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Effect of ultrasound on the extraction of total anthocyanins from *Purple Majesty* potato

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ABSTRACT

This study examined anthocyanin extraction using the application of ultrasound to raw freeze dried, microwaved and raw sliced *Purple Majesty* potato, a new pigmented potato variety rich in anthocyanins. A 20 kHz probe was used for the sonication at 3 different amplitudes (30%, 50% and 70%) and ethanol in water at different ratios (50:50 and 70:30 v/v) was used for the extraction. Anthocyanin extraction from raw freeze dried purple potato was optimal at an ethanol:water ratio (70:30; v/v) after 5 min of ultrasonication, while the least amount of anthocyanins was extracted from raw sliced potatoes. The application of microwaves (as a pre-treatment) before the UAE resulted in an increase in the amount of anthocyanins extracted and a decrease in the amount of solvent used. Analysis of variance showed that potato form, ultrasonication time, ultrasonication amplitude and solvent ratio as well as two and three way interactions between some of these factors had a very significant effect ($p < 0.000$) on the amount of anthocyanins extracted.

1. Introduction

Anthocyanins are water soluble flavonoids, which are responsible for the bright colouration in fruits and vegetables. This class of phytochemicals are used in the food and pharmacological industries because of their anti-oxidant, anti-inflammatory, anti-microbial and natural pigment properties. The extraction of anthocyanins can be performed using conventional solvent extraction, however this method has limitations and disadvantages. Recently, other techniques, for instance, microwave assisted extraction (MAE), pulsed-electric-fields and ultrasound assisted extraction (UAE) have been proposed for the extraction of these compounds. UAE has become an alternative technology to the thermal food techniques, both for preservation and extraction of bioactive compounds. Ultrasound technology has been investigated as an alternative for conventional thermal processes for a number of years and it has been employed as an alternative (or complementary) technique to some conventional food processes because of the minimal detrimental effects on food components (including bioactive compounds) and on enzyme and microbiological activity [1–9]. Extraction of compounds from plants often involves the use of different solvents commonly in combination with heat. However, better extraction techniques are required, preferably utilising more environmentally friendly processes such as a decrease in solvent consumption, lower temperatures and less time-consuming techniques. In fact, emerging techniques, such as microwave assisted extraction (MAE), supercritical fluid extraction, pressurized liquid extraction (PLE), pulsed-electric-field-assisted extraction (PEF) and ultrasound assisted extraction (UAE) have been developed for extraction of bioactive compounds from plant sources [10–16]. Additionally, UAE has been recognised as a green extraction technique which readily fits into at least three of the "six principles of Green Extraction of Natural Products", such as the use of green solvents; reduction of energy consumption and time; decrease in the number of unit processes; increased yields; and production of non-denatured products [17]. Furthermore, the use of UAE has been reviewed, along with other green techniques, and has been shown to have a number of advantages in terms of intensification of extraction, reduction in energy use and, interestingly, production of a better quality of extract when compared to simple maceration [18]. UAE has been employed to enhance the extraction of bioactive compounds and is used by pharmacological and food industries to obtain compounds having antioxidant, antimicrobial, anti-inflammatory and/or anticancer potential [19–27].

Potato (*Solanum tuberosum*) is one of the principal food crops in the world and the tubers are a good source of carbohydrates

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(starch), proteins and vitamin C and, as a product of plant origin, they also contain secondary metabolites (phytochemicals). Polyphenolic compounds are a large group of phytochemicals, and depending on their chemical structure, they can be divided into flavonoids, phenolic acids, tannins, stilbenes and lignans [28]. Anthocyanins (classified as flavonoids) are responsible for the colour found in pigmented potatoes and as they possess interesting dye properties, these compounds are used in the food industry as "natural" food additives [29]. Potato is a relatively cheap crop and waste from potato products could potentially be utilised in order to extract bioactive compounds. The current work investigates the effect of ultrasound assisted extraction on the amount of anthocyanin extracted from *Purple Majesty* potatoes. The variables studied were extraction time (5, 30 and 60 min), ultrasound amplitudes (30%, 50% and 70%), solvent ratios (ethanol:water; 50:50 and 70:30; v/v) and different "forms" of potato (raw freeze dried; microwaved and raw sliced potato).

2. Materials and methods

2.1. Chemicals and reagents

Hydrochloric acid was provided by Rathburn Chemical (Walkerburn, Peebleshire, UK) and ethanol, di-sodium hydrogen phosphate, citric acid and methanol were purchased from Fisher Scientific (Loughborough, UK).

2.2. Purple Majesty potato and conditions

Purple Majesty potatoes were kindly supplied by Albert Bartlett Ltd, Scotland, UK and stored in a cool, dark room throughout the experiments. Potatoes were used in the following "forms": (i) raw freeze dried; (ii) microwaved and (iii) raw sliced [indicated in the figures as form 0, 1 and 2 respectively]. In order to obtain the freeze dried form, raw purple potatoes (unpeeled) were cut, frozen and then freeze dried for 72 h (Micro-Modulyo freeze dryer) and the resultant powder was stored at −20 °C until required. Microwaved potato was obtained by microwaving a whole potato (approximately 100 g) for 2 min at 900 W. Raw potatoes were prepared by simply cutting into 5 mm thick slices. All the extractions were performed using 5 g of the three forms of potatoes mixed with extraction solution (200 mL) which consisted of a mixture of ethanol and water at two different ratios (50:50 and 70:30 v/v), indicated in the figures as solvent ratio 0 and 1, respectively. Each potato form was sonicated in each solvent and all the samples were filtered before analysis.

2.3. Thermal assisted extraction (control)

This experiment is used as a control to rule out the effect of rising temperature during the ultrasonication process on the levels of anthocyanins extracted. The assisted thermal extraction was performed (using the different forms of potato, solvent ratios and extraction time) by immersing the potato samples in a 400 mL beaker in a water bath at 33 °C (this was the temperature reached by the extraction performed by UAE).

2.4. Ultrasound assisted extraction (UAE)

UAE was performed using a Misonix Ultrasonic Liquid Processor fitted with a 1.3 cm Titanium probe operating at 20 kHz in the continuous mode at 30%, 50% and 70% amplitude. This corresponded to 23, 28 and 35 W of thermal energy as measured by calorimetry. The probe was immersed in 200 mL of extraction solvent containing 5 g of potato in a 400 mL beaker and the probe was positioned 20 mm from the bottom. In order to control the increase of the temperature during sonication the beaker was placed in a 2 L bath filled with ice and water and the temperature was maintained at 33 ± 2 °C for each experiment. Samples were removed at regular time intervals (5, 30, 60 and 120 min), filtered and analysed for total anthocyanins content (TA).

2.5. Determination of total anthocyanins

The pH shift method was used to estimate the anthocyanin content in the samples of purple potato as previously described [30]. The absorbance, at 700 nm, of the extracted samples (after filtration) was measured at both pH 0.6 and pH 3.5 using a spectrophotometer (Shimadzu UV-1650PC) (which allows background correction) and 520 nm (to determine the anthocyanin content) against a blank of solvent mix used for extraction. In order to obtain the absorbance (A) related to the total anthocyanins the following equation was used:

$$A = (A_{520} - A_{700})\mathrm{pH}_{0.6} - (A_{520} - A_{700})\mathrm{pH}_{3.5} \quad (1)$$

Considering the Beer–Lambert law the concentration of total anthocyanins (g/L) was calculated according to Eq. (2).

$$\text{Anthocyanin concentration (g/L)} = (A^{*}MW)^{*}\varepsilon^{-1*}1^{-1} \quad (2)$$

where, A is the absorbance (calculated from Eq. (1)), MW is the molecular weight of a reference pigment (cyanindin-3-glucoside; 449.2 g/mol), ε is the molar absorptivity of the reference anthocyanin (extinction factor 26,900 L cm^{-1} mol^{-1}), and l is the optical path length in centimetres (1 cm). Considering the dry (when applicable) and fresh weight (FW) of the different samples, the total anthocyanin content was calculated and expressed as mg cyanindin-3-glucoside/kg FW.

2.6. Data analysis

Analysis of variance using General linear model (GLM – Minitab version 14, Minitab Inc., US) was used to investigate and model the response variables studied (in this case the amount of anthocyanin extracted) as a function of ultrasonication amplitude, extraction time and solvent to water ratio used during the extraction process of the three different forms of potatoes. The experimental design was orthogonal and GLM applied a regression approach by which each response variable was regressed on the factors studied based on a user specified hierarchical model. In this case, the effects of the main factors (potato form, set at three levels – raw freeze dried, microwaved and raw sliced; extraction time, set at four levels – 5, 30, 60 and 120 min; ultrasonication amplitude, set at three fixed levels: 30%, 50% and 70% and solvent to water ratio, set at two levels of ethanol to water ratio of 50:50 and 70:30) as well as the two way and three way interactions between these factors were elucidated.

Data presented on the tables are the mean ($n = 3$) ± standard deviation (SD), which were calculated using functions in Microsoft Excel®.

3. Results and discussion

Table 1 shows the amount of TA obtained from raw freeze dried potato using UAE. With 50:50 ethanol:water, the UAE showed little or no advantage over thermal treatment (control) but a higher ethanol:water ratio favoured the extraction process resulting in a threefold increase in the anthocyanins obtained compared to 50% ethanol. The amount of anthocyanins obtained after 5 min of ultrasound treatment was very similar at the three amplitudes chosen but UAE was around 70% better than the purely thermal treatment.