrices (Ali, 2015; Maria et al., 2019). On the other hand, López-Berenguer et al. (2007) reported a high mineral retention in microwaved broccoli under different conditions. In the present study, it was found that mineral content of broccoli was stable, which agrees with the report by Lopez-Berenguer et al. (2007). Not adding water for microwaving could be the reason why the mineral content remained unchanged (Maria et al., 2019).

4. Conclusions

This is the first study to report the effect of microwave bag cooking on main bioactive compounds of broccoli florets. Microwave bag cooking allowed to preserve health potential of broccoli florets, for a cooking time of 5 min. Broccoli florets cooked in bag kept total glucosinolate content and

antioxidant capacity (determined by DPPH assay) compared to conventionally microwaved broccoli. These results suggest that volatilization of glucosinolates predominate over thermal degradation. Glucosinolate profile remained unchanged after microwaving. Hydroxycinnamic acids content was reduced during cooking, regardless of cooking method and time applied. Thermal degradation seems to be the main phenomenon in the loss of these compounds.

Results obtained using domestically and industrially processed broccoli were in agreement.

In conclusion, this study shows that microwave bag cooking can preserve potential health benefit of broccoli florets, being a fast and easy cooking method, which fulfills modern consumer needs.

Conducting further studies in order to determine a possible effect of interaction of weight loss and texture on GSL content should be considered. The effect of microwave bag cooking on sensory attributes and harmful compounds content should also be considered in future studies.

Acknowledgements

This work was supported by Comisión Sectorial de Investigación Científica, Universidad de la República. Authors are indebted to Agencia Nacional de Innovación e Investigación for granting Erika Paulsen a PhD scholarship and an internship at Phytochemistry and Healthy Foods Laboratory of the Department of Food Science and Technology at CEBAS-CSIC (scholarship reference: POS_EMHE_2018_1_1007740).

Samples of broccoli florets from industry were a generous gift from Verdisfresh - Grupo Alimentario Citrus (GAC) (Valencia, Spain). We would like to thank Ms. Elena Bahon for the logistics and collaboration in the preparation and supply of samples.

Authors would like to express their gratitude also to Paula García-Ibañez, Ph.D student who helped in the experimental tasks and preparation of samples for analysis.

References

Ali, A. M. (2015). Effect of Food Processing Methods on the Bioactive Compound of Cauliflower, *93*(1), 117–131.

Armesto, J., Gómez-Limia, L., Carballo, J., & Martínez, S. (2019). Effects of different cooking methods on the antioxidant capacity and flavonoid, organic acid and mineral contents of Galega Kale (Brassica oleracea var. acephala cv. Galega). *International Journal of Food Sciences and Nutrition*, *70*(2), 136–149. http://doi.org/10.1080/09637486.2018.1482530

Baenas, N., Marhuenda, J., García-Viguera, C., Zafrilla, P., & Moreno, D. A. (2019). Influence of cooking methods on glucosinolates and isothiocyanates content in novel cruciferous foods. *Foods*, *8*(7), 1–9. http://doi.org/10.3390/foods8070257

Baenas, N., Moreno, D. A., & Garc, C. (2012). Selecting Sprouts of Brassicaceae for Optimum Phytochemical.

Baenas, N., Villaño, D., García-viguera, C., & Moreno, D. A. (2016). Optimizing elicitation and seed priming to enrich broccoli and radish sprouts in glucosinolates. *FOOD CHEMISTRY*, *204*, 314–319. http://doi.org/10.1016/j.foodchem.2016.02.144

Barakat, H., & Rohn, S. (2014). Effect of different cooking methods on bioactive compounds in vegetarian, broccoli-based bars. *Journal of Functional Foods*, *11*(C), 407–416. http://doi.org/10.1016/j.jff.2014.10.009

Campos, D., Aguilar-Galvez, A., García-Ríos, D., Chirinos, R., Limaymanta, E., & Pedreschi, R. (2019). Postharvest storage and cooking techniques affect the stability of glucosinolates and myrosinase activity of Andean mashua tubers (Tropaeolum tuberosum). *International Journal of Food Science and Technology*, *54*(7), 2387–2395. http://doi.org/10.1111/ijfs.14150

Chandrasekaran, S., Ramanathan, S., & Basak, T. (2013). Microwave food processing-A review. *Food Research International*, *52*(1), 243–261. http://doi.org/10.1016/j.foodres.2013.02.033

Collado, E., Venzke Klug, T., Martínez-Hernández, G. B., Artés-Hernández, F., Martínez-Sánchez, A., Aguayo, E., … Gómez, P. A. (2019). Nutritional and quality changes of minimally processed faba (Vicia faba L.) beans during storage: Effects of domestic microwaving. *Postharvest Biology and Technology*, *151*(January), 10–18. http://doi.org/10.1016/j.postharvbio.2019.01.008

dos Reis, L. C. R., de Oliveira, V. R., Hagen, M. E. K., Jablonski, A., FlÔres, S. H., & de Oliveira Rios, A. (2015). Carotenoids, flavonoids, chlorophylls, phenolic compounds and antioxidant activity in fresh and cooked broccoli (Brassica oleracea var. Avenger) and cauliflower (Brassica oleracea var. Alphina F1). *LWT - Food Science and Technology*, *63*(1), 177–183. http://doi.org/10.1016/j.lwt.2015.03.089

Floegel, A., Kim, D., Chung, S., Koo, S. I., & Chun, O. K. (2011). Journal of Food Composition and Analysis Comparison of ABTS / DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods §. *Journal of Food Composition and Analysis*, *24*(7), 1043–1048. http://doi.org/10.1016/j.jfca.2011.01.008

Francisco, M., Velasco, P., Moreno, D. A., García-Viguera, C., & Cartea, M. E. (2010). Cooking methods of Brassica rapa affect the preservation of glucosinolates, phenolics and vitamin C. *Food Research International*, *43*(5), 1455–1463. http://doi.org/10.1016/j.foodres.2010.04.024

Guo, Q., Sun, D. W., Cheng, J. H., & Han, Z. (2017). Microwave processing techniques and their recent applications in the food industry. *Trends in Food Science and Technology*, *67*, 236–247. http://doi.org/10.1016/j.tifs.2017.07.007

Jones, R. B., Frisina, C. L., Winkler, S., Imsic, M., & Tomkins, R. B. (2010). Cooking method significantly effects glucosinolate content and sulforaphane production in broccoli florets. *Food Chemistry*, *123*(2), 237–242. http://doi.org/10.1016/j.foodchem.2010.04.016

López-Berenguer, C.; Carvajal, M.; Moreno, D.A.; García-Viguera, C. (2007). Effects of

Microwave Cooking Conditions on Bioactive Compounds Present in Broccoli Inflorescences. *J. Agric. Food Chem.*, *55*, 10001–10007.

Lu, Y., Pang, X., & Yang, T. (2020). Microwave cooking increases sulforaphane level in broccoli. *Food Sci Nutr.*, *8*(February), 2052–2058. http://doi.org/10.1002/fsn3.1493

Maria, A., Lima, S., Oliveira, L., David, J. M., Luis, S., & Ferreira, C. (2019). Mineral content in mustard leaves according to the cooking method. *Food Chemistry*, *273*(December 2017), 172–177. http://doi.org/10.1016/j.foodchem.2017.12.042

Mena, P., García-Viguera, C., Navarro-Rico, J., Moreno, D. A., Bartual, J., Saura, D., & Martí, N. (2011). Phytochemical characterisation for industrial use of pomegranate (Punica granatum L.) cultivars grown in Spain. *Journal of the Science of Food and Agriculture*, *91*(10), 1893–1906. http://doi.org/10.1002/jsfa.4411

Ou, B., Huang, D., Hampsch-Woodill, M., Flanagan, J. A., & Deemer, E. K. (2002). Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: A comparative study. *Journal of Agricultural and Food Chemistry*, *50*(11), 3122–3128. http://doi.org/10.1021/jf0116606

Pellegrini, N., Chiavaro, E., Gardana, C., Mazzeo, T., Contino, D., Gallo, M., … Porrini, M. (2010). Effect of different cooking methods on color, phytochemical concentration, and antioxidant capacity of raw and frozen brassica vegetables. *Journal of Agricultural and Food Chemistry*, *58*(7), 4310–4321. http://doi.org/10.1021/jf904306r

Ruiz, A., Aguilera, A., Ercoli, S., Parada, J., Winterhalter, P., Contreras, B., & Cornejo, P. (2018). Effect of the frying process on the composition of hydroxycinnamic acid derivatives and antioxidant activity in flesh colored potatoes. *Food Chemistry*, *268*(March), 577–584. http://doi.org/10.1016/j.foodchem.2018.06.116

Rybarczyk-plonska, A., Fiskaa, S., Iren, G., Borge, A., Bengtsson, G. B., Kåre, M., & Wold, A.

(2016). Postharvest Biology and Technology Glucosinolates in broccoli (Brassica oleracea L . var . italica) as affected by postharvest temperature and radiation treatments. *Postharvest Biology and Technology*, *116*, 16–25. http://doi.org/10.1016/j.postharvbio.2015.12.010

Soares, A., Carrascosa, C., & Raposo, A. (2017). Influence of Different Cooking Methods on the Concentration of Glucosinolates and Vitamin C in Broccoli. *Food and Bioprocess Technology*, *10*(8), 1387–1411. http://doi.org/10.1007/s11947-017-1930-3

Tabart, J., Pincemail, J., Kevers, C., Defraigne, J. O., & Dommes, J. (2018). Processing effects on antioxidant, glucosinolate, and sulforaphane contents in broccoli and red cabbage. *European Food Research and Technology*, *244*(12), 2085–2094. http://doi.org/10.1007/s00217-018-3126-0

Vallejo, F.; Tomás-Barberán, F.A.; García-Viguera, C. (2002). Glucosinolates and vitamin C content in edible parts of broccoli florets after domestic cooking. *Eur. Food Res. Technol.*, *215*, 310–316. http://doi.org/10.1007/s00217-002-0560-8

Vallejo, F., Tomás-Barberán, F. A., & García-Viguera, C. (2003). Phenolic compound contents in edible parts of broccoli inflorescences after domestic cooking. *Journal of the Science of Food and Agriculture*, *83*(14), 1511–1516. http://doi.org/10.1002/jsfa.1585

Wu, X., Zhao, Y., Haytowitz, D. B., Chen, P., & Pehrsson, P. R. (2019). Effects of domestic cooking on flavonoids in broccoli and calculation of retention factors. *Helyon*, *5*(January). http://doi.org/10.1016/j.heliyon.2019.e01310

Xu, Y., Chen, Y., Cao, Y., Xia, W., & Jiang, Q. (2016). Application of simultaneous combination of microwave and steam cooking to improve nutritional quality of cooked purple sweet potatoes and saving time. *Innovative Food Science and Emerging Technologies*, *36*, 303–310. http://doi.org/10.1016/j.ifset.2016.07.014

Yuan, G. F., Sun, B., Yuan, J., & Wang, Q. M. (2009). Effects of different cooking methods on

health-promoting compounds of broccoli. *Journal of Zhejiang University: Science B*, *10*(8), 580–588. http://doi.org/10.1631/jzus.B0920051

Zhao, C., Liu, Y., Lai, S., Cao, H., Guan, Y., San Cheang, W., … Xiao, J. (2019). Effects of domestic cooking process on the chemical and biological properties of dietary phytochemicals. *Trends in Food Science and Technology*, *85*(January), 55–66. http://doi.org/10.1016/j.tifs.2019.01.004

Zhong, X., Dolan, K. D., & Almenar, E. (2015). Effect of steamable bag microwaving versus traditional cooking methods on nutritional preservation and physical properties of frozen vegetables: A case study on broccoli (Brassica oleracea). *Innovative Food Science and Emerging Technologies*, *31*, 116–122. http://doi.org/10.1016/j.ifset.2015.07.002

Zhong, X., Siddiq, M., Sogi, D. S., Harte, B., Dolan, K. D., & Almenar, E. (2017). Effect of microwave steamable bag design on the preservation of ascorbic acid and antioxidant capacity and on the physical properties of cooked frozen vegetables: A case study on broccoli (Brassica oleracea). *LWT - Food Science and Technology*, *83*, 165–171. http://doi.org/10.1016/j.lwt.2017.05.018

Tables

Table 1. Weight loss (WL) and broccoli temperature after cooking for each treatment performed. Data are expressed as means ± SD (n = 3). Different letters within the same column indicate significant differences according to Tukey's test ($p < 0.05$).

I. Microwave cooking using domestic processed broccoli				
Sample code	WL (%)		Product temp (°C)	
MW3	21.1 ± 1.3	b	87.5 ± 3.5	b
MWB3	8.3 ± 0.3	d	96.0 ± 1.4	a
MW5	28.2 ± 0.1	a	91.0 ± 1.4	ab
MWB5	14.1 ± 0.3	c	97.0 ± 0.0	a
II. Microwave cooking using broccoli processed in industry				
Sample code	WL (%)		Product temp (°C)	
IMW3	20.9 ± 0.0	b	87.0 ± 0.0	b
IMWB3	4.1 ± 1.1	d	96.0 ± 0.0	a
IMW5	33.5 ± 0.0	a	92.0 ± 0.0	ab
IMWB5	12.2 ± 0.0	c	96.0 ± 0.0	a

~~I. Microwave cooking using domestically processed broccoli~~				
~~Sample code~~	~~WL (%)~~		~~Product temp (°C)~~	
~~MW3~~	~~21.1 ± 1.3~~	~~b~~	~~87.5 ± 3.5~~	~~b~~
~~MWB3~~	~~8.3 ± 0.3~~	~~d~~	~~96.0 ± 1.4~~	~~a~~
~~MW5~~	~~28.2 ± 0.1~~	~~a~~	~~91.0 ± 1.4~~	~~ab~~
~~MWB5~~	~~14.1 ± 0.3~~	~~c~~	~~97.0 ± 0.0~~	~~a~~
~~II. Microwave cooking using broccoli preprocessed in industry~~				
~~Sample code~~	~~WL (%)~~		~~Product temp (°C)~~	
~~IMW3~~	~~20.9 ± 0.0~~	~~c~~	~~87.0 ± 0.0~~	~~b~~
~~IMW5~~	~~33.5 ± 0.0~~	~~d~~	~~92.0 ± 0.0~~	~~ab~~
~~IMWB3~~	~~4.1 ± 1.1~~	~~a~~	~~96.0 ± 0.0~~	~~a~~
~~IMWB5~~	~~12.2 ± 0.0~~	~~b~~	~~96.0 ± 0.0~~	~~a~~

Figure Captions

Fig. 1. Diagram of experimental design. Letters between parentheses are the codes used to refer to each treatment.

Fig. 2. Total GSL content (µmol g^{-1}). A) Domestically processed broccoli florets: uncooked (F), microwaved broccoli under different conditions (MW3: conventional microwaving for 3 min, MW5: conventional microwaving for 5 min, MWB3: microwave bag cooking for 3 min, MWB5: microwave bag cooking for 5 min) and microwaved broccoli after 2 d of refrigerated storage (suffix "-2d"). B) Broccoli preprocessed in industry: uncooked (IF), conventional microwaving for 3 (IMW3) and 5 min (IMW5) and microwave bag cooking for 3 (IMWB3) and 5 min (IMWB5). Data are expressed as means ± SD (n = 3). Different letters indicate significant differences between treatments (Tukey's test, $p < 0.05$). "*" indicates significant difference between treatment and uncooked samples (Dunnett's test, $\alpha = 0.05$).

Fig. 3. HCAs content (µmol g^{-1}). A) Domestically processed broccoli florets: uncooked (F), microwaved broccoli under different conditions (MW3: conventional microwaving for 3 min, MW5: conventional microwaving for 5 min, MWB3: microwave bag cooking for 3 min, MWB5: microwave bag cooking for 5 min) and microwaved broccoli after 2 d of refrigerated storage (suffix "-2d"). B) Broccoli preprocessed in industry: uncooked (IF), conventional microwaving for 3 (IMW3) and 5 min (IMW5) and microwave bag cooking for 3 (IMWB3) and 5 min (IMWB5). Data are expressed as means ± SD (n = 3). Different letters indicate significant differences between treatments (Tukey's test, $p < 0.05$). "*" indicates significant difference between treatment and uncooked samples (Dunnett's test, $\alpha = 0.05$).

Fig. 4. Mean value of log CFU/g for aerobic mesophilic bacteria and aerobic psychrophilic bacteria in domestically processed broccoli: uncooked (F), microwaved broccoli under different conditions (MW3: conventional microwaving for 3 min, MW5: conventional microwaving for 5 min, MWB3: microwave bag cooking for 3 min, MWB5: microwave bag cooking for 5 min) and microwaved broccoli after 2 d of refrigerated storage (suffix "-2d"). Vertical bars represent standard deviation (n = 3) and different letters indicate significant differences according to Tukey's test ($p < 0.05$). "*" indicates significant difference between treatment and uncooked samples (Dunnett's test, $\alpha = 0.05$).

Fig. 5. Antioxidant capacity (µmol TE g^{-1}) measured by DPPH method of broccoli preprocessed in industry: uncooked (FI), conventional microwaving for 3 (IMW3) and 5 min (IMW5) and microwave bag cooking for 3 (IMWB3) and 5 min (IMWB5). Data are expressed as means ± SD (n = 3). Different letters indicate significant differences between treatments (Tukey's test, $p < 0.05$). "*" indicates significant difference between treatment and uncooked samples (Dunnett's test, $\alpha = 0.05$).

Supplementary material

Tables

Table S1. Intact GSL detected in broccoli florets (*Brassica oleracea* var. *Italica* cv. Parthenon) in ESI negative mode. Rt=retention time.

Code	Glucosinolate	Semisystematic name	Class	Rt (min)	[M-H]- (m/z)	MS2 and MS3
GIB	Glucoiberin	3-methylsulfinylpropyl-gsl	aliphatic	4.4	422	259 and 97
GRA	Glucoraphanin	4-methylsulfinylbutyl-gsl	aliphatic	5.2	436	
HGB	4-Hydroxyglucobrassicin	4-hydroxy-3-indolylmethyl-gsl	indolic	8.1	463	
GB	Glucobrassicin	3-indolylmethyl-gsl	indolic	16.6	447	
GST	Gluconasturtin	2-phenylethyl-gsl	aromatic	19.5	422	
MGB	4-Methoxyglucobrassicin	4-methoxy-3-indolylmethyl-gsl	indolic	19.8	477	
NGB	Neoglucobrassicin	N-methoxy-3-indolylmethyl-gsl	indolic	21.1	477	447, 259 and 97

Table S2. Individual GSL content (μmol g^{-1}) of fresh broccoli and broccoli microwaved under different conditions. Data are expressed as means ± SD (n=3).

Sample code	GIB	GRA	HGB	GB	GST	MGB	NGB
F	11.70 ± 0.62	2.97 ± 0.51	2.22 ± 0.12	5.27 ± 0.32	1.94 ± 0.12	1.11 ± 0.13	6.78 ± 0.91
MW3	10.14 ± 1.75	3.22 ± 0.35	2.18 ± 0.17	4.98 ± 0.60	2.04 ± 0.16	1.10 ± 0.09	5.33 ± 0.63
MW5	7.39 ± 0.81	2.94 ± 0.05	1.92 ± 0.11	4.57 ± 0.23	1.79 ± 0.09	1.02 ± 0.04	6.80 ± 0.31
MWB3	7.90 ± 0.70	3.31 ± 0.21	2.26 ± 0.13	4.96 ± 0.69	2.17 ± 0.10	1.02 ± 0.13	5.23 ± 0,91
MWB5	10.01 ± 1.51	4.10 ± 0.58	2.21 ± 0.24	4.80 ± 0.78	3.12 ± 0.82	1.01 ± 0.19	6.22 ± 1.83
MW3-2d	8.03 ± 0.63	3.72 ± 0.24	1.97 ± 0.18	4.09 ± 0.35	1.73 ± 0.12	0.88 ± 0.05	4.23 ± 0.43
MW5-2d	6.20 ± 0.80	3.92 ± 0.47	2.18 ± 0.17	4.73 ± 0.76	2.18 ± 0.22	1.06 ± 0.16	4.93 ± 1.13
MWB3-2d	7.75 ± 1.74	4.26 ± 0.48	1.87 ± 0.11	4.31 ± 0.26	1.77 ± 0.01	0.81 ± 0.03	4.48 ± 0.48
MWB5-2d	6.60 ± 0.35	4.13 ± 0.28	2.11 ± 0.08	4.64 ± 0.30	2.24 ± 0.07	0.92 ± 0.06	4.49 ± 0.23
Sample code	GIB	GRA	HGB	GB	GST	MGB	NGB
IF	9.37 ± 0.73	8.18 ± 0.93	2.14 ± 0.24	3.86 ± 0.37	1.73 ± 0.13	0.85 ± 0.03	4.16 ± 0.12
IMW3	10.16 ± 1.59	8.58 ± 1.44	2.23 ± 0.31	3.49 ± 0.21	1.81 ± 0.26	0.98 ± 0.16	2.72 ± 0.39
IMW5	4.95 ± 0.03	7.83 ± 0.26	1.73 ± 0.03	2.95 ± 0.21	1.40 ± 0.08	0.78 ± 0.03	3.35 ± 0.17
IMWB3	9.39 ± 0.43	7.59 ± 0.24	1.94 ± 0.03	2.86 ± 0.35	1.62 ± 0.17	0.75 ± 0.10	1.86 ± 0.72
IMWB5	8.25 ± 2.00	8.23 ± 1.76	1.87 ± 0.28	2.98 ± 0.55	1.60 ± 0.21	0.80 ± 0.18	2.54 ± 0.46

Table S3. Mineral content of broccoli (*Brassica oleracea* var. *Italica* cv. Parthenon) preprocessed in industry, fresh and microwaved under different conditions. Data are expressed as means ± SD (n=3).

Treatment	P (g/100g)	K (g/100g)	Na (g/100g)	Ca (g/100g)	Mg (g/100g)	Fe (mg/Kg)	Mn (mg/Kg)	Zn (mg/Kg)	Cu (mg/Kg)	B (mg/Kg)
IF	0.74 ± 0.02	2.7 ± 0.1	0.23 ± 0.01	0.36 ± 0.01	0.19 ± 0.01	53 ± 1	40 ± 1	62 ± 4	5.6 ± 0.4	25 ± 1
IMW3	0.71 ± 0.14	2.7 ± 0.1	0.21 ± 0.01	0.40 ± 0.02	0.19 ± 0.01	51 ± 2	38 ± 1	45 ± 1	4.5 ± 0.2	27 ± 1
IMW5	0.70 ± 0.03	2.8 ± 0.1	0.15 ± 0.01	0.36 ± 0.01	0.20 ± 0.01	51 ± 1	36 ± 1	55 ± 3	5.8 ± 0.1	26 ± 1
IMWB3	0.79 ± 0.09	2.7 ± 0.1	0.19 ± 0.01	0.32 ± 0.02	0.18 ± 0.01	54 ± 2	33 ± 1	45 ± 2	4.6 ± 0.7	25 ± 1
IMWB5	0.68 ± 0.09	2.8 ± 0.1	0.20 ± 0.05	0.37 ± 0.01	0.19 ± 0.01	58 ± 11	37 ± 3	47 ± 2	4.7 ± 0.4	26 ± 2

Figures

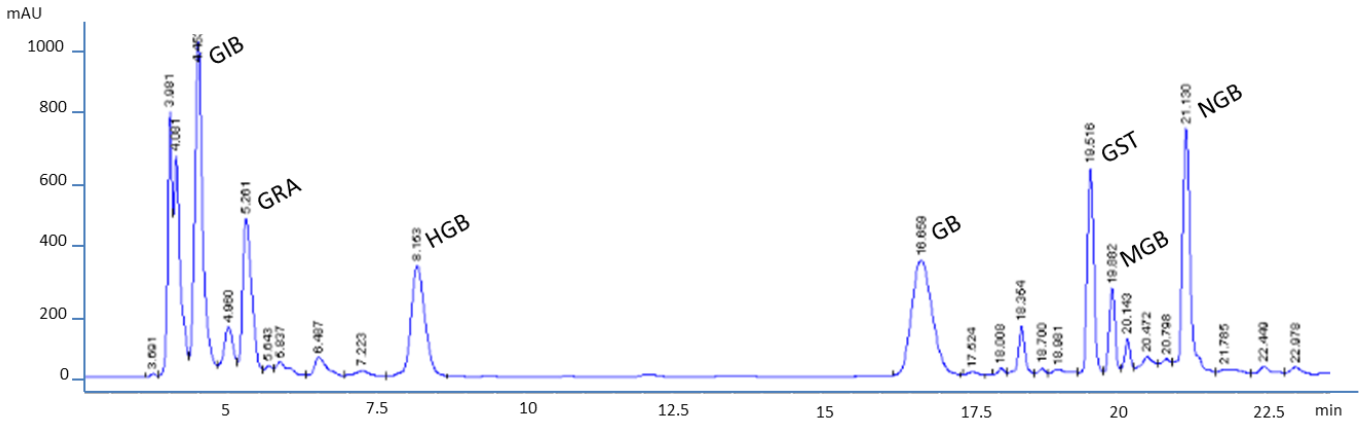


Figure S1. HPLC-DAD chromatogram of glucosinolate profile for fresh broccoli florets (*Brassica oleracea* var. *Italica* cv. Parthenon). Detection at 227 nm. Peaks: (GIB) glucoiberin, (GRA) glucoraphanin, (HGB) 4-hydroxyglucobrassicin, (GB) glucobrassicin, (GST) gluconasturtin, (MGB) 4-methoxyglucobrassicin, (NGB) neoglucobrassicin.

Click here to access/download;Figure;Figure 1 V03.docx

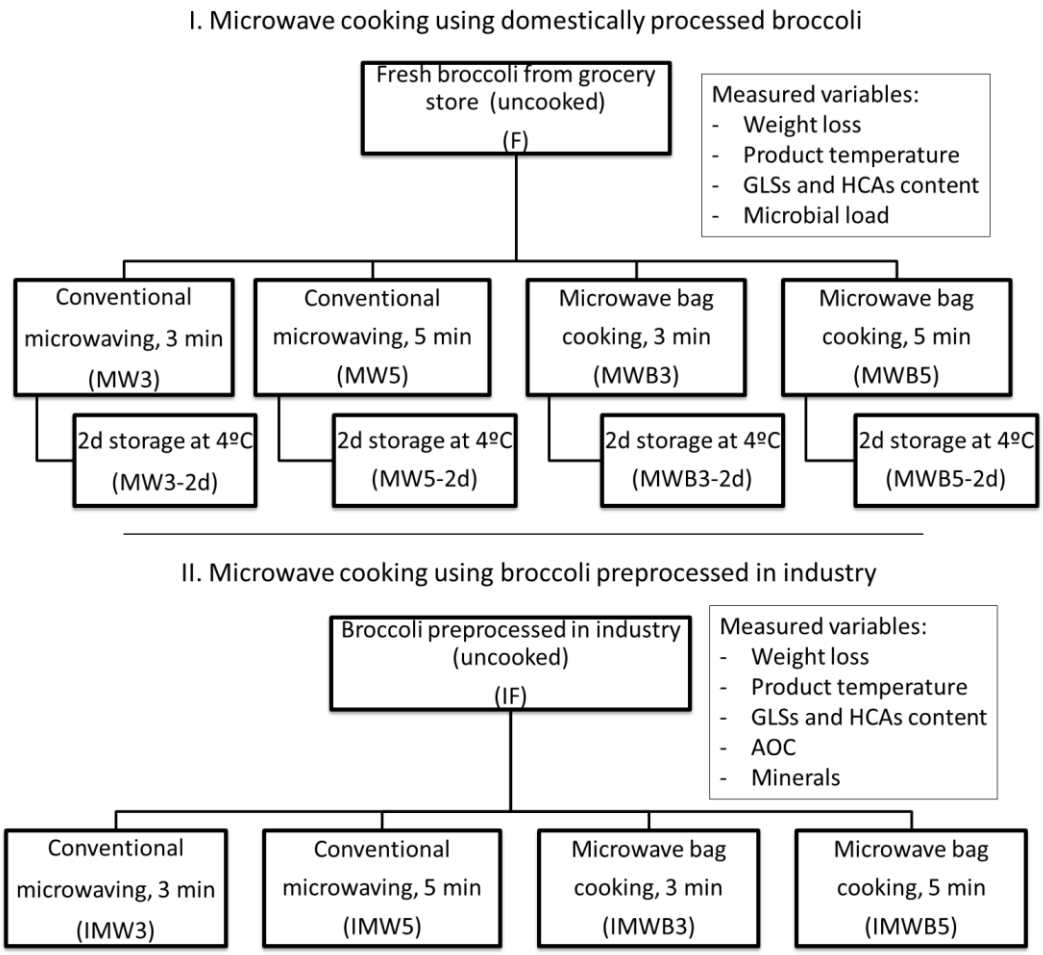


Figure 1.

Click here to access/download;Figure;Figure 2 V03.docx

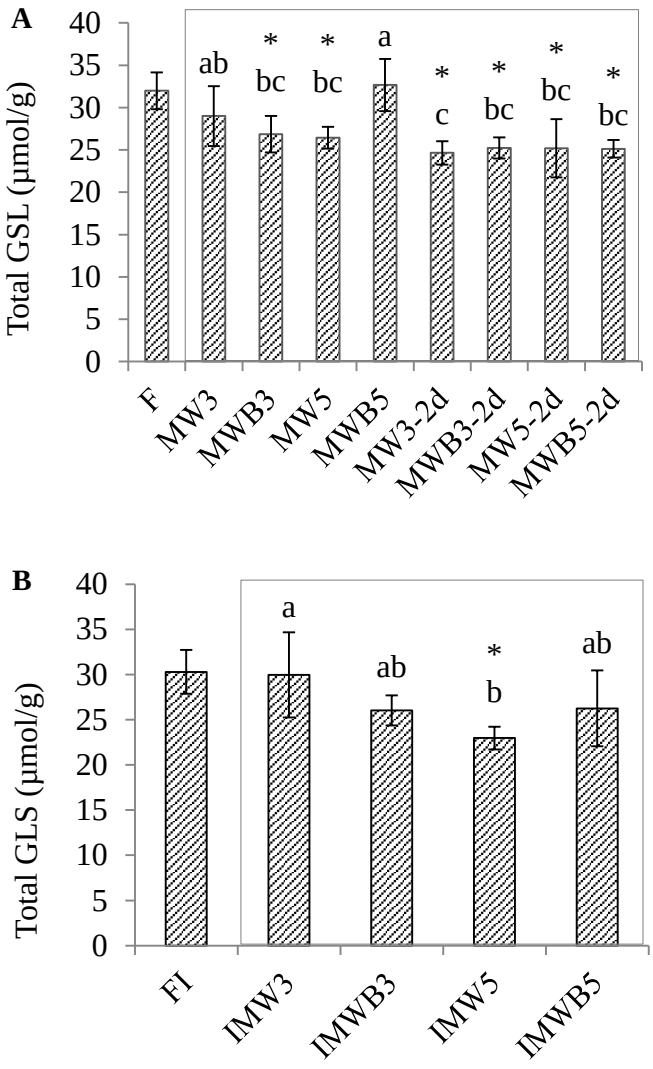


Figure 2.

Click here to access/download;Figure;Figure 3 V03.docx

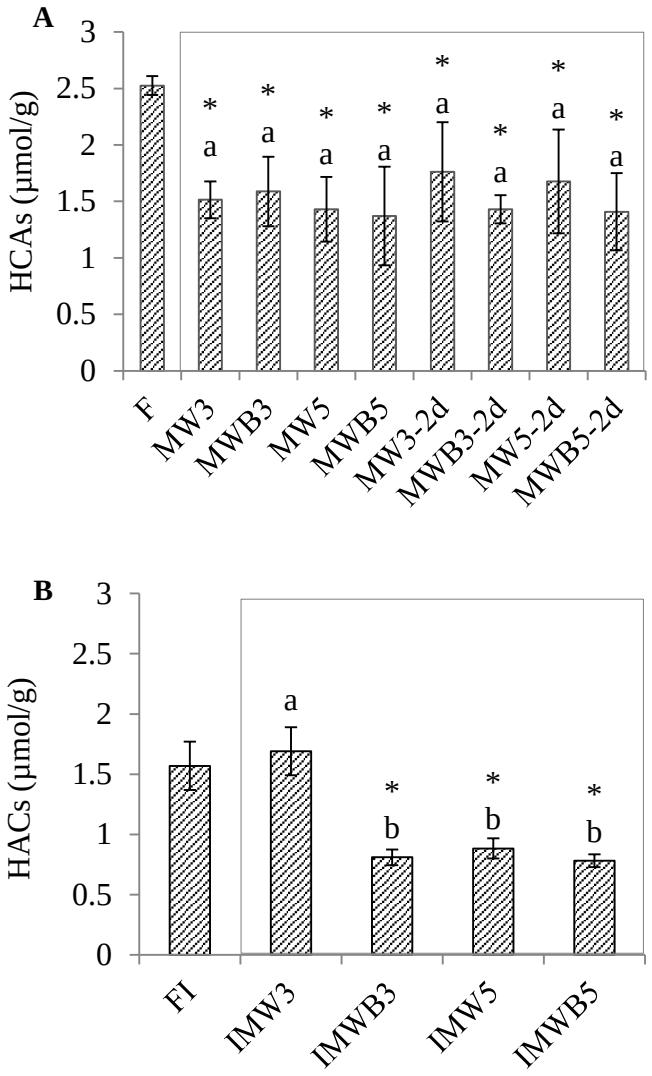


Figure 3.

Click here to access/download;Figure;Figure 4 V03.docx

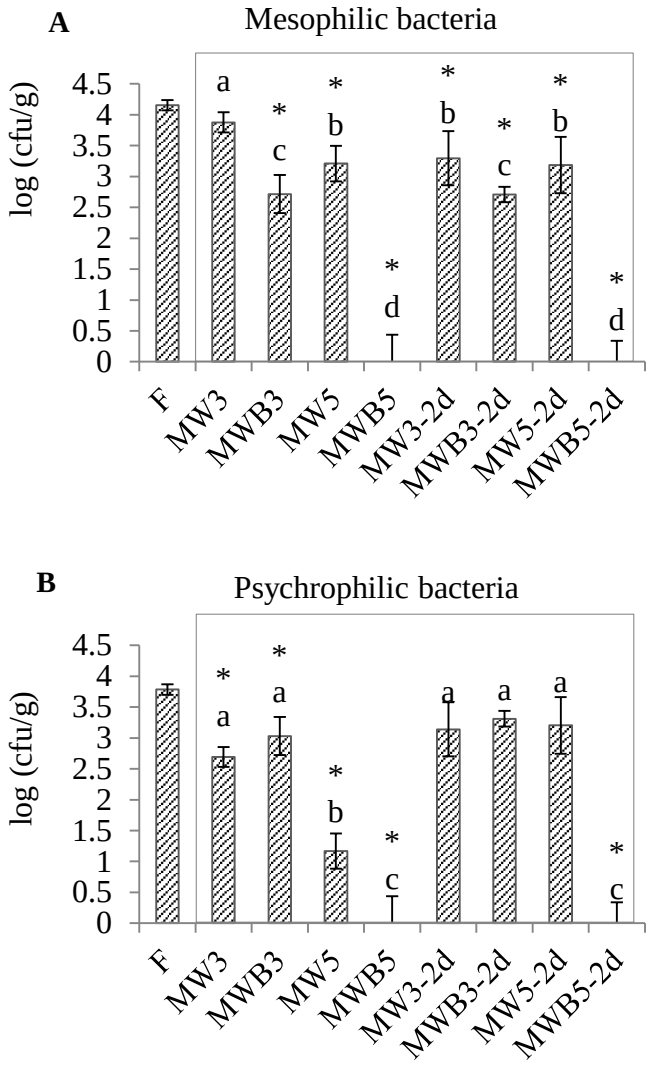


Figure 4.

Click here to access/download;Figure;Figure 5 V03.docx

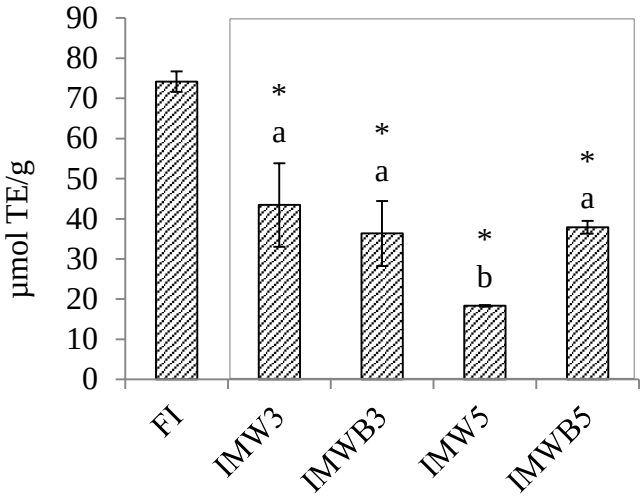
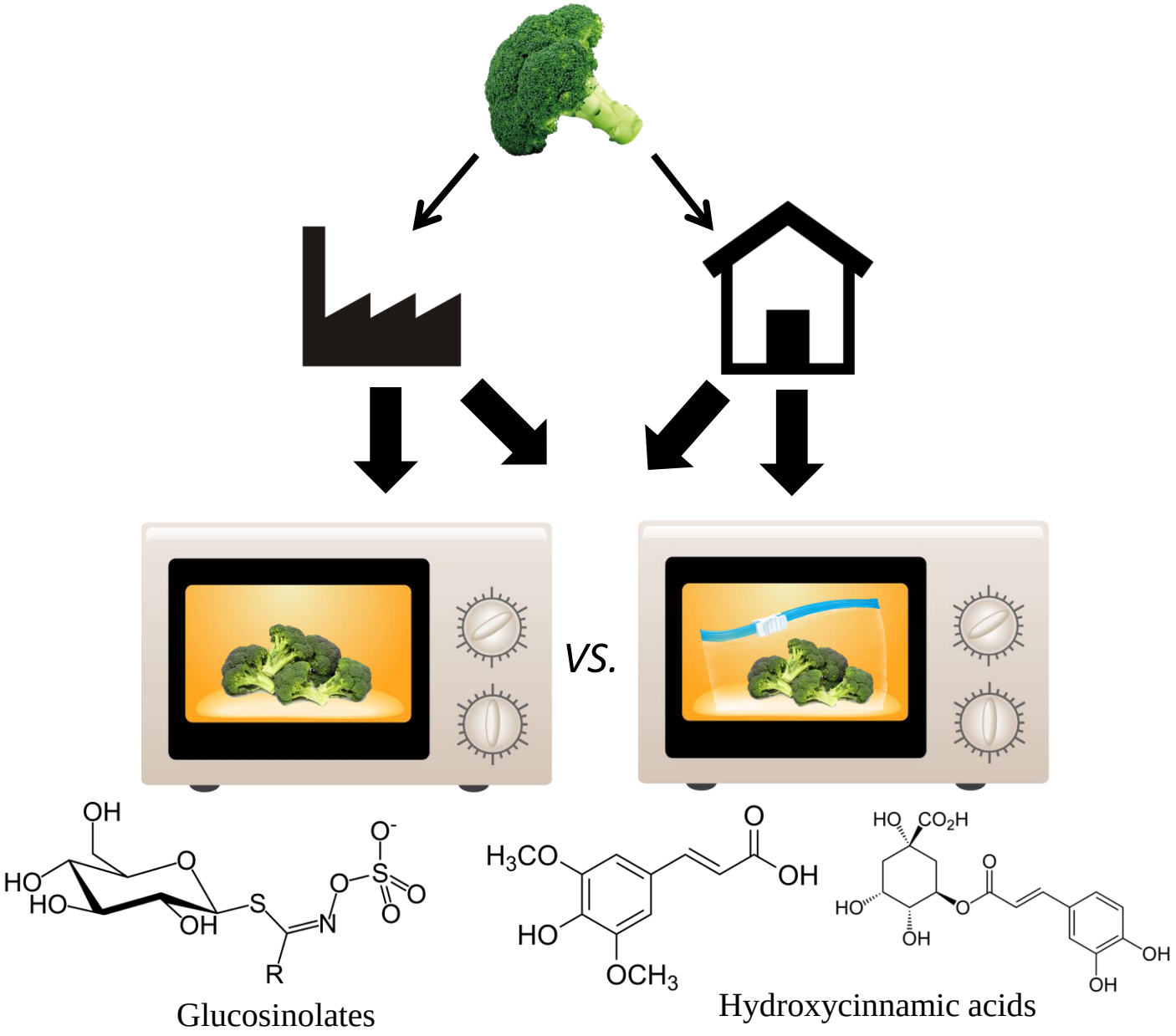


Figure 5.

*Graphical Abstract



Credit Author Statement

Erika Paulsen: Formal analysis, Investigation, Writing - Original Draft, Visualization

Diego A. Moreno: Conceptualization, Methodology, Resources, Writing-Reviewing and Editing, Supervision

Paula M. Periago: Resources, Reviewing

Patricia Lema: Writing-Reviewing and Editing, Supervision

*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.

Erika Paulsen
Instituto de Ingeniería Química - Facultad de Ingeniería
Universidad de la República, Montevideo, Uruguay
erikap@fing.edu.uy