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Characteristics of organic acids in the fruit of different pumpkin species

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ABSTRACT

The aim of the research was to determine the composition of organic acids in fruit of different cultivars of three pumpkin species. The amount of acids immediately after fruit harvest and after 3 months of storage was compared. The content of organic acids in the examined pumpkin cultivars was assayed using the method of high performance liquid chromatography (HPLC). Three organic acids (citric acid, malic acid, and fumaric acid) were identified in the cultivars, whose content considerably varied depending on a cultivar. Three-month storage resulted in decreased content of the acids in the case of cultivars belonging to *Cucurbita maxima* and *Cucurbita pepo* species, while a slight increase was recorded for *Cucurbita moschata* species.

1. Introduction

Due to the constantly increasing pollution of the environment (Szczurek & Maciejewska, 2013), as well as the fast pace of life, people attempt to find ways for healthier eating. Therefore, there is a tendency to find raw materials rich in most beneficial nutrients as far as their health – supporting properties are concerned while consumers more eagerly choose vegetables of high nutritional and biological value. Pumpkin is one of the vegetables which are still underrated by consumers, as well as food growers. Its fruit can be a healthy and valuable component of a number of dishes and fruit products (Nawirska-Olszańska, Biesiada, Kucharska, & Sokół-Łętowska, 2012). This vegetable is particularly valuable as it contains high quantity of carotenoids in its flesh (Biesiada, Nawirska, Kucharska, & Sokół-Łętowska, 2009), including β-carotene, lutein, and violaxantine. In addition, many cultivars of winter squash are characterised by considerable content of vitamin C (Biesiada, Nawirska, Kucharska, & Sokół-Łętowska, 2011). An extraordinary trait of pumpkin is its low caloric value, since its flesh, depending on variety, contains merely 15–25 kcal in 100 g, and thanks to the presence of numerous, easily digestible nutrients it has become an advantageous component of slimming diets. It regulates metabolism, lowers glucose level in blood, possesses detoxicating as well as slightly dehydrating properties. Another attributed function of pumpkin species is defense against cancer (Astorg, 1999).

Organic acids are widely distributed in fruits and vegetables. The quantity of organic acids varies considerably among vegetable species and cultivars (biotic factors) and it is also influenced by local (abiotic) factors such as climate, soil, etc. The content of organic acids tends to decrease throughout the course of vegetable development (Saccani et al., 1995; Saradhuldhat & Paull, 2007).

Vegetables generally contain low concentrations of organic acids, nevertheless the acid components merit study owing to their important role as natural antimicrobial agents and flavour enhancers. The nature and concentration of organic acids are important factors influencing organoleptic characteristics of fruit and vegetables, namely their flavour (Picha, 1985). The effect of organic acids on flavour principally results in tissue acidity, which translates into the perception of sweetness. In fact, the ratio of organic acid content to that of sugar is used as an indicator of fruit and vegetable ripeness (Kuti, 1992). The relative amounts and the presence of each of the components were considered useful in taxonomic studies and as means to evaluate food processing. Acids fulfill various functions in food products. Apart from affecting food flavour, they also stimulate the growth of microorganisms, coagulate proteins and perform buffering function. Organic acids present in fruit and vegetables, mainly malic and citric acid, exert an alkalising effect on the human body, inhibit the growth of undesirable microflora, as well as exert influence on the course of metabolic processes (Silva, Andrade, Goncalves, et al., 2004; Valentão et al., 2005). Additionally, organic acids due to their antioxidant activity play a protective role against various diseases. Not only ascorbic acid but also other organic acids, except for oxalic acid, improve the absorption of nonheme iron from plant foods (Silva, Andrade, Valentão, et al., 2004). Organic acids in vegetables enhance food

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flavour, as well as stimulate action of digestive glands and intestinal peristalsis.

In vegetables, organic acids are present in a bound form (except for tomatoes and rhubarb), while in fruits - in a free form. Hydracids (citric, malic, tartaric, succinic, and oxalic) can be found mainly in fruits, providing a characteristic flavour and causing decrease in pH values. In vegetables the acids occur in the form of salts (potassium, calcium, and sodium). The very fact of their presence in vegetables and fruits is definitely advantageous, as they inhibit the growth of microorganisms and facilitate the preservation process both in of fruits and vegetables.

The quantity of organic acids is highest when a fruit is unripe, and after that the content of acids decreases (ripening, storage). Acidity of unripe fruit ranges from 0.2% to 3% (for apples and pears it amounts to 0.2–0.8%, for fruit with a stone 0.8–1.5%, for berries 1–3%, and for citrus it is 5%). In sour varieties of apples more than 90% total acids belong to malic acid, while the rest belongs to citric and other acids. Similar composition of acids can be found in plums, cherries and sour cherries. In berries the citric acid is dominant, e.g. in black currants it ranges from 21.5 to 28.2 mg/kg, in red currants 16.2–22.8 mg/kg, in strawberries 6.6–8.7 mg/kg. Acidity of fruits is higher than that of vegetables, except for rhubarb whose acidity is fairly high – up to 1.6% (Gillooly et al., 1983). Among vegetables, the highest quantity of total organic acids have Brussel sprouts, parsnip, tomato and eggplant fruit (0.4–0.6 g/100 g FW).

The content of free titratable acids in vegetables is 0.2–0.4 g/100 g fresh tissue, which is low in comparison with fruits. Accordingly, pH is relatively high (5.5–6.5), with several exceptions such as tomato or rhubarb. Other acids of the citric acid cycle are present in negligible amounts. Oxalic acid occurs in larger amounts in some vegetables. Among the 18 vegetables tested the predominant acids are malic and citric acid and in most cases malic acid was the most abundant. Succinic, fumaric, and quinic acids are widely spread, while tartaric acid was found in carrots, lettuce, endives, chicory and celery. Other acids such as malonic, shikimic and t-aconitic acids occur sporadically. Lactic acid was not detected in any vegetable species (Belitz, Grosch, & Schieberle, 2004).

According to other authors, organic acids in fruit included malic, citric, quinic, shikimic, lactic, tartaric, fumaric and succinic acids (Ruhl & Herrmann, 1985). However, not many authors investigated the content of organic acids in fruit of different pumpkin species within a single cultivation region (Gil et al., 2000; Kafkas, Kosar, Türemis, & Baser, 2006; Moing et al., 2001; Sturm, Koron, & Stampar, 2003). To our knowledge there is no up-to-date study concerning organic acids in pumpkin after harvest and after 3-month storage.

2. Materials and methods

2.1. Chemicals

The organic acid kit and sulfuric acid were from Sigma–Aldrich (Germany).

2.2. Pumpkin cultivation

The aim of the study was to determine the content of organic acids in eight pumpkin cultivars: Ambar, Bambino, and Uchiki Kuri belonging to *Cucurbita maxima* species, the *Cucurbita pepo* species was represented by Danka, Jet F_1, Makaronowa Warszawska (spaghetti type) and Miranda (hulles Styrian type), and Butternut cultivar of *C. moschata* species. All the pumpkin cultivars were grown in the Research-Experimental Station at Psary, belonging to Wroclaw University of Environmental and Life Sciences. Soil under the cultivation was fertilised with nitrogen in the form of ammonium nitrate at 160 kgN/ha. The experiment was set in the random block design in four replications. The area of one block was 9 m^2, the planting was 1 m × 1.5 m. Four-week seedlings were planted in the third week of May and harvested in the second decade of September 2011. Fully ripened fruits were chosen for chemical analysis, five fruits from each replication. The investigation was carried out in the laboratory of Fruit and Vegetable Technology of Wroclaw University of Environmental and Life Sciences in September and December of 2011. Pumpkin fruit was kept in cold store at 10 °C.

2.3. Preparation of samples

Pumpkin puree was prepared by mixing in Thermomix TM (Vorverk) for 10 min at a temperature of 90 °C and stored at −18 °C until it was subjected to analyses. First, it underwent basic analyses. In order to assay acids with the use of HPLC, the samples were prepared according to the following procedure: fruit puree (10 g) was diluted to 50 ml with double-distilled water and boiled in 95 °C for 5 min, and clarified by centrifugation at 6000*g* for 15 min. The extract was filtered through 0.45 μm Millipore filters and 20 μl sample was used for HPLC analysis of organic acid.

2.4. Chemical analysis of pumpkin

Dry matter content was determined using the standard AOAC method (AOAC, 1995). The method consists in drying at defined pressure and temperature until the sample attains constant mass. In the present study the soluble solids content was determined using a digital Pocket PAL-1 refractometer made by ATAGO (Japan). The measuring range of the apparatus was between 0 and 53 Brix degrees with automatic temperature compensation ranging from 10 to 75 °C. Pumpkin puree reaction was determined with a pH-meter PX-processor PM-600 made by TMS Electronics. Sugars were assayed according to the Lane–Eynon method (PN-90A-75101/07).

2.5. Analysis of organic acids

Organic acids were determined by the HPLC method, isocratically, using 0.001 N sulfuric acid, at 210 nm wave length and flow of 0.6 ml min^{-1} (Sturm et al., 2003). The assay was done with the use of liquid chromatographer produced by Dionex (USA), equipped with LED model detector Ultimate 3000. The latter one cooperated with the following devices: LPG-3400A pump, EWPS-3000SI autosampler, TCC-3000SD column thermostat and the Chromeleon v.6.8 computer software. Separation was conducted on Aminex HPH-87 H (300 × 7.8 mm) column with IG Cation H (30 × 4.6) precolumn of Bio-Red firm, at a temperature of 65 °C.

2.6. Statistical analysis

Analysis of variance was conducted using ANOVA procedures. Statistical analysis was performed with Statistica 8.0 (StatSoft Poland). Significant differences ($P \leqslant 0.05$) between the mean values were determined using Duncan's Multiple Range Test.

3. Results and discussion

The examined pumpkin cultivars characterised diverse content of dry matter (Table 1). Within both the *C. maxima* and *C. pepo* species the content of dry matter differed significantly. For Ambar cultivar it amounted to 19.3%, which was about twice the values determined for Jet F_1 (9.5%) and Uchiki Kuri (9.4%), while the lowest values belonged to Bambino (4.5%) and Miranda (3.6%) culti-