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Original research article

Effect of cultivar and storage temperature on identification and stability of polyphenols in strawberry cloudy juices

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ABSTRACT

The effect of strawberry (*Fragaria x ananassa* Duchesne) cultivars (cvs) and storage temperature on the stability of polyphenols in cloudy juices was determined. Identification of phenolic compounds by ultra performance liquid chromatography-electrospray ionization/mass spectrometry (UPLC–ESI/MS), as well as quantitative analysis by ultra-performance liquid chromatography–photodiode array–fluorescence (UPLC–PDA–FL), were carried out on fresh and stored products (6 months, 4 °C and 20 °C) from 7 different strawberry cvs. A total of 32 polyphenolic compounds were identified: flavan-3-ols (6), anthocyanins (8), flavonols (4) and flavones (3), hydroxycinnamic acids (4), and ellagic acid derivatives (7). Total polyphenol content ranged from 642.08 mg/L in fresh 'Florence' cv. juice to 296.72 mg/L in 'Honeoye' cv. juice after 6 months of storage at 20 °C. Anthocyanins (max. 161.40 mg/L in 'Honeoye' juice) demonstrated the greatest degree of degradation (31–100%, depending on the molecular structure and temperature). Cyanidin-3-malonylglucoside was less labile than 3-glucoside. In the case of pelargonidin glycosides, 3-glucoside was more stable. The degradation process was the least advanced in 'Kimberly' cv. juice (58.53% at 4 °C). Storage conditions also influenced the decrease in *p*-coumaroyl-hexose, and quercetin-glucuronide content. However, low temperatures limited this process. This was especially noticeable in 'Florence' and 'Honeoye' cvs. In turn, proanthocyanidins showed the most stability and were the main polyphenols identified in juices (212–434 mg/L). Generally, it was observed that the total content of polyphenols and their stability in juices was dependent on the cultivar, and the duration of storage conditions.

1. Introduction

According to the European Commission report on the European Union (EU) fruit and vegetables sector in the period 2011–2013, the EU produced 1070 thousand tons of strawberries per year (on average). Spain, Poland and Germany accounted for 57% of the total production (27%, 16% and 14%, respectively). Some 5.5% of strawberry production was exported outside the EU, with 2.7% to Russia (European Union Commission, 2014). Strawberries are primarily regarded as an excellent dessert fruit and are mainly consumed fresh. The surpluses of this popular raw material are used mainly in the preparation of juice concentrate or frozen products. Some of the best ways to preserve these fruits include freezing drying, and making jams or jellies (Harris and Mitcham, 2007). Strawberries are also used in the preparation of alcoholic beverages (including wine), purees, clear juices (Sharma et al., 2009), nectars (Gössinger et al., 2009), cloudy juices (Oszmiański et al., 2007), and as an ingredient in smoothies (Keenan et al., 2010).

In addition to their exceptional flavor and aromatic qualities, strawberries and their products are characterized by high nutritional value and concentration of phytochemicals. One of the most characteristic compounds found in strawberries in a high concentration is ellagic acid, which exists in free form, as a glycoside or linked with glucose as ellagitannins esterified (da Silva Pinto et al., 2008). Ellagic acid shows anticancer properties via its ability to inhibit cell division and induce apoptosis in tumor cells (Han et al., 2006; Narayanan et al., 1999). Moreover, its anti-inflammatory effects (Corbett et al., 2010) and antioxidant properties (Aiyer et al., 2008) have also been confirmed. In terms of the health benefits of strawberries, equally important are the anthocyanins-mainly pelargonidin glycosides-which contribute to the healthy benefits of strawberries (da Silva et al., 2007). These compounds are the most abundant flavonoids in strawberry fruits,

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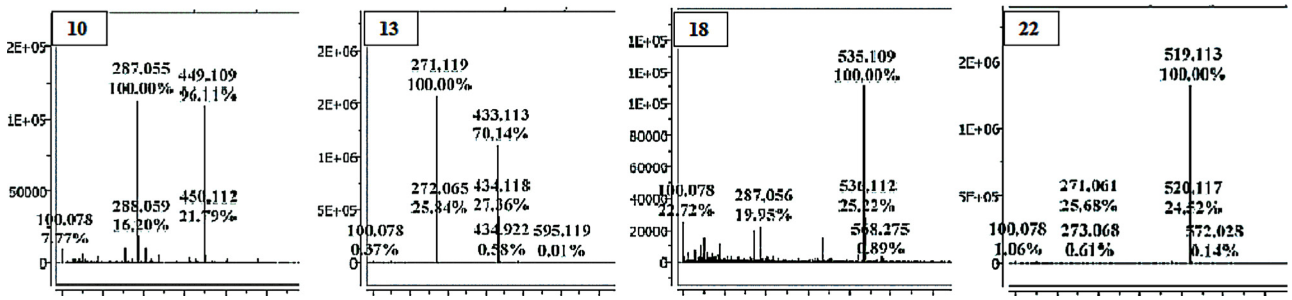

Fig. 2. UPLC–MS spectrum of major anthocyanins in strawberry juices.

followed by flavanols (catechins), and flavonols (quercetin and kaempferol glycosides) (da Silva Pinto et al., 2007).

In spite of the density of bioactive compounds, it is difficult to produce high quality 100% strawberry juice, and this still poses a major challenge for food technologists. Strawberry anthocyanins are less stable than those in many other fruits. Thus, rapid handling and storage under vacuum conditions are recommended during processing (Bates et al., 2001). According to Gössinger et al. (2009), processing companies try to extend the shelf life of strawberry products by adding colorants from natural (chokeberry, elderberry, black currant) or synthetic sources (e.g. cochenille red). However, in accordance with EU legislation, the use of these additives is strictly forbidden in the production of fruit juices and nectars. The appealing red color of strawberry juice is a highly attractive quality that becomes extensively degraded during heat processing (Rodrigo et al., 2007). Pasteurization and other heat treatment methods are typically used in the fruit processing industry. Another quality problem is the formation and subsequent precipitation of ellagic acid in strawberry juices. While ellagic acid is a desirable phytochemical in strawberries, in juice it forms an unsightly, powdery sediment (Bates et al., 2001; Musingo et al., 2001).

The requirements for high quality in strawberry juices combined with rigorous, precise categories of juice-type products has meant that ever more research is being conducted on the effects of non-processing factors (e.g. fruit cultivar) on the nutritional, health, or sensory value of these products. Our earlier studies Teleszko and Wojdyło (2015); Wojdyło et al. (2014), other studies, including those by Fredericks et al. (2013) or Cordenusni et al. (2002), showed significant differentiation in the content of phytochemicals between cultivars of each fruit species.

Here we present the study on determination of the effect of strawberry cultivars on the quality of cloudy juices, measured after processing and after 6-month storage at 4 °C and 20 °C. The strawberry juices were compared for the content and stability of polyphenolic compounds (flavan-3-ols (6), anthocyanins (8), flavonols (7), hydroxycinnamic acids (4), ellagitannins (5) and ellagic acid + conjugates (2)).

2. Material and methods

2.1. Reagents and chemicals

Acetic acid, methanol, and phloroglucinol (1,3,5-trihydroxybenzene) with HPLC purity were purchased from Sigma-Aldrich (Steinheim, Germany). Analytical standards of (+)-catechin, (−)-epicatechin, *p*-coumaric acid, ellagic acid, quercetin-and kaempferol-3-*O*-glucoside, cyanidin- and pelargonidin-3-*O*-glucoside, pelargonidin-3-*O*-galactoside and 3-*O*-rutinoside were purchased from Extrasynthese (Lyon, France). Acetonitrile for ultra-phase liquid chromatography (UPLC; gradient grade) and ascorbic acid were from Merck (Darmstadt, Germany). UPLC grade water prepared with an HLP SMART 1000 s system (Hydrolab, Gdan'sk, Poland) was additionally filtrated through a 0.22 μm membrane filter immediately before use.

2.2. Strawberry cultivars for juice production

Fruits of 7 strawberry cultivars (cvs: 3 kg per cvs.) were collected in Andrzej Zwolski's commercial strawberry plantation in Smolna, Poland (51°09′24″N, 17°25′56″E) during the 2014 season: 'Honeoye', 'Flair', 'Florence', 'Kimberly', 'Pandora', 'Roxana', and 'Rumba' (*Fragaria x ananassa* Duchesne). Fully expanded, mature, and undamaged fruits, minus their stems, were softly hand washed and in this form the strawberries were used in the production of juices.

2.3. Cloudy strawberry juice technology on a laboratory scale

Fruits were disintegrated and heated (75 °C, 10 s) in a Thermomix appliance (Vorwerk, Wuppertal, Germany). The pulp was pressed in a basket press (SSRE, Warsaw, Poland) at a piston thrust of 5000 kg/cm^2 for 5 min. Fresh cloudy juice was heated in the Thermomix to 90 °C for 2 min, poured into colorless 80 mL jars, left for pasteurization (10 min) and cooled to 20 °C. Strawberry juice was prepared in three replicates. Juices were analyzed both directly after processing and after 6-month storage at 4 °C and 20 °C, no light exposure.

2.4. Analysis of polyphenol compounds via ultra-performance liquid chromatography–photodiode array-fluorescence (UPLC-PDA-FL)

2 mL of various cloudy strawberry juices were centrifuged for 10 min at 15,000 x *g* at 4 °C and filtered with a Millex Samplicity® Filters System (Merck Millipore, Darmstadt, Germany). The analytical column was kept at 30 °C in the column oven, whereas the samples were kept at 4 °C. The mobile phase was composed of solvent A (4.5% formic acid) and solvent B (acetonitrile). The program began with isocratic elution with 99% A (0–1 min), and then a linear gradient was used for 12 min, lowering A to 0%; from 12.5 to 13.5 min, this returned to the initial composition (99% A), and then held constant to re-equilibrate the column. The UPLC–PDA (UPLC system Acquity; Waters Corp., Milford, MA, USA) spectra were measured over range of 200–600 nm in steps of 2 nm. The runs were monitored at the following wavelengths: flavan-3-ols and ellagitanins at 280 nm, hydroxycinnamates at 320 nm, flavonol glycosides at 360 nm, and anthocyanins at 520 nm. Calibration curves at concentrations ranging from 0.05 to 5 mg/mL ($r^2 \leq 0.9998$) were produced from (+)-catechin, (−)-epicatechin, *p*-coumaric acid, ellagic acid, quercetin-and kaempferol-3-*O*-