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ISOPRENOID CONTENT OF PEACH *PRUNUS PERSICA* AND APRICOT *PRUNUS ARMENIACA* FRUIT CUTICULAR WAXES

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ABSTRACT

Peach (*Prunus persica* L. Batsch) and apricot (*Prunus armeniaca* L.) are very popular drupe-type fleshy fruits from Rosaceae family, important to the agricultural economies of many countries. The majority of Rosaceae fruits are characterized with a relatively high content of isoprenoids (triterpenoids and steroids) occurring in their surface cuticular waxes. However, the data concerning the occurrence of these compounds in peaches and apricots are scarce. The aim of the present study was the determination of isoprenoid content in the cuticular waxes of peach var. 'Redhaven' and apricot var. 'Somo' cultivated in Poland. Chloroform-soluble wax extracts obtained from fruit samples were fractionated with the use of preparative adsorption chromatography and analyzed by gas chromatography-mass spectrometry (GC-MS). The profile of the identified compounds was similar in both fruits. However, some quantitative and qualitative differences were noticed. Triterpenoid acids (betulinic, oleanolic and ursolic acids) were the predominating fraction of isoprenoids identified in cuticular waxes of both fruits, however, they were 4-fold more abundant in peach. Moreover, dihydroxy acid of oleanane-type, maslinic acid, was identified only in peach, whereas ursane-type corosolic acid only in apricot wax. The fraction of the neutral triterpenoids was similar, composed primarily of oleanane- and ursane-type alcohols and aldehydes. Typical profile of steroids was identified in both fruits, however, stigmasterol was dominating in peach, whereas sitosterol in apricot. The total contents of isoprenoids in peach (approx. 90 µg/mg of wax extract) and apricot (approx. 27 µg/mg) cuticular waxes were found to be significantly less than in other Rosaceae fruits.

Keywords: *apricot, cuticular wax, peach, steroids, triterpenoids.*

INTRODUCTION

Occurrence of isoprenoids, including triterpenoids and steroids, in fruit surface cuticular wax is a well-known feature; however, our understanding of the relevance of these compounds for fruit quality traits is still rather preliminary. Regarding various biological activities of triterpenoids, their presence in fruit cuticle might be of interest due to their potential role in protection against abiotic and biotic stresses, mechanical toughness of the fruit peel, surface texture, general fruit appearance, post-harvest quality and shelf-life duration (Szakiel *et al*. 2012; Trivedi *et al*. 2019; Lara *et al*. 2015, 2019). Moreover, anti-inflammatory, antimicrobial, antiviral, cardio- and hepatoprotective, and potentially anti-cancer properties of triterpenoids make their occurrence in fruits valuable for the consumers (Szakiel *et al*. 2012; Dashbaldan *et al*. 2020). As constituents of cuticular waxes, triterpenoids and steroids can be very abundant in some fruits, while occurring only in trace amounts in others (Dashbaldan *et al*. 2019). The diversity of fruit cuticular wax composition deserves to be further explored, particularly regarding edible fleshy fruits. The Rosaceae family is one of the major angiosperm families, with many economically important fruit crop plants bearing various types of fruits, e.g., follicles, capsules, nuts, achenes, drupes and accessory fruits (Butkevičiūtė *et al*. 2018; Dashbaldan *et al*. 2020, 2021). Many Rosaceae fruits have been characterized in terms of isoprenoid content of their cuticular waxes, however, the data concerning the triterpenoid and steroid content in peaches (*Prunus persica* L. Batsch) and apricots (*Prunus armeniaca* L.) are scarce. Meanwhile, peach and apricot are very popular drupe-type fleshy fruits, important to the agricultural economies of many countries. Therefore, the aim of the present study was the qualitative and quantitative determination of isoprenoid content in the cuticular waxes of peach var. 'Redhaven' and apricot var. 'Somo' cultivated in Poland, through targeted GC-MS metabolomic profiling of chloroform-soluble wax extracts.

MATERIAL AND METHODS

Peach (*Prunus persica* L. Batsch) var. 'Redhaven' and apricot (*Prunus armeniaca* L.) var. 'Somo' were cultivated in a private orchard in Rudno (51°471 N, 15°410 E), western Poland (Lubusz Voivodeship). The samples of fully ripened fruit were collected in 2020 in three independent replicates (i.e., from different trees) for each species. Cuticular waxes were extracted as described previously (Dashbaldan *et al.* 2019, 2020) by incubation of entire intact fruit in appropriate volume of chloroform (permitting the full dipping of fruit sample) with gentle stirring for 30 s at room temperature.

Evaporated extracts of chloroform-soluble cuticular waxes were fractionated by adsorption preparative thin-layer chromatography (TLC) on 20 cm × 20 cm glass plates coated manually with silica gel 60H (Merck). The solvent system chloroform:methanol 97:3 (v/v) was applied for developing. Two fractions were obtained as described earlier (Woźniak *et al*. 2018): free (non-esterified) steroids and triterpenoids, and triterpenoid acids. Fractions were eluted from the gel in diethyl ether. Subsequently, fractions containing free neutral triterpenes and sterols (R_F 0.3-0.9) were directly analyzed by GC-MS, whereas fractions containing triterpene acids (R_F 0.2-0.3) were methylated with diazomethane. Nitrosomethylurea (2.06 g) was added to a mixture of 20 mL of diethyl ether and 6 mL of 50% aqueous KOH, and the organic layer was then separated from the aqueous layer. Samples containing triterpenoid acids were dissolved in 2 mL of the obtained solution of diazomethane in diethyl ether,and held at 2 °C for 24 h.

Analyses were performed with the use of an Agilent Technologies 7890A gas chromatograph (GC–MS) (Perlan Technologies). The system was equipped with a 5975C mass selective detector, a G4513A autosampler, and a 30 m × 0.25 mm i.d., 0.25-μm, HP-5MS UI column (Agilent Technologies, Santa Clara, CA, USA). The following temperature program was applied: the start at 160 °C (2 min), an increase to 280 °C at 5 °C/min, and the final temperature of 280 °C held for 44 min. The other employed parameters were as follows: the carrier gas (helium, 1 mL/min), inlet and FID (flame ionization detector) temperature 290 °C; quadrupole temperature 150 °C; ion source temperature 230 °C; EI ionization energy 70 eV; scan range, m/z 33–500; MS transfer line temperature 275 °C; FID gas: hydrogen 30 mL·min−1; air 400 mL/min. Wiley 9th ED. and NIST 2008 Lib. SW Version 2010 were used in GC-MS data analysis. Individual compounds were identified by comparing their mass spectra with library data, and/or their chromatographic mobility and corresponding mass spectra with those of authentic standards. Quantitation was conducted with a FID detector and performed using an external standard method based on calibration curves determined for authentic standards of ursolic acid methyl ester, α-amyrin and stigmasterol. Data were presented as the means ± standard deviation of three independent samples and subjected to one-way analysis of variance (ANOVA), the differences between means were evaluated using Duncan's multiple-range test. Statistical significance was considered to be obtained at $p<0.05$ (Dashbaldan *et al*. 2021).

RESULTS AND DISCUSSION

The composition of the identified triterpenoids and steroids was similar in chloroform-soluble cuticular wax extracts obtained from both peach and apricot fruit samples. The list of the compounds and the results of the quantification of the individual compounds are presented in Table 1.

Table 1. The content of triterpenoids and steroids in peach (*Prunus persica* L. Batsch) var. 'Redhaven' and apricot (*Prunus armeniaca* L.) var. 'Somo' fruit cuticular waxes. Results are referenced to wax extract mass and expressed as the mean ± SD of three independent samples.

Compound	Peach 'Redhaven'	Apricot 'Somo'
	Content (µg/mg wax extract)	
Triterpenoid acids:		
betulinic acid	1.84 ± 0.20[a*]	4.64 ± 0.48[b]
oleanolic acid	38.42 ± 4.06[a]	2.64 ± 0.30[b]
ursolic acid	40.83 ± 5.01[a]	9.29 ± 1.14[b]
olean-2,12-dien-28-oic acid	1.22 ± 0.08[a]	0.36 ± 0.05[b]
ursa-2,12-dien-28-oic acid	2.94 ± 0.22[a]	0.81 ± 0.10[b]
corosolic acid	n.d.**	1.08 ± 0.08
maslinic acid	0.42 ± 0.04	n.d.
sum of triterpenoid acids	*85.67*	*20.88*
Neutral triterpenoids		
α-amyrin	1.05 ± 0.12[a]	0.61 ± 0.07[b]
β-amyrin	0.23 ± 0.02[a]	0.22 ± 0.03[a]
α-amyrenone	0.07 ± 0.01[a]	0.11 ± 0.01[b]
erythrodiol	0.08 ± 0.02[a]	0.06 ± 0.01[a]
uvaol	0.36 ± 0.04[a]	0.19 ± 0.02[b]
oleanolic aldehyde	0.13 ± 0.04[a]	0.29 ± 0.03[b]
ursolic aldehyde	0.35 ± 0.05[a]	0.46 ± 0.06[a]
sum of neutral triterpenoids	*2.27*	*1.94*
Steroids:		
campesterol	0.39 ± 0.05[a]	0.23 ± 0.04[b]
cholesterol	0.06 ± 0.01[a]	0.31 ± 0.03[b]
sitosterol	0.05 ± 0.01[a]	2.66 ± 0.32[b]
stigmasterol	0.97 ± 0.12[a]	0.10 ± 0.01[b]
sitostenone	0.05 ± 0.01[a]	0.25 ± 0.03[b]
tremulone	0.07 ± 0.01[a]	1.05 ± 0.12[b]
sum of steroids	*1.63*	*4.60*
Total	89.57	27.42

*Means in rows not sharing a common letter are significantly different ($p<0.05$).
**n.d., not detected.

As in the majority of other Rosaceae (Dashbaldan *et al*. 2020, 2021), triterpenoid acids belonging to the three types of carbon skeletons, i.e., lupane-, oleanane- and ursane-type groups, were found; with their typical representatives, namely betulinic, oleanolic and ursolic acids. Moreover, the analogs of oleanolic and ursolic acids with an additional double bond in position 2, i.e., olean-2,12-dien-28-oic acid and ursa-2,12-dien-28-oic acids, were also identified. The difference in triterpenoid composition between peach and apricot was the presence of 2,3-dihydroxy analogs of oleanolic and ursolic acids; i.e., oleanane-type maslinic acid was identified in peach, whereas ursane-type corosolic acid in apricot. In turn, no compositional differences were noticed in the fractions of the neutral triterpenoids and steroids. The basic ursane- and oleanane-type alcohols with one hydroxyl group (α- and β-amyrins) and two hydroxyl groups (uvaol and erythrodiol), as well as respective aldehydes (ursolic and oleanolic) were identified in both peach and apricot cuticular waxes, accompanied by one ketone, α-amyrenone. The steroid fraction was composed of typical sterols, i.e., campesterol, cholesterol, sitosterol and stigmasterol, as well as two steroid ketones, sitostenone and tremulone.The total contents of both groups of isoprenoids, i.e., triterpenoids and steroids, were found to be relatively low in peach and apricot cuticular waxes as compared to other Rosaceae fruit crops analyzed previously, e.g., apple (Andre *et al*. 2012; Butkevičiūtė *et al*. 2018; Sut *et al*. 2019), black chockeberry (Dashbaldan *et al.* 2020), cherry (Peschel *et al.* 2007), pear (Kolniak-Ostek 2016), plum (Huang *et al*. 2022) or rose (Dashbaldan *et al*. 2020, 2021). The total isoprenoid content in peach cuticular waxes did not exceed 100 µg/mg, i.e., 10% of chloroform-soluble wax extract, whereas in such fruits like apple it could reach 50% of the extract. The total isoprenoid content in apricot cuticular waxes was 3.3-fold lower than in peach.

Triterpenoid acids constituted the major fraction of cuticular wax isoprenoids, reaching 96% and 76% of the total content of all quantified compounds in peach and apricot, respectively. In turn, steroids were the minor fraction (1.8%) in peach fruit cuticular waxes, whereas they constituted 17% in apricot waxes.

Ursolic acid was the predominating compound in both peach and apricot cuticular waxes (48% and 44% of the fraction of triterpenoid acids, respectively). The second abundant acid was oleanolic acid (45% of the fraction) in peach, whereas betulinic acid (22%) in apricot. Among the neutral triterpenoids, α-amyrin was the most abundant in both fruits (46% and 31% of the fraction in peach and apricot, respectively). In turn, the predominating sterol was stigmasterol in peach (60% of steroid fraction), whereas sitosterol in apricot (58%).

The results obtained in this study and reported previously (Dashbaldan *et al*. 2020) point to some similarities in the isoprenoid content of fruit cuticular waxes in Rosaceae crops. One of such common features is the presence of lupane-, oleanane- and ursane-type triterpenoids, whereas in other taxonomic families, e.g., Ericaceae, the composition of triterpenoids was found to be more complex, containing compounds of several other carbon skeletons (Dashbaldan *et al*. 2019). Another common feature is the predominance of the triterpenoid acid fraction, observed in numerous Rosaceae fruit crops bearing various fruits as drupes (Peschel *et al*. 2007, Lino *et al*. 2020, Huang *et al*. 2022) or accessory fruits (Andre *et al.* 2012, Butkevičiūtė *et al.* 2018, Kolniak-Ostek 2016, Sut *et al*. 2019, Dashbaldan *et al*. 2020).

However, comparing the isoprenoid content in such Rosaceae drupes as cherry (Peschel *et al*. 2007), plum (Huang et al. 2022) or even nectarine (Lino et al. 2020), revealed that the amounts of these compounds (particularly triterpenoids) in peach and apricot cuticular waxes are significantly lower. It might be due to the fruit surface properties and the morphological features of epidermis (e.g., the presence of trichomes forming a dense indumentum, in which such volatile compounds as monoterpenoids can be accumulated). Perhaps it is also related to the general plant defense strategy, based either on the accumulation of triterpenoids (in a free form, or as glycosides called saponins) or other bioactive constituents belonging to various classes of plant metabolites.

The observed difference between the isoprenoid content in cuticular waxes of peach and apricot revealed that it is difficult to correlate this feature directly with plant taxonomy. The significant differences in triterpenoid and steroid profiles exist even within one genus of Rosaceae family, i.e., Prunus. Moreover, such differences can be observed also among different varieties and cultivars of crop plants (Andre *et al*. 2012; Butkevičiūtė *et al.* 2018; Kolniak-Ostek 2016; Sut *et al*. 2019; Huang *et al.* 2022), therefore, the taxonomic classification of any fruit crop might not be relevant to predict the isoprenoid content of its cuticular waxes, and it requires a detailed case study.

CONCLUSIONS

This report presents the isoprenoid content of peach and apricot fruit cuticular waxes, and it complements the data concerning the occurrence of these compounds in surface layer of Rosaceae fruit crops. Some described features, e.g., the presence of lupane-, oleanane- and ursane-type compounds, the predominance of the triterpenoid acid fraction, seem to be typical for the majority of Rosaceae fruits characterized so far. However, the total isoprenoid contents in peach and apricot cuticular waxes are significantly lower than in other Rosaceae fruits, including other drupes. Therefore, it can be concluded that the isoprenoid content of fruit cuticular waxes is related mainly to the fruit surface properties and the morphological features of epidermis (e.g., the presence of surface trichomes), whereas it is difficult to clearly connect the isoprenoid composition and quantity with the fruit crop taxonomy or the type of fruit (e.g., drupes, accessory fruit, berries).

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