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Assessment of the differences in the physical, chemical and phytochemical properties of four strawberry cultivars using principal component analysis

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ABSTRACT

The worldwide established strawberry cultivar 'Albion' and three recently introduced cultivars in Europe: 'Monterey', 'Capri', and 'Murano', grown hydroponically, were studied to ascertain the influence of cultivar and harvesting date on the physical, chemical, antioxidant and phytochemical properties of their fruits. Interrelationships of investigated parameters and these cultivars were investigated by the statistical approach of principal component analysis (PCA). Results indicated that cultivar had a more significant effect on the analyzed parameters than harvesting date. Thus grouping of the variables in a PCA plot indicated that each cultivar has specific characteristics important for consumer or industrial use. Cultivar 'Monterey' was the richest in phytochemical contents and consequently in antioxidant activity, 'Albion' showed the highest contents of total soluble solids, titratable acidity content and ascorbic acid, 'Capri' had the highest value of firmness, while 'Murano' had lighter color in comparison to others. Potential use of these cultivars has been assessed according to these important measured attributes.

1. Introduction

The strawberry (*Fragaria x ananassa* Duch.) fruits are one of the most popular and widely consumed berries worldwide, due to the sweet flavor and preferred organoleptic properties. According to the Food and Agriculture Organization (FAO) of the United Nations, world strawberries production was increased more than 80% in the last 20 years reaching over 4.5 million tons in 2012 (FAOSTAT, 2013). Fresh market strawberries is account for about 80% of total strawberries production, while the rest are intended for industrial processing purposes, such as the production of yogurts, jams, jellies, dessert toppings, etc. Color, firmness and chemical composition of fruits are considered the main quality parameters that consumer acceptance depends on (Ornelas-Paz et al., 2013). Fruit flavor is mostly determined by contents of total acids, total soluble solids and their ratio (Krüger et al., 2012). The color in strawberries is caused by the accumulation of anthocyanins, polyphenolic compounds which contribute beneficial health properties (Piljac Žegarac et al., 2012; Strathearn et al., 2014). Strawberries are fruits known also as a good source of other polyphenolic compounds such as flavan-3-ols, ellagitannins, cinnamic acid conjugates, flavanols, ellagic acid conjugates, etc. (Giampieri, Alvarez-Suarez, & Battino, 2014; Kosinska, Diering, Prim, Heritier, & Andlauer, 2013). One of the aspects of major nutritional relevance of strawberries is the high content of vitamin C, but additionally they contain an extensive range of other vitamins such as thiamin, riboflavin, niacin, and vitamin B6 and fat-soluble vitamins, including carotenoids, vitamin A, vitamin E and vitamin K (Alvarez-Suareza et al., 2014; Giampieri et al., 2015). Content of beneficial health compounds in strawberry could vary depending on the genotype (Kosinska, Diering, Prim, Heritier, & Andlauer, 2013), factors related to the growing environment (Krüger et al., 2012; Ulrich & Olbricht, 2014), ripening stage (Ornelas-Paz et al., 2013) and postharvest storage (Piljac-Žegarac & Šamec, 2011; Šamec & Piljac-Žegarac, 2015).

All phytochemicals present in strawberries exert a synergistic and cumulative effect on human health promotion and on the prevention of various diseases such as inflammation, cardiovascular diseases, obesity, metabolic syndrome, certain types of cancers and even neurological diseases (Giampieri et al., 2015). The polyphenols are mainly known for their anti-inflammatory and antioxidant actions, but recent studies have demonstrated that

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their biological activities also spread to other pathways involved in cellular metabolism and cellular survival (Chiva-Blanch & Visioli, 2012; Forbes-Hernández et al., 2014; Giampieri et al., 2014). According to the recent review article by Giampieri et al. (2015), strawberry phenolics, in addition to their ability to act as free radicals scavengers, are able to modulate the expression of genes involved in metabolism, cell survival, proliferation and antioxidant defense, and they protect and repair DNA damage.

Due to their huge commercial and economic impacts, strawberries can be considered the most studied berry from nutritional, genomic, or agronomic points of view (Giampieri et al., 2014). Recently, market requirements have focused on strawberry fruits with good appearance, taste and shelf life, while industrial requirements include strawberries with balanced physical and chemical properties as well as higher antioxidant compound content. The main effort of producers is to manage environmental factors during fruit production to gain stability of the mentioned properties (Krüger et al., 2012). One possible solution is use of soilless protected cultivation of strawberries (Neocleous, 2012). This type of production, in addition to facilitating the consumption of fresh strawberries all year round, can enable the industrial production of 'seasonal desserts' (which require the use of fresh strawberries) throughout the entire year.

In the present study we evaluated physical and phytochemical parameters of four strawberry cultivars grown in soilless media and, out of season, with the aim of assessing specific quality parameters for each studied cultivar. We analyzed data using principal component analysis, a statistical tool which allowings for visualization of the interrelationships of the investigated parameters and the four studied cultivars: 'Albion', 'Monterey', 'Capri', and 'Murano'. Additionally, in order to determine how harvesting time influences fruit quality we analyzed fully mature fruits at three harvest dates.

2. Material and methods

2.1. Plant material and growing conditions

The plants were grown in the year 2013, in the greenhouse of the private company GIS-IMPRO, situated in Vrbovec, Croatia. Cold-stored frigo plants of four cultivars: 'Albion', 'Capri', 'Monterey' and 'Murano' were planted on 18th of March 2013, in plastic bags ($0.12 \times 0.20 \times 1.00$ m) filled with coconut coir and double rows of 12 planting holes for strawberry production (SIMONETTI ADAMO s.r.l.). Distance between the middle of bags was 1.00 m, with plant density at 12 plants per square meter. A drip irrigation system with spaghetti drip tubes was used. The standard nutrient solution for strawberries had an EC of 1.2–1.5 dS m^{-1} and a pH from 5.0 to 5.5. Bumblebees (*Bombus terrestris*) "NATUPOL Beehive" from the Koppert B.V. (The Netherlands) was used for pollination. Temperature (°C) and insolation (J cm^{-2} d^{-1}) were recorded continuously in the greenhouse during strawberry production (Supplement material Table 1).

Fruits were harvested when they reached fully red color. Harvesting was performed early in the morning on the following dates: 09th October (I), 29th October (II) and 15th November (III) in 2013. Immediately after harvesting, fruits were subjected to analyses of physical and chemical properties such as fruit color, firmness, total soluble solid (TSS), titratable acidity (TA), and pH value. Fruits intended for the phytochemical analyses were stored at −80 °C until use.

2.2. Determination of physical and chemical fruit properties

For determining fruit color, weight and firmness, 20 individual fruits were evaluated. The same fruits were then mashed, homogenized and used for determination of total soluble solid content (TSS), titratable acidity (TA), and pH value. Four replicates were measured for each of these analyses.

Fruit color was measured using a ColorTec PCM colorimeter (ColorTec Associates Clinton, New Jersey, USA) and expressed as CIE *Lab* (CIELab) values. Color is defined three- dimensionally using the *L*, *a*, *b* notation. The *L* axis represents lightness of the color (the lower the value, the darker the color). *L* is orthogonal to a chromaticity plane defined by two perpendicular axes, *a* and *b*. The *a* axis represents the balance between red (positive values) and green (negative values) and the *b* axis the balance between yellow (positive values) and blue (negative values). These coordinates give access to new indices, the hue angle (h = arctan b/a), which represents the basic color.

Firmness was measured using a penetrometer PCE-PTR 200 Penetrometer (PCE Instruments UK Limited) with 6 mm probe. Firmness value for each individual fruit was the average of two measurements made at opposite fruit sides at the equatorial fruit zone and expressed as kg cm^{-2}. The juice from each fruit was extracted and was used for determination of TSS (°Brix) using a refractometer ATAGO PAL-1 (Atago Co., Ltd., Tokyo, Japan) according to Mitcham, Cantwell, and Kader (1996).

TA was determined by juice titration with 0.1 N NaOH and expressed in percent of citric acid per 100 ml of juice (Lacey, Hancock, & Ramsey, 2009).

2.3. Total ascorbic acid determination

Total ascorbic acid (vitamin C) was determined using dinitrophenylhydrazine (DNPH) (Terada, Watanabe, Kunitomo, & Hayashi, 1978). Frozen fruits (1 g) were homogenized with 10 ml of mixture of 5% metaphosphoric acid and 10% acetic acid solution using vortex and then centrifuged. Further, 2 mL of supernatant were mixed with 115 μL of bromine water for oxidation of ascorbic acid to dehydroascorbic acid. To remove the excess of bromine, 65 μL of 10% thiourea was added. This was followed by the addition of 500 μL of DNPH solution and incubation at 37 °C for 3 h. After cooling the samples on ice, 2.5 mL of 85% H_2SO_4 was added which led to the formation of a red complex and the absorbance was determined spectrophotometrically at 521 nm. The calibration curve was prepared using ascorbic acid (5–100 mg/L) and results were expressed in mg/100 g FW.

2.4. Polyphenols determination

For polyphenols analysis, about 20 frozen strawberry fruits were quickly homogenized in a kitchen blender and supplemented with 80% methanol (10 ml per g of fruit). Following extraction for 15 min using ultrasonic bath, samples were subjected to a rotation homogenizer for 2 h, and were centrifuged for 20 min at 4600 rpm (Eppendorf Centrifuge 5415C, Germany). All extractions were done in triplicate. The obtained supernatant was used for phytochemical analysis.

The total phenolic (TP) content was determined by using Folin–Ciocalteu method (Singleton & Rossi, 1965) in the final reaction volume of 2 mL. Gallic acid was used to prepare the calibration curve and the results were expressed on a fresh weight basis as an mg gallic acid equivalents/100 g of samples (mg GA/100 g FW).

The total flavonoid (TF) content was determined according to the $AlCl_3$ colorimetric assay (Zhishen, Mengcheng, & Jianming, 1999) adapted for small volumes (Šamec, Piljac-Žegaraca, Bogovic, Habjanic, & Grúz, 2011). The added reagent volumes were proportionally reduced so that the final reaction volume amounted to 2 mL and could be prepared in disposable plastic cuvettes. Catechin was used as a standard and the results were expressed as