Accepted Manuscript

Cooking methods of Brassica rapa affect the preservation of glucosinolates, phenolics and vitamin C

Marta Francisco, Pablo Velasco, Diego A. Moreno, Cristina Garcia-Viguera, María Elena Cartea

PII: S0963-9969(10)00128-6
DOI: 10.1016/j.foodres.2010.04.024
Reference: FRIN 3192

To appear in: *Food Research International*

Received Date: 18 February 2010
Accepted Date: 28 April 2010

Please cite this article as: Francisco, M., Velasco, P., Moreno, D.A., Garcia-Viguera, C., Cartea, a.E., Cooking methods of Brassica rapa affect the preservation of glucosinolates, phenolics and vitamin C, *Food Research International* (2010), doi: 10.1016/j.foodres.2010.04.024

Cooking methods of Brassica rapa affect the preservation of glucosinolates, phenolics and vitamin C

Marta Francisco*[a], Pablo Velasco[a], Diego A. Moreno[b], Cristina Garcia-Viguera[b], María Elena Cartea[a]

a. Misión Biológica de Galicia (CSIC), PO Box 28, E-36080· Pontevedra, Spain

b. Department of Food Science and Technology, CEBAS-CSIC, Campus Universitario de Espinardo, PO Box 164, Espinardo, E-30100 Murcia, Spain

*** Corresponding author:**

Marta Francisco

Address: Misión Biológica de Galicia (CSIC), PO Box 28, E-36080· Pontevedra, Spain

Tel: 0034-986854800

Fax: 0034-986841362

E-mail: mfrancisco@mbg.cesga.es

ABSTRACT

Cooking *Brassica* vegetables as a domestic processing method has a great impact on health- promoting bioactive compounds: glucosinolates (GLS), flavonoids, hydroxycinnamic acids, and vitamin C. In Galicia (NorthWestern Spain), one of the most consumed horticultural crops is *Brassica rapa*, by using the leaves (turnip greens) and the young sprouting shoots (turnip tops) in different culinary preparations. In order to determine the effect of cooking, on turnip greens and turnip tops, bioactive GLS, flavonoids, hydroxycinnamic acids and vitamin C were analysed and simultaneously determined. The level of retention of each individual compound after cooking procedures was evaluated in the edible organs, and we also in the cooking water, in order to compare their composition to a fresh uncooked control. Steaming, conventional boiling, and high-pressure cooking, traditional processing methods of this kind of vegetables, were the three domestic processing methods used in this work. Results showed that total GLS and phenolics were significantly affected by the cooking procedure and the loss rate varied among individual compounds. Steaming was the method that better preserved GLS and phenolic compounds. Conventional boiling and high-pressure cooking methods presented similar rate of losses of total GLS content (64%) and total phenolic content (more than 70%). Degradation among glucosinolate classes, aliphatic or indolic, was similar. The total flavonoids lost in turnip greens were 64% and 67% for conventional boiling and high-pressure, respectively. The main losses were caused by leaching into the cooking water. The concentration of vitamin C suffered a drastic loss in the process of sample handling and after cooking. Despite the fact that any cooking procedure affected negatively the nutritional composition of the turnip greens and tops, our results showed high retentions of individual compounds in steaming, and the lowest retentions were obtained in the traditional high-pressure

cooking. High retention of health-promoting compounds in the cooking water should be considered for increasing the intake of properties of *Brassica rapa.*

Keywords: *Brassica rapa*; domestic processing, steaming, boiling, high-pressure cooking; glucosinolates; flavonoids; hydrocycinnamic acids; vitamin C

1. INTRODUCTION

The *Brassicaceae* family includes a wide range of horticultural crops, many of them with economic significance and extensively consumed as commodities and used in the industry worldwide. *Brassica rapa* is one of the oldest cultivated vegetables that has been used for human consumption since prehistoric times (Liang, Kim, Lefeber, Erkelens, Choi & Verpoorte, 2006) which comprises several morphologically diverse crops, including Chinese cabbage, pak choi, turnip and broccoletto, as well as oilseeds that include yellow and brown sarsons (Gómez-Campo, 1999). In the coldest regions of Portugal and Spain the edible parts of *B. rapa* includes turnip greens and turnip tops for culinary profit as well as turnips for fodder (Padilla et al., 2005) and they constitute a unique supply of vegetables during the winter (Rosa, 1997). Turnip greens are the leaves harvested in the vegetative period while turnip tops are the fructiferous stems with the flower buds and the surrounding leaves which are consumed before opening and while still greens. Turnip edible parts are commonly consumed as a boiled vegetable generally as meat companions.

The consumption of *Brassica* vegetables has been related to human health and to reduction of the risk of certain cancers and cardiovascular diseases. This association is often attributed to the presence of glucosinolates (GLS), phenolic compounds and vitamins (Podsedek, 2007; Sies & Stahl, 1995; Traka & Mithen, 2009; Verhoeven, Verhagen, Goldbohm, vandenBrandt & vanPoppel, 1997).

Thermal treatment causes denaturation of enzymes that can catalyse breakdown of nutrients and phytochemicals. When *Brassica* vegetables are chewed or cut, tissues will disrupt and the GLS will come into contact with myrosinase (thioglucoside glucohydrolase EC 3.2.1.147), leading the conversion to isothiocyanates, nitriles,

thiocyanates, epithionitriles, oxazolidine-2-thiones, and epithioalkanes (Grubb & Abel, 2006). The number of hydrolysis products, mostly formed simultaneously during storage and processing, as well as the myrosinase activity of the intestinal microbial floral may affect to the total content and bioavailability of these compounds (Verkerk et al., 2009).

It has been generally shown that conventional cooking methods such as boiling, steaming, pressure cooking and microwaving reduce the intake of glucosinolates by approximately 30 to 60%, depending on the method, intensity and type of compound (Rangkadilok et al., 2002; Rodrigues & Rosa, 1999; Verkerk & Dekker, 2004; Verkerk, Dekker & Jongen, 2001). Some reports have focused mainly on the preservation of phenolic compounds in broccoli and vitamin C in broccoli and Brussels sprouts (Czarniecka-Skubina, 2002; Howard, Wong, Perry & Klein, 1999; Vallejo, Tomás-Barberán & García-Viguera, 2003; Zhang & Hamauzu, 2004). These studies reported that steaming led to the retention of the highest levels of flavonoids and hydroxycinnamic acids in broccoli. On the contrary, cooking from 3 to 15 min by microwave and conventional boiling caused losses on phenolic content approximately 30 to 90%. Related to vitamin C were reported losses from 3 to 10% after cooking Brussels sprouts in a microwave oven and pressure cooker (Czarniecka-Skubina, 2002). Conventional cooking in broccoli florets at 0.5, 1.5 and 5 min caused loss by 19.2%, 47.5%, and 65.9% of vitamin C, respectively (Zhang & Hamauzu, 2004).

At the Misión Biológica de Galicia (CSIC), a collection of local varieties of *B. rapa* [*rapa* group] is kept as part of the *Brassica* genus germplasm bank. In previous reports, this collection was evaluated based on nutritional traits (Francisco, Moreno, Cartea, Ferreres, García-Viguera & Velasco, 2009; Padilla, Cartea, Velasco, de Haro & Ordas, 2007). Since these crops are thermally processed prior to consumption, the objective of

this study was to determine the changes on the content of total and individual GLS, flavonoids, hydroxycinnamic acids and vitamin C in a representative set of turnip greens and turnip tops with three different cooking methods: high-pressure cooking, steaming and conventional boiling.

2. MATERIAL AND METHODS

2.1. Plant material. Five local varieties of *B. rapa* were evaluated in this study. From these, four varieties were chosen based on their agronomic performance for turnip tops and/or turnip greens and one variety derived from three cycles of masal selection by fresh yield. The varieties were evaluated in 2007 at two environments in northwestern Spain: Oroso (A Coruña) (43°1'N, 8°26'W, 280 m.a.s.l.) and Guitiriz (Lugo) (43°12'N, 7°53'W, 516 m.a.s.l.). Both environments represent standard *B. rapa* production areas in NW Spain. The varieties were planted in multipot-trays and seedlings were transplanted into the field at the five or six leaves stage. Transplanting dates were on the 01th and 04th September in Oroso and Guitiriz, respectively. Varieties were transplanted in a randomized complete block design with three replications. The experimental plots consisted of three rows with 10 plants per row. Rows were spaced 0.8 m apart and plants within rows 0.5 m apart. Cultural operations, fertilization, and weed control were made according to local practices. Leaf harvest ranged from 44 to 64 days after planting while sprouting shoot harvest ranged from 127 to 229 days after planting according to the maturity cycle of each variety at the optimum time for consumption.

2.2. Processing. Three different cooking methods were tested: conventional boiling, steaming and high-pressure cooking. A total of 1.5 Kg of leaves (turnip greens) and sprouting shoots (turnip tops) of each variety and environment were randomly selected. Samples were immediately transported on ice to the laboratory, where they were vacuum packed, frozen, and stored for further cooking. For turnip greens, three cooking procedures were carried out replicated two times in each variety sample and environment. For turnip tops, only samples from Lugo were used due to low yields from Santiago and two methods were performed (conventional boiling and steaming). Each

126 sample was divided in several portions of 150 g for subsequent cooking and the analysis
127 of health-promoting bioactives. For each variety, two portions of 150g were kept as
128 uncooked fresh control. The cooking settings (time, temperature and water) were chosen
129 according to recipes. For conventional boiling, fresh portion was added to 1500 mL of
130 boiling water and cooked for 15 min. For high-pressure cooking, the leaves were fully
131 dipped in 1500 mL of cold water and cooked during 5 min under high-pressure in a
132 pressure cooker (Fagor^{TM} Rapid-Express, Fagor Electrodomésticos S.C., Mondragon,
133 Guipuzkoa, Spain). For steaming, the portion of vegetable was placed on a steaming
134 rack over boiling water in a closed water bath (1500 mL) during 15 min. Of each
135 method, 45 mL of the cooking water was kept for further analysis. After cooking and
136 drained, cooked portions, water samples and fresh control were flash frozen using liquid
137 N_2 and kept at −80 °C prior to their lyophilization (Christ Alpha 1-4D, Christ, Osterode
138 am Harz, Germany). The dried material was powdered using an IKA-A10 (IKA-Werke
139 GmbH and Co.KG) mill and the powder was used for analysis.
140
141 **2.3. Extraction and determination of GLS and phenolic compounds**. The HPLC
142 gradient for glucosinolate and phenolic analyses is a multi-purpose chromatographic
143 method that simultaneously separates glucosinolates and phenolics (Bennett et al.,
144 2003) and it was recently applied to Galician turnip tops and greens (Francisco,
145 Moreno, Cartea, Ferreres, García-Viguera & Velasco, 2009). Briefly, a portion of 150
146 mg of each sample were extracted in 4 mL of 70% MeOH at 70 °C for 30 min with
147 vortex mixing every 5 min to facilitate the extraction. The samples were centrifuged
148 (13000g, 15 min), and 1 mL of supernatant was collected to completely remove
149 methanol using a sample concentrator (DB-3D, Techne, UK) at 70 ºC. The dry material
150 obtained was redissolved in 1mL of ultrapure water and filtered through a 0.20 µm

151 syringe filters (Acrodisc® Syringe Filters, Pall Life Sciences). Chromatographic
152 analyses were carried out on a Luna C18 column (250 mm × 4.6 mm, 5 µm particle
153 size; Phenomenex, Macclesfield, UK). The mobile phase was a mixture of (A) ultrapure
154 water/trifluoro acetic acid (TFA) (99.9:0.1) and (B) methanol/TFA (99.9:0.1). The flow
155 rate was 1 mL min^{-1} in a linear gradient starting with 0% B at 0–5 min, reaching 17% B
156 at 15–17 min, 25% B at 22 min, 35% B at 30 min, 50% B at 35 min, 99% B at 50 min
157 and at 55-65 min 0% B. The injection volume was 20 µL and chromatograms were
158 recorded at 330 nm for phenolics derivatives and 227 nm for GLS in a Model 600
159 HPLC instrument (Waters) equipped with a Model 486 UV tunable absorbance detector
160 (Waters). Glucosinolates were quantified using sinigrin (sinigrin monohydrate from
161 Phytoplan, Diehm and Neuberger GmbH, Heidelberg, Germany) as standard. Caffeoyl-
162 quinic and p-coumaroyl-quinic acids derivatives were quantified as chlorogenic acid (5-
163 caffeoyl-quinic acid, Sigma-Aldrich Chemie GmbH, Steinheim, Germany), flavonoids
164 as kaempferol 3-rutinoside (Extrasynthese, Genay, France) and sinapic acid and
165 derivatives as sinapic acid (Sigma).
166
167 **2.4. Extraction and determination of vitamin C**. Ascorbic (AA) and dehydroascorbic
168 (DHAA) acid contents were determined as described by Zapata and Dufour (1992) with
169 some modifications (Gil, Ferreres & Tomas-Barberan, 1999; González-Molina, Moreno
170 & García-Viguera, 2008). For the determination in fresh, 5 g of fresh weight sample
171 were homogenised in a an Ultra-Turrax T25 (Janke & Kunkel, Germany) for 30 s on an
172 ice bath with 20 mL extractant solution, consisting of MeOH and H_2O (5:95), and 2.1%
173 (v:v) dissolved citric acid, 0.05% (v:v) EDTA, and 0.01% (v:v) NaF. For freeze-dried
174 samples 50 mg were homogenized in a vortex stirrer for 20 s with 10 mL of extractant
175 solution. The homogenate was filtered through a four-layer cheesecloth. The extract (1

mL) was centrifuged (3600g for 15 min at 4 °C), and the supernatant was recovered and filtered through a C18 Sep-Pack cartridge (Waters, Milford, MA) previously activated with 10 mL of methanol followed by 10 mL of deionized water, and then 10 mL of air. The collected extract was filtered through a 0.45 µm polyethersulfone filter (Millex-HV, Millipore, Bedford, MA). Then, 250 µL of 1,2-phenilenediamine dihydrochloride (OPDA) solution (18.8 mM) were added to 750 µL of extract for dehyhroascorbic acid derivatization into the fluorophore 3-(1,2-dihydroxietyl)furo[3,4-b]quinoxaline-1-one (DFQ). After 37 min in darkness, the samples were analyzed by HPLC. Ascorbic acid and dehydroascorbic acid was evaluated using an HPLC system (Merck-Hitachi, Tokyo, Japan), equipped with a L-6000 pump, injection valve and sample loop 20 µL (Rheodyne, CA, USA) and coupled to a L-4000 UV detector . Samples were analysed on a Lichrospher 100 RP-18 reversed-phase column (250 x 4mm, particle size 5 µm) (Teknokroma, Barcelona, España) with a C_{18} precolumn (Teknokroma, Barcelona, España). The mobile phase was MeOH/H_2O (5:95, v/v), 5 mM cetrimide, and 50 mM KH_2PO_4 (pH = 4.59). The flow rate was kept at 0.9 mL min^{-1}. The detector wavelength was initially set at 348 nm, and after DFQ eluted, it was manually shifted to 261 nm, for ascorbic acid detection. L-AA y el L-DHAA were identified and quantified by comparison with pattern areas from L-AA and L-DHAA.

2.5. Statistical analyses. All analyses were made separately for each plant organ (turnip greens and turnip tops). The content of each metabolite (individual and total GLS and phenolic compounds) was determined in two ways: i) in the fresh (raw) and cooked vegetable tissue and ii) in the sum in the cooked vegetable tissue plus the cooking water (CW). Individual analyses of variance were performed for each compound. Varieties were considered as random factors. Comparison of means among cooking methods was

made by Fisher's protected least significant difference (LSD) at P=0.05 (Steel, Torrie & Dickey, 1997). All statistical analyses were made using SAS (SAS Institute, 2007).

3. RESULTS AND DISCUSSION

3.1. Effect of cooking on total and individual glucosinolates (GLS)

3.1.1. Effect on vegetable tissues

Total GLS content in *B. rapa* varieties was very similar in both organs (12.99 μmol/g^{-1} dw in fresh turnip greens and 12.84 μmol/g^{-1} dw in fresh turnip tops). Seven major GLS were found in both organs: progoitrin (PRO), gluconapin (GNA), glucobrassicanapin (GBN), 4-hydroxyglucobrassicin (4-OHGBS), glucobrassicin (GBS), neoglucobrassicin (NGBS) and gluconasturtiin (GNT). Aliphatic GLS were the most abundant (66% of total GLS) followed by indolic (25%) and aromatic (9%). In agreement with data published by other authors (Francisco, Moreno, Cartea, Ferreres, García-Viguera & Velasco, 2009; Kim, Kawaguchi & Watanabe, 2003; Padilla, Cartea, Velasco, de Haro & Ordas, 2007) the predominant GLS in *B. rapa* crops was GNA, which represents 51% and 77% of total GLS and total aliphatic contents, respectively.

In turnip greens, significant differences among cooking methods were found for all GLS ($P \leq 0.01$). Varieties did not show any significant differences among them. The variety × cooking method interaction was not significant for any GLS, which is indicative of the stability of different genotypes. In the same way, in turnip tops, significant differences among cooking methods were found for total GLS content ($P \leq 0.01$) as well as for most of the individual GLS. Varieties were significantly different for GBS and total GLS content. Differences in harvest time according to the maturity state of each variety could influence the final content of GLS. No GLS showed any significant variety × cooking method interaction.

Total and individual GLS concentrations were significantly reduced by the cooking method used and these losses were similar in turnip greens and turnip tops

(Table 1, Figure 1). Conventional boiling and high-pressure methods presented similar loss rate, by about 64% of total GLS content in comparison with fresh samples. Rosa and Heaney (1993) and Pereira et al. (2002) found losses from 40 to 80% of total GLS in Portuguese cabbage after boiling. Similar degratadion rates of total GLS contents (58-77%) were described by Song and Thornalley (2007) after boiling differents brassicas during 30 min. Ciska and Kozlowska (2001) also observed a time course decrease of GLS content from 35% after 5 min of cooking to 87% after 30 min in white cabbage. In coincidence with previous results in broccoli (Vallejo, Tomás-Barberán & García-Viguera, 2002; Volden, Wicklund, Verkerk & Dekker, 2008), in the present work the steaming method was found to be the preferred cooking method for better preservation (or higher level of retention of) individual GLS content, because the losses ranged only by 9% in turnip greens and 21% in turnip tops (Figure 1).

After cooking, the relative distribution of the three classes of GLS (aliphatic, indolic, and aromatic) did not change (Table 1). In turnip greens, the total aliphatic GLS content was reduced by 14% in steamed, a 60% in conventional boiling, and by 61% in high-pressure cooking. Similarly, in turnip tops, the aliphatic GLS content reductions were 25% in steamed, and 63% in conventional boiling. In turnip greens, total indole GLS content was reduced by about 60%, both after high-pressure and conventional boiling cooking, while in boiled turnip tops this loss was a 52%. Aliphatic GLS are generally reported as being more thermostable than indole GLS and under different cooking treatments (Ciska & Kozlowska, 2001; Goodrich, Anderson & Stoewsand, 1989; Vallejo, Tomás-Barberán & García-Viguera, 2002). However, in this work we found similar degradation rates between total aliphatic and total indole GLS eventhough the loss rates varied among individual GLS. GNA, the most abundant aliphatic GLS, was reduced after steaming by 14% and 23% in turnip greens and turnip tops,

253 respectively, while it was reduced about 60% after high-pressure and conventional
254 boiling cookings in both turnip tissues (Figure 1). Loss rates of PRO were notably
255 higher in turnip greens than in turnip tops. The greatest reductions after high-pressure
256 and conventional boiling were found for two indolic GLS (4-OHGBS and GBS) and for
257 the aromatic GNT with losses close to 100%. Other authors found that GBS, PRO and
258 4-OHGBS are very susceptible to heat treatments showing a great reduction after
259 cooking (Rosa & Heaney, 1993; Volden, Wicklund, Verkerk & Dekker, 2008). In the
260 edible part of steamed turnip greens, we found an increase of 85% on the initial value of
261 the indolic 4-OHGBS. The increase of GLS levels after steaming was reported
262 previously (Gliszczynska-Swiglo, Ciska, Pawlak-Lemanska, Chmielewski, Borkowski
263 & Tyrakowska, 2006) and also Verkerk and Dekker (2004) found more than 70%
264 higher levels of indolic GLS after microwave treatment who explained it by an increase
265 in chemical extractability from the plant tissue after heating.
266
267 3.1.2. Effect on the summatory of vegetable tissues and cooking water (CW)
268 Glucosinolates are water-soluble compounds and are usually lost during
269 conventional cooking because of leaching into surrounding water due to cell lysis.
270 Analysis of the water remains after boiling indicated that all GLS were leached out into
271 the cooking water (CW). The analysis of GLS in CW of turnip greens and CW of turnip
272 tops showed significant differences among cooking methods for total GLS content ($P \leq$
273 0.01) as well as for same GLS. Other GLS did not show any significant differences
274 among cooking methods indicating low or no degradation of these compounds.
275 After steaming, total GLS content of CW in both plant organs was not
276 significantly different from the total GLS content in fresh vegetables (Table 2, Figure
277 2), which means that the amounts of GLS recovered were not significantly different

278 from the initial GLS content of the fresh vegetable. On the contrary, after conventional
279 boiling and high-pressure, there were recovered 67% and 52%, respectively of the total
280 GLS content in fresh turnip greens (Table 2, Figure 2). In turnip tops, this recovery was
281 62% after conventional boiling (Table 4, Figure 2). The most stable GLS in both plant
282 organs after cooking were GBN, 4-OHGBS and NGBS. In turnip greens, total
283 recoveries of compounds with the largest reductions i.e. PRO, GBS and GNT were
284 35%, 41%, and 13%, respectively after conventional boiling, and 67%, 29%, and 4%
285 after high pressure cooking. Different behaviour was found for 4-OHGBS, which
286 suffered high reductions after cooking and it was recovered completely into the cooking
287 water. In turnip tops, the highest loss after conventional boiling was detected in GNT
288 which was recovered only 21%. These results are not consistent with other studies in
289 which recoveries were over 80% for all GLS (Rosa & Heaney, 1993; Vallejo, Tomás-
290 Barberán & García-Viguera, 2002; Volden, Wicklund, Verkerk & Dekker, 2008). GLS
291 losses can be explained because the breakdown of cellular membranes during cooking
292 allows the contact between glucosinolates and myrosinase. The myrosinase mediated
293 hydrolysis of glucosinolates generates an unstable aglycone intermediate,
294 thiohydroxamate-O-sulfonate, which is immediately converted to a wide range of
295 bioactive metabolites, including isothiocyanates, thiocyanates, nitriles and oxazolidines
296 (Bones & Rossiter, 1996; Fenwick, Heaney & Mullin, 1983). Some of them are volatile
297 metabolites associated with the typical bitter and hot flavour of *Brassica* foods
298 (Fenwick, Heaney & Mullin, 1983). Isothiocyanates and indoles exhibit protective
299 activities against many types of cancer in humans (Fahey, Zalcmann & Talalay, 2001;
300 Mithen, Faulkner, Magrath, Rose, Williamson & Márquez, 2003; Zhang & Talalay,
301 1994).
302

3.2. Effect of cooking on phenolic compounds

3.2.1. Effect on vegetable tissues

The HPLC-DAD analysis allowed the quantification of 14 phenolic compounds including flavonoids, quinic acid derivatives and sinapic acids derivatives: kaempferol-3-O-sophoroside-7-O-glucoside (F1); kaempferol-3-O-(caffeoyl)sophoroside-7-O-glucoside (F2); kaempferol-3-O-(sinapoyl)sophoroside-7-O-glucoside (F3); kaempferol-3-O-(feruloyl)sophoroside-7-O-glucoside (F4); kaempferol-3-O-(*p*-coumaroyl)sophoroside-7-O-glucoside (F5); kaempferol-3,7-di-O-glucoside (F6); isorhamnetin-3,7-di-O-glucoside (F7); 3-caffeoyl quinic acid (3CQAc); 3-*p*-coumaroyl quinin acid (3*p*CoQAc); sinapic acid (SA); 1,2-disinapoylgentiobioside (A1); 1-sinapoyl-2-feruloylgentiobioside (A2); 1, 2, 2´-trisinapoylgentiobioside (A3); 1,2´-disinapoyl-2-feruloylgentiobioside (A4). Results of total phenolic content revealed higher amount of these compounds in turnip greens (31.51 μmol/g^{-1} dw), than in turnip tops (14.80 μmol/g^{-1} dw). These differences are probably due to the high amount of SA in turnip greens, compound present in lower quantities in turnip tops. Total phenolic content found in our study was similar to those found in turnip tops by other authors (Fernandes, Valentão, Sousa, Pereira, Seabra & Andrade, 2007; Francisco, Moreno, Cartea, Ferreres, García-Viguera & Velasco, 2009; Sousa et al., 2008).

In turnip greens, the analysis of variance showed significant differences among cooking methods ($P \leq 0.01$) for all of the flavonoids and hydroxycinnamic acids evaluated. No significant differences among varieties were found for any compound. Variety × cooking method interaction was significantly different ($P \leq 0.01$) for A1, total quinic acids derivatives, total phenolics and 3CQAc may be due to similar degradation rates found between high-pressure and conventional boiling methods. In turnip tops, the analysis of variance for phenolic compounds showed significant differences between

328 cooking methods for total phenolic compounds and for most individual compounds ($P \leq$
329 0.05). No significant differences among varieties were found for any compound. Variety
330 × cooking method interaction was significantly different ($P \leq 0.01$) for F2, F6 and A4.
331 After cooking, total phenolics content in turnip greens was reduced in 15%, 75%
332 and 72% in steaming, high-pressure and conventional boiling, respectively (Figure 1).
333 In turnip tops, total phenolics were reduced 35% in steaming and 73% in conventional
334 boiling (Figure 1). During steaming, the temperature is lower than in the other two
335 methods and the edible portions were not into contact with the cooking water.
336 Therefore, the phenolic content was less affected. In agreement with Wachtel-Galor et
337 al. (2008), boiling and high-pressure cooking had strong effects on total phenolics
338 content (Table 1). The depletion of total phenolics content after cooking could be due to
339 their breakdown or by leached into the cooking water (Vallejo, Tomás-Barberán &
340 García-Viguera, 2003).
341 The amount of favonoid glycosides lost in the cooked tissue of turnip greens
342 were 5%, 64% and 67% for steaming, conventional boiling and high-pressure,
343 respectively. In turnip tops, the loss of flavonoid glycosides was a 36% after steaming
344 and a 72% after conventional boiling (Figure 1). Our results indicate higher levels of
345 total flavonoids in the edible part after cooking than those previously reported by Price
346 et al. (1998) and Vallejo et al. (2003) which found that boiled broccoli lost a 80% of its
347 initial flavonoid content. This better retention in turnip could be explained by the
348 different flavonoid profile of *B. oleracea* and *B. rapa*. The studies mentioned before are
349 focused on total phenolic content on broccoli but, as far as we are aware, there are no
350 data avaliable about rates of degradation on individual flavonoids presents on brassica
351 vegetables after domestic cooking. Regarding to individual flavonoids, in the present
352 work we focused on the study of seven major flavonoids of *B. rapa* (Table 1).

353 Compunds F1, F2, F3, F4, F5 and F6 are flavonoids derivatives from kaempferol that
354 have been described in other brassica vegetables such as cabbage, pak choi and broccoli
355 (Ferreres et al., 2006; Harbaum, Hubbermann, Wolff, Herges, Zhu & Schwarz, 2007;
356 Vallejo, Tomas-Barberan & Ferreres, 2004). Compound F7 is a flavonoid derived from
357 isorhamnetin that was described in high quantities in *B. rapa* crops (Francisco, Moreno,
358 Cartea, Ferreres, García-Viguera & Velasco, 2009).
359 Results showed that the same cooking method have different effects on different
360 types of flavonoids, even within the same class. Besides, the loss rates of individual
361 flavonoids varied among cooking methods and plants stages. High losses, from 80 to
362 90% were detected on F5 after high-pressure and conventional boiling. Compound F3
363 has different behavior between cooking methods. After conventional boiling more than
364 86% of F3 was lost, however after high-pressure the same compound was the less
365 reduced, only by 47%. In turnip greens F6 and F7 showed good retention levels with
366 losses between 55-60% after both cooking methods, conventional boiling and high-
367 pressure. After steaming, low hydroxycinnamic acid levels were lost in both plant
368 organs, between 0 and 15% of total quinic acids derivatives and between 22 and 35% of
369 total sinapic acid derivatives (Figure 1). These minor losses could be due because
370 during steaming inactivation of oxidative enzymes occurs (Vallejo, Tomás-Barberán &
371 García-Viguera, 2003). By contrast, high-pressure and conventional boiling produced
372 losses close to 100% of total quinic acids derivatives in turnip greens (Table 1, Figure
373 1). In turnip tops, 3CQAc and 3*p*CoQAc did not show significant losses after
374 conventional boiling. Total sinapic derivatives were lost about 80% in both organs after
375 high-pressure and conventional boiling (Table 1, Figure 1). The loss rates of
376 hydroxycinnamic acids found in this work were higher than those reported in boiled
377 broccoli by other authors (Gliszczynska-Swiglo, Ciska, Pawlak-Lemanska,

Chmielewski, Borkowski & Tyrakowska, 2006; Price, Casuscelli, Colquhoun & Rhodes, 1998; Vallejo, Tomás-Barberán & García-Viguera, 2003). In plants, phenolic compounds occur in soluble forms as well as in combination with cell wall components. Hence, large surface area in contact with the cooking water at high temperature and the long cooking time may have been responsible of the disruption of the cell walls and the compound breakdown causing greater losses of these compounds.

3.2.2. Effect on the summatory of vegetable tissues and cooking water (CW)

The study of CW indicated that all phenolic compounds were leached after boiling (Table 2, Figure 2). The analysis of variance of phenolic content in CW showed that in turnip greens there were significant differences among cooking methods for total phenolics content and for most of phenolic compounds ($P \leq 0.01$). On the contrary, the analysis of turnip tops did not show differences among cooking methods, which means that the amounts of phenolic compounds recovered were not significantly different from the initial phenolic content of the fresh vegetable. Results showed that total flavonoid recoveries were 100% in steaming samples. After cooked at high-pressure and conventional boiling increases from 5 to 70% in CW in both plant organs were found (Table 2, Figure 2). The deacylated compounds F1, F6 and F7 are the main contributors to the increase in the concentration of flavonoids in CW respect to the fresh portion due to a greater amount of these flavonoids into the processing water. The high retention of these compounds may be due the conversion of acylated flavonoids into their glycosylated form. Contray to this, some hydroxycinnamic acids were lost during the cooking process (Table 2, Figure 2). In turnip greens, after high-pressure only a 32% of total quinic acids derivatives were recovered while in turnip tops increased the amount of 3CQAc and 3*p*CoQAc specialy in CW of high-pressure cooking. Total sinapics in

403 turnip greens were recovered by 80%, 32%, and 18% after steaming, high-pressure, and
404 conventional boiling, respectively. In turnip tops, almost all hydroxycinnamic acids
405 were recovered. Total phenolics levels were recovered almost 100% in both plants
406 organs except after high-pressure cooking. Traditional home cooking of turnip greens
407 and turnip tops is carried out under long cooking times. Zhang and Hamauzu (2004)
408 showed that a 10-fold (from 0.5 to 5 min) prolongation of the conventional cooking
409 time caused up 2-fold total phenolic losses in broccoli and, therefore stability of
410 phenolics strongly depended on cooking time.
411
412 **3.3. Effect of cooking on Vitamin C**
413 The concentration of vitamin C (ascorbic acid, the predominant form of vitamin C) was
414 dramatically reduced by the processing method. The content of vitamin C in fresh turnip
415 greens and turnip tops was 62 mg/100g fw and 46 mg/100g fw, respectively. Similar
416 results were described by Mondragón-Portocarnero et al. (2006) in fresh turnip greens.
417 The fresh material suffered various manipulations before analysis (i.e., freezing, freeze-
418 drying, and grounding) that definitively affected the content of vitamin C in the samples
419 causing a dramatic lost respect to the fresh material (96%). With respect to cooked
420 samples, as expected, vitamin C was decreased after all cooking methods. After
421 steaming treatment, the loss was 64% respect to untreated fresh material and after high-
422 pressure and conventional boiling, vitamin C was not found in the edible parts.
423 Mondragón-Portocarnero et al. (2006) reported loss by 61% after blanching turnip
424 greens in water for 2 min. Other authors showed that the content of ascorbic acid in
425 broccoli declined dramatically during cooking (Vallejo, Tomás-Barberán & García-
426 Viguera, 2002; Zhang & Hamauzu, 2004) having the cooking time a higher influence on
427 ascorbic acid level than any cooking method (Zhang & Hamauzu, 2004). The results

obtained in the present study showed that the content of ascorbic acid not only was declined dramatically during the cooking but also in the process of sample handling.

4. CONCLUSIONS

Brassica foods include different crops such us cabbage, cauliflower, broccoli, Brussels sprouts, turnips and kale. These vegetables are consumed all year around, and represent worldwide used ingredients of different salads either as raw or frozen vegetables or after domestic processing (cooking). Conventional methods of cooking reduce the intake of potentially health-promoting compounds. Most of reports that studied the effects of cooking methods on *Brassica* vegetables are focused mainly on the preservation of total GLS and phenolic compounds. In this work we conducted a comprehensive study about more than 20 individual GLS and phenolic compounds. The quantification was carried out with a multipurpose method for the simultaneous identification of GLS and phenolics. Results have given us information on the effect of cooking on flavonoids levels, some of them have been studied for first time in this work. It can be concluded that steaming cooking resulted in high retention of the GLS and phenolic compounds. No contact of the vegetables with water during steaming prevents leaching and solubilization of these metabolites in the cooking water. The other two methods caused similar loss rates, although in high-pressure method, plant material was less time into contact with water. Varieties were affected in the same way by the cooking methods.

In this study we found that the greatest loss of vitamin C happened throughout sample management. This indicates that not only the cooking process but also the manipulation affects the retention of ascorbic acid in the tissues, due to its high degree of water solubility and low stability.

Thus, an appropriate method might be sought for *B. rapa* domestic processing is key to better retain its nutritional value at the maximum level. Our study may help consumers to make their choice of the cooking practices to retain the nutritional quality of turnip greens and turnip tops. In this regards, it is likely that *B. rapa* vegetables cooked by steaming will be better for human consumption than other cooking methods. Although since both phenolic compounds and GLS were present in high quantities in the cooking water after boiling and high-pressure, the use of this water for either soups or gravies should also be considered for increasing the intake of these health-beneficial compounds into the diet.

Acknowledgements

Research supported by the Xunta de Galicia (PGIDIT06RAG40302PR) and Excma. Diputación Provincial de Pontevedra. Marta Francisco acknowledges an I3P fellowship from the CSIC. The authors thank Rosaura Abilleira and Susana Calvo for all the invaluable help in the laboratory work.

Literature cited

Bennett, R. N., Mellon, F. A., Foidl, N., Pratt, J. H., Dupont, M. S., Perkins, L., & Kroon, P. A. (2003). Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees *Moringa oleifera* L. (horseradish tree) and *Moringa stenopetala* L. Journal of Agricultural and Food Chemistry, 51(12), 3546-3553.

Bones, A. M., & Rossiter, J. T. (1996). The myrosinase-glucosinolate system, its organisation and biochemistry. Physiologia Plantarum, 97(1), 194-208.

Ciska, E., & Kozlowska, H. (2001). The effect of cooking on the glucosinolates content in white cabbage. European Food Research and Technology, 212(5), 582-587.

Czarniecka-Skubina, E. (2002). Effect of the material form, storage and cooking methods on the quality of Brussels sprouts. Polish Journal of Food and Nutrition Sciences, 11/52(3), 75-82.

Fahey, J. W., Zalcmann, A. T., & Talalay, P. (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry, 56(1), 5-51.

Fenwick, G. R., Heaney, R. K., & Mullin, W. J. (1983). Glucosinolates and their breakdown products in food and food plants. Crc Critical Reviews in Food Science and Nutrition, 18(2), 123-201.

Fernandes, F., Valentão, P., Sousa, C., Pereira, J. A., Seabra, R. M., & Andrade, P. B. (2007). Chemical and antioxidative assessment of dietary turnip (*Brassica rapa* var. *rapa* L.). Food Chemistry, 105(3), 1003-1010.

Ferreres, F., Sousa, C., Vrchovska, V., Valentão, P., Pereira, J. A., Seabra, R. M., & Andrade, P. B. (2006). Chemical composition and antioxidant activity of tronchuda cabbage internal leaves. European Food Research and Technology, 222(1-2), 88-98.

Francisco, M., Moreno, D. A., Cartea, M. E., Ferreres, F., García-Viguera, C., & Velasco, P. (2009). Simultaneous identification of glucosinolates and phenolic compounds in a representative collection of vegetable *Brassica rapa*. J Chromatogr A, 1216(38), 6611-6619.

Gil, M. I., Ferreres, F., & Tomas-Barberan, F. A. (1999). Effect of postharvest storage and processing on the antioxidant constituents (flavonoids and vitamin C) of fresh-cut spinach. Journal of Agricultural and Food Chemistry, 47(6), 2213-2217.

Gliszczynska-Swiglo, A., Ciska, E., Pawlak-Lemanska, K., Chmielewski, J., Borkowski, T., & Tyrakowska, B. (2006). Changes in the content of health-promoting compounds and antioxidant activity of broccoli after domestic processing. Food Additives and Contaminants, 23(11), 1088-1098.

Gómez-Campo, C. (1999). Origin and domestication. In Gómez-Campo, C. Biology of Brassica Coenospecies pp. 33-52): Elsevier, Amsterdam.

González-Molina, E., Moreno, D. A., & García-Viguera, C. (2008). Genotype and harvest time influence the phytochemical quality of Fino lemon juice (Citrus limon (L.) Burm. F.) for industrial use. Journal of Agricultural and Food Chemistry, 56(5), 1669-1675.

Goodrich, R. M., Anderson, J. L., & Stoewsand, G. S. (1989). Glucosinolate changes in blanched broccoli and Brussels sprouts. Journal of Food Processing and Preservation, 13(4), 275-280.

Grubb, C. D., & Abel, S. (2006). Glucosinolate metabolism and its control. Trends in Plant Science, 11(2), 89-100.

Harbaum, B., Hubbermann, E. M., Wolff, C., Herges, R., Zhu, Z., & Schwarz, K. (2007). Identification of flavonolds and hydroxycinnamic acids in pak choi varieties (Brassica campestris L. ssp chinensis var. communis) by HPLC-ESI-MSn and NMR

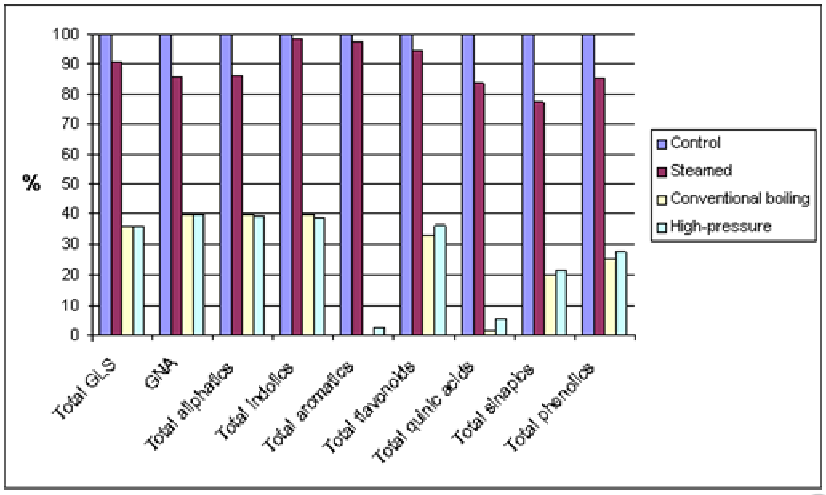
and their quantification by HPLC-DAD. Journal of Agricultural and Food Chemistry, 55, 8251-8260.
Howard, L. A., Wong, A. D., Perry, A. K., & Klein, B. P. (1999). Beta-carotene and ascorbic acid retention in fresh and processed vegetables. Journal of Food Science, 64(5), 929-936.
Kim, S. J., Kawaguchi, S., & Watanabe, Y. (2003). Glucosinolates in vegetative tissues and seeds of twelve cultivars of vegetable turnip rape (*Brassica rapa* L.). Soil Science and Plant Nutrition, 49(3), 337-346.
Liang, Y. S., Kim, H. K., Lefeber, A. W. M., Erkelens, C., Choi, Y. H., & Verpoorte, R. (2006). Identification of phenylpropanoids in methyl jasmonate treated *Brassica rapa* leaves using two-dimensional nuclear magnetic resonance spectroscopy. Journal of Chromatography A, 1112(1-2), 148-155.
Mithen, R., Faulkner, K., Magrath, R., Rose, P., Williamson, G., & Márquez, J. (2003). Development of isothiocyanate-enriched broccoli, and its enhanced ability to induce phase 2 detoxification enzymes in mammalian cells. Theoretical and Applied Genetics, 106(4), 727-734.
Mondragón-Portocarrero, A., Pena-Martínez, B., Fernández-Fernández, E., Romero-Rodríguez, A., & Vázquez-Oderiz, L. (2006). Effects of different pre-freezing blanching procedures on the physicochemical properties of *Brassica rapa* leaves (Turnip Greens, Grelos). International Journal of Food Science and Technology, 41(9), 1067-1072.
Padilla, G., Cartea, M. E., Velasco, P., de Haro, A., & Ordas, A. (2007). Variation of glucosinolates in vegetable crops of *Brassica rapa*. Phytochemistry, 68(4), 536-545.
Pereira, F. M. V., Rosa, E., Fahey, J. W., Stephenson, K. K., Carvalho, R., & Aires, A. (2002). Influence of temperature and ontogeny on the levels of glucosinolates in broccoli (*Brassica oleracea* var. *italica*) sprouts and their effect on the induction of mammalian phase 2 enzymes. Journal of Agricultural and Food Chemistry, 50(21), 6239-6244.
Podsedek, A. (2007). Natural antioxidants and antioxidant capacity of Brassica vegetables: A review. Lwt-Food Science and Technology, 40(1), 1-11.
Price, K. R., Casuscelli, F., Colquhoun, I. J., & Rhodes, M. J. C. (1998). Composition and content of flavonol glycosides in broccoli florets (*Brassica olearacea*) and their fate during cooking. Journal of the Science of Food and Agriculture, 77(4), 468-472.
Rangkadilok, N., Tomkins, B., Nicolas, M. E., Premier, R. R., Bennett, R. N., Eagling, D. R., & Taylor, P. W. J. (2002). The effect of post-harvest and packaging treatments on glucoraphanin concentration in broccoli (*Brassica oleracea* var. *italica*). Journal of Agricultural and Food Chemistry, 50(25), 7386-7391.
Rodrigues, A. S., & Rosa, E. A. S. (1999). Effect of post-harvest treatments on the level of glucosinolates in broccoli. Journal of the Science of Food and Agriculture, 79(7), 1028-1032.
Rosa, E. A. S. (1997). Glucosinolates from flower buds of Portuguese Brassica crops. Phytochemistry, 44(8), 1415-1419.
Rosa, E. A. S., & Heaney, R. K. (1993). The effect of cooking and processing on the glucosinolate content: Studies on four varieties of Portuguese cabbage and hybrid white cabbage. Journal of the Science of Food and Agriculture, 62(3), 259-265.
SAS Institute. (2007). The SAS System. SAS Online Doc. HTML Format Version Eight: SAS Institute Inc. Cary, North Carolina. USA.
Sies, H., & Stahl, W. (1995). Vitamin E and vitamin C, Beta-Carotene, and other carotenoids as antioxidants. American Journal of Clinical Nutrition, 62(6), S1315-S1321.

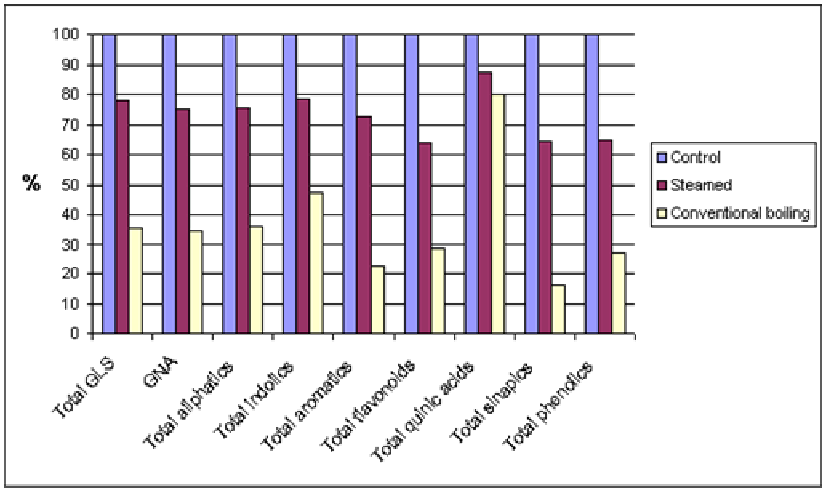
Song, L. J., & Thornalley, P. J. (2007). Effect of storage, processing and cooking on glucosinolate content of Brassica vegetables. Food and Chemical Toxicology, 45(2), 216-224.
Sousa, C., Taveira, M., Valentão, P., Fernandes, F., Pereira, J. A., Estevinho, L., Bento, A., Ferreres, F., Seabra, R. M., & Andrade, P. B. (2008). Inflorescences of Brassicacea species as source of bioactive compounds: A comparative study. Food Chemistry, 110(4), 953-961.
Steel, R. D. G., Torrie, J. H., & Dickey, D. A. (1997). Principles and procedures in statistics: a biometrical approach, 3rd ed: McGraw Hill, New York.
Traka, M., & Mithen, R. (2009). Glucosinolates, isothiocyanates and human health. Phytochemistry Reviews, 8(1), 269-282.
Vallejo, F., Tomas-Barberan, F. A., & Ferreres, F. (2004). Characterisation of flavonols in broccoli (*Brassica oleracea* L. var. *italica*) by liquid chromatography-UV diode-array detection-electrospray ionisation mass spectrometry. Journal of Chromatography A, 1054(1-2), 181-193.
Vallejo, F., Tomás-Barberán, F. A., & García-Viguera, C. (2002). Glucosinolates and vitamin C content in edible parts of broccoli florets after domestic cooking. European Food Research and Technology, 215(4), 310-316.
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.
Verhoeven, D. T. H., Verhagen, H., Goldbohm, R. A., vandenBrandt, P. A., & vanPoppel, G. (1997). A review of mechanisms underlying anticarcinogenicity by brassica vegetables. Chemico-Biological Interactions, 103(2), 79-129.
Verkerk, R., & Dekker, M. (2004). Glucosinolates and myrosinase activity in red cabbage (*Brassica oleracea* L. var. *capitata* f. rubra DC.) after various microwave treatments. Journal of Agricultural and Food Chemistry, 52(24), 7318-7323.
Verkerk, R., Dekker, M., & Jongen, W. M. F. (2001). Post-harvest increase of indolyl glucosinolates in response to chopping and storage of Brassica vegetables. Journal of the Science of Food and Agriculture, 81(9), 953-958.
Verkerk, R., Schreiner, M., Krumbein, A., Ciska, E., Holst, B., Rowland, I., De Schrijver, R., Hansen, M., Gerhauser, C., Mithen, R., & Dekker, M. (2009). Glucosinolates in Brassica vegetables: the influence of the food supply chain on intake, bioavailability and human health. Molecular Nutrition & Food Research, 53 Suppl 2, S219.
Volden, J., Wicklund, T., Verkerk, R., & Dekker, M. (2008). Kinetics of changes in glucosinolate concentrations during long-term cooking of white cabbage (*Brassica oleracea* L. ssp *capitata* f. alba). Journal of Agricultural and Food Chemistry, 56(6), 2068-2073.
Wachtel-Galor, S., Wong, K. W., & Benzie, I. F. F. (2008). The effect of cooking on Brassica vegetables. Food Chemistry, 110(3), 706-710.
Zapata, S., & Dufour, J. P. (1992). Ascorbic, dehydroascorbic and isoascorbic acid simultaneous determinations by reverse phase ion interaction HPLC. Journal of Food Science, 57(2), 506-511.
Zhang, D. L., & Hamauzu, Y. (2004). Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. Food Chemistry, 88(4), 503-509.
Zhang, Y. S., & Talalay, P. (1994). Anticarcinogenic activities of organic isothiocyanates: chemistry and mechanisms. Cancer Research, 54(7), S1976-S1981.

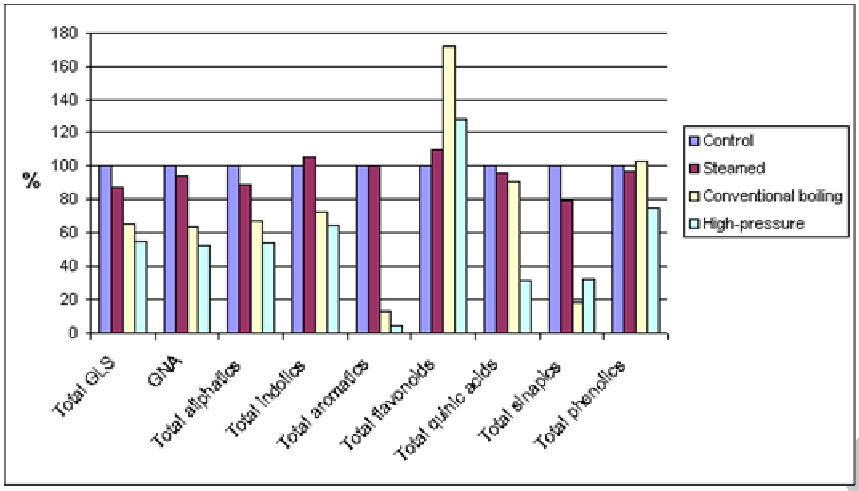
Figure captions

Figure 1. Content (% of the fresh uncooked control) of the main compounds after different cooking methods in the edible parts of turnip greens (a) and turnip tops (b).

Figure 2. Content (% of the fresh uncooked control) of the main compounds after different cooking methods in CW turnip greens (a) and CW turnip tops (b).







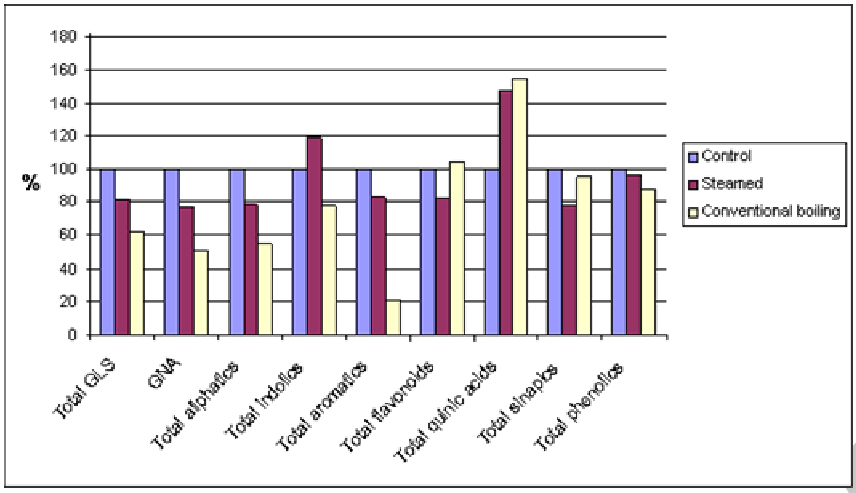


Table 1. Mean (μmol/g^{-1} dw) for the individual and total GLS, flavonoid and hydroxycinnamic acid content in turnip greens and turnip tops before (control) and after three cooking methods.

	Turnip greens					Turnip tops			
	Control	**Steaming**	**Conventional boiling**	**High-pressure**	**LSD (5 %)**	**Control**	**Steaming**	**Conventional boiling**	**LSD (5 %)**
GLS									
PRO	0.83a	0.57ab	0.30bc	0.23c	0.28	0.13a	0.12a	0.05a	0.44
GNA	6.27a	5.38b	2.48c	2.49c	0.80	7.25a	5.43a	2.48b	2.47
GBN	1.44a	1.43a	0.61b	0.63b	0.28	1.31a	1.01a	0.60b	0.44
Total aliphatics	8.55a	7.38b	3.38c	3.35c	1.12	8.69a	6.56a	3.13b	2.08
4-OHGBS	0.47b	0.87a	0.09c	0.26c	0.26	0.42a	0.40a	0.00a	0.37
GBS	1.50a	1.14b	0.35ce	0.20c	0.17	1.54a	0.90b	0.52b	0.42
NGBS	1.24a	1.16a	0.83b	0.80b	0.21	1.18a	1.16a	0.97a	0.25
Total indolics	3.21a	3.17a	1.27b	1.25b	0.40	3.14a	2.46b	1.49c	0.45
GNT	1.23a	1.20a	0.00b	0.03b	0.18	1.21a	0.88a	0.27b	0.52
Total GLS	12.99a	11.80a	4.66b	4.64b	1.32	12.84a	10.02b	4.56c	2.75
Flavonoids									
F1	2.18a	2.32a	0.85b	089b	0.31	2.00a	1.48a	0.65b	0.01
F2	1.85a	1.67a	0.57b	0.57b	0.22	1.47a	1.21a	0.51b	0.28
F3	1.23a	1.41a	0.16c	0.65b	0.27	1.93a	1.44a	0.60b	0.49
F4	1.44a	1.38a	0.50b	0.64b	0.20	1.75a	1.22a	0.57b	0.56
F5	0.96a	0.86a	0.40b	0.19c	0.17	0.97a	0.37b	0.11b	0.35
F6	1.75a	1.47a	0.78b	0.78b	0.52	2.14a	1.37b	0.70c	0.62
F7	3.29a	3.55a	1.32b	1.28b	0.46	1.76a	1.15ab	0.52b	0.71
Total flavonoids	13.85a	13.10a	4.58b	5.00b	1.63	13.10a	8.40b	3.70c	3.13
Hydroxycinnamics									
3CQAc	0.41a	0.31b	0.01c	0.03c	0.06	0.19a	0.33a	0.16a	0.21
3*p*CoQAc	0.33a	0.32a	0.00b	0.01b	0.05	0.15a	0.26a	0.16a	0.19
Total quinic acids	0.75a	0.63b	0.01c	0.04c	0.09	0.40a	0.35a	0.32a	0.39
SA	12.27a	9.58b	3.04c	3.26c	1.58	0.68a	0.52a	0.25b	0.18
A1	1.48a	0.93b	0.14c	0.07c	0.30	0.21a	0.13b	0.00c	0.46
A2	1.73a	1.60a	0.12b	0.19b	0.3	0.25a	0.17a	0.00b	0.0

					0				9
A3	1.68a	0.51b	0.01c	0.03c	0.11	0.17a	0.14a	0.00b	0.11
A4	0.43a	0.24b	0.03c	0.04c	0.08	0.08a	0.01b	0.00c	0.01
Total sinapics	16.59 a	12.86b	3.33c	3.60c	0.62	1.78a	1.15b	0.29c	0.35
Total phenols	31.51 a	26.87b	7.93c	8.67c	3.37	14.80 a	9.60b	4.02c	3.23

PRO: progoitrin; GNA: gluconapin; 4-OHGBS: 4-hydroxyglucobrassicin; GBN: glucobrassicanapin; GBS: glucobrassicin; GNT: gluconasturtiin; NGBS: neoglucobrassicin; F1: kaempferol-3-O-sophoroside-7-O-glucoside; F2: kaempferol-3-O-(caffeoyl)sophoroside-7-O-glucoside; F3: kaempferol-3-O-(sinapoyl)sophoroside-7-O-glucoside; F4: kaempferol-3-O-(feruloyl)sophoroside-7-O-glucoside; F5: kaempferol-3-O-(p-coumaroyl)sophoroside-7-O-glucoside; F6: kaempferol-3,7-di-O-glucoside; F7:isorhamnetin-3,7-di-O-glucoside; 3CQAc: 3-caffeoyl quinic acid; 3*p*CoQAc: 3-p-coumaroylquinin acid; SA: sinapic acid; A1: 1,2-disinapoylgentiobioside; A2: 1-sinapoyl-2-feruloylgentiobioside; A3: 1, 2, 2´-trisinapoylgentiobioside; A4: 1,2´-disinapoyl-2-feruloylgentiobioside
Means with the same letter in the same row are not significant different.

Table 2. Mean (μmol/g^{-1} dw) for the individual and total GLS, flavonoid and hydroxycinnamic acid content in CW of turnip greens and turnip tops as compared to the uncooked tissue (control) and after three cooking methods.

	Turnip greens					**Turnip tops**			
	Control	**Steaming (CW)**	**Conventional Boiling (CW)**	**High-pressure cooking (CW)**	**LSD (5 %)**	**Control**	**Steaming (CW)**	**Conventional Boiling (CW)**	**LSD (5 %)**
GLS									
PRO	0.83a	0.63ab	0.56ab	0.29b	0.38	0.13a	0.22a	0.10a	0.47
GNA	6.27a	5.44a	4.05b	3.40b	0.93	7.25a	5.58b	3.68c	2.70
GBN	1.44a	1.52a	1.13a	0.90a	0.25	1.31a	1.07a	0.97a	0.49
Total aliphatics	8.55a	7.59a	5.74b	4.59b	0.95	8.69a	6.87b	4.75c	2.10
4-OHGBS	0.47a	0.93a	0.38a	0.57a	0.29	0.42a	1.15a	0.23a	0.27
GBS	1.50a	1.23b	0.62c	0.43c	0.27	1.54a	1.16a	1.01a	0.57
NGBS	1.24a	1.21a	1.32a	1.06a	0.25	1.18a	1.44a	1.20a	0.20
Total indolics	3.21a	3.37a	2.32b	2.06b	0.46	3.14a	3.75a	2.44b	0.62
GNT	1.23a	1.27a	0.16b	0.05b	0.20	1.21a	1.01a	0.26b	0.49
Total GLS	12.99a	12.26a	8.24b	6.71c	1.51	12.84a	10.42ab	7.93b	3.10
Flavonoids									
F1	2.18b	2.61b	4.20a	4.50a	0.54	2.00a	2.30a	2.45a	0.86
F2	1.85a	1.91a	1.74a	0.79b	0.64	1.47a	1.10a	1.89a	0.70
F3	1.23b	1.44ab	1.90a	1.18b	0.61	1.93a	1.44a	1.07a	0.45
F4	1.44b	1.74ab	2.16a	1.33b	0.59	1.75a	1.22a	1.13a	0.58
F5	0.96a	1.09b	1.98a	0.47c	0.49	0.97a	0.51a	0.37a	0.61
F6	1.75b	1.83b	4.13a	3.63a	0.92	2.14a	2.01a	2.96a	0.72
F7	3.29c	4.02c	6.90a	5.35b	1.11	1.76a	1.52a	1.84a	0.62
Total flavonoids	13.85b	15.20b	23.81a	17.76b	4.10	13.10a	10.75a	13.40a	3.13
Hydroxycinnamics									
3CQAc	0.41a	0.35a	0.24ab	0.10b	0.17	0.19a	0.33a	0.30a	0.22
3*p*CoQAc	0.33a	0.37a	0.44a	0.14b	0.14	0.15a	0.26a	0.32a	0.24
Total quinic acids	0.75a	0.72a	0.68a	0.24b	0.31	0.40a	0.59a	0.62a	0.46
SA	12.27a	9.74b	5.07c	4.72c	1.65	0.68a	0.75a	1.40a	0.28
A1	1.48a	0.96c	0.26c	1.11b	0.32	0.21a	0.13a	0.04a	0.09

A2	1.73a	1.64a	0.36b	0.27b	0.34	0.25a	0.16a	0.06a	0.09
A3	1.68a	0.54b	0.20c	0.12c	0.14	0.17a	0.14a	0.07a	0.10
A4	0.43a	0.24b	0.15b	0.18b	0.11	0.08a	0.07a	0.01a	0.03
Total sinapics	16.59 a	13.11b	3.04c	5.40c	1.96	1.78a	1.38a	1.70a	0.50
Total phenols	31.51 a	30.40a	32.40a	23.44b	5.08	14.80 a	14.20a	13.00a	3.33

PRO: progoitrin; GNA: gluconapin; 4-OHGBS: 4-hydroxyglucobrassicin; GBN: glucobrassicanapin; GBS: glucobrassicin; GNT: gluconasturtiin; NGBS: neoglucobrassicin; F1: kaempferol-3-O-sophoroside-7-O-glucoside; F2: kaempferol-3-O-(caffeoyl)sophoroside-7-O-glucoside; F3: kaempferol-3-O-(sinapoyl)sophoroside-7-O-glucoside; F4: kaempferol-3-O-(feruloyl)sophoroside-7-O-glucoside; F5: kaempferol-3-O-(p-coumaroyl)sophoroside-7-O-glucoside; F6: kaempferol-3,7-di-O-glucoside; F7:isorhamnetin-3,7-di-O-glucoside; 3CQAc: 3-caffeoyl quinic acid; 3*p*CoQAc: 3-p-coumaroylquinin acid; SA: sinapic acid; A1: 1,2-disinapoylgentiobioside; A2: 1-sinapoyl-2-feruloylgentiobioside; A3: 1, 2, 2´-trisinapoylgentiobioside; A4: 1,2´-disinapoyl-2-feruloylgentiobioside
Means with the same letter in the same row are not significant different.