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Influence of cooking on the levels of bioactive compounds in *Purple Majesty* potato observed *via* chemical and spectroscopic means

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ABSTRACT

Tubers rich in phytochemicals can exhibit a potential health benefit. This work aims at studying the relative effect of different domestic cooking techniques by monitoring the level of total phenolic compounds (TP), total anthocyanins (TA) and anti-oxidant activity (AOA) on a variety of pigmented potatoes. Raw purple potatoes are a good source of anthocyanins (219 mg/kg FW) and the level of these compounds increased using different cooking techniques, with the exception of baking. However, the levels of phenolic compounds (originally 209 mg GAE/100 g FW) decreased in the cooked potatoes. Although potatoes contain different antioxidants in this work the antioxidant activity seems to be related to the levels of phenolic compounds present in the pigmented potato. The fact that some of the compounds present fluoresce enabled both steady state and time-resolved fluorescence techniques to be assessed as a non destructive means of monitoring. This elucidated the presence of different components (*via* spectral deconvolution and time-resolved emission spectra). Their relative contribution to the fluorescence emission was found to be affected by the different cooking process, with a longer wavelength emission appearing to relate to reflect the presence of anthocyanins.

1. Introduction

Potato (*Solanum tuberosum*) is one of the most important food crops in the world and the tubers are a good source of carbohydrates (starch), proteins and vitamin C. As a product of plant origin they also contain secondary metabolites (phytochemicals), which are proven to have health benefits (Ezequiel, Singh, Sharma, & Kaur, 2013); meaning that potatoes can be considered a functional food. Polyphenolic compounds are a large group of phytochemicals and depending on their chemical structure they can be divided into the following classes; flavonoids, phenolic acids, tannins, stilbenes and lignans (Ignat, Volf, & Popa, 2011). Polyphenols are known for their anti-oxidant, anti-inflammatory and anti-microbial properties (Brown, 2005; Dziri et al., 2012; Thompson et al., 2009). Research has showed that their consumption can decrease the risk of chronic diseases, such as heart disease, type 2 diabetes and cancer (Han et al., 2006; Sancho & Pastore, 2012; Teow et al., 2007; Thompson et al., 2009). Within European markets white potatoes are very popular, but in recent years different coloured varieties of potatoes are appearing. These include; red skin/white flesh, red skin/red flesh, purple skin/marble flesh and purple skin/purple flesh. *Purple Majesty* potato (purple skin/purple flesh) is a cultivar registered on the British Potato Variety Database that has been introduced in Scotland. The interest in studying this variety stems from the tubers' richness in phytochemicals, including high levels of anthocyanins. Stushnoff et al. (2008) analysed several existing cultivars including *Purple Majesty* potato in order to characterise the antioxidant profile for the Colorado potato breeding program. These researchers determined the total phenolic content, antioxidant capacity and also investigated the levels of Vitamin C of the selected cultivars. Their study identified chlorogenic acid isomers as being the main phenolic compound present in *Purple Majesty*. The study provided also anthocyanin's profile of the *Purple Majesty* potato (the same cultivar used on the present work) they identified 5 petunidin glycoside and a single glycoside of each of the delphinidin, peonidin and malvidin aglycones. However, there appears to be little data concerning the domestic usage, ie effect of cooking techniques on the levels of total phenolics (TP), anthocyanins (AA) and anti-oxidant activity (AOA) on this specific variety produced in UK.

Anthocyanins (classified as flavonoids) are responsible for the colour found in the pigmented potatoes. Their stability can, however, be affected by several factors such as pH, light, oxygen, enzyme activity, concentration, ascorbic acid and sugars (Cavalcanti, Santos, & Meireles, 2011; Patras, Brunton, O'Donnell,

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& Tiwari, 2010). It should be noted that the content of phenolic compounds/anthocyanins and their stability is also dependent on factors such as genotype, agronomic factors, storage conditions after the harvest, processing and cooking methods (Brown, Durst, Wrolstad, & De Jong, 2008; Burmeister et al., 2011; Eichhorn & Winterhalter, 2005; Ieri, Innocenti, Andrenelli, Vecchio, & Mulinacci, 2011; Lachman et al., 2012; Stushnoff et al., 2008). Although there are several studies demonstrating the effect of these compounds on health, which is responsible in classifying potatoes as a functional food, we need to take in consideration that the levels of polyphenols found in raw and cooked tubers will be different. As well as assessing the quantity of anthocyanins via the pH shift method (Moncada et al., 2004; Petrov, Gomes, Parola, & Pina, 2009; Quina et al., 2009), the fact that they can exhibit fluorescence, the behaviour of which depends on their form (Moreira et al., 2003), provides a means by which to assess the influence of cooking method on this class of compounds. In fact we have previously used time-resolved fluorescence techniques to study anthocyanins in purple potato, both using freeze dried powder and tuber slices via time-resolved fluorescence microscopy (Lemos, Aliyu, & Hungerford, 2012).

The stability and form of anthocyanins is highly dependent on their local environment, particularly in relation to pH (Fossen, Cabrita, & Andersen, 1998; Petrov et al., 2009; Tirupula, Balem, Yanamala, & Klein-Seetharaman, 2009) and we have previously used this in a fluorescence microscopy study (Lemos et al., 2012) to assess the location of these compounds in potato slices. The use of time-resolved fluorescence, shows promise as the fluorescence lifetime is independent of concentration, thus not affected by photobleaching (these compounds can be easily photodegraded) enabling a non destructive and potentially "remote" sensing of the sample to be carried out. In this work we assess the effect of cooking on the relative overall levels of bioactive components and make use of fluorescence spectroscopy to help elucidate any relative changes in the form of the fluorescing compounds induced by cooking.

2. Materials and methods

2.1. Chemicals and reagents

Hydrochloric acid (HCl) was provided by Rathburn Chemical (Walkerburn, Peebleshire, UK); Ethanol, Di-sodium hydrogen phosphate (Na_2HPO_4), Methanol, Acetone, Acetic acid (glacial), Di-sodium carbonate (Na_2CO_3), were purchased from Fisher Scientific (Loughborough, UK) and Citric acid, Folin's reagent, Gallic acid and Ferrous sulphate ($FeSO_4$) were obtained from Sigma (Poole, Dorset, UK).

2.2. Tubers and cooking techniques

Purple Majesty potatoes were kindly supplied by Albert Bartlett Ltd, Scotland, UK and stored in a cool and dark room throughout the experiments. This variety is registered in the British Potato Variety Data Base as follows (i) Parentage – All blue X ND2008-2; (ii) Breeder – San Luis Valley Research Center: (iii) Breeder's agent – Albert Bartlett and Son Ltd, Airdrie. This cultivar has been produced and marketed in the UK.

Tubers were cooked unpeeled using different cooking techniques and the time established according to tenderness. This was assessed by piercing the potatoes with a fork (as usually done in domestic cooking). The following cooking times/temperatures were used: 25 min (boiling); 35 min (steaming); 1 h at 200 °C (baking) and 5 min 900 W (Microwave). Samples of raw and cooked potatoes were frozen overnight and freeze dried (using a Micro-Modulyo) for 72 h. Freeze dried samples were kept at −20 °C until further analysis.

2.3. Determination of the level of bioactive components and antioxidant activity

The pH shift method adapted from Ribereau-Gayon and Stone Street (1965) was used to estimate the anthocyanin content in the samples of purple potato and has been previously employed in other works (Lachman et al., 2012). In order to determine the total anthocyanin content, 0.1 g of the dried purple potato was re-suspended in 20 mL of 50% methanol:water (v/v) mixture and then filtered. The absorbance of each sample at both pH < 1.0 and pH 3.5 was measured in a spectrophotometer (Shimadzu UV-1650PC) at 700 nm (which allows background correction) and 520 nm (to determine the anthocyanin content) against a blank. 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):

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

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 (extinction factor 26,900 L cm^{-1} mol^{-1}), l is the optical path length in centimetres (1 cm). Then, and considering the dry and fresh weight (FW) of the different samples, the total anthocyanin content was calculated and expressed as mg Cyanindin-3-glucoside/kg FW.

The total quantity of phenolics was measured by the Folin–Ciocalteu method. Briefly, 0.1 g of freeze dried potato was re-suspended in 20 mL of 50% methanol:water (v/v). This solution was filtered and 100 μL was added to 5 mL of a 1:10 dilution of Folin–Ciocalteu reagent and 0.9 mL of distilled water. After 5 min 3.5 mL of a Na_2CO_3 (115 g L^{-1}) was added and the mixture was left in the dark, at room temperature, for 2 h. The absorbance of the solution was measured at a wavelength of 765 nm against a blank. The optical density was compared to a standard curve ($y = 0.001x$; $R^2 = 0.998$) prepared with 50–500 mg L^{-1} of gallic acid and the result expressed as mg L^{-1} gallic acid equivalents (GAE). Results are expressed as mg GAE/100 g FW.

Antioxidant activity was determined using ferric reducing antioxidant potential (FRAP). In summary, 0.1 g of dried purple potato was re-suspended in 20 mL of 80:20 (v/v) acetone/water. 30 μl of this sample was added to 1 mL of FRAP reagent and incubated in a water bath at 37 °C for 4 min followed by measurement of absorbance at 593 nm against a blank. The Optical density was compared to the standard curve ($y = 0.4895x$; $R^2 = 0.9931$) for ferrous sulphate ($FeSO_4$) solutions, with concentrations between 0 and 10.0 mM. Results are presented as Fe II (mM) produced/100 g FW.

2.4. Fluorescence measurements

Time-resolved fluorescence measurements (using time-correlated single-photon counting) were performed on a HORIBA Scientific DeltaFlex equipped with a DeltaDiode laser excitation sources. Time-resolved emission spectra were obtained recording time-resolved fluorescence decays at 5 nm intervals for fixed durations over the required wavelength range. This equipment has a nominal time resolution of 25 ps. The resulting decays were then "sliced" in the wavelength-intensity plane at different time intervals, using DataStation software, combining the specified temporal data to produce the corresponding emission spectra. These are shown nor-