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Phenolic contents in fruit juices of plums with different skin colors

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Summary

Polyphenols in fruits are of increasing interest for consumers and for plant scientists because of their health beneficial potential and their role in plant physiology and disease resistance. Anthocyanins contribute significantly to the attractive pigmentation of red and blue plums. Mirabelles and several reineclaudes do usually not accumulate anthocyanins in the skin. Is this linked to a general low phenolic level? Both the health aspect and the pigmentation are interesting traits for the breeder. For this purpose, rapid analytical methods are necessary. One time consuming step is the extraction of polyphenols. However, fruit juices are easily produced and are anyhow used for estimation of quality traits such as sugars and acidity. Here we show that HPLC analysis of plum juices represent the phenolic profiles of the whole fruits. We analysed the phenolic patterns of juices from 43 plum varieties with yellow, blue and dark blue fruit skins. In most cases, a weak red pigmentation co-occurs with a low total phenol level. However, there are exceptions that may help the breeder to combine yellow fruit skin with a high level of health beneficial phenolic compounds by using the appropriate donor genotypes. The method described here offers a valuable tool for selection.

Introduction

Polyphenols are among the most important non-nutritive secondary compounds with antioxidant, anti-inflammatory and anti-cancerogenic activity in plant food (Middleton et al., 2000). Depending on ethnic origin and eating habit, the daily uptake of phenolic compounds ranges between 5 - 125 mg per person (Manach et al., 2004). Fruits contribute significantly to this uptake. The annual fruit consumption in Germany is about 110 kg per person including an estimated plum percentage of 1% (Ludwig-Ohm and Dirksmeyer, 2013). Since plums are a seasonal product, the consumption during summer and autumn may be above this mean value. Furthermore, based on many studies it is assumed that polyphenols play a role in plant defense and resistance against biotic and abiotic stressors (Treutter, 2005; Lattanzio et al., 2008). The bioactivity of the diverse phenolic compounds depends on their respective structures. It is therefore of interest to know both the quantitative as well as the qualitative composition of phenolic constituents in fruits. European plum varieties exhibit a great diversity concerning their phenolic profiles both in the fruit skin (Treutter et al., 2012) and the flesh (Jaiswal et al., 2013). More than 40 individual phenolic compounds were identified; among these are 19 chlorogenic acids, 9 flavonols, 10 flavan-3-ols (Jaiswal et al., 2013) as well as 5 anthocyanins (Usenik et al., 2008; Treutter et al., 2012).

Both the health aspect and the pigmentation are interesting traits for the breeder. Because of their beneficial effects on human health, the content and profiles of phenolic compounds in fruits are of increasing consumer's interest and fruit breeders are more and more asked for this new quality character of their products. Thus, knowledge about the inheritance of the different phenolic classes is useful for the plum breeder. However, harvest time is a period of full workload since many hundreds of varieties have to be evaluated for fruit quality traits. Thus, sampling of analytical probes cannot be extended too much. Fruit juices are easily prepared and are used for the analyses of soluble solids, acid concentration and pH. The final analytical method for phenolic profiling is surely HPLC. However, the time consuming extraction of fruits with organic solvents is a big deal and should be avoided. Therefore, we attempted to use the fruit juice, which is routinely prepared, for phenolic profiling.

Material and methods

Sample preparation

In 2013, fruits from trees grown in the experimental orchard of the School of Life Sciences Weihenstephan at Freising were harvested in a ripe stage as usual for the fresh market. The varieties of European plums analysed in this study belong to the species *Prunus domestica* L. Some myrobalanes (*P. cerasifera* L.) are also included. The fruits were grouped according to their skin colour. One group consists of mostly yellow plums with sometimes reddish skin spots including a mirabelle, reineclaudes and one Myrobalane-plum. These were 'Autumn Compote', 'Bellamira' (mirabelle), 'Colora', 'Early Transparent', 'Eibensbacher Aprikosenpflaume', 'Goldzwetsche', 'Große Grüne Reneklode' (Green Gage), 'Liegels Gelbe', 'Ontariopflaume', P 34-56, 'Reine Claude Diane', 'Tatjana' (Myrobalane plum). In a second group the varieties exhibiting blue or deep red skin color were combined: 'Angelina Burdette', 'Auerbacher', 'Blaue Wiener' (Myrobalane plum), 'Cacaks Späte', 'Carpatin', 'Docera 5' (Myrobalane hybrid), 'Elena', 'Felsina', 'Gabrowska', 'Haganta', 'Isjumnaja', 'Liablu', Ortenauer, P 63-143-94, 'Topking', 'Topper', 'Toptaste'. The third group consists of varieties with dark blue skin: AGRI 2000 10/91, 'Bühler' (clones Doll and Meier), 'Cacaks Beste', 'Cacaks Julia', 'Cacaks Schöne', 'Hanka', 'Hauszwetsche' (clones Schüfer and Wolff), 'Maria Novella', 'Oneida', 'Topend Plus', 'Topgigant Plus', 'Tophit Plus', 'Topfive'.

From 10 randomly selected fruits per variety the stones were removed and the flesh including the skin was crushed and the juice obtained using a household juicer. The juice was frozen and stored at -20 °C. Before further analysis, the frozen juice was melted in a cooled ultrasonic water bath at 4 °C and then centrifuged twice for 30 min. with 10,000 g at 4 °C. 1 ml of the clear supernatant was subjected onto a SPE cartridge prepacked with 1 ml C18 (Bond Elute, Agilent Chem). The sugars are not retained and elute immediately. Further 0.5 mL water was added onto the cartridge. The corresponding fraction contained already about 10 to 15% of the total neochlorogenic acid which was calculated from the neochlorogenic acid of all fractions. The remaining phenolic compounds were eluted with 1 ml methanol.

The fractions containing the phenolic compounds were evaporated to dryness with a vacuum centrifuge (Univapo, UniEquipe) and redissolved in 250 µL methanol containing 0.05 mg/mL 3-methoxyflavone as internal standard. This solution was directly used for

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HPLC analysis. In case of very high concentrations of neochlorogenic acid the sample had to be diluted and a second HPLC run was necessary.

The phenolic compounds were identified according to their UV-/VIS-absorbance spectra and their retention time in comparison to previously obtained information (TREUTTER et al., 2012; JAISWAL et al., 2013). The hydroxycinnamic acids include 3-caffeoylquinic acid (= neochlorogenic acid), 4-caffeoylquinic acid, 5-caffeoylquinic acid, 3-caffeoylshikimic acid, 3-*p*-coumaroylquinic acid, 3-feruloylquinic acid. Four flavonols were found which are glycosides of quercetin (JAISWAL et al., 2013) as well as the anthocyanins cyanidin-3-rutinoside, peonidin-3-rutinoside, cyanidin-3-glucoside, peonidin-3-glucoside. Among the flavan-3-ols, catechin and epicatechin were identified. Besides these two monomers, a number of 22 proanthocyanidins were detected. 13 of them are supposed to consist only of epicatechin units as stated earlier (JAISWAL et al., 2013). The remaining oligomers are assumed to consist of catechin units or are mixed-type procyanidins consisting of catechin and epicatechin units; they are summated as groups.

High performance liquid chromatography

The HPLC system and the gradient elution system were described by TREUTTER et al. (2012). Chlorogenic acids were quantified at 320 nm, flavonols at 350 nm and anthocyanins at 540 nm; for quantification they were calculated as chlorogenic acid, rutin and cyanidin-3-glucoside, respectively. For the selective detection of the flavan-3-ols a post-column derivatisation method was used with p-dimethylaminocinnamaldehyde as the reagent (TREUTTER, 1989). The reaction products were detected at 640 nm. Catechin and epicatechin were calculated as their respective standards; for quantification of procyanidins the response factor of procyanidin B2 was used.

Results and discussion

Comparison of phenolic profiles from fruit juices with fruit skin and flesh

We prepared juices from the plum varieties 'Green Gage' (green skinned fruit), 'Tatjana' (red skinned fruit), 'Ortenauer' (blue skinned fruit) and 'Toptaste' (Dark blue skinned fruit) and estimated the concentrations of anthocyanins, flavonols, flavan-3-ols, and hydroxycinnamic acids. Fig. 1a shows the differing profiles of the varieties' juices. In order to check if these profiles may be representative for the whole fruit, which is essential for the interpretation by the breeder, we compared these profiles with the results of former studies. In those experiments fruit skin (TREUTTER et al., 2012) or fruit flesh (JAISWAL et al., 2013) were exhaustively extracted with methanol. The corresponding profiles are given in Fig. 1b and 1c. For calcu-

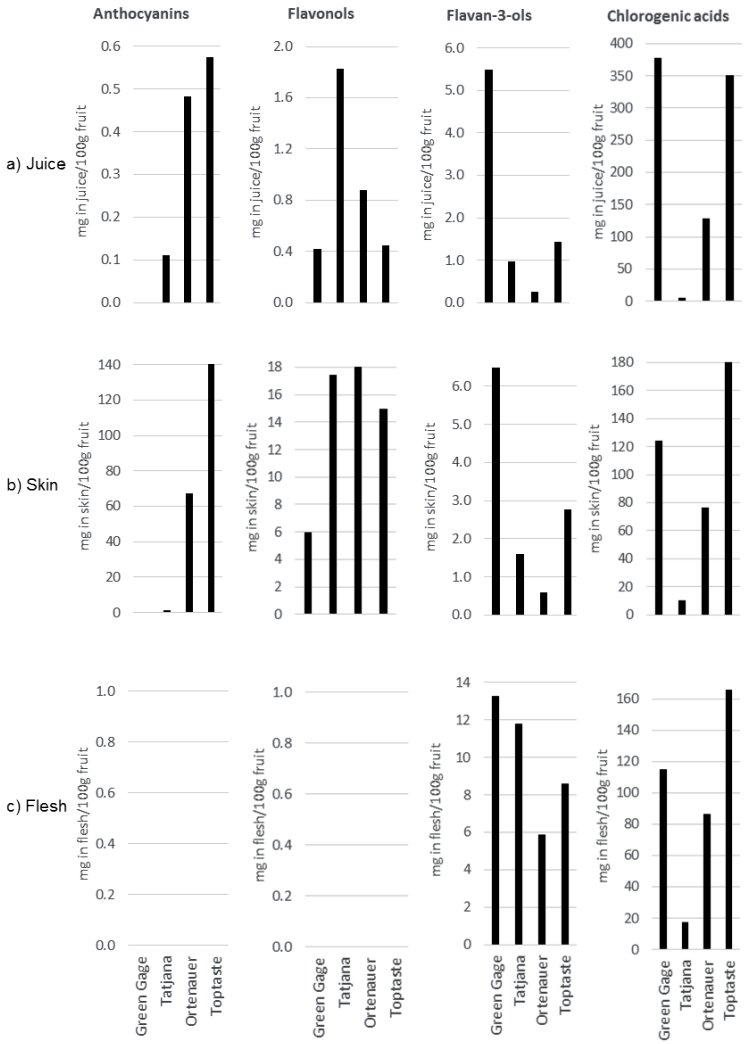

Fig. 1: Phenolic profiles of plum fruit juices (a) in comparison to fruit skin (b) and fruit flesh (c). Data of (b) and (c) are calculated from TREUTTER et al (2012) and JAISWAL et al. (2013), respectively.

lation of the contents per 100 g fresh fruit the following estimation was used: skin 15%, flesh 85%, juice 46%. When comparing the profiles of skin and flesh with that of the corresponding juice it is obvious that the juice's profile combines those of both skin and flesh. Even the anthocyanins and the flavonols that are restricted in the skin are found in the juice. Hydroxycinnamic acids and flavan-3-ols occur in skin and flesh and the profile differences between the varieties are represented by the juices' profiles. Thus, the quantitative phenolic profiles of the plum fruit juices are a useful tool for the breeder to roughly estimate phenolic patterns of plum fruits for fruit quality purpose or for getting an idea about heredity and biosynthesis. The absolute amount of most phenolic compounds extracted by the simple juice-making is lower than the sum of the content in the respective tissues. The quantitative differences may also due to the year of harvesting the fruits, which were different. Thus differing weather conditions have to be considered. For Flavan-3-ols and flavonols this could also be explained by the exhaustive extraction of skin and flesh with methanol. The low anthocyanin levels in the juice might be due to the instability of these molecules in aqueous solution. Only the chlorogenic acids represent the content of flesh plus skin.

Phenolic profiles of fruit juices of plums with different skin colours

It is often stated that anthocyanin rich fruits and berries have a pronounced health beneficiary status, which is only partially related to the anthocyanin contents since non-visible phenolic compounds exhibit also health-promoting activities (AIYER et al., 2012; BRADISH et al., 2011; KAUME et al., 2011; RODRIGUEZ-MATEOS et al., 2011). It is furthermore not known if a high anthocyanin content is generally linked to a high total phenol level. This may be true for some berry fruits which accumulate red pigments in all fruit cells. However, this may not be the case for plums which accumulate anthocyanins only in the skin. In order to clarify these questions, 43 plum varieties were divided into three groups according to their skin colour. 12 varieties are combined to a yellow group (Y). The skin of the corresponding ripe fruits is green to yellow with some of them show reddish sprinkles. The blue-skinned group (B) include 16 varieties, and the third group shows dark blue skin (DB).

Comparing the total phenolic contents (Fig. 2) it can be seen that indeed most of the yellow varieties have lower levels than the blue and dark blue ones with now difference between blue and dark blue varieties. This difference is not due to the anthocyanins but to the hydroxycinnamic acids which in most juices represent more than 95% of the phenolic content. There is no quantitative difference of flavan-3-ols and flavonols between the three plum groups. The anthocyanin concentrations represent more or less the blue/dark blue grouping, which was visually made.

The predominant anthocyanin in most juices is cyanidin 3-rutinoside (Fig. 3) followed by peonidin 3-rutinoside according to USENIK et al. (2008) and TREUTTER et al. (2012). The juices of the dark blue varieties (DB) show the highest values of these cyanidin-glycosides as compared to the blue-skinned group (B) wheres no such tendency was found for the peonidin-glycosides. Trace amounts of anthocyanins were detected in the yellow varieties as expected. However, it has to be noted that even small amounts of red pigments from lightly red sprinkled fruit skins can be found in the respective juices. Only peonidin-3-glucoside remained obviously below the detection limit in those juices.

The level of total flavan-3-ols as indicated in Fig. 2 is mainly due to the sum of procyanidins consisting of at least one catechin-type unit (C-/C-E-Procyanidins) and the monomer catechin (Fig. 4). Epicatechin and its oligomers (E-Procyanidins) play a minor role in most of the juices. This confirms former fruit flesh analyses (JAISWAL et al., 2013).

Among the hydroxycinnamic acids, the neochlorogenic acid (3-caffeoylquuinic acid) is predominating in all juices (Fig. 5). Among the minor hydroxycinnamic acids, the 3-p-coumaroylquinic acid also exhibits lowest concentrations in the yellow group. There are no general group differences for 3-feruloylquinic acid, 3-caffeoylshikimic acid, 5-caffeoylquinic acid and 4-caffeoylquinic acid (Fig. 5).

Conclusion

Red pigmentation is known to be dominantly inherited with an additive effect of genes (HARTMANN and NEUMÜLLER, 2009; ALEHINA, 1978). Many of the blue skinned varieties seem to be heterozygote concerning this trait since crossings among them may produce descendants with yellow fruit skin which are homozygote related to this character. Further chemical characterization is necessary to understand the relevant biosynthetic steps that regulate the pigmentation and the total phenol level. The results presented here seem to confirm

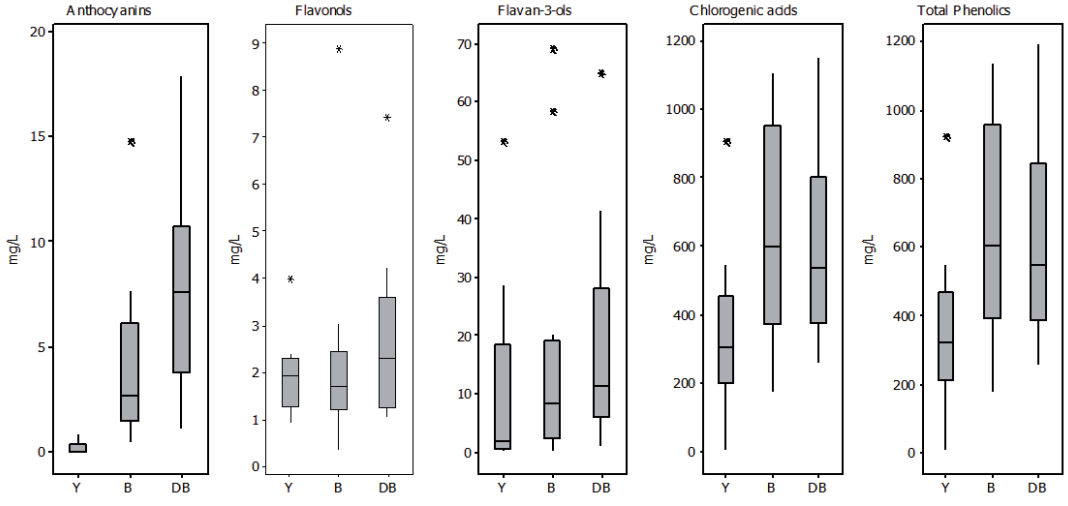

Fig. 2: Box plots of total phenolic contents (mg/L), total hydroxycinnamic acids, total flavan-3-ols, total anthocyanins, and total flavonols in juices of plum varieties with yellow (Y), blue (B) and dark blue (DB) skin. Quantification was performed by HPLC and the individual components were summed up for getting the corresponding total values. Respect the different scales. The box plots show the meridian (horizontal line), the interquartile range box between the first and the third quartile, and outliers (asterisk). The upper and lower whiskers extend to the highest and lowest data value, respectively.

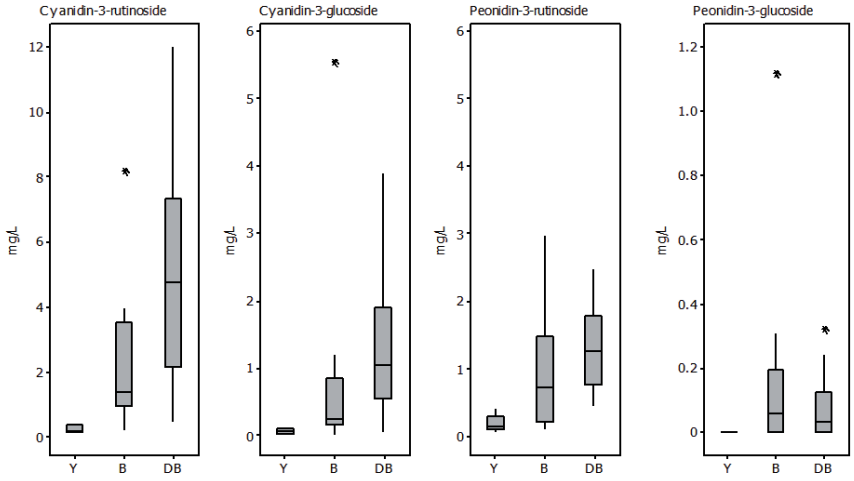

Fig. 3: Box plots of concentrations (mg/L) of individual anthocyanins in juices of plum varieties with blue (B), dark blue (DB) and yellow (Y) skin.

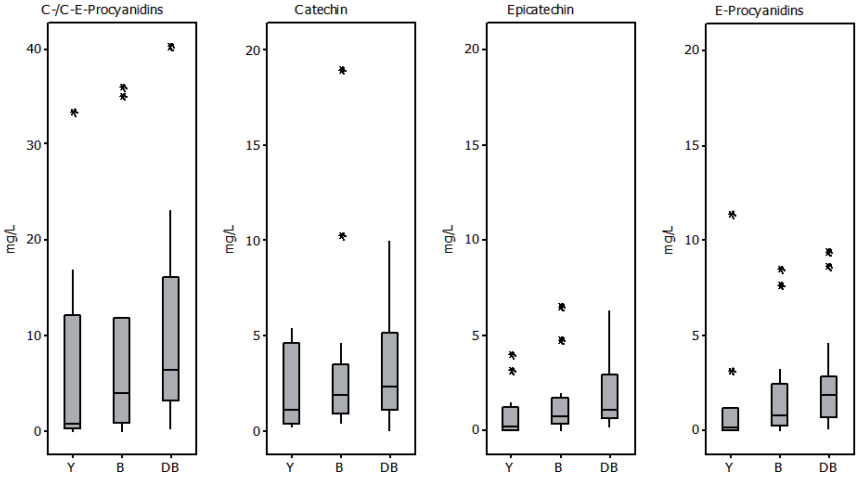

Fig. 4: Box plots of concentrations (mg/L) of the flavan-3-ols catechin and epicatechin and the corresponding proanthocyanidins consisting of epicatechin-type units (E-Procyanidins) or of catechin and mixed catechin-/epicatechin-type units (C-/C-E-Procyanidins) in juices of plum varieties with yellow (Y), blue (B) and dark blue (DB) skin.

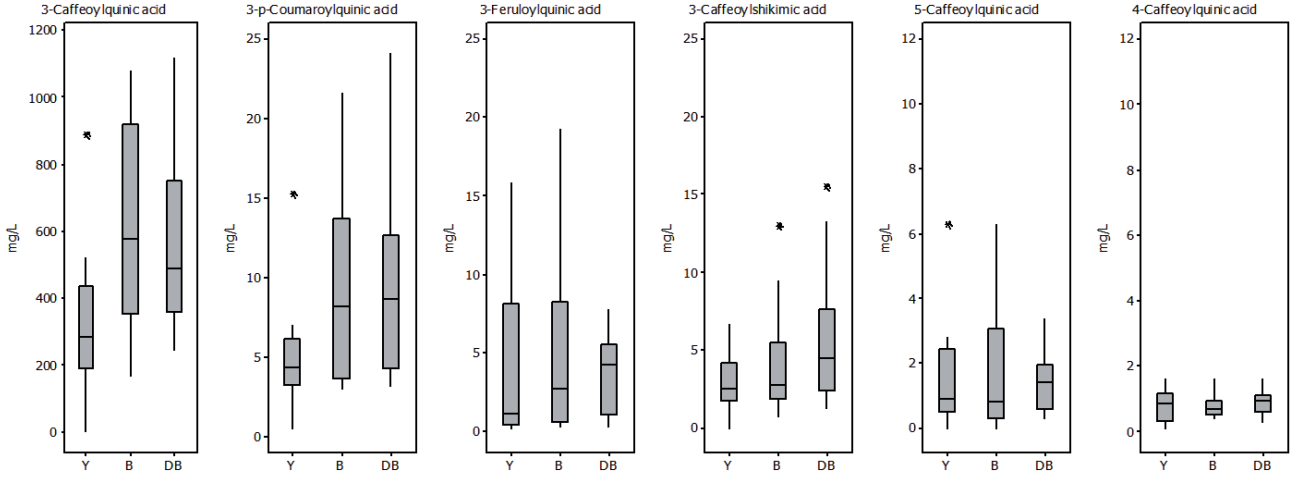

Fig. 5: Box plots of concentrations (mg/L) of individual hydroxycinnamic acids in juices of plum varieties with yellow (Y), blue (B) and dark blue (DB) skin.

the tendency that weak red pigmentation of plum skin corresponds to low total phenol concentration in the juice. However, exceptions seem to be possible as indicated by an extreme value and by overlapping boxes in Fig. 2. It may be a challenge for the breeder to combine the desired yellow fruit skin of a mirabelle or a reineclaude, for instance, with a high content of health-related chlorogenic acids. Further studies are necessary to find the genetic donors of the respective traits.

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