Effects of domestic processing methods on the phytochemical content of watercress (Nasturtium officinale)

Title

Effects of domestic processing methods on the phytochemical content of watercress *(Nasturtium officinale)*.

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Abstract

The impact of conventional cooking and processing methods on total phenols, antioxidant activity, carotenoids and glucosinolates of watercress was evaluated. Boiling significantly decreases phenolic content, antioxidant activity and recoverable glucosinolates, however it increases the carotenoid concentrations of watercress as compared to the raw vegetable. Cooking by microwaving and steaming maintains the majority of phytochemicals in comparison to the fresh material, suggesting that they should be used as the preferred methods of watercress preparation. Boiling of watercress should be avoided to ensure maximum ingestion of watercress-derived beneficial phytochemicals.

Keywords

Watercress; Brassica; Processing; Phytochemicals; Phenolics; Carotenoids; Glucosinolates; Flavonols

1.0 Introduction

Watercress (*Nasturtium officinale)* belongs to the family of *Brassicaceae* together with broccoli, cabbage, mustard and Brussels sprouts. Epidemiological studies associate a higher intake of Brassica vegetables, such as watercress, with a reduced risk of various types of cancers (Verhoeven, Goldbohm, vanPoppel, Verhagen & vandenBrandt, 1996). Watercress is an exceptional source of natural, bioactive compounds for which research has highlighted a favourable role in anti-genotoxic and anti-cancer processes both *in vivo* and *in vitro* (Boyd, McCann, Hashim, Bennett, Gill & Rowland, 2006; Gill, Haldar, Boyd, Bennett, Whiteford, Butler, et al., 2007; Rose, Faulkner, Williamson & Mithen, 2000). The health

benefits of watercress have been attributed to phytochemicals including glucosinolates, carotenoids and flavonoid compounds.

Watercress, and essentially all members of the *Brassicaceae* family, have been identified as a rich source of glucosinolates (Bell & Wagstaff, 2014). Glucosinolates are hydrolysed to isothiocyanates by the action of the enzyme myrosinase (β-thiglucoside glucohydrolase; EC 3.2.3.1), upon cell tissue damage such as mastication, chopping or cooking. This group of plant bioactive compounds is responsible for the characteristic pungent taste that Brassica vegetables possess. Gluconasturtiin (2-phelylethyl glucosinolate) is the most prominent glucosinolate in watercress (Boyd, et al., 2006; Gill, et al., 2007) with a range of aliphatic and indole glucosinolates adding to its glucosinolate profile.

High concentrations of carotenoids and flavonol compounds are also contained in watercress. Carotenoids with well established health benefits such as β-carotene, lutein and zeaxanthin are abundant in watercresss (Hart & Scott, 1995). Flavonols like quercetin, kaempferol and isorhamnetin, make up the polyphenolic core of watercress (Martinez-Sanchez, Gil-Izquierdo, Gil & Ferreres, 2008). Polyphenols have attracted great importance due to their many health benefits related to cardiovascular function, antioxidant and anticancer activity (Morel, Lescoat, Cillard, & Cillard, 1994 Doostdar, Burke, & Mayer, 2000; Galati, Teng, Moridani, Chan, & O'Brien, 2000).

While watercress is widely consumed raw in salads, it is becoming increasingly popular in cooked foods such as soups, smoothies and also wilted in pasta and meat dishes. Annual retail sales of watercress in the United Kingdom amounted to 40 million pounds in 2015. Sales of food products with cooked or processed watercress as the main ingredient have taken off the last few years,

representing approximately 50% of total watercress sales (S. Rothwell, Vitacress salads LTD, personal communication, March 10, 2016). Culinary processing is the source of several complex biochemical and physical alterations, modifying the phytochemical constituents of vegetables, ultimately resulting in nutritional changes (Palermo, Pellegrini & Fogliano, 2014).

To our knowledge, phytochemical characterisation of watercress subjected to different culinary treatments has not been explored to date. The present research was undertaken to elucidate the effects of five common cooking methods on the phytochemical profile of watercress and formulate suggestions for the most appropriate method for consuming watercress for maximum nutrient ingestion.

2.0 Materials and methods

2.1 Plant Material

Fresh watercress samples were provided from VITACRESS LTD (Andover, Hampshire, UK), transferred to the laboratory and stored at 4 °C for up to 24 hours until all watercress processing analyses were performed. Only samples free from mechanical damage were used in the experiments. All analyses were performed in triplicate using the same batch of plant material to minimise variation in our results.

2.2 Reagents & Chemicals

All chemicals were obtained from Sigma Aldrich (Poole, UK), unless otherwise stated.

2.3 Domestic Processing

The effect of domestic processing on the phytochemical content and antioxidant activity of watercress was examined by cooking of the plant material by boiling, microwaving, steaming, chopping and blending with water to make a watercress smoothie. Processing treatments and cooking times used were decided upon general consumer preferences and after online search of watercress recipes as well as using past research papers looking at the effects of domestic processing in other types of Brassica vegetables. 100 g portions of watercress were used for each replicate *(n=3).* Temperature data for boiling and steaming treatments were recorded throughout cooking, using a temperature logger (Squirrel OQ610-S, Grant instruments, UK) and a type T thermocouple.

2.3.1 Boiling (n=3): 500 ml of tap water was brought to boil (90 °C) in a stainless steel pot and watercress was boiled for 2, 5 and 10 min. Watercress was removed from the boiling water and water used for cooking was kept at -20 °C for analysis.

2.3.2 Microwaving (n=3): Fresh watercress was placed in plastic trays, then transferred to a domestic microwave oven (Panasonic, UK) and cooked at full power (1400 W) for 1, 2 and 3 min.

2.3.3 Steaming (n=3): A domestic steamer (Russel Hobbs, UK) was pre-heated at 100 °C with 500 ml water at its base. Watercress was placed in the steamer and cooked for 5, 10 and 15 min.

2.3.4 Chopping (n=3): 100 g of watercress was transferred to a food processor (Waring Commercial, New York, USA) and chopped for 30 secs at full speed. To study the effect of storage time on the phytochemical content, the chopped watercress was left on the bench at room temperature (21 °C) for 0, 10, 30, 60

and 120 min to replicate how watercress can be treated at home when chopped in salads or other dishes and not consumed immediately after preparation.

2.3.5 Watercress smoothie (n=3): 100 g of the plant material was transferred to a juice maker (Vitamix, Total Nutrition Centre, UK), 200 ml of water was added and the watercress was blended for 30 secs at full power. The effect of storage time was also examined by leaving the smoothie on the bench at room temperature (21 °C) for 0, 10, 30, 60 and 120 min.

After processing, all samples were immediately frozen in liquid nitrogen then freeze-dried (Christ A 2-4 LD, Christ, Germany); ground to fine powder using a coffee bean grinder (De'Longhi, Italy), vacuum packed and stored at -20 °C.

2.4 Preparation of watercress extracts

2.4.1 Crude methanol (MeOH) extracts: The method used for the preparation of the extracts was adapted from Bell *et al*. (Bell, Oruna-Concha & Wagstaff, 2015) Briefly, 40 mg of ground watercress powder was heated in a dry-block at 75 °C for 2 min to inactivate myrosinase enzyme. Preheated (70 °C) 70% (v/v) MeOH (1 ml) was then added to each sample and placed in a water bath for 20 min at 70 °C. Samples were then centrifuged for 5 min at 6,000 rpm and the supernatant was transferred to fresh tubes. The final volume was adjusted to 1 ml with 70% (v/v) MeOH and stored at -20 °C until the day of analysis. MeOH extracts were used for the FRAP assay, total phenols as well as flavonols and glucosinolates identification and quantification.

2.4.2 Acetone extracts: Total and specific carotenoids were determined in acetone watercress extracts. Watercress powder (25 mg) was weighed out in Falcon tubes (12 ml) previously wrapped in aluminium foil to minimise the degradation of carotenoids by ultra-violet light. Acetone (4 ml) was added to the powder and the

samples were shaken for 15 min at 8000 rpm. Following centrifugation at 4000 rpm for 5 min, the supernatant was transferred to a clean tube and the process was repeated (4 ml acetone for the second time and 2 ml the third time) until a colourless supernatant was obtained. The combined supernatants were transferred in fresh tubes and the final volume was adjusted to 10 ml with 100% acetone.

2.5 Determination of total phenolics

Total phenols were measured using the method developed by Singleton and Rossi (Singleton & Rossi, 1965) with slight modifications. Briefly, 0.2 ml of the MeOH watercress extract (Section 2.4) or blank was added to 6.0 ml of distilled water in volumetric flasks and mixed with 0.5 ml of Folin - Ciocalteu reagent. A sodium carbonate solution 20% (v/v) (1.5 ml) was added to the mixture and the volume was adjusted to 10 ml. Absorbance was read after incubation of the samples for two hours at room temperature, at 760 nm using a UV-Vis Spectrophotometer (UV-VIS, Perkin Elmers, UK). A standard curve was made using gallic acid in the following concentrations: 0, 50, 100, 150, 250, 500, 750 &1000 mg/L and total phenols were measured as gallic acid equivalents (R^2 > 0.99).

2.6 FRAP (Ferric Reducing Antioxidant Power) assay

Antioxidant activity of the samples was determined using the FRAP assay based on an adapted version of the method developed by Benzie and Strain (Benzie & Strain, 1996). The FRAP reagent was made by mixing 25 ml of 300 mM acetate buffer (pH 3.6), 2.5 ml 10 mM 2,4,6-tripyridyl-s-triazine solution (TPTZ) and 2.5 ml of freshly prepared ferric chloride hexahydrate ($FeCl_3 \cdot 6H2O$). A standard curve

was made using L-Ascorbic acid in the following concentrations: 0, 10, 50, 100, 250, 500, 750, 1000 µmol/L (R^2 > 0.99). Each MeOH extract (Section 2.4) or standard (10 µl) was combined with 300 µl of the FRAP reagent and 100 µl of the mixture was transferred in duplicate in a 96-well plate. Absorbance was measured immediately using a plate reader (Tecan GENios, Geneva, Switzerland) at 595 nm.

2.7 Total carotenoids

An aliquot of the acetone extracts prepared as previously described (Section 2.4) was used to quantify the total carotenoid content of the samples spectrophotometrically. Absorbance was measured at 470, 645 and 662 nm in a spectrophotometer (UV-VIS, Perkin Elmers, UK). The total amount of carotenoids was calculated according to the following equations by Lichtenthaler (Lichtenthaler & Buschmann, 2001).

$$C_a = 11.24\, A_{662} - 2.04\, A_{645}$$

$$C_b = 20.13\, A_{645} - 4.19\, A_{662}$$

$$C_{a+b} = 7.05\, A_{662} + 18.09\, A_{645}$$

$$C_{x+c} = \frac{1000\, A_{470} - 190\, C_a - 63.14\, C_b}{214}$$

*Chlorophyll a (C_a), Chlorophyll b (C_b), Total Chlorophylls (C_{a+b}), Total Carotenoids (C_{x+c}). Equations are based on specific absorption coefficients for 100% acetone. The pigment concentrations obtained by inserting the measures absorbance values are µg/ml plant extract solution.

185 *2.8 Quantification of carotenoids via HPLC*

186 To determine the amount of lutein, zeaxanthin and β-carotene present, the
187 acetone extracts were used (Section 2.4). Carotenoids were quantified using the
188 method developed by Guiffrida *et al*. (Giuffrida, Dugo, Torre, Bignardi, Cavazza,
189 Corradini, et al., 2013) with modifications.10 ml of the extract was mixed with 10
190 ml of diethyl ether, 10 ml of water and 5ml of 10% (v/v) NaCl. Two layers were
191 formed and the lower - acetone phase was discarded. The upper layer containing
192 the ether was collected in a glass vial and anhydrous Na_2SO_4 was added to it to
193 remove any moisture from the solution. The ether phase was transferred to a
194 clean glass vial, the volume was adjusted to 10ml with diethyl ether and the
195 solution was condensed under nitrogen gas. The dry residue was then
196 reconstituted in 1 ml of methyl tert- butyl ether (MTBE):MeOH (1:1, v/v), filtered
197 using 0.22 µm syringe driven filter unit and analysed by HPLC. The analyses
198 were performed using an YMC30 column (5 µm 250 x 4.6 mm) on a HP Agilent
199 1050 series HPLC system. The mobile phases used were as follows: Eluent A,
200 consisting of MeOH:MTBE:H_2O (82:16:2 v/v/v) and Eluent B, consisting of
201 MeOH:MTBE:H_2O (23:75:2 v/v/v). The analyses followed a gradient program for
202 the mobile phases, 0 min 0% B, 20 min 0% B, 80 min 70% B, 90 min 70% B. The
203 protocol used a 1 mL/min flow rate and a 100 µL injection volume. UV-vis spectra
204 were gathered in the range of 190-600 nm and the chromatograms were analysed
205 at 450 nm. Identification was based on retention times by comparison with HPLC
206 grade standards of lutein, zeaxanthin and β-carotene (Extrasynthese, France).

207 *2.9 Identification and quantification of glucosinolates and flavonols via LC-*
208 *MS/MS*

209 Methanol extracts, prepared as described above, were used for the quantification

of glucosinolates and flavonols in the samples (Section 2.4.1). 1ml of each extract was filtered using a 0.22 µm syringe driven filter unit (Millex; EMD Millipore, Billerica, MA, USA) and then diluted using 9ml LC-MS grade water. For the quantification of glucosinolates and flavonols, external calibration curves of 12 mM sinigrin hydrate and isorhamnetin standards were prepared using the following concentrations (56 $ng.\mu l^{-1}$, 42 $ng.\mu l^{-1}$, 28 $ng.\mu l^{-1}$, 14 $ng.\mu l^{-1}$, 5.6 $ng.\mu l^{-1}$, $R^2 > 0.99$). Glucosinolates and flavonols were analysed by LC-MS/MS using an Agilent 1200 LC system coupled to an Agilent 1100 series LC/MSD mass trap spectrometer. Separation conditions of samples and MS analysis settings used are identical to those described by Bell, et al. (2015) . Glucosinolates were quantified at 229 nm and flavonols at 330 nm. The identification was performed using the compounds nominal mass and the analysis of their fragmentation patterns, and also by the comparison with previously published data. All data were analysed using Agilent ChemStation.

2.10 Statistical Analysis

The results are presented as the mean of three biological replicates (n = 3) for each sample. One-way ANOVA and Dunnett's multiple comparisons test were used for comparison of all treatments related to the raw watercress. These analyses were carried out using GraphPad Prism version 5.0a for Mac OS X,GraphPad software (Version 5.0a La Jolla, California, USA). Principal component analysis (PCA) and correlation analysis were performed using XL Stat (Version 2016 Addinsoft, New York City, New York, USA).

3.0 Results and Discussion

3.1 Total phenols content

Fresh watercress had the highest amount of total phenols (14.86 ± 2.02 mg GAE g^{-1} DW) compared to the processed samples (Figure 1A). Our results are in agreement with that of Aires, Carvalho, Rosa and Saavedra (2013) who found the phenolic content of watercress to be 14.00 ± 0.03 mg GAE g^{-1} DW. In comparison to other vegetables in the Brassica family, watercress is a rich source of phenolic compounds. It has a similar amount to kale (16.67 ± 0.67 mg GAE g^{-1} DW) (Hagen, Borge, Solhaug & Bengtsson, 2009) and it is much higher than broccoli and cabbage which have a lower phenolic content that being 8.86 mg and 5.6 mg GAE g^{-1} DW respectively (Gliszczynska-Swiglo, Ciska, Pawlak-Lemanska, Chmielewski, Borkowski & Tyrakowska, 2006; Puupponen-Pimiä, Häkkinen, Aarni, Suortti, Lampi, Eurola, et al., 2003).

Boiling of watercress resulted in a significant decrease ($P<0.05$) in the total phenolic content in comparison with the fresh samples. Total phenolic losses ranged from 49% to 71% in the samples boiled for 2 and 10 minutes respectively. Microwaving and steaming for up to 5 minutes did not significantly affect the phenolic content of watercress ($P>0.05$). Likewise, blending with water to make a watercress smoothie and chopping did not have a significant effect on the total phenolic content in the watercress. However, storage of the smoothies and the chopped watercress samples for 120 minutes at room temperature resulted in a significant reduction of the phenolics from 13.65 ± 1.56 to 10.76 ± 1.15 mg GAE g^{-1} DW and from 10.55 ± 1.48 to 8.65 ± 2.29 mg GAE g^{-1} DW respectively (Figure 1A).

256 Our results are corroborated by previous studies showing that boiling of Brassica
257 vegetables can lead to significant time dependant losses of phenolics whereas
258 microwaving and steaming led to only minor decreases in the phenolic content of
259 broccoli (Turkmen, Sari & Velioglu, 2005; Zhang & Hamauzu, 2004), red cabbage
260 (Podsedek, Sosnowska, Redzynia & Koziolkiewicz, 2008) and cauliflower (Natella,
261 Belelli, Ramberti & Scaccini, 2010). During the process of cooking, phenolic
262 compounds appear to be highly reactive undergoing several changes including
263 their release from bound forms, oxidation, degradation and polymerisation
264 (Gliszczynska-Swiglo, et al., 2006).
265 The losses during boiling can be attributed to water-soluble compounds leaching
266 into the water used for boiling or due to breakdown of these compounds during
267 thermal processing. Indeed, analysis of the water used in the boiling experiments
268 (9.35 ± 0.12 mg GAE g^{-1} DW) for total phenolics revealed that phenols had
269 leached into the boiling water. The total amount of phenols in the water used in
270 boiling and the remaining phenol content of watercress was no different from the
271 total phenols in raw watercress. The minimal effect of microwaving and steaming
272 on the phenolic compounds is potentially a result of limited or no contact of the
273 samples with water and also the inactivation of oxidative enzymes preventing the
274 disruption of phenolic biosynthesis and degradation (Vallejo, Tomás-Barberán &
275 García-Viguera, 2003).

276 *3.2 Flavonols identification and quantification*

277 Flavonol profiling of watercress revealed three main derivatives namely
278 kaempferol, quercetin and isorhamnetin as well as feruloyl, ceffeoyl, p-coumaroyl
279 and sinapoyl glucosides attached to kaempferol and quercetin. Kampferol-3-
280 diglucoside-7-glucoside was the most abundant flavonol detected (3.76 ± 0.09 mg

281 g^{-1} DW). The flavonols identified in the fresh watercress leaves are similar to those
282 defined by Martinez-Sanchez, et al. (2008).
283 Domestic processing of watercress resulted in a significant decrease in the levels
284 of all quantified flavonols (Table 1). The only exception was Q 3,4'diGlc-3'-
285 (p.coum-Glc) + K 3,4'-diGlc which appeared to be the most stable of all flavonols
286 and were only significantly affected by boiling ($P<0.05$). Total flavonol losses
287 suggest that these compounds are particularly sensitive to all cooking regimes
288 used. Boiling for 10 minutes nearly depleted all watercress samples of flavonols in
289 a time dependent manner. The unstable nature of flavonols was also apparent in
290 chopped watercress and watercress smoothie with the levels going down to 3.42 ±
291 0.32 and 4.11 ± 0.36 mg g^{-1} DW respectively as compared to the total amount of
292 flavonols in the fresh samples (10.70 ± 1.07mg g^{-1} DW, $P<0.001$). Similarly to total
293 phenols, the highest retention of flavonols was observed in the microwaved
294 watercress followed by steamed.

295 *3.3 Carotenoid content*

296 In contrast to the previous assays, boiling of watercress resulted in an increased
297 concentration of total measurable carotenoids, from 2.35 ± 0.22 mg g^{-1} DW in the
298 fresh samples to 3.13 ± 0.20 mg g^{-1} DW after 2 minutes of cooking and up to 3.28
299 ± 0.30 mg g^{-1} DW after 5 minutes of boiling (Table 1). Microwaving and steaming
300 did not have a significant impact on the level of total carotenoids ($P>0.05$). On the
301 other hand, the watercress smoothie had significantly lower total carotenoid
302 content, with the levels decreasing from 1.54 ± 0.21 to 1.11± 0.08 mg g^{-1} DW after
303 60 minutes of storage at ambient temperature. A similar decreasing trend was
304 observed in the chopped watercress samples.

305 The individual carotenoids identified and quantified in our watercress samples
306 were β-carotene, lutein and zeaxanthin and they all resulted in distinct responses
307 upon domestic processing. β-carotene was the most abundant of the three
308 quantified carotenoids (0.95 ± 0.08 mg g^{-1} DW) and its levels significantly
309 increased after thermal treatment of the watercress samples. Boiling for 5 minutes
310 resulted in β-carotene being significantly increased up to 1.75 ± 0.09 mg g^{-1} DW
311 as compared to the raw samples ($P<0.001$). In the microwaved watercress
312 samples β-carotene was increased up to 1.48 ± 0.26 mg g^{-1} DW ($P<0.01$) and in
313 the samples steamed for 15 minutes levels went up to 1.54 ± 0.07 mg g^{-1} DW
314 ($P<0.001$). β-carotene was decreased in the watercress smoothie only after
315 storage for 30 and 60 and 120 minutes ($P<0.01$) therefore, immediate
316 consumption of a watercress smoothie ensures sufficient intake of β-carotene. No
317 significant differences were found in the chopped samples.
318 Lutein content of fresh watercress samples was 0.24 ± 0.02 mg g^{-1} DW and it
319 exhibited the highest degree of stability after watercress processing. It was
320 significantly increased only after 5 minutes of boiling going up to 0.36 ± 0.02 mg g^{-1} DW ($P<0.05$). Significant decreases in lutein were only observed in the smoothie
322 after 120 minutes of storage ($P<0.05$). Zeaxanthin concentration in fresh
323 watercress was notably lower than β-carotene and lutein (0.02 ± 0.00 mg g^{-1} DW).
324 It was dramatically affected by boiling with increases higher than 6 and 3 times, as
325 compared to fresh watercress, after boiling for 5 minutes and steaming for 10
326 minutes respectively.
327 Increases in the carotenoid contents of other Brassica vegetables such as
328 broccoli, Brussels sprouts, cabbage and cauliflower upon boiling and steaming
329 have been reported by a number of research groups (Bernhardt & Schlich, 2006;

330 Gliszczynska-Swiglo, et al., 2006; Hart, et al., 1995). Elevations in the measurable
331 carotenoid concentrations after thermal treatments can be explained by changes
332 in the plant cell wall due to the breakdown of cellulose as well as improved
333 extractability of carotenoids from the plant as a result of the denaturation of
334 carotenoid-protein complexes due to thermal processing (Khachik, Beecher, Goli
335 & Lusby, 1991).

336 *3.4 Glucosinolate identification and quantification*

337 Gluconasturtiin was the most abundant glucosinolate in fresh and cooked
338 watercress samples followed by the indole glucosinolates: glucobrassicin, 4-
339 methoxyglucobrassicin, 4-hydroxyglucobrassicin and the aliphatic glucosinolate
340 glucoibarin (Table 3). The profile characterised here is similar to that previously
341 defined by Boyd, et al. (2006); Gill, et al. (2007).

342 Glucosinolate quantification revealed a major impact of cooking on the levels of
343 these phytochemicals. Boiling reduced the levels of total glucosinolates by up to
344 63% and led to significant loses of all the individual glucosinolates identified in this
345 study ($P<0.001$). Considerable glucosinolate losses after boiling of Brassica
346 vegetables like broccoli, cauliflower and Brussels sprouts, have also been
347 observed in other studies performed by a number of research groups (Song &
348 Thornalley, 2007; Vallejo, Tomás-Barberán & Garcia-Viguera, 2002). Heat
349 application combined with cooking in water can result in depletion of
350 glucosinolates in Brassica as a result of enzyme activity modification and
351 thermally induced breakdown processes (Jones, 2007; Palermo, et al., 2014).
352 Boiling of watercress in water caused significant loss of glucosinolates that most
353 likely have leached into the cooking water. Similar conclusions were drawn by
354 Song, et al. (2007) who showed that boiling of Brassica vegetables leads to

355 significant leaching of glucosinolates in the boiling water. Jones (2007) have
356 shown that the glucosinolate losses in Brassica vegetables are positively
357 correlated with the cooking time.
358 Microwaving and steaming had a subtle effect on glucosinolate concentrations
359 with minor losses at the longest cooking duration, as compared to the other
360 treatments. Microwaving and steaming for 2 or 5 minutes did not result in major
361 losses of total glucosinolates suggesting that these cooking methods will ensure a
362 higher retention rate of these phytochemicals. Our results are in agreement with
363 that of Song, et al. (2007) who examined the impact of different cooking methods
364 on broccoli, brussels sprouts, cauliflower and green cabbage. This observation is
365 likely due to denaturation and subsequent deactivation of the myrosinase enzyme,
366 which depletes glucosinolates in favour of their hydrolysis to isothiocyanates, after
367 application of high temperatures during cooking (Verkerk, vanderGaag, Dekker &
368 Jongen, 1997). We found that cooking by steaming resulted in a slight increase in
369 gluconasturtiin concentrations from 1.76 to 2.04 mg g^{-1} DW ($P<0.05$) and it can
370 therefore be considered as the preferred method of watercress consumption to
371 maximise gluconasturtiin levels. Elevated gluconasturtiin concentrations upon
372 steaming are also reported by Gliszczynska-Swiglo, et al. (2006) in broccoli.
373 Increases in other glucosinolates in Brassica vegetables subjected to steaming
374 have been also been noted in a number of studies (Pellegrini, Chiavaro, Gardana,
375 Mazzeo, Contino, Gallo, et al., 2010; Vallejo, et al., 2002). The inactivation of
376 myrosinase at the high temperatures such as the ones reached during steaming,
377 can temporarily cease the conversion of glucosinolates to isothiocyanates
378 (Vallejo, et al., 2002) a process which can be undertaken post ingestion*, in vivo*,
379 by the action of the endogenous bacterial myrosinase in the gut (Rouzaud, Rabot,

380 Ratcliffe & Duncan, 2003). Furthermore, heat application leads to plant cell
381 structure disintegration allowing glucosinolates to be released from their bound
382 forms on the plant cell wall making these compounds more recoverable during
383 extraction (Gliszczynska-Swiglo, et al., 2006). Steaming is performed without
384 direct contact of the plant material and water, preventing the leaching of
385 glucosinolates into it.
386 Homogenisation by blending watercress with water to create a smoothie resulted
387 in dramatic reductions in glucosinolates stemming mainly from the complete loss
388 of gluconasturtiin ($P<0.001$). Upon chopping losses ranged from 35% to 46% after
389 120 minutes of storage at room temperature. Chopping of vegetables before
390 consumption is a regular practise and this can lead to decreased glucosinolate
391 content since they are exposed to myrosinase for conversion to isothiocyanates.
392 This was reflected in our results and those of others (Smith, Mithen & Johnson,
393 2003; Song, et al., 2007), and it was particularly apparent in the gluconasturtiin
394 quantification. When watercress was homogenised to create a smoothie,
395 gluconasturtiin was completely lost and the levels of other glucosinolates were
396 significantly diminished. Our results are comparable with results from a study
397 performed by Smith, et al. (2003) where homogenisation for juice extraction from
398 Brussels sprouts led to loss of glucosinolates which were converted to
399 isothiocyanates and other breakdown products due to the exposure of
400 glucosinolates to myrosinase enzyme. Song, et al. (2007) observed that shredding
401 of Brassica vegetables and subsequent storage at ambient temperature results in
402 major losses of glucosinolates with concurrent formation of isothiocyanates.
403 Isothiocyanates such as PEITC are highly volatile compounds therefore they are
404 prone to evaporation as observed by Rose, et al. (2000) who did not detect PEITC

405 in watercress aqueous extracts. However, Ji, Kuo and Morris (2005) noted that
406 PEITC remains stable in aqueous buffers with a half-life of 56 h at ambient
407 temperature. This suggests that smoothies or juices made from watercress, which
408 is rich in PEITC, should be freshly consumed after preparation to ensure adequate
409 ingestion.

410 *3.5 Antioxidant activity*

411 The antioxidant activity of all watercress samples was determined using the FRAP
412 assay (Figure 1B). Fresh watercress had an antioxidant activity of 74.54 ± 10.81
413 μmol AAE g^{-1} DW. Watercress was found to have the highest antioxidant activity
414 when compared to spinach, rocket and mizuna (Martinez-Sanchez, et al., 2008;
415 Payne, Mazzer, Clarkson & Taylor, 2013).
416 Boiling dramatically decreased the antioxidant capacity of watercress over time as
417 compared to raw watercress, with losses reaching 67% of total antioxidant activity
418 for samples cooked for 10 minutes (Figure 1B). Antioxidant activity analysis of the
419 cooking water showed that the losses observed during boiling are due to leaching
420 of antioxidant compounds in the water (46,03 ± 9.42 μmol AAE g^{-1} DW). In
421 contrast, microwaving and steaming of watercress did not result in any significant
422 losses. Chopping and blending to smoothie had no significant impact on the
423 antioxidant activity of the samples, however storage of these samples at room
424 temperature for 30 or 120 minutes resulted in a significant decrease in antioxidant
425 activity. Chopping and blending to smoothie reduced the antioxidant activity to
426 42.84 ± 8.00 and 48.47 ± 9.63 μmol AAE g^{-1} DW at 120 minutes of storage
427 respectively. The antioxidant activity of raw and cooked samples followed a similar
428 trend to that found for total phenols with a significant correlation between these
429 measures (R^2 = 0.759, $P<0.05$).

In a study carried out by Ismail, Marjan and Foong (2004) it was found that boiling for 1 minute significantly decreased the antioxidant activity of kale, but not that of cabbage. Zhang and Hamauzu Zhang, et al. (2004) showed that after boiling and microwaving, broccoli lost 65% and 65.3% of its total antioxidant activity respectively.

Since the antioxidant activity of plants may be defined by the concentration of phenols and ascorbic acid in combination with other phytochemicals, leaching of these compounds into the boiling water, or oxidation and degradation of them during cooking, can lead to lower antioxidant activity of watercress (Gliszczynska-Swiglo, et al., 2006; Vallejo, et al., 2003).

3.6 Watercress phytochemical profile modifications upon cooking

PCA revealed distinct phytochemical profiles for watercress cooked using different regimes (Figure 2). The profiles obtained from microwaved and steamed watercress closely resembled that of fresh watercress with these cooking methodologies positively correlating with the phenolics, carotenoids and glucosinolate concentrations. In stark contrast, boiled watercress has a phytochemical profile very different from that of fresh watercress characterised by elevated carotenoid amounts (R^2= 0.668) and significant losses in glucosinolates and flavonols, which essentially result in compromised antioxidant activity (R^2= -0.596). Chopped watercress and watercress smoothie samples have similar phytochemical profiles and separate from the fresh samples on the first principal component characterised by losses of all the phytochemicals quantified in our study. Cooking time appears to be negatively correlated with microwaving, boiling and steaming but exposure of chopped samples and watercress smoothie to ambient temperature for extended time periods does not appear to have a

particular impact on the measureable phytochemicals in these samples, expect in the total phenolic content of stored chopped watercress. Antioxidant activity as measured by the FRAP assay, exhibits a significant positive correlation with microwaving (R^2= 0.452) driven by higher concentrations of glucosinolates and flavonols suggesting that it should be the preferred method of watercress preparation when it is not consumed raw.

4.0 Conclusions

This study clearly demonstrates that health-promoting compounds in watercress are significantly influenced by domestic processing methods. Cooking by microwaving and steaming preserves the levels of most phytochemicals in watercress. Domestic processing can have a detrimental effect on the bioactives which may be responsible for the health promoting properties of watercress. Satisfactory retention of beneficial phytochemicals in watercress may be achieved by avoiding boiling which results in a compromised phytochemical profile.

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592

593 **Figure 1 (A)** Total phenols content in raw and processed samples expressed as
594 gallic acid equivalents (GAE) in mg $g^{-1}$of dry weight (DW). **(B)** FRAP-assay results
595 for the measurement of the antioxidant activity in raw and cooked watercress
596 samples. Results are presented as ascorbic acid equivalents (AAE) in mg g^{-1} of
597 DW. Data is mean of three biological replicates + SD. Significance: *, $P < 0.05$; **,
598 $P < 0.01$; *** $P < 0.001$ as compared to carotenoid content of raw watercress. (BD:
599 Boiled, MW: Microwaved, ST: Steamed, SM: Smoothie, CH: Chopped**.**

600 **Figure 2** PCA scores of all cooked samples **(**□) and loadings plot for all
601 quantified phytochemicals (**O**). Abbreviations: 4-MGB, 4-methoxyglucobrassicin;
602 4-HGB, 4-hydroxyglucobrassicin; KSG, K 3-(sinp-Glc)-4'Glc; KSTG, K 3-(sinp-
603 triGlc)-7-Glc; QDGCG, QCSG, Q 3-(caf-Glc)-3'-(sinp-Glc)-4'-Glc; KDG, K 3-diGlc-
604 7-Glc; IG, I 3-Glc**;** KFTG, K 3-(fer-triGlc)-7 Glc; QCG+KDG Q 3,4'diGlc-3'-(p.coum-
605 Glc) + K 3,4'-diGlc.

606 **Table 1** Concentration of individual and average total flavonols in raw and
607 processed watercress samples. Data is presented in mg g^{-1} of DW (mean ± SD).
608 Experiment was performed with three biological replicates per group. Significance:
609 *, $P < 0.05$; **, $P < 0.01$; *** $P < 0.001$ as compared to flavonoid content of raw
610 watercress. Abbreviations: K, kaempferol; I, isorhamnetin; Q, quercetin; Glc;

611 glucoside, fer, feroloyl; sinp, sinapoyl; p.coum, p-coumaroyl; caf, caffeoyl.
612 [a]Flavonols co-elute.

613

614 **Table 2.** Quantification of total and specific carotenoids, in raw and processed
615 watercress samples. Data is presented as absolute carotenoid concentration in
616 mg g^{-1} of DW (mean ± SD). Experiment was performed with three biological
617 relicates per group. Significance: *, $P < 0.05$; **, $P < 0.01$; $^{***}P < 0.001$ as
618 compared to carotenoid content of raw watercress. [a] Total amount of carotenoids
619 measured spectrophotometrically.

620 **Table 3** Concentration of individual and average total glucosinolates in raw and
621 processed watercress samples. Data is presented in mg g^{-1} of DW (mean ± SD).
622 Experiment was performed with three biological replicates per group. Significance:
623 *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ as compared to carotenoid content of raw
624 watercress.

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