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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.

1. 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 et al., 2006; Gill 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-thioglucoside 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 (Doostdar, Burke, & Mayer, 2000; Galati, Teng, Moridani, Chan, & O'Brien, 2000; Morel, Lescoat, Cillard, & Cillard, 1994).

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

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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. 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 h 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 s 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 s 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, Oruna-Concha, and 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 6000 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 (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