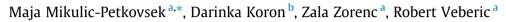
# Food Chemistry 215 (2017) 41-49

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

# Do optimally ripe blackberries contain the highest levels of metabolites?



 $kg^{-1}$  FW).

ABSTRACT

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# ARTICLE INFO

Article history: Received 24 May 2016 Received in revised form 21 July 2016 Accepted 26 July 2016 Available online 27 July 2016

Keywords: Blackberry fruits Fruit quality Maturity stages Color parameters Sugars Acids Phenolic compounds Antioxidant activity

# 1. Introduction

The demand and interest for fresh blackberry fruits has increased among the consumers over the past few years. The reason may be ascribed to a higher degree of health conscious dietary practices, which associate regular consumption of small berry fruits with improved physical conditions related to their strong antioxidant properties (Oszmianski et al., 2015).

Fully ripe blackberries are much appreciated for fresh consumption as they develop a characteristically pleasant flavour. The fruit is also used for processing into juices, purees, spreads and concentrates or it can be fresh frozen to prolong its availability. In the food industry blackberries are often used for dietary supplements, ice cream, jam and other sweets. In blackberries water represents almost 90% of the fruit, other share is depicted by carbohydrates, proteins and fibres (Kaume, Howard, & Devareddy, 2012).

Several compounds contribute to the organoleptic properties of fruits, such as sugars, organic acids, polyphenols and volatile aromas (Sweetman, Sadras, Hancock, Soole, & Ford, 2014). The content of organic acids and the ratio between sugars and organic acids greatly influence the taste of the fruit (Milivojevic et al., 2011). The concentration of sugars and the pH value usually increase during fruit ripening. Contrary, the content of organic acids decreases (Siriwoharn, Wrolstad, Finn, & Pereira, 2004). The acidity is reduced from 2% to 3% (under-ripe fruit) to approximately 1% or below (blackberry fruit with a shiny black color) (Siriwoharn et al., 2004). Ascorbic acid represents an important share of organic acids in blackberries. Its content is significantly higher compared to other fruit species, which are often intended for fresh consumption (e.g. apples, pears) (Lee & Kader, 2000).

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Five blackberry cultivars were selected for the study ('Chester Thornless', 'Cacanska Bestrna', 'Loch Ness',

'Smoothstem' and 'Thornfree') and harvested at three different maturity stages (under-, optimal- and

over-ripe). Optimally ripe and over-ripe blackberries contained significantly higher levels of total sugars

compared to under-ripe fruit. 'Loch Ness' cultivar was characterized by 2.2-2.6-fold higher levels of total

sugars than other cultivars and consequently, the highest sugar/acids ratio. 'Chester Thornless' stands out as the cultivar with the highest level of vitamin C in under-ripe ( $125.87 \text{ mg kg}^{-1}$ ) and optimally mature

fruit (127.66 mg kg<sup>-1</sup>). Maturity stage significantly affected the accumulation of phenolic compounds.

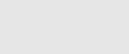
The content of total anthocyanins increased for 43% at optimal maturity stage and cinnamic acid

derivatives for 57% compared to under-ripe fruit. Over-ripe blackberries were distinguished by the high-

est content of total phenolics (1251–2115 mg GAE kg<sup>-1</sup> FW) and greatest FRAP values (25.9–43.2 mM TE

In addition to primary metabolites, the taste of blackberries is significantly affected by high contents of polyphenolics such as anthocyanins, ellagic acid, tannins, gallic acid and quercetin derivatives (Mitic et al., 2013; Veberic et al., 2014). Their contents change during the progress of ripening and the highest differences in phenolic concentration were observed in the last stages of fruit maturity (Siriwoharn et al., 2004). High levels of phenolic compounds in blackberries contribute to their notable antioxidant activity (the capability of slowing down or prevent the oxidation of molecules) which groups them among fruit highly recommended in everyday diet. It has been reported, that phenolic compounds are responsible for several health benefits and may decrease the risk of different chronic diseases such as cardiovascular disease, cancer, diabetes and various types of inflammation (Skrovankova, Sumczynski, Mlcek, Jurikova, & Sochor, 2015).

The concentration of bioactive compounds in blackberry fruits differs considerably among individual cultivars and largely depends on the environmental and climatic conditions (lightness, soil, nutrients etc.) of the planting site. The growing season and







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degree of fruit ripeness additionally alter the phenolic composition of blackberry fruit (Acosta-Montoya et al., 2010; Zielinski et al., 2015). Fruits in the fully ripe stage also develop superior taste to that of under-ripe fruits (Kruger, Schopplein, Rasim, Cocca, & Fischer, 2003).

Blackberry color changes during fruit development and ripening. Fruit color is modified from green (firm, immature fruit), to red (fruit in the initial stages of maturity) and black (final ripening stage), when the fruit softens (Chen, Yu, Tang, & Wang, 2012). Blackberry fruits lose their glossy appearance in late maturity stages and are easily detached from the pedicel (Perkins-Veazie, Clark, Huber, & Baldwin, 2000). If fruits are picked when incompletely colored or even when they develop intense black color prior to full ripeness they are characterized by low sugars/organic acids ratio and taste sour. The optimum picking time for blackberries is thus very hard to define and no suitable methods have been established for quick assessment of fruit ripeness. Blackberry fruits are frequently picked too early or too late and their quality is impaired.

In the current study we aimed to establish whether it would be possible to use the analyzed levels of primary and secondary metabolites in the fruits for the prediction of their ripeness. Non-destructive methods would enable rapid assessment of fruit composition and determine the optimum harvest time of blackberries on the basis of our results. Some studies (Siriwoharn et al., 2004; Zielinski et al., 2015) on blackberry fruits at different maturity stages are available, but in our research a detailed HPLC-MS analysis of individual phenolic compounds, individual sugars and organic acids, fruit color characteristics, soluble solids content and antioxidant capacity were performed on different blackberry cultivars, which are frequently cultivated in the last years in intensive orchards. The experiment included five blackberry cultivars grown predominantly for fresh consumption and also for processing. Fruit were picked at their slightly under-ripe stage, in optimal ripeness and at slight over-ripe maturity stage and their color parameters, pH of juice, soluble solids, the content of individual sugars and organic acids were subsequently analyzed. Moreover, a precise analysis of individual phenolic components was performed using HPLC-DAD-MS<sup>n</sup>. The content of total phenolics and antioxidant activity as ferric reducing antioxidant power were determined spectrophotometrically.

# 2. Materials and methods

#### 2.1. Plant material

'Chester Thornless', 'Cacanska Bestrna', 'Loch Ness', 'Smoothstem' and 'Thornfree' blackberry cultivars were planted in 2007 in the experimental station of Agricultural Institute of Slovenia, located east of Ljubljana (latitude: 46° 10′ N, longitude: 14° 41′ E). Plants were spaces 1.3 m apart in the row and trained on a trellis system with supporting wires. Each plant developed three to five central canes, which were cut back during the summer. Lateral branches were also cut back to keep the plant canopy from becoming too vigorous and enable optimal fruit ripening.

Blackberry fruit were harvested on August 5th, 2015 at three stages of ripeness: slightly under-ripe fruit, optimally ripe fruit and slightly over-ripe blackberries. All fruits were harvested on the outside part of the plant canopy to ensure equal light availability. Blackberries were divided to different treatments based on their color, organoleptic properties, force needed for their separation from the pedicel and long term experiences. Under-ripe fruits were dark-red to black near the stem and were removed from the branches with some difficulty; optimally ripe fruits were black, glossy and easily picked from the branches; over-ripe fruits were black, without the characteristic gloss and fell from the branches at the slightest touch. Only undamaged fruits were selected for the analysis. Fruit characteristics (fruit color, soluble solids and pH value of blackberry juice) were measured immediately after harvest and blackberry extracts were prepared in order to determine the content of vitamin C, sugars, organic acids and phenolics. Extracts of individual cultivars were prepared in ten replicates for each treatment by combining several blackberries. For each replicate approximately 100 g of fruits were used.

# 2.2. Chemicals

The following standards were used for the determination of sugars and organic acids: fructose, glucose and sucrose; ascorbic, citric, malic and fumaric acid from Fluka Chemie (Buchs, Switzerland); tartaric and shikimic acid from Sigma-Aldrich Chemie (Steinheim, Germany). The following standards were used for the quantification of phenolic compounds: neochlorogenic (3-caffeoylquinic) acid. ellagic acid, cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, pelargonidin-3-O-glucoside, luteolin-7-O-glucoside and rutin (quercetin-3-O-rutinoside) from Sigma-Aldrich Chemie; (+) catechin from Roth (Karlsruhe, Germany), (-) epicatechin, quercetin-3-O-galactoside, quercetin-3-O-glucoside, p-coumaric acid, procyanidin B1 and kaempferol-O-glucoside from Fluka Chemie; quercetin-3-O-xyloside from Apin Chemicals (Abingdon, UK); isorhamnetin-3-O-glucoside from Extrasynthese (Genay, France). Methanol for the extraction of phenolics was acquired from Sigma-Aldrich Chemie. The chemicals for the mobile phases were HPLC-MS grade acetonitrile and formic acid from Fluka Chemie. Water for the mobile phase was double distilled and purified with the Milli-Q system (Millipore, Bedford, USA). For the total phenolic content, Folin-Ciocalteu phenol reagent (Fluka Chemie), sodium carbonate (Merck, Darmstadt, Germany), gallic acid (Sigma-Aldrich Chemie) and ethanol (Sigma-Aldrich Chemie) were used. For the antioxidant capacity 6-hydroxy-2,5,7,8-tetramethylchro man-2-carboxylic acid (Trolox), acetic acid, 2,4,6-tripyridyl-1,3,5triazine (TPTZ), FeCl<sub>3</sub>  $\times$  6H<sub>2</sub>O, hydrochloric acid, and methanol were purchased from Sigma-Aldrich.

### 2.3. Fruit characteristics

Soluble solids content (°Brix) and pH values were evaluated in blackberry juice obtained by crushing the berries. Soluble solids were determined with a digital hand held refractometer (30PX, Mettler Toledo, United States) and pH value was assessed with a pH-meter (Cond 720, WTW, Germany). Blackberry surface color was measured using a portable colorimeter (Konica Minolta, Tokyo, Japan) and values were expressed in L\*, a\* and b\* color parameters. The lightness dimension L\* ranges from 0 (pure black) to 100 (the reference white), while negative values of parameter a\* represent green color, a\* positive describes red and b\* negative values mean blue and b<sup>\*</sup> positive yellow color. Hue ( $h^{\circ}$ ) and chroma (C<sup>\*</sup>) describe the basic tone of a color and the brightness of the color. The values for hue angle are in range from 0 to 360°, where  $0^\circ$  = red,  $90^\circ$  = yellow,  $180^\circ$  = green, and  $270^\circ$  = blue. When C\* (color intensity) increases, color becomes more intense (McGuire, 1992). Soluble solids and pH value of blackberry juice were measured in ten replications and fruit color parameters were monitored on thirty blackberry fruits for each treatment and individual cultivar.

## 2.4. Extraction and determination of sugars and organic acids

Primary metabolites (sugars and organic acids) were analyzed in blackberry fruit according to the protocol described by Mikulic-Petkovsek et al. (2013). 5 g of fruits were immersed in 25 mL of double distilled water and macerated with a homogenizer Download English Version:

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