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Impact of processing of red beet on betalain content and antioxidant activity

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ABSTRACT

Red beet has high concentration betalains that are used as food colorants and food additives due to their health promoting properties. Redbeet is generally processed before consumption which influences the stability of betalains in turn which affects the acceptability and health properties. The objective of the study was to investigate the influence of processing techniques as microwaving, boiling, roasting and vacuuming on the red beet. The impact of processing was evaluated on the basis of belatains content and antioxidant activity of the processed samples. With spectrophotometric results betalains content were found to add up to 20% with vacuum treatment and also with microwave treatment of 900 W and 1800 W for 30 s up to7% and 19% respectively, however there was decrease in boiling and roasting treatments. With HPLC analyses the content of betanin seems to increase in microwave treatments with 450 W and 900 W but reduced with 1800 W. In the case of antioxidant activity there was 2 to 3 fold increase in boiling, roasting and microwave treatments as compared to the control.

1. Introduction

Colors are important quality indicators that determine the consumer acceptance of foods. In recent days market for application of synthetic colorants has decreased in favor of natural colorants (Fletcher, 2006). Fruits and vegetables are good sources of natural colorants. However, the natural colors have disadvantages as higher cost and reduced stability (Herbach, Stintzing, & Carle, 2006). It is a common practice that food from plant origin is generally processed by thermal methods to extend their shelf life, to improve flavor and to enhance the palatability. Due to processing and storage there are significant changes in betalains. According to Howard (2008) in the recent times consumption of processed foods have been increasing in greater amounts along with fresh fruits and vegetables. Betalains are water-soluble nitrogen-containing pigments, found in high concentrations in red beet (*Beta vulgaris*). Betalains consist of two sub--classes: betacyanins (red-violet pigments) and betaxanthins (yellow-orange pigments) (Delgado-Vargas, Jiménez, & Paredes-López, 2000; Stintzing & Carle, 2004). They have antimicrobial and antiviral effects (Strack, Vogt, & Schliemann, 2003) and also can inhibit the cell proliferation of human tumor cells (Reddy, Ruby, Lindo, & Nair, 2005).

Betalains can be used as food additives which either avoid the food discoloration or to enrich food. The use of betalains as food colorant is approved by European Union and betalains are labeled as E-162. Betalains are particularly suited for use colouring food products (Von Elbe, Maing, & Amundson, 1974) (Cai, Sun, Schliemann, & Corke, 2001). (Roy, Gullapalli, U.R., & R., 2004) Although anthocyanins are the most wide spread and mostly used natural pigments covering the red purple color range, betalains are more stable to pH and temperature. Betalains exhibit broad pH stability which are suited for low-acid foods where coloring with anthocyanins usally not possible. (Stintzing & Carle, 2004) For the yellow orange color range carotenoids are the natural pigments but due to poor solubility in water, betaxanthin could be used in application as yellow orange food colorants in situations (Azeredo, 2009). Betalain pigment mixtures can be used as a natural additive for food, drugs and cosmetic products in the form of beet juice concentrate or beet powder (Dörnenburg & Knorr, 1996). Consumption of red beet which are rich source of antioxidants can contribute to protection from age-related diseases. According to Vinson, Hao, Su, and Zubik (1998) Žitňanová et al. (2006) red beet is one of the most potent vegetables with respect to antioxidant activity. Betacyanins are a group of compounds exhibiting antioxidant and radical-scavenging activities (Escribano, Pedreño, García-Carmona, & Muñoz, 1998; Pedreno & Escribano, 2000). They also inhibit cervical ovarian and bladder cancer cells in vitro (Zou et al., 2005). Red beet also can be used as antioxidants (Georgiev et al., 2010). Netzel et al. (2005) reported that the ingestion of a single dose of red beet juice resulted in an increase of antioxidant compounds

Abbreviations: AOA, antioxidant activity; MW, microwave; PV, vacuum.

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including betalains in urinary excretion. Betalains and other phenolic compounds presented in red beet decreases oxidative damage of lipids and improves antioxidant status in humans. Antioxidant activity in red beet is associated involvement of antioxidants in the scavenging of free radicals and consequently in the prevention of diseases like cancer, cardiovascular diseases (Delgado-Vargas et al., 2000). Antioxidant activity was also reported to enrich human low-density lipoproteins by betalains which increase resistance to oxidation (Tesoriere, Allegra, Butera, & Livrea, 2004). According to Gentile, Tesoriere, Allegra, Livrea, and Alessio (2004) betalains exhibit anti-inflammatory effects, antiradical and antioxidant activity.

According to Stintzing and Carle (2006) maximizing betalain yield during extraction and processing is prerequisite. Extracted betalains are susceptible to pH, water activity, and exposure to light, oxygen, metal ions, temperature and enzymatic activities However temperature is the most decisive factor for betalain decomposition within the optimal pH. However, processing changes the content of betalains and consequently food color as well as the antioxidant activity. The susceptibility of betalains to the above mentioned factors restricts their use as food colorants. The aim of our study was to investigate the influence of thermal processes as microwave, boiling, roasting and vacuuming, on the betalain content and antioxidant activity of the red beet.

2. Material and methods

2.1. Plant materials

The fresh red beet plants were obtained from the local vegetable market in Berlin, Germanyand stored at 4 °C. The experiments were generally performed immediately after procurement.

2.2. Sample preparation

The red beet roots were washed and chopped into small pieces using magic mix, mechanical chopper.

For microwave irradiation was done with Microwave-Panasonic NE-1846, each sample of 10 g was placed in microwavable dishes and they were treated at 450, 900 and 1800 W for 10, 20, and 30 s.

In case of boiling 50 g of sample was treated at medium direct heat at 80 °C over a pan and stirred frequently at 30 rpm. An equal quantity of water was added to samples and heated for 60, 120, 180 sec variations.

With roasting 50 g of sample was treated at medium direct heat over a pan and stirred frequently at 30 rpm for uniform heat distribution for 60, 120, 180 s variations.

For vacuum treatment Komet plus Vacu 23 was used, about 30 g sample was packed in polypropylene bags and vacuumed at medium 94% and high 99% vacuum conditions.

All the samples after the respective treatment were cooled to room temperature, homogenized and freeze dried. The dried samples were taken for further extraction studies. All the experiments were done in triplicates.

2.3. Extraction of betalains

0.1 g of freeze dried samples were dissolved in 10 ml of 50% ethanol, were agitated for 10 s and the homogenate was centrifuged at 6000 rpm for 10 min. The supernatant was collected as it is after centrifugation and the same was repeated for 2 more times to ensure maximum extraction of betalains. The supernatant was further used for determination of betalains.

2.4. Determination of betalain compounds with spectrophotometric analses

The content of betaxanthins and betacyanins in the extracts was determined spectrophotometrically at 538 nm and 480 nm with a UV–Vis spectrometer, respectively according to the methods of Stintzing, Schieber, and Carle (2003). The absorbance reading obtained was used to calculate the betalain concentration for each sample. The betalain content (BC) was calculated as BC (mg/L) = [(A × DF × MW × 1000)/(e × l)], where A is the absorption, DF the dilution factor and l the pathlength (1 cm) of the cuvette. For quantification of betacyanins and betaxanthins, the molecular weights (MW) and molar extinction coefficients (e) (MW = 550 g/mol; e = 60,000 L/mol cm in H2O) and (MW = 308 g/mol; e = 48,000 L/mol cm in H2O) were applied.

2.5. Determination of betalain compounds with HPLC analyses

HPLC analyses were performed on HPLC (Dionex Summit P680A HPLC-System), equipped with P680 HPLC pump, ASI-100 automated sample injector, The analytical column Merck Lichrocart 250 × 4 RP-18 e (5 μm) was operated at 30 °C. Solvents were 0.2% (v/v) formic acid in water (A) and acetonitrile (B). At a flow rate of 1 mL/min, simultaneous monitoring was performedat 538 nm and 476 nm for betalains.

2.6. Antioxidant activity of extracts

The antioxidant activity of the extracted betalain samples was determined by DPPH method. The DPPH assay (Lee et al., 2003) was utilized with some modifications. The stock reagent solution (1×10^{-3} M) was prepared by dissolving 22 mg of DPPH in 50 mL of methanol and stored at −20 °C until use. The working solution (6×10^{-5} M) was prepared by mixing 6 mL of stock solution with 100 mL of methanol to obtain an absorbance value of 0.8 ± 0.02 at 515 nm, as measured using a spectrophotometer. Extracts each of 0.1 ml were vortexed for 30 s with 3.9 ml of DPPH solution and left to react for 30 min, after which the absorbance at 515 nm was recorded. A control with no added extract was also analyzed. The DPPH solution with no added extract was analyzed as control.

Scavenging activity was calculated as follows:

$$\text{DPPH radical}-\text{scavenging activity}(\%) = \left[\left(A_{\text{control}} - A_{\text{sample}}\right) / A_{\text{control}}\right] \times 100$$

where A is the absorbance at 515 nm.

2.7. Statistical analysis

All analyses were performed in triplicate and data reported as mean ± standard deviation (SD). Data were subjected to analysis of variance (ANOVA) ($P<0.05$). Results were processed by Excel (Microsoft Office 2007) and SPSS Version 17.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussions

The stability of betalains at different treatment conditions were investigated. Inititally plant material was macerated, freeze dried and betalains were extracted with 30%, 50%, 70% ethanol and with distilled water. Efficacy of extraction was compared with different ethanol concentrations to standardize the optimal concentration for the extraction of betalains. And 50% ethanol was found to be optimal. In the literature we have found the different methods, *e.g.*Delgado-Vargas et al. (2000) recommended 20 to 50% of ethanol for the complete extraction of betalains. Our results of 50% ethanol are correlating the previous studies done. In our experiments 30% ethanol showed the increased extractability, while 50% ethanol showed higher stability of betalains as compared to other concentrations. For processing experiments the solvent mixtures of 50% ethanol concentration were utilized.