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Original Research Article

Characterization and differentiation of botanical and geographical origin of selected popular sweet cherry cultivars grown in Greece

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ABSTRACT

Four sweet cherry cultivars grown in northern Greece (Kordia, Regina, Skeena, Mpakirtzeika) were characterized and differentiated according to both botanical and geographical origin (Skeena cultivar). Conventional quality parameter analyses applied to achieve differentiation included: a) conventional quality parameters namely, titratable acidity (TA), pH and total soluble solids (TSS) measurement of mechanical properties-penetration, b) analysis of glucose and fructose (major sugars) using High Performance Liquid Chromatography-Refractive Index Detector (HPLC-RID), c) identification and semi-quantification of volatile compounds using Solid Phase Micro Extraction in combination with Gas Chromatography/Mass Spectrometry (SPME-GC/MS) and d) analysis of minerals using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). Data were analysed using Multivariate Analysis of Variance (MANOVA) and Linear Discriminant Analysis (LDA). Results showed that the combination of volatile compounds-conventional properties-minerals provided the highest correct prediction rate of 97.4% for botanical differentiation while for the geographical differentiation of the Skeena cultivar, conventional parameters and volatile compounds provided a 94.9% and 95.0% correct prediction rate respectively.

1. Introduction

Cherries are an important fruit crop of Greece, with an annual production of 45,000–60,000 tons (7%–8% of EU production). As a result, Greece ranks No. 12 globally in cherry production (Kazantzis and Chatzicharisis, 2011). Cherries are thought to be native to Europe and western Asia but are now widely grown around the world (Lezzoni, 2008). They belong to the *Rosaceae* family, genus *Prunus*, subspecies *Cerasus*.

In 1992, through Regulation 2081/92, the European Union (EU, 1992a) first introduced the scheme on the Protection of Geographical Indication (PGI) and Designation of Origin (PDO) for agricultural products and foodstuffs. Likewise, Regulation 2082/92 (EU, 1992b) covered concepts for certifications of specific character for agricultural and food products. In 2006, the EU updated above regulations with R. 510/06 and 509/06 respectively (EU, 2006a,b).

A ripe cherry fruit has a bright shiny pale to deep red or even purple colour, usually with a thin skin. Because of their colour, aroma, taste and health beneficial antioxidant properties, cherries are greatly appreciated worldwide (Crisosto et al., 2003; Serradilla et al., 2012). Among the classical sweet cherry cultivars grown in Greece, the Skeena cultivar of Canadian origin, gives a very large, red, shiny skin, kidney-shaped fruit of very firm texture destined for fresh consumption. The Regina cultivar of German origin, gives a medium to large, red, heart-shaped fruit of firm texture, rich in volatile compounds and TSS. The third cultivar, Kordia of Bohemian (Czech) origin, gives a large, deep red heart-shaped fruit of firm texture, rich in sugars and metals. Lastly, the Mpakirtzeika cultivar of Greek origin, gives a large, red, heart-shaped fruit of firm texture with a characteristic sweet-sour taste of low acidity and a high concentration of phenolic compounds.

In terms of sensory quality, the main cherry attributes include skin colour, sweetness (sugar content), sourness (organic acid content), fruit firmness, fruit weight, and particular aroma even though the compounds contributing to fruit odour comprise a very small portion of fruit weight (0.001-0.01% fresh weight basis) (Zhang et al., 2007). Aroma is one of the most valued attributes of sweet cherries which may influence consumer acceptance of the fruit. Cherries are also a good source of phenolic compounds (ca.150 mg Gallic Acid Equivalents /kg

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Fresh Weight (FW)) made up mainly of hydroxycinnamates, anthocyanins, flavan-3-ols and flavonols (Gonçalves et al., 2004a,b). Such compounds are responsible for the strong antioxidant activity of cherry fruit (Yoo et al., 2010).

Sugars also, play an important role in the quality of sweet cherry fruits as they are balanced with acid to contribute to fruit flavour. In sweet cultivars, the level of sugars can be as high as 25 g/100 g of fruit (Girard and Kopp, 1998). Five different sugars are normally found in sweet cherries namely, glucose, sucrose, fructose, maltose, and sorbitol. However the main sugars found in sweet cherries are glucose and fructose which account for approximately 90% of total sugars of the fruit (Usenik et al., 2008).

Based on the above, the objectives of the present study were to characterize different cherry cultivars grown in Greece and to differentiate cherries according to a) botanical origin (for the Mpakirtzeika, Regina, Kordia and Skeena cultivars) and b) geographical origin (for the Skeena cultivar). A further objective was to investigate possible, specific characteristics of the Mpakirtzeika cultivar in relation to those of the other three cultivars in an effort to possibly file a petition to Greek authorities in order to obtain either a PDO or PGI status for this Greek cherry cultivar. To the best of our knowledge, there are no reports in the literature on the differentiation of either botanical or geographical origin of the above sweet cherry cultivars.

2. Materials and methods

2.1. Samples

A total of 78 sweet cherry samples were collected from northern Greece as follows: 14 samples of the Regina cultivar from Voio - Kozani, 12 samples of the Kordia cultivar from Kozani, 12 samples of the Mpakirtzeika cultivar from Edessa and 40 samples of the Skeena cultivar (12 from Edessa, 14 from Kozani and 14 from Kato Milia - Pieria). The climate in Edessa and Kato Milia-Pieria is mild and temperate. Both areas have a significant amount of rainfall during the year. The average annual temperature is between 13–14 °C. The average annual rainfall is between 435–487 mm. Respectively, the climate in Voio - Kozani is cooler as the area is partly mountainous but also considered temperate. Regarding rainfall is similar to that of Edessa and Pieria. The average annual temperature is in this area is between 10–13 °C. It should be noted that the three above areas are approximately 100 km apart from each other. Cherry management measures were the same in all three above areas as specific cherry orchards were selected for the study.

Samples were collected during two periods May 20-June 30, 2015 (39 samples) and 2016 (another 39 samples) at the stage of full ripeness. Specifically, cherry samples were collected form specific cherry orchards in Edessa, Kozani and Pieria in 2015. Respective samples were collected form same cherry orchards in 2016. They were placed in rectangular plastic cups (500 g/cup) and transferred to the laboratory within four hours after collection.

2.2. Determination conventional quality parameters

Three independent samples of twenty fruits, each from the four cultivars, were homogenized after removing the pit in a Moulinex model LM811D blender (Bagnolet, France). TSS were measured at 20 °C using a model ATC, Atago portable refractometer (Atago, Tokyo, Japan) and the results were expressed as °Brix. The pH was measured using a model HD 3456.2, Delta OHM pH-meter (Padova, Italy). A portion of 10 g of homogenized sample were centrifuged at 8000 rpm for 10 min at 4 °C (Biofuge Primo R, Heraeus, Kendro Laboratory Products GmbH, Hanau, Germany) and the supernatant was used for the determination of TA as follows: 5 mL of the supernatant were diluted with distilled water at 1:10 ratio and this solution was titrated with 0.1 N NaOH pro analysis (Merck, Darmstadt, Germany). TA was expressed as Malic Acid Equivalents (MAE) per 100 g FW. All measurements were carried out in triplicate. Fruit diameter was measured using a Mitutoyo model 530 Series vernier calliper (Aurora, IL, USA). Each measurement was performed five times.

An Instron Universal Testing Machine, model 4411 (Instron Corp., Bucks, UK) was used for the measurement of mechanical properties. A specified cylindrical probe (cherry pitter needle) with a blunt tip was used (diameter = 8 mm) (Instron Corp., Bucks, UK). The penetration of the samples was carried out with a crosshead loading rate of 20 mm/min. Force required (Load) to separate the pit from the fruit was recorded (N). Each measurement was performed five times.

2.3. Determination of minerals using ICP-OES

A sample portion of 0.5 g was weighed into a TFM Teflon vessel for the microwave digestion. Aliquots of 7 ml of nitric acid 65% suprapur grade (Merck, Darmstadt, Germany) and 1 mL of 30% hydrogen peroxide for analysis (Merck, Darmstadt, Germany) were added. The vessel was tightly sealed and was then introduced into the microwave system. The digestion was initiated at 0 °C and temperature was linearly increased to 200 °C and 1000 W within a 10 min period. The procedure continued for 20 min at 200 °C and 1000 W. After cooling at room temperature, the sample was transferred into a 20 mL volumetric flask with distilled water. Aliquots of the latter were analyzed using ICP-OES. Elemental analysis was carried out on a Thermo Scientific IRIS Intrepid II XDL inductively coupled plasma-atomic emission spectrometer (Thermo Electron Corporation, Waltham, MA, USA). Detailed ICP-OES instrumentation and method analytical characteristics were as follows: radio frequency power: 1150 W, plasma gas (argon) flow rate: 16.0 L/min, nebulizer gas flow rate: 0.65 L/min, carrier gas flow rate: 0.5 L/min, sample injection volume: 1.85 L/min, instrument temperature: 34 °C, dual view direction, mode: axial. All measurements were carried out in triplicate.

2.4. Determination of sugars using HPLC-RID

Sample preparation was as follows: each cherry once ground, was placed in plastic centrifuge tubes of capacity 50 mL, and centrifuged for 15 min at 10 °C in 8000 rpm (Biofuge Primo R, Heraeus, Kendro Laboratory Products GmbH, Hanau, Germany). The juice was collected and diluted 1:1 with water. The main sugars of cherries are glucose and fructose. The analysis was based on the method of Kelebek et al., (2009), with some modifications. Analysis was performed using a Shimadzu HPLC unit (LC-20AD, Shimadzu, Kyoto, Japan) equipped with a refractive index detector (RID-10 A, Shimadzu, Kyoto, Japan) and a thermostated oven held at 80 °C (CTO-10 A, Shimadzu, Kyoto, Japan). Sugars were separated on an Aminex HPX-87C column (250 × 4.0 mm, particle size 9 μm, Bio-Rad, Hercules, CA, USA). The mobile phase was acetonitrile for HPLC (Merck, Darmstadt, Germany) 30% in ultrapure water (Merck, Darmstadt, Germany). The flow rate was 0.3 mL/min and the elution program for the separation of sugars lasted 12 min. The operating pressure was 86 bar and temperature was 80 °C. All measurements were carried out in triplicate. Quantification of glucose and fructose in the samples was achieved using calibration curves prepared from standard solutions of HPLC grade glucose (Merck, Darmstadt, Germany) and HPLC grade fructose (Merck, Darmstadt, Germany) (2.0–10.0 g/100 mL for fructose and 7.0–20.0 g/100 mL for glucose).

2.5. Identification and semi-quantification of volatile compounds with SPME-GC/MS

The analysis of volatile compounds was based on the work of Vavoura, et al., (2015). All measurements were carried out in triplicate.

2.6. Statistical analysis

Analytical data were treated using the SPSS 23.0 Statistics software