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# Changes in the content and composition of anthocyanins in red cabbage and its antioxidant capacity during fermentation, storage and stewing

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ABSTRACT

The effect of fermentation, storage and stewing on the content and composition of anthocyanins as well as antioxidant capacity of red cabbage was studied. The observation of anthocyanins profile by HPLC-DAD-MS/MS was conducted. Red cabbage products contained twenty different nonacylated and acylated anthocyanins with main structure of cyanidin-3-diglucoside-5-glucoside. Treatments applied affected concentration and profile of red cabbage anthocyanins. Anthocyanins content was reduced by 24%, 25% and 34% in fermented and stewed (30 and 60-min) red cabbage, respectively. The intensity of anthocyanins degradation during storage depended on the process length. Derivatives of cyanidin-3-diglucoside-5-glucoside acylated with sinapic acid were characterised by the highest losses. Five assays were used to analysis of antioxidant capacity. Fresh red cabbage had stronger antioxidant capacity in comparison to fermented, stored and stewed red cabbage. The study has shown that red cabbage products are valuable vegetables for daily consumption in fresh, fermented, stored as well as stewed form.



## 1. Introduction

Anthocyanins content and profile as well as antioxidant capacity of both fruits and vegetables strongly depend on genetic and environmental conditions, however food processing and storage conditions also constitute major influential factors. Fruits and vegetables are often subjected to various types of processing in order to obtain more suitable and attractive food products, as well as to achieve longer and stable storage capacity. It has to be noted however that during treatment the stability of anthocyanins is dependent on their structure, plants' matrix and the process environment. Temperature, length of the process, presence of oxygen, light, plant enzymes and microorganism activities, as well as accompanying substances and pH value affect the half-life of anthocyanins (Clifford, 2000).

Anthocyanins are characterised by complex patterns of hydroxylation, methoxylation, glycosylation, and acylation (Wu & Prior, 2005). These factors are linked to plant species to form a characteristic pattern of anthocyanins. Glycosylation and acylation of anthocyanins raise their stability through intra-molecular and/or inter-molecular co-pigmentation, and self-association reactions (Bąkowska-Barczak, 2005). Therefore, acylated anthocyanins with a high degree of glycosylation being a source of colour and bioactivity may maintain the desired stability during food processing.

Anthocyanins are absorbed by humans in the aglycone, glucosidic and acylated forms (Charron, Clevidence, Britz, & Novotny, 2007). It has been indicated that anthocyanins consumed do not have any toxic, teratogenic and mutagenic properties even at high doses of these compounds (Clifford, 2000). The intake of anthocyanins has been considered to exert a beneficial effect on human health (Zafra-Stone et al., 2007), however mechanisms of this action have not been entirely explained. These natural red colourants have been demonstrated to have anticancer, cardioprotective, antineurodegenerative, vision improving and diabetes preventing activities (De Pascual-Teresa & Sanchez-Ballesta, 2008).

Red cabbage is gaining popularity all over the world and is eaten raw and after both technological and home treatment. Red cabbage is an attractive for consumers not only because of its crucial dietetic and taste values, but also its intense purple/red colour. It has been indicated that anthocyanins are responsible for formation of this colour. As was presented in the previous study (Podsedek, 2007), anthocyanins are one of the major groups of phytochemicals in red cabbage. The concentration of anthocyanins in red cabbage is relatively large and varies significantly in plants grown in different years. From nine to thirty-six different anthocyanin derivatives have been detected in various red cabbages. Among them, a large number occurs in acylated forms. Red cabbage varieties are characterised by a specific and individual profile

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of anthocyanins (Charron et al., 2007; Pliszka, Huszcza-Ciolkowska, Mieleszko, & Czaplicki, 2009; Wu & Prior, 2005). In addition, in the previous work, it has been found that red cabbage has its own characteristic antioxidant capacity where the kind of acylation affects the antioxidant activity of acylated anthocyanins (Wiczkowski, Szawara-Nowak, & Topolska, 2013). Published reports prove that red cabbage is considered to be a vegetable of a considerably high antioxidant activity (Hassimotto, Genovese, & Lajolo, 2005; Wu et al., 2004).

Taking the above into account, measurement of anthocyanins content and determination of their profile appearing in products after treatment are essential requirements for exploring the fate of anthocyanins during processing, as well as for development of suitable procedures to reduce the degradation of these red natural compounds. Examination of the manner in which red cabbage is processed and consumed has to be accompanied by the consideration of its role in preventing diseases and obtaining maximum health effects. We have therefore investigated the effects of fermentation, storage and stewing processes on the red cabbage anthocyanin concentration and antioxidant activity. The composition of individual red cabbage anthocyanins in fresh, fermented and stewed products by means of HPLC-DAD and HPLC-MS/MS methods was also determined. Five different assays were used for determination of antioxidant capacity in red cabbage products.

## 2. Materials and methods

### 2.1. Reagents

2,2′-Azobis(2-amidopropane) hydrochloride (AAPH), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma Chemical Co. (Sigma Chemical Co., St. Louis, MO). Sodium fluorescein was obtained from Fluka (Buchs, Switzerland). ACW (hydrophilic condition) and ACL (lipophilic condition) kits (model no. 400.801) for the photochemiluminescence (PCL) assay were received from Analytik Jena AG (Jena, Germany). Cyanidin aglycone was obtained from Extrasynthese (Genay, France). All other reagents of gradient-grade including acetonitrile, methanol, trifluoroacetic and formic acid were purchased from Merck KGaA (Dramstad, Germany). Water was purified with a Mili-Q system (Millipore, Bedford, MA).

### 2.2. Plant material and processes

Red cabbage (*Brassica oleracea* L. var. capitata L. f. rubra) plants of the cultivar Langedijker Polona were grown in the experimental fields of the Research Centre for Cultivar Testing (COBORU, Szczecin-Dąbie, Poland). The plants harvested were planted in 2009. Before cutting the bulbs (7 bulbs) in a shredder into ~2 mm thick strips their dried outer leaves were removed. After mixing red cabbage strips, three samples of 200 g were taken to determine the initial composition and content of anthocyanins and antioxidant properties. Subsequently, samples were immediately frozen together with liquid nitrogen. After lyophilisation, the samples were pulverised and stored at −80 °C until the analysis. The remaining shredded red cabbage was divided into two parts: 10% – process of stewing and 90% – process of fermentation.

#### 2.2.1. Fermentation and storage processes

The shredded red cabbage was thoroughly mixed with grated carrots (1%) and NaCl (3%), and after mixing, the whole was transferred to three traditional stoneware pots to run three independent fermentations. For the first 3 days cabbage pots were kept at a temperature of 24 °C and for further 11 days at a temperature of about 18 °C. For the proper conduct of fermentation process, the cabbage was pricked in order to remove releasing fermentation gases. During the fermentation process changes of pH (Radiometer PHM85, Denmark) were measured. The results obtained clearly showed that the process was run properly (Table 1). After 14 days, the process of fermentation was ended and the sauerkraut juice was collected. Next, the samples from each stoneware pot (200 g) mixed with the proportional volume of the sauerkraut juice collected were taken to determine the composition and content of anthocyanins as well as antioxidant properties of fermented red cabbage. Next, the samples were immediately frozen together with liquid nitrogen and then lyophilisated. The samples obtained were pulverised and stored at −80 °C until the analysis. The remaining red cabbage from each stoneware pot mixed with the proportional volume of the sauerkraut juice collected was transferred into five Weck jars (volume of 900 mL). The jars were filled, by strongly pressing down, to 1 cm from their upper edges and stored at ~4 °C in a refrigerator. The composition and content of anthocyanins and antioxidant properties of fermented red cabbage were analysed after 7, 30, 60, 90, and 180 days of storage. At the due time the samples from jars were immediately frozen together with liquid nitrogen and then lyophilisated. The samples obtained were pulverised and stored at −80 °C until the analysis.

#### 2.2.2. Stewing process

The shredded red cabbage samples (200 g) were placed into a stainless steel pot with a small volume of boiling distilled water (50 mL) and covered with lid. After bringing the water to boil, red cabbage was stewed for 30 min or 60 min, and mixed from time to time. Following this procedure, the stewed red cabbage was immediately frozen together with liquid nitrogen and then lyophilisated. The samples obtained were pulverised and stored at −80 °C until the analysis.

### 2.3. Extraction and chromatographic analysis

Extraction and analysis of anthocyanins in red cabbage products were carried out as described previously by Wiczkowski et al. (2013). About 0.05 g of dried and pulverised red cabbage tissues were extracted by 30 s sonication (VC 750, Sonics & Materials, USA) with 1 mL of mixture consisting of methanol/water/trifluoroacetic acid (0.58/0.38/0.04, v/v/v). Subsequently, the mixture was

**Table 1**
The changes of environmental pH value during process of fermentation and storage.

| Time of fermentation and storage | pH value ± SD |
|---|---|
| Shredded red cabbage | 6.21 ± 0.02 |
| 1 Day of fermentation | 5.81 ± 0.02 |
| 2 Day of fermentation | 4.80 ± 0.01 |
| 3 Day of fermentation | 4.20 ± 0.03 |
| 4 Day of fermentation | 4.08 ± 0.01 |
| 5 Day of fermentation | 4.00 ± 0.01 |
| 6 Day of fermentation | 3.90 ± 0.01 |
| 7 Day of fermentation | 3.81 ± 0.02 |
| 8 Day of fermentation | 3.79 ± 0.03 |
| 9 Day of fermentation | 3.78 ± 0.03 |
| 10 Day of fermentation | 3.82 ± 0.02 |
| 11 Day of fermentation | 3.80 ± 0.01 |
| 12 Day of fermentation | 3.81 ± 0.02 |
| 13 Day of fermentation | 3.78 ± 0.02 |
| 14 Day of fermentation | 3.79 ± 0.02 |
| 7 Days of storage | 3.98 ± 0.03 |
| 30 Days of storage | 3.99 ± 0.02 |
| 60 Days of storage | 3.98 ± 0.01 |
| 90 Days of storage | 3.99 ± 0.02 |
| 180 Days of storage | 4.01 ± 0.01 |