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

Authors Natasa Giallourou¹, Maria Jose Oruna-Concha¹, Niamh Harbourne ²

Affiliations
¹ Department of Food and Nutritional Sciences, School of Chemistry, Food and Pharmacy, University of Reading, Whiteknights campus, Reading, United Kingdom,
² Institute of Food and Health, School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland

Corresponding author
Natasa Giallourou
Department of Food and Nutritional Sciences, School of Chemistry, Food and Pharmacy, University of Reading, Whiteknights, Reading RG6 6AP, United Kingdom.
E-mail: n.giallourou@pgr.reading.ac.uk
Telephone: +44 (0) 7761402005
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-phenylethyl 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 watercress (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$_3$·6H$_2$O). 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.
2.8 Quantification of carotenoids via HPLC

To determine the amount of lutein, zeaxanthin and β-carotene present, the acetone extracts were used (Section 2.4). Carotenoids were quantified using the method developed by Guiffrida et al. (Guiffrida, Dugo, Torre, Bignardi, Cavazza, Corradini, et al., 2013) with modifications. 10 ml of the extract was mixed with 10 ml of diethyl ether, 10 ml of water and 5 ml of 10% (v/v) NaCl. Two layers were formed and the lower - acetone phase was discarded. The upper layer containing the ether was collected in a glass vial and anhydrous Na₂SO₄ was added to it to remove any moisture from the solution. The ether phase was transferred to a clean glass vial, the volume was adjusted to 10 ml with diethyl ether and the solution was condensed under nitrogen gas. The dry residue was then reconstituted in 1 ml of methyl tert- butyl ether (MTBE):MeOH (1:1, v/v), filtered using 0.22 μm syringe driven filter unit and analysed by HPLC. The analyses were performed using an YMC30 column (5 μm 250 x 4.6 mm) on a HP Agilent 1050 series HPLC system. The mobile phases used were as follows: Eluent A, consisting of MeOH:MTBE:H₂O (82:16:2 v/v/v) and Eluent B, consisting of MeOH:MTBE:H₂O (23:75:2 v/v/v). The analyses followed a gradient program for the mobile phases, 0 min 0% B, 20 min 0% B, 80 min 70% B, 90 min 70% B. The protocol used a 1 mL/min flow rate and a 100 μL injection volume. UV-vis spectra were gathered in the range of 190-600 nm and the chromatograms were analysed at 450 nm. Identification was based on retention times by comparison with HPLC grade standards of lutein, zeaxanthin and β-carotene (Extrasynthese, France).

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

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.μl⁻¹, 42 ng.μl⁻¹, 28 ng.μl⁻¹, 14 ng.μl⁻¹, 5.6 ng.μl⁻¹, R² > 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).
Our results are corroborated by previous studies showing that boiling of Brassica vegetables can lead to significant time dependant losses of phenolics whereas microwaving and steaming led to only minor decreases in the phenolic content of broccoli (Turkmen, Sari & Velioglu, 2005; Zhang & Hamauzu, 2004), red cabbage (Podsedek, Sosnowska, Redzynia & Koziolkiewicz, 2008) and cauliflower (Natella, Beel, Ramberti & Scaccini, 2010). During the process of cooking, phenolic compounds appear to be highly reactive undergoing several changes including their release from bound forms, oxidation, degradation and polymerisation (Gliszczyńska-Swiglo, et al., 2006).

The losses during boiling can be attributed to water-soluble compounds leaching into the water used for boiling or due to breakdown of these compounds during thermal processing. Indeed, analysis of the water used in the boiling experiments (9.35 ± 0.12 mg GAE g⁻¹ DW) for total phenolics revealed that phenols had leached into the boiling water. The total amount of phenols in the water used in boiling and the remaining phenol content of watercress was no different from the total phenols in raw watercress. The minimal effect of microwaving and steaming on the phenolic compounds is potentially a result of limited or no contact of the samples with water and also the inactivation of oxidative enzymes preventing the disruption of phenolic biosynthesis and degradation (Vallejo, Tomás-Barberá & García-Viguera, 2003).

3.2 Flavonols identification and quantification
Flavonol profiling of watercress revealed three main derivatives namely kaempferol, quercetin and isorhamnetin as well as feruloyl, caffeoyl, p-coumaroyl and sinapoyl glucosides attached to kaempferol and quercetin. Kampferol-3-diglucoside-7-glucoside was the most abundant flavonol detected (3.76 ± 0.09 mg
The flavonols identified in the fresh watercress leaves are similar to those defined by Martinez-Sanchez, et al. (2008).

Domestic processing of watercress resulted in a significant decrease in the levels of all quantified flavonols (Table 1). The only exception was Q 3,4’diGlc-3’-(p.coum-Glc) + K 3,4’-diGlc which appeared to be the most stable of all flavonols and were only significantly affected by boiling (P<0.05). Total flavonol losses suggest that these compounds are particularly sensitive to all cooking regimes used. Boiling for 10 minutes nearly depleted all watercress samples of flavonols in a time dependent manner. The unstable nature of flavonols was also apparent in chopped watercress and watercress smoothie with the levels going down to 3.42 ± 0.32 and 4.11 ± 0.36 mg g\(^{-1}\) DW respectively as compared to the total amount of flavonols in the fresh samples (10.70 ± 1.07mg g\(^{-1}\) DW, P<0.001). Similarly to total phenols, the highest retention of flavonols was observed in the microwaved watercress followed by steamed.

3.3 Carotenoid content

In contrast to the previous assays, boiling of watercress resulted in an increased concentration of total measurable carotenoids, from 2.35 ± 0.22 mg g\(^{-1}\) DW in the fresh samples to 3.13 ± 0.20 mg g\(^{-1}\) DW after 2 minutes of cooking and up to 3.28 ± 0.30 mg g\(^{-1}\) DW after 5 minutes of boiling (Table 1). Microwaving and steaming did not have a significant impact on the level of total carotenoids (P>0.05). On the other hand, the watercress smoothie had significantly lower total carotenoid content, with the levels decreasing from 1.54 ± 0.21 to 1.11± 0.08 mg g\(^{-1}\) DW after 60 minutes of storage at ambient temperature. A similar decreasing trend was observed in the chopped watercress samples.
The individual carotenoids identified and quantified in our watercress samples were β-carotene, lutein and zeaxanthin and they all resulted in distinct responses upon domestic processing. β-carotene was the most abundant of the three quantified carotenoids (0.95 ± 0.08 mg g⁻¹ DW) and its levels significantly increased after thermal treatment of the watercress samples. Boiling for 5 minutes resulted in β-carotene being significantly increased up to 1.75 ± 0.09 mg g⁻¹ DW as compared to the raw samples (P<0.001). In the microwaved watercress samples β-carotene was increased up to 1.48 ± 0.26 mg g⁻¹ DW (P<0.01) and in the samples steamed for 15 minutes levels went up to 1.54 ± 0.07 mg g⁻¹ DW (P<0.001). β-carotene was decreased in the watercress smoothie only after storage for 30 and 60 and 120 minutes (P<0.01) therefore, immediate consumption of a watercress smoothie ensures sufficient intake of β-carotene. No significant differences were found in the chopped samples.

Lutein content of fresh watercress samples was 0.24 ± 0.02 mg g⁻¹ DW and it exhibited the highest degree of stability after watercress processing. It was 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 after 120 minutes of storage (P<0.05). Zeaxanthin concentration in fresh watercress was notably lower than β-carotene and lutein (0.02 ± 0.00 mg g⁻¹ DW). It was dramatically affected by boiling with increases higher than 6 and 3 times, as compared to fresh watercress, after boiling for 5 minutes and steaming for 10 minutes respectively.

Increases in the carotenoid contents of other Brassica vegetables such as broccoli, Brussels sprouts, cabbage and cauliflower upon boiling and steaming have been reported by a number of research groups (Bernhardt & Schlich, 2006;
Gliszczynska-Swiglo, et al., 2006; Hart, et al., 1995). Elevations in the measurable carotenoid concentrations after thermal treatments can be explained by changes in the plant cell wall due to the breakdown of cellulose as well as improved extractability of carotenoids from the plant as a result of the denaturation of carotenoid-protein complexes due to thermal processing (Khachik, Beecher, Goli & Lusby, 1991).

3.4 Glucosinolate identification and quantification

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

Glucosinolate quantification revealed a major impact of cooking on the levels of these phytochemicals. Boiling reduced the levels of total glucosinolates by up to 63% and led to significant loses of all the individual glucosinolates identified in this study (P<0.001). Considerable glucosinolate losses after boiling of Brassica vegetables like broccoli, cauliflower and Brussels sprouts, have also been observed in other studies performed by a number of research groups (Song & Thornalley, 2007; Vallejo, Tomás-Barberán & Garcia-Viguera, 2002). Heat application combined with cooking in water can result in depletion of glucosinolates in Brassica as a result of enzyme activity modification and thermally induced breakdown processes (Jones, 2007; Palermo, et al., 2014). Boiling of watercress in water caused significant loss of glucosinolates that most likely have leached into the cooking water. Similar conclusions were drawn by Song, et al. (2007) who showed that boiling of Brassica vegetables leads to
significant leaching of glucosinolates in the boiling water. Jones (2007) have shown that the glucosinolate losses in Brassica vegetables are positively correlated with the cooking time. Microwaving and steaming had a subtle effect on glucosinolate concentrations with minor losses at the longest cooking duration, as compared to the other treatments. Microwaving and steaming for 2 or 5 minutes did not result in major losses of total glucosinolates suggesting that these cooking methods will ensure a higher retention rate of these phytochemicals. Our results are in agreement with that of Song, et al. (2007) who examined the impact of different cooking methods on broccoli, brussels sprouts, cauliflower and green cabbage. This observation is likely due to denaturation and subsequent deactivation of the myrosinase enzyme, which depletes glucosinolates in favour of their hydrolysis to isothiocyanates, after application of high temperatures during cooking (Verkerk, vanderGaag, Dekker & Jongen, 1997). We found that cooking by steaming resulted in a slight increase in gluconasturtiin concentrations from 1.76 to 2.04 mg g\textsuperscript{-1} DW (P<0.05) and it can therefore be considered as the preferred method of watercress consumption to maximise gluconasturtiin levels. Elevated gluconasturtiin concentrations upon steaming are also reported by Gliszczynska-Swiglo, et al. (2006) in broccoli. Increases in other glucosinolates in Brassica vegetables subjected to steaming have been also been noted in a number of studies (Pellegrini, Chiavaro, Gardana, Mazzeo, Contino, Gallo, et al., 2010; Vallejo, et al., 2002). The inactivation of myrosinase at the high temperatures such as the ones reached during steaming, can temporarily cease the conversion of glucosinolates to isothiocyanates (Vallejo, et al., 2002) a process which can be undertaken post ingestion, \textit{in vivo}, by the action of the endogenous bacterial myrosinase in the gut (Rouzaud, Rabot, 16
Furthermore, heat application leads to plant cell structure disintegration allowing glucosinolates to be released from their bound forms on the plant cell wall making these compounds more recoverable during extraction (Gliszczynska-Swiglo, et al., 2006). Steaming is performed without direct contact of the plant material and water, preventing the leaching of glucosinolates into it.

Homogenisation by blending watercress with water to create a smoothie resulted in dramatic reductions in glucosinolates stemming mainly from the complete loss of gluconasturtiin ($P<0.001$). Upon chopping losses ranged from 35% to 46% after 120 minutes of storage at room temperature. Chopping of vegetables before consumption is a regular practise and this can lead to decreased glucosinolate content since they are exposed to myrosinase for conversion to isothiocyanates. This was reflected in our results and those of others (Smith, Mithen & Johnson, 2003; Song, et al., 2007), and it was particularly apparent in the gluconasturtiin quantification. When watercress was homogenised to create a smoothie, gluconasturtiin was completely lost and the levels of other glucosinolates were significantly diminished. Our results are comparable with results from a study performed by Smith, et al. (2003) where homogenisation for juice extraction from Brussels sprouts led to loss of glucosinolates which were converted to isothiocyanates and other breakdown products due to the exposure of glucosinolates to myrosinase enzyme. Song, et al. (2007) observed that shredding of Brassica vegetables and subsequent storage at ambient temperature results in major losses of glucosinolates with concurrent formation of isothiocyanates. Isothiocyanates such as PEITC are highly volatile compounds therefore they are prone to evaporation as observed by Rose, et al. (2000) who did not detect PEITC
in watercress aqueous extracts. However, Ji, Kuo and Morris (2005) noted that PEITC remains stable in aqueous buffers with a half-life of 56 h at ambient temperature. This suggests that smoothies or juices made from watercress, which is rich in PEITC, should be freshly consumed after preparation to ensure adequate ingestion.

3.5 Antioxidant activity

The antioxidant activity of all watercress samples was determined using the FRAP assay (Figure 1B). Fresh watercress had an antioxidant activity of $74.54 \pm 10.81 \mu mol \ AAE \ g^{-1} \ DW$. Watercress was found to have the highest antioxidant activity when compared to spinach, rocket and mizuna (Martinez-Sanchez, et al., 2008; Payne, Mazzer, Clarkson & Taylor, 2013).

Boiling dramatically decreased the antioxidant capacity of watercress over time as compared to raw watercress, with losses reaching 67% of total antioxidant activity for samples cooked for 10 minutes (Figure 1B). Antioxidant activity analysis of the cooking water showed that the losses observed during boiling are due to leaching of antioxidant compounds in the water ($46.03 \pm 9.42 \mu mol \ AAE \ g^{-1} \ DW$). In contrast, microwaving and steaming of watercress did not result in any significant losses. Chopping and blending to smoothie had no significant impact on the antioxidant activity of the samples, however storage of these samples at room temperature for 30 or 120 minutes resulted in a significant decrease in antioxidant activity. Chopping and blending to smoothie reduced the antioxidant activity to $42.84 \pm 8.00$ and $48.47 \pm 9.63 \mu mol \ AAE \ g^{-1} \ DW$ at 120 minutes of storage respectively. The antioxidant activity of raw and cooked samples followed a similar trend to that found for total phenols with a significant correlation between these 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 (R² = 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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References


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

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

**Table 1** Concentration of individual and average total flavonols in raw and processed watercress samples. Data is presented in mg g⁻¹ of DW (mean ± SD). Experiment was performed with three biological replicates per group. Significance: *, P < 0.05; **, P < 0.01; *** P < 0.001 as compared to flavonoid content of raw watercress. Abbreviations: K, kaempferol; I, isorhamnetin; Q, quercetin; Glc;
glucoside, fer, feroloyl; sinp, sinapoyl; p.coum, p-coumaroyl; caf, caffeoyl.

*Flavonols co-elute.

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

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