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Profiling phenolics, soluble sugars and carotenoids in different cultivars of European plum (*Prunus domestica* L.)

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1. Abstract

European plum is a species that shows variability regarding its fruit quality traits, such as skin and flesh color, sweetness, acidity, fruit flesh consistency and ripening period. Therefore, it can be expected that plums also differ in their concentrations of phenolic compounds, soluble sugars and carotenoids. The aim of this study was to establish differences in the concentration of these compound between fruits of *Prunus domestica* in cultivars with different skin color, subspecies and harvest period, via HPLC analysis.

A general profile for the concentrations of phenolic compounds, sugars and carotenoids in European plum was determined. The most abundant phenolic compounds were hydroxycinnamic acids, mainly due to the high concentration of neochlorogenic acid (8.54 - 408.52 µg/g). Regarding soluble sugars, sucrose (0.001 - 0.01 g/ml) and glucose (0.001 - 0.006 g/ml) showed the highest concentrations, depending on the cultivar. In the case of carotenoids, the colorless carotenoids were the most abundant such as phytoene isomers (0.08 - 3.54 µg/g) and phytofluene isomeres (0 - 0.49 µg/g). Within the colored carotenoids, the carotenes were dominant, which was mainly due to the high concentration of ß-carotene (0.07 - 4.58 µg/g). An interesting correlation between sorbitol and anthocyanin content was identified, both compounds can accumulate under drought stress conditions.

When comparing the metabolite levels in fruits of cultivars with different skin colors, plums with purple skin showed the greatest differences in the concentrations of phenolic compounds, soluble sugars and carotenoids. Yellow, green-yellow and green skinned cultivars tended to have higher concentrations of sucrose (0.001 - 0.012 g/ml), while purple skinned plums had higher concentrations of glucose (0.001 - 0.006 g/ml), fructose (0.001 - 0.004 g/ml) and sorbitol (0 - 0.005 g/ml). Samples from yellow skinned fruits also showed lower levels of hydroxycinnamic acids (8.54 - 182.68 µg/g), while green-yellow and green skinned plums tended to have higher concentrations of these compounds (9.87 - 380.00 µg/g). All fruit skin categories had similar amounts of colored carotenoids.

Regarding subspecies, not many differences were observed between the categories. Samples from the subspecies *domestica* and *italica var. claudiana* showed the greatest variability in the concentrations of carotenoids and phenolic compounds. The highest concentrations of sucrose and lower concentrations of all other sugars were found in samples of the subspecies *intermedia var. ovoidea* and *prisca*.

Metabolite levels changed during harvest season. Early-season ripening plums tended to have higher concentrations of fructose, xanthophylls, colorless carotenoids and other apolar colorless compounds, but lower concentrations of hydroxycinnamic acids, other phenolic compounds and sucrose. The amount of flavan-3-ols was higher in the first harvest period (0 - 68.65 µg/g in early-season ripening plums and 0 - 7.93 µg/g in late-season ones). Mid-season fruits contained higher concentrations of sorbitol, glucose and carotenes.

The content of phenolics, carotenoids and sugars in the fruits was also affected by environmental conditions. Each cultivar and also each compound group and subgroup reacted differently. This was confirmed by analyzing samples collected in different years, harvest periods and from trees growing in different locations. The concentration of xanthophylls in fruits of 'Mirabelle Wallenberg' in 2014 was higher (3.47 µg/g) than in 2015 (2.30 µg/g), while for 'Hanka' fruits, the concentration in 2014 was lower (2.61 µg/g) than in 2015 (3.50 µg/g). Even the compound profile of a cultivar can change, depending on the environmental conditions. This was the case for the sugars profiles in fruits from 'Hauszwetschge Schüfer' growing in different sectors, where trees growing in sector-N had lower concentrations of sorbitol (0.026 µg/g) than fructose (0.034 µg/g). In fruits from trees growing in sector-S the concentration of sorbitol was higher (0.048 µg/g) than that of fructose (0.024 µg/g).

Samples from the cultivar 'Angelina Burdette' differed from the rest because they showed higher concentrations of anthocyanins and more peonidin-glycosides than cyanidin-glycosides. High contents of flavan-3-ols (130.54 µg/g), chlorophylls (0.69 µg/g) and xanthophylls (1.29 µg/g) accumulated in the fruits of this cultivar. On the other hand, very low to average concentrations of carotenes (1.25 µg/g) and colorless compounds (0.44 µg/g) were found compared to the rest of the samples.

This study thus provides a comprehensive analysis of different plum varieties harvested in different years, at different ripening dates and which were grown under different environmental conditions. The results demonstrate the biological variability of the bioactive plant metabolites, which are important for future breeding initiatives.

2. Zusammenfassung

Die Europäische Pflaume (*Prunus domestica*) ist eine Art, die hinsichtlich ihrer Fruchtqualitätsmerkmale große Schwankungen aufweist. Daher ist zu erwarten, dass sich Pflaumen auch in ihren Konzentrationen an phenolischen Verbindungen, löslichen Zuckern und Carotinoiden unterscheiden. Das Ziel dieser Studie war es, Unterschiede in der Konzentration dieser Verbindung zwischen Früchten von Prunus domestica in Sorten mit unterschiedlicher Hautfarbe, Unterart und Erntezeit mittels HPLC-Analyse festzustellen.

Im Laufe dieser Arbeit wurde ein allgemeines Profil für die Konzentrationen von Phenolverbindungen, Zuckern und Carotinoiden in Europäischen Pflaumen erstellt. Die am häufigsten vorkommenden phenolischen Verbindungen waren Hydroxyzimtsäuren, hauptsächlich aufgrund der hohen Konzentration an Neochlorogensäure (8,54 - 408,52 µg/g). In Bezug auf lösliche Zucker zeigten Saccharose (0,001 - 0,01 g/ml) oder Glucose (0,001 - 0,006 g/ml) die höchsten Konzentrationen abhänging von der Sorte. Bei Carotinoiden waren die farblosen Carotinoide am häufigsten anzutreffen, wie Phytoenisomere (0,08 - 3,54 µg/g) und Phytofluenisomere (0 - 0,49 µg/g). Innerhalb der farbigen Carotinoide dominierten die Carotine, was hauptsächlich auf die hohe Konzentration an ß-Carotin (0,07 - 4,58 µg/g) zurückzuführen war. Es wurde eine interessante Korrelation zwischen Sorbit- und Anthocyaningehalt festgestellt, wobei beide Verbindungen unter Trockenstressbedingungen akkumulierten können.

Beim Vergleich der Metabolitwerte in Früchten von Sorten mit unterschiedlichen Schalenfarben zeigten purpurfarbenen Pflaumen die höchsten Unterschiede in den Konzentrationen der Phenolverbindungen, löslichen Zuckern und Carotinoide. Gelbe, grün-gelbe und grünhäutige Sorten zeigten tendenziell höhere Konzentrationen an Saccharose (0,001 - 0,012 g/ml), während purpurhäutige Pflaumen höhere Konzentrationen an Glucose (0,001 - 0,006 g/ml), Fructose (0,001 - 0,004 g/ml) und Sorbit (0 - 0,005 g/ml) aufwiesen. Proben von Früchten mit gelber Schale zeigten auch geringere Mengen an Hydroxyzimtsäuren (8,54 - 182,68 µg/g), während grün-gelbe und grünhäutige Pflaumen tendenziell höhere Konzentrationen dieser Verbindungen aufwiesen (9,87 - 380,00 µg/g). Alle Fruchtschalen-Kategorien hatten ähnliche Mengen an farbigen Carotinoiden.

In Bezug auf Unterarten konnten nicht viele Unterschiede zwischen den Kategorien festgestellt werden. Die höchsten Konzentrationen an Saccharose und

niedrigere Konzentrationen an allen anderen Zuckern wurden in Proben der Unterart *intermedia var. ovoidea* und *prisca* gefunden.

Die Metabolitenspiegel unterschieden sich in Früchte von Sorten, die an unterschiedlichen Zeiten geerntet wurden. Frühsaison reifende Pflaumen wiesen tendenziell höhere Konzentrationen an Fructose, Xanthophyllen, farblosen Carotinoiden und anderen unpolaren farblosen Verbindungen auf, jedoch geringere Konzentrationen an Hydroxyzimtsäuren, anderen phenolischen Verbindungen und Saccharose. Die Menge an Flavan-3-olen war höher in der erste Ernteperiode (0 - 68.65 µg/g). Früchte, die in der Mitte der Saison geerntet wurden enthielten höhere Konzentrationen an Sorbit, Glucose und Carotine.

Der Gehalt an Phenolen, Carotinoiden und Zuckern in den Früchten wurde auch durch die Umweltbedingungen beeinflusst. Jede Sorte und auch jede Verbindungsgruppe und Untergruppe reagierte unterschiedlich. Dies wurde durch die Analyse von Proben bestätigt, die in verschiedenen Jahren, zu verschiedenen Erntezeiten und von Bäumen an verschiedenen Standorten geerntet wurden. Beispielsweise war die Konzentration von Xanthophyllen in Früchten von 'Mirabelle Wallenberg' im Jahr 2014 höher (3,47 µg/g) als im Jahr 2015 (2,30 µg/g), während die Konzentration in Hanka-Früchten im Jahr 2014 niedriger war (2,61 µg/g) als in 2015 (3,50 µg/g). Auch der Metabolitenspiegel einer Sorte kann sich durch die Umgebungsbedingungen verändern. Dies ist der Fall beim Zuckerprofil in Früchten von Hauszwetschge Schüfer, wobei bei Bäumen, die in Sektor-N wuchsen, die Konzentration von Sorbit niedriger war als die von Fructose. In Früchten von Bäumen, die in Sektor-S wachsen, war die Konzentration von Sorbit höher als die von Fructose.

Proben der Sorte 'Angelina Burdette' unterschieden sich deutlich von den anderen Kultivaren, da sie höhere Konzentrationen an Anthocyanen und mehr Peonidin-Glycoside als Cyanidin-Glycoside aufwiesen. In den Früchten dieser Sorte reicherten sich hohe Gehalte an Flavan-3-olen (130,537 µg/g), Chlorophyllen (0,685 µg/g) und Xanthophyllen (1,288 µg/g) an. Andererseits wurden im Vergleich zu den übrigen Proben sehr niedrige bis durchschnittliche Konzentrationen an Carotinen (1,249 µg/g) und farblosen Verbindungen (0,440 µg/g) gefunden.

Diese Studie liefert somit eine umfassende Analyse verschiedener Europäischer Pflaumensorten, die in verschiedenen Jahren, zu unterschiedlichen Reifeterminen und unter verschiedenen Umweltbedingungen geerntet wurden. Die Ergebnisse zeigen die biologische Variabilität der bioaktiven Pflanzenmetaboliten, die für zukünftige Züchtungsinitiativen für die Entwicklung verbesserter Pflaumensorten wichtig sind.

3. Introduction

The European plum (*Prunus domestica* L.) is a species with a large phenotypic variation of its fruit quality traits such as fruit skin and flesh color, size, sweetness, acidity and ripeness date. However, one of the less studied species from the genus *Prunus* (Urrestarazu et al., 2017).

Plum fruits can be consumed fresh, as jams, preserved, pastry, dumplings or even pickled (Bhutani and Joshi, 1995; Delucchi, 2011; Lim, 2012; Roussos et al., 2016). One of the most important processed product are dried plums, also known as prunes. Plums can also be used to make juice or fermented juice to produce plum wine and distilled brandy. From the seeds, prune kernel oil can be elaborated. Even flowers can be eaten in salad, ice cream and tea (Delucchi, 2011; Lim, 2012).

Several health promoting properties have been related with European plum (Igwe and Charlton, 2016). Although more detailed studies are needed, plums have promising health benefits in cancer treatment, improvement of cognition, reduction of anxiety-related behavior, reduction of cardiovascular risk, laxative effect, prevention and treatment of osteoporosis, anti-hypercholesterolemic activity and others. Some of these effects can be explained by the antioxidant activity of the compounds present in the fruit like anthocyanins and hydroxycinnamic acids (Ahmed et al., 2010; Bouayed et al., 2007; Igwe and Charlton, 2016; Kosar et al., 2010; Lim, 2012; Shukitt-Hale et al., 2009; Stacewicz-Sapuntzakis, 2013; Stacewicz-Sapuntzakis et al., 2001).

Dried plums are known as a source of energy in form of simple sugars that does not produce a rapid rise in blood sugar concentration (Stacewicz-Sapuntzakis et al., 2001). Some flavonoids detected in seed kernel of *P. domestica* showed significant antifungal activity (Mahmood et al., 2010).

3.1. *Prunus domestica* L.: The European plum

3.1.1. Botanical features of the European plum

Plum trees are branched, deciduous, growing from 4 to 15 m high. Branches can be glabrous with a red-brownish or greyish-green color, with some spines or spineless, sometimes pubescent (Delucchi, 2011; Faust and Surányi, 1998; Lim, 2012; Roussos et al., 2016; Scholz and Scholz, 1995). The bark is smooth at the beginning and then longitudinally cracked. Winter buds are red-brownish and usually glabrous (Delucchi, 2011). The trees are robust and vigorous. They need cold winters, short springs and

warm summers to have a proper productivity. For dormancy break, a vernalization of 250 to 500 chill hours (temperatures between 0 to 7.2 °C) is needed. The trees are normally not sensitive to frost, only during flowering in spring a frost event can damage the flowers and negatively affect the fruit production yield. They grow well in sunny locations with well-drained, fertile soil. Establishment on a gentle slope can protect the flowers from frost during the sensitive period. They are more tolerant to damp conditions than apricots, but still they prefer well-drained soil (Lim, 2012).

Plum leaves are simple and alternate with a 1 - 3 cm long pubescent petiole. They are elliptic to obovate, 3 - 12 cm long, 1.6 - 6 cm broad, deep green, glabrous on the upper side, pubescent bellow, with a cuneate base and a pair nectaries, five to seven pairs of secondary veins, with the border notched to serrate sometimes with glandular spines. Stipules are linear or oblong-triangular, deciduous, short, with glandular margin and acuminate apex.

The flowers emerge in spring before the appearance of the leaves. They can be solitary or disposed in fascicles of two to four flowers at the lateral buds of branchlets. The best quality of flowers and fruits are produced in two-year old branchlets. Flowers are perfumed, have a diameter of 10 - 15 mm when fully open and a pedicel 5 - 30 mm long. The hypanthium is pubescent. The five sepals are ovate with acute apex and imbricate. Flowers have also five white or greenish-white petals, obovate with a rounded to obtuse tip, imbricate on the rim to the hypanthium. The androecium has 20 - 30 stamens with unequal filaments organized in two whorls. The gynoecium is composed by one carpel forming an uninoculated ovary in superior position, glabrous to villous, and a terminal and elongated style (Delucchi, 2011; Friedrich, 1993; Lim, 2012; Scholz and Scholz, 1995; Sterling, 1953).

The fruit is a drupe, 2 - 6 cm long, globose, sub-globose, ellipsoidal or ovoid-oblong, sulcate, glaucous with a characteristically whitish wax-bloom and red, purple, purple-black, green, yellow or golden yellow skin (epicarp) color. The mesocarp is fleshy, yellow or reddish, normally not splitting at ripeness. The endocarp is lignified, ellipsoid, laterally compressed forming what is also called stone, pit or kernel. One almond similar seed can be found inside. This seed has no albumen, a thin episperm, mostly containing cyanogenic glycosides and fatty oil, membranous seed coat and flat cotyledons. The fruit is juicy, with a sweet to tart taste or slightly bitter (Bhutani and Joshi, 1995; Delucchi, 2011; Lim, 2012; Roussos et al., 2016; Scholz and Scholz, 1995; Sterling, 1953).

3.1.2. Taxonomy

European plum is the common name for the species *Prunus domestica* L. It is also frequently called plum, which leads to confusion with other species with similar characteristics that share this common name like Japanese plum (*P. salicina*) and myrobalan or cherry plum (*P. cerasifera*) (Lim, 2012). Other common names for European plum in English are: common plum, gage, garden plum, prune, prune plum damsons, and bullaces green gage (Lim, 2012; Zohary, 1992); in German: Bauernpflaume, Echte Pflaume, Hauspflaume, Haferschlehe, Kultur Pflaume, Pflaume, Pflaumenbaum, Zwetsche, Zwetschge, Zwispeln, Reneklode, Mirabelle, Kriechele, Spilling, and Ziparte (Lim, 2012; Scholz and Scholz, 1995); and in Spanish: ciruela europea, pruna (Lim, 2012), reina claudia (Hueso Martín and Cueva González, 2014) and ciruela pasa (Urfalino and Worlock, 2014). In this document, whenever plum is mentioned it refers to *P. domestica*, unless something different is stated. Some synonyms for *P. domestica* are: *Prunus communis* Huds., *Prunus domestica* var. *damascena* Ser., *Prunus oeconomica* Borkh., *Prunus sativa* subsp. *domestica* (L.) Rouy & Camus, and *Prunus domestica* subsp. *oeconomica* (Borkh.) C.K.Schneid (Delucchi, 2011). This species belongs to the genus *Prunus*, subgenus Prunophora, section Euprunus (true plums), subfamily Prunoidae, family Rosaceae (Roussos et al., 2016; Scholz and Scholz, 1995). It is a species that shows a broad morphological variability and therefore many varieties have been described (Scholz and Scholz, 1995). The taxonomy of the genus *Prunus* including *P. domestica* has been reviewed. An identification key for subspecies translated from the original in German is displayed in Supplementary Material 1, p. 114. For *P. domestica* the following subspecies have been described:

- **subsp. *domestica***: true plum, plum. One-year old shoots are mainly glabrous. Fruits of this group are long to egg shaped, both ends of the fruit are thinner than the center, 4 - 8 cm long. The fruit skin color is blue to bluish-black, with wax-bloom giving the characteristically grayish color. Fruit flesh is firm with a moderate sweet taste. The pit is egg to half-moon formed, flat and pointed to both ends, with a bumpy-net surface that separates easy from the fruit flesh (freestone) (Scholz and Scholz, 1995).

- **subsp. *insititia***: bullace, wild damson, bokar plum. Shoots are pubescent until they are two years old, mostly spiny. Fruits are round to tear formed, 1.5 - 3 cm long, bluish-black with blood red juice. The stone is symmetric, round to egg shaped, clingstone (almost not separable from the flesh), both ends pointed, not keeled, with a smooth surface and a deep ridge furrow with or without comb strokes. Trees of this subspecies are mostly used for production of Persian gum and seed oil (Scholz

and Scholz, 1995). It has even been described as a species itself by some authors (Kárpáti, 1967). Reales *et al* (2009) compared different Eurasian *Prunus* species and suggested that *P. insititia* cannot be distinguished from *P. domestica* and, therefore, it is confirmed to be part of the same species.

- **subsp*. intermedia***: egg plums. They are similar to subsp. *domestica* with the difference that one-year shoots are pubescents. Also, the fruits are long to egg formed, round on both ends or on the peduncular side thinner, 4 - 8 cm long, with blue, violet, red or yellow skin color. Fruit flesh is mostly soft and juicy. The stone is long-egg shaped, similar to subsp. *domestica,* wrinkled to smooth and clingstone. Different botanical varieties have been described for this subspecies: var. *culinaria, mamillaris, ovoidea, oxycarpa* (Scholz and Scholz, 1995).

- **subsp. *italica***: gages. This subspecies' shoots have no spines and one- or two-year old shoots are glabrous. Fruits are round, 3 - 5 cm in diameter, with skin in greenish-yellow, yellow, blue or red color. Pulp is mostly juicy and not suitable for drying. Fruit pit is almost round with highly woven sides and clingstone. Some botanical varieties described for this group are: *subrotunda* and *claudiana.*

- **subsp. *pomariorum***: spilling plums. It is similar to subsp. *insititia*, but with spineless shoots (Scholz and Scholz, 1995). The fruits are long (2 - 3.5 cm), pointed on both sides, epicarp is yellow, red or blue, early ripening and very soft fleshed. The stone is also pointed on both sides, flat, with a slight curvature and freestone (Scholz and Scholz, 1995).

- **subsp. *prisca***: zipartes, zibartes. They are characterized by spined shoots. Fruit is round, 2 - 3 cm in diameter, with fruit skin color blue, black, bluish-red, greenish-yellow or yellow with reddish cheeks. The endocarp is round to egg formed, wrinkled, only in ripe or overripe fruits easily separated from the pulp (Scholz and Scholz, 1995).

- **subsp. *syriaca***: yellow plums and mirabelles. It has spineless shoots and one-year old shoots are hairy. Fruits are round, 2 - 3 cm diameter, deep yellow colored skin, frequently red or green dotted. The fruit flesh is very sweet, proper for drying, with a stone, which is round-egg formed that separates easily from the pulp (freestone) (Scholz and Scholz, 1995).

Other classifications have been proposed separating the different cultivars into four groups (Faust and Surányi, 1998; Roussos et al., 2016):

- **Prunes:** are the most important of these groups in a commercial perspective. Fruits are freestone, oval, dark blue or purple, firm, thick and with a high sugar content. They are suitable for drying even without removing the pit.

- **Reine Claude**: are round fruits with high sugar content and freestone. The fruit skin is green to yellow, ranging to slight red or golden colors. This sweet and juicy fruits are suitable for fresh consumption or canning.

- **Yellow Egg**: are large, long to oval fruits, yellow skin and flesh mainly used for canning.

- **Lombard**: large, oval, reddish fruits used for fresh consumption.

3.1.3. Origin of *P. domestica*

It is proposed that the European plum originated in the region Caucasus and Asia Minor. Nonetheless no wild forms of this species have been described (Lim, 2012; Scholz and Scholz, 1995). Thus, the origin of this species is still unclear (Lim, 2012; Zohary, 1992). One theory proposes that it derives from natural allopolyploid (amphiploid) crosses of diploid cherry plum (*Prunus cerasifera*) and tetraploids sloe (*Prunus spinosa*) (Crane and Lawrance, 1931; Lim, 2012; Rybin, 1936). Spontaneous hybridization happens naturally between *P. cerasifera* and *P. spinosa* in the Caucasus, where these species grow together. Nevertheless, these hybrids are highly sterile. Directed interspecific crossings, done by Rybin (1936), of two *P. cerasifera* x one *P. spinosa* produced one hexaploid and many triploids individuals. This hexaploid was assumed to be a re-synthesis of *P. domestica* (Rybin, 1936; Zohary, 1992). Another argument supporting this theory is that the variation displayed by *P. domestica* in various traits like fruit ground color and anthocyanin content could only be explained by a combination of *P. cerasifera* and *P. spinosa* (Crane and Lawrance, 1931; Zohary, 1992).

Beridze and Kvatchadze (1981) and later Zohary (1992) proposed an alternative theory for the origin of *P. domestica* as a result of intraspecific crossings within *P. cerasifera* (2n, 4n, 6n) (Lim, 2012; Zohary, 1992). Zohary also rejected the previous theory arguing that, although Rybin did find one hexaploid individual by crossing *P. cerasifera* and *P. spinosa*, he did not cross this individual with *P. domestica* to test the chromosome homology between them. He also contradicted the findings of Crane and Lawrence (1931; Zohary, 1992), arguing that wild forms of *P. cerasifera* show enough variation in traits like fruit size and color to explain the variation observed in *P. domestica*. In addition, *P. cerasifera* is chromosomally polymorphic having diploid,

tetraploid and hexaploid chromosome races occurring naturally (Beridze and Kvatchadze, 1981; Watkins, 1981; Zohary, 1992). He also noted that some *P. domestica* fruits, especially those from the subsp. *insititia*, like damsons, bullaces and greengages, are very similar to *P. cerasifera* fruits in morphology and taste. On the contrary, *P. spinosa* fruits are very different to those of *P. domestica* in taste and form, with the only similar characteristic being the deep purple color of the fruit skin. In addition, spontaneous hybrids of *P. domestica* and *P. spinosa* produce highly sterile hybrids (5x), sometimes called *Prunus x fructicans* Weihe (Weimarck, 1942; Zohary, 1992). Finally, tetraploid progeny of crossings between *P. domestica* and *P. cerasifera* show balanced chromosome behavior (Darlington, 1930; Zohary, 1992).

Recent phylogenetical analyses on the Eurasian plums considering chloroplast DNA sequences done by Reales *et al* (2009) grouped *P. domestica* and *P. cerasifera* in the same clade, whereas *P. spinosa* was in a different one. This suggested a closer relationship between the first two than with the later. Nevertheless, the authors also indicated that participation of *P. spinosa* in the formation of *P. domestica* could not be discarded, because this study only considered chloroplast and therefore, maternal lineage only (Reales et al., 2009). This was also confirmed by Horvath et al (2011).

3.1.4. Distribution and commercialization of *P. domestica*:

As already stated, the European plum is supposed to originate from the Caucasus and Asia Minor. The first report of this species in Central Europe dates from about 500 BC (Faust and Surányi, 1998; Lim, 2012; Roussos et al., 2016). Nowadays, it is distributed globally in warm-temperate regions on all continents (Lim, 2012). In Chile, plums were introduced in the mid 16th century brought by the Spanish *conquistadores*. Since then, they were cultivated in closed gardens called *quintas* or *huertas* and as hedgerows. Cultivars with black, red or white fruits have been described on governmental registers of that time. They were cultivated in the whole country, but the best quality fruits were obtained mainly in the central part of the territory and they were consumed as fresh and dried fruits (Gay, 2009; Lacoste et al., 2011). Nowadays in Chile, almost all commercially cultivated *P. domestica* trees are from the cultivar 'D'Agen' (98.94%) and 'President' (0.9%) (ODEPA, 2004). In Argentina, *P. domestica* has been described as naturalized in some places, growing wild within the native forest (Delucchi, 2011). Something similar can be observed in Chile, where the European plum trees growing in private gardens were left there when the houses where abandoned. Years later, the native species started naturally reforesting the place and the fruit trees were incorporated into the forest. Because mostly small local nurseries are selling *P. domestica* in Chile, it is highly probable that they derived from the plants brought by the

European immigrants during the *Conquista* and later during the *Colonia* (1598 - 1810) (Valko, 2010).

Regarding Germany, there is not much information about how many cultivars are grown nowadays in the country. Crossing database information from the German *Bundessortenamt* (Federal Cultivar Office) (BSA, 1997; BSA, 2018) and the *Deutsche Genbank Obst* (German Fruit Genbank) (DGO, 2018) state that 209 cultivars are protected by the DGO and 43 modern commercial cultivars are described. The total area of plums (including damsons, mirabelle plums and green gages) cultivated in 2017 in Germany was 4838 ha (Statistisches-Bundesamt, 2018). No detailed information was found on hectares or percentages of cultivated areas of individual varieties.

Table 1: Production, import and export of fresh plums, sloes, and dried prunes Worldwide in 2016.

Item	Element	Ranking	Country	Amount (tonnes)	%
Plums and sloes	Production	1	China, mainland	6,663,165	55.29
		2	Romania	512,975	4.26
		3	Serbia	463,115	3.84
		6	Chile	294,873	2.45
		30	Germany	37,783	0.31
			Global total	**12,050,799**	**100**
	Export Quantity	1	Chile	115,187	16.12
		2	Spain	109,328	15.3
		3	South Africa	63,872	8.94
		21	Germany	4,897	0.69
			Global total	**714,359**	**100**
	Import Quantity	1	Russian Federation	58,400	8.14
		2	Germany	56,635	7.9
		3	United Kingdom	53,300	7.44
		132	Chile	15	<0.01
			Global total	**716,869**	**100**
Plums dried (prunes)	Export Quantity	1	Chile	70,102	34.96
		2	USA	38,036	18.97
		3	Argentina	31,003	15.46
		9	Germany	3,135	2.56
			Global total	**200,511**	**100**
	Import Quantity	1	USA	19,247	9.9
		2	Russian Federation	13,116	6.74
		3	Germany	12,567	6.46
		29	Chile	1,453	0.75
			Global total	**194,503**	**100**

The first three countries, Chile and Germany are shown. It is important to consider that the values for fresh fruits include European (*P. domestica*) and Japanese plum (*P. salicina*) Source: FAOSTAT (2016).

Statistics about production and global trade of plums mainly include mixed information about *P. domestica* and *P. salicina*. A summary of the data presented by FAOSTAT (2016) for the year 2016 is shown in Table 1. Analyzing the worldwide production of both species of plums in the year 2016, China (mainland) was the largest

producer with 55.29% of the global production of fresh plum and sloe, followed by Romania (4.26%) and Serbia (3.84%). Chile was placed in the 6th position with 2.45% and Germany in the 30th place with less than 1%. Although Chile showed a low world production of fresh plum, it was the strongest exporter in 2016 with 16.12% of global world export, followed by Spain (15.3%) and South Africa (8.94%). Germany was placed 21th with less than 1%. The country that imported most fresh plums in 2016 was the Russian Federation (8.14%), Germany was placed second (7.9%) and United Kingdom third (7.44%). Considering the commercialization of dried plums (prunes), Chile was also the top exporter of prunes with 34.96%, United States of America was placed second (18.97%), followed by Argentina (15.46%). German prune exports represented 2.56% of world prune trade. Looking at which countries imported prunes in 2016, United States of America ranked first (9.9%), Russian Federation second (6.74%) and Germany third (6.74%) whereas, Chile imported less than 1% of the total imported dried plums in 2016.

3.1.5. Nutritional values of plums and effect on human health:

General nutritional values of plums reported by USDA (2016) are summarized in Table 2 to Table 5. These data mix information on *P. domestica* and *P. salicina*. A summary of data regarding content of sugars, phenolics and carotenoids in *P. domestica* whole fruit (excluding the pit) are displayed in Table 6. The most abundant component of plums is water (87.23%) followed by carbohydrates (11.42%). Within Vitamins, the most abundant are vitamin C, K and choline. The predominant fatty acids are monounsaturated ones and the predominant amino acid is aspartic acids.

Table 2: General nutrient values of fresh plum (*P. domestica* and *P. salicina*).

Nutrient	Unit	Value per 100 g		Std. Error
Water	g	87.23	±	0.328
Energy	kcal	46	±	--
Protein	g	0.7	±	0.034
Total lipid (fat)	g	0.28	±	0.045
Ash	g	0.37	±	0.008
Carbohydrate, by difference	g	11.42	±	--
Fiber, total dietary	g	1.4	±	0.075

The pit is not considered. Source: USDA (2016)

Table 3: Vitamins in fresh plum (*P. domestica* and *P. salicina*)

Vitamins	Unit	Value per 100 g		Std. Error
Vitamin C, total ascorbic acid	mg	9.5	±	0.847
Thiamin	mg	0.028	±	0.004
Riboflavin	mg	0.026	±	0.001
Niacin	mg	0.417	±	0.027
Pantothenic acid	mg	0.135	±	0.009
Vitamin B6	mg	0.029	±	0
Folate, total	µg	5	±	0.125
Choline, total	mg	1.9	±	--
Vitamin E (alpha-tocopherol)	mg	0.26	±	0.064
Tocopherol, gamma	mg	0.08	±	0.076
Vitamin K (phylloquinone)	µg	6.4	±	0.929

The pit is not considered. Source: USDA (2016)

Table 4: Lipids in fresh plum (*P. domestica* and *P. salicina*)

Lipids	Unit	Value per 100 g		Std. Error
Fatty acids, total saturated	g	0.017	±	--
Fatty acids, total monounsaturated	g	0.134	±	--
Fatty acids, total polyunsaturated	g	0.044	±	--
Phytosterols	mg	7	±	--

The pit is not considered. Source: USDA (2016)

Table 5: Amino acids in fresh plum (*P. domestica* and *P. salicina*)

Amino Acids	Unit	Value per 100 g		Std. Error
Tryptophan	g	0.009	±	--
Threonine	g	0.01	±	--
Isoleucine	g	0.014	±	--
Leucine	g	0.015	±	--
Lysine	g	0.016	±	--
Methionine	g	0.008	±	--
Cysteine	g	0.002	±	--
Phenylalanine	g	0.014	±	--
Tyrosine	g	0.008	±	--
Valine	g	0.016	±	--
Arginine	g	0.009	±	--
Histidine	g	0.009	±	--
Alanine	g	0.028	±	--
Aspartic acid	g	0.352	±	--
Glutamic acid	g	0.035	±	--
Glycine	g	0.009	±	--
Proline	g	0.027	±	--
Serine	g	0.023	±	--

The pit is not considered. Source: USDA (2016)

Table 6: Concentrations of phenolic compounds, soluble sugars and carotenoids reported for fruits of several *P. domestica* fruits cultivars.

Compound	Unit	Value per 100 g fruit
phenolic compounds	**g**	**1.11**
hydroxycinnamic acids and derivates	g	0.316-7.452
anthocyanins	g	0.0044-0.3285
flavan-3-ols/proanthocyanidin	g	0.469-2.044
flavonols	g	0.0014-0.124
benzoic acid derivates	g	0.0013
soluble sugars	**g**	**9-19**
sucrose	g	1-9
glucose	g	1-11.5
fructose	g	1-3
sorbitol	g	1-5
xylitol	g	0-0.0053
carotenoids	**g**	**0.00042-0.021**
carotenes	g	0.00002-0.00554
free xanthophylls	g	0.0004-0.1554
esterified xanthophylls	g	0-0.00186
colorless carotenoids	g	0-0.00064

Only reports for whole fruit are displayed in the table. Sources: Breithaupt and Bamedi (2001), Curl (1963), García-Mariño et al. (2008), Gross (1987), INRA et al. (2013), Jovanovic-Malinovska et al. (2014), Khachik et al. (1991), Khallouki et al. (2012), Lombardi-Boccia et al. (2004), Mäkinen and Söderling (1980), Mangels et al. (1993), Mattila et al. (2006), NAL (2018), Nunes et al. (2008), Slimestad et al. (2009), Stacewicz-Sapuntzakis et al. (2001), Usenik et al. (2007), Usenik et al. (2008), Usenik et al. (2009), Usenik et al. (2013), Treutter et al. (2012).

3.2. Bioactive compounds in fleshy fruits

3.2.1. Phenolic compounds

Phenolic compounds are a group of bioactive compounds that have at least one aromatic ring and one hydroxyl group attached to it (Brielmann et al., 2006; Crozier, 2003). This aromatic ring (or rings) can present different degrees of hydroxylation (Vicente et al., 2009). These compounds are broadly spread throughout the plant kingdom (Crozier, 2003; Strack, 1997; Strack and Wray, 1993; Tsao and Deng, 2004; Vicente et al., 2009). Most of them derive from the shikimate and phenylpropanoid pathway. Compounds in this group are structurally very variable ranging from simple compounds with a single phenolic ring to complex and large polymers like proanthocyanidins (Crozier, 2003).

In fruits they tend to accumulate more in the peel than in the flesh (Vicente et al., 2009). Their role in plants include predator deterrents, UV-protection and flower/fruit pigmentation. They can also be related to taste and astringency in plant products (Vicente et al., 2009).

Considering their structure, they can be divided into phenolic acids, flavonoids and other compounds (Vicente et al., 2009).

3.2.1.1. Phenolic acids

They are two types of phenolic acids: benzoic acid derivates and cinnamic acid derivates:

- **Benzoic acid derivates**, also called hydroxybenzoates have a structure of C6-C1 and are composed by a benzoic ring and a carboxyl group (Crozier, 2003; Macheix et al., 1990). Some common benzoic acids are p-hydroxybenzoic, vanillic, syringic and gallic acid (Figure 1a) (Brielmann et al., 2006; Macheix et al., 1990; Vicente et al., 2009). They can be found glycosylated or bound to acids and alcohols (Macheix et al., 1990). Gallic acid and its derivatives have a major role in the formation of galls in plants as a consequence of parasitic insect attacks (Crozier, 2003; Gross, 1992). It is also the basic unit of the dimer ellagic acid and the polymers pentagalloylglucose and gallotannins. Ellagic acid itself is the basic unit for ellagitannins. Gallotannins and ellagitannins are considered hydrolysable tannins (Crozier, 2003; Macheix et al., 1990).

- **Cinnamic acid derivates,** or hydroxycinnamates have a C6-C3 structure, and are based on cinnamic acid with different hydroxylations and methylations of the aromatic ring. Some examples of cinnamic acid derivatives are p-coumaric, caffeic (Figure 1b), ferulic and sinapic acid (Macheix et al., 1990). The most abundant one in fruits is caffeic acid (Crozier, 2003; Mattila et al., 2006; Vicente et al., 2009), followed by coumaric acid (Rice-Evans et al., 1997; Vicente et al., 2009). Additional modifications that can be present are glycosylation or bonding to other organic molecules like flavonoids and lignins. In fruits, hydroxycinnamic acids esterified with another organic compound are very common, for example neochlorogenic acid (caffeic acid + quinic acid, Figure 1c). Coumarins derive from hydroxycinnamic acids (Macheix et al., 1990).

3.2.1.2. Flavonoids

Flavonoids are polyphenolic compounds with a skeleton of 15 carbons (C_6-C_3-C_6) arranged in aromatic rings and a three-carbon bridge (Crozier, 2003). The A-ring originates from the malonate pathway. The B-ring and the carbon bridge constituting the C-ring form a phenylpropanoid unit, synthetized from phenylalanine via the shikimic acid pathway (Crozier, 2003; Macheix et al., 1990).

Flavonoids are usually bound to sugar in the form of glycosides. They are also frequently hydroxylated in the 3-, 5- and 7- positions. These two features enhance the water solubility of the compounds. (Brielmann et al., 2006; Crozier, 2003; Vicente et al., 2009). Glycosylation also reduce their antioxidant activity (Vicente et al., 2009). Other

modifications like methylation and isopentylation make these compounds more lipophilic (Crozier, 2003).

From all phenolics, flavonoids are the most common and are found throughout the plant kingdom. They accumulate in the epidermis of leaves and fruits playing roles in plants such as UV protection, pigmentation, stimulation of nitrogen fixing nodules and disease resistance (Crozier, 2003; Koes et al., 1994; Pierpoint, 2000).

Flavonoids can be subclassified based on their structural features in: flavonols, flavones, flavanones, isoflavones, anthocyanins, flavan-3-ols, aurones, dihydroflavones, flavan-3,4-diols, chalcones and dihydrochalcones (Crozier, 2003).

- **Flavonols** contain a central ring of 3-hydroxypyran-4-one (Rice-Evans et al., 1997; Vicente et al., 2009). They are frequently present as O-glucosides, commonly at the 3-position (Crozier, 2003; Herrmann, 1976). Most common flavonols are hydroxylated on the 5-, 7- and 4'-position (like kaempferol) with other modifications in 3'- and/or 5'-positions. Common modifications include further hydroxylations and methylations (Crozier, 2003). In fruits, they are mainly present in the epidermis because they are mostly synthetized in response to light (Vicente et al., 2009). The most common flavonols found in fruits are quercetin, kaempferol and their 3-O-monoglycosides like rutin (quercetin + glucose + rhamnose, Figure 1d) (Macheix et al., 1990).

- **Flavones** have a similar structure like flavonols, but they do not have a hydroxyl group in the 3-position (Crozier, 2003; Macheix et al., 1990; Vicente et al., 2009). They frequently have modifications in position 7, 5, and 4'. Some possible modifications are hydroxylation, methylation, O- and C-alkylation or O- and C-glycosylation. Nevertheless, they are also common in their free forms. Flavones are less common in plants species than flavonols and occur in significant amounts in celery, parsley and herbs. One known flavone in herbs is apigenin (Figure 1e). They do not occur in fruits, with the only exception of citrus fruits (Crozier, 2003; Macheix et al., 1990; Ooghe et al., 1994).

- **Flavanones** are early products of the flavonoid pathway. Their main characteristic is that they do not have a double bond between C2-C3 and they have a chiral center at carbon C2 (Crozier, 2003; Macheix et al., 1990; Vicente et al., 2009). One example for this compound class is naringenin (Figure 1f). They are highly reactive and are reported to undergo modifications like hydroxylation, glycosylation and O-methylation. Flavanones are common in citrus fruits, for example the flavanone-glycosides hesperidin and naringin (Figure 1g). The sugar bound to the flavanone

can affect the taste of the compound. For example, flavanone rutinosides are tasteless. On the contrary, flavanone neohesperidosides are bitter (Bohm et al., 1998; Crozier, 2003; Macheix et al., 1990).

- **Isoflavones** are characterized by the attachment of the B-ring in the C3 position instead of C2, as is the case with most other flavonoids. Common modifications on isoflavones are hydroxylations, methylations and prenylations that lead to the formation of other isoflavonoids like coumestans (Figure 1h), rotenoids and pterocarpans (Crozier, 2003; Dewick, 2002). They are common in species of the family Fabaceae, but not in fleshy fruits (Macheix et al., 1990). Nevertheless, some studies report low concentrations of these compounds in *Prunus* species like apricot, cherry, peach and the European plum (green, yellow and purple skinned) (Kuhnle et al., 2009). Isoflavonoids present in soya, alfalfa and clovers have been reported to act as phytoestrogens (Crozier, 2003; Vicente et al., 2009), because they seem to block ovulation due to mimicking the effects of the steroidal hormone estradiol. They are proposed to reduce incidence of prostate and breast cancers (Crozier, 2003).

- **Anthocyanidins'** basic structure is the flavylium cation (Pervaiz et al., 2017; Vicente et al., 2009). Most frequent anthocyanidins are pelargonidin, cyanidin, delphinidin, peonidin, petunidin and malvidin. In nature, these compounds are mainly found in conjugation with sugars, and are called anthocyanins, like cyanidin 3-O-glucoside (Figure 1j). Anthocyanins are responsible for the red, purple and blue colors in some plant tissues (Crozier, 2003; Khoo et al., 2017; Macheix et al., 1990; Vicente et al., 2009). Anthocyanidins can also conjugate with hydroxycinnamates and organic acids like malic and acetic acid. The most common conjugations are located in 3-position. Nevertheless they can also be found in positions 5, 7, 3', and 5' (Crozier, 2003). The role of these compounds in plants are protection against excessive light by shading leaf mesophyll cells. They also have a key role in attraction of pollinators and seed dispersers (Crozier, 2003; Vicente et al., 2009).

- **Flavan-3-ols,** also called flavanols, are structurally similar to flavonols but they do not have a carbonyl group in position C4 and they lack the double bond between position 2 and 3 in the C ring (Vicente et al., 2009). Most common flavan-3-ol in fruits are catechin (Figure 1i), epicatechin, gallocatechin and epigallocatechin. They are mainly found free rather than glycosylated or esterified. Flavan-3-ols are common in grapes and in some species of the genus *Prunus* (Macheix et al., 1990). A frequent modification of the molecule is galloylation forming compounds like epigallocatechin-gallate and gallocatechin-gallate. These compounds are present in

green tea and are presumed to be responsible for health beneficial effects on humans (Chacko et al., 2010; Crozier, 2003; Menet et al., 2004). In black tea, flavan-3-ol-gallates degrade during fermentation leading to the formation of theaflavins, theasinensins and thearubigins (Del Rio et al., 2004; Leung et al., 2001; Menet et al., 2004; Tanaka et al., 2005), which have also been reported to have positive effects on human health (Aron and Kennedy, 2008; Crozier, 2003; Del Rio et al., 2004; Fuchs et al., 2014; Hisanaga et al., 2014; Leung et al., 2001; Lombardo et al., 2018; Miyata et al., 2013; Weerawatanakorn et al., 2015). Flavan-3-ols can polymerize into oligomers or polymers called proanthocyanidins or condensed tannins (Vicente et al., 2009).

- **Aurones'** structure differs from other flavonoids, because instead of having a hexacyclic C-ring, they have a pentacyclic ring in that position (National-Center-for-Biotechnology-Information, 2018). They are yellow pigments mainly present in species from Asteraceae and Fabaceae species. One example of this compound class is aureusidin (Figure 1k), present in Garden Snapdragon (*Antirrhinum majus*) (Nakayama et al., 2001). Although they are uncommon in fleshy fruits, studies on litchi fruits indicate that aurone similar substances occur in fruits but they have not been accurately described (Macheix et al., 1990).

3.2.1.3. Other phenolics

This section groups polyphenolics that are not phenolic acids and do not have the skeleton structure present in flavonoids. They include stilbenes, lignans, coumarins, quinones, diarylheptanoids and polymers like lignins, hydrolysable tannins and proanthocyanidins (Vicente et al., 2009).

- **Stilbenes** have a C6-C2-C6 skeleton. They are known to be phytoalexins produced in response to fungal, bacterial and viral attack (Crozier, 2003). The most common compound is resveratrol (Figure 1l), which is synthetized in grape as a response to pathogens attack and environmental changes. This compound has also been reported in blueberries (Vicente et al., 2009).

- **Lignans** are di-phenolic compounds derived from two cinnamyl units. One example is secoisolariciresinol diglucoside (Figure 1m) that can be found in flaxseed (Strandas et al., 2008). They are found in cereals and legumes, but rarely in fruits. (Liu et al., 2006; Macheix et al., 1990; Vicente et al., 2009), although some reports of lignans in *Prunus* species have been published (Tetens et al., 2017).

- **Lignin** is a phenolic polymer constituent of the cell wall in plants, formed by three monomeric precursors: coumaryl, sinapyl and coniferyl alcohols (Figure 2a, b and

c). The monomeric precursors are not frequently found in the edible part of fruits (Macheix et al., 1990; Vicente et al., 2009). Nevertheless, they have been reported in pears and kiwis (Bunzel et al., 2005).

- **Coumarins** are lactones derived from hydroxycinnamic acids by cyclization between hydroxy and carboxy groups (Huang et al., 2009; Macheix et al., 1990). They are characterized by a different degree of oxygenation of the benzopyrene moiety (Huang et al., 2009). In fruits they are frequently found in species from the Rutaceae family (mainly in the genus *Citrus*) and Apiaceae (Huang et al., 2009; Macheix et al., 1990). Some coumarins have been detected also in species of the family Rosaceae, genus *Prunus* like scopoletin (Figure 2d) in European plum, although at very low concentrations (Macheix et al., 1990).

- **Diarylheptanoids** are biphenolic compounds with O-methoxy phenolic groups connected by a heptane (7 carbon) chain (González-Albadalejo et al., 2015; Priyadarsini, 2014). The most popular compound in this group is curcumin (Figure 2e) present in *Curcuma longa* root (Priyadarsini, 2014). These compounds are uncommon in fruits, but they have been reported in Yunnan banana (*Musa itinerans*) (Liu et al., 2014), white cardamom (*Amomum kravanh*) (Yin et al., 2013) and black cardamom (*Alpinia oxyphylla*) (Bian et al., 2013). Contradictory reports have been published on its health effect in humans (Amalraj et al., 2017; Fadus et al., 2017; Morales et al., 2017; Nelson et al., 2017).

- **Hydrolysable tannins** are polymers of gallic acid (gallotannins) or ellagic acid (ellagitannins) with a central sugar core, like in 1, 2, 3, 4, 6-pentagalloyl glucose (Figure 2f). They are present in pomegranate with glucose in the center as in punicalin and punicalagin (Macheix et al., 1990; Quideau et al., 2011).

- **Condensed tannins** or proanthocyanidins are polymers of flavan-3-ols mostly with a C4 to C8 bond (Macheix et al., 1990; Quideau et al., 2011). Depending on the type of bond between the monomers, different types of polymers can be described. Proanthocyanin dimers type B (Figure 2g) are composed by two flavan-3-ol units with a single bond between the C4 of one molecule and the C8 or the C6 of the other (2ß→8 or 2ß→6). Type A proanthocyanidins dimers are composed by two double bonds: one between C2 of one molecule with the oxygen in position 7 of the other molecule and the second between C4 of the first molecule with C8 of the second (2ß→O7; 4ß→8). Further polymerization occurs by the successive addition of flavan-3-ols and formation of C4-C8 bonds or C4-C6 bonds. But also mixed substitutions can exist (Haslam, 1998) Proanthocyanidins can form polymers with up to 50 units (Crozier, 2003). They are present in many fruits such as grape,

Japanese persimmon, apple, blackcurrant, blueberry and cranberry (Macheix et al., 1990).

3.2.2. Soluble sugars

Carbohydrates are the second most abundant components in plants after water, constituting 50 to 80% of the total dry weight. Simple carbohydrates, like soluble sugars, are direct products of the dark phase of photosynthesis. In fruits, the main soluble sugars present are sucrose, glucose and fructose. All three of them are water soluble and are mainly responsible for the sweet taste of fruits and some vegetables. The concentration of sucrose, glucose and fructose changes during fruit ripening. In some fruits such as apple, pear, grape and strawberry glucose and fructose are more concentrated. In other fruits like melon, banana, peach and pineapple sucrose is the predominant sugar (Maness, 2010; Vicente et al., 2009). Other sugars which might be present at trace levels are xylose, arabinose, mannose, galactose and maltose (Salunkhe et al., 1991; Vicente et al., 2009). Sorbitol is a sugar alcohol frequently found in the Rosaceae family, including species of the genus *Malus*, *Prunus* and *Sorbus* (Loescher et al., 1982; Vicente et al., 2009).

Sugars play a substantial role in plant health, as structural compounds, energy reserves and as signaling molecules (Fellman and Loescher, 1987; Maness, 2010; Singh and Malhotra, 2000; Vicente et al., 2009). Sugar alcohols, such as sorbitol, mannitol and xylitol, have been shown to play a major role in plants' adaptation to salt or drought stress. These compounds are considered compatible solutes and can accumulate in high concentrations without affecting cell metabolism. Plants under salt or drought stress can accumulate sugars alcohols to cope with the osmotic stress derived from these environmental conditions (Bielski, 1982; Lo Bianco and Rieger, 2002).

The soluble sugar content is a relevant quality trait in dried plum production. During the drying step in the production of prunes, three main chemical processes may affect the sugars present: acid hydrolysis of sucrose, glucose and fructose interactions with nitrogen containing compounds like proteins or free amino acids (Maillard reaction) and caramelization (Sabarez et al., 2000; Wilford et al., 1997). Sucrose, as a non-reducing sugar, does not participate directly in the Maillard reaction. Nevertheless, sucrose can be hydrolyzed to fructose and glucose and both reducing sugars are reactants in the Maillard reaction. As a consequence, special care should be taken during the drying process in order to avoid or minimize hydrolysis. (Wilford et al., 1997). On the other hand, sorbitol, does not take part in the Maillard reaction, because it lacks the necessary carbonyl group and it does not caramelize as easy as the other sugars

present in plum. These factors prevent the excessive browning of prunes and allow a high content of sugars after the drying process. (Wilford et al., 1997; Cinquanta et al., 2002).

In human health, soluble sugars have a direct impact as an energy source for metabolic processes. Sorbitol and xylitol are interesting sweeteners for the food industry, because they are non-cariogenic and their metabolism is insulin independent making them suitable alternatives for sugars in diabetic diets (Schiweck et al., 2012). Another interesting feature of sorbitol is its laxative effect at low doses (70 g/day) (Cinquanta et al., 2002). In addition, there are reports of sorbitol intolerance in adults (Jain et al., 1987; Jain et al., 1985).

Soluble sugars can be divided in monosaccharides, disaccharides and polysaccharides by their degree of polymerization. Sugar alcohols, i.e. polyhydric alcohols obtained from monosaccharides, are a special category.

- Monosaccharides: This type of soluble sugars contain a single aldehyde or ketone functional group like glucose (Figure 2h) and fructose (Figure 2i) (Brielmann et al., 2006).

- Disaccharides: They result from the condensation of two molecules of monosaccharides. Sucrose (Figure 2l) is the sweetest of the disaccharides composed by one molecule of glucose and one of fructose (Brielmann et al., 2006).

- Polysaccharides: Polysaccharides are high molecular weight compounds, due to the polymerization of numerous monosaccharide molecules. One example is cellulose (Figure 2m) (Brielmann et al., 2006).

- Sugar alcohols: These sugars are acyclic polyols with similar functions to sucrose in the plant. They are formed by reduction of the carbonyl group and a monosaccharide in a hydroxyl group. Examples are sorbitol (Figure 2j) and xylitol (Figure 2k) (Loescher et al., 1982; Singh and Malhotra, 2000).

3.2.3. Carotenoids

Carotenoids are tetraterpenoids with a molecular structure consisting of a polyene hydrocarbon chain composed by eight isoprene units (C_{40}). The first four isoprene units are linked head-to-tail. At the center of the molecule, the forth and the fifth isoprene units are bonded tail-to-tail and the last four units are linked tail-to-head. Therefore, this structure can be also considered a symmetrical tetraterpene constituted by two C_{20} units linked tail-by-tail (Brielmann et al., 2006; Britton, 1993; Britton, 1995; Crozier, 2003; Kohler, 1995; Rodriguez-Amaya, 2016).

Carotenoids are extremely hydrophobic and lipophilic substances. Even glycosides are almost insoluble in water. In living organisms, they are often associated with a protein that stabilizes them in aqueous systems. An example of this is the association with proteins of the photosynthetic system (Britton, 1993). The structure of carotenoids can be acyclic, if they do not possess rings, such as phytoene, phytofluene and lycopene (Figure 3a, b and c). Other carotenoids are cyclic, containing one (monocyclic) or two cycles (dicyclic) at the ends of the molecule, as in the case of γ–carotene and ß-carotene (Figure 3d), respectively. The ring is mostly composed by six atoms. Occasionally, it may consist of five atoms, such as capsanthin and capsorubin, which occur in red pepper (Britton, 1993; Britton et al., 2004; Rodriguez-Amaya, 2016). Acyclic carotenoids have a planar configuration, while cyclic carotenoids do not. In the later, the rings adopt the half chair position. If the ring has a bulky substituent, this will take an equatorial or pseudo equatorial position (Britton, 1993).

Another basic feature of carotenoids is the conjugated double bond system, where the π-electrons delocalize throughout the polyene chain (Rodriguez-Amaya, 2016). This conjugated-double-bond system is responsible for the color and antioxidant capacity of these compounds. The polyene conjugated double bond chain also plays a role in the energy transfer process during photosynthesis and makes the molecule susceptible to oxidation and free radical reactions, which explains the instability of carotenoids, specially under oxidizing conditions (Britton, 1993).

These compounds are not only present in photosynthetic organs of plants like leaves and young stems, but also in non-photosynthetic organs like colored fruits, flowers and roots, giving them yellow, orange or red colors (Britton, 1993; Rodriguez-Amaya, 2016). They may be present in free form or associated with proteins resulting in a wide range of possible colors (Britton, 1993). They have major roles in plants, acting as accessory light-harvesting pigments in photosynthesis extending the range of absorbed light (Brielmann et al., 2006; Crozier, 2003). By coloring of fruits and flowers, they act as attractants of pollinators and seed dispersers (Brielmann et al., 2006). They can also be found on photosynthetic membranes of phototropic bacteria and cyanobacteria and in non-phototrophic organism like bacteria, fungi, and animals. Nevertheless, all carotenoids found in animals have their origin in a phototrophic organism, for example plants (Britton, 1993). In humans they are dietary antioxidants that protect against cancer and they are precursors of vitamin A, especially ß-carotene (Crozier, 2003).

Carotenoids can be classified into two groups depending on the absence or presence of oxygen in the molecule (Britton, 1993; Britton, 1996; Rodriguez-Amaya, 2016).

- Carotenes: They are oxygen free carotenoids (Britton, 1993; Britton, 1996; Rodriguez-Amaya, 2016). They can be linear (as lycopene, Figure 3c), monocyclic (γ–carotene) or bicyclic (ß-carotene, Figure 3d). They are less polar than oxygenated xanthophylls (Britton, 1993; Rodriguez-Amaya, 2016; Saini and Keum, 2018). Colorless carotenoids such as phytoene and phytofluene can also be considered carotenes, as they do not have oxygens in their structures. Nevertheless, because they have different chromatographic properties they are considered as a separated group in this study.

- Xanthophylls: These compounds include oxygenated carotenoids, for example lutein (Figure 3e). The oxygen atom can be present as hydroxyl-, aldehyde-, keto-, carboxy-, carbomethoxy-, epoxy- or lactone group. Hydroxyl groups can also be esterified or glycosylated (Britton, 1993; Britton, 1996; Rodriguez-Amaya, 2016). The incorporation of polar functional groups into the molecule increases the polarity of these compounds and therefore, xanthophylls are more polar than carotenes (Saini and Keum, 2018). Xanthophylls in green tissues like leaves and fruits (kiwis) are mostly present in the free form (Gross, 1987; Kobori and Amaya, 2008; Rodriguez-Amaya, 2016). In colored fruits they appear usually esterified with fatty acids. They can be monoesters if only one of the hydroxyl groups is attached to a fatty acid, or diesters if both are esterified (Mercadante et al., 2017; Saini and Keum, 2018). The fatty acids can be saturated (lauric, mystric, palmitic, stearic acid) or unsaturated (oleic, linoleic or alpha-linoleic acids) (Mercadante et al., 2017). This esterification process occurs during fruit ripening. Acylated xanthophylls are more lipophilic, which facilitates the accumulation in the chromoplast during fruit development (Gross, 1987). The most common xanthophyll esters found in European plum are lutein diesters, although ß-cryptoxanthin monoesters have been described as well (Breithaupt and Bamedi, 2001; Khachik et al., 1991).

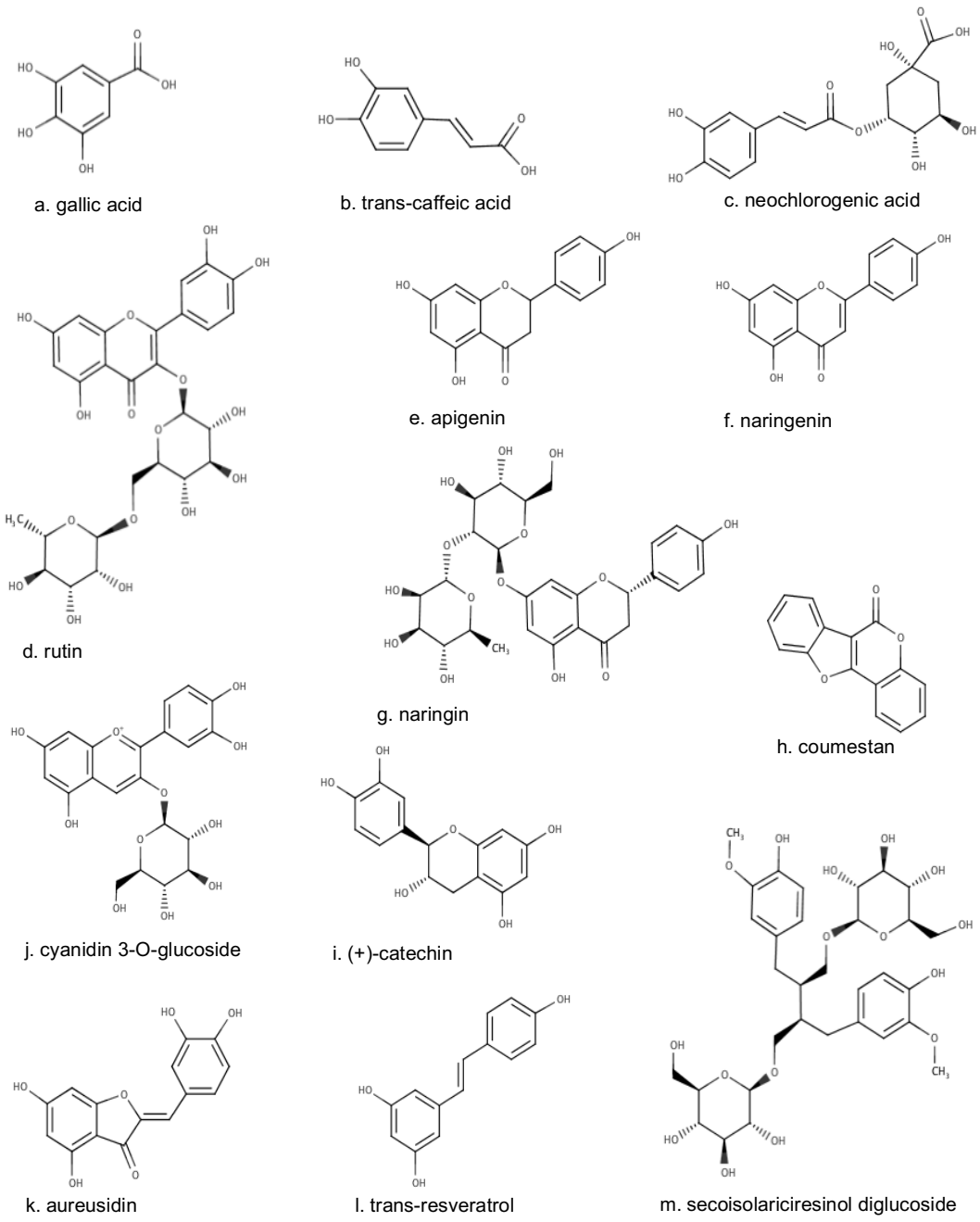


Figure 1: Chemical structures of selected phenolic compounds.

Phenolic acids: a. benzoic acid derivative, b. and c. cinnamic acid derivates. Flavonoids: d. flavonol, e. flavone, f. and g. flavanones, h. isoflavone, i. anthocyanin, j. flavan-3-ol, k. aurone and other phenolics: l. stilbene and m. lignan. PubChem (https://pubchem.ncbi.nlm.nih.gov), software MarvinSketch 18.28©, ChemAxon Ltd (http://ww.chemaxon.com).

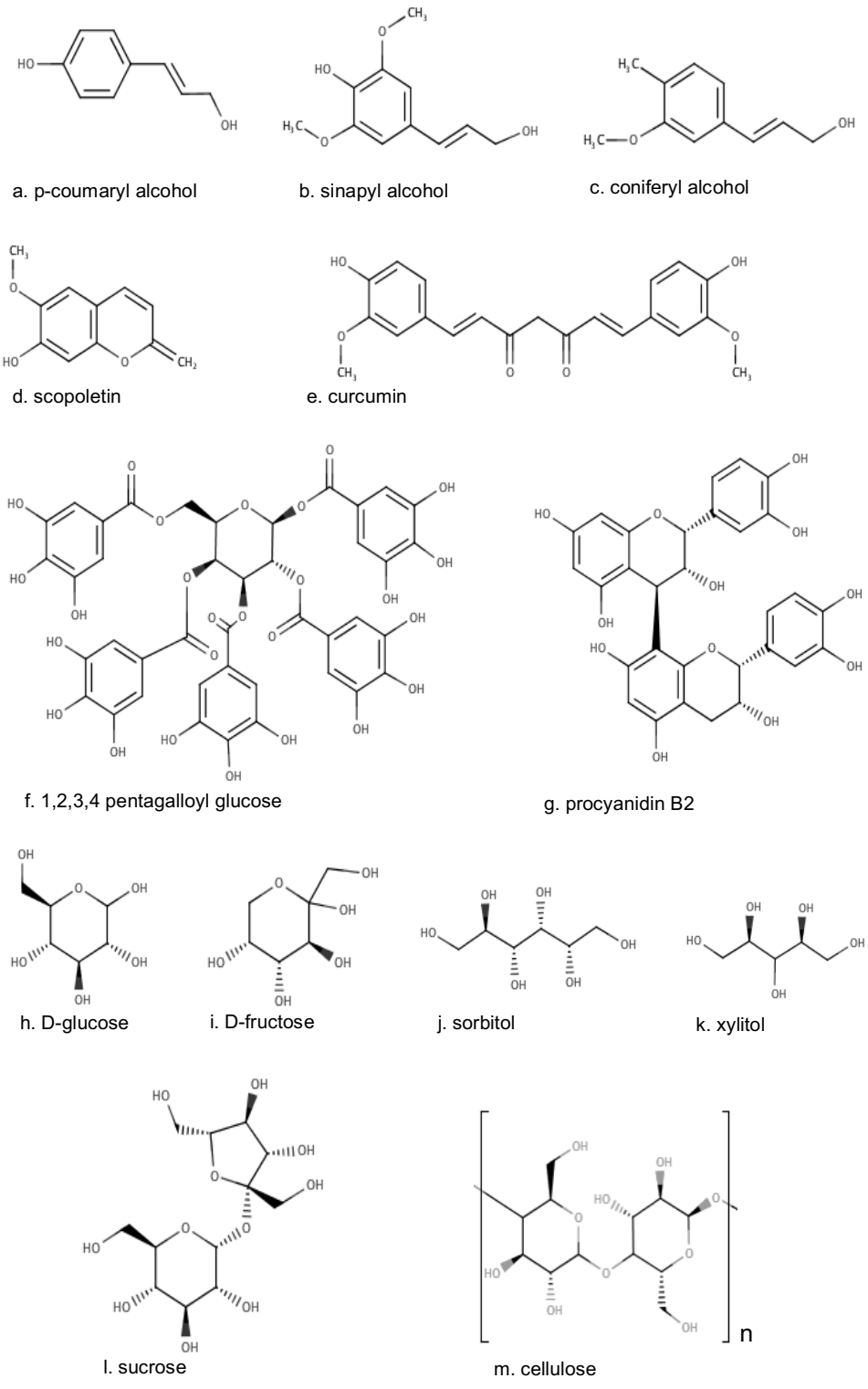
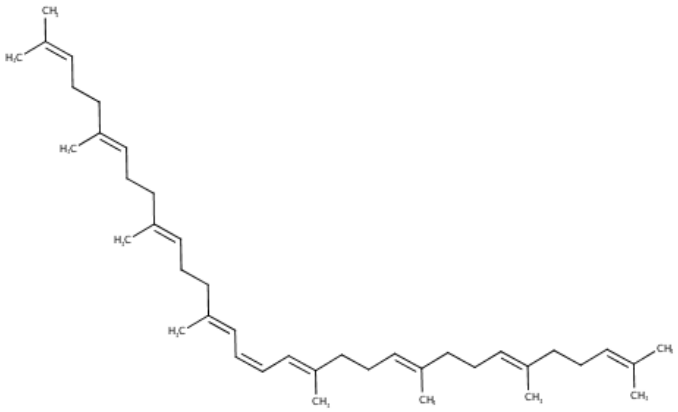
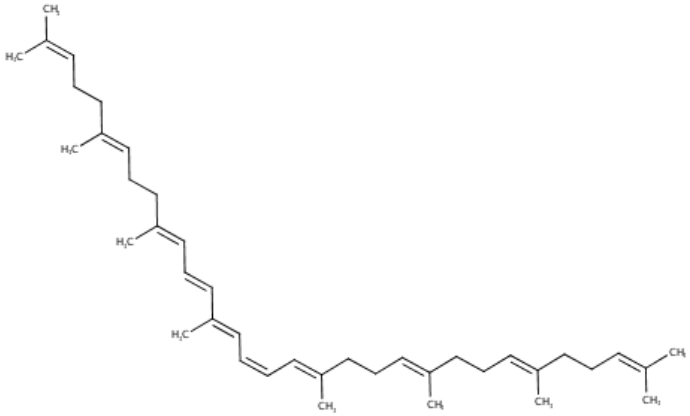


Figure 2: Chemical structures of phenolics and soluble sugars.

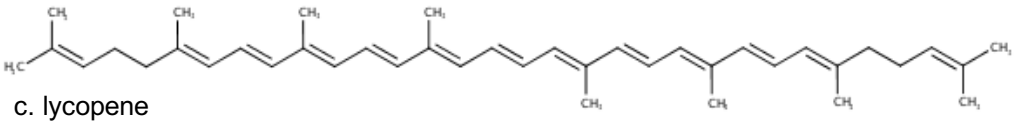
Phenolics: a., b. and c. monomeric precursors of lignin, d. coumarin, e. diarilheptanoids, f. hydrolizable tannin, g. proanthocyanidin. Soluble sugars: h. and i. monosaccharides, j. and k. sugar alcohols, l. disaccharides and m. polysaccharide. PubChem (https://pubchem.ncbi.nlm.nih.gov), software MarvinSketch 18.28©, ChemAxon Ltd (http://ww.chemaxon.com).



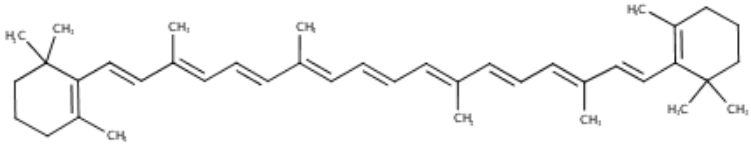
a. 15-cis-phytoene



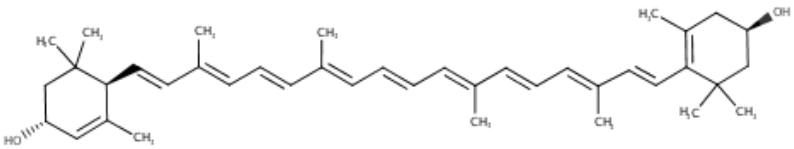
b. 15-cis-phytofluene



c. lycopene



d. ß-carotene



e. lutein

Figure 3: Chemical structures of carotenoids.

Carotenoid subgroups: a. and b. colorless carotenoids, c. linear carotene, d. cyclic carotene and e. cyclic xanthophyll. PubChem (https://pubchem.ncbi.nlm.nih.gov), software MarvinSketch 18.28©, ChemAxon Ltd (http://ww.chemaxon.com).

3.2.4. Regulation of the biosynthesis of the studied compounds

The basic biosynthetic pathways for soluble sugar, phenolic acids, flavonoids and carotenoids have been elucidated, but a number of questions remain to be clarified. Biosynthetic pathways of these compound classes in plants are summarized in Figure 4 to Figure 6.

The biosynthesis of **carbohydrates and sugars** is directly dependent of the photosynthesis rate. In this process, CO_2 from the atmosphere is assimilated and reduced to triose phosphate, the starting molecule for carbohydrate synthesis. The consumption of triose phosphate for starch or soluble sugar biosynthesis is in equilibrium with the regeneration of ribulose-1,5-bisphosphate. A complex regulatory network allows parallel response to different abiotic and biotic stimuli such as environmental, metabolic and physiological conditions. Carbohydrate biosynthesis can be regulated by photosynthesis rate, consumption rate of triose phosphate, and physiological control, mainly regulated by the relationship between sink and source organs (Hofius and Börnke, 2007; Rolland et al., 2006).

Sucrose synthesis and transport can also affect carbohydrate accumulation and plant development. The sucrose biosynthetic pathway comprises various steps catalyzed by enzymes, most of them are complex and regulated on multiple levels. Two key enzymes have been described to regulate this process: fructose-1,6-biphosphatase FBPase (catalyzes the synthesis of fructose-6-phosphate from fructose-1,6-biphosphate) and sucrose phosphatase SPP (catalyzes the hydrolysis of sucrose-6-phosphate to form sucrose). Both steps are essentially irreversible and therefore they prevent the reverse direction of the reactions back to triose phosphate (Hofius and Börnke, 2007; Rolland et al., 2006).

Sucrose is the main sugar transported in plants (Hofius and Börnke, 2007). Some sugar alcohols like sorbitol and mannitol can also be transported through the phloem (Fellman and Loescher, 1987; Singh and Malhotra, 2000). Transfer of sugars from the synthesis cell at the source organ to the phloem and the subsequent unloading at the target cell of the sink organ can happen via the apoplastic or symplastic route depending on the species (Frommer and Sonnewald, 1995; Hofius and Börnke, 2007; McCauley et al., 1992; Rolland et al., 2006; Singh and Malhotra, 2000; van Bel et al., 1992; Vicente et al., 2009). The transport itself is proposed to happen through a pressure generated flow gradient between the source and the sink tissue (Hofius and Börnke, 2007; Singh and Malhotra, 2000). The mechanism determining the sink demand of an organ in a plant has not been completely elucidated but two main theories have been postulated to explain it: hormonal regulation (for example cytokinin with a

positive influence on photosynthesis rate) and photosynthesis inhibition by the end-products (Goldschmidt and Huber, 1992; Hofius and Börnke, 2007; Wareing et al., 1968).

Once sucrose has been imported into the cytoplasm of the sink organ cells, it can be cleaved into glucose and fructose or other derivates by cytoplasmic or vacuolar invertases (Hofius and Börnke, 2007; Rolland et al., 2006). Further modifications (Figure 4), lead to GGPP the starting molecule of the carotenoids pathway (Figure 6) via the mevalonate or non-mevalonate pathway and to phenylalanine: the starting molecule of the phenylpropanoid pathway (Figure 5).

Sucrose and its derivates can also act as signaling molecules. The main receptors of sugar signals in plants are hexokinases for glucose and SNF1-protein kinases for sucrose (Jang, 1997).

As already mentioned, the main enzymes of the **phenylpropanoid (phenolic acid and flavonoid)** pathway have been described (Figure 5). The starting molecule of this pathway is L-phenylalanine which is transformed to cinnamic acid catalyzed by phenylalanine ammonia lyase PAL. From cinnamic acid, one synthesis branch leads to hydroxycinnamic acids, another to benzoic acids and a third to flavonoids. The first compound of the flavonoid biosynthesis pathway is a chalcone, which results from the addition of three malonyl-CoA to a p-coumaroyl-CoA molecule catalyzed by chalcone synthase CHS. Further transformations lead to flavones, flavonols, and leucocyanidins. Leucocyanidins are precursors of anthocyanidins and of some flavan-3-ols like catechin. Anthocyanidins are precursors of flavan-3-ols like epicatechin and also of glycosylated anthocyanidins, called anthocyanins. The anthocyanins can undergo further modifications like methylations. Proanthocyanidins result from the polymerization of flavan-3-ols. Some authors suspect that this synthesis takes place in the vacuole (Dixon et al., 2004; Koes et al., 2005; Zhao et al., 2010) while others locate it in a special organelle derived from chloroplasts called tannosome (Brillouet et al., 2013a; Brillouet et al., 2014; Brillouet et al., 2013b).

Some common regulation patterns have been described for some *Prunus* species like peach (*P. persica*), sweet cherry (*P. avium*) and the European plum (*P. domestica*). In most of the cultivars analyzed, the key enzyme of the flavonoid pathway was CHS. In those cultivars containing anthocyanins also glycosyltransferase UFGT has a relevant role in flavonoid accumulation. In the European plum, two isomers of UFGT were described: UFGT1 was responsible for glycosylation of flavonols and UFGT2 for anthocyanins (Selvaraj et al., 2016). When flavonols accumulated in the fruits, the enzyme flavonol synthase FLS was the key enzyme while anthocyanidin

reductase ANR and leucoanthocyanidin reductase were important enzymes for the production flavan-3-ols and proanthocyanidins (Liu et al., 2013; Ravaglia et al., 2013; Selvaraj et al., 2016; Selvaraj, 2014; Tuan et al., 2015; Wei et al., 2015).

It has been already demonstrated in different plant species, that the transcriptional regulation of the phenylpropanoid pathway is controlled by the MBW complex composed by three transcription factor groups R2R3-MYB + bHLH + WD40 (Jaakola, 2013). In a MBW complex, the MYB protein seems to define the target gene specificity of the complex (Hichri et al., 2011). Other MYB protein families such as the R3-MYB type, have been described to inhibit flavonoid accumulation by reducing the expression of the genes coding for the pathway enzymes (Dubos et al., 2008; Matsui et al., 2008).

In the case of the **carotenogenesis** enzymes, most of them have already been described (Figure 6) (Fraser et al., 1994; Moise et al., 2013; Nisar et al., 2015). Common model species for the analysis of carotenoids are tomato, *Citrus spp* and *Arabidopsis thaliana* (Bemer et al., 2012; Itkin et al., 2009; Kato et al., 2004; Martel et al., 2011; Rodrigo et al., 2013; Vrebalov et al., 2009; Vrebalov et al., 2002; Wang et al., 2014; Zhang et al., 2012). Carotenoid biosynthesis in *Prunus* species had been less intensively studied than the flavonoid pathway. Kita et al (2007) analyzed the expression of the carotenoid pathways genes in Japanese apricot, *P. mume*.

The first compounds of the carotenogenic pathway (Figure 6) are two molecules of geranylgeranyl diphosphate GGPP that are fused to form one molecule of phytoene. This GGPP molecules can have two origins: the mevalonate and non-mevalonate pathway. A series of desaturations in different parts of the phytoene molecule result in linear carotenoids with increasing number of conjugated double bonds. First colorless compounds (phytoene and phytofluene) and then the colored ones (lycopene) are formed. After lycopene, the pathway splits in two directions. One of them leads to compounds like α-carotene and lutein, while the other branch leads to compounds like β-carotene, β-cryptoxanthin and zeaxanthin. Within the second branch the xanthophyll cycle is placed and this pathway can also lead to abscisic acid synthesis. The first steps in both branches are cyclizations on the extreme ends of the molecules leading to cyclic carotenes (α- and β-carotene). Then, the molecules are hydroxylated forming xanthophylls like lutein and zeaxanthin. Further modifications are possible in carotenoids such as the formation of epoxides, glycosides, ethers, aldehydes, ketones and xanthophylls bonded to one or two fatty acids (esterified xanthophylls) (Ruiz-Sola and Rodriguez-Concepcion, 2012).

One of the key enzymes for the regulation of this pathway in different species is lycopene beta-cyclase LCYb, because the suppression of the corresponding gene led to the accumulation of lycopene in sweet orange (*Citrus sinensis*) (Lu et al., 2016) and grapefruit (*Citrus paradisi*) flavedo (Alquézar et al., 2009; Mendes et al., 2011). On the other hand, the induction of its expression led to the accumulation of downstream carotenoids like ß,ß-xanthophylls (Kato et al., 2004; Rodrigo et al., 2013; Zhang et al., 2012). Nevertheless, the mechanism of transcription regulation of the carotenogenesis genes is still unclear (Lu et al., 2018).

One group of transcription factors that have been described to regulate carotenogenesis are MADS-box proteins (MADS6 in orange and RIN, TAGL1 and FUL1/2 in tomato). They can directly induce the expression of constitutive genes of the carotenoid biosynthesis or control the expression and activity of other transcription factors like of HY5, RAP2.2, and PIF1. Some of them are regulated by hormones such as ethylene and influenced by the environment (Bemer et al., 2012; Chung et al., 2010; Itkin et al., 2009; Lu et al., 2018; Martel et al., 2011; Vrebalov et al., 2009; Vrebalov et al., 2002; Wang et al., 2014). Other transcription factors like NAC proteins (in *Carica papaya* and tomato) can influence directly the transcription of structural genes, of other transcription factors or bind to other proteins like EIN to regulate indirectly the transcription of promoters (Fu et al., 2016; Fu et al., 2017; Kou et al., 2018; Lu et al., 2018). Even R2R3-MYB factors, commonly related to flavonoid biosynthesis regulation have been found to regulate carotenoid biosynthesis, for example MYB68 in the flavedo of *Citrus reticulata* (Zhu et al., 2017) and RCP1 in *Mimulus lewisii*. This last protein induced carotenoid accumulation during flower development and downregulated anthocyanin production, although parallel regulation in the same organ is rare (Sagawa et al., 2016).

Some transcription factors that indirectly control carotenoid accumulation by regulating physiological processes like fruit ripening have been described such as TAGL1 (Itkin et al., 2009; Vrebalov et al., 2009), ERF6 (Lee et al., 2012), GLK2 (Powell et al., 2012), and CubHLH1 (Endo et al., 2016).

The regulation of the synthesis of sugars, phenolic compounds and carotenoids is part of a complex interconnected metabolism net, which has not yet been completely elucidated. For example, sugars, mainly sucrose, have been described to have an influence on flavonoid and carotenoid accumulation. Most studies dealing with the effects of soluble sugars on flavonoids and phenolic acids focused on anthocyanin accumulation. In grape berry skin, the expression of genes related with anthocyanin biosynthesis, such as *DFR*, seem to be correlated with sucrose accumulation (Boss et

al., 1996; Gollop, 2002). Similar effects have been described in other species, such as in leaves of *Egeria densa* (Momose and Ozeki, 2013), *Populus* leaves (Arnold et al., 2004) and girdled maple trees (Murakami et al., 2008), shoots of Carob (*Ceratonia siliqua*) (Vinterhalter et al., 2007), apple (Liu et al., 2017) and *Arabidopsis* seedlings (Mita et al., 1997; Ohto et al., 2001; Solfanelli, 2006; Teng, 2005). Therefore, this regulation pathway has been designated as sucrose-induced anthocyanin accumulation (Teng, 2005). Sucrose can influence the expression of genes of the flavonoid pathway and their transcription factors in a differential manner (Liu et al., 2017; Shi et al., 2014; Solfanelli, 2006; Teng, 2005). The effect of sucrose on flavonoid accumulation is modulated by controlling the expression of sucrose-nonfermenting (SnF1)-related kinases (SnRK1s) (Baena-González et al., 2007). These proteins have been reported to have a role in major developmental processes in plants and also in reactions to stress conditions (Baena-González et al., 2007; Radchuk et al., 2009). They interact and respond to hormones like auxin, cytokinin and abscisic acid, being involved in sugar-hormone crosstalk signaling pathway (Radchuk et al., 2009). The effect of sucrose on SnRK1.1 is concentration dependent. A high concentration of sucrose enhanced anthocyanin concentration in *Arabidopsis* (*AtSnRk1.1*) and apple (*MdSnRK1.1*) (Baena-González et al., 2007; Liu et al., 2017). It also increased proanthocyanidin concentration in apple (Liu et al., 2017). An oversupply of sucrose in apple led to an inactivation of the MdSnRK1.1 protein and therefore reduced the anthocyanin accumulation (Baena-González et al., 2007; Liu et al., 2017). In addition, overexpression of KIN10 (a SnRK1 kinase) suppressed expression of transcription factor *MYB75/PAP1* associated with anthocyanin accumulation, thereby inhibiting anthocyanin biosynthesis (Baena-González et al., 2007).

The influence of sugar on carotenoid biosynthesis was investigated to a lesser extent than on flavonoid biosynthesis. It has been reported, that sucrose accumulation promotes carotenoid accumulation by inducing transformation of chloroplast into chromoplast in citrus fruits (Huff, 1983; Huff, 1984; Zhang et al., 2012) and tomato (Telef et al., 2006). This effect may depend on ethylene for some citrus species (Iglesias et al., 2001). Also sugar alcohols can have a regulatory effect on carotenoid accumulation. Sorbitol can negatively regulate the synthesis of chlorophylls and carotenoids in a concentration dependent manner in maize seedlings during greening (Swati and Meeta, 2016). Mannitol also induced carotenoid accumulation in *in-vitro* juice sacs of mandarin, orange and lemon (Zhang et al., 2012). The regulatory mechanism of carotenoid concentration of mannitol and sucrose was different at the transcriptional level (Zhang et al., 2012).

As it has already mentioned, hormones have an influence on flavonoid and carotenoid accumulation in a sucrose dependent or independent way. The hormonal influence on flavonoid accumulation is mainly based on the regulation of the expression of biosynthesis genes and their promoters or inhibitors (Das et al., 2012; Deikman and Hammer, 1995; El-Kereamy et al., 2003; Jeong et al., 2010). For example, jasmonic acid (Das et al., 2012; Loreti et al., 2008; Shan et al., 2009), gibberellic acid and abscisic acid (Kim et al., 2006; Mori et al., 2005) have a positive effect on flavonoid accumulation. On the other hand, abscisic acid and gibberellic acid have been reported to suppress carotenoid accumulation in citrus fruits (Zhang et al., 2012).

Reports on the effects of ethylene, auxin and cytokinin on flavonoid accumulation are contradictory (Craker and Wetherbee, 1973; El-Kereamy et al., 2003; Kang and Burg, 1973; Loreti et al., 2008; Mori et al., 1994). In the case of carotenoid biosynthesis, ethylene has a promoting effect by inducing accumulation of lycopene or of downstream carotenoids depending on the species and the organ analyzed (Alba et al., 2005; Fraser et al., 1994; Kita et al., 2007; Kou et al., 2018; Ma et al., 2014; Ronen et al., 2000). Some MADS-box transcription factors (RIN, TAGL1, FUL1/2, RAP2.2) are ethylene responsive in fruits such as tomato, although the presence of the hormone is not mandatory but synergistic (Bemer et al., 2012; Itkin et al., 2009; Lu et al., 2018; Martel et al., 2011; Vrebalov et al., 2009; Vrebalov et al., 2002; Wang et al., 2014; Welsch et al., 2007). For auxin it was shown that it also indirectly regulates biosynthesis by regulating ethylene production (Hao et al., 2015; Lu et al., 2018). The mechanism underlying this regulation is still unknown (Lu et al., 2018).

Environmental effects on flavonoid and carotenoid accumulation have also been reported. Light-regulated flavonoid and carotenoid biosynthesis is mainly controlled by phytochromes (red/far red-light receptors) and cryptochromes (blue-light receptors). These photoreceptors perceive and transduce the light signals to control physiological processes in plants (Llorente et al., 2017). Two flavonoid biosynthesis transcription factors that are known to be regulated by phytochromes are PIF3 and HY5 (Gyula et al., 2003; Shin et al., 2007). Both transcription factors promote anthocyanin accumulation through collaborative transcriptional regulation of anthocyanin biosynthesis genes (Shin et al., 2007). HY5 can also be regulated by cryptochrome signals (Gyula et al., 2003). High light conditions can also favor the accumulation of flavonoids by inhibiting the expression of negative transcription factors like MYBL2 (Dubos et al., 2008). In the case of carotenoids, HY5, PAR1 and PIF1 also modulate carotenoid accumulation in response to light (Lu et al., 2018; Toledo-Ortiz et al., 2014). PIF1 is a transcription factor repressed by phytochrome-mediated light signals and belongs to the same family as PIF3, which plays a role in flavonoid biosynthesis

(Llorente et al., 2016; Llorente et al., 2017; Toledo-Ortiz et al., 2010). HY5 and PAR1 are antagonists of PIF1. They bind to PIF1 and release an expression promoter of *PSY*, the first enzyme of the carotenoid biosynthesis. Hence PIF1 inhibition promotes carotenoid accumulation (Llorente et al., 2017; Toledo-Ortiz et al., 2014). Consequently carotenoids accumulation is induced in response to light and temperatures signals (Toledo-Ortiz et al., 2014). Also, cryptochromes can regulate carotenoid biosynthesis. They inhibit a HY5-repressor complex called COP1-DDB1-CUL4, which indirectly induces carotenoid accumulation. COP1 mediated HY5 degradation happens in dark conditions, in absence of blue light (Llorente et al., 2017). Some phytochrome encoding genes in tomato fruit (SlPHY) influence carotenoid accumulation in a light dependent manner by regulating chloroplast division and maturation into chromoplast (Bianchetti et al., 2018).

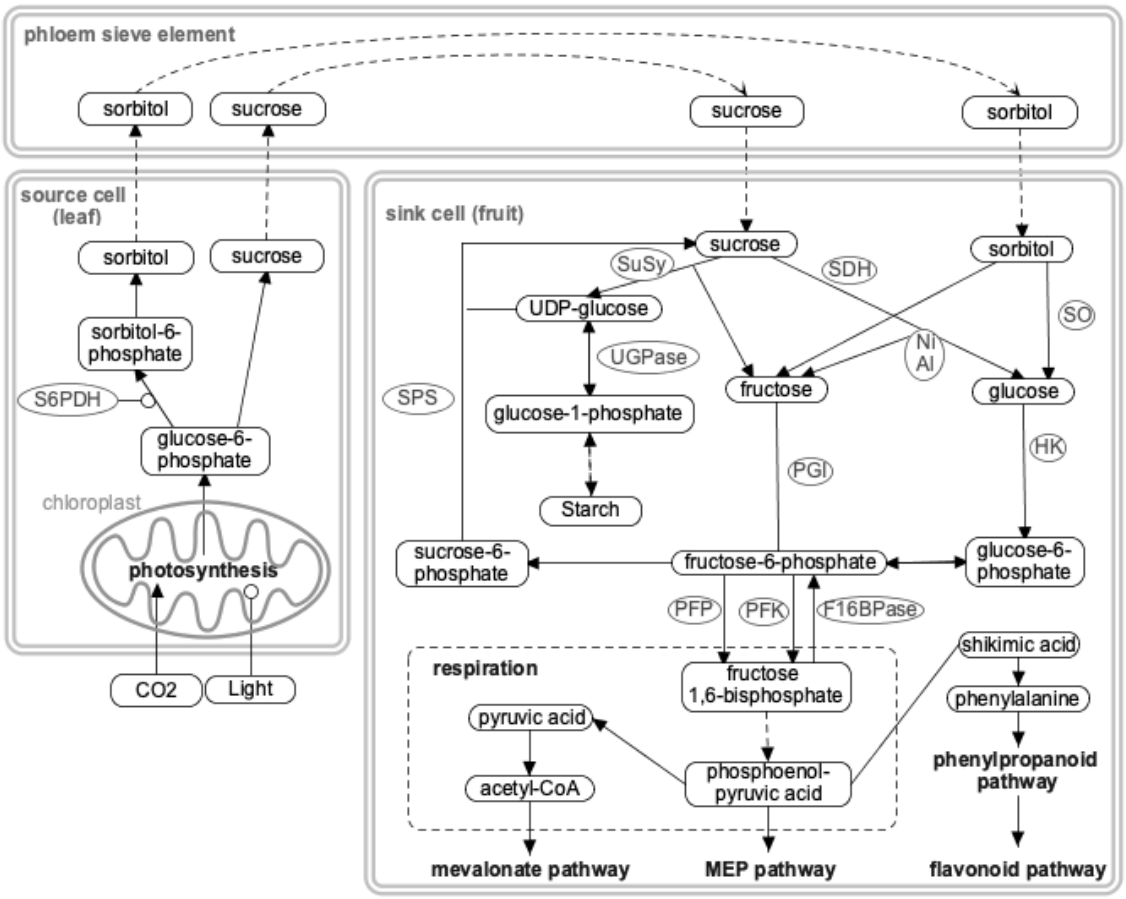


Figure 4: Sugar biosynthesis pathway

Acetyl-CoA: acetyl-Coenzyme A, AI: acid invertase, F16BPase: fructose 1,6 biphosphatase, HK: hexokinase, MEP: 2-C-methyl-D-erythritol 4-phosphate, NI: neutral invertase, PFK: ATP-phosphofructokinase, PFP: PPi-phosphofructokinase, PGI: phosphoglucose isomerase, S6PDH: sorbitol-6-phosphate dehydrogenase, SDH: sorbitol dehydrogenase, SO: sorbitol oxydase, SPS: sucrose-6-phosphate synthase, SuSy: Sucrose synthase, UGPase: UDP-glucose pyrophosphorylase. Adapted from Desnoues et al. (2014); Escobar Gutiérrez and Gaudillère (1996); (Li et al., 2012); Rolland et al. (2006); Teo et al. (2006); Williamson et al. (2002); Yamaki (2010); Zhang et al. (2017).

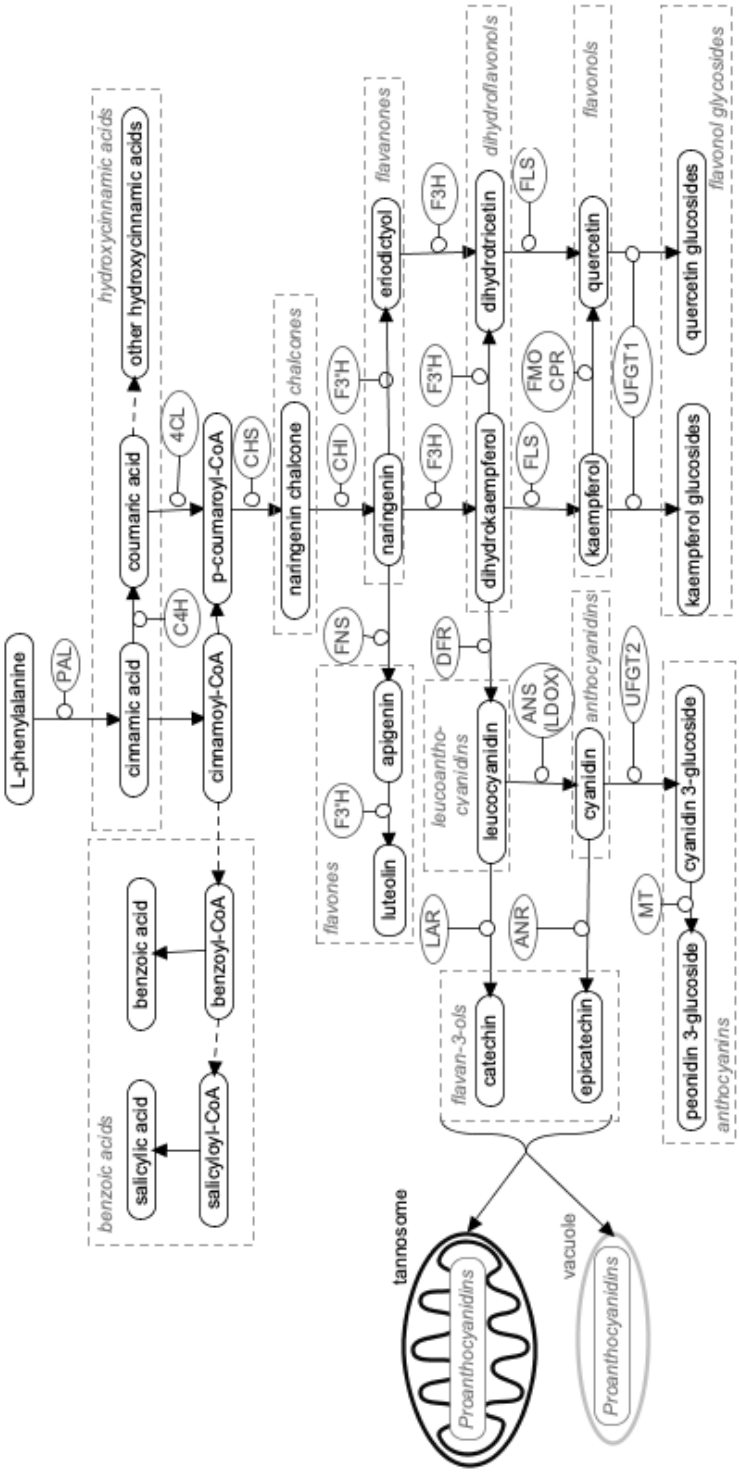


Figure 5: Phenylpropanoid and flavonoid pathway

4CL: 4-coumaric acid:CoA ligase, ANS: anthocyanidin synthase, ANR: anthocyanidin reductase, C4H: cinnamate-4-hydroxylase, CHS: chalcone synthase, CHI: chalcone isomerase, CPR: cytochrome P450 reductase, DFR: dihydroflavon-4-reductase, F3'H: flavonoid 3'-hydroxylase, F3H: flavonoid 3-hydroxylase, FLS: flavonol synthase, FMO: flavonoid monooxygenase, LAR: leucoanthocyanidin reductase, LDOX: leucoanthocyanidin dioxygenase, OMT: methyltransferase, PAL: phenylalanine ammonia lyase, UFGT: UDP-glucose: flavonoid-3-O-glucosyltransferase. Benzoic and hydroxycinnamic acids biosynthetic pathways are simplified and therefore no enzymes or organelles are indicated. Adapted from Brillouet et al. (2013a); Brillouet et al. (2014); Brillouet et al. (2013b); Chong (2001); Dixon et al. (2004); Falcone Ferreyra et al. (2012); Harakava (2005); Haslam (1998); Koes et al. (2005); Nesi et al. (2001); Winkel-Shirley (2001); Zhao et al. (2010)

Nutrient depleted soils resulted in increased flavonoid accumulation. Transcription factors like WD40 (TTG1), bHLH (GL3) and MYB (PAP1 and PAP2) also responded to nutrient depletion (reviewed by Lillo et al., 2008). For carotenoids, some reports indicate that fertilization with nitrogen or sulfur showed no significant difference in the carotenoid content of spinach (*Spinacia oleracea*) leaves (Reif et al., 2012). It has also been reported that in spinach leaves with a proper nitrogen nutrition under high light intensity, the carotenoid (violaxanthin, astaxanthin, zeaxanthin) and chlorophyll concentrations remained unchanged. However, in plants exposed to limited nitrogen supply, higher light intensity reduced chlorophyll and carotenoid concentrations (Verhoeven et al., 1997).

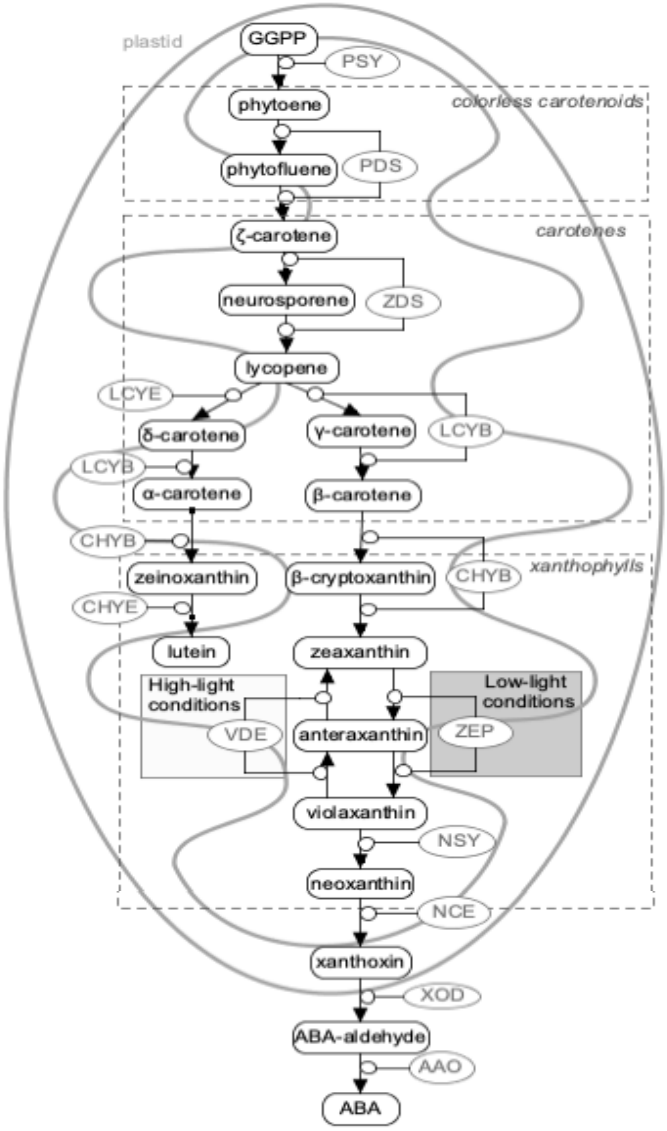


Figure 6: Carotenoid biosynthesis pathway
AAO: ABA-aldehyde oxidase, ABA: abscisic acid, GGPP: geranylgeranyl diphosphate, CHYB: carotene beta hydroxylase, CHYE: carotene epsilon hydroxylase, LCYB: lycopene beta cyclase, LCYE: lycopene epsilon cyclase, NSY: neoxanthin synthase, NCE: neoxanthin oxidase, PDS: phytoene desaturase, PSY: phytoene synthase, VDE: violaxanthin de-epoxidase, XOD: xanthoxin oxidase, ZDS: zeta-carotene desaturase, ZEP: zeaxanthin epoxidase. Adapted from Hsieh (2005); Kita et al. (2007); Kuzuyama (2014); Liu et al. (2015); Rodriguez-Concepcion (2002); (Soetaert et al., 2013); Telef et al. (2006); Zhang et al. (2012); Zhang et al. (2016)

Not many reports have been found to explain the effect of drought stress on flavonoid accumulation. In *Arabidopsis* plants undergoing drought stress, soluble sugars and anthocyanin concentration increased and negatively correlated with leaf water content (Sperdouli and Moustakas, 2012). In the case of carotenoids, some reports on the effect of water stress and salinity in tomato, show contradictory results. In this species, the effect of the lycopene accumulation depends on the resistance of the cultivar (Atkinson et al., 2011; Borghesi et al., 2011; De Pascale et al., 2015; Krauss et al., 2006; Pernice et al., 2010; Riggi et al., 2008; Sánchez-Rodríguez et al., 2012).

Finally, regarding the effect of temperature on flavonoid biosynthesis, few reports could be found. Xie *et al* (2012) showed that cold stress (low environmental temperatures) promotes anthocyanin accumulation in apple, due to induction of bHLH transcription factor *MdbHLH3* (Xie et al., 2012). Carotenoid biosynthesis is sensitive to temperature, being higher at an optimal range of 17-23°C in the case of tomato. Lycopene is especially sensitive to temperatures in tomato and Japanese persimmon (*Cydonia oblonga*) (Dumas et al., 2003; Niikawa et al., 2008).

3.3. Aims of this study

From all cultivated *Prunus* species is *P. domestica* one of the less studied regarding their metabolite content. This species is also frequently confused with *P. salicina*, as both share the common name “plum”. Therefore, reported nutritional values frequently include both species, generating confusion. Another fact that leads to confusion is that the origin of the species has still not been elucidated and considering the high variability of its fruits it is important to define which traits/features characterize this species.

Therefore, the main goal of this study was to determine the phenolics, soluble sugars and carotenoids profiles of *Prunus domestica* fruits from cultivars and of different subspecies which show different fruit features. The cultivars were selected to represent the fruit trait variability of this species. Special interest was placed on analyzing not only purple skinned cultivars, but also non-anthocyanic fruits. Some of the cultivars selected are commercially cultivated for the German market, but also some older and non-commercial cultivars, as the ones listed in the Deutsche Gene Bank Obst (DGO), were included. This was important, because market trends define which fruit traits are preferred and which new cultivars are breeded. Nevertheless, older and non-commercial cultivars have different characteristics that might be interesting for future breeding programs.

After defining a metabolite profile for *P. domestica*, the metabolite levels were compared considering the fruit skin color, cultivars subspecies and ripening date to determine to which extend these factors play a role to differentiate the different genotypes.

4. Materials and Methods

4.1. Plant material

Ripe plum fruits were randomly collected from different cultivars growing in the experimental field of the Associate Professorship of Fruit Science at the Technical University of Munich (TUM) in Freising (Figure 7, GoogleMaps: 48°24'08.7"N, 11°43'20.2"E; 48.402424, 11.722287). This field was divided in sectors for practical and administrative reasons. For the pulp analysis of samples from 2012 to 2014, fruits were collected mainly from sector-B, -C, -N and -S. Samples harvested in 2016 were collected from trees growing in sector-B and -C. For analysis of sugars and phenolics, a comparison between fruits from the same cultivar growing in different locations was performed. In this case, samples from two sections were collected: sector-N (in the north and uphill part of the field) and sector-S (in the south and downhill part). In addition, fruits from another experimental field (sector-U) were harvested. This field is located in Dürnast 2, 85354, Freising (Figure 7, GoogleMaps: 48°24'18.6"N, 11°41'26.3"E; 48.405174, 11.690646).

Figure 7: Maps of the experimental fields and their respective sectors.
Blue markers identify different field sectors. Red markers show reference adresses near the experimental fields. 1: Liesel-Beckmann-Straße 1, 85354 Freising, Germany; 2: Dürnast 2, 85354 Freising, Germany. Source of the maps: Google Maps.

In the harvest years 2012 and 2013, 30 fruits per cultivar were collected. During the years 2014 and 2015, ten fruits per cultivar were harvested. Finally, in harvest season 2016, three biological repetitions of five fruits were collected from each cultivar. These five fruits were taken from two trees growing next to each other. Plum cultivars used in this study are described in Table 7. Pictures from fruits of cultivars used for all 2016 analyses are shown in Figure 8. Most of them are from the species *P. domestica*, except for 'Liegels Gelbe' which is a *P. cerasifera* cultivar (INRA, 2018) and 'Tatjana'. It is still discussed if 'Tatjana' is a *P. cerasifera* (INRA, 2018) genotype or a hybrid between

P. cerasifera x *P. salicina* (Xuan et al., 2011). The subspecies to which each variety belonged were identified by literature search. If no literature reference was found, the cultivars were classified using the identification key for *P. domestica* published in Scholz and Scholz (1995, Supplementary Material 1).

Figure 8: Diversity of European plum fruits used for analyses in 2016.

Fruit size are not on exact scale and colors might slightly variate, as pictures were taken on the laboratory at the respective harvest date but with different light conditions. **Cultivars**: 1: 'Oullins Reneklode', 2: 'Aprikoosprium', 3: 'Belle Thuin', 4. 'Rote Eierpflaume Blazek', 5: 'Haferpflaume', 6: 'Ontariopflaume', 7: 'Grosse Grüne Renekolde', 8: 'Colora', 9: 'Frühe Mirabelle', 10: 'Mirabelle aus Nancy', 11: 'Tipala', 12: 'Angelina Burdette', 13: 'Maria Novella', 14: 'Eibenbacher Aprikosenpflaume', 15: 'Presenta' , 16: 'Mirabelle Wallenberg', 17: 'Hauszwetschge Schüffer' , 18: 'Topend Plus', 19: 'Zwintschers Frühe', 20: 'Katinka', 21: 'Jojo', 22: 'Haroma', 23: 'Cacaks Schöne', 24: 'Haganta', 25: 'Ortenauer', 26: 'President'.

The collected fruits were transported in ventilated plastic bags to the laboratory of the Professorship of Fruit Science at TUM in Freising. They were pitted and blended into juice. In the harvest seasons 2012 and 2013, fruits were juiced using a household juicer (Design Juicer Advanced Pro 40133, Gastroback, Germany). Fruits were cut in halves. One set of 30 half fruits was taken to push away the remnants of the previous fruits before the sample juice was prepared. The other set of halves was used to obtain the pulp sample. This took place within the framework of the TUM breeding programme in cooperation with BayOZ for the annual characterisation of the quality characteristics of the varieties. As part of this project, fruits were evaluated for their physical characteristics, attractiveness, taste, pH of the juice, acidity, and sweetness (Brix). After these analyses, the resulting juices were stored at 4 °C and at the end of the day they were transferred to -23 °C.

In the years 2014 to 2016, harvesting and sample preparation were carried out differently. Fruits were harvested and pitted. Both halves of the fruits were used to obtain the pulp. An immersion blender or hand blender (ESGE Zauberstab, Switzerland) was used because the juicer used in the previous years separated fibers and skin from the juice. As anthocyanins and carotenoids are expected to be present mainly in the skin, the separation of it may affect the results. By using an immersion blender, the resulting sample is more a pulp than a juice. In addition, the immersion blender is easier and faster to clean between samples, even with a large number of samples. After blending, samples were stored at 4 °C and at the end of the day at -23 °C.

4.2. Sample Preparation

Frozen samples were thawed in a cooled ultrasonic bath (Sonorex Digitec, Bandelin Electronic, Germany). For samples from 2012, 2013, 2014 and 2015, the juice/pulp was completely thawed before using it for the next extraction steps. After thawing the sample, a defined volume was taken for each extraction method. Therefore, the results were expressed in mass (weight) of compound per volume of sample. To avoid oxidation of the metabolites, samples from 2016 were not completely thawed in the ultrasonic bath. They were only sonicated until a sorbet like (ice crystals) consistency was reached and then the samples were scratched out and transferred to fresh tubes for further extraction. If oxidation happened, this part of the sample was discarded. Because the volume of ice crystals is larger than the one of water, and it was impossible to obtain the same consistency every time, the samples from 2016 were weighed to obtain the same amount of sample material for all cultivars. Hence, results of samples from 2016 are expressed in mass of compound per mass of sample.

4.3. Standard Substances

The following standard substances were used for identification and quantification of the compounds present in the samples:

- phenolic acids and flavonoids: B2, vicenin 2, flavone, chlorogenic acid (Sigma-Aldrich, Germany), rutin, (+)-catechin, (-)-epicatechin (Carl Roth, Germany), cyanidin-3-O-rutinoside, cyanidin 3-O-glucoside, peonidin 3-O-glucoside, peonidin 3-O-rutinoside, 3-methoxyflavon (Extrasynthese, France) as an internal standard, and neochlorogenic acid (Phytolab, Germany).

- soluble sugars: D-sorbitol, D-(+)-glucose, D-(-)-fructose, xylitol, sucrose, myo-inositol (Sigma-Aldrich, Germany), and mannitol (Thermo Fisher Scientific, USA).

- carotenoids and chlorophylls: ß-carotene, lutein, lycopene (Extrasynthese, France), ß-cryptoxanthin (APIN Chemicals, UK), chlorophyll a, and chlorophyll b (Sigma-Aldrich, Germany)

- Sudan-I (Honeywell Fluka, USA).

Table 7: Plum cultivars used in this research with fruit characteristics, harvest date, harvest period, subspecies, common names and whether they have been included in the DGO list of conservation of valuable varieties.

Cultivar Name	Cultivar Code	Harvest Date 2012	2013	2014	2015	2016	Field sector	Skin color	Fruit flesh color	Subspecies	Subsp. Code	Harvest period	English Common Name	German Common Name	DGO
AGRI 2000 10/92	AG	22.08.	17.09.	28.08.			G	purple	green	*domestica (1)*	D	-	European plum	Zwetschge	no
Angelina Burdette	AB	21.08.	30.08.	11.08.		18.08.	C	purple	yellow-green	*italica var. subrotunda (1)*	IS	3	Gage	Rundpflaume	yes
Aprikoosprium	AP					18.08.	S	green	green	*italica var. claudiana (1)*	IC	3	Green Gage	Reineclaude	no
Auerbacher	Au	04.09.	17.09.	02.09.			C	purple	yellow-green	*domestica (1)*	D	-	European plum	Zwetschge	yes
Bellamira	Be	20.08.					B	yellow-red	yellow	*syriaca (1)*	S	-	Yellow plum	Mirabelle	no
Belle de Thuin	BT					18.08.	C	green-yellow	green	*intermedia var. ovoidea (1)*	I	3	Egg-plum	Eierpflaume	yes
Cacaks Fruchtbare (Čačanska Rodna)	CF	3.09.	12.09.	02.09.			B	purple	yellow-green	*domestica (1)*	D	-	European plum	Zwetschge	no
Cacaks Julia (Čačanska Julia)	CJ	6.08.	26.08.	29.07.			N	purple	yellow-green	*domestica (1)*	D	-	European plum	Zwetschge	no
Cacaks Schöne (Čačanska Lepotica)	CS	13.08.	30.08.	11.08.		18.08.	B	purple	yellow-green	*domestica (1)*	D	3	European plum	Zwetschge	yes
	CS-N			29.07.			N								
	CS-U			29.07.			U								
Colora	C	13.08.	30.08.	11.08.		18.08.	B	yellow-red	yellow-green	*intermedia var. ovoidea (1)*	I	3	Egg-plum	Eierpflaume	no
Eibenbacher Aprikosenpflaume	EA	6.08.	19.08.	04.08.		17.08.	N	wine red	yellow	*italica var. subrotunda (1)*	IS	3	Gage	Rundpflaume	yes
Elena	E	18.09.	30.09.	22.09.			B	purple	yellow-green	*domestica (1)*	D	-	European plum	Zwetschge	no
Frühe Mirabelle P-2778	FM					06.08.	C	yellow-red	yellow	*syriaca (2)(3)*	S	2	Yellow plum	Mirabelle	no
Goldzwetsche	G	18.09.	25.09.	02.09.			C	yellow-red	yellow	*intermedia var. ovoidea (1)*	I	-	Egg-plum	Eierpflaume	yes
Grosse Grüne Reneklode	GR	22.08.	04.09.	28.08.		02.09.	B	green-purple	green	*italica var. claudiana (3)*	IC	4	Green Gage	Reineclaude	yes
Haferpflaume	Hf					02.09.	C	green-yellow	yellow-green	*prisca (1)*	P	4	Damson	Ziparte	no
Haganta	Hg	8.09.	30.09.	15.09.		16.09.	B	purple	yellow-green	*domestica (1)*	D	5	European plum	Zwetschge	no
Hanka	Hk	6.08.	30.08.	04.08.	11.08.		B	purple	yellow-green	*domestica (1)*	D	-	European plum	Zwetschge	no
Haroma	Hm	18.09.	25.09.	08.09.		09.08.	B	purple	orange	*domestica (1)*	D	4	European plum	Zwetschge	no
	Hm-S			08.09.			S								
	Hm-U			09.09.			U								
Hauszwetschge Schüfer	HZ	10.09.	30.09.	11.09.		16.09.	B	purple	yellow-green	*domestica (2) (3)*	D	5	European plum	Zwetschge	no
	HZ-N			11.09.			N								
	HZ-S2			15.09.			S								
Jojo	Jo					08.09.	B	purple	yellow-green	*domestica (1)*	D	4	European plum	Zwetschge	no

Continued on the next page

Table 7, continued from the previous page

Cultivar Name	Cultivar Code	Harvest Date 2012	2013	2014	2015	2016	Field sector	Skin color	Fruit flesh color	Specie or Subspecies	Subsp. Code	Harvest period	English Common Name	German Common Name	DGO
Jubileum	Ju	22.08.	12.09.	25.09.			C	purple	yellow	*domestica (1)*	D	-	European plum	Zwetschge	no
Katinka	Ka		12.08.		31.07.	29.07.	B	purple	yellow-green	*domestica (1)*	D	1	European plum	Zwetschge	no
Liegels Gelbe*	LG			08.09.			C	yellow-red	yellow	**P. cerasifera (4)*		-	Cherry plum		yes
Maria Novella	MN					17.08.	N	blue	yellow-green	*italica var. subrotunda (1)*	IS	3	Gage	Rundpflaumen	no
Mirabelle aus Nancy 1510	My					02.09.	B	yellow-red	yellow	*syriaca (2)(3)*	S	4	Yellow plum	Mirabelle	yes
Mirabelle Wallenberg	MW			29.07.	04.08.	29.07.	C	yellow-red	yellow	*syriaca (2)(3)*	S	1	Yellow plum	Mirabelle	yes
Ontariopflaume	On					17.08.	C	green-yellow	yellow-green	*italica var. claudiana (1)*	IC	3	Green Gage	Reineclaude	yes
Ortenauer	Or	10.09.	25.09.	04.09.		08.09.	B	purple	yellow-green	*domestica (2)(3)*	D	3	European plum	Zwetschge	yes
Oullins Reneklode	Ou					18.08.	C	green	green	*italica var. claudiana (1)*	IC	3	Green Gage	Reineclaude	yes
Presenta	Pa	25.09.	07.10.	22.09.		23.09.	B	purple	yellow-green	*domestica (1)*	D	5	European plum	Zwetschge	no
President	Pt	18.09.	25.09.	22.09.		30.09.	C	purple	yellow	*domestica (1)*	D	5	European plum	Zwetschge	yes
Rote Eierpflaume Blazek	RE					17.08.	N	red	yellow	*intermedia var. ovoidea (1)*	I	3	Egg-plum	Eierpflaume	yes
Tatjana**	Tj	6.08.	12.08.	18.08.		24.08.	N	purple	yellow-red	***P. salicina x P. cerasifera (5)*		-			
Tipala	Ti			04.08.		12.08.	C	yellow-red	yellow	*domestica*	D	2	European plum	Zwetschge	no
Topend Plus	Te	01.10.	30.09.	22.09.			B	purple	green	*domestica (1)*	D	-	European plum	Zwetschge	no
Topfive	TF	03.09.	04.09.	18.08.			B	purple	yellow-green	*domestica (1)*	D	-	European plum	Zwetschge	no
Tophit Plus	TP	25.09.	01.10.	22.09.		30.09.	B	purple	yellow-green	*domestica (1)*	D	5	European plum	Zwetschge	no
Zwintschers Frühe	ZF			14.07.	24.07.	22.07.	B	purple	yellow-green	*domestica (1)*	D	1	European plum	Zwetschge	yes

(1) determined by identification key from Scholz and Scholz (1995); (2) Scholz and Scholz (1995); (3) Hanke and Flachowsky (2017); (4) INRA (2018); (5) Xuan et al. (2011).

4.4. Analysis of phenolic compound

4.4.1. Extraction of phenolic acids and flavonoid

The extraction method was based on the protocol published by Goldner et al. (2015). This method was modified to reduce the loss of neochlorogenic acids caused by the overloading of the solid phase extraction column in the original method.

As described in 3.2, the juice or pulp sample was thawed. An aliquot of the sample was transferred to a 15 ml tube and centrifuged at 10,000 *g* and 4 °C for 10 min (Rotina 380R, Hettich Lab Technology, Germany). Two ml of the clear supernatant was transferred to an Eppendorf tube centrifuged at 10,000 *g* and 4 °C for 10 min (Mikro 22R, Hettich Lab Technology, Germany). One mL of the supernatant was used for solid phase extraction (SPE).

SPE cartridges prepacked with C18 material (Bond Elute, 100 mg, 1 ml, Agilent Chem) were used to perform the extraction of phenolic compounds and to remove the sugars present in the sample. The SPE cartridge was washed with 3 ml of methanol and conditioned with 6 ml of distilled water. One ml of the clear sample was filled into the cartridge. The first fraction was collected for a second extraction. The phenolic compounds were eluted from the cartridge by using 0.5 ml of methanol containing 5% distilled water:formic acid (95:5) and 3-methoxyflavon at a concentration of 0.025 mg/ml as an internal standard. The extraction was repeated with the same volume of solvent. Both eluted methanolic fractions were collected in the same Eppendorf tube and stored on ice. The first fraction was used for a second extraction. A new conditioned cartridge was loaded with the first fraction and eluted as described above.

All eluents from both extraction steps were collected and evaporated in a vacuum centrifuge (Univapo, UniEquipe, Germany). The samples were resuspended in 250 µl methanol containing 5% distilled water:formic acid (95:5). Afterwards, the samples were stored at -20°C. HPLC results were expressed in µg/ml.

For samples from 2016, 2 ml of the thawed sample was transferred to an Eppendorf tube and weighed. Therefore, the results were expressed in µg/mg.

4.4.2. RP-HPLC analysis

The method is based on the one published by Goldner et al. (2015) with some modifications. The RP-HPLC consisted of an Autosampler 465 (Kontron Instruments, Germany), 2 HPLC gradient pumps model 422 (Kontron Instruments, Germany), and a photodiode array detector Kontron 540 (Kontron Instruments, Germany). Flavan-3-ol

and proanthocyanidins were detected by post column derivatization with p-dimethylaminocinnamaldehyde (DMACA) as reagent, as described in Treutter (1989). The post-column derivatization system was composed of a HPLC gradient pump Gynkotek 300c (Gynkotek, Germany) for samples from 2012-2014 and a Kontron 422 gradient pump (Kontron Instruments, Germany) for samples from 2016, a reactor of knitted PTFE capillary (10 m x 0.5 mm ID) and a Kontron Detector 432 (Kontron Instruments) measuring the reaction products at 640 nm.

A SpeedCore (PFP, 2.6 µm, 150 x 4.6mm ID, Fortis Technologies) column was used with the following gradient of methanol (B) and formic acid (A): 5% B, 30 min; 5-10% B, 20 min; 10% B, 20 min; 10-20% B, 20 min; 20-30% B, 20 min; 30%-40% B, 20 min; 40-50% B, 10 min; 50-90% B, 10 min and 90-100% B, 5 min. Injection volume was 10 µl.

4.4.3. Quantification of phenolic compounds

Quantification was done using the software Geminyx III (Goebbels) and Microsoft Excel (Microsoft).

Formula 1 was used to determine the concentration of each compound.

$$\text{Concentration} = \frac{\text{Area}_{(\text{Compound})} * \text{RF}_{(\text{Compound})}}{\text{Area}_{(\text{Internal Standard})} * \text{RF}_{(\text{Internal Standard})}} * \text{Conc}_{(\text{Internal Standard})} * \text{MF}$$

$\text{Area}_{(\text{internal standard})}$ = Area of the internal standard peak in the chromatogram
$\text{RF}_{(\text{internal standard})}$ = Response Factor of the internal standard
$\text{Area}_{(\text{compound})}$ = Area of the peak of the compound of interest in the chromatogram
$\text{RF}_{(\text{compound})}$ = Response Factor of the compound of interest. If the response factor was not known or the compound was still unidentified, a response factor of a similar compound was used. Values for different response factors can be found inTable 9.
$\text{Conc}_{(\text{internal standard})}$= Concentration of the internal standard solution used for the extraction (0.025 mg/ml).
MF= Multiplication factor: How much of the internal standard solution is used to extract 1 g (or 1 ml) of sample. In this study, the value was 0.5 ml

Formula 1: Quantification of phenolic compounds

For quantification, the following compounds were used and their response factors determined: chlorogenic acid at 320 nm for calculating the concentrations of hydroxycinnamic acids and at 280 nm for the quantification of other phenolics; neochlorogenic acid at 368 nm for neochlorogenic acid; vicenin 2 at 320 nm for flavones; rutin at 350 nm for flavonols; cyanidin 3-O-glucoside at 540 nm for anthocyanins; B2 at 640 nm for flavan-3-ols and proanthocyanins; catechin at 640 nm for catechin and epicatechin at 640 nm for epicatechin. For the measurements of samples from 2016, a new column was used, and response factors were measured and calculated again. Also, there were some changes in the standards used to calculate the response factors for the quantification. Vicenin 2 was replaced by flavone for the quantification of flavones and cyanidin 3-O-glucoside was replaced by cyanidin 3-O-rutinoside for the quantification of anthocyanins. Also, another wavelength was measured (430 nm) to

detect one unknown yellow compound present in some cultivars (ONT01). It was quantified using cyanidin 3-O-rutinoside at 430 nm. Response factor values can be found in Table 9.

4.5. Sugar analysis

4.5.1. Extraction of soluble sugar

After sample preparation as described in 4.2, the samples were centrifuged at 10,000 *g* and 4 °C for 10 min (Mikro 22R, Hettich Lab Technology, Germany). An aliquot of the clear supernatant was diluted 1:10000 and used for the HPLC analysis.

4.5.2. HPLC-ECD analysis

Ten µl of the diluted supernatant was injected into HPLC-ECD (High performance liquid chromatography-electrochemical detection). The HPLC system consisted of a pump (GP50 Gradient Pump, Dionex Corporation, USA), an automatic sample injector (model 231, Gilson Abimed Systems, Germany) and an electrochemical detector with a gold electrode (ED40, Dionex Corporation). The columns used for separation of the sugars were a CarboPac PA100 Guard Column (4 x 50 mm) and an analytical column (4 x 250 mm) both from ThermoFischer Scientific (USA). The mobile phase used to elute sugars was 100 mM NaOH at a flow rate of 1 ml/min. The integration and quantification were done with the software Chromeleon Version 6.80 from Dionex Corporation.

4.5.3. Quantification

For quantification, authentic standards (sorbitol, glucose, fructose and sucrose) were used as external standards. The formula to calculate the concentration was Formula 2.

$$\text{Concentration} = \text{Area of the peak} * \text{RF of the standard} * \text{DF}$$

DF = Dilution factor: 10,000
RF = Response Factor

Formula 2: Quantification of soluble sugars

4.6. Analysis of carotenoids

4.6.1. Extraction of carotenoids

Ten ml of the fruit pulp was mixed with 20 ml of extraction solution (hexane:ethanol:acetone; 50:25:25, v/v/v) including the internal standard (Sudan I) at a concentration of 0.0001 mg/ml in a 50 ml tube. After vortexing and ultrasonication at 4 °C in darkness, samples were centrifuged (Rotina 380R, Hettich Lab Technology,

Germany) at 5,000 rpm and 4 °C for 10 min. The upper layer (hexane extract) was transferred to a round-bottom flask and concentrated to dryness with a rotary evaporator (Rotavapor 110, Büchi, Germany). The water bath temperature set at 40 °C, pressure of the system: 335 mbar.

The round-bottom flask was washed three times with 500 µl hexane. The fractions were combined and evaporated in a vacuum centrifuge (Univapo, UniEquipe, Germany) and resuspended with 100 µl ethyl acetate.

Saponification is a method frequently used as a part of the extraction of carotenoids to purify the sample from chlorophylls and fatty acids, including release from esterified xanthophylls. This extraction step showed no positive effect for this study (Haager, 2015).

4.6.2. RP-HPLC

The HPLC system used for this study consisted of a Quaternary Pump P580 (Dionex, USA), UV/VIS Detector UVD 170S/340S (Dionex, USA), Autosampler Gina 50 (Dionex, USA). Carotenoids were detected at a wavelength of 450 nm. The software used was Chromeleon Version 6.80 (Thermo Scientific Dionex, USA). The chromatographic column was YMC Carotenoid C30, 250 x 4.6 mm I.D., S-5 µm (YMC Europe GmbH). Ten µl of the extract was injected. The flow rate was 1 ml/min. The following gradient was used with solvent A: methyl *tert.*-butyl ether (MTBE, Carl Roth) and solvent B: methanol (Backer): 100% B, 0-12 min; 85% B, 40 min; 80% B, 50-60 min; 65% B, 90-110 min; 20% B, 120-125 min. For samples from 2016 an isocratic elution method was used with 5% MTBE.

4.6.3. Quantification of carotenoids

Formula 3 was used for the quantification of each carotenoid. For the quantification of lutein, the response factor of lutein was used. For all other compounds the response factor of ß-carotene was used (Table 10). It is important to note, that colorless compounds were quantified using ß-carotene at 280 nm as reference substance. Since ß-carotene has a low absorbance coefficient at 280 nm, where the colorless compounds have the highest values, it is expected that the concentrations of these compounds have been overestimated in this study. Proper reference substances such as phytoene or phytofluene were not available at the time when these analyses were performed.

$$\text{Concentration} = \frac{\text{Area}_{(\text{Compound})} * \text{RF}_{(\text{Compound})}}{\text{Area}_{(\text{Internal Standard})} * \text{RF}_{(\text{Internal Standard})}} * \text{Conc}_{(\text{Internal Standard})} * \text{MF}$$

$\text{Area}_{(\text{internal standard})}$ = Area of the internal standard peak in the chromatogram
$\text{RF}_{(\text{internal standard})}$ = Response Factor of the internal standard
$\text{Area}_{(\text{compound})}$ = Area of the peak of the compound of interest in the chromatogram
$\text{RF}_{(\text{compound})}$ = Response Factor of the compound of interest. If the response factor was not known, a response factor of a similar compound was used (Table 10).

$\text{Conc}_{(\text{internal standard})}$ = Concentration of the internal standard solution used for the extraction (0.0002 mg/ml)
MF = Multiplication factor: How much of the internal standard solution is used to extract 1 g (or 1 ml) of sample: 3 ml

Formula 3: Quantification of carotenoids

4.7. Statistical Analysis

For the samples of harvest years 2012, 2013 and 2014, the data were expressed as mean and standard deviation for the three years. For the comparison of cultivars harvested in 2016, the mean and standard deviation were calculated for three biological repetitions. Each biological repetition was extracted and measured twice and the average of the two measurements was calculated.

To establish differences in the phenolic content between the cultivars a One-Way Analysis of Variance (one way-ANOVA) with Duncan Multiple Range test and a significance level α of $p < 0.05$ was applied using SPSS® Statistics 21.0.0.0 (IBM®). If only two samples were available to compare (comparing polyphenol concentrations of the same cultivar growing in different places), means were compared using a t-test for independent samples and calculated using SPSS® Statistics 21.0.0.0 (IBM®).

Normal distribution was assumed, because the distribution of compound concentrations within the same species/cultivar is often normally distributed (Granato et al., 2014). Column graphs were done using Excel® 16.16.1 (Microsoft®).

When comparing compound concentrations in fruits showing different skin colors, subspecies and harvest periods, normal distribution could not be assumed. Therefore, boxplots were used to compare compound content in different groups. These boxplots were elaborated with SPSS® Statistics 21.0.0.0 (IBM®).

Principal component analysis (PCA) was done using Past 3.14 (Hammer et al., 2001). For each PCA analysis a scatterplot or biplot (including vectors) with Principal component 1 (PC1) in the x-axis and Principal component 2 (PC2) in the y-axis was generated. Direction vectors for each compound used for the PCA are displayed in the biplot.

5. Results

5.1. Phenolic acids and flavonoids in plum fruits

5.1.1. Polyphenolic profile in *P. domestica* samples harvested in 2012-2014

The phenolic compounds found in the 19 cultivars selected for this study could be subdivided into the following groups according to their spectral properties: hydroxycinnamic acids, other phenolic compounds (benzoic acid derivates), anthocyanins, flavan-3-ols/proanthocyanidins, flavonols and flavones. Some compounds were identified by co-chromatography, comparing the UV/vis spectrum and retention time of the metabolite with those of an authentic reference. A list of structurally identified compounds is shown in Table 11 (Supplementary Material 3). Unknown metabolites were grouped according to their UV/vis spectra and retention time. Examples of each compound group with their spectrum can be found in Table 12 (Supplementary Material 3).

Hydroxycinnamic acids (HCAs) were one of the identified compound groups. In the juice samples from the harvest season 2012-2014, 24 HCAs were detected. Neochlorogenic acid was identified and eluted at 15 min. The other 23 compounds were denominated HCAs with a number indicating their order of appearance. At the end of the chromatogram, substances with an absorbance maximum at 320 nm and a spectrum similar to hydroxycinnamic acids were detected. These compounds were probably acylated hydroxycinnamic acids. The ones eluting between 80 to 110 min were grouped under the name HCA G01 and those with a retention time between 120 and 145 min as HCA G02.

Another group detected was anthocyanins, composed by five compounds. Four of them were identified by comparison of their UV/vis spectra, retention time and literature reports (Goldner et al., 2015; Treutter et al., 2012; Usenik et al., 2009) as cyanidin 3-O-rutinoside (84 min), cyanidin 3-O-glucoside (93 min), peonidin 3-O-rutinoside (102 min) and peonidin 3-O-glucoside (107 min). The fifth compound was not identified and named as Antho 04 (101 min). This compound occurred in small amounts if it was present.

Twenty-five compounds were found after post column derivatization with DMACA. Two monomers (flavan-3-ols) were identified by their spectra and retention time comparison with those of authentic reference materials and literature reports (Jaiswal et al., 2013; Treutter et al., 2012), namely catechin (29 min) and epicatechin

(61 min). The other compounds were classified as proanthocyanins based on previous findings (Jaiswal et al., 2013) and named as PA with a consecutive number. Some of these peaks seemed to be composed by more than one compound. Nevertheless, they were considered as one, because they could not be identified separately. Considering the results published by Jaiswal et al. (2013), catechin dimers and trimers elute from the column between 0 and 40 min, dimers of catechin, epicatechin or both between 20 and 50 min, and epicatechin dimers, epicatechin trimers and tetramers (with catechin and/or epicatechin) between 50 min and 90 min. Compounds eluting between 80 and 110 min are most probably A-type proanthocyanidins. Based on these findings, proanthocyanidin compounds where grouped in three groups. Proanthocyanidin like compounds showing retention times between 0 and 50 min (PA01-PA09) were grouped as PAG01, proanthocyanins between 10 and 90 min (PA10-PA22) were assigned to PAG02, and the two last compounds (PA23-PA24) were grouped as PAG03.

Compounds showing an absorption maximum between 250 and 299 nm and not reacting with DMACA during post column derivatization were classified as other phenolic compounds. Compounds in this group were probably benzoic acid derivatives like gallic acid and protocatechuic acid. Twenty-eight compounds belong to this category and have been designated as OPC with a sequential number. Many compounds in this category elute early, but they could not be identified individually. Therefore, all OPC compounds occurring before 8 min were grouped under the name OPC G01.

Regarding flavonols, 13 compounds were detected and named as FL with a consecutive number. The fourth flavonol (118 min) was tentatively identified as rutin (quercetin 3-O-rutinoside), based on retention time, spectrum and literature data (Jaiswal et al., 2013; Treutter et al., 2012). Compound FL13 had a different spectrum and a longer retention time than the other flavonols. However, it showed an absorption maximum at 350 nm; hence it was classified in this group and referred to as late flavonol.

Finally, four compounds were detected in the group of flavones. The first two, FN01 (53.43 min) and FN02 (58.88 min), had typical spectra and retention times for flavones. The last two compounds, which showed slightly different spectra and retention times compared to flavones, were classified as late flavones. Identification of these compounds was not done.

5.1.2. Polyphenolic profiles in *P. domestica* samples harvested in 2016

Since some changes were made to the HPLC equipment between the measurements of 2012-2014 and 2016 it was not possible to relate all compounds found in samples 2012-2014 with those of the 2016 harvest period. For that reason, the compounds have different denominations and numbers than those described before.

The same groups of compounds described for samples from 2012-2014 were found in samples of this harvest season: hydroxycinnamic acids (HC), other phenolic compounds (OP), anthocyanins (ANT), flavon-3-ols and proanthocyanidins (PAN), flavonols (FNL) and flavones (FVN). One extra group with only one compound was added. The spectrum of this compound had absorption maxima at 274 nm and 430 nm, which means that it is a yellow colored compound. Structural identification was not possible. Therefore, it was called ONT01, because it was first detected in the cultivar 'Ontariopflaume'.

In the case of hydroxycinnamic acids, 25 compounds were detected including neochlorogenic acid (16 min). As described for the 2012-2014 samples, many compounds with a spectrum similar to HCs eluted late (after 118 min). They were probably acylated hydroxycinnamic acids. In this case all those compounds were grouped in a single group called HCS.

A total of 28 other phenolic compounds were detected and named as OP with a sequential number.

Also, the same four anthocyanins as in 2012-2014 samples were detected in 2016: cyanidin 3-O-glucoside (84 min), cyanidin 3-O-rutinoside (93 min), peonidin 3-O-glucoside (102 min) and peonidin 3-O-rutinoside (107 min). A fifth compound which could not be identified was named ANT 05 (97 min) and could only be detected in 'Cacaks Schöne' and 'Tophit Plus'.

The flavan-3-ol and proanthocyanidin pattern was similar to the one described for samples 2012-2014, but during harvest season 2016, 29 compounds could be found instead of 25. The same monomers (catechin and epicatechin) were identified. All the other compounds were classified as proanthocyanidins based on literature (Jaiswal et al., 2013). As it was done for the previous sample set, they were classified in three groups: PAs G01 (retention time 0 to 45 min) including dimers (catechin-catechin, catechin-epicatechin and epicatechin-catechin) and catechin trimers, PAs G02 (retention time 45 to 95 min) including tetramers and epicatechin dimers and trimers and PAs G03, which was composed of A-type proanthocyanidins.

As with the 2012-2014 samples, rutin (115 min) was identified. Also, the compound named as late flavonol was found. Nevertheless, only nine flavonols were detected instead of 13 compounds found in the previous sample set. Finally, only two flavones were detected in the season 2016. The first compound was a flavone (52 min) named FVN 01. The second one is a late flavone (145 min) that was named FVN 02. It had a spectrum similar to those described in the previous sample set.

5.1.3. Concentrations of phenolic compounds in cultivars of *P. domestica* in 2012-2014

The mean concentration of phenolic compounds in fruit juices from the 19 cultivars analyzed during the harvest seasons 2012-2014 was 1501.91 µg/ml, ranging from 118.51 µg/ml in 'Cacaks Julia' to 3000.83 µg/ml in 'Topfive' (Figure 9a).

The highest concentrations were determined for hydroxycinnamic acids, which accounted for 52-86 % of the total phenols in the samples of each cultivar (Figure 9a). The main compound of this group was neochlorogenic acid representing 29-86% of the hydroxycinnamic acids and 18-69.9% of the total phenolic compounds (Figure 9a-b). This implies that the total content of phenolics in fruits was mainly determined by the content of hydroxycinnamic acids, in particular by the content of neochlorogenic acid. The fruit samples of 'Cacaks Julia' had the lowest concentration of neochlorogenic acid (61.90 µg/ml) and total hydroxycinnamic acids (87.78 µg/ml) followed by samples of 'Jubileum', 'Cacaks Fruchtbare' and 'Ortenauer' with a content of neochlorogenic acid between 82.58 and 120.35 µg/ml and a content of hydroxycinnamic acids between 256.74 and 289.72 µg/ml. The highest concentrations of neochlorogenic acid and hydroxycinnamic acids were found in fruits of 'Topfive', 'Tophit Plus', 'Topend Plus' and 'Angelina Burdette' with a concentration range of 1621.69-1950.48 µg/ml and 2194.28-2385.89 µg/ml, respectively.

The second most abundant phenolic compound group found in the fruit pulps were the other phenolic compounds (Figure 9a, c). As it was mentioned before, this group includes benzoic acid derivatives and other compounds with absorption maxima between 250 and 299 nm, excluding flavan-3-ols and proanthocyanidins. These other phenolic compounds represent 6.11 to 44.18% of the total phenolic compounds. The lowest concentration was found in 'Cacaks Julia' (19.46 µg/ml) and the highest in 'Topfive' (356.36 µg/ml). It is important to consider that the concentration of other phenolic compounds in fruits of 'Cacaks Julia' was 8-fold lower than in the next low concentrated cultivar 'Goldzwetsche' and almost 19-fold lower than the highest concentrated cultivar. The mean value for all cultivars was 198.83 µg/ml. The ANOVA

results confirmed that ‘Cacaks Julia’ was the only cultivar that showed a significant statistical difference to the other.

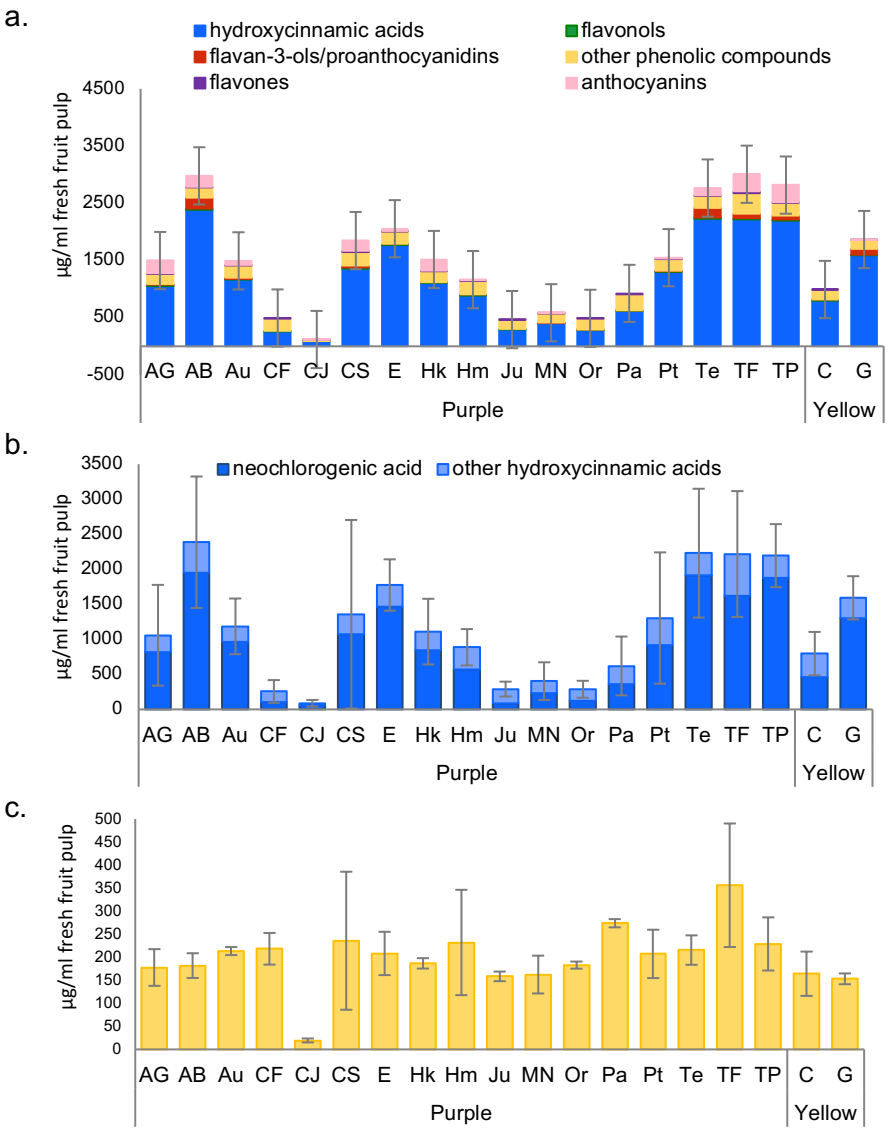


Figure 9: Mean concentrations and composition of total phenolic compounds (a), hydroxycinnamic acid (b) and other phenolic compounds (c) in fruits harvested in 2012-2014.

The error bars represent the standard deviations of total compound group concentration. Analysis of statistical significance is displayed in Table 19. **Cultivars** in the first row of the x-axis, AG: ‘AGRI 2000 10/92’, AB: ‘Angelina Burdette’, Au: ‘Auerbacher’, CF: ‘Cacaks Fruchtbare’, CJ: ‘Cacaks Julia, CS: ‘Cacaks Schöne’, E: ‘Elena’, Hk: ‘Hanka’, Hm: ‘Haroma’, Ju: ‘Jubileum’, MN: ‘Maria Novella’, Or: ‘Ortenauer’, Pa: ‘Presenta’, Pt: ‘President’, Te: ‘Topend Plus’, TF: ‘Topfive’, TP: ‘Tophit plus’, C: ‘Colora’ and G: ‘Goldzwetschge’. **Fruit skin color** is shown in the lower row of the x-axis. Further information about the cultivars are displayed in Table 7.

Of all cultivars analyzed during 2012-2014, 14 showed detectable amounts of anthocyanins: 13 purple skinned and one yellow skinned fruit cultivar. The mean concentration was 86.57 µg/ml, ranging from 0 µg/ml in ‘Cacaks Fruchtbare’, ‘Jubileum’, ‘Ortenauer’, ‘Presenta’ and ‘Colora’ to 299.10 µg/ml in ‘Topend Plus’ and 298.10 µg/ml in ‘Topfive’ (Figure 10a). The most abundant anthocyanin in terms of quantity was cyanidin 3-O-rutinoside with values between 1.26 µg/ml in ‘Goldzwetsche’ and

207.56 µg/ml in ‘Topend Plus’. In contrast, Antho 04 showed values lower than 1 µg/ml. Of the anthocyanins identified, the lowest concentration was found for peonidin 3-O-glucoside. In the cultivars ‘Angelina Burdette’, ‘Topend Plus’, ‘Tophit Plus’, ‘Topfive’, ‘AGRI 2000 10/91’, ‘Cacaks Schöne’ and ‘Cacaks Julia’, cyanidin 3-O-glucoside showed the second highest concentration. On the other hand, peonidin 3-O-rutinoside was the second most abundant anthocyanin in ‘Auerbacher’, ‘Hanka’, ‘Elena’ and ‘Haroma’.

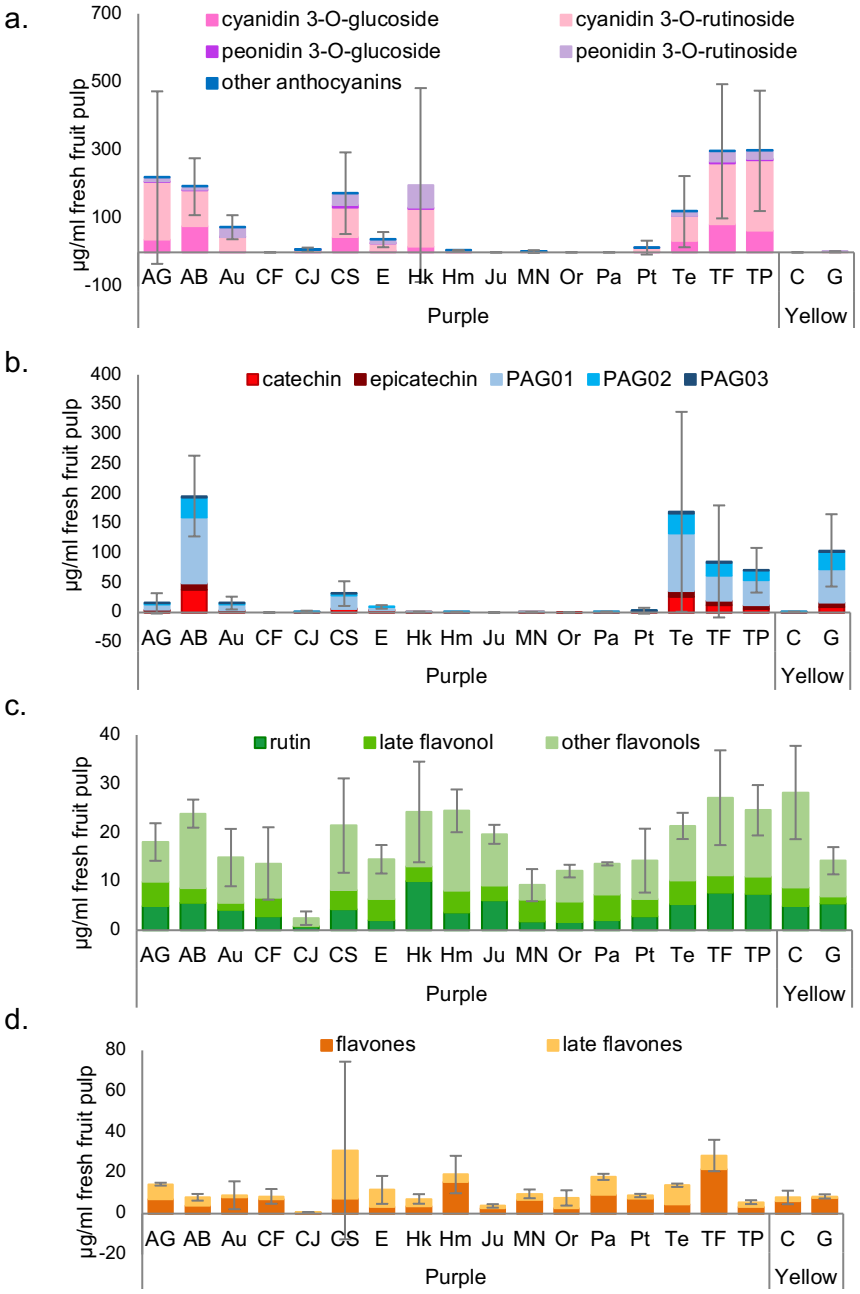


Figure 10: Mean concentrations and composition of anthocyanins (a), flavan-3-ols/proanthocyanidins (b), flavonols (c) and flavones (d) in fruits from harvest 2012-2014.

The error bars represent the standard deviations of total compound group concentration. Analysis of statistical significance is displayed in Table 19. **Cultivars** in the first row of the x-axis, AG: ‘AGRI 2000 10/92’, AB: ‘Angelina Burdette’, Au: ‘Auerbacher’, CF: ‘Cacaks Fruchtbare’, CJ: ‘Cacaks Julia, CS: ‘Cacaks Schöne’, E: ‘Elena’, Hk: ‘Hanka’, Hm: ‘Haroma’, Ju: ‘Jubileum’, MN: ‘Maria Novella’, Or: ‘Ortenauer’, Pa: ‘Presenta’, Pt: ‘President’, Te: ‘Topend Plus’, TF: ‘Topfive’, TP: ‘Tophit plus’, C: ‘Colora’ and G: ‘Goldzwetschge’. **Fruit skin color** is shown in the lower row of the x-axis. Further information about the cultivars are displayed in Table 7.

The analysis of flavan-3-ol and proanthocyanidin (PAs) content revealed some differences between varieties (Figure 10b). Two of them, ‘Jubileum’ and ‘Cacaks Fruchtbare’, showed no detectable amounts of these compounds. The other 17 cultivars contained flavan-3-ol and proanthocyanidins in a range from 0.03 µg/ml in ‘Ortenauer’ and 190.66 µg/ml in ‘Angelina Burdette’. The mean concentration of all cultivars was 36.90 µg/ml. The cultivars producing the highest amounts of these compounds were ‘Angelina Burdette’, ‘Topend Plus’ and ‘Goldzwetsche’. In the 17 cultivars containing these compounds, catechin was more abundant than epicatechin. Catechin was found in concentrations between 0.03 and 38.03 µg/ml. The concentrations of epicatechin ranged between 0.02 and 10.62 µg/ml. For some cultivars, the most abundant compound was PA02 with a concentration of up to 64.47 µg/ml. As described in section 5.1.1, the corresponding peak is apparently composed by more than one compound.

The concentrations of flavonols ranged from 1.75 µg/ml in ‘Cacaks Julia’ to 23.42 µg/ml in ‘Colora’. The highest content was determined for rutin with concentrations that varied from 0.71 µg/ml in ‘Cacaks Julia’ to 10.04 µg/ml in ‘Hanka’ (Figure 10c).

The compounds with the lowest contents were flavones with values between 0.59 µg/ml in ‘Cacaks Julia’ and 30.94 µg/ml in ‘Cacaks Schöne’. A similar concentration was detected in ‘Topfive’ (28.48 µg/ml). Interestingly, the concentrations of the late flavone in ‘Cacaks Schöne’ was higher than the total value of the early flavones. The opposite was found for ’Topfive’ (Figure 10d).

The analysis of the statistical significance of the values shown in Figure 9 and Figure 10 is presented in Table 19 (Supplementary Material 5).

It should be noted that the standard deviation of the concentrations was large for some cultivars (Figure 9a). This implies a high variation in the phenolic content during different harvest seasons. Therefore, there are few statistical differences between the cultivars. Figure 11 shows the respective results for the years 2012, 2013 and 2014. Clear differences in the concentrations of metabolites were observed between years. For some cultivars, samples from 2012 and 2013 tend to contain lower concentrations of metabolites. For others, such as ‘Cacaks Schöne’, the concentration of total phenolic compounds was higher in the samples harvested in 2012. Nine cultivars had the highest phenolic concentration in 2013. When looking at the concentration of the different groups of phenolic compounds, no clear trend over the years was observed. For the cultivar ‘Topfive’ all groups were higher concentrated in 2014. This was also observed in ‘Topend Plus’ with the exception of flavonols, which were higher concentrated in

2012. In the cultivar ‘Hanka’ total phenolics and hydroxycinnamic acids were higher in 2013, other phenolic compounds were higher in 2012 and the other phenolic groups were higher in 2014.

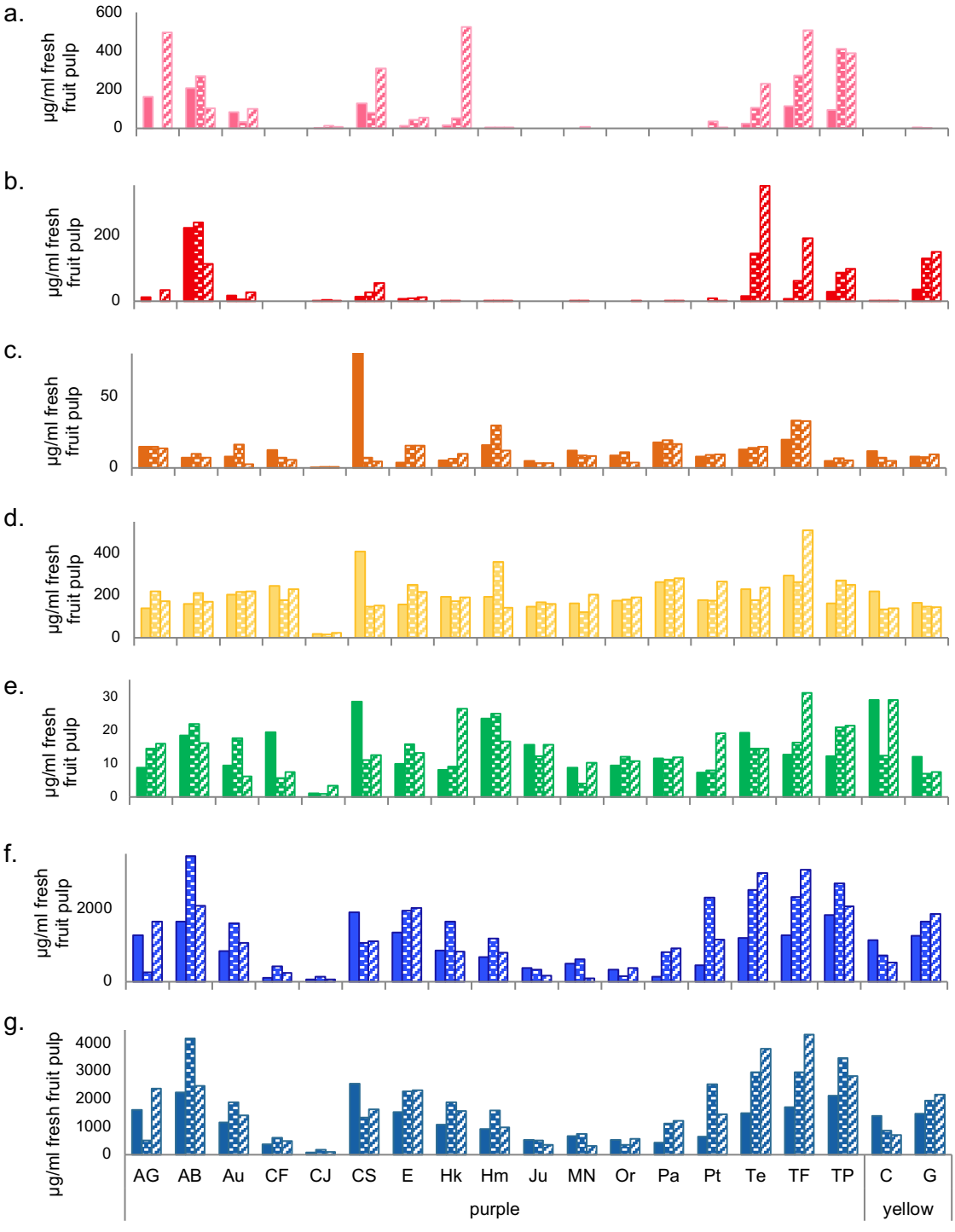


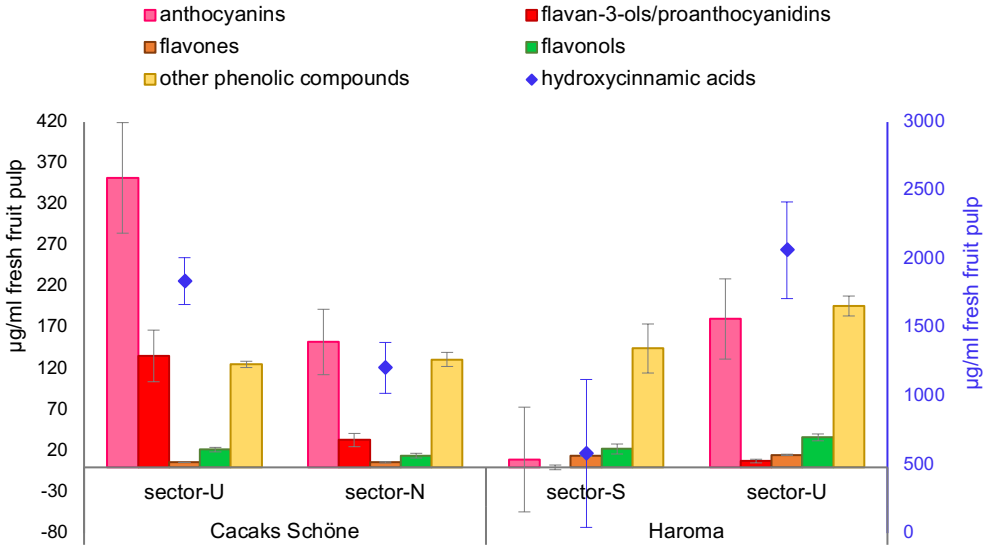
Figure 11: Phenolic compounds in samples from different harvest years.

Harvest years: 2012 (■), 2013 (▩) and 2014 (▨). Phenolic compounds: a. anthocyanins, b. flavan-3-ols, c. flavones, d. other phenolic compounds, e. flavonols, f. hydroxycinnamic acids and g. total phenolic compounds. **Cultivars** in the first row of the x-axis, AG: ‘AGRI 2000 10/92’, AB: ‘Angelina Burdette’, Au: ‘Auerbacher’, CF: ‘Cacaks Fruchtbare’, CJ: ‘Cacaks Julia, CS: ‘Cacaks Schöne’, E: ‘Elena’, Hk: ‘Hanka’, Hm: ‘Haroma’, Ju: ‘Jubileum’, MN: ‘Maria Novella’, Or: ‘Ortenauer’, Pa: ‘Presenta’, Pt: ‘President’, Te: ‘Topend Plus’, TF: ‘Topfive’, TP: ‘Tophit plus’, C: ‘Colora’ and G: ‘Goldzwetschge’. **Fruit skin color** is shown in the lower row of the x-axis. Further information about the cultivars are displayed in Table 7.

5.1.4. Concentration of phenolic compounds in fruits of the same cultivar of trees grown in different places within the same region in 2014

Fruits were harvested from trees growing in both experimental orchards (Figure **7**). In the experimental orchard near Liesel-Beckmann-Straße 1, sector-N was located uphill, while sector-S was downhill. On the other hand, sector-U was located about 3 km apart, in the experimental orchard next to Dürnast 2.

a.



b.

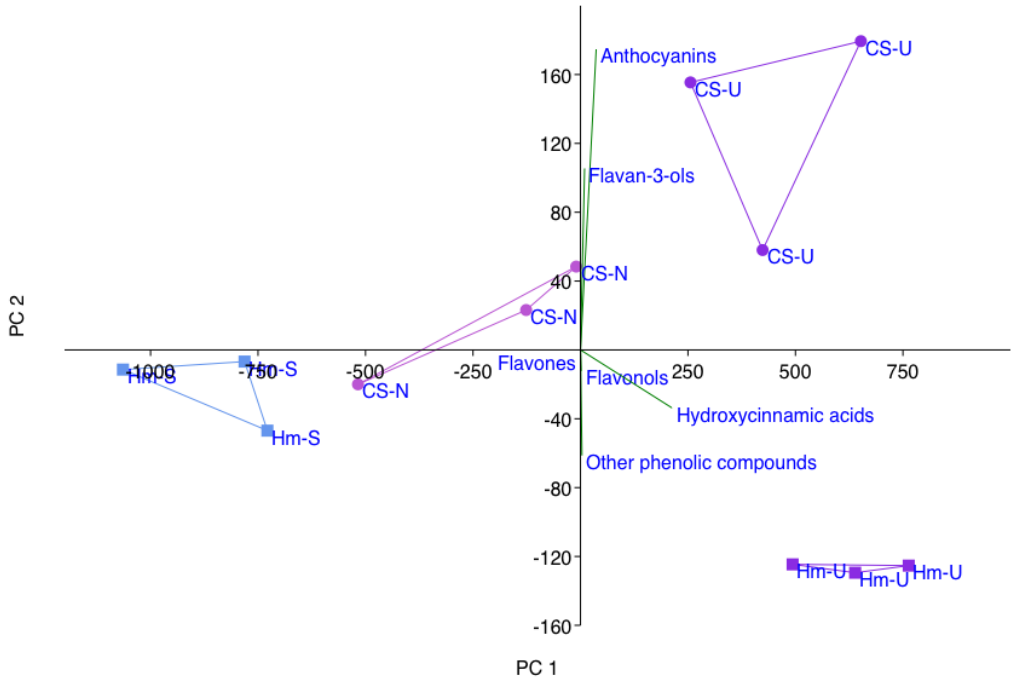


Figure 12: Concentrations of phenolic compounds in fruits of ‘Cacaks Schöne’ and ‘Haroma’ harvested in 2014 from trees growing in different locations of the experimental orchards.

a. Mean concentration of different phenolic compound groups. Values of hydroxycinnamic acid concentration are plotted with a different scale (right axis) than the other compounds (left axis). Analysis of statistical significance is displayed in Table 15. b. Principal component analysis. The error bars represent the standard deviations. Green lines represent the vectors conforming the biplot. Convex hulls group samples of the same location and cultivar repetitions. Cultivars: Hm: ‘Haroma’ and CS: ‘Cacaks Schöne’. Harvest locations: U: sector-U; N: sector-N and S: sector-S.

Samples from ‘Cacaks Schöne’ trees growing in sector-N contained significantly less total phenolic compounds, hydroxycinnamic acids, anthocyanins and flavan-3-ols/proanthocyanidins than those growing in sector-U (Figure 12a). This result was confirmed by t-test analysis (Table 15, Supplementary Material 5). A similar tendency was observed when comparing flavonols, but this was not statistically significant. Other phenolic compounds and flavones showed similar concentrations in fruits from both places. For fruits of the cultivar ‘Haroma’ (collected from sector-S and -U), samples from sector-U had a higher total polyphenol content than those from sector-S with a statistical significance confirmed by t-test. This was also true for hydroxycinnamic acids, anthocyanins and flavan-3-ol, flavones and flavonols. For both cultivars, trees growing in sector-U contained higher concentrations of total phenolics, hydroxycinnamic acids, anthocyanins and flavan-3-ols/PAs than those grown in sector-N or sector-S.

A principal component analysis PCA was performed for all biological repetitions of each cultivar and each location (Figure 12b). PC1 explains 96.86% of the variance with hydroxycinnamic acids having the highest contribution. PC2 explains only 2.46% of the variance. Anthocyanins and flavan-3-ols contributed most to this component. In the biplot each cultivar/location combination was grouped and separated from the others. It could be concluded that ‘Haroma’ samples tend to contain less phenolics than the ‘Cacaks Schöne’ samples independent of the location. Also, the spread of the repetitions over the axes was different for each combination. ‘Haroma’ samples appeared tighter grouped than ‘Cacaks Schöne’ for both locations.

5.1.5. Concentrations of phenolic in cultivars of *P. domestica* harvested in 2016

The concentration patterns detected in the 2012-2014 samples was similar to the patterns observed in the 2016 samples. The most abundant metabolites in the 2016 samples were hydroxycinnamic acids with concentrations between 24.19 µg/g in ‘Oullins Reneklode’ and 621.07 µg/g in ‘President’ (Figure 13). The total content of phenolic compounds was mostly determined by the concentration of hydroxycinnamic acids. Neochlorogenic acid was the major metabolite of this group and of all compounds that were detected. The mean concentration of neochlorogenic acid of all cultivars was 125.37 µg/g and ranged from 8.54 µg/g in ‘Tipala’ to 408.52 µg/g in ‘President’. This compound represented 15-79% of the total hydroxycinnamic acids concentration.

The second most abundant group of compounds in terms of quantity was other phenolic compounds (Figure 13). The mean concentration of all cultivars was 56.47 µg/g and ranged from 12.99 µg/g in ‘Presenta’ to 125.50 µg/g in ‘Große Grüne Reneklode’.

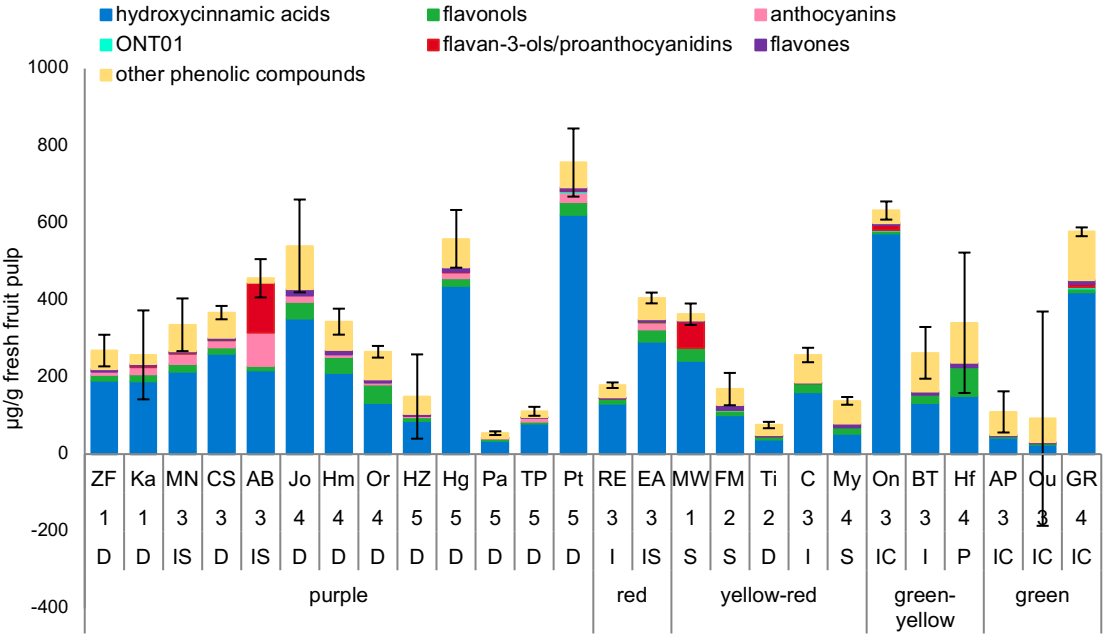


Figure 13: Mean concentrations of total phenolic compounds and composition in fruits harvested in 2016.

The error bars represent the standard deviations of the total phenolic concentration. Analysis of statistical significance is displayed in Table 20. Cultivars in the first row of the x-axis, ZF: 'Zwintschers Frühe', Ka:' Katinka', MN: 'Maria Novella', CS: 'Cacaks Schöne' AB: 'Angelina Burdette', Jo: 'Jojo', Hm: 'Haroma', Or: 'Ortenauer', HZ: 'Hauszwetschge Schüfer', Hg: 'Haganta', Pa: 'Presenta', TP: 'Tophit Plus', Pt: 'President', RE: 'Rote Eierpflaume Blazek', EA: 'Eibenbacher Aprikosenpflaume', MW: 'Mirabelle Wallenberg', FM: 'Frühe Mirabelle P-2778', Ti: 'Tipala', C: 'Colora', My: 'Mirabelle aus Nancy 1510', On: 'Ontariopflaume', BT: 'Belle de Thuin', Hf: 'Haferpflaume', AP: 'Aprikoosprium', Ou: 'Oullins Reneklode' and GR: 'Grosse Grüne Reneklode'. **Harvest period** in the second row: (1) 15-31 July; (2) 1-15 August; (3) 16-30 August; (4) 1-15 September and (5) 16-31 September. **Subspecies** in the third row of the x-axis, D: *domestica*, IS: *italica var. subrotunda*, S: *syriaca*, I: *intermedia var. ovoidea*, IC *italica var. claudiana*, P: *prisca*. **Fruit skin color** is shown in the lower row of the x-axis. Further information about the cultivars are displayed in Table 7.

Figure 14: Fruits with coloration in flesh and skin

Anthocyanic coloration in yellow skinned fruits from 'Mirabelle Wallenberg' (a), 'Colora' (b) and 'Tipala' (c) and in fruit flesh from 'Angelina Burdette' (d).

Anthocyanins were detected in 15 of the 26 cultivars, mainly in those with purple/red skinned fruits. The mean concentration of this compound group of all cultivars was 9.77 µg/g (range: 0-86.76 µg/g). In one red skinned cultivar, 'Rote Eierpflaume Blazek', no anthocyanins were detected. Of the yellow and green skinned cultivars only 'Mirabelle Wallenberg' (Figure 14a) contained detectable amounts of anthocyanin with 0.17 µg/g of cyanidin 3-O-glucoside (Figure 13). Other yellow skinned cultivars showed red coloration in parts of the skin, but no anthocyanins were detected, for example in 'Tipala' (Figure 14c) and 'Colora' (Figure 14b). The cultivar 'Angelina Burdette', had the

highest concentration of anthocyanins. This difference was statistically significant (Table **20**, Supplementary Material). Cyanidin 3-O-glucoside and cyanidin 3-O-rutinoside showed the highest concentrations. In 'Angelina Burdette' the concentration of peonidins was relatively low in comparison to the high content of cyanidins. During sample preparation, it could be observed that anthocyanins were also present in the fruit flesh (Figure 14a). The most abundant anthocyanin was cyanidin 3-O-rutinoside (up to 45.14 µg/g in 'Angelina Burdette'). 'Maria Novella' produced only 1/3 of the cyanidin 3-O-rutinoside concentration detected in 'Angelina Burdette'. Peonidin 3-O-glucoside showed the lowest concentrations (0-2.25 µg/g). In some cultivars, the second most abundant anthocyanin was peonidin 3-O-rutinoside and for others cyanidin 3-O-glucoside.

The concentration of flavan-3-ols/proanthocyanidins ranged from 0 to 130.54 µg/g (Figure 13). Catechin (0-26.02 µg/g) was always more abundant than epicatechin (0-7.01 µg/g). In addition, high levels of CAT 02 (0-24.15 µg/g) and CAT 03 (0-26.93 µg/g) were determined. As explained previously, peaks CAT 02 and CAT 03 probably consisted of more than one substance. The cultivar with the highest concentration of flavan-3-ol/PAs was 'Angelina Burdette'.

Flavonols showed a concentration between 3.01 µg/g in 'Aprikoosprium' and 74.26 µg/g in 'Haferpflaume' (Figure 13). The most abundant compound was rutin with a maximal value of 35.88 µg/g in 'Jojo'. Flavones were present in small amounts and showed values from 0 µg/g in 'Angelina Burdette' to 16.27 µg/g in 'Jojo' (Figure 13). The unidentified yellow compound named ONT 01 was detected in ten cultivars: 'Maria Novella', 'Cacaks Schöne', 'Presenta', 'Tophit Plus', 'President', 'Eibenbacher Aprikosenpflaume', 'Mirabelle Wallenberg', 'Frühe Mirabelle P-2778', 'Ontariopflaume' and 'Große Grüne Reneklode' and its concentration ranged from 0 to 2.9 µg/g. The cultivar with the highest concentration of ONT01 was 'President'.

To evaluate the influence of each phenolic compound group on cultivar differentiation, a PCA was performed (Figure 15, Figure 17 and Figure 19). PC1 explained 91.23% of the variance. Hydroxycinnamic acids had the highest loading contribution within PC1 and therefore they were the most dominant factor. PC2 explained 5.4% of the variance. The most dominant vectors in PC2 were other phenolic compounds in one direction and flavan-3-ols/proanthocyanidins and anthocyanins in the opposite direction. The vector of flavan-3-ols/proanthocyanidins was longer than the one of anthocyanins, implying more discriminating power. Also boxplot analyses were performed for all compound groups with the cultivars grouped by skin color, subspecies and harvest period (Figure 16, Figure 18 and Figure 20).

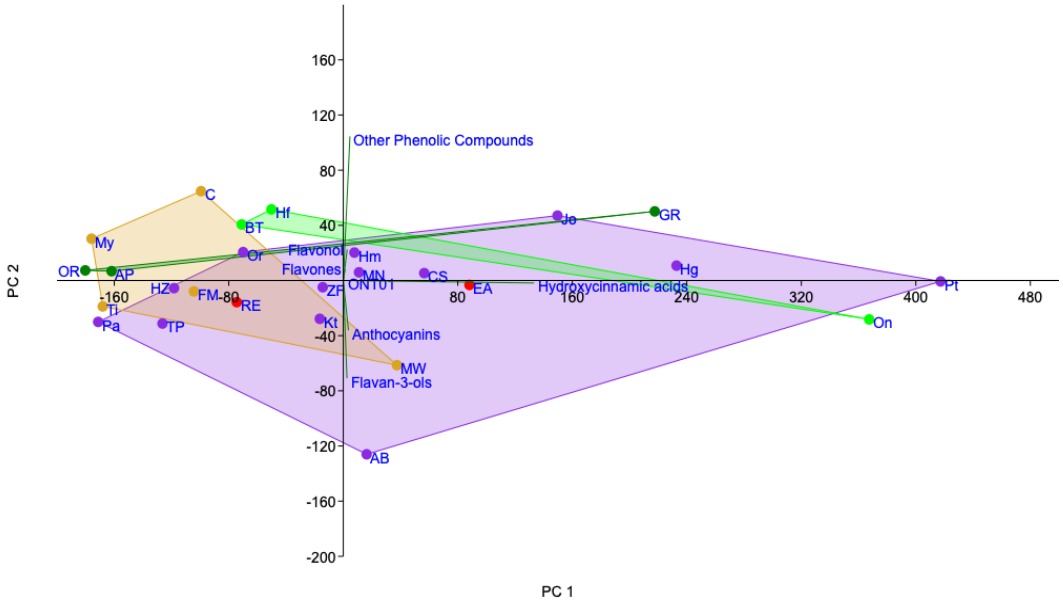


Figure 15: PCA of cultivars and concentration of phenolic compound groups grouped by their fruit skin colors.

The first two principal components are displayed on the biplot. Color of the markers and convex hulls represent the **fruit skin color**: purple, red, yellow-red, green-yellow and green. Green lines represent the vectors conforming the biplot. **Cultivar** abbreviations, ZF: 'Zwintschers Frühe', Ka:' Katinka', MN: 'Maria Novella', CS: 'Cacaks Schöne' AB: 'Angelina Burdette', Jo: 'Jojo', Hm: 'Haroma', Or: 'Ortenauer', HZ: 'Hauszwetschge Schüfer', Hg: 'Haganta', Pa: 'Presenta', TP: 'Tophit Plus', Pt: 'President', RE: 'Rote Eierpflaume Blazek', EA: 'Eibenbacher Aprikosenpflaume', MW: 'Mirabelle Wallenberg', FM: 'Frühe Mirabelle P-2778', Ti: 'Tipala', C: 'Colora', My: 'Mirabelle aus Nancy 1510', On: 'Ontariopflaume', BT: 'Belle de Thuin', Hf: 'Haferpflaume', AP: 'Aprikoosprium', Ou: 'Oullins Reneklode' and GR: 'Grosse Grüne Reneklode'. Further information of the cultivars can be found in Table 7.

When grouping all cultivars regarding their skin color (Figure 15 and Figure 16), some differences between groups were detected. The most variable group was the one of purple skinned fruits with the largest convex hull tending to the right part of the biplot, where the concentration of hydroxycinnamic acids was higher (Figure 15). This hull intercepts all other group hulls at least at one point. Considering the boxplots (Figure **16**b-e) of the most important vectors of the PCA (hydroxycinnamic acids, other phenolic compounds, anthocyanins and flavan-3-ols/proanthocyanidins), purple skinned cultivars showed the greatest variation in the concentration of these metabolites (considering the outliers). Within this skin color category, the cultivar 'President' was an upper outlier for hydroxycinnamic acid concentration and 'Angelina Burdette' was an extreme upper outlier for anthocyanin and flavan-3-ol/proanthocyanidin concentration. This can also be observed in the PCA where these two cultivars are separated from the others in the direction of the respective vector. For the compound groups with less contribution in the PCA, flavonol, flavone and the unidentified yellow compound ONT01 the respective boxplots (Figure 16f-h) showed also a great variation within the purple skinned cultivars. In the case of ONT01, 'Maria Novella' was an upper outlier and 'Presenta' was an extreme upper outlier. Red skinned cultivars clustered around the

PC1 axis and as described previously for the boxplot analysis, they contained less of other phenolic compounds and higher concentrations of anthocyanins than cultivars with green-yellow and green skinned fruits (Figure 16c-d). Regarding yellow skinned fruit cultivars, it was found that they group on the left part of the plot with lower concentrations of hydroxycinnamic acids (Figure 15). This was confirmed in the boxplot showing the hydroxycinnamic acid concentrations (Figure 16b). The only exception was 'Mirabelle Wallenberg', which was an upper outlier regarding hydroxycinnamic acids. This cultivar was also an extreme upper outlier for anthocyanin and flavan-3-ol/proanthocyanidin concentration. In the case of flavones, 'Frühe Mirabelle P-2778' was an upper outlier within this fruit skin color category (Figure 16g). Green and green-yellow skinned fruit cultivars showed very thin and long hulls in PCA, spreading over the plot (Figure 15). This was a consequence of one cultivar in each group that was placed away from the other samples of the group: 'Große Grüne Reneklode' for green skinned fruits and 'Ontariopflaume' for green-yellow skinned fruits. Nevertheless, they were not identified as outliers during the boxplot analysis. Analyzing the concentration of flavonols (Figure 16f), green skinned fruits were the only ones that differed from the other categories showing lowest concentrations.

Looking at the PCA in Figure 17 where samples are grouped by the subspecies, it was observed that *subsp. domestica* (*D*) and *subsp. italica var. claudiana* (*IC*) showed the broadest distribution in the plot. They showed a similar distribution. The largest differences between them were observed on the PC1 axis, which is mainly influenced by the concentration of hydroxycinnamic acids. Samples from *subsp. intermedia var. ovoidea* (*I*), cluster in a small hull on the left of the PCA (Figure 17) with low concentration of hydroxycinnamic acids. These differences were confirmed by the boxplot analysis (Figure 18a-b), because the concentration ranges of hydroxycinnamic acids of *D* and *IC* are large and the one of *I* was small. In the case of samples of *subsp. italica var. subrotunda* (*IS*), they showed a large range of concentrations of anthocyanins and flavan-3-ols/proanthocyanidins (Figure 18d-e), which is mainly caused by the high concentrations in 'Angelina Burdette' (Figure 17). For other phenolic compounds, flavonols, flavones and ONT01 no large differences between subspecies were observed (Figure 18c, f-h). The cultivar 'President' was an extreme upper outlier for ONT01 concentration. Not much information of the *subsp. prisca* (*P*) could be obtained by these analyses, because there was only one sample for this category.

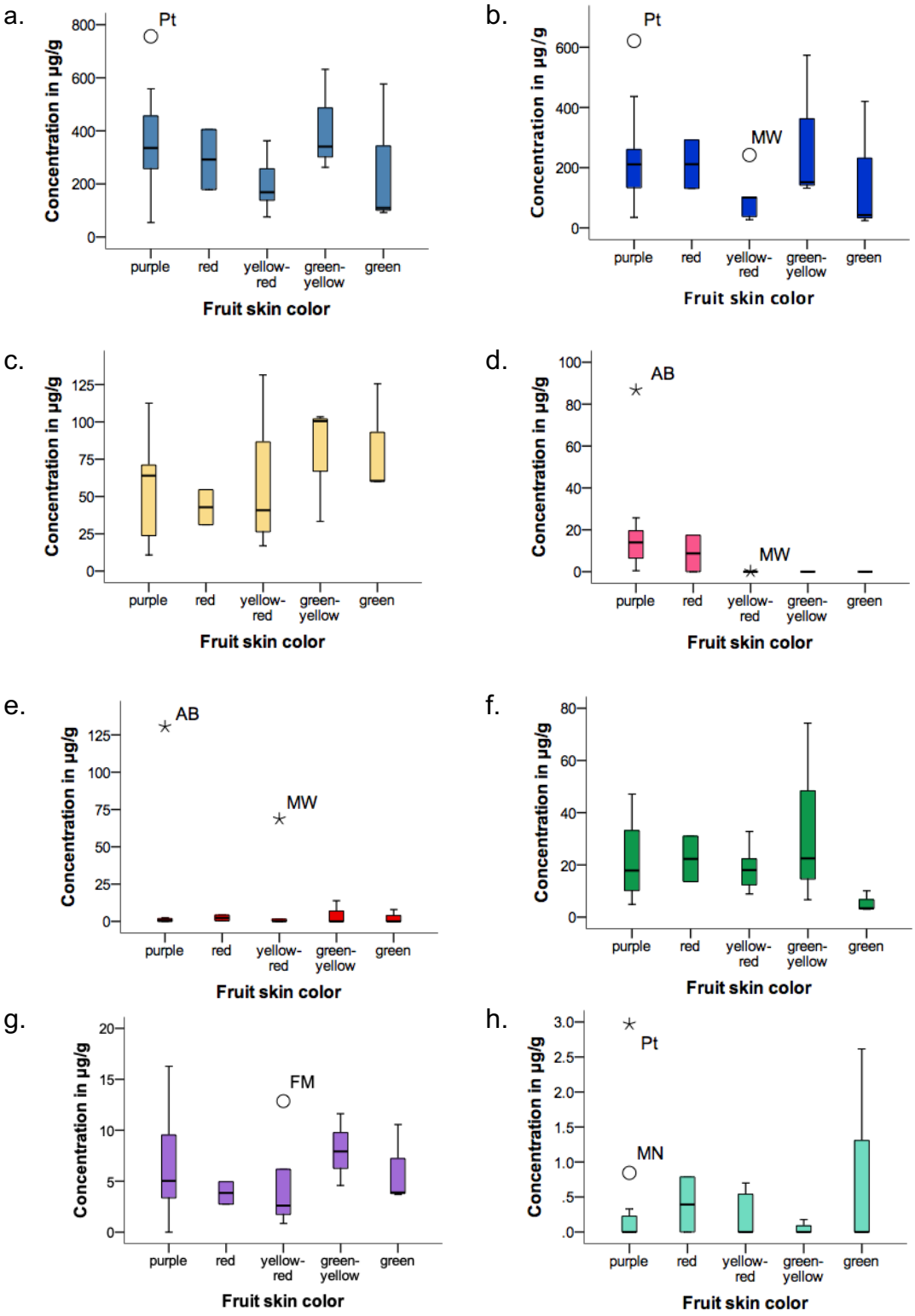


Figure 16: Boxplots of the concentrations of total phenolics and phenolic compound groups in fruits of *P. domestica* cultivars grouped by color of the fruit skin.
a. Total phenolic compounds, b. hydroxycinnamic acids, c. other phenolic compounds, d. anthocyanins, e. flavan-3-ols/proanthocyanidins, f. flavonols, g. flavones and h. unidentified yellow compound ONT01. In the boxplots, empty circle (○) shows an outlier and a star (∗) shows extreme outliers**. Outlier cultivar names**, Pt: 'President', AB: 'Angelina Burdette', MW: 'Mirabelle Wallenberg' and MN: 'Maria Novella'. Further information about these cultivars can be found in Table 7.

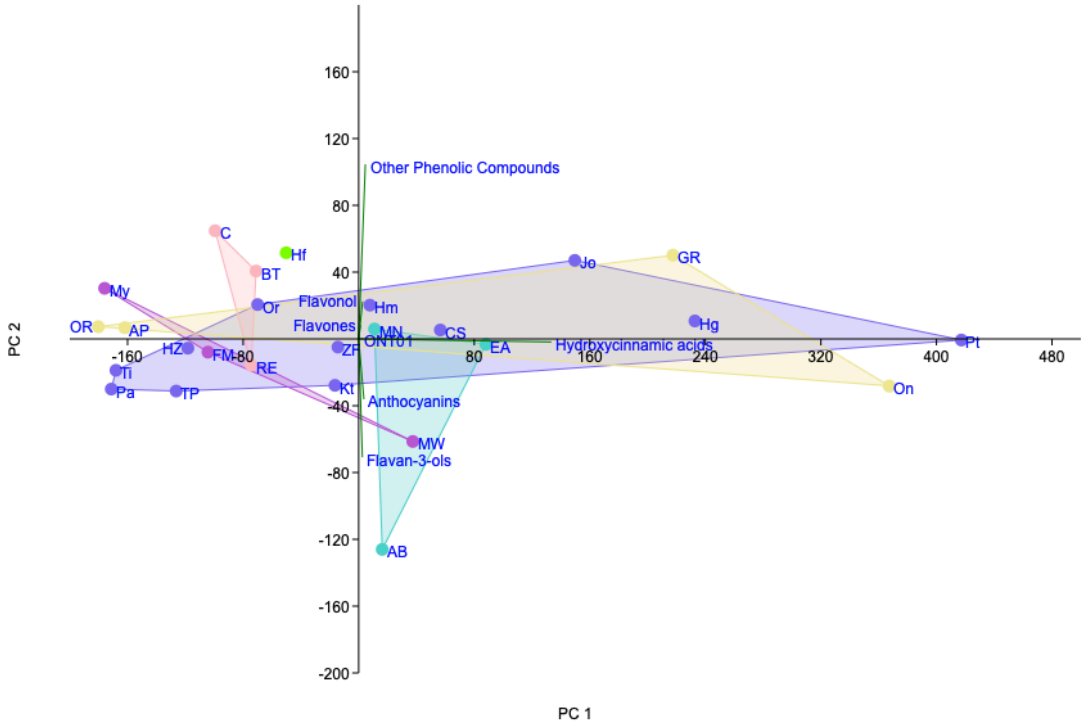


Figure 17: PCA of the concentrations of phenolic compound groups grouped by subspecies.

The first two principal components are displayed in the biplot. Color of the markers and convex hulls represent a **subspecies**: : subsp. *domestica,* : subsp. *italica var. subrotunda*, : subsp. *italica var. claudiana*, : subsp. *syriaca*,: subsp. *intermedia var. ovoidea*,: subsp. *prisca*. Green lines represent the vectors conforming the biplot. **Cultivar** abbreviations: ZF: 'Zwintschers Frühe', Ka:' Katinka', MN: 'Maria Novella', CS: 'Cacaks Schöne' AB: 'Angelina Burdette', Jo: 'Jojo', Hm: 'Haroma', Or: 'Ortenauer', HZ: 'Hauszwetschge Schüfer', Hg: 'Haganta', Pa: 'Presenta', TP: 'Tophit Plus', Pt: 'President', RE: 'Rote Eierpflaume Blazek', EA: 'Eibenbacher Aprikosenpflaume', MW: 'Mirabelle Wallenberg', FM: 'Frühe Mirabelle P-2778', Ti: 'Tipala', C: 'Colora', My: 'Mirabelle aus Nancy 1510', On: 'Ontariopflaume', BT: 'Belle de Thuin', Hf: 'Haferpflaume', AP: 'Aprikoosprium', Ou: 'Oullins Reneklode' and GR: 'Grosse Grüne Reneklode'. Further information of the cultivars can be found in Table 7.

In Figure 19 the same PCA plot shown in Figure 15 was displayed again but with the samples grouped by their harvest period, represented by the different colors of the markers and the convex hull lines. The largest convex hull is the one containing the samples of harvest period 3. This group is the most heterogenous of all. Looking at the corresponding boxplots (Figure 20b-h) of the harvest period 3, 'Ontariopflaume' was an upper outlier for hydroxycinnamic acids and flavan-3-ols/proanthocyanidins and it was located on the right of the PCA (Figure 19). The cultivar 'Angelina Burdette' was an extreme upper outlier for anthocyanin and flavan-3-ols/proanthocyanidins and 'Colora' was an upper outlier for other phenolic compounds (Figure 20c-e). This was also reflected in the PCA with these two cultivars placed separated from the others in the direction of the respective vector (Figure 19). Other outliers within this harvest categories were 'Belle de Thuin' as an upper outlier for flavone concentration, 'Maria Novela' and 'Eibenbacher Aprikosenpflaume' as upper outliers for ONT01 (Figure 20g-h).

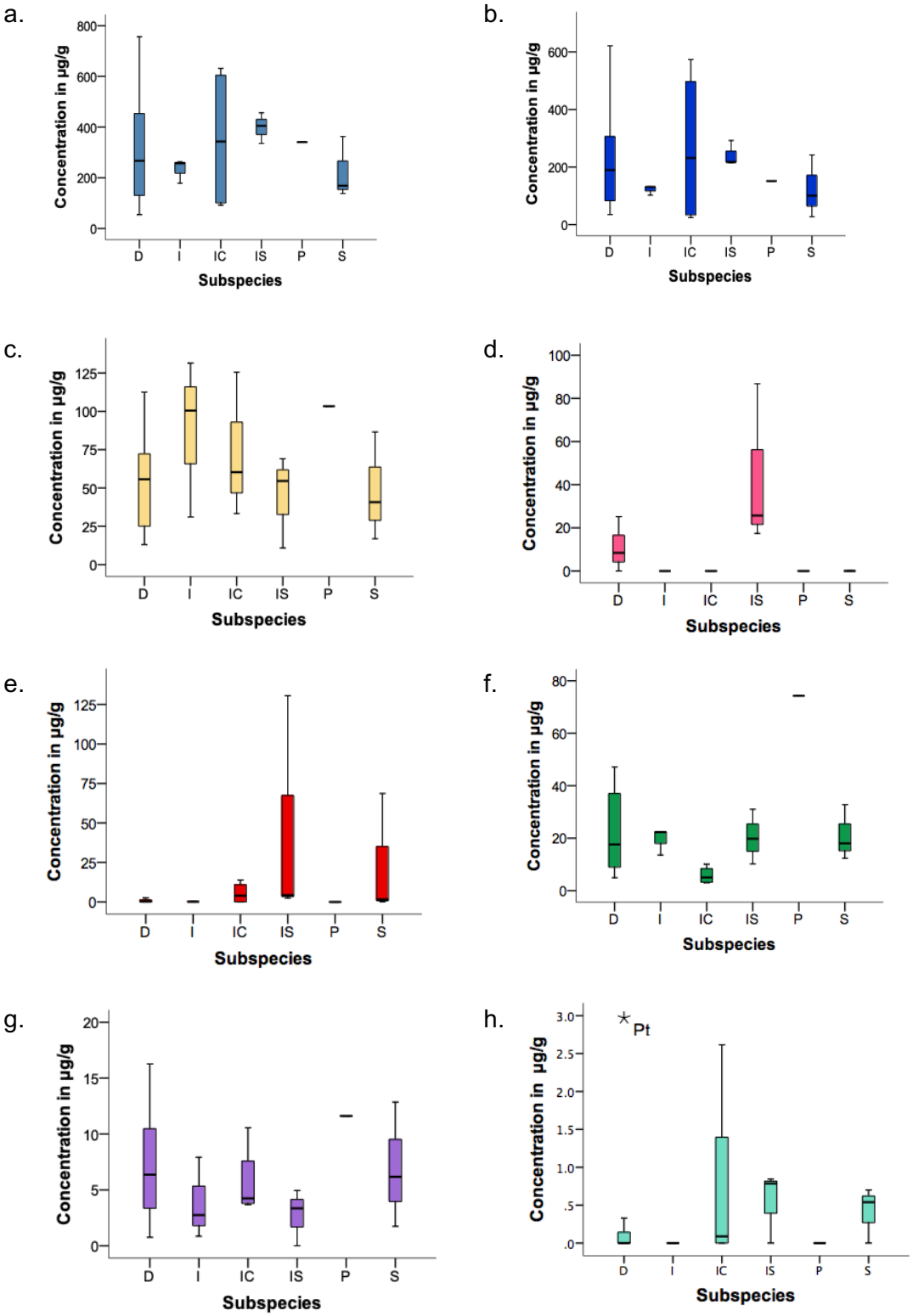


Figure 18: Boxplots of the concentrations of total phenolics and phenolic compound groups in fruits of *P. domestica* cultivars grouped by subspecies.
a. Total phenolic compounds, b. hydroxycinnamic acids, c. other phenolic compounds, d. anthocyanins, e. flavan-3-ols/proanthocyanidins, f. flavonols, g. flavones and h. unidentified yellow compound ONT01. In the boxplots, empty circle (○) shows an outlier and a star (∗) shows extreme outliers. **Outlier cultivar names:** Pt: 'President'. Further information about these cultivars can be found in Table 7.

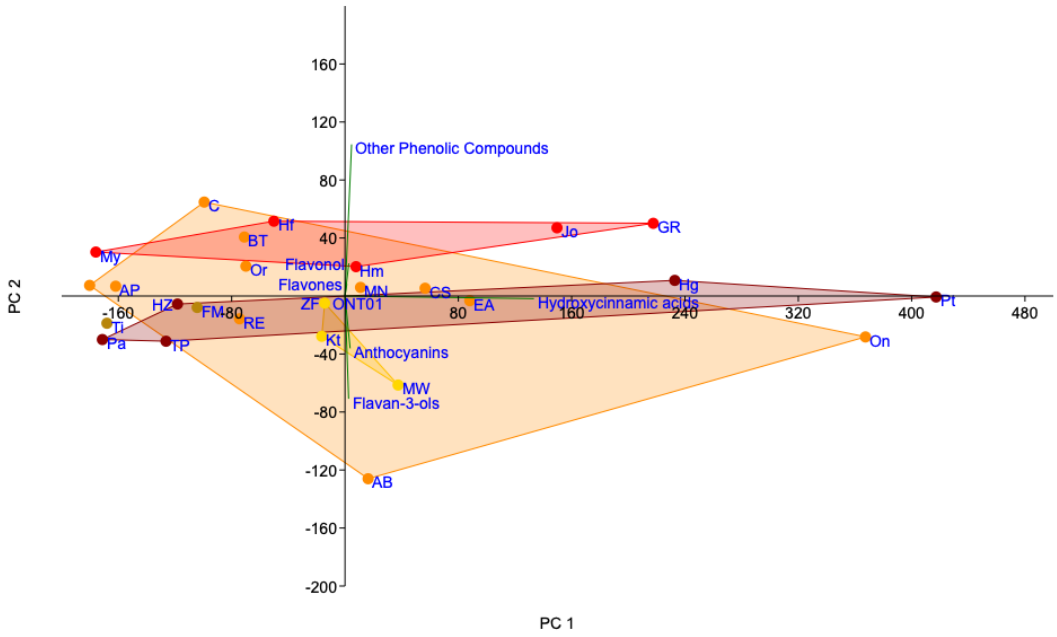


Figure 19: PCA of cultivars and concentrations of phenolic compound groups grouped by harvest period

The first two principal components are displayed in the biplot. Color of the markers and convex hulls represent a **harvest period category**: harvest period 1 (15-31 July), harvest period 2 (1-15 August), harvest period 3 (16-30 August), harvest period 4 (1-15 September) and harvest period 5 (16-31 September). Green lines represent the vectors conforming the biplot. **Cultivar** abbreviations: ZF: 'Zwintschers Frühe', Ka:' Katinka', MN: 'Maria Novella', CS: 'Cacaks Schöne' AB: 'Angelina Burdette', Jo: 'Jojo', Hm: 'Haroma', Or: 'Ortenauer', HZ: 'Hauszwetschge Schüfer', Hg: 'Haganta', Pa: 'Presenta', TP: 'Tophit Plus', Pt: 'President', RE: 'Rote Eierpflaume Blazek', EA: 'Eibenbacher Aprikosenpflaume', MW: 'Mirabelle Wallenberg', FM: 'Frühe Mirabelle P-2778', Ti: 'Tipala', C: 'Colora', My: 'Mirabelle aus Nancy 1510', On: 'Ontariopflaume', BT: 'Belle de Thuin', Hf: 'Haferpflaume', AP: 'Aprikoosprium', Ou: 'Oullins Reneklode' and GR: 'Grosse Grüne Reneklode'. Further information of the cultivars can be found in Table 7.

Regarding early harvested fruits from harvest periods 1 and 2, they are grouped on the lower part of the PCA near to the axis of PC1 (Figure 19). This means that the concentrations of other phenolic compounds tended to be lower in later harvest periods, which is also visible in the boxplots (Figure 20c). The concentration of other phenolic compounds was highest in harvest period 4 (Figure 20c) and therefore, samples of this group cluster on the upper part of the PCA in the direction of the corresponding vector (Figure 19). This harvest group has some outliers. 'Große Grüne Reneklode' was an extreme upper outlier for flavan-3-ol and ONT01 (Figure 20e and h), 'Jojo' was an upper outlier for flavones and 'Mirabelle aus Nancy' was a lower outlier for flavones (Figure 20g). Samples of harvest period 5 had lower concentrations of other phenolic compounds than harvest period 4 (Figure 20c) and they showed the broadest range of hydroxycinnamic acid concentrations (Figure 20b). Consequently, this harvest group is located in the PCA (Figure 19) under the convex hull of harvest period 4. Also, this harvest group includes the cultivar with the highest concentration of hydroxycinnamic acids ('President'). This cultivar was an extreme upper outlier for ONT01 concentration.

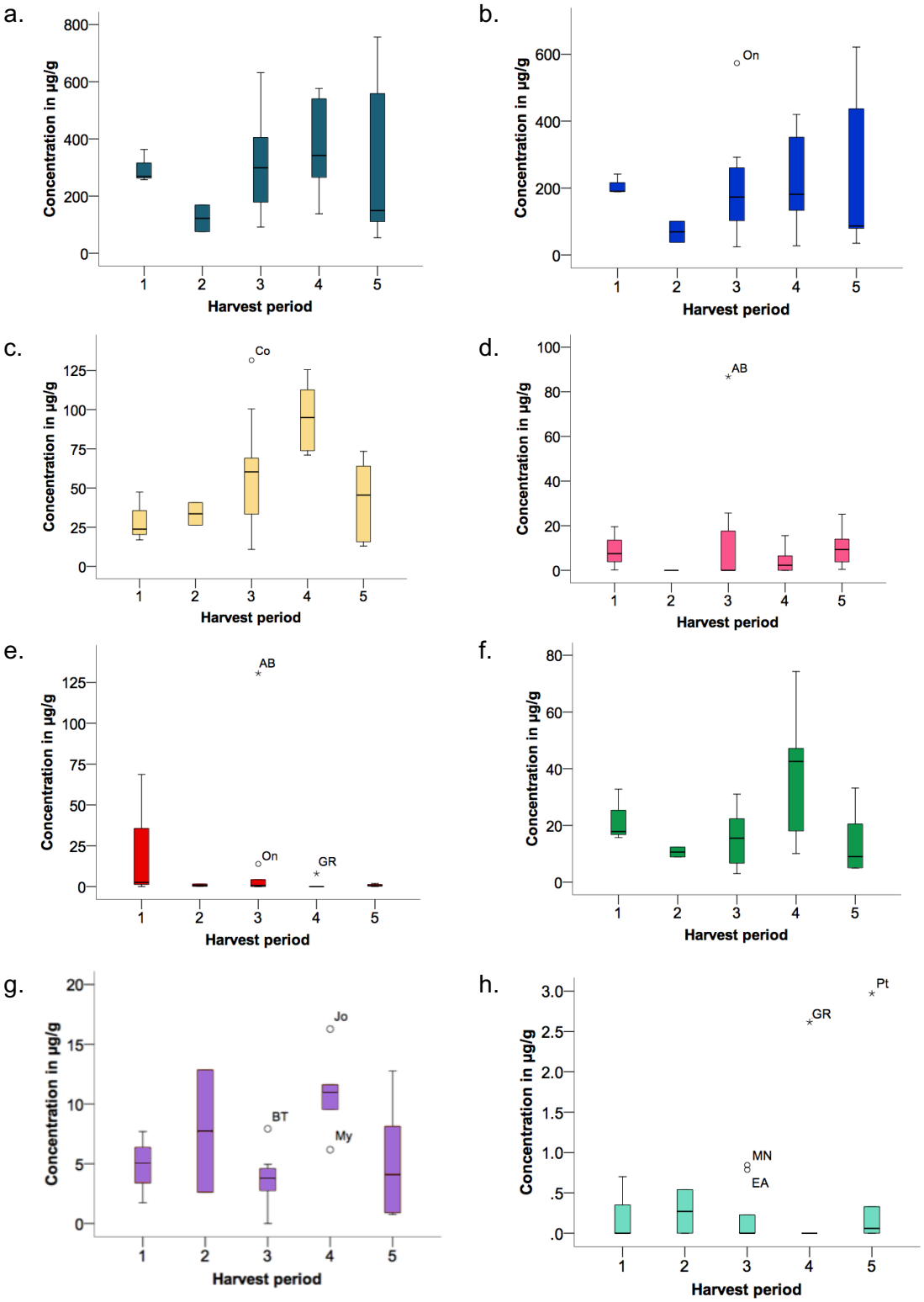


Figure 20: Boxplots of concentrations of total phenolics and phenolic compound groups in fruits of *P. domestica* cultivars grouped by harvest period

a. Total phenolic compounds, b. hydroxycinnamic acids, c. other phenolic compounds, d. anthocyanins, e. flavan-3-ols/proanthocyanidins, f. flavonols, g. flavones and h. unidentified yellow compound ONT01. In the boxplots, empty circle (○) shows an outlier and a star (∗) shows extreme outliers. **Harvest period category**: harvest period 1 (15-31 July), harvest period 2 (1-15 August), harvest period 3 (16-30 August), harvest period 4 (1-15 September) and harvest period 5 (16-31 September). **Outlier cultivar** names: On: 'Ontariopflaume', Co: 'Colora', AB: 'Angelina Burdette', BT: 'Belle de Thuin', Jo: 'Jojo', My: 'Mirabelle aus Nancy', MN: 'Maria Novella', EA: 'Eibenbacher Aprikosenpflaume', GR: 'Grosse Grüne Reneklode', Pt: 'President'. Further information about these cultivars can be found in Table 7.

5.2. Sugars in plum fruits

5.2.1. Sugar profiles in *P. domestica* samples harvested in 2012-2014 and 2016

The sugars detected in the 2012-2014 and in 2016 samples were identical. In all samples four compounds were identified: sorbitol, glucose, fructose and sucrose. In the year 2016 an extra compound was found in some samples eluting earlier than sorbitol. This compound eluted at a similar time to polyols such as mannitol, xylitol and myo-inositol. Structural identification was not possible; therefore, this compound was named S01.

5.2.2. Sugars concentrations in cultivars havested in 2012-2014

The total amount of sugars in the samples analyzed in 2012-2014 ranged from 0.1 g/ml in 'Tatjana' fruits, to 0.25 g/ml in 'Grosse Grüne Reneklode' fruits (Figure 21). The lowest concentration was detected in 'Tatjana' samples, the only cultivar that was not *P. domestica*. Looking at the ANOVA results (Table 16), groups were not clearly separated based on the total sugar concentration. This was also true for sucrose, glucose and sorbitol. The two cultivars with the highest concentration were yellow-red skinned ('Bellamira') and green skinned ('Grosse Grüne Reneklode').

The highest concentrations were determined for sucrose and ranged from 0.05 g/ml in 'Haganta' to 0.12 g/ml in fruit samples of 'Colora' and 'Bellamira' (Figure 21).

The concentration of glucose varied between 0.01 g/ml in 'Tatjana' to 0.06 g/ml in 'Harbella' samples, being the second most abundant sugar in 11 of the 13 cultivars analyzed (Figure 21). Although no statistical difference was found, purple skinned cultivars tended to have higher concentrations than yellow and green skinned ones (Table 16).

For fruits from cultivars 'Bellamira' and 'Grosse Grüne Reneklode', the second most abundant sugar was sorbitol. The concentrations of this compound varied between 0.01 g/ml in 'Colora' and 0.07 g/ml in 'Grosse Grüne Reneklode' fruit pulp (Figure 21). Fructose concentrations ranged in fruit pulp from 0.01 g/ml in 'Tatjana' samples to 0.05 g/ml in 'Harbella' samples (Figure 21). Three clear groups could be defined due to the one-way ANOVA results (Table 16). Cultivars with low concentrations of fructose in the fruits were: 'Auerbacher', 'Cacaks Julia', 'Tatjana', and 'Colora'. The cultivars 'Cacaks Fruchtbare', 'Cacaks Schöne', 'Haganta', 'Haroma', 'Hauszwetschge Schüfer', 'Bellamira', 'Goldzwetschge' and 'Grosse Grüne Reneklode' had a medium concentration of fructose. Finally, 'Harbella' was the only cultivar with a high concentration of fructose in the fruit pulp.

The only green skinned plum and gage analyzed in this harvest period was ‘Grosse Grüne Reneklode’. This cultivar had a high content of sorbitol compared to the fructose and glucose concentrations (Figure 21).

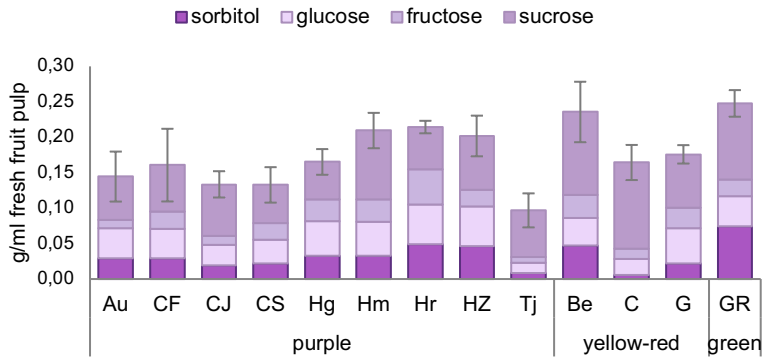


Figure 21**:** Mean concentrations and composition of soluble sugars in fruit samples of different cultivars harvested in 2012 to 2014.
The error bars represent the standard deviations of the total soluble sugar concentration. Analysis of the statistical significance is displayed in Table 16. **Cultivars** in the first row of the x-axis, Au: ‘Auerbacher’, CF: ‘Cacaks Fruchtbare’, CJ: ‘Cacaks Julia, CS: ‘Cacaks Schöne’, Hg: ‘Haganta’, Hm: ‘Haroma’, Hr: ‘Harbella’, HZ: ‘Hauszwetschge Schüfer’, Tj: ‘Tatjana’, Be: ‘Bellamira’, C: ‘Colora’, G: ‘Goldzwetschge’ and GR: ‘Große Grüne Reneklode’. **Fruit skin color** in the second row of the x-axis. Further information about the cultivars are displayed in Table 7.

5.2.3. Sugar concentrations in fruits of the same cultivar of trees grown in different places within the same region in 2014

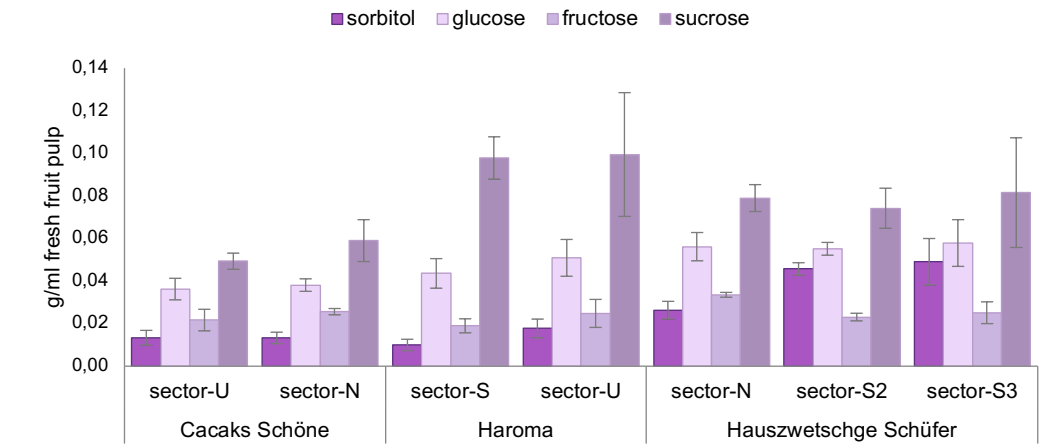
The sugar concentrations of selected cultivars growing in different locations (Figure 7) were analyzed, to observe the effect of microclimatic changes on the concentration of sugars in the fruit. To determine significant differences between samples, t-tests were performed in the case of ‘Cacaks Schöne’ and ‘Haroma’. To compare the three samples of ‘Hauszwetschge Schüfer’ one-way ANOVA was applied (Table 17).

Looking at the total sugar content of ‘Cacaks Schöne’ fruits grown in sector-U or sector-N no significant difference was found (Figure 22a). The same was true for fruits of ‘Haroma’ grown in sector-S and sector-U and for ‘Hauszwetschge Schüfer’ fruits growing in sector-N and sector-S. One statistically significant difference was observed for the fructose content in ‘Hauszwetschge Schüfer’ grown in sector-N. Their fructose content was higher than the content in those fruits grown in sector-S. Also, the sorbitol content in ‘Hauszwetschge Schüfer’ fruits grown in sector-N was significantly lower than in those fruits grown in sector-S.

In a PCA plot of all repetitions (Figure 22b), samples of the same cultivar tended to group independent from the location where the fruits grew (blue circles). In addition, the spread of the repetitions was different for each cultivar. PC1 explained 64.43% of the variance, with sucrose as the compound with the major contribution. On the other

hand, PC2 was responsible for 30.8% of the variance with sorbitol as the most dominant compound.

a.



b.

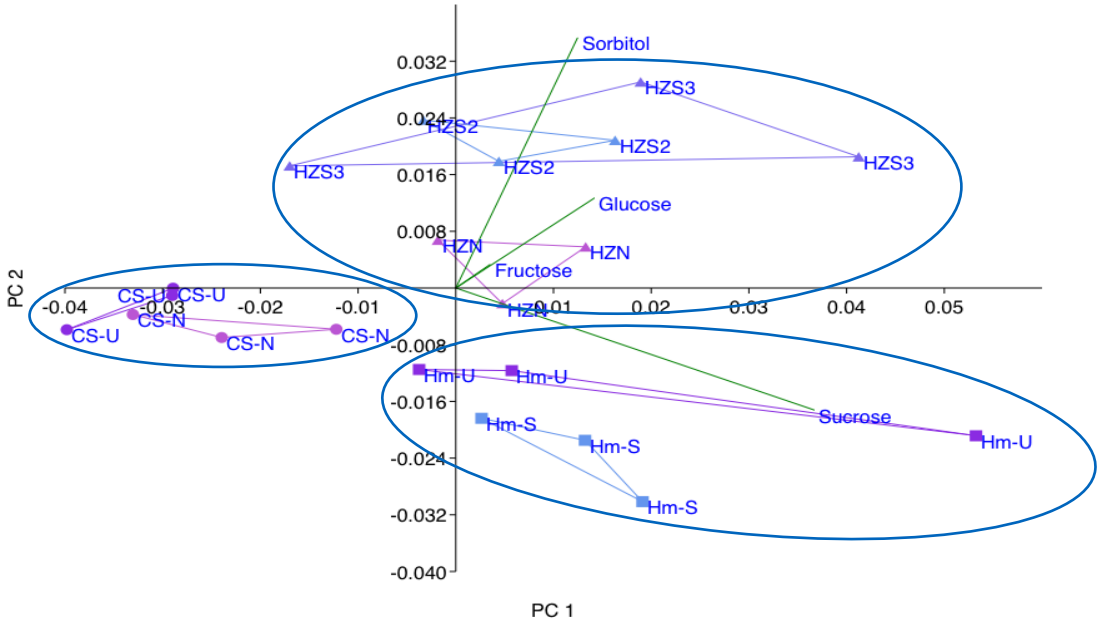


Figure 22: Concentrations (a) and principal component analysis (b) of soluble sugars in fruit pulp of three plum cultivars grown in different locations.
a. The error bars represent the standard deviations. Analysis of statistical significance is displayed in Table **17**. b. Principal component analysis: color of the markers represent different **harvest locations**, U: sector-U; N: sector-N and S: sector-S (numbers indicate the field row). The form of the marker represents the cultivar: ●: 'Cacaks Schöne' (CS), ■: 'Haroma' (Hm) and ▲: 'Hauszwetschge Schüfer' (HZ). Green lines represent the vectors conforming the biplot. **Convex hulls** group samples of the same location and cultivar repetitions of the same cultivar grown in the same location. Blue circles group samples of the same cultivar.

5.2.4. Sugar concentrations in samples of the 2016 harvest

The concentrations of soluble sugars in 26 cultivars harvested in 2016 was analyzed. The lowest concentration of total sugar was found in 'Zwintschers Frühe' (0.005 g/ml) and the highest concentration in 'Angelina Burdette' (0.02 g/ml) as shown in Figure 23.

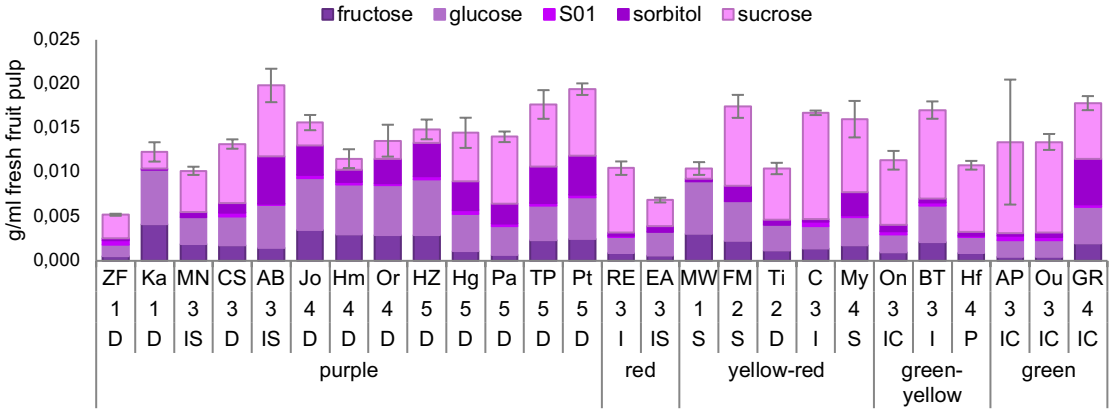


Figure 23: Mean concentrations and composition of total soluble sugars in fruit samples harvested in 2016.

The error bars represent the standard deviations of total sugar concentration. Analysis of statistical significance is displayed in Table 20. **Cultivars** in the first row of the x-axis, ZF: 'Zwintschers Frühe', Ka:' Katinka', MN: 'Maria Novella', CS: 'Cacaks Schöne' AB: 'Angelina Burdette', Jo: 'Jojo', Hm: 'Haroma', Or: 'Ortenauer', HZ: 'Hauszwetschge Schüfer', Hg: 'Haganta', Pa: 'Presenta', TP: 'Tophit Plus', Pt: 'President', RE: 'Rote Eierpflaume Blazek', EA: 'Eibenbacher Aprikosenpflaume', MW: 'Mirabelle Wallenberg', FM: 'Frühe Mirabelle P-2778', Ti: 'Tipala', C: 'Colora', My: 'Mirabelle aus Nancy 1510', On: 'Ontariopflaume', BT: 'Belle de Thuin', Hf: 'Haferpflaume', AP: 'Aprikoosprium', Ou: 'Oullins Reneklode' and GR: 'Grosse Grüne Reneklode'. **Harvest period** in the second row: (1) 15-31 July; (2) 1-15 August; (3) 16-30 August; (4) 1-15 September and (5) 16-31 September. **Subspecies** in the third row of the x-axis, D: *domestica*, IS: *italica var. subrotunda*, S: *syriaca*, I: *intermedia var. ovoidea*, IC *italica var. claudiana*, P: *prisca*. **Fruit skin color** is shown in the last row of the x-axis. Further information about the cultivars are displayed in Table 7.

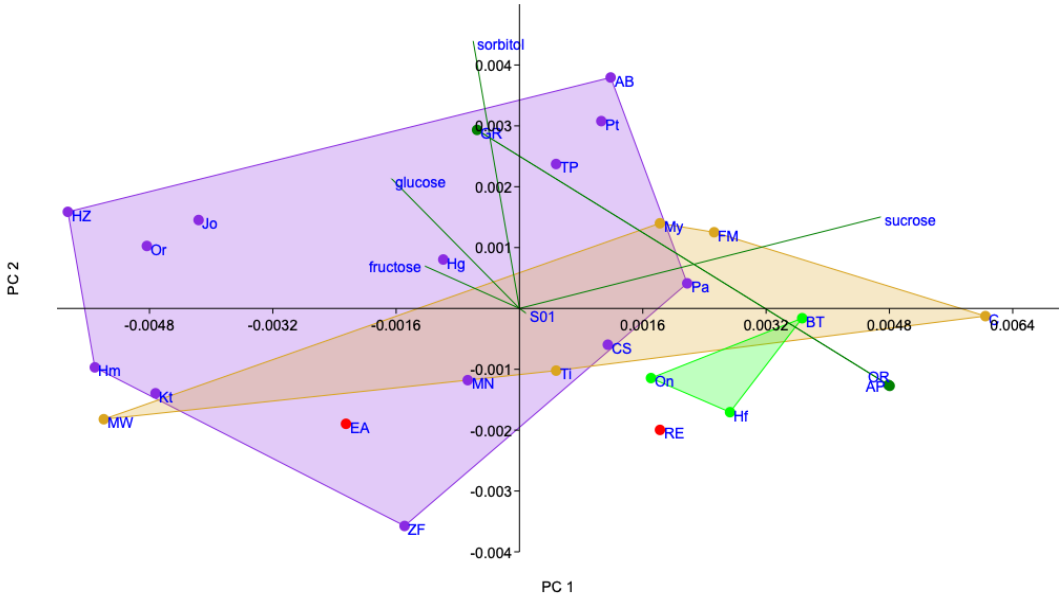


Figure 24: PCA of cultivars and concentrations of sugars grouped by their fruit skin color.

The first two principal components are displayed in the biplot. Color of the markers and convex hulls represent the color of **fruit skin** color: purple, red, yellow-red, green-yellow and green. Green lines represent the vectors conforming the biplot. Cultivar abbreviations, ZF: 'Zwintschers Frühe', Ka:' Katinka', MN: 'Maria Novella', CS: 'Cacaks Schöne' AB: 'Angelina Burdette', Jo: 'Jojo', Hm: 'Haroma', Or: 'Ortenauer', HZ: 'Hauszwetschge Schüfer', Hg: 'Haganta', Pa: 'Presenta', TP: 'Tophit Plus', Pt: 'President', RE: 'Rote Eierpflaume Blazek', EA: 'Eibenbacher Aprikosenpflaume', MW: 'Mirabelle Wallenberg', FM: 'Frühe Mirabelle P-2778', Ti: 'Tipala', C: 'Colora', My: 'Mirabelle aus Nancy 1510', On: 'Ontariopflaume', BT: 'Belle de Thuin', Hf: 'Haferpflaume', AP: 'Aprikoosprium', Ou: 'Oullins Reneklode' and GR: 'Grosse Grüne Reneklode'. Further information of the cultivars can be found in Table 7.

Five different sugars were detected namely fructose, glucose, sucrose, sorbitol and an unknown sugar S01 (Figure 23). For 20 of the 26 cultivars analyzed, sucrose showed the highest concentration in the fruit pulp. The concentration of this compound ranged from 0.001 g/ml in fruit pulp of 'Mirabelle Wallenberg' to 0.01 g/ml in 'Colora'. In fruits of the other six cultivars, glucose was the most abundant sugar (0.001 to 0.006 g/ml).

The concentration of fructose ranged from 0.0005 g/ml ('Aprikoosprium') to 0.004 g/ml ('Katinka'), sorbitol from 0.0002 g/ml ('Katinka') to 0.005 g/ml ('Angelina Burdette') and S01 from 0 g/ml (in six cultivars) to 0.0005 g/ml ('Aprikoosprium').

The biplots presented in Figure 24, Figure 26 and Figure 28 are PCAs for the concentration of each sugar in each cultivar. PC1 represents almost 70% of the variance with sucrose as the most dominant compound, while PC2 represented 21.69% of the variance with sorbitol as major factor. On the left part of the graphic, six cultivars are grouped and separated from the others. Boxplot analyses were performed for all compounds with the cultivars grouped by skin color, subspecies and harvest period (Figure 25, Figure 27 and Figure 29). Outliers observed in the boxplots did not clearly separate them from the other cultivars in the biplot.

In Figure 24 all cultivars with the same fruit skin color are grouped within the same convex hull. The cultivars with purple skinned fruits show the largest spread over the plot, meaning that they were the most heterogeneous group of all. This was also observed in the boxplot analysis (Figure 25), where this group showed the widest range (considering the outliers) for all compounds. This group clustered in the left-upper side of the plot (Figure 24), with lower concentrations of sucrose and higher concentrations of glucose, fructose and sorbitol, which was also observed in the boxplots (Figure 25b-d). Within this fruit skin category, the cultivar 'Zwintschers Frühe' was a lower outlier for total sugar concentration (Figure 25a). Also 'Katinka' and 'Maria Novella' were lower outliers for the unidentified sugar S01 (Figure 25f). Yellow skinned cultivars also showed a broad distribution, but grouped in the right part of the biplot (Figure 24) and in the upper part of the boxplot (Figure 25b), tending to higher concentrations of sucrose. In the case of green-yellow skinned fruits, samples contained higher concentrations of sucrose and low concentrations of other sugars, as it could be observed in the boxplots (Figure 24) and in the biplot (Figure 25b-e). Green skinned fruit samples were widely spread in the plot. Two samples grouped on the left side with highest concentrations of sucrose, the third sample of this group ('Grosse Grüne Reneklode') was placed in the upper part of the plot near PC2 axis, with a high concentration of sorbitol (Figure 24). This was confirmed in the boxplots, where this group had a high concentration of

sucrose (Figure 25b), a wide range of sorbitol concentrations (Figure 25d) and a rather low concentration of glucose and fructose (Figure 25c and e).

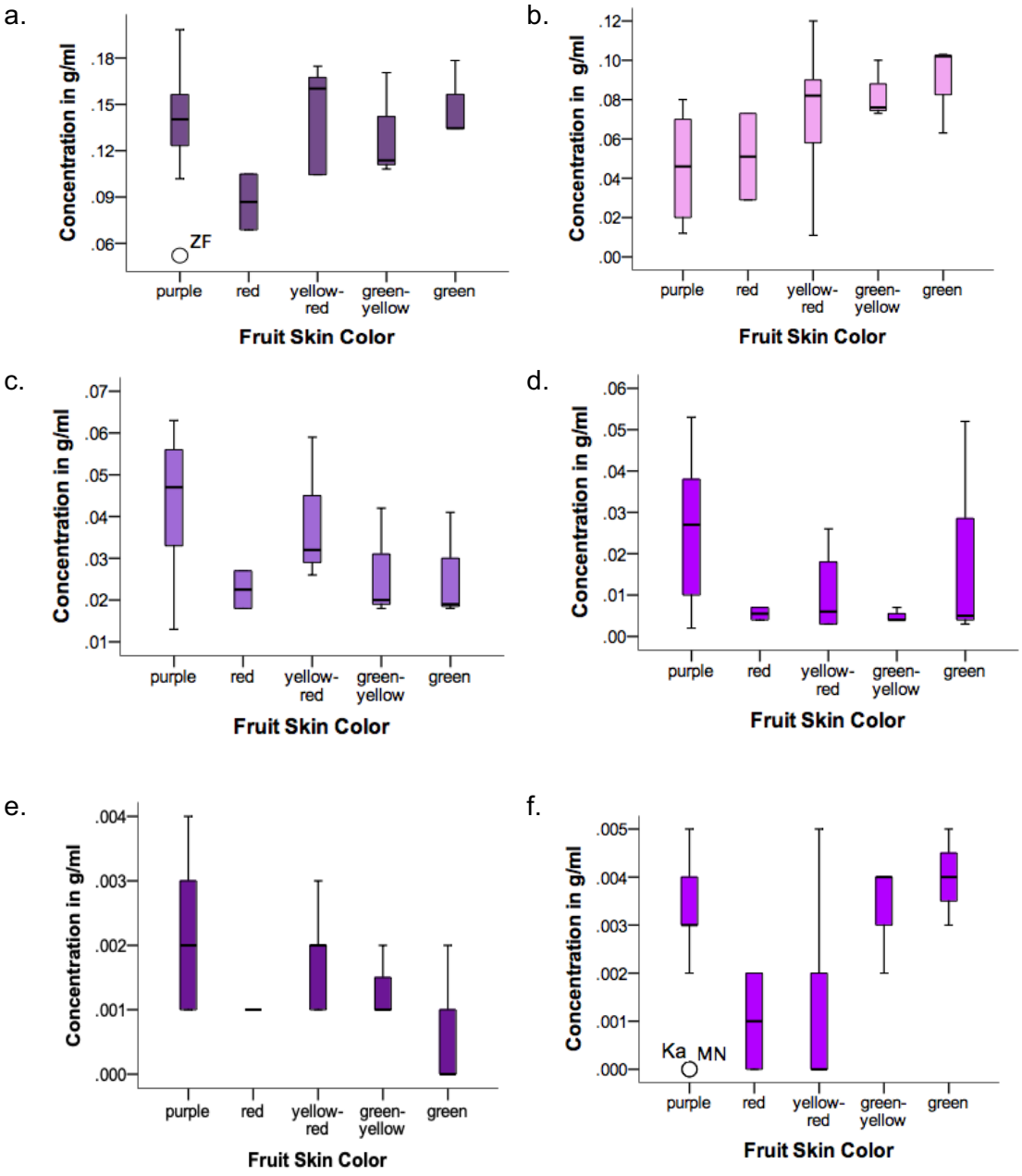


Figure 25: Boxplots of mean concentrations of total sugars and individual sugars in fruits of *P. domestica* cultivars grouped by the color of the fruit skin.
a. Total sugar, b. sucrose, c. glucose, d. sorbitol, e. fructose and f. S01. In the boxplots, an empty circle (○) shows an outlier and a star (∗) shows extreme outliers. Outlier cultivar names, ZF: 'Zwintschers Frühe', Ka: 'Katinka' and MN: 'Maria Novella'. Further information about these cultivars can be found in Table 7.

The two red skinned samples had the lowest concentration of fructose, glucose and sorbitol (Figure 25c-e). In the biplot (Figure 24) they were grouped in the lower part of the plot, one with high concentration of sucrose on the right and the other one on the left with low concentration of sucrose. The contribution of the vector S01 was too low to see the differences between skin color categories in the biplot (Figure 24). In the boxplot

analysis, it was observed that purple, green-yellow and green had higher concentrations of this compound than red and yellow-red (Figure 25f).

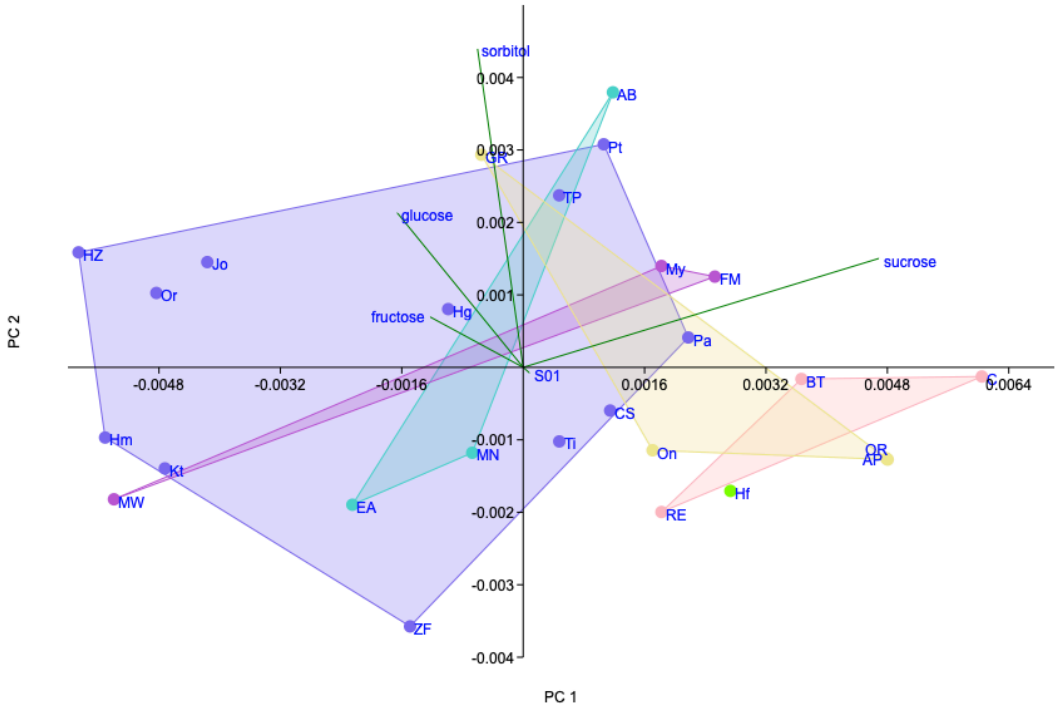


Figure 26: PCA of concentrations of different sugars grouped by subspecies.

The first two principal components are displayed on the biplot. Color of the markers and convex hulls represent a **subspecies**: : subsp. *domestica,* : subsp. *italica var. subrotunda*, : subsp. *italica var. claudiana*, : subsp. *syriaca*,: subsp*. intermedia var. ovoidea*,: subsp. *prisca.* Green lines represent the vectors conforming the biplot. **Cultivar** abbreviations: ZF: 'Zwintschers Frühe', Ka:' Katinka', MN: 'Maria Novella', CS: 'Cacaks Schöne' AB: 'Angelina Burdette', Jo: 'Jojo', Hm: 'Haroma', Or: 'Ortenauer', HZ: 'Hauszwetschge Schüfer', Hg: 'Haganta', Pa: 'Presenta', TP: 'Tophit Plus', Pt: 'President', RE: 'Rote Eierpflaume Blazek', EA: 'Eibenbacher Aprikosenpflaume', MW: 'Mirabelle Wallenberg', FM: 'Frühe Mirabelle P-2778', Ti: 'Tipala', C: 'Colora', My: 'Mirabelle aus Nancy 1510', On: 'Ontariopflaume', BT: 'Belle de Thuin', Hf: 'Haferpflaume', AP: 'Aprikoosprium', Ou: 'Oullins Reneklode' and GR: 'Grosse Grüne Reneklode'. Further information of the cultivars can be found in Table 7.

Grouping the samples in the PCA by subspecies revealed some differences (Figure 26). Samples of cultivars of *subsp. domestica* (*D)* are widespreaded in the plot grouped on the left part of the plot, with low sucrose concentrations. This convex hull intersected almost all other groups at least at one point. The only exceptions were *subsp.* intermedia var. ovoidea (*I)* and *subsp. prisca (P)* that had a higher concentration of sucrose (Figure 27b). The cultivar 'Zwintschers Frühe' was a lower outlier for total sugar concentration (Figure 27a) and 'Katinka' and 'Tipala' were lower outliers for the unidentified sugar S01 (Figure 27f). Cultivars of *subsp. italica var. subrotunda (IS)* and *subsp. syriaca (S)* had thin and long convex hulls (Figure 26). This was mainly due to one cultivar placed opposite than the others. In the case of *S*, two cultivars 'Frühe Mirabelle-P2778' and 'Mirabelle aus Nancy' grouped on the upper right part of the plot while the third cultivar of this group was placed in the left lower part ('Mirabelle Wallenberg') with low concentrations of sucrose and other sugars (Figure 26). In the boxplot for sucrose

(Figure 27b) it was observed that the median is shifted to higher concentrations and the lower whisker is longer than the upper one. In *IS*, two cultivars were placed in the left lower part of the biplot (Figure 26), while the third one ('Angelina Burdette') was placed on the opposite upper right part, with higher concentrations of sorbitol than the others of the group.

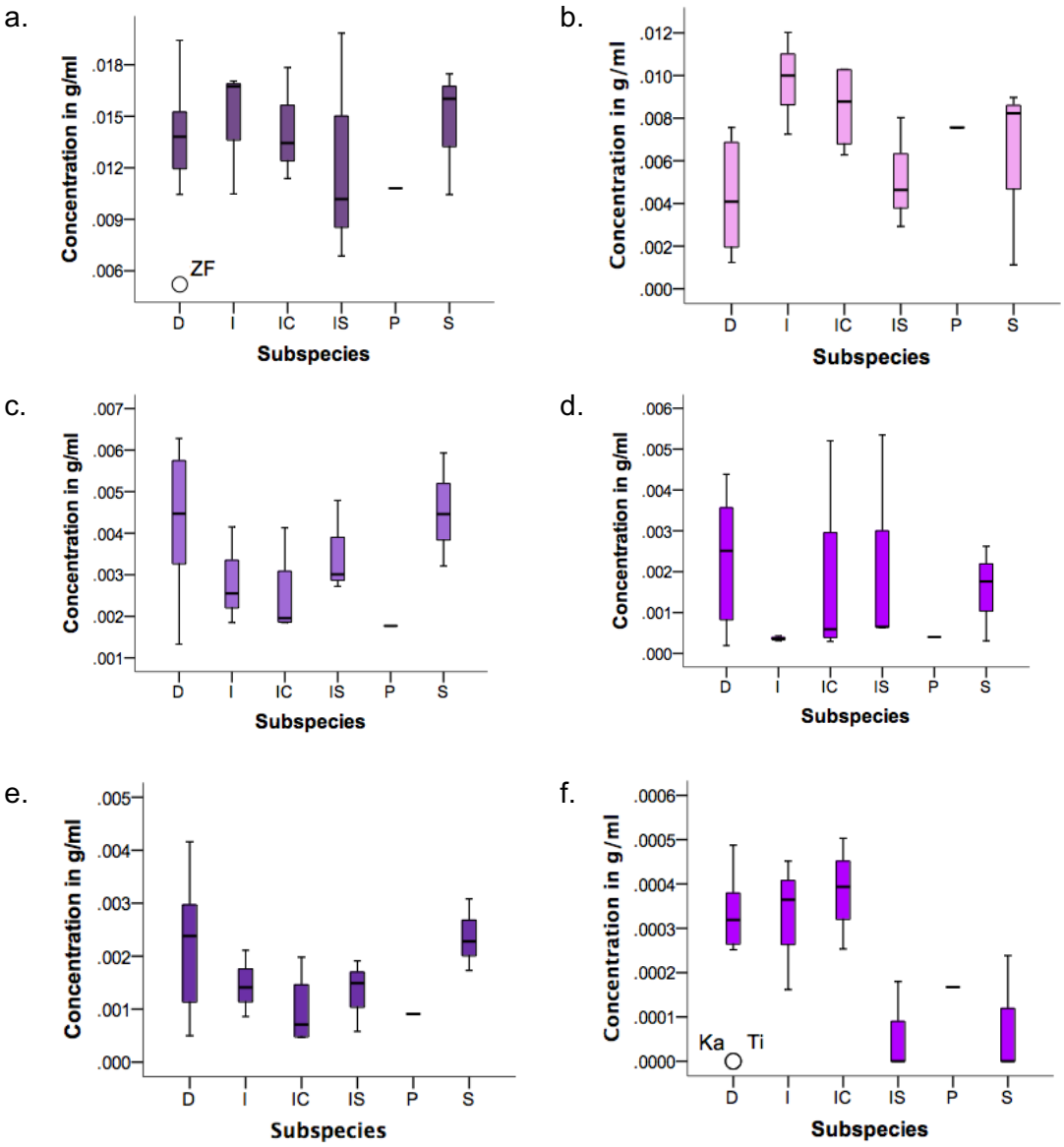


Figure 27: Boxplots of concentrations of total sugars and individual sugars in fruits of *P. domestica* cultivars grouped by subspecies.
a. Total sugar, b. sucrose, c. glucose, d. sorbitol, e. fructose and f. S01. In the boxplots, an empty circle (○) shows an outlier and a star (∗) shows extreme outliers. Outlier cultivar names, ZF: 'Zwintschers Frühe', Ka: 'Katinka' and Ti: 'Tipala'. Further information about these cultivars can be found in Table 7.

This was also observed in the boxplot (Figure 27d), where the upper whisker of *IS* was longer than the lower whisker. Something similar was observed in *IC*, where three cultivars were placed on the lower right part of the plot with high sucrose concentration and low concentration of other sugars. The fourth cultivar of this group ('Grosse Grüne Reneklode') was placed on the upper part of the plot near the PC2 axis

with high concentrations of sorbitol (Figure 26 and Figure 27d). The subspecies *I* produced the highest concentrations of sucrose and lower concentrations of sorbitol and glucose (Figure 26 and Figure 27b-d).

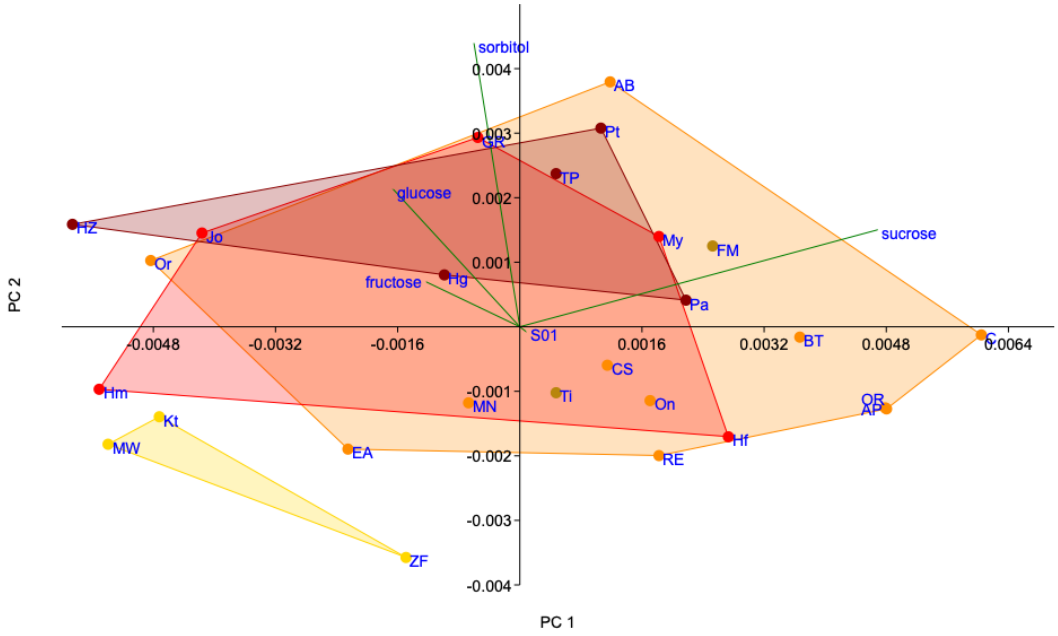


Figure 28: PCA of cultivars and concentrations of sugars grouped by harvest period.
The first two principal components are displayed in the biplot. Color of the markers and convex hull represent a **harvest period category**: harvest period 1 (15-31 July), harvest period 2 (1-15 August), harvest period 3 (16-30 August), harvest period 4 (1-15 September) and harvest period 5 (16-31 September). Green lines represent the vectors conforming the biplot. **Cultivar** abbreviations: ZF: 'Zwintschers Frühe', Ka:' Katinka', MN: 'Maria Novella', CS: 'Cacaks Schöne' AB: 'Angelina Burdette', Jo: 'Jojo', Hm: 'Haroma', Or: 'Ortenauer', HZ: 'Hauszwetschge Schüfer', Hg: 'Haganta', Pa: 'Presenta', TP: 'Tophit Plus', Pt: 'President', RE: 'Rote Eierpflaume Blazek', EA: 'Eibenbacher Aprikosenpflaume', MW: 'Mirabelle Wallenberg', FM: 'Frühe Mirabelle P-2778', Ti: 'Tipala', C: 'Colora', My: 'Mirabelle aus Nancy 1510', On: 'Ontariopflaume', BT: 'Belle de Thuin', Hf: 'Haferpflaume', AP: 'Aprikoosprium', Ou: 'Oullins Reneklode' and GR: 'Grosse Grüne Reneklode'. Further information of the cultivars can be found in Table 7.

When the samples were analyzed accorrding to their harvest date (Figure 28), samples of harvest period 1 were separated from the others as they were grouped in the left lower part of the biplot, with low concentrations of sucrose and sorbitol as it was confirmed by boxplot analysis (Figure 29a, b, d). The convex hull of harvest periods 3 and 4 were the largest of all groups (Figure 28). This variation was mainly explained by the variation of the glucose and sorbitol concentration and in harvest period 4 by sucrose (Figure 29). In harvest period 3 one lower outlier, 'Angelina Burdette', was detected for the unidentified sugar S01. Two two samples of harvest period 2 grouped on the right part of the biplot, namely 'Tipala' with lower concentrations of all sugars than 'Frühe Mirabelle P-2778' (Figure 28). Finally, samples of harvest period 5 were grouped in the upper part of the plot with higher concentrations of sucrose (Figure 28) and confirmed in the boxplot (Figure 29b). One extreme upper outlier of the sorbitol concentrations was cultivar 'President'.

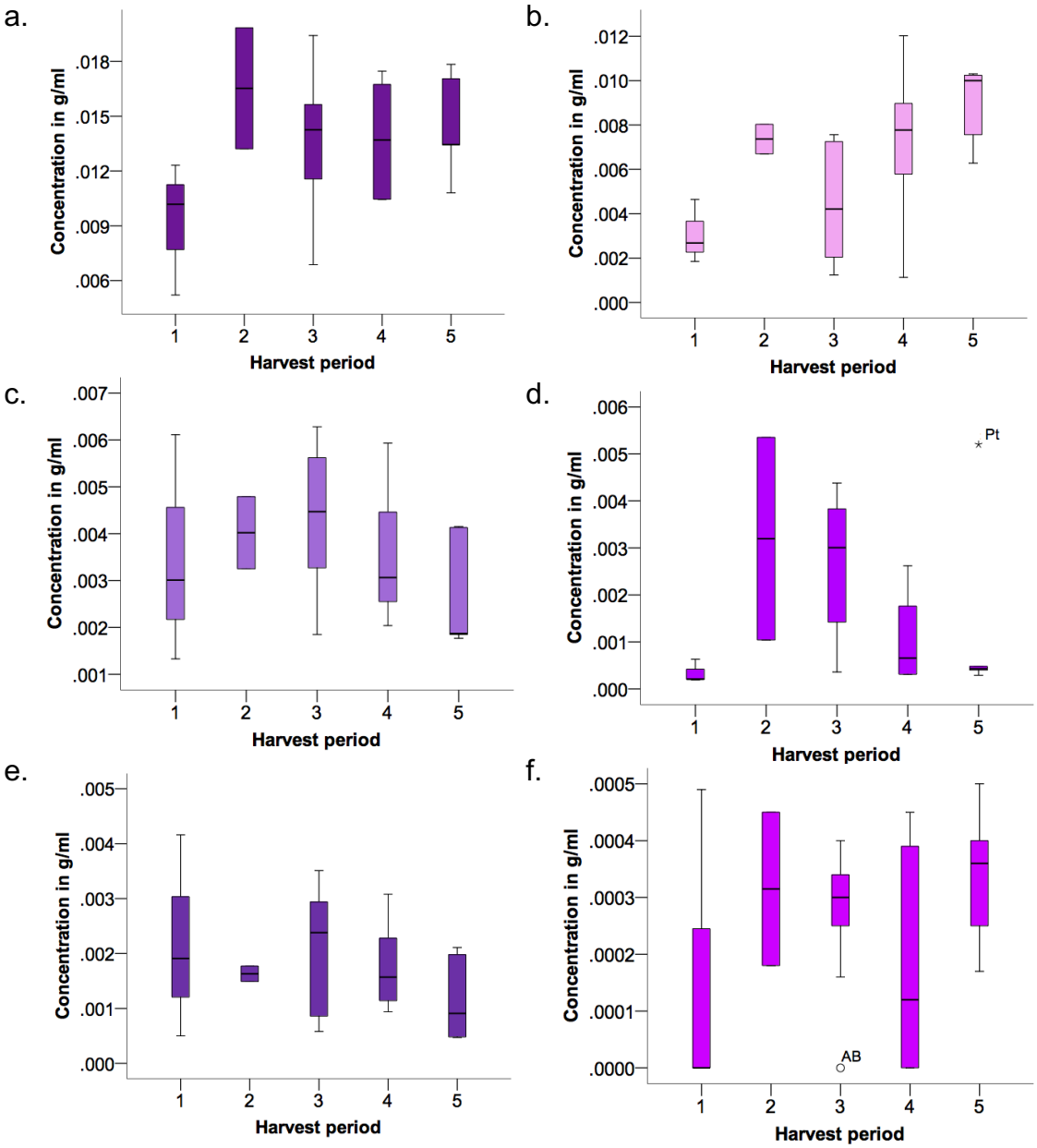


Figure 29: Boxplots of concentrations of total sugars and individual sugars in fruits of *P. domestica* cultivars grouped by harvest period.

a. Total sugar, b. sucrose, c. glucose, d. sorbitol, e. fructose and f. S01. **Harvest period category**: harvest period 1 (15-31 July), harvest period 2 (1-15 August), harvest period 3 (16-30 August), harvest period 4 (1-15 September) and harvest period 5 (16-31 September). In the boxplots, an empty circle (○) shows an outlier and a star (∗) shows extreme outliers. Outlier cultivar names, Pt: 'President', AB: 'Angelina Burdette'. Further information about these cultivars can be found in Table 7.

5.3. Carotenoids in plum fruits

5.3.1. Carotenoid profiles in *P. domestica* samples harvested in 2012-2015 measured with a gradient elution method

A list of identified carotenoids are shown in Table 13 (Supplementary Material 4). Unknown metabolites were grouped according to their UV/vis spectra and retention time. Examples of each compound group with their spectrum can be found in Table 14 (Supplementary Material 4).

Carotenoid analysis in samples from 2012 to 2015 revealed 40 peaks at a wavelength of 450 nm: eight carotenes, 13 free xanthophylls, 15 esterified xanthophylls and four chlorophylls. Additionally, twelve peaks were detected at 280 nm: four phytoene isomers, two phytofluene isomers, five other colorless compounds and one group of colorless compounds. Unidentified compounds were named with different letters regarding their compound group, with a sequential number within the group: colored carotenoids were referred to as C, phytoene isomers as PH, phytofluene isomers as PF, chlorophylls as CHL and other colorless carotenoids as CC. By means of co-chromatography the following compounds were tentatively identified: all-trans-lutein (C04), all-trans-ß-carotene (C21) and all-trans-ß-cryptoxanthin (C14). Lycopene was identified based on its characteristic UV/vis spectrum which shows a third maximum at 500 nm, and because it was the last carotenoid to elute from the column. It could not be determined which isomer of lycopene was detected (Gupta et al., 2015). Peaks CHL01, CHL02, CHL03, and CHL04 were classified as chlorophylls or chlorophyll derivatives because of their characteristic UV/vis spectra (Table 14; Supplementary Material 4). Structural identification was not possible. Neither the extraction method nor the analysis methods was optimized for chlorophylls, because the aim of this study was the determination of carotenoids in plums. Isomerization and degradation of chlorophyll can therefore be expected.

All metabolites that eluted before ß-cryptoxanthin were classified as xanthophylls, with ß-cryptoxanthin being the least polar xanthophyll. Metabolites eluting after ß-cryptoxanthin (C14) were classified as non-polar carotenoids that included carotenes and esterified xanthophylls. Saponification experiments (Haager, 2015) showed that carotenoids C23 to C37 were esterified xanthophylls. Different isomers of ζ-carotene, δ-carotene, γ-carotene and neurosporene elute at this retention time as well. The only carotene eluting later than ß-carotene after saponification was C22. This compound was tentatively identified as ζ-carotene due to it UV/Vis spectrum and the

elution order described by Britton et al. (2004); Gupta et al. (2015); Melendez-Martinez et al. (2015).

At 280 nm, colorless carotenoids were detected. By means of comparison with published spectra (Gupta et al., 2015) (Table 13; Supplementary Material 4), PH01, PH02, PH03 and PH04 were identified as phytoene isomers with PH03 being the most abundant one. PF01 and PF02 are phytofluene isomers. All other compounds detected at this wavelength had a simple spectrum with one maximum at 275 nm, 268 nm, 295 nm or 256 nm. These compounds could not be structurally identified and were designated as other colorless compounds (OCC). Other colorless compounds eluting before the internal standard (Sudan I, retention time: 6 min) were grouped under the name GOCC01.

5.3.2. Carotenoid profiles in *P. domestica* samples harvested in 2016 measured with an isocratic elution method

Using an isocratic elution method, fewer compounds were eluted and they showed lower peak heights compared with the gradient elution. In samples isocratically analyzed, all carotenoids that eluted after C22 with gradient elution were missing when isocratic elution was used. The missing compounds were mainly esterified xanthophylls and lycopene isomers. Despite of this, the main carotenoids such as lutein and ß-carotene could still be determined. Fourteen free xanthophylls and eight carotenes were detected. Spectra and elution times of the identified compounds and of representative metabolites of each group are shown in Table 13 and Table 14 (Supplementary Material 4).

Six colorless carotenoids were detected at 280 nm, one less than with gradient elution. In addition, four other colorless compounds were detected instead of five. Five chlorophylls were detected at 450 nm, one more than with the previous method and two additional chlorophylls were detected at 280 nm.

5.3.3. Carotenoid concentrations in cultivars of *P. domestica* harvested in 2014

Ten *P. domestica* cultivars with different fruit skin colors were analyzed. Five of them were purple skinned: ‘Angelina Burdette’ (AB), ‘Hanka’ (Hk), ‘Haroma’ (Hm), ‘Katinka’ (Kt), and ‘Zwintschers Frühe’ (ZF). The other five cultivars had yellow skinned fruits, some with red spots: ‘Colora’ (C), ‘Goldzwetschge’ (Go), ‘Liegels Gelbe’ (LG), ‘Mirabelle Wallenberg’ (MW), and ‘Tipala’ (Ti).

The highest total carotenoid concentrations were found in ‘Katinka’ (71.538 µg/ml) and ‘Hanka’ (35.030 µg/ml) (Figure 30a). The rest of the cultivars

showed carotenoid concentrations between 0.311 µg/ml in ‘Angelina Burdette’ and 13.601 µg/ml in ‘Liegels Gelbe’ and 13.689 µg/ml in ‘Tipala’.

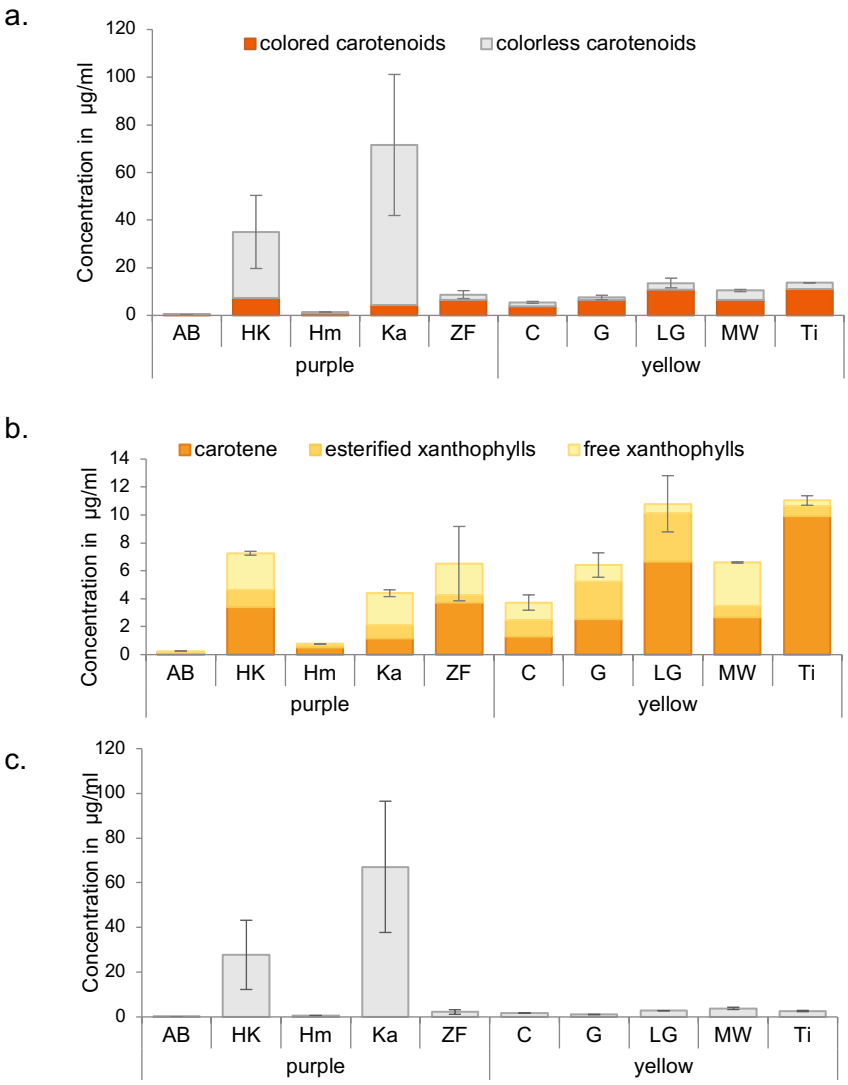


Figure 30: Mean concentrations and composition of total carotenoids (a), colored carotenoids (b) colorless carotenoids (c) of ten *P. domestica* cultivars harvested in 2014. The error bars represent the standard deviations of the sum of all compounds. Analysis of statistical significance is displayed in Table 18. **Cultivars** in the first row of the x-axis, AB: ‘Angelina Burdette’, Hk: ‘Hanka’, Hm: ‘Haroma’, Ka:’ Katinka’, ZF: ‘Zwintschers Frühe’, C: ‘Colora’, G: ‘Goldzwetschge’, LG: ‘Liegels Gelbe’, MW: ‘Mirabelle Wallenberg’, and Ti: ‘Tipala’. **Fruit skin color** is shown in the second row of the x-axis. Further information about the cultivars are displayed in Table 7.

Colorless carotenoids were the most important compound group in ‘Katinka’ and ‘Hanka’ (Figure 30a and c), but not for the other samples. The difference was statistically significant (Table 18, Supplementary material 5).

Regarding the concentrations of total colored carotenoids (Figure 30b) and considering the statistical analysis results (Table 18, Supplementary material 5), three main groups were defined: ‘Angelina Burdette’ and ‘Haroma’ with low concentrations of colored carotenoids; ‘Tipala’ and ‘Liegels Gelbe’ with high amounts of colored

carotenoids and the others with medium levels of colored carotenoids. The concentrations ranged from 0.294 to 11.033 µg/ml.

Within the colored carotenoids (Figure 30b), three groups were identified: free xanthophylls, esterified xanthophylls and carotenes. The first group included the polar carotenoids, the two last non-polar carotenoids. For some cultivars like 'Tipala', 'Liegels Gelbe' and 'Haroma', the concentration of non-polar carotenoids was higher than those of the polar metabolites. In 'Katinka', 'Colora', 'Goldzwetschge' and 'Mirabelle Wallenberg' fruits, polar carotenoids predominated.

The carotene concentrations of the cultivars are shown in Figure 31a. The cultivar with the highest concentration of carotenes was 'Tipala' (9.900 µg/ml), followed by 'Liegels Gelbe' (6.630 µg/ml). The lowest concentration was detected in 'Angelina Burdette' (0.131 µg/ml). These differences were statistically significant (Table 18, Supplementary material 5). The major compound within the carotenes was ß-carotene with a mean value of 2.439 µg/ml. The cultivar with the highest content of ß-carotene was 'Tipala' (8.137 µg/ml), followed by 'Liegels Gelbe' (4.688 µg/ml). 'Angelina Burdette' had the lowest concentration (0.062 µg/ml). The other cultivars accumulated low to average concentrations of this compound.

Free xanthophylls represented more than 50% of the total xanthophylls in 'Angelina Burdette', 'Hanka', 'Katinka', 'Zwintschers Frühe' and 'Mirabelle Wallenberg' (Figure 31b). In 'Colora' the amount of free and esterified xanthophylls was almost identical (51% and 49% respectively). In 'Angelina Burdette', no esterified xanthophylls could be detected. In contrast, in 'Haroma', 'Goldzwetschge', 'Liegels Gelbe' and 'Tipala' esterified xanthophylls were more abundant than the free ones. Of the free xanthophylls, lutein was the most important compound in terms of quantity with an average concentration of all cultivars of 1.037 µg/ml. This compound was also the second most abundant of all colored carotenoids after ß-carotene. The cultivar with the highest amount of lutein was 'Mirabelle Wallenberg' (2.378 µg/ml) followed by 'Hanka' (1.836 µg/ml), 'Zwintschers Frühe' (1.857 µg/ml) and 'Katinka' (1.575 µg/ml). The cultivars with the lowest concentration of lutein were 'Haroma' (0.027 µg/ml) and 'Angelina Burdette' (0.118 µg/ml). The other free xanthophylls showed a mean concentration range from 0.004 µg/ml (C13) to 0.093 µg/ml (C10). Within the esterified xanthophylls, the most important compound in terms of quantity was C34 (mean concentration 0.2 µg/ml) and the least one was C38 (mean: 0.014 µg/ml).

a.

b.

c.

d.

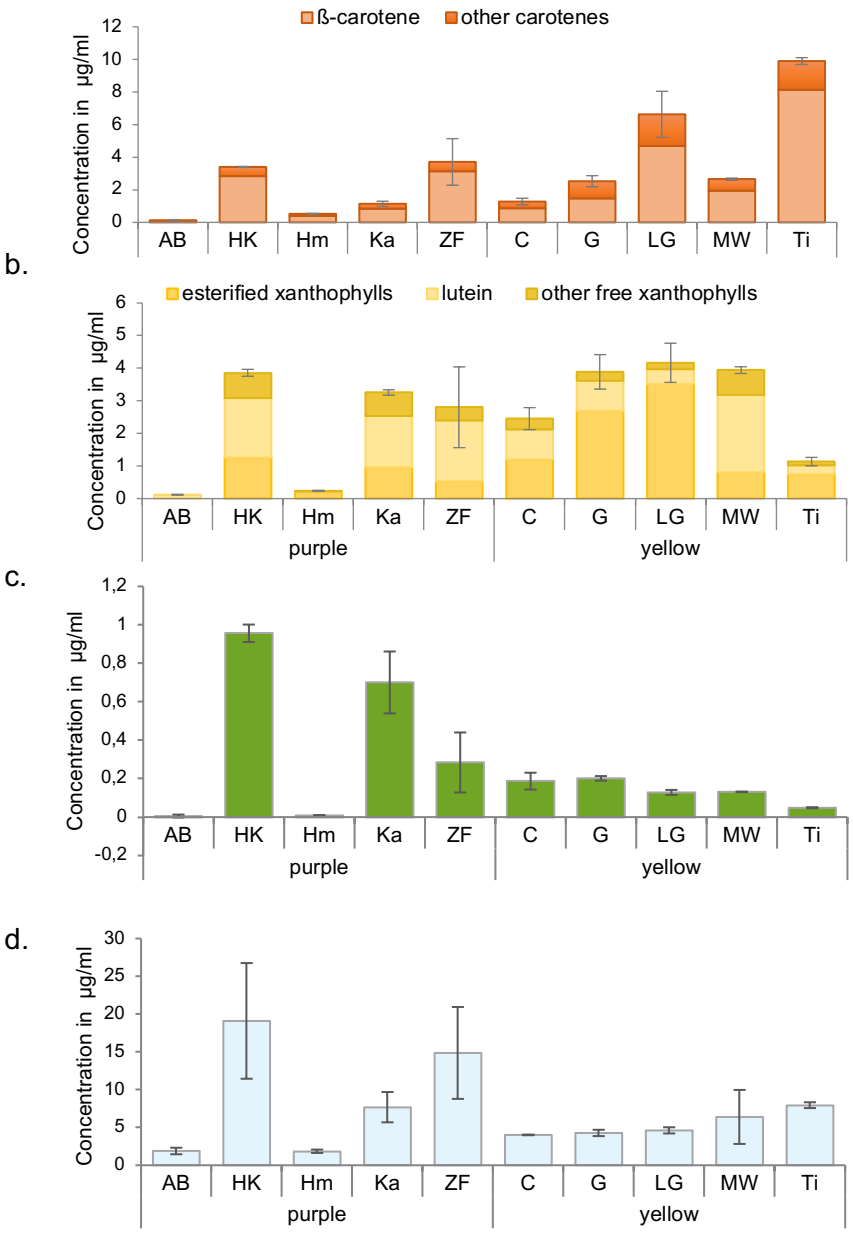


Figure 31: Concentrations and composition of carotenes (a), xanthophylls (b), chlorophylls (c), and other colorless compounds (d) of ten *P. domestica* cultivars harvested in 2014.

The error bars represent the standard deviations of the sum of all compounds. Analysis of statistical significance is displayed in Table 18. **Cultivars** in the first row of the x-axis, AB: 'Angelina Burdette', Hk: 'Hanka', Hm: 'Haroma', Ka:' Katinka', ZF: 'Zwintschers Frühe', C: 'Colora', G: 'Goldzwetschge', LG: 'Liegels Gelbe', MW: 'Mirabelle Wallenberg', and Ti: 'Tipala'. **Fruit skin color** is shown in the second row of the x-axis. Further information about the cultivars are displayed in Table 7.

Other colorless compounds could not be structurally identified, but they were detected in considerable amounts from 1.890 µg/ml to 19.103 µg/ml (Figure 31d). The cultivar that presented the highest concentration of these compounds was 'Hanka' (19.103 µg/ml) followed by 'Zwintschers Frühe' (14.864 µg/ml). 'Haroma' (1.850 µg/ml)

and ‘Angelina Burdette’ (1.885 µg/ml) contained the lowest concentrations of these compounds.

Finally, chlorophylls were detected at concentrations from 0.005 µg/ml (‘Angelina Burdette’) and 0.008 µg/ml (‘Haroma’) to 0.956 µg/ml (‘Hanka’) and 0.700 µg/ml (‘Katinka’) (Figure 31c).

5.3.4. Storage stability of the carotenoids

Fruit pulp from fruits of four cultivars, ‘Hanka’, ‘Katinka’, ‘Zwintschers Frühe’ and ‘Mirabelle Wallenberg’, harvested in the years 2012, 2013, 2014 and 2015 were stored at -20°C and analyzed in the year 2015. This experiment was performed to determine the storage stability of the carotenoids.

Samples stored for a prolonged period contained lower levels of carotenoids (Figure 32). One exception were free xanthophylls of ‘Mirabelle Wallenberg’ in the years 2014 and 2015. The concentration was higher in the sample of 2014.

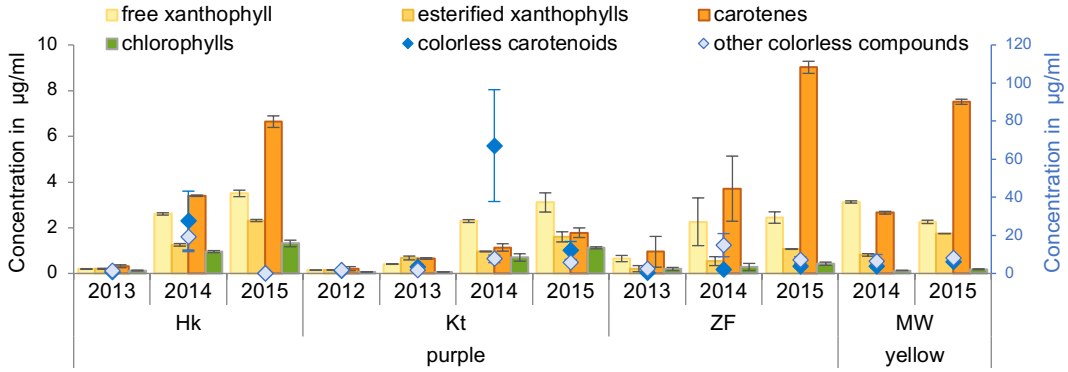


Figure 32: Mean concentrations of total carotenoids, colored and colorless carotenoids from fruits of different cultivars and different harvest years.

The error bars represent the standard deviations. Values of colorless carotenoids and other colorless compounds are plotted with different scale (right axis) than the rest of the compounds (left axis). Mean concentrations of total carotenoids (a), colored (b) and colorless carotenoids (c). **Cultivars** in the first row of the X-axis, Hk: ‘Hanka’, Ka:’ Katinka’, ZF: ‘Zwintschers Frühe’ and MW: ‘Mirabelle Wallenberg’. **Fruit skin color** is shown in the last row of the x-axis. Further information about the cultivars are displayed in Table 7.

5.3.5. Carotenoid concentrations in cultivars of *P. domestica* in 2016

The cultivar with the highest concentration of total carotenoids was ‘Tipala’ (7.413 µg/g) followed by ‘Colora’ (6.232 µg/g) and ‘Zwintschers Frühe’ (6.387 µg/g) (Figure 33). The lowest concentration was detected in ‘Presenta’ (0.365 µg/g) and ‘Haferpflaume’ (0.671 µg/g). The mean concentration of all cultivars was 2.961 µg/g. Looking at possible statistically significant differences (Table 20, Supplementary material 5), no clear separation of groups was found. Mean concentration of colorless carotenoids was 0.990 µg/g for carotenes 1.299 µg/g and xanthophylls 0.672 µg/g (Figure 33). For other colorless compounds and chlorophylls, the mean concentrations

were 3.293 µg/g and 0.090 µg/g, respectively. Colorless carotenoids and other colorless compounds were the most abundant in most cultivars.

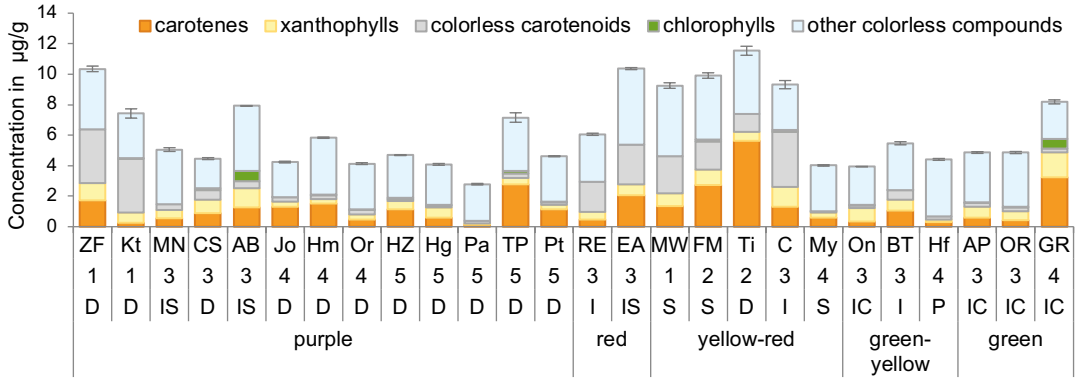


Figure 33: Mean concentrations and composition of total carotenoids including chlorophylls, colorless carotenoids and other colorless compounds in fruits harvested in 2016.

The error bars represent the standard deviations of the concentration of total polar compounds (carotenoids, chlorophylls and other colorless compounds). Analysis of statistical significance is displayed in Table 20. **Cultivars** in the first row of the x-axis, ZF: 'Zwintschers Frühe', Ka:' Katinka', MN: 'Maria Novella', CS: 'Cacaks Schöne' AB: 'Angelina Burdette', Jo: 'Jojo', Hm: 'Haroma', Or: 'Ortenauer', HZ: 'Hauszwetschge Schüfer', Hg: 'Haganta', Pa: 'Presenta', TP: 'Tophit Plus', Pt: 'President', RE: 'Rote Eierpflaume Blazek', EA: 'Eibenbacher Aprikosenpflaume', MW: 'Mirabelle Wallenberg', FM: 'Frühe Mirabelle P-2778', Ti: 'Tipala', C: 'Colora', My: 'Mirabelle aus Nancy 1510', On: 'Ontariopflaume', BT: 'Belle de Thuin', Hf: 'Haferpflaume', AP: 'Aprikoosprium', Ou: 'Oullins Reneklode' and GR: 'Grosse Grüne Reneklode'. **Harvest period** in the second row: (1) 15-31 July; (2) 1-15 August; (3) 16-30 August; (4) 1-15 September and (5) 16-31 September. **Subspecies** in the third row of the x-axis, D: *domestica*, IS: *italica var. subrotunda*, S: *syriaca*, I: *intermedia var. ovoidea*, IC *italica var. claudiana*, P: *prisca*. **Fruit skin color** is shown in the last row of the x-axis. Further information about the cultivars are displayed in Table 7.

Cultivar 'Mirabelle aus Nancy' (0.079 µg/g) contained the lowest concentration of colorless carotenoids. The highest concentration was found in 'Colora' (3.631 µg/g), 'Zwintschers Frühe' (3.531 µg/g) and 'Katina' (3.521 µg/g). The only yellow-red skinned cultivar that accumulated low concentrations of colorless carotenoids was 'Mirabelle aus Nancy'. Both red skinned cultivars analyzed contained a high concentration of colorless carotenoids. Most purple skinned cultivars had a low concentration of colorless carotenoids with the exception of 'Katinka' and 'Zwintschers Frühe'. In addition, cultivars with yellow-green and green colored fruit skin produced low concentrations of these compounds.

On the other hand, colored carotenoid concentration ranged from 0.025 µg/g in 'Presenta' to 6.209 µg/g in 'Tipala' with a mean concentration of 1.971 µg/g. Cultivars that showed higher levels of total carotenoids, had also higher concentrations of carotenes and free xanthophylls. The only exceptions were 'Tophit Plus' and 'Tipala' that showed high levels of carotenes but low contents of xanthophylls.

As in the 2014 samples, the main compound within the carotenes was ß-carotene. (mean concentration: 0.850 µg/g, ranging from 0.065 to 4.580 µg/g) and within the free xanthophylls it was lutein (mean concentration: 0.394 µg/g, ranging from 0.044 to

0.940 µg/g). The cultivar with the lowest concentration of both carotenoids was ‘Presenta’. The highest concentration of ß-carotene was found in the cultivar ‘Tipala’, whereas the highest amount of lutein was detected in ‘Angelina Burdette’.

The total level of other colorless compounds varied only slightly. The mean concentration of all cultivars was 3.293 µg/g. Cultivar ‘Cacaks Schöne’ contained the lowest concentration (1.951 µg/g) and ‘Eibenbacher Aprikosenpflaume’ the highest (5.007 µg/g). The mean concentrations of chlorophylls was 0.090 µg/g, ranging from 0 to 0.685 µg/g. Two cultivars showed a high amount of chlorophylls: ‘Angelina Burdette’ (0.630 µg/g) and ‘Große Grüne Reneklode’ (0.685 µg/g).

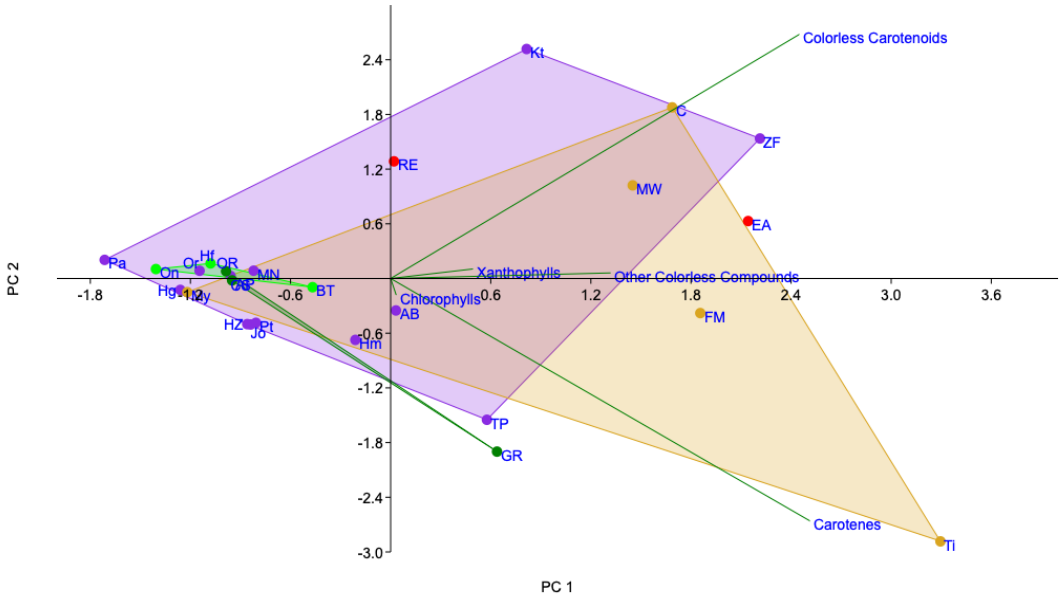


Figure 34: PCA of the cultivars and concentrations of carotenoid groups, other colorless compounds and chlorophylls of cultivars grouped by their fruit skin color.

The first two principal components are displayed in the biplot. Color of the markers and convex hulls represent the color of **fruit skin color**: purple, red, yellow-red, green-yellow and green. Green lines represent the vectors conforming the biplot. **Cultivar** abbreviations, ZF: ‘Zwintschers Frühe’, Ka:’ Katinka’, MN: ‘Maria Novella’, CS: ‘Cacaks Schöne’ AB: ‘Angelina Burdette’, Jo: ‘Jojo’, Hm: ‘Haroma’, Or: ‘Ortenauer’, HZ: ‘Hauszwetschge Schüfer’, Hg: ‘Haganta’, Pa: ‘Presenta’, TP: ‘Tophit Plus’, Pt: ‘President’, RE: ‘Rote Eierpflaume Blazek’, EA: ‘Eibenbacher Aprikosenpflaume’, MW: ‘Mirabelle Wallenberg’, FM: ‘Frühe Mirabelle P-2778’, Ti: ‘Tipala’, C: ‘Colora’, My: ‘Mirabelle aus Nancy 1510’, On: ‘Ontariopflaume’, BT: ‘Belle de Thuin’, Hf: ‘Haferpflaume’, AP: ‘Aprikoosprium’, Ou: ‘Oullins Reneklode’ and GR: ‘Grosse Grüne Reneklode’. Further information of the cultivars can be found in Table 7.

A PCA was performed with all compounds grouped in the subcategories colorless carotenoids, carotenes, xanthophylls, chlorophylls and other colorless compounds (Figure 34, Figure 36 and Figure 38). PC1 explained 51% of the variance and PC2 34%, with colorless carotenoids and carotenes as the compound groups with the highest contribution to both principal components. In addition, boxplot analyses were done with the cultivars grouped by skin color, subspecies and harvest period (Figure 35, Figure 37 and Figure 39).

In Figure 34, the convex hulls represent the color of the fruit skin. Most of the samples were tightly grouped on the left part of the plot with similar concentrations of all compounds. This was also observed in the boxplot analysis (Figure 35). The widest spreads of samples were found in purple and yellow skinned fruit samples (Figure 34). In purple skinned fruits, this was mainly due to the broad range of concentrations of colorless carotenoids (including outliers). In yellow-red skinned fruits not only colorless carotenoids but also carotenes (including outliers) showed a large concentration variation (Figure 35). Within this group 'Zwintschers Frühe' was an upper outlier for total carotenoids and colored carotenoids and it was an extreme upper outlier for colorless carotenoids. 'Katinka' was also an extreme outlier for colorless carotenoids. For colored carotenoids and carotenes 'Tophit Plus' was an upper outlier. 'Angelina Burdette' was an upper outlier for xanthophyll concentration and an extreme upper outlier for chlorophylls. 'Mirabelle aus Nancy' was a lower outlier for total carotenoids and 'Tipala' was an upper outlier for colored carotenoids and carotenes. Green and green yellow skinned cultivars were grouped, where most of the samples are, at the left part of the plot near PC1 axis (Figure 34) and do not differ from the other categories for most analyzed compounds (Figure 35). Green skinned cultivars (Figure 35f), showed the highest concentration of chlorophyll and the widest concentration range of all fruit skin color categories. Red skinned samples were found in the upper right part of the biplot (Figure 34). 'Eibenbacher Aprikosenpflaume' had higher concentrations of carotenoids than 'Rote Eierpflaume Blazek' (Figure 35a-e). Only green skinned cultivars showed high concentrations and high variability of the concentration of chlorophylls.

Figure 36 presents a PCA plot of all carotenoid groups, chlorophylls and other colorless compounds from samples organized by their subspecies. The largest convex hull was the one of *subsp. domestica* (*D)*, which intercepted almost all samples from the other groups. This shows that there are no major differences between the groups, as found in the boxplot analysis. (Figure 37). The cultivars 'Zwintschers Frühe and 'Katinka' were extreme upper outliers for colorless carotenoids and 'Tipala' was an extreme upper outlier for colored carotenoids and carotenes. Most of the samples of the *subsp. italica var. claudiana* (*IC)* were grouped on the left part of the biplot (Figure 36) as they contained lower concentrations of colorless carotenoids than the other subspecies (Figure 37b). Samples of *subsp. italica var. subrotunda* (*IS)* and *subsp. intermedia var. ovoidea* (*I)* were located in the center and upper part of the biplot (Figure 36), tending to higher concentrations of colorless carotenoids than carotenes (Figure 37b and d). Cultivars of *subsp. syriaca* (*S)* were clustered on the right of the biplot (Figure 36).

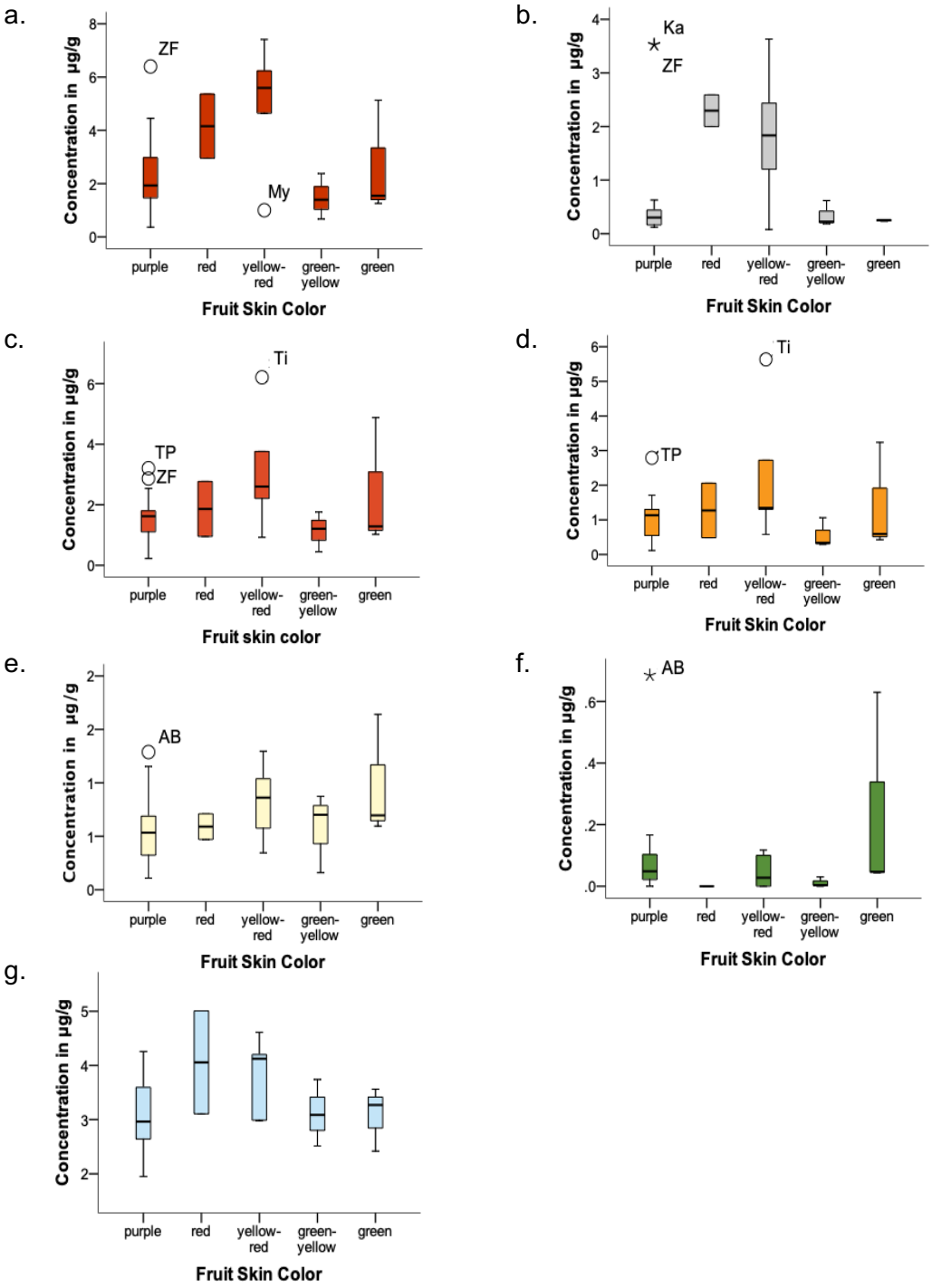


Figure 35: Boxplots of the concentrations of total carotenoids and carotenoid groups in fruits of *P. domestica* cultivars grouped by the color of the fruit skin.
a. Total carotenoids, b. colorless carotenoids, c. colored carotenoids, d. carotenes, e. xanthophylls, f. chlorophylls and g. other colorless compounds. In the boxplots, empty circle (○) shows an outlier and a star (✳) shows extreme outliers. Outlier cultivar names, ZF: 'Zwintschers Frühe', My: 'Mirabelle aus Nancy', Ka: 'Katinka', Ti: 'Tipala', Ou: 'Oullins Reneklode', TP: 'Tophit Plus' and AB: 'Angelina Burdette'. Further information about these cultivars can be found in Table 7.

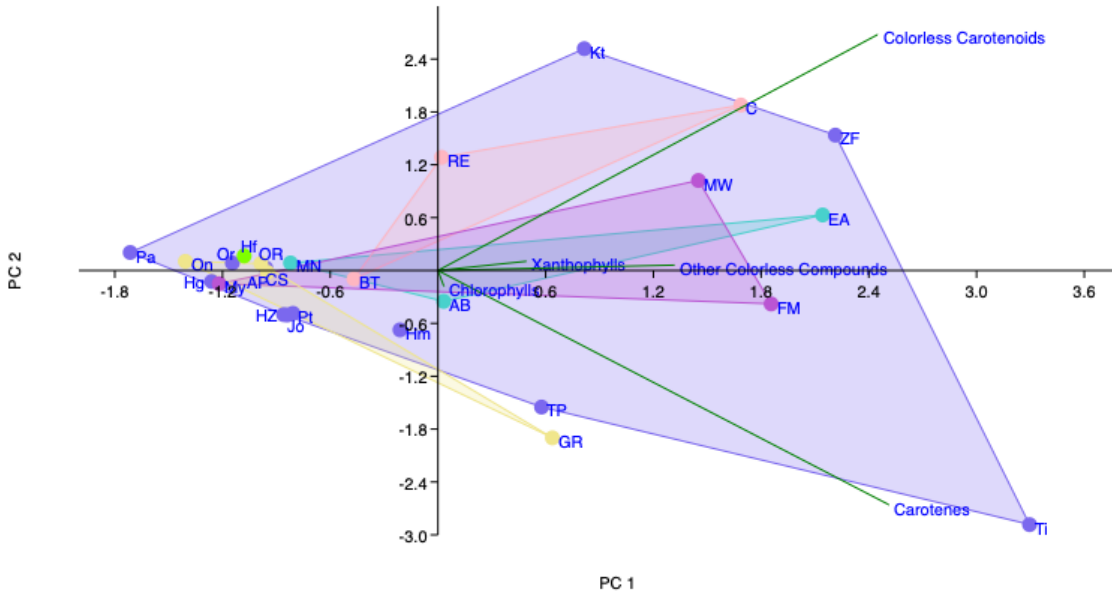


Figure 36: PCA of the concentrations of carotenoid subgroups, colorless compounds and chlorophylls grouped by subspecies.

The first two principal components are displayed in the biplot. Color of the markers and convex hulls represent a **subspecies**: ■: subsp. *domestica,* ■: subsp. *italica var. subrotunda*, ■: subsp. *italica var. claudiana*, ■: subsp. *syriaca*,■: subsp. *intermedia var. ovoidea*,■: subsp. *prisca*. Green lines represent the vectors conforming the biplot. **Cultivar** abbreviations: ZF: 'Zwintschers Frühe', Ka:' Katinka', MN: 'Maria Novella', CS: 'Cacaks Schöne' AB: 'Angelina Burdette', Jo: 'Jojo', Hm: 'Haroma', Or: 'Ortenauer', HZ: 'Hauszwetschge Schüfer', Hg: 'Haganta', Pa: 'Presenta', TP: 'Tophit Plus', Pt: 'President', RE: 'Rote Eierpflaume Blazek', EA: 'Eibenbacher Aprikosenpflaume', MW: 'Mirabelle Wallenberg', FM: 'Frühe Mirabelle P-2778', Ti: 'Tipala', C: 'Colora', My: 'Mirabelle aus Nancy 1510', On: 'Ontariopflaume', BT: 'Belle de Thuin', Hf: 'Haferpflaume', AP: 'Aprikoosprium', Ou: 'Oullins Reneklode' and GR: 'Grosse Grüne Reneklode'. Further information of the cultivars can be found in Table 7.

This difference could not be confirmed by the boxplot analysis (Figure 37). The concentration of chlorophylls was similar in the subspecies, only *IC* and *IS* had higher concentrations and showed a larger variability (Figure 37f). Subspecies *D, I* and *IC* had lower concentrations of other colorless compounds than *IS, P* and *S* (Figure 37g).

Samples that were harvested early in the season had higher concentrations of total carotenoids than the samples harvested later (Figure 38). This was confirmed in the boxplot analysis (Figure 39a) and was mainly due to high concentrations of colorless carotenoids especially in harvest period 1 (Figure 39b) and to a lesser extent of high concentrations of xanthophylls (Figure 39e). On the other hand, samples of harvest period 2 had higher concentrations of total carotenoids mainly because of their high concentrations of carotenes (Figure 39d). The samples from harvest group 3 showed the largest convex hull and therefore it was the group with the largest variability, due to the variability of the concentrations of the colorless carotenoids (Figure 38 and Figure 39b).

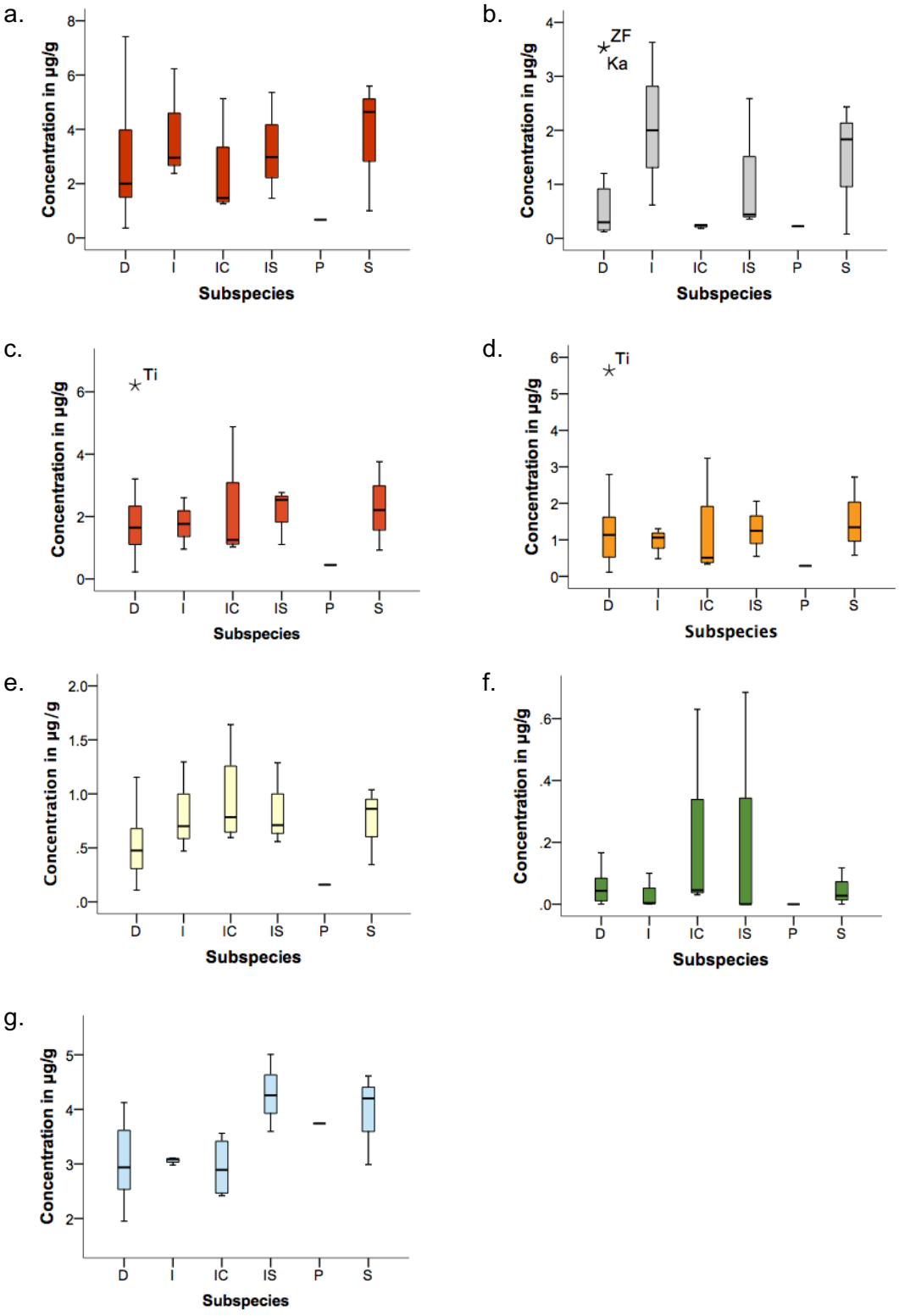


Figure 37: Boxplots of the concentrations of total carotenoids and carotenoid groups in fruits of *P. domestica* cultivars grouped by subspecies.
a. Total carotenoids, b. colorless carotenoids, c. colored carotenoids, d. carotenes, e. xanthophylls, f. chlorophylls and g. other colorless compounds. In the boxplots, a star (∗) shows extreme outliers. Outlier cultivar names, ZF: 'Zwintschers Frühe', Ka: 'Katinka' and Ti: 'Tipala'. Further information about these cultivars can be found in Table 7.

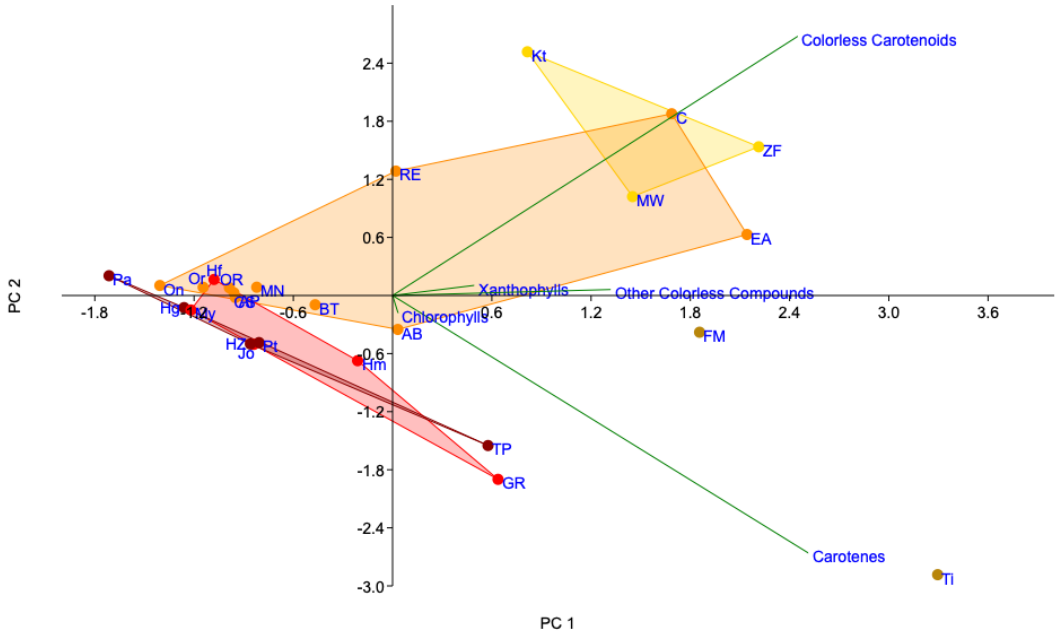


Figure 38: PCA of the cultivars and concentrations of carotenoid subgroups, other colorless compounds and chlorophylls of cultivars grouped by harvest period

The first two principal components are displayed in the biplot. Color of the markers and convex hulls represent a **harvest period**: harvest period 1 (15-31 July), harvest period 2 (1-15 August), harvest period 3 (16-30 August), harvest period 4 (1-15 September) and harvest period 5 (16-31 September). Green lines represent the vectors conforming the biplot. **Cultivar** abbreviations: ZF: 'Zwintschers Frühe', Ka:' Katinka', MN: 'Maria Novella', CS: 'Cacaks Schöne' AB: 'Angelina Burdette', Jo: 'Jojo', Hm: 'Haroma', Or: 'Ortenauer', HZ: 'Hauszwetschge Schüfer', Hg: 'Haganta', Pa: 'Presenta', TP: 'Tophit Plus', Pt: 'President', RE: 'Rote Eierpflaume Blazek', EA: 'Eibenbacher Aprikosenpflaume', MW: 'Mirabelle Wallenberg', FM: 'Frühe Mirabelle P-2778', Ti: 'Tipala', C: 'Colora', My: 'Mirabelle aus Nancy 1510', On: 'Ontariopflaume', BT: 'Belle de Thuin', Hf: 'Haferpflaume', AP: 'Aprikoosprium', Ou: 'Oullins Reneklode' and GR: 'Grosse Grüne Reneklode'. Further information of the cultivars can be found in Table 7.

Eibenbacher Aprikosenpflaume' and 'Colora' were upper outliers for total carotenoids (Figure 39a). The cultivar 'Angelina Burdette' was an extreme upper outlier for the chlorophyll concentration. 'Eibenbacher Aprikosenpflaume' was an upper outlier for other colorless compounds and 'Cacaks Schöne' was a lower outlier for this compound group (Figure 39f-g). The lowest concentrations of all compound groups were detected in samples of harvest period 4 and 5 (Figure 38 and Figure 39). Within harvest period 4, the cultivar 'Grosse Grüne Reneklode' was an upper outlier for total carotenoids and carotenes and an extreme upper outlier for colored carotenoids, xanthophylls and chlorophylls. In addition, 'Mirabelle aus Nancy' was a lower outlier for colorless carotenoids and 'Haferpflaume' was a lower outlier for xanthophylls. For harvest period 5, the cultivar 'Tophit Plus' was an extreme upper outlier for total carotenoids, colorless carotenoids, colored carotenoids and carotenes and it was an upper outlier for other colorless compounds. 'Presenta' was a lower outlier for total carotenoid concentration and for colored carotenoids (Figure 39). The chlorophyll concentration was similar at all harvest periods (not considering the outliers) and other colorless carotenoids tend to decrease during the harvest season (Figure 39f-g).

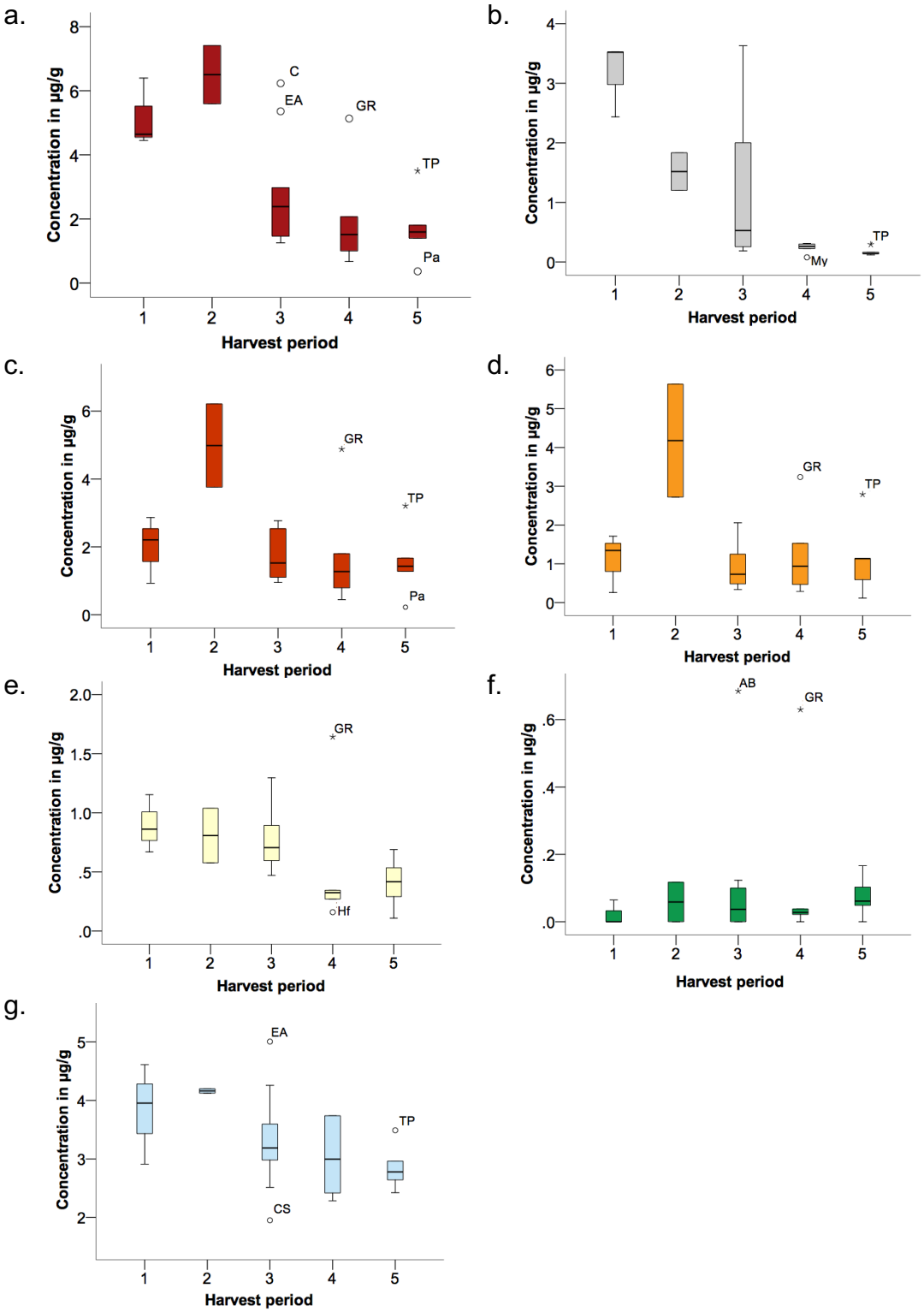


Figure 39: Boxplots of the concentrations of total carotenoids and carotenoid groups in fruits of *P. domestica* cultivars grouped by harvest period.

a. Total carotenoids, b. colorless carotenoids, c. colored carotenoids, d. carotenes, e. xanthophylls, f. chlorophylls and g. other colorless compounds. Harvest period category: harvest period 1 (15-31 July), harvest period 2 (1-15 August), harvest period 3 (16-30 August), harvest period 4 (1-15 September) and harvest period 5 (16-31 September). In the boxplots, a star (∗) shows extreme outliers. Outlier cultivar names, C: 'Colora', EA: 'Eibenbacher Aprikosenpflaume', GR: 'Grosse Grüne Reneklode', TP: 'Topend Plus', Pa: 'Presenta', My: 'Mirabelle aus Nancy', Hf: 'Haferpflaume', AB: 'Angelina Burdette', CS: 'Cacaks Schöne' Further information about these cultivars can be found in Table 7.

5.4. Correlation of the content of sugars, phenolic compounds and carotenoids in plum samples from the 2016 harvest

Pearson correlation was calculated for possible combinations between the following factors (Table 8): individual sugars (fructose, sucrose, glucose, sorbitol and S01), compound groups (monosaccharides, disaccharides and sugar alcohols) and total sugar concentrations. For carotenoids and phenolic compounds no single compounds were included for the calculations, only compound group concentrations. In Table 8, all green colored values show positive correlations and red colored values represent negative correlations. Light green and light red marker values had a significance 0.05 while for darker colors the significance was 0.01.

In order to define which compound groups were determinant to differentiate between cultivars, a PCA was performed using the converted values for individual compounds in the case of sugar (sucrose, glucose, fructose, sorbitol, S01), and compound subgroups for phenolics (anthocyanin, flavan-3-ols/PAs, flavonol, hydroxycinnamic acids, flavones, ONT01, other phenolic compounds) and carotenoids (xanthophylls, carotenes, colorless carotenoids, other colorless compounds and chlorophylls) (Figure 40). Instead of using the concentration values of each compound the percentage quantity ratio of each metabolite was used. This data conversion was done to normalize the data, because concentration ranges between compound groups (sugars, phenolic compounds and carotenoids) were different on many folds. Using the concentration for this analysis would overestimate the importance of sugars because they were highly concentrated in all cultivars, although they do not show big concentration differences between cultivars. PC1 explained 49% of the variance with flavan-3-ols/PAs, anthocyanins and chlorophylls as the factors with the major contribution. On the other hand, PC2 explained 20% of the variation with ONT01 as the most determinant factor. In this analysis the most cultivars appeared tightly grouped within the 95% of variance. Nevertheless, some cultivars were distributed outside the 95% of variance: ‘Angelina Burdette’ in the vector direction of anthocyanins and flavan-3-ols/PAs (PC1), ‘President’ and ‘Grosse Grüne Reneklode’ in the vector direction of ONT01 (PC2). ‘Mirabelle Wallenberg’ was not grouped with the majority of cultivars, but still within the 95% of variance.

Table 8: Pearson correlation values for compound subgroups, compound groups and total amounts of major groups in samples from 2016 harvest.

	sucrose	fructose	glucose	S01	sorbitol	disaccharides	monosaccharides	sugar alcohols	total soluble sugar	anthocyanins	ONT01	flavan-3-ols	flavones	flavonol	hydroxycinnamic acids	other phenolic compounds	total phenolic compounds	xanthophylls	carotenes	colorless carotenoids	chlorophylls	other colorless compounds	total carotenoids
fructose	**-.541****																						
glucose	**-.563****	**.899****																					
S01	,344	-,271	-,262																				
sorbitol	-,058	,275	**.481***	,121																			
disaccharides	**1.000****	**-.541****	**-.563****	,344	-,058																		
monosaccharides	**-.568****	**.963****	**.984****	-,272	**.408***	**-.568****																	
sugar alcohols	-,024	,244	**.448***	,217	**.995****	-,024	,375																
total soluble sugar	**.468***	,308	**.399***	,214	**.701****	**.468***	,371	**.711****															
anthocyanins	-,066	,101	,246	-,142	**.449***	-,066	,192	**.427***	,278														
ONT01	,006	,134	,136	-,137	**.431***	,006	,139	**.411***	,296	,079													
flavan-3-ols	-,035	,056	,247	-,231	,307	-,035	,174	,279	,222	**.737****	-,002												
flavones	-,238	,289	,262	,066	,173	-,238	,279	,177	,069	-,186	,146	-,347											
flavonol	-,334	,291	,211	-,177	-,088	-,334	,249	-,104	-,169	-,032	,028	-,073	**.549****										
hydroxycinnamic acids	-,197	,140	,165	,092	,312	-,197	,159	,316	,087	,243	**.604****	,108	,357	,196									
other phenolic compounds	,213	,049	-,066	,334	,036	,213	-,020	,068	,204	-,260	,189	-,381	**.544****	**.444***	,157								
total phenolic compounds	-,178	,182	,216	,078	,364	-,178	,208	,366	,161	,362	**.576****	,229	**.414***	,339	**.963****	,292							
xanthophylls	,193	-,129	-,103	,076	,080	,193	-,117	,086	,128	,215	,225	,388	-,127	-,386	,187	,126	,228						
carotenes	-,058	,015	,043	-,290	,160	-,058	,032	,129	,033	-,072	,238	-,001	,003	-,199	-,075	-,071	-,102	,307					
colorless carotenoids	-,154	,034	-,100	-,258	**-.511****	-,154	-,047	**-.528****	**-.417***	-,066	-,118	,024	-,210	-,019	-,128	-,119	-,143	**.408***	,138				
chlorophylls	,158	,056	,196	,010	**.673****	,158	,144	**.663****	**.550****	**.579****	,346	**.608****	-,092	-,263	,177	,034	,282	**.638****	,243	-,161			
other colorless compounds	-,148	-,164	-,097	**-.557****	-,261	-,148	-,127	-,311	-,363	,187	-,047	,382	-,189	,125	-,245	-,348	-,195	,102	,362	,379	-,036		
total carotenoids	-,090	,005	-,053	-,312	-,192	-,090	-,031	-,219	-,203	-,042	,115	,086	-,146	-,203	-,086	-,089	-,103	**.612****	**.737****	**.751****	,170	**.459***	
total color carotenoids	,002	-,022	,009	-,235	,163	,002	-,004	,137	,064	-,004	,271	,106	-,032	-,281	-,015	-,028	-,027	**.546****	**.965****	,234	**.390***	,346	**.818****

Green colors show positive correlations and red color negative correlations. Levels of significance: ** =0.01; *=0.05.

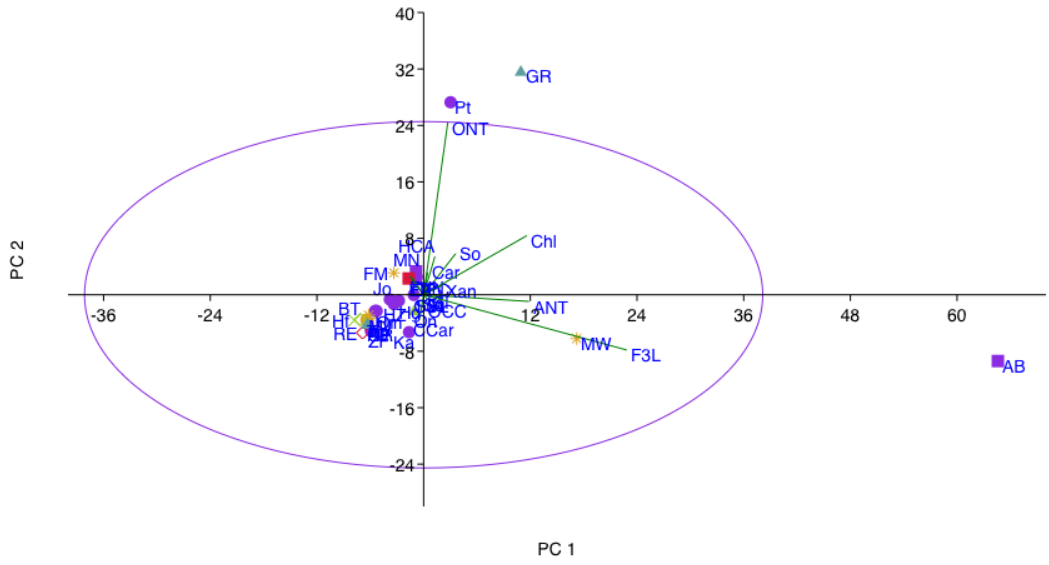


Figure 40: PCA percentage index of sugars, phenolic compounds and carotenoid subgroups of the 26 cultivars from 2016 harvest.

Color of the markers represent the color of **fruit skin color**. The form of the marker represents the **subspecies**: ●:subsp. *domestica*, ■:subsp. *italica var. subrotunda*, ▲:subsp. *italica var. claudiana*, ✷:subsp. *syriaca*, ◊: subsp. *intermedia var. ovoidea*, X: subsp. *prisca*. Green lines represent the vectors conforming the biplot and purple oval groups 95% of the variance. **Cultivars** on the first row of the X-axis, ZF: 'Zwintschers Frühe', Ka:' Katinka', MN: 'Maria Novella', CS: 'Cacaks Schöne' AB: 'Angelina Burdette', Jo: 'Jojo', Hm: 'Haroma', Or: 'Ortenauer', HZ: 'Hauszwetschge Schüfer', Hg: 'Haganta', Pa: 'Presenta', TP: 'Tophit Plus', Pt: 'President', RE: 'Rote Eierpflaume Blazek', EA: 'Eibenbacher Aprikosenpflaume', MW: 'Mirabelle Wallenberg', FM: 'Frühe Mirabelle P-2778', Ti: 'Tipala', C: 'Colora', My: 'Mirabelle aus Nancy 1510', On: 'Ontariopflaume', BT: 'Belle de Thuin', Hf: 'Haferpflaume', AP: 'Aprikoosprium', Ou: 'Oullins Reneklode' and GR: 'Grosse Grüne Reneklode'. Compounds and compound group abbreviation: Su: sucrose, Fr: fructose, Gl: glucose, S01: unknown sugar alcohol, So: sorbitol, ANT: anthocyanins, ONT: unknown yellow compound ONT01, F3L: flavan-3-ols/proanthocyanidins, FVN: flavones, FVL: flavonols, HCA: hydroxycinnamic acids, OPC: other phenolic compounds, Xan: xanthophylls, Car: carotenes, CCar: colorless carotenoids Chl: chlorophyll and OCC: other colorless compounds. Further information of the cultivars can be found in Table 7.

6. Discussion

6.1. Phenolic acids and flavonoids in plum fruits

Various studies have already been conducted on the quality and quantity of different phenolic compounds in *P. domestica*. These studies analyzed different parts of the fruit such as the skin (Khallouki et al., 2012; Nunes et al., 2008; Raynal et al., 1989; Treutter et al., 2012; Usenik et al., 2013), the flesh (Jaiswal et al., 2013; Khallouki et al., 2012; Nunes et al., 2008; Raynal et al., 1989) and the whole fruit (INRA et al., 2013; Lombardi-Boccia et al., 2004; Mattila et al., 2006; Miletic et al., 2013; NAL, 2018; Piga et al., 2003; Rop et al., 2009; Slimestad et al., 2009; Stacewicz-Sapuntzakis, 2013; Treutter et al., 2012; Usenik et al., 2008; Usenik et al., 2009) by means of different methods. The results were given in different units, like mg/100 g fresh weight (FW), mg/kg, mg/kg dry weight (DW), %, mg of gallic acid equivalents (GAE)/gFW, g/kg FW, mg/g DW, mg tannic acid equivalents/100g, μg/g FW and mg/fruit. This makes a comparison between studies almost impossible. A mean concentration of 1,100 µg/g FW total phenolic compounds in whole plums has been previously reported (USDA, 1984 and Stacewicz-Sapuntzakis, 2013). This value is the mean concentration for *P. domestica* and *P. salicina*. The values in the present study ranged from 54.33 µg/g FW to 756.33 µg/g FW, which are lower than those reported by USDA. A later study (Usenik et al., 2013) reported a total phenolics concentration of 500 and 8,900 µg/g FW in the fruit flesh and skin of 'Jojo', respectively and of 1,000 and 3,500 µg/g FW in the flesh and skin of 'Haganta', respectively. The fruit of both cultivars had a purple skin. In the present study the whole fruit pulp was used. Since the fruit samples consisted mainly of pulp, it is justified to compare the concentrations of total phenolic compounds of the pulp samples with those of the fruit flesh. In this case, the values for 'Jojo' were very similar to the values obtained in present study (465 µg/g in fruit flesh and 540 µg/g in fruit pulp), while for 'Haganta' the value was 50% lower in the present study (899 µg/g in fruit flesh and 558.39 µg/g in fruit pulp). Nunes et al. (2008) reported for one yellow skinned cultivar 'Mirabelle', 1,357 µg/g FW in the flesh and 3,981 µg/gFW in the skin and for one green skinned cultivar 'Green Gage' 2,794 µg/gFW in the flesh and 5,685 µg/g FW in the skin. No information about the cultivars was provided. The three 'Mirabelle' cultivars and the four 'Green Gage' cultivars analyzed in present study for the fruit pulp were much lower than the values reported by Nunes et al. (2008). Possible explanations for these differences between studies may be differences in analytical methods, but also the environmental conditions in which the fruit developed may have an impact on the overall polyphenolic concentration.

The main phenolic concentration was similar in both sample sets. The hydroxycinnamic acids were the most abundant group in terms of quantity with neochlorogenic acid as the most predominant compound. The second most important compound group was other phenolic compounds followed by anthocyanins and flavan-3-ol/proanthocyanidins for 2012-2014 samples. In the 2016 sample set, the mean concentration of flavan-3-ol/proanthocyanidins was higher than those of anthocyanins. This might be because 2012-2014 cultivars were mainly purple skinned while in the 2016 sample set more non-anthocyanic cultivars (yellow-red, green-yellow and green skinned cultivars) were included. It can be concluded that in anthocyanin containing fruits, this compound group is the third most abundant compound in terms of quantity followed by flavan-3-ols/proanthocyanidins. In those cultivars with yellow-red, green-yellow or green skinned fruits, flavan-3-ols/proanthocyanidins was the third most important group. The flavonols were in both samples sets the forth most abundant group followed by flavones.

The values for the concentration of hydroxycinnamic acids of the studied cultivars in 2016 were between 24.19 µg/g FW in 'Oullins Reneklode' and 621.07 µg/g FW in 'President'. Usenik et al. (2008) reported higher values of these compounds in fruits of four *P. domestica* cultivars 'Jojo', 'Valor', 'Cacanska rodna' ('Cacaks Fruchtbare' in German) and 'Cacanska najbolja' (`Cacaks Beste' in German) with a concentration in a range of 316 to 1823 µg/gFW. The most abundant compound in almost all the samples was identified as neochlorogenic acid. This was also the only compound of this group that was identified in this study. The following hydroxycinnamic acids have been reported to be present in *P. domestica* whole fruit: neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, p-coumaroyl quinic acid, p-coumaric acid, caffeic acid, ferulic acid, sinapic acid, 3-O-coumaroylquinic acid, feruloyl quinic acid, caffeoyl shikimic acid, 3-feruoylquinic acid, 5-feruoylquinic acid and p-coumaric acid derivates (Jaiswal et al., 2013; Khallouki et al., 2012; Lombardi-Boccia et al., 2004; Miletic et al., 2013; Nunes et al., 2008; Piga et al., 2003; Raynal et al., 1989; Treutter et al., 2012; Usenik et al., 2008; Usenik et al., 2013).

Twenty-eight compounds were classified as other phenolic compounds. The group of early other phenolic compounds GOPC01 was not detected in the 2016 samples. The concentration of these compounds ranged from 59.88 µg/g to 12.99 µg/g. Identification of the metabolites was not performed, but simple phenolic compounds reported for *P. domestica* fruits were vanillic acid, (Mattila et al., 2006), protocatechuic acid and gallic acid (Miletic et al., 2013).

Five anthocyanins were detected in the 2012-2014 and 2016 sample sets. The most abundant compounds were identified as cyanidin 3-O-rutinoside, cyanidin 3-O-

glucoside, peonidin 3-O-rutinoside and peonidin 3-O-glucoside. These four compounds have been previously reported for *P. domestica* (Piga et al., 2003; Slimestad et al., 2009; Treutter et al., 2012; Usenik et al., 2008; Usenik et al., 2013; Usenik et al., 2009). The other anthocyanin detected in this study had a low concentration and could not be identified. Usenik et al. (2009) reported the presence of cyanidin 3-O-xyloside in the fruits. However, they reported that cyanidin 3-O-xyloside co-eluted with cyanidin 3-O-glucoside, therefore, we exclude cyanidin 3-O-xyloside as structure of the unknown anthocyanin.

The concentration of anthocyanins detected in this study ranged from 0 to 86.76 µg/g FW. Reported values for anthocyanins in plums, measured in the whole fruit, ranged from 4.4-328.5 µg/g (Slimestad et al., 2009; Usenik et al., 2013; Usenik et al., 2009). It has been shown that these compounds accumulate in the skin of plums with red and purple skins, in which the concentrations range from 623 to 1627 µg/g FW (Raynal et al., 1989; Treutter et al., 2012; Usenik et al., 2013). They have not been detected or have been detected only in trace amounts in yellow-skinned plums of the 'Mirabelle' variety (Khallouki et al., 2012; Nunes et al., 2008) and 'Miragrande' (Treutter et al., 2012) and in the green skinned cultivar 'Grosse Grüne Reneklode/Green Gage' (Nunes et al., 2008; Treutter et al., 2012). Studies focusing only on the analysis of fruit flesh report no anthocyanins for *P. domestica* cultivars (Jaiswal et al., 2013; Khallouki et al., 2012; Nunes et al., 2008; Usenik et al., 2013). Some fruits analyzed in this study contained anthocyanins in the fruit flesh such as 'Angelina Burdette'.

Highest concentrations were determined for cyanidin 3-O-rutinoside in all cultivars. This agrees very well with literature reports for the whole fruit and the skin. The lowest concentrations in the samples were determined for peonidin 3-O-glucoside in accordance with literature data for whole fruit and skin. The second most abundant anthocyanin in some cultivars was cyanidin 3-O-glucoside and for others it was peonidin 3-O-rutinoside. Previous reports have reported the same (Slimestad et al., 2009; Treutter et al., 2012; Usenik et al., 2008; Usenik et al., 2013; Usenik et al., 2009). 'Angelina Burdette' had the highest concentration of anthocyanin and was an outlier of the purple skinned plums. In this cultivar in the year 2016, the concentration of peonidin-glycosides was relatively low in comparison to cyanidin glycosides. 'Presenta' and 'Rote Eierpflaume Blazek' showed low concentrations of anthocyanins although these cultivars have fruits with purple and red skin. Goldner et al. (2015), compared the phenolic contents detected in different parts of plum fruits. As anthocyanins accumulate in fruit skin but not in the flesh, the anthocyanins present in the skin can be diluted with the flesh when homogenizing the whole fruit. The only cultivars with a light red coloration in the skin of the fruits (yellow-red, green-yellow and green) and detectable concentrations of

anthocyanins were ‘Mirabelle Wallenberg’ in the 2016 sample set and ‘Goldzwetschge’ in 2012-2014. ‘Mirabelle’ and other yellow plums synthetized anthocyanins when the fruit was exposed to direct sunlight. These anthocyanins were diluted in the fruit juice to trace levels and, for this reason, no other yellow-red cultivar showed anthocyanins, although, a red coloration can be observed in some parts of the fruit skin.

Twenty-five flavan-3-ols and proanthocyanidins were detected in 2012-2014 samples and 29 in 2016 samples. Only catechin and epicatechin were identified in both sample sets. Flavan-3-ol and proanthocyanidins previously reported for plum are (-)-catechin, (-)-epicatechin, proanthocyanidin dimers, proanthocyanidin trimers, proanthocyanidin 4-6mers, proanthocyanidin 7-10mers, proanthocyanidin polymers (>10mers), procyanidin dimer B1, procyanidin dimer B2, procyanidin dimer B7, procyanidin dimer B3, procyanidin dimer B4, procyanidin dimer B5, procyanidin trimer ECC, procyanidin trimer C and other unidentified proanthocyanidins. Most of these compounds occur at high concentration in the skin of the fruit (Khallouki et al., 2012; Lombardi-Boccia et al., 2004; Nunes et al., 2008; Slimestad et al., 2009; Treutter et al., 2012; Usenik et al., 2008; Usenik et al., 2013). In this study, catechin showed a higher concentration (0-26.02 µg/g) than epicatechin (0-7.01 µg/g), which is in accordance with reported values for catechin ranging from 15-121 µg/g FW in the flesh (Nunes et al., 2008) and 0-290 µg/g FW in the fruit skin. For epicatechin, the values reported were 0-79 µg/g FW in the flesh and 0-41 µg/gFW in the fruit skin (Nunes et al., 2008; Treutter et al., 2012).

The concentrations of flavonols ranged from 0 to 16.27 µg/g. Other studies reported concentrations of flavonols in plum ranging from 3.01 to 74.26 µg/g whole fruit (Miletic et al., 2013; Piga et al., 2003; Slimestad et al., 2009; Usenik et al., 2008; Usenik et al., 2013). Higher concentrations have been reported for the skin from 110-1,431 µg/g, while in the flesh a concentration of 3 µg/g was found (Khallouki et al., 2012; Lombardi-Boccia et al., 2004; Nunes et al., 2008; Treutter et al., 2012; Usenik et al., 2013). Thirteen flavonol-like compounds were detected in the 2012-2014 sample set nine in 2016. The most abundant compound was identified as rutin (quercetin 3-O-rutinoside) in accordance with literature data. In addition to rutin, other flavonols have been reported for plums: quercetin, myricetin, kaempferol, hyperin (quercetin 3-O-galactoside), isoquercitrin (quercetin 3-O-glucoside), isorhamnetin 3-O-glucoside, isorhamnetin 3-O-rutinoside and an unidentified isorhamnetin-glycoside and two unidentified quercetin-glycosides (Khallouki et al., 2012; Nunes et al., 2008; Slimestad et al., 2009; Treutter et al., 2012; Usenik et al., 2008; Usenik et al., 2013).

Four flavones were identified in 2012-2014 samples (two early and two late flavones) and two in 2016 (one early and one late flavone). The concentration found in the 2016 samples ranged from 1.2 to 124 µg/g. The only study on flavones in the skin of European plums comes from Treutter et al (2012) and showed a range of 2-102 µg/g.

Finally, no reference was found for a compound similar to the yellow unidentified compound ONT 01 in plum. Although this compound was detected in ten cultivars, its concentration ranged only from 0 and 2.9 µg/g. Yellow phenolic compounds described in literature, that might correspond to this compound are chalcones and aurones (Harborne, 1994).

Concentration differences between samples harvested in different years could not be related to sample degradation during storage (Figure 11). As all samples were extracted at a similar date, lower concentrations in older samples were expected, as it was observed for some cultivars. But for other cultivars, fruit samples in 2012 had a higher concentration of total phenolic compounds than in 2013 and 2014 ('Cacaks Schöne'). Fruit samples of nine cultivars from 2013 had a higher concentration than the samples from other harvest years. This might indicate an influence of the climatic conditions on the phenolic content in plums. In some cultivars, fruit samples had higher concentrations of total phenolics and all phenolic groups in 2013 ('Angelina Burdette', 'Tophit Plus', 'Elena' and 'Haroma'). In other cultivars, only the concentrations of total phenolics, flavan-3-ol/proanthocyanidins and anthocyanidins in the fruit pulp were higher in 2013, while the concentrations of other phenolic compound groups were not ('AGRI 2000 10/91'). For some cultivars, concentrations of phenolic compound groups were higher in fruit samples from other harvest years than 2013. For example, the concentrations of flavan-3-ol/proanthocyanidins were higher in samples from 2014 in four cultivars ('Topend Plus', 'Topfive' 'Tophit Plus' and 'Goldzwetsche'). Since the concentrations of metabolites in older samples were generally not lower than the concentrations in the samples of recent harvest years, the degradation of compounds during storage may not be the main factor for the differences between samples from different years.

These differences seem related to different weather conditions between the years. However, climatic fluctuations alone cannot explain the variations in concentration between years, since not all varieties and all compounds in the same harvest year have the highest concentrations. From this it can be concluded that there is a different sensitivity to climatic conditions in each variety and for each group. This suggests a genetic factor that influences the sensitivity of the accumulation of phenolic compounds in each variety to environmental changes.

Such a seasonal effect has already been described by Miletic et al., (2012) for another plum cultivar called 'Stanley'. In this cultivar, different total phenolic and anthocyanin content was found comparing three harvest years (2008 to 2010). The influence of the harvest season on the phenolic concentration and composition has also been reported for other fruits like apples, pears (Kevers et al., 2011) and blackberries (Connor et al., 2002). Apricot varieties were also reported to have different sensitivity to weather conditions with respect to the accumulation of fruit phenols. Leccese et al. (2012) reported a correlation between weather conditions and total phenolic compound accumulation in some cultivars, like 'Pisana', 'San Castrese', 7C 20/3 and 'Dulcinea'. A higher concentration of phenolic compounds was detected in a year with less rainfall at harvest. For other cultivars, like 'Farmingdale' and 'Thyrinthos', no correlation was found with rainfall before harvest. A similar effect of season on phenolic compound concentration was reported in three cultivars of murtilla (*Ugni molinae* Turcz) by Alfaro et al. (2013). These authors reported a significant difference in the accumulation of polyphenols over five years (2006, 2007, 2008, 2009 and 2011) showing a positive correlation between rainfall and phenolic concentration.

A different sensitivity to climatic conditions in different cultivars but also of different compound groups has been reported in four apple cultivars ('Golden Delicious', 'Jonagold', 'Elstar' and 'Cox's Orange') harvested during the years 1997, 1998, and 1999 (van der Sluis 2001). Fruits from the cultivars 'Golden Delicious' and 'Jonagold' showed no significant variation in the concentration of chlorogenic acid, catechin and cyanidin-galactoside between harvest years. On the other side, phlorizin and quercetin glycoside did show seasonal variation. In apples of the cultivar 'Elstar', only phlorizin showed some significant difference over the years. Fruits from the cultivar 'Cox's Orange' were the most sensitive with respect to the year of harvest. The concentration of chlorogenic acid, catechin, quercetin glycoside and phlorizin showed a significant seasonal variation. Some blueberries cultivars showed a significant difference in the concentration of total phenolics, anthocyanins, flavonols and hydroxycinnamic acids between harvest years. In twelve of 18 cultivars studied by Howard et al. (2003), the anthocyanin concentration seemed to be the most sensitive. Other compounds like hydroxycinnamic acids and flavonols showed significant differences in only seven cultivars (Howard et al., 2003). These results indicate that the phenolic concentration and composition in different fruits is affected by climatic conditions during the fruit growing season and by a genetic component.

When the cultivars where grouped by skin color and subspecies in a boxplot analysis, no clear tendency was observed, although some individual differences could be seen. Purple skinned samples showed the largest variation in the concentration of

different compound groups, tending to higher concentrations of hydroxycinnamic acid, while yellow skinned cultivars tend to lower concentrations. Red and purple skinned cultivars tend to have higher concentrations of anthocyanins and flavan-3-ols/proanthocyanidins than yellow-red, green-yellow and green skinned cultivars.

A PCA analysis of samples organized by their subspecies did not shows many differences between them. Most of the differences were in the hydroxycinnamic acids concentrations. The largest variations in concentration of these compounds were *domestica* (*D*) and *italica var. claudiana* (*IC*). An interesting case was the cultivar 'Angelina Burdette', which had a very high concentration of anthocyanins and flavan-3-ol/PAs compared to the other purple skinned cultivars.

Samples that ripened early in the harvest season tend to have lower concentrations of hydroxycinnamic acids and other phenolic compounds than later ones. The range of the flavonol concentration tended to be larger in the late harvest season. Flavan-3-ols/proanthocyanidins were more abundant in terms of quantity in the early harvest season than in all the other harvest periods. As it has been already discussed, these differences might be related to the different environmental conditions during each harvest periods and the differential sensitivity of each cultivar to them.

6.2. Sugars in plum fruits

Sucrose, glucose, fructose and sorbitol were found in plums in the 2012-2014 and 2016 samples. This agrees with published reports for *P. domestica* (Forni et al., 1992; Wilford et al., 1997). In samples from 2016, a fifth compound was detected: S01. Three polyols had a similar retention time: mannitol, myo-inositol and xylitol. As xylitol is the only one of these three polyols that has been reported previously in plums, it was tentatively identified (Mäkinen and Söderling, 1980). Other sugars reported in plums are maltose in fresh fruits (Mäkinen and Söderling, 1980; Richmond et al., 1981) and galactitol in processed fruits (Stacewicz-Sapuntzakis et al., 2001). These compounds were not detected in this study.

The concentrations of each compound in 2012-2014 compared with 2016 were similar. Total sugar concentration ranged from 0.05 to 0.25 g/ml. The concentration of sucrose ranged from 0.001 to 0.012 g/ml; glucose from 0.02 to 0.06 g/ml; fructose 0.005 to 0.05 g/ml and sorbitol from 0.002 to 0.07 g/ml. S01 was detected with a concentration range between 0 and 0.005 g/ml in 2016. Different studies have previously addressed the content of sugars in the European plum. Some studies indicated the sugar content in % of the fresh weight (Bozhkova, 2014; Forni et al., 1992; Richmond et al., 1981;

Wilford et al., 1997). All these studies found similar values for total sugar content (6.96-14.7%), sucrose (2.15-9.82%), glucose (2.02-5.46%), fructose (0.76-5.45%) and sorbitol (1-5.33%). Other studies indicated the concentration of sugars in g/100g of fresh matter (García-Mariño et al., 2008; Stacewicz-Sapuntzakis et al., 2001) or g/100g of dry matter (Miletic et al., 2013) or g/fruit (Rees, 1958).

The sugar types which are accumulated in the ripe fruit depend on the species. Sucrose is higher concentrated in fruits like peach, pineapple while in apple, pears and strawberries the monomers glucose and fructose are more abundant (Vicente et al., 2009). In the case of plums, most studies reported higher concentration of glucose than sucrose in ripe fruits (Table 6) (Forni et al., 1992; García-Mariño et al., 2008; Rees, 1958; Richmond et al., 1981; Usenik and Marn, 2017).

Glucose was always more abundant than fructose (Figure 23), which is in accordance with previous reports for European plum (Forni et al., 1992; García-Mariño et al., 2008; Rees, 1958; Richmond et al., 1981; Stacewicz-Sapuntzakis et al., 2001; Usenik and Marn, 2017).

Wilford et al. (1997) reported that in *P. domestica* the concentration of sorbitol and fructose was similar. In the present study, this fact was confirmed for five of 26 cultivars analyzed in 2016: 'Jojo', 'Ortenauer', 'Eibenbacher Aprikosenpflaume', 'Ontariopflaume' and 'Oullins Reneklode'. The concentration of sorbitol should be considered as an important trace factor for plums production and breeding. It is interesting for the prune elaboration industry as it does not caramelize easily during fruit dehydration resulting in higher sugar content in the end product and, therefore in a sweeter taste (Cinquanta et al., 2002; Wilford et al., 1997). Sugar alcohols in general are also relevant for the food industry as they are processed independently from insulin in humans. As a consequence, they are suited for a diabetic diet (Schiweck et al., 2012). However, it should not be forgotten that sorbitol can be laxative at low doses (70 g/day) (Cinquanta et al., 2002).

Sugar concentrations and profiles in *P. domestica* fruits and other *Prunus* species, have shown to be dependent on climatic conditions. Cultivars and compounds have different sensibilities to the same growing conditions (Bielski, 1982; Dugalic et al., 2014; Rees, 1958). When comparing fruits of the same cultivar growing in different places of the experimental orchard, the concentrations of most soluble sugars show no differences between locations. Only 'Hauszwetschge Schüfer' showed statistically significant differences in the concentration of sorbitol and of fructose between locations. These findings suggest that different cultivars and sugars have different sensitivities to environmental conditions. It has been reported previously that this concentration pattern

does not only depend on the cultivar but also on its sensibility to environmental factors. Fruits of the cultivar 'Sugar' growing in three different places (Bologna, Cagliari and Forli) had not only different amounts of sugars but also the composition was different. For two of them, sucrose was the most abundant sugar in mature fruits. For the third, it was glucose (Forni et al., 1992).

Sorbitol has been described as an important compound in drought or salt stress reactions by facilitating osmotic adjustment of the cells (Lo Bianco et al., 2000; Williamson et al., 2002). Although none of the trees used for this analysis were exposed to drought or salt stress, differences in irrigation and radiation between sites are conceivable. Fructose and sorbitol accumulation appears to be more sensitive to differences in environmental conditions between sites.

The absence of clear correlations of fruit skin color and subspecies with sugar content and composition might be explained by the low number of cultivars for this analysis.

Some differences in sugar concentrations were observed between different harvest periods. Total sugar, sucrose and S01 concentrations tended to increased towards the end of the season. On the other hand, glucose and sorbitol concentrations tended to be higher in cultivars harvested during the mid of the season, than those early or late harvested. Fructose concentration in harvested fruits tended to decrease during the season. The influence of environmental conditions and cultural practices on the accumulation of soluble sugars has also been reported for tomato (Beckles et al., 2011; Nookaraju et al., 2010) and grape (Dai et al., 2011; Kuhn et al., 2013).

6.3. Carotenoids in plum fruits

The most obvious difference between the results of sample sets 2014 and 2016 is the fact that in 2016 the esterified xanthophylls could not be detected due to a change in the chromatographic system. Therefore, 15 esterified xanthophylls were detected in the samples from 2014 but not in samples from 2016. In both samples sets eight carotenes were detected. On the other hand, 13 free xanthophylls in samples of 2014 and 14 in samples of 2016. Considering colorless carotenoids, four phytoene isomers were detected in samples 2014 and three in samples from 2016. In both samples sets two phytoene isomers were detected. Five other colorless compounds and one group of compounds were detected in samples 2014. In 2016, four other colorless compounds were found. Finally, four chlorophylls were detected in samples from 2014 and six in samples of 2016.

Concentrations of carotenoids, chlorophylls and other colorless compounds from sample sets 2012-2015 and 2016 were similar. In 2014, the total carotenoid concentration ranged from 0.3 to 71.538 µg/ml. Two cultivars showed a very high concentration of total carotenoids in comparison with the cultivar producing the third highest concentration: 'Hanka' (35.538 µg/ml), 'Katinka' (71.538 µg/ml) and 'Tipala' (13.689 µg/ml). In these cultivars, also the concentrations of colorless carotenoids were very high in the year 2013. These cultivars were outside the average range of the other cultivars. In 2016, concentrations from 0.365 µg/ml to 7.412 µg/g ('Tipala') were determined. These values were within the range of values reported before. Curl (1963) and Gross (1984) reported total contents of carotenoids (including colorless carotenoids) in plums (*P. domestica* cv. 'Saghiv' and cv. 'Italian prunes', and *P. insititia* cv. 'Mirabelle') in a range of 7.5 to 21 µg/g fresh weight.

The concentrations of colored carotenoids ranged from 0.294 to 11.033 µg/ml (2014) and 0.25 to 6.209 µg/g (2016). 'Tipala' (2014 and 2016) and 'Liegels Gelbe' (2014) showed the highest concentrations. The lowest concentrations were detected in 'Hanka' and 'Angelina Burdette' (2014) and 'Presenta' (2016). 'Angelina Burdette' in 2014 showed the lowest concentration but this was not the case in 2016. The concentrations of colored carotenoids in both harvest years was lower than those published (2.98 to 19.7 µg/g) (Curl, 1963; Gross, 1987; Mangels et al., 1993).

Carotenes showed a concentration range between 0.131 and 9.90 µg/ml in 2014 and 0.116 an 5.633 µg/g in 2016. The major carotene was ß-carotene ranging in 2014 from 0.062 µg/ml ('Angelina Burdette') to 8.137 µg/ml ('Tipala') and in 2016 from 0.065 µg/g ('Presenta') to 4.580 µg/g ('Tipala'). All the values found in the present study agree with previously reported values for carotenes (0.191 to 5.6 µg/g) and ß-carotene (0.18 to 5.6 µg/g) (Breithaupt and Bamedi, 2001; Curl, 1963; Gross, 1984; Khachik et al., 1991; Lombardi-Boccia et al., 2004; Mangels et al., 1993; Moutounet, 1976; Stacewicz-Sapuntzakis, 2013).

The content of free xanthophylls in 2014 ranged from 0.118 µg/ml to 3.129 µg/ml and in 2016 from 0.108 to 1.641 µg/g. The most abundant compound in this group and the second most abundant compound in the group of colored carotenoids was lutein ranging from 0.027 µg/ml ('Haroma') to 2.378 µg/ml ('Mirabelle Wallenberg') in 2014 and from 0.044 µg/g ('Presenta') to 0.940 µg/g ('Angelina Burdette') in 2016. The reported values for this compound group (1.08-15.5 µg/g) are higher than those found in the present study (Curl, 1963; Gross, 1984; Mangels et al., 1993).

In the case of esterified xanthophylls, data were only obtained in 2014. The concentrations ranged from 0 to 3.525 µg/ml. Information on these compounds in *P.*

domestica fruits is scarce. Concentrations of 1.86 µg/g and 2.51 µg/g were found in a red skinned European plum and a yellow skinned, respectively (Breithaupt and Bamedi, 2001).

The concentration of colorless carotenoids in the fruits samples in 2014 ranged from 0.061 µg/ml to 3.831 µg/ml. Few studies reported the presence of colorless carotenoids in the European plum. Concentrations of 0.50 to 0.64 µg/ml were found (Curl, 1963; Gross, 1987). These values were lower than those determined in this study. In general, colorless carotenoids are not so well studied as colored ones and are not described in most food carotenoid databases. The interest in these compounds is rising, due to some studies suggesting health benefits (Melendez-Martinez et al., 2015). Since other colorless carotenoids were not identified, it was not possible to find literature on these compounds.

When the samples were grouped by their harvest period concentrations of colorless carotenoids, xanthophylls and other colorless compounds in ripe fruits tended to decrease during the season (Figure 38, Figure 39). In the case of total carotenoids, colored carotenoids and carotenes, the concentration in ripe fruits during the season increased from the first to the second harvest period and then it decreased towards the last harvest period (Figure 38, Figure 39). Carotenoids accumulation has been reported to depend on genetic factors, but also on cultural practices and environmental conditions, especially light (Bianchetti et al., 2018; Cronje et al., 2013; Lado et al., 2015; Lado et al., 2016; Llorente et al., 2017; Pizarro and Stange, 2009).

6.4. Correlation between compound groups and differentiation between cultivars

Pearson correlation analysis showed some obvious positive correlations (Table 8). Compound subgroups correlate positive with the individual compounds of which they are composed such as: disaccharides correlated positively with sucrose, monosaccharides with glucose or fructose, sugar alcohol with sorbitol, total colored carotenoids with total xanthophylls and total colored carotenoids with total carotenes. Sucrose, glucose and sorbitol showed a positive correlation with total sugars (significance 0.05). These soluble sugars were three out of the five soluble sugars detected in plum. They contributed significantly to the total concentration of soluble sugars.

The positive Pearson correlation coefficient of total carotenes with total color carotenoids (0.965) was higher than the one of total xanthophyll with total color

carotenoids (0.546) although both have the same level of significance 0.01. This was due to the fact that ß-carotene was the most abundant compound in most of the cultivars.

Sucrose concentration correlated negatively with the concentrations of its monomers fructose and glucose (significance of 0.01). Sugars are transported through the phloem mainly in form of sucrose. When sucrose arrives in the cytoplasm of the cells at the sink organ, such as fruits, it can accumulate as sucrose or be cleaved into glucose and fructose (Hofius and Börnke, 2007; Rolland et al., 2006). The correlation of the fructose with glucose concentration is positive because both derive from the degradation of sucrose (correlation coefficient 0.899; significance 0.01).

A positive correlation (significance 0.05) was found between glucose and sorbitol concentration (in additon, glucose with sugar alcohols; monosaccharides with sorbitol). Sorbitol, once imported from the phloem into the sink cell, can be metabolized into glucose (Figure 4) (Desnoues et al., 2014).

A positive correlation coefficient was also calculated by Pearson analysis for concentrations of sorbitol and anthocyanins and the yellow compound ONT01 (significance 0.05). The positive regulatory influence of sucrose on anthocyanin and flavonoid accumulation has been previously reported in grape (Boss et al., 1996; Gollop, 2002) and apple (Liu et al., 2017). However, reports on the influence of sugar alcohols such as sorbitol are scarce. Most authors have demonstrated that sugar alcohols do not affect flavonoid accumulation (Liu et al., 2017; Teng, 2005; Tholakalabavi et al., 1994; Vinterhalter et al., 2007). Some authors even proposed that sugar alcohols inhibit flavonoid accumulation at certain concentrations (Góraj-Koniarska and Saniewski, 2015). It has also been reported that anthocyanin can accumulate as a result of osmotic (drought) stress (Chalker-Scott, 1999; Do and Cormier, 1991; Kovinich et al., 2015). Sorbitol also accumulated under osmotic stress situations, as part of the mechanism to regulate osmotic equilibrium (Williamson et al., 2002). Therefore, a correlation of the concentration of both compounds can be expected, although the accumulation of these compounds do not directly affect each other.

For sorbitol (total sugar alcohols and total sugars) a statistical significative positive correlation was found with chlorophyll concentration and a negative one with colorless carotenoids. It has already been shown that chlorophyll and carotenoids decreased as a function of sorbitol concentration in leaf segments of etiolated maize seedling (Swati and Meeta, 2016).

Anthocyanin concentration correlated positively with flavan-3-ol/PAs concentration. Other positive correlations between phenolic compound groups were

established for anthocyanins with flavones, flavonols, other phenolic compounds (simple phenolics), and hydroxycinnamic acids. These compounds share a common pathway and CHS, an early enzyme of this pathway, has been described as the key regulatory enzyme in various *Prunus* species. Therefore, up-regulation of the early pathway steps resulted in more precursor compounds available for later pathway steps (Liu et al., 2013; Ravaglia et al., 2013; Selvaraj et al., 2016; Selvaraj, 2014; Tuan et al., 2015; Wei et al., 2015).

The concentration of the yellow compound ONT01 correlated positively with hydroxycinnamic acids and total phenolic compounds. This compound has only been found in a few cultivars, which tend to have a high concentration of total phenolic compounds. These results suggest that ONT01 is mainly detectable in cultivars with high concentrations of phenolics, but it could also be present in traces in the others.

Anthocyanins and flavan-3-ol/PAs concentrations correlated positively with chlorophyll concentration. This means that fruits with high levels of chlorophyll at maturity had also high levels of anthocyanins and flavan-3-ols. Most authors reported that chlorophyll concentration tends to decrease towards maturity (Bian et al., 2011; Hempel et al., 2014; Li and Yuan, 2013; Schweiggert et al., 2011). Nevertheless, some authors observed that the retention of chloroplasts during ripening could be an advantage for ripe fruits as they actively carry out photosynthesis under high light conditions. However, this has been demonstrated for fruits that remain green at maturity, and not for those with anthocyanin coloration (Blanke and Lenz, 1989; Cipollini and Levey, 1991).

There was also a positive correlation between chlorophylls with xanthophylls concentrations, which might be explained by the fact that both compound groups do not accumulate in ripe plum fruits. Chlorophyll is degraded during conversion of chloroplasts into chromoplasts and xanthophylls are mainly converted into esterified xanthophylls during fruit ripening (Bian et al., 2011; Egea et al., 2011; Gross, 1987; Hempel et al., 2014; Hornero-Mendez and Minguez-Mosquera, 2000; Lado et al., 2015; Li and Yuan, 2013; Mercadante et al., 2017; Schweiggert et al., 2011).

Between carotenoid subgroups, only positive correlations were observed. Other colorless compounds also positively correlated with total carotenoids. Thus, they presumably are precursors of carotenoids.

PCA analysis was performed to find metabolites to differentiate *P. domestica* (Figure 40). The only cultivars that appeared separated from the rest were ‘Mirabelle Wallenberg’, ‘Angelina Burdette’, ‘President’ and ‘Grosse Grüne Reneklode’. The most important vectors explaining the separation were flavonoids such as flavan-3-ols/PAs,

anthocyanins and ONT01 as groups, or as individual compounds. Carotenoids only contributed to PC3 and PC4 which explained 3 to 7% of the variance (data not shown). Soluble sugars and hydroxycinnamic acids had less influence on the differentiation of the cultivars despite their high concentration.

In the present study the metabolite profiles of phenolic compounds, soluble sugars and carotenoids in *Prunus domestica* were determined. In the future, further varieties and subspecies as well as different fruit tissues should be analysed to confirm these results. Genetic expression studies would also be interesting to investigate correlations of gene expression and accumulation of metabolites in different varieties. Finally, further studies on colorless carotenoids and the identification of other colorless compounds are necessary to clarify the actual concentration of these compounds in plum fruits.

7. Supplementary Material

7.1. Supplementary Material 1: Identification key for *P. domestica* subspecies

For the classification of cultivars by subspecies for which no reference was found in the literature, the following identification keys were used. It was translated from the original German published in Scholz and Scholz (1995)

1 Shrubs or small trees, thorny, fruit more or less spherical.....6

1* Trees more or less thornless.....2

2 One-year branches are glabrous, fruit long egg-shaped with both ends thinner than the center, 4-8 cm long, blue-black, coated by wax bloom, fruit flesh firm, thickness of the stone is 47-58% of the stone-length.....**subsp. *domestica***

2* One-year branches are more or less hairy (trichomes).....3

3 Fruit and stone almost spherical, thickness of the stone is 72-97% of the stone-length.....5

3* Fruit mostly oval-shaped, stone oval, flat, thickness of the stone is 45-65% of the stone
length.....4

4 Fruit 4-8 cm long, both ends rounded (or only the Stem end thinner), blue, violet, red, yellow, fruit flesh sweet and soft..... .....**subsp. *intermedia***

4* Fruit 2-3.5 cm long, yellow, red, blue, fruit flesh very soft, stone very flat.....**subsp. *pomariorum***

5 Fruit 3-5 cm long (primitive cultivar also smaller), yellow, green, blue, red, clingstone (the flesh adheres to the stone).....**subsp. *italica***

5* Fruit 2-3 cm long, yellow, with red dots, fruit flesh very sweet, freestone (the fruit flesh does not adhere to the stone).....**subsp. *syriaca***

6 Branches with trichomes until the second year, fruit 1.5-3 cm long, black-blue, with blood-red juice, thickness of the stone is 56-76% of the stone-length.....**subsp. *insititia***

6* Branches are green at the beginning, fruit 1-3 cm long, blue, black, yellow (with a red cheek), thickness of the stone is 73-79% of the stone-length.....**subsp. *prisca***

7.2. Supplementary Material 2: Response factors for quantification

Table 9: Response factors for phenolics used for quantification.

Samples	Compound	Wavelength (nm)	RF
2012, 2013 and 2014	procyanidin B2	640	0.47E-05
	catechin	640	0.24E-05
	epicatechin	640	0.17E-05
	cyanidin-3-O-glucoside	540	26.59E-05
	rutin	350	4.21E-05
	chlorogenic acid	280	5.11E-05
		320	2.89E-05
	neochlorogenic acid	320	5.01E-05
		368	65.93E-05
	vicenin 2	350	3.77E-05
	3-methoxyflavon	280	3.11E-05
2016	procyanidin B2	640	0.47E-05
	catechin	640	0.24E-05
	epicatechin	640	0.17E-05
	cyanidin 3-O-rutinoside	540	2.52E-05
		430	5.85E-05
	rutin	350	2.32E-05
	chlorogenic acid	280	5.11E-05
		320	2.89E-05
	neochlorogenic acid	320	1.39E-05
		368	1.92E-04
	flavone	350	1.08E-05
	3-methoxyflavon	280	1.64E-05

Table 10: Response factors for carotenoid, chlorophyll and other colorless compounds used for quantification.

Samples	Compound	Wavelength (nm)	RF
2012, 2013 and 2014 (gradient elution)	sudan-I	450	2.99124E-05
	ß-carotene	450	1.64947E-05
	lutein	450	1.56E-05
2016 (isocratic elution)	sudan-I	450	2.99124E-05
		280	7.67399E-05
	ß-carotene	450	1.64947E-05
		280	0.000109007
	lutein	450	1.56E-05
		280	1.02E-04

7.3. Supplementary Material 3: List of phenolic compounds

Table 11: Structurally identified phenolics in plums.

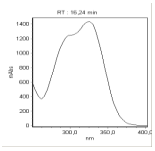
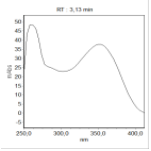
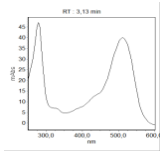
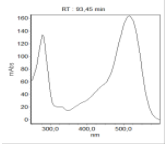
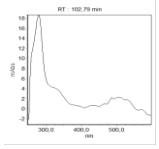
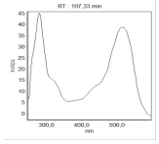
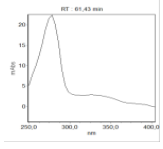
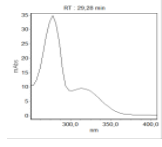
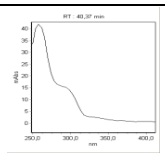
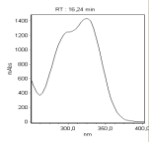
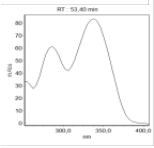
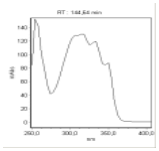
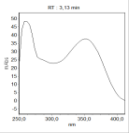
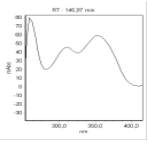
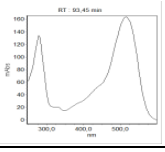
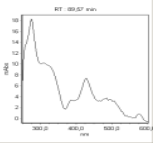
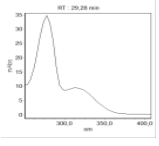
compound name	spectrum	maxima (nm)	retention time (min)
320 nm			
neochlorogenic acid		326	14.64
350 nm			
rutin		262+350	118.13
540 nm			
cyanidin 3-O-glucoside		278+510	84.7
cyanidin 3-O-rutinoside		278+514	93.49
peonidin 3-O-glucoside		278+486+510	102.79
peonidin 3-O-rutinoside		278+514	107.36
640 nm (post column derivatization)			
epicatechin		278	61,57
catechin		278	29,35

Table 12: Unknown phenolics were grouped according to their spectral properties into other phenolics, hydroxycinnamic acids, flavones, late flavones, flavonols, late flavonols, anthocyanins, proanthocyanidins, and an unidentified yellow compound, which was found in samples of the year 2016.

group name	example spectrum
280 nm	
other phenolic compounds (benzoic acid derivates)	
320 nm	
hydroxycinnamic acids	
flavones	
late flavones	
350 nm	
flavonols	
late flavonols	
540 nm	
anthocyanins	
430 nm	
unidentified yellow compound (ONT01)	
640 nm (post column derivatization)	
flavan-3-ols/proanthocyanidins	

7.4. Supplementary Material 4: List of carotenoids

Table 13: Structurally identified carotenoids in plums

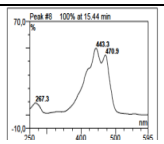
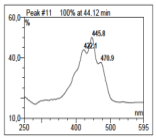
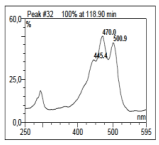
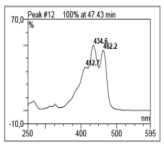
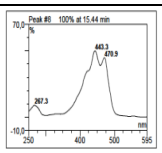
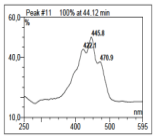
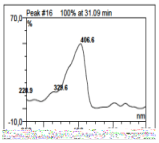
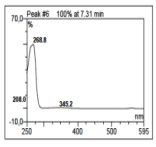
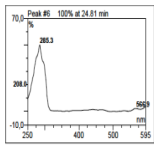
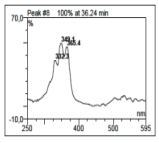
compound name	spectrum	maxima (nm)	Retention time in each elution method (min)	
			gradient	isocratic
		450 nm		
Lutein		267+443+470	18.68	15.44
ß-carotene		422+445+470	44.12	69.35
lycopene		445+470+500	118.9	not eluted
ß-cryptoxanthin		412+434+462	47.43	62.73

Table 14: Unknown carotenoids and polar compounds were grouped according to their spectral properties and into xanthophylls, carotenes, colorless carotenoids, chlorophylls, other colorless compounds.

group name	example spectrum
450 nm	
free and esterifierd xanthophylls	
carotenes	
chlorophylls	

group name	example spectrum
280 nm	
other colorless compounds	
colorless carotenoids (phytoene isomers)	
colorless carotenoids (phyofluene isomers)	

7.5. Supplementary Material 5: Statistical significance

Table 15: Statistical significance of the different concentrations of phenolic compound groups for samples harvested in 2014.

Compound Subgroup	CS-U	CS-N	Hm-S	Hm-U
anthocyanins	a	b	a	b
flavan-3-ol/Pas	a	b	a	b
flavones	a	a	a	a
flavonols	a	a	a	a
hydroxycinnamic acids	a	b	a	b
other phenolic compounds	a	a	a	a
total phenolic compounds	a	b	a	b

Calculated by t-test with a Levene's test. Only different letters within a row and within a cultivar represent significant difference.

Table 16: Statistical significance of the different contents of soluble sugars for samples harvested in 2012-2014.

Com-pound	Auerbacher	Bellamira	Cacaks Fruchtbare	Cacaks Julia	Cacaks Schöne	Colora	Gold-zwetschg	Grosse Grüne Reneklod	Haganta	Harbella	Haroma	Haus-zwetschge Schüfer	Tatjana
sucrose	a	d	ab	ab	a	d	abc	cd	a	a	bcd	abc	ab
fructose	a	b	b	a	b	a	b	b	b	c	b	b	a
glucose	de	cde	de	bc	bcd	ab	ef	de	ef	f	ef	f	a
sorbitol	abcdef	de	abcde	ab	abcd	a	abc	f	bcde	e	bcde	cde	ab
total sugar	ab	e	bc	ab	ab	bcd	bcd	e	bcd	de	cde	cde	a

Calculated by one-way ANOVA. Only different letters within a row represent significant difference.

Table 17: Statistical significance of the different concentrations of each soluble sugar for samples harvested in 2014 growing in different field sectors.

Compound Subgroup	CS-U	CS-N	Hm-S	Hm-U	HZ-S2	HZ-N	HZ-S3
sucrose	a	a	a	a	a	a	a
fructose	a	a	a	a	a	b	a
glucose	a	a	a	a	a	a	a
sorbitol	a	a	a	a	a	b	b
total sugar	a	a	a	a	a	a	a

Statistical significance was calculated for CS' and 'Hm' by t-test with a Levene's test for and for 'HZ' by one-way ANOVA. Only different letters **within a row and within a cultivar** represent significant difference.

Table 18: Statistical significance of the different concentrations of each carotenoid group in samples harvested on 2014.

Compound Subgroup	Zwint-schers Frühe	Mirabelle Wallen-berg	Gold-zwetschge	Haroma	Liegels Gelbe	Katinka	Hanka	Tipala	Colora	Angelina Burdette
xanthophylls	c	d	b	a	ab	c	cd	a	b	a
colorless carotenoids	a	a	a	a	a	c	b	a	a	a
chlorophyll	d	abcd	cd	ab	abcd	e	f	abc	bcd	a
other colorless compounds	bc	ab	a	a	a	ab	c	ab	a	a
carotenes	c	bc	bc	a	d	ab	c	e	ab	a
esterified xanthophylls	abc	cd	e	ab	f	cd	d	bcd	d	a
xanthophylls	bc	cd	cd	a	d	bcd	cd	a	b	a
color carotenoids	cd	cd	cd	a	e	bc	d	e	b	a
total carotenoids	a	ab	a	a	ab	c	b	ab	a	a

Calculated by one-way ANOVA. Only different letters **within a row** represent significant difference.

Table 19: Statistical significance of the different concentrations of each phenolic compound group in samples harvested in 2012-2014.

Compound Subgroup	AGRI 2000 10/92	Angelina Burdette	Auerbacher	Cacaks Fruchtbare	Cacaks Julia	Cacaks Schöne	Colora	Elena	Goldzwetschge	Hanka	Haroma	Jubileum	Maria Novella	Ortenauer	Presenta	President	Topend Plus	Topfive	Tophit Plus
anthocyanins	ab	ab	a	a	a	ab	a	a	a	ab	a	a	a	a	a	a	ab	b	b
flavan-3-ol/Pas	ab	d	ab	a	a	ab	a	a	bcd	a	a	a	a	a	a	a	cd	abc	ab
flavones	abc	ab	ab	ab	a	c	ab	abc	ab	a	abc	a	ab	ab	abc	ab	abc	bc	a
flavonols	bcde	bcde	abcd	abcd	a	bcde	e	bcde	abc	bcde	de	bcde	ab	abcd	abcd	abcd	bcde	cde	bcde
hydroxycinnamic acids	abcdef	h	bcdefg	ab	a	defg	abcdef	fgh	efgh	abcdef	abcdef	abc	abcd	abc	abcde	cdefg	gh	gh	gh
other phenolic compounds	b	b	b	b	a	b	b	b	b	b	b	b	b	b	bc	b	b	bc	b
total phenolic compounds	bcde	f	bcde	abc	a	cdef	abcd	def	abcd	bcde	abcd	ab	abc	ab	abcd	bcde	ef	f	f

Calculated by one-way ANOVA. Only different letters **within a row** represent significant difference.

Table 20: Statistical significance of the different concentrations of different metabolites in samples harvested in 2016.

Compound Group	Compound Subgroup	Angelina Burdette	Aprikoosprium	Belle de Thuin	Cacaks Schöne	Colora	Eibenbacher Aprikosenpflaume	Frühe Mirabelle P2778	Grosse Grüne Reneklode	Haferpflaume	Haganta	Haroma	Hauszwetschge Schüffer	Jojo	Katinka	Maria Novella	Mirabelle aus Nancy	Mirabelle Wallenberg	Ontariopflaume	Ortenauer	Oullins Reneklode	Presenta	President	Rote Eierpflaume Blazek	Tipala	Topend Plus	Zwintschers Frühe
Soluble Sugars	Sucrose	ghi	j	j	defg	k	b	ij	def	ghi	cd	ab	ab	ab	ab	c	hi	a	fghi	ab	j	ghi	ghi	fghi	cde	defg	ab
	Fructose	cdef	a	fgh	efg	cde	ab	gh	efgh	abc	abcd	ij	ij	j	h	efgh	defg	j	abc	ij	a	ab	hi	abc	bcd	gh	ab
	Glucose	hi	abc	gh	efg	bcde	bcde	h	gh	ab	gh	ij	j	j	j	def	efg	j	abcd	ij	abc	efg	hi	abc	cdef	fgh	a
	S01	b	h	cdefg	fgh	fgh	a	a	bcd	b	efgh	bcde	cdef	cdef	a	a	bc	a	defgh	bcd	efgh	bcde	cde	b	a	cdefg	gh
	Sorbitol	i	a	a	bc	a	ab	d	i	a	f	cd	g	f	a	ab	e	a	ab	e	a	e	h	a	ab	gh	a
	Total Sugar	**i**	**cdefg**	**fghi**	**cdefg**	**fghi**	**ab**	**ghi**	**bcde**	**bcd**	**cdefg**	**bcde**	**cdefgh**	**defghi**	**cdef**	**bc**	**efghi**	**bc**	**bcde**	**cdefg**	**cdefg**	**cdefg**	**hi**	**bc**	**bc**	**ghi**	**a**
Phenolic Compounds	Anthocyanins	f	a	a	cde	a	cde	a	a	a	bcde	abc	ab	bcde	de	e	a	a	a	ab	a	a	e	a	a	abcd	abcd
	ONT	a	a	a	ab	a	e	cd	f	a	a	a	a	a	a	e	a	de	ab		a	a	g	a	a	bc	a
	Flavan-3-ol/PAs	f	a	a	ab	a	b	ab	c	a	ab	a	a	a	ab	ab	a	e	d	a	a	a	ab	a	a	a	a
	Flavones	a	abc	cdef	abc	a	abcd	gh	efg	fg	gh	fg	abc	h	abcd	abc	bcde	ab	abc	defg	abc	a	cdefg	ab	ab	a	cdef
	Flavonols	ab	a	abcd	abcd	abcd	abcd	abc	ab	e	abcd	bcd	ab	cd	abcd	abcd	abcd	abcd	a	de	a	a	abcd	abc	ab	a	abcd
	Hydroxycinnamic acids	efg	ab	abcde	fgh	abcd	gh	abcd	i	bcdef	i	defg	abc	hi	cdefg	efg	a	efg	j	abcde	a	a	j	abcde	a	abc	cdefg
	Other Phenolic Compounds	a	defg	hijk	efg	k	cdefg	abcdef	k	ijk	fghi	fghi	abcdef	jk	abc	fgh	ghij	ab	abcde	fghi	defg	ab	efh	abcde	abcd	ab	bcdef
	Total Phenolic Compound	**defg**	**a**	**abcd**	**cdef**	**abcd**	**def**	**abc**	**def**	**bcde**	**fg**	**bcde**	**ab**	**efg**	**abcd**	**bcde**	**ab**	**cdef**	**gh**	**abcd**	**a**	**a**	**h**	**abc**	**a**	**a**	**abcd**
Carotenoids	Xanthophylls	l	fghij	fghij	jk	l	ghij	kl	m	ab	fghij	abc	cdefg	abcde	fghij	cdefg	abcde	hijk	ijk	abcde	efghi	a	abcd	cdefg	defgh	bcdef	l
	Carotenes	cdef	abcd	bcde	abcde	cdef	fg	gh	h	ab	abcd	ef	bcde	cdef	ab	abcd	abcd	def	ab	abcd	abc	a	bcde	abcd	i	gh	ef
	Colorless Carotenoids	a	a	a	a	e	cd	bc	a	a	a	a	a	a	de	a	a	c	a	a	a	a	a	bc	ab	a	de
	Chlorphylls	c	ab	a	ab	ab	a	ab	c	a	ab	ab	ab	a	ab	a	ab	a	ab	ab	ab	a	ab	a	a	ab	a
	Other Colorless Compounds	hij	bcdefgh	bcdef	a	bcdef	j	hij	ab	efghi	abcd	efghi	abcde	ab	abcdef	defgh	bcdef	ij	abc	bcdef	defgh	ab	abcdef	bcdefg	ghij	cdefgh	fghi
	Color Carotenoids	efg	bcd	cde	cde	efg	fg	h	i	ab	bcd	cde	cde	cde	abc	abc	abc	def	abcd	abc	abc	a	bcd	abc	j	gh	fgh
	Total Carotenoids	**cde**	**abc**	**bcd**	**bcd**	**hij**	**ghi**	**ghi**	**ghi**	**ab**	**abc**	**abcd**	**abc**	**abcd**	**efg**	**abc**	**ab**	**fgh**	**abc**	**ab**	**abc**	**a**	**abc**	**cde**	**j**	**def**	**ij**

Calculated by one-way ANOVA. Only different letters **within a row** represent significant difference.

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8. Abbreviations:

#
4CL: 4-coumaric acid:CoA ligase

A
ABA: abscisic acid
AI: Acid invertase
ANOVA: analysis of variance
ANR: anthocyanidin reductase
ANS: anthocyanidin synthase
ARF2a y 2b: AUXIN RESPONSE FACTOR 2a and 2b (transcription factor)

B
BayOZ: Bayerischer Obstzentrum
BC: before Christ
bHLH: basic Helix-Loop-Helix

C
C-INV: cytoplasmic invertase
C1 referring chemical structures means the Carbon and its respective number position.
C40 number of carbons in eight isoprene units.
C4H: cinnamate-4-hydroxylase
CHI: chalcone isomerase
CHS: chalcone synthase
CHYB: carotene beta hydroxylase
CHYE: carotene epsilon hydroxylase
cm centimeter
CoA: Coenzyme A
Conc: concentration
COP1: CONSTITUTIVE PHOTOMORPHOGENIC 1 (transcription factor)
CPR: cytochrome P450 reductase
CUL4: Cullin 4 (transcription factor)

D
DDB1: DNA damage-binding protein 1 (transcription factor)
DFR: dihydroflavon-4-reductase
DM: dry matter
DMACA: p-dimethylaminocinnamaldehyde

E
EIN: (transcription factor)
ERF6: ETHYLENE RESPONSE FACTOR 6 (transcription factor)

F
F16BPase: fructose 1,6 biphosphatase
F3'H: flavonoid 3'-hydroxylase
F3H: flavonoid 3-hydroxylase
FBPase: fructose 1,6-biphosphatase
FLS: flavonol synthase
FMO: flavonoid monooxygenase
FUL1/2: FRUITFULL-like MADS-box 1 (transcription factor)
FW fresh weight

G
GAE: gallic acid equivalents
GGPP: geranylgeranyl diphosphate,
GLK2: Golden 2-like 2 (transcription factor)
GmbH: Gesellschaft mit beschränkter Haftung

H
ha: hectares
HK: hexokinase
HPLC-ECD: high pressure liquid chromatography electrochemical detection
HPLC: high pressure liquid chromatography
HY5: ELONGATED HYPOCOTYL 5 (transcription factor)

I
I.D.: inner diameter
KIN10: SNF1 kinase homolog 10
LAR: leucoanthocyanidin reductase
LCYB: lycopene beta cyclase
LCYE: lycopene-epsilon cyclase
LDOX: leucoanthocyanidin dioxygenase

M
m meter
MADS-box
mbar: millibar
MBW: MYB-bHLH-WD40 complex
MF: multiplication factor
min: minutes
ml: milliliter
mm millimeters
MTBE: Methyl-tert-butylether
MYB: MYB transcription factor
MYBL2: MYB-related protein B (transcription factor)

N
NAC: (transcription factor)
NI: neutral invertase
nm: nanometer
NSY: neoxanthin synthase

O
OMT: Methyltransferase

P
PA: proanthocyanidin
PAL: phenylalanine ammonia lyase
PAP1: production of anthocyanin pigment 1 (transcription factor)
PAR1: protease-activated receptor 1 (transcription factor)
PAs: proanthocyanidins
PC1: principal component 1
PC2: principal component 2
PCA: principal component analysis
PDS: phytoene desaturase
PDV2: plastid division protein 2 (transcription factor)
PELAN: PETAL LOBE ANTHOCYANIN (transcription factor)
PFK: ATP-phosphofructokinase
PFP: material de la columna
PFP: PPi-phosphofructokinase
PGI: phosphoglucose isomerase
PIF1 and PIF3: phytochrome interacting transcription factor 1 and 3
PSY: phytoene synthase
PTFE: polytetrafluoroethylene

R
R2R3-MYB: transcription factor family
R3-MYB: transcriptor factor family
RAP2.2: transcription factor

RCP1: root cap 1(transcription factor)
RF: response factor
RIN. RIPENING INHIBITOR (transcription factor)
RP-HPLC: reverse phase HPLC
rpm: revolutions per minute

S
S6PDH: sorbitol-6-phosphate dehydrogenase
SDH: sorbitol dehydrogenase
SNF1: sucrose non-fermenting 1
SnRK1s: sucrose non-fermenting 1 related kinases
SO: sorbitol oxidase
SPE: solid phase extraction
SPP: sucrose phosphate phosphatase
spp: various species
SPS: sucrose phosphate synthase
SPS: sucrose-P synthase
Subsp.: Subspecies
SuSy: sucrose synthase

T
TAG1: TOMATO-AGAMOUS-LIKE1 (transcription factor)
TTG1: TRANSPARENT TESTA GLABRA 1 (transcription factor)
TUM: Technische Universität München

U
UDP: uridine diphosphate
UFGT: UDP-glucose: flavonoid-3-O-glucosyltransferase
UGPase: UDP-glucose pyrophosphorylase
UV: ultraviolet radiation

V
V-INV: vacuolar invertase
var. botanical variety
VDE: violaxanthin de-epoxidase

W
WD40: WD40 repeat

Z
ZDS: zeta-carotene desaturase
ZEP: zeaxanthin epoxidase

µ
µl: microliter

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