

LIPID COMPOSITION OF SWEET CHERRIES

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Abstract—Lipid comprises about 0.1% of the fresh weight of cherries. The major components of the neutral lipid fraction are wax and sterol esters, sitosterol, oleanolic and ursolic acids. The glycolipid fraction is comprised of monoglycosyldiacylglycerol, diglycosyldiacylglycerol and acylated sterol glycoside, while phosphatidylcholine and phosphatidylethanolamine are the major phospholipids. Palmitic, oleic, linoleic and linolenic acids comprise over 85% of the total fatty acids in each lipid fraction.

INTRODUCTION

Although the role of cuticular lipids in fruit drying, and that of membrane lipids in the storage of chilling sensitive tissues is well recognized [1, 2], studies on fruit lipids have been mainly confined to the detection and isolation of individual components. This communication presents an analysis of the lipids present in freshly-harvested sweet cherries, *Prunus avium* (L.) L.

RESULTS AND DISCUSSION

Extraction of fresh cherries yielded 123 ± 3 mg of lipid per 100 g of fruit, with a lipid phosphorus content of 2.1% and a lipid sugar content of 5.0%. The fractionation of the total lipid extract on acid-Florisil yielded fractions containing neutral lipid ($31.2 \pm 0.7\%$), glycolipid ($23.5 \pm 1.9\%$) and phospholipid ($45.3 \pm 1.7\%$).

The neutral lipid component was further fractionated on Florisil-7% H_2O . The major species obtained were wax esters, sterol esters, free sterols and triterpene acids. The ester fraction (11.8 mg per 100 mg total lipid) contained 1.2 mg sterol per 100 mg total lipid and free sterol comprised 6.6 mg per 100 mg total lipid. In both cases sitosterol accounted for over 95% of the sterol present, with small amounts of campesterol. A substantial portion of the neutral lipid fraction (12.5 mg per 100 mg total lipid) which was eluted with MeOH showed properties consistent with those of triterpene acids. The fraction migrated as a single spot on TLC in both neutral lipid solvents with an R_f identical to that of oleanolic and ursolic acids, and after methylation with CH_2N_2 its R_f was identical to that of methyl oleanolate and methyl ursolate. Examination of the methylated material by GLC resolved the fraction into two components with retention times identical to that of methyl oleanolate (RR , cholestane 5.60) and methyl ursolate (RR , 6.42). The ratio of ursolic acid to oleanolic acid in the cherry lipid was 6.7:1.

The glycolipid fraction contained three major components, an acylated sterol glycoside (2.6 mg sugar per

100 mg total lipid), a monoglycosyldiacylglycerol (0.4 mg sugar per 100 mg total lipid) and a diglycosyldiacylglycerol (1.62 mg sugar per 100 mg total lipid). As was the case in the neutral lipid fractions, sitosterol comprised over 95% of the sterol component of the sterol glycoside, but no glycosides of oleanolic or ursolic acids were detected.

Phosphatidylcholine (1.16 mg P per 100 mg total lipid) and phosphatidyl ethanolamine (0.48 mg P per 100 mg total lipid) comprised 80% of the phospholipid fraction with small amounts of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylserine and phosphatidylinositol.

The fatty acid composition of the total lipid and sub-fractions is shown in Table 1. The major component in

Table 1. Fatty acid composition of cherry lipids

Acid	Total lipids	Neutral lipids	Glycolipids	Phospholipids
14:0	0.7	1.8	0.9	0.6
16:0	19.7	26.4	17.2	18.8
16:1	0.4	0.7	0.4	0.2
17:0	0.4	0.4	0.4	0.3
17:1	0.2	0.3	0.3	0.2
18:0	6.7	8.1	6.9	6.3
18:1	33.5	30.5	29.3	35.2
18:2	19.0	15.2	15.8	20.8
18:3	18.4	15.2	28.1	16.5
20:0	0.9	1.3	0.7	1.1
Σ Saturated acids	28.4	38.0	26.1	27.1

all cases was 18:1, with substantial amounts of 16:0, 18:1, 18:2, and 18:3 in each fraction although variations are present in the content of individual acids between the three fractions. For example, the neutral lipid has a higher concentration of saturated fatty acids than the glyco- and phospho-lipids, whereas the glycolipid has the highest content of 18:3, an acid characteristic of

plant galactosyldiacylglycerols [3]. No elaeostearic acid could be detected in any fraction, although it had been previously reported to comprise 13.5% of the oil from sweet cherries [4].

The most characteristic features of the lipids of sweet cherries appears to be the content of free sterol, acylated sterol glycoside and the triterpene acids, ursolic and oleanolic acids. Ursolic acid has been previously detected in the leaves of wild black cherry trees (*P. serotina*) [5], and oleanolic acid in plums (*P. domestica*) [6]. The leaves of peach trees (*P. persica*) have been reported to contain ursolic acid and sitosterol [7]. The role of these compounds is not clear but in commercial drying of fruits such as plums [6] and grapes [8] the presence of cuticular lipids including sterols and triterpene acids exerts a marked effect on the drying rate of fruit. By contrast the susceptibility of fruits and vegetables to chilling injury during storage appears to be related to properties of membrane lipids [1, 2] such as the glycolipids and phospholipids, and the role of these compounds in low-temperature storage of cherries is currently being evaluated.

EXPERIMENTAL

Lipid extraction. Sweet cherry fruit (*P. avium* cv. Ron's Seedling grafted on cv. Mazzard rootstocks) were harvested at full ripeness, cooled to 2° and extracted within 24 hr. All extractions and analyses were performed in triplicate. Lipids were extracted from 100 g aliquots of fresh depitted cherry tissue by the method of Bligh and Dyer [9]. Two mg of butylated hydroxytoluene were added to prevent oxidation of polyunsaturated fatty acids. Non-lipid contaminants were removed by Sephadex treatment [10].

Column chromatography. The total lipid extract was fractionated on columns of acid-washed Florisil [11]. Neutral lipids and pigments were eluted with CHCl_3 (8 ml/g of adsorbent) followed by $\text{Me}_2\text{CO}-\text{CHCl}_3$ (15:85) (5 ml/g). Glycolipids were eluted with Me_2CO (10 ml/g) and phospholipids with MeOH (10 ml/g). Neutral lipids were further fractionated on columns of Florisil-7% H_2O [12]. Sterol esters and wax were eluted with Et_2O -petrol (1:9) (6 ml/g adsorbent), free sterols with Et_2O -petrol (1:1) (6 ml/g) and triterpene acids with MeOH (5 ml/g).

TLC. Thin layer chromatography was carried out on Si Gel G plates using as solvents hexane- Et_2O - HOAc (70:30:1) [13] and CHCl_3 [14] for neutral lipids, $\text{Me}_2\text{CO}-\text{C}_6\text{H}_6-\text{H}_2\text{O}$ (91:30:8) [15] for glycolipids and $\text{CHCl}_3-\text{MeOH}-\text{H}_2\text{O}$ (65:25:4) for phospholipids. Neutral lipids, sterols and triterpene acids were detected with the ferric chloride reagent [16], glycolipids with Schiff reagent [17] and phospholipids with Zinzadze

reagent [18]. Analytical TLC was carried out on Si Gel HR plates using the technique of Roughan and Batt for glycolipids [19] and that of Kuiper *et al.* [20] for phospholipids.

GLC. Fatty acid methyl esters prepared as described previously [21] were analyzed on 1800 \times 4 mm columns packed with 25% diethyleneglycol-succinate-2%, phosphoric acid on 100-120 mesh Gas Chrom P. Sterols and triterpene acid methyl esters were analyzed on 180 \times 0.4 cm columns packed with 3% OV-101 on 100-120 mesh Gas Chrom Q. Cholestane was included as internal standard and the detector response calculated using pure sterol or triterpene acid methyl ester standards [22]. Quantitative analyses were performed with an electronic integrator.

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