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Characterization of phenolics, glucosinolates and antioxidant activity of beverages based on apple juice with addition of frozen and freeze-dried curly kale leaves (*Brassica oleracea* L. var. *acephala* L.)

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ABSTRACT

The aim of this study was to determine the polyphenols, glucosinolates and ascorbic acid content as well as antioxidant activity of beverages on the base of apple juice with addition of frozen and freeze-dried curly kale leaves. Upon enrichment with frozen (13%) and freeze-dried curly kale (3%), the naturally cloudy apple juice was characterized by an increase in phenolic compounds by 2.7 and 3.3-times, accordingly. The antioxidant activity of beverages with the addition of curly kale ranged from 6.6 to 9.4 μmol Trolox/mL. The obtained beverages were characterized glucosinolates content at 117.6–167.6 mg/L and ascorbic acid content at 4,1–31,9 mg/L. The results of sensory evaluation of colour, taste and consistency of apple juice and beverages with the addition of kale did not differ significantly prior to pasteurization ($P \leq 0.05$), whereas after the pasteurization the evaluated factors decreased significantly.

1. Introduction

In development of novel foodstuffs exhibiting bioactive properties a particularly important role is played by fruit and vegetables and their processed products, being rich sources of biologically active compounds. Epidemiological studies show a positive correlation between consumption of fruit and vegetables and prevention of diseases, such as atherosclerosis, cancer, diabetes, arthritis and many others (Borek, 2005; Kaur & Kapoor, 2002; van Poppel, Verhoeven, Verhagen, & Goldbohm, 1999). Plant metabolites, such as polyphenols and glucosinolates are among the most commonly studied biologically active compounds (Kaur & Kapoor, 2002; Verkerk et al., 2009). In case of vegetables, the role of cabbage vegetables in the prevention of several diseases is very notable. Furthermore, from a nutritional point of view, this type of vegetables is a crucial part of human diet. It has been established that consumption of cabbage vegetables may decrease the risk of cancer and cardiovascular diseases (Zhang & Tang, 2007). The sulfur compounds present in cabbage vegetables – glucosinolates, which play a major role in the metabolism of cancer cells, are secondary plant metabolits, which are derivatives of amino acids with sulphlur and thioglucosidic moieties (Agerbirk & Olsen, 2012). Recent studies provide evidence regarding a correlation between the possibility of lowering the risk of some cancer types (i.e. breast, cervix, prostate, lung, stomach or colon) and increased consumption of cabbage vegetables (Sapone et al., 2007; Verkerk et al., 2009). Curly kale is a particular vegetable in this group, due to the presence of several beneficial compounds. Aside from a high glucosinolates content, the biological value of curly kale is increased by a relatively high content of vitamins, especially vitamin C, and considerable amounts of phenolic compounds, including quercetin and kaempferol – flavonoids, which along with carotenoids are responsible for the high antioxidant activity of this raw material (Kaur & Kapoor, 2002). Kale is the best source among cabbage vegetables of vitamin A (0,8 mg/100 g edible portion), B_1 (0,11 mg), B_2 (0,2 mg), B6 (0,35 mg), E (1,7 mg) also contains folic acid and niacin. In addition, kale is characterized by a high content of essentials minerals, both macro and micronutrients, especially K (188–873 mg), Ca (50–550 mg), Mg (20–126 mg), Fe (1,7–2,1 mg) and Cu (50–120 mg) (Rosa & Heaney, 1996; Thavarajah et al., 2016). The studies of Chung, Lee, and Sung (2002) indicate that consumption of curly kale reduces the amount of emerging N-nitrosodimethylamine, a carcinogenic compound which is formed in the human organism upon consumption of nitrate- and amine compounds-rich food. Currently there are numerous medical studies conducted around the world regarding on the anticarcinogenic properties of curly kale (Borek, 2005; Chung et al., 2002; van Poppel et al., 1999). They are focused on the possibility of reducing

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the risk of certain types of cancer (Sapone et al., 2007). Furthermore, the recent reports suggest that the glucosinolates degradation products may also play a significant role in the prevention of cardiovascular system diseases, ie. hypertension, atherosclerosis, due to antioxidant and anti-inflammatory properties. Kim, Sun, Kwon, Park, and Lee-Kim (2008) indicate that suppelentation of the diet with curly kale juice results in a beneficial fatty acid profile by influencing the HDL-cholesterol level and glutathione peroxidase activity in men with hyperlipidemia, thereby reducing the risk of coronary heart disease in these patients.

Currently, the use of curly kale as a food raw material is limited, its industrial processing is also low (mainly by freezing). Fresh curly kale is seldom consumed due to low sensory appeal. The proposed introduction of curly kale in the form of a beverage based on apple juice allows to obtain a food product with attractive sensory quality and beneficial composition of bioactive compounds (phenolic acids, flavonoids, glucosinolates). Apple juice seems to be a good base for the new product, since it is often consumed and is a potent source of bioactive compounds by itself. The characteristic compounds for naturally cloudy apple juice include catechins, procyanidins, consisting primarily of (−)-epicatechin units and hydroxycinnamic acids (mainly chlorogenic and dihydrochalcones) (Kolniak-Ostek, Oszmiański, & Wojdyło, 2013).

The aim of this study was to determine the content of biologically active compounds (vitamin C, polyphenols and glucosinolates) and antioxidant activity of a beverage based on apple juice with the addition of frozen and freeze-dried curly kale leaves. Additionally, the bioactive compounds content in the beverage was compared in the fresh form and after thermal processing.

2. Materials and methods

2.1. Chemicals

Ultra pure water was produced in the laboratory (Direct-Q UV3 Water Purification System Millipore, Billerica, USA). Sodium acetate ($C_2H_3NaO_2$) was obtained from Chempur (Piekary Śląskie, Poland). HPLC grade acetonitrile (CH_3CN), methanol (MeOH) and meta-phosphoric acid (HPO_3) were obtained from Sigma Aldrich Chemie GmbH (Steinheim, Germany). 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), (+)-catechin, DEAE Sephadex A-25, L-ascorbic acid ($C_6H_8O_6$), 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^+$), sulphatase from *Helix pomatia* and standards for phenolic compounds were purchased from Sigma Aldrich Chemie Co. (St. Louis, USA). Monobasic potassium phosphate (KH_2PO_4) was obtained from Sigma Aldrich Chemie Co. (Tokyo, Japan). Potassium persulphate ($K_2O_8S_2$), chlorogenic acid and L-dithiothreitol were purchased from Sigma Aldrich Chemie Co. (Buchs, Switzerland). Imidazole ($C_3H_4N_2$) was obtained from Merck Schuchardt OHG (Hohenbrunn, Germany) and glucotropaeolin from Roth (Karlsruhe, Germany).

2.2. Raw material

Curly kale *(Brassica oleracea* L. var. *sabellica* L.) cv (Reflex) was purchased from an agricultural-gardening farm in Przeclaw, near Poznań (Poland). The raw material was subjected to freezing and freeze-drying directly after harvesting. Apple juice was obtained from (Shampion) apples originating from the Agricultural and Pomiculture Experimental Station in Przybroda. The raw material was processed directly after harvesting.

2.3. Technological process

2.3.1. Storage of curly kale intermediate products

Curly kale was collected in the form of whole plants. Afterwards the leaves were separated and the stems were discarded. The leaves were sorted, cleaned and cut into approx. 3 cm fragments. The prepared raw materials were used to obtain intermediate products in a frozen and freeze-dried form.

2.3.2. Freezing process

In order to avoid qualitative changes during freezing, the process was conducted in a two-step manner. During the first step the raw material was submerged in liquid nitrogen (−196 °C) for 3 min. During the second step the raw material was kept at −50 °C for 24 h. Afterwards, it was comminuted (while in the frozen state) using a Termomix laboratory mill (Wuppertal, Vorwerk, Germany). The raw material was thawed until it was added to the apple juice.

2.3.3. Freeze-drying process

Prior to freez-drying the raw material was frozen as described above in order to obtain a frozen intermediate product and afterwards it was subjected to freeze-drying using a Labconco Corporation Freeze Dryer 6L (Kansas City, USA) at a pressure of 13.3 Pa for 48 h at shelf temperature of 5 °C (first step) and further dried at shelf temperature of 25 °C. After freeze-drying products were comminuted (Termomix laboratory mill).

2.3.4. Preparation of the apple juice-based beverages

The apples were sorted, cleaned and comminuted using a food processor (Zelmer, type 986.87, Rzeszów, Poland), then subjected to pressing using a Para-press laboratory press (Arauner Kitzingen, Kitzingein, Germany) at 0.3 MPa for 10 min (the pressing efficiency was at 58%). The juice was the base for beverages obtained by addition of curly kale intermediate products and mixing in a Termomix laboratory mill. Two compositions were obtained: 87% of apple juice with 13% of frozen curly kale (17% dry matter) and 97% of apple juice with 3% of freeze-dried curly kale (95% dry matter). In order to improve the consistency, the beverages were subjected to homogenization using a flow homogenizer Super-Dispax SD 41 (IKA-Labor Technik, Jane & Kunkel GMBH & Co.KG, Staufen, Germany) at 0.1 mm gap size. The beverages were subjected to thermal processing using pasteurization at 90 °C for 5 min and hot fill bottling into glass bottles (0,33 L), then cooled to 20 °C.

2.4. Extraction and determination of phenolic compounds

In order to determine the polyphenols content a 10 ml aliquot was collected from the prepared samples. The samples homogenized with an IKA T-25 homogenizer (IKA-WERKER, Staufen, Germany), with 50 ml 700 g/L methanol at room temperature. The homogenates were shaken for 15 min in a mechanical Water Bath Shaker 357 (Elpin, Lubawa, Poland) and centrifuged for 30 min using a MPW-351R centrifuge (Warszawa, Poland) at 4000x*g*. The procedure was perform twice. The supernatants were combined and evaporated in a vacuum evaporator with water bath Büchi R-205 (Flawil, Switzerland) at 40 °C. Condensed extracts were diluted to 25 ml with ultra pure water (Vallejo, Tomás-Barberán, & García-Viguera, 2002).

Determination of phenolic compounds was performed using the LC Agilent Technologies 1200 Rapid Resolution (Waldbronn, Germany) system equipped with a UV–Vis detector (DAD 1260,Waldbronn, Germany) and a Poroshell 120, SB-C18 column (4.6 × 150 mm, 2.7 μm) (Wilmington, USA). Separation was performed in a reverse phase system using gradient elution. The mobile phase was performed by 60 g/L acetic acid in 2 mmol