The effect of the ripening stage on the total phenolics content in selected plum cultivars

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Abstract
The total phenolics (TP) content was determined in the fruits of the plum cultivars 'Čačanska lepotica', 'Tophit' and 'Valor', during the ripening period. The TP in plum extracts were determined by the Folin-Ciocalteu assay. At the commercial ripeness stage, the TP content was 84-127 mg of gallic acid equivalents/100 g of fresh weight. During the last five days on-tree ripening, no statistically significant differences in the content of TP were observed in the cultivar 'Čačanska lepotica', whilst in 'Valor', which was studied for a period of 19 days, the content detected at the last sampling date was significantly higher than the values determined on the previous sampling dates. 'Tophit' exhibited the lowest TP content at the first sampling date.

Key words: plums (Prunus domestica L.), ripening, total phenolics

Introduction
Fruit ripening is a very complex process. A large number of biochemical, physiological, and structural changes occur during the ripening of fruits. Some of the biochemical changes are the synthesis and actions of those hormones responsible for ripening, the degradation of chlorophyll, the biosynthesis of carotenoids, anthocyanins, essential oils, and the metabolisms of those sugars, acids and volatile compounds involved during taste and flavour development (Abeles and Takeda, 1990).

Phenolic compounds are important secondary aromatic metabolites synthesized by plants during normal development, as well as in response to stress conditions (UV radiation, infection by pathogens and parasites, wounding, etc). Phenolic compounds are the important components of many fruits, vegetables and beverages, in which they are closely associated with colour, flavour, odour, bitterness, and astringency. They have attracted the attention of researchers, consumers and producers, due to their possible health-promoting effects (Shahidi and Naczk, 2004).

The phenolic classes commonly found in fruits are hydroxycinamic acids, hydrolyzable tannins, anthocyanins, hydroxybenzoic acids, flavonols, flavan-3-ols, and condensed tannins (proanthocyanidins). The predominant hydroxycinnamate in plums is chlorogenic acid, whilst neochlorogenic acid and cryptochlorogenic acid are found in lower concentrations (Fang et al., 2002). Flavonols such as quercetin-3-rutinoside, and flavan-3-ols such as catehin and epicatehehin, have been identified (Kim et al., 2003, Tomás-Barberán et al., 2001).

Several methods for the assessment of total phenolic content in biological or food extracts are based on reactions with a colorimetric reagent (such as Folin-Ciocalteu reagent). The Folin-Ciocalteu assay is reproducible and simple. It has been, and is still, widely-used despite the fact that other components in the sample extract (ascorbic acid, aromatic amines, or sugars) can interfere the determination (Singelton and Rossi, 1965).

Although it is well-known that plums are a good source of phenolic compounds, there is a limited amount of information on the content of phenolics during ripening. The purpose of this study was to determine the content of total phenolics and to investigate any variation in the content of total phenolics with cultivar and ripening stage. From a traditional horticultural, the fresh fruit market and processing industry point of view, studying the total phenolic content during the fruit ripening may not be of practical interest, since plums
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have to be harvested in a certain fruit ripening stage, however, in our view this opens the possibility of reaching new markets which are focused mainly on the content of bioactive compounds with health promoting effects.

Materials and methods

Fruit sampling

In 2007, the fruits of three different plum cultivars (*Prunus domestica* L.) were collected from a five-year experimental orchard at the Fruit Growing Centre, Maribor. The experiment consisted of ten trees for each cultivar. Fifty similar plums were randomly picked for each cultivar at 2-3 day intervals (Table 1). The plums were randomly-divided into five replicates of 10 fruits, pitted by hand, dipped in liquid nitrogen, and homogenized using a blender (Grindomix GM 200, Retsch). Three grams of homogenized sample were used for the extraction.

<table>
<thead>
<tr>
<th>Cultivar</th>
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<th>VIII.</th>
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<tr>
<td>'Čačanska lepotica'</td>
<td>30.7</td>
<td>1.8</td>
<td>3.8</td>
<td>/</td>
<td>/</td>
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<tr>
<td>'Tophit'</td>
<td>5.9</td>
<td>7.9</td>
<td>10.9</td>
<td>12.9</td>
<td>/</td>
<td>/</td>
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<tr>
<td>'Valor'</td>
<td>10.8</td>
<td>14.8</td>
<td>16.8</td>
<td>20.8</td>
<td>22.8</td>
<td>24.8</td>
<td>27.8</td>
<td>29.8</td>
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Determination of total phenolics

The phenolics were quantified using the Folin-Ciocalteu phenol reagent. Three grams of homogenized plums were extracted following the procedure described by Pérez-Jimenez and Saura-Calixto (2005). The extract was diluted in a 100 mL volumetric flask, with deionized water. One mL of diluted extract was added to a 50 mL volumetric flask filled with approx. 20 mL of deionized water, then one mL of Folin-Ciocalteu's reagent was added and allowed to react for three mins before the addition of 7.5 mL 20% Na2CO3. The mixture was diluted to 50 mL with water, mixed thoroughly, and then incubated for 2 h at room temperature. A blank containing 1 mL of diluted extraction solution, and standard solutions of gallic acid (10-50 mg/L), were prepared following the same procedure. The absorbance was measured at 750 nm. The samples were extracted and analysed in triplicate.

Statistical analysis

The results obtained were analysed using the SPSS program (version 17.0 for Windows, SPSS, Chicago, IL). The analysis of variance was performed and the means were compared with the Duncan’s test. The significant difference was statistically considered at the level of *p* < 0.05.

Results and discussion

From the literature data TA, TSS and their ratio are the most reliable parameters to determine consume ripeness, since TSS significantly correlated with the perception of sweetness, flavour and plum aroma intensity and the ratio TSS: TA with sweetness and flavour intensity. Due to these facts, we decided to determine TSS and TA and to use the ratio of TSS:TA as the ripening index (data not shown).

The content of total phenolics during the ripening period of plums, is shown in Figures 1-3. The first sampling of the plums of 'Čačanska lepotica' was performed four days before the fruits reached eating quality. The content of total phenolics was the lowest at the second sampling date with 76 mg GAE/100 g FW. The total phenolic content detected on the last sampling date (83 mg GAE/100 g FW) was insignificantly different to the value detected on the first sampling date. Usenik et al. (2008) observed that the last month of the ripening period had no influence on the content of phenolics in the plum cultivars 'Jojo', 'Čačanska najbolja', 'Valor', and 'Čačanska rodna'.
Figure 1. Total phenolics content during the last five days of ripening of the cultivar 'Čačanska lepotica'. Error bars represent standard deviation (n=3).

Figure 2. Total phenolics content during the last week of ripening of the plum cultivar 'Tophit'. Error bars represent standard deviation (n=3).

Figure 3. Total phenolics content during the last 19 days of ripening of the cultivar 'Valor'. Error bars represent standard deviation (n=3).
The total phenolics regarding the cultivar 'Tophit' increased by approximately 25%, from the lowest value at the first sampling date (76 mg GAE/100 g FW) to the second sampling date, and this value was insignificantly different to those obtained at the last sampling date.

From among the cultivars studied, the 'Valor' exhibited the highest total phenolics content throughout the last 19 days of ripening. As with cultivar 'Tophit', the lowest value was detected on the first sampling date (92 mg GAE/100 g FW). No increase in total phenolics content was observed during the next 17 days. At the last sampling date total phenolics content was significantly higher (127 mg GAE/100 g FW) compared to the values detected before the fruits reached eating quality.

When comparing the total phenolics content during the ripening of three studied plum cultivars, it can be concluded that there was no clear trend. The results obtained are in agreement with those previously reported for nectarine, peach, and plums by Tomás-Barberán et al. (2001) who also observed no clear trend in the phenolics content during ripening.

On the last sampling dates, which corresponded to the eating qualities of the fruits, the total phenolics content of various plum cultivars were within the range from 83 to 127 mg GAE/100 g FW. There was a significant difference at the level of p < 0.05 regarding the total phenolics content among the plum cultivars. The study showed that the cultivar 'Valor' had the highest total phenolics content and was in agreement with the work of Usenik et al. (2008) who reported the highest phenolics content determined within the same cultivar. In general, the total phenolics content as measured in the studied cultivars was within the range previously reported for twenty plums genotypes (Vasantha Rupasinghe et al., 2006), and lower compared to the values reported by Chun et al. (2003) and Kim et al. (2003), who studied different plum cultivars.

**Conclusion**

In this study, we determined the total phenolics content in the extracts of plum cultivars 'Čačanska lepotica', 'Tophit', and 'Valor' during on-tree ripening. This is an aspect which has rarely been investigated. The results showed that the phenolics content depends on the cultivar. From among the cultivars studied, the total content of phenolic compounds was highest in 'Valor'. During ripening, there was no clear trend in the total phenolics content. A significant increase in the total phenolics content between the first and last sampling dates was observed in cultivars 'Tophit' and 'Valor', whilst the cultivar 'Čačanska lepotica' did not show any difference during the last four days of on-tree ripening.

In cultivars 'Tophit' and 'Valor', which were studied during longer ripening period the trend of increase in phenolic content was confirmed. That indicates that early picking satisfies only tradesman demands and deprives the consumer of potential enjoyment in full developed taste and nutrition value.

**References**


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