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LIPID EVOLUTION DURING DEVELOPMENT AND RIPENING OF PEACH FRUITS

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Abstract—Changes in the phospholipid, glycolipid, diacyl and triacylglycerol, free fatty acid and free sterol classes were examined during the development and ripening of *Prunus persica* cv. Redhaven peach fruit. Total lipid and lipid class contents decreased until 100 days after full bloom (AFB). While phospholipids and glycolipids decreased in the following stages, diacyl- and triacylglycerols increased. The analysis of each lipid class showed that the predominant fatty acids were 16:0 and 18:2. The double bond index showed, in each class, a general increase during maturation. The free sterol content continuously declined during maturation with the exception of samples at 100 days AFB, which showed an increase in the free sterol to phospholipid molar ratio. The main changes observed in lipids occurred mostly between the climacteric and postclimacteric stages.

INTRODUCTION

Physical, biochemical and physiological changes which occur during development of fruits imply intracellular variations that play an important role in the different distribution of metabolites in the cells.

It has been hypothesized [1] that membrane changes at the onset of ripening increase membrane permeability, resulting in decompartmentation of cellular components and increased catabolic processes, thus initiating fruit senescence. In avocado and apple fruits [2, 3], membrane changes become pronounced as the fruit reached the climacteric. Ripening has also been considered as a process of cell differentiation, at early stages of which the synthesis of specific enzymes occurs and cell metabolism is tightly controlled [4]. One of the major components of biomembranes are polar lipids; their physical state and composition influence both structural and functional properties of biological membranes [5]. The associations between membrane lipids and proteins, enzyme activity, transport capacity and permeability are all affected by the composition and phase properties of the membrane lipids [6–8]. During ripening of tomato fruit a decrease in phospholipids (PL) has been reported [9], while, in a study on ripening of apple fruit [10], decreases in monogalactosyldiacylglycerol (MGDG), and digalactosyldiacylglycerol (DGDG), which are lipids associated with plastid membranes, were found. Lipid changes, occurring during ripening of apple fruit, result in an increase in the free sterol (FS) to phospholipid molar ratio (FS/PL) and a decrease in the double bond index (DBI) of PL [3]. The increase in tissue leakage found in banana fruit during ripening was correlated with changes in membrane lipid composition [11].

Unlike fruits of other species, changes in lipids during the development and ripening of peach fruit have not been studied. Thus, the aim of the present work was to follow the changes in the most important lipid classes during their development and ripening.

RESULTS

Dry wt increased continuously from 0.5 g (49 days AFB) to 14.2 g (113 days AFB). In the first exponential growth stage (49 days AFB) [12], the peach mesocarp contained high quantities of total lipids as well as of all the lipid classes, free fatty acids (FFA) excluded (Table 1). Whereas FFA remained constant, all the other lipid classes decreased thereafter, although with different trends. PL and glycolipids (GL) decreased by *ca* 70 and 54%, respectively, from 49 to 65 days AFB, and by an additional 23 and 50% from 65 to 113 days AFB (Table 1). The neutral lipids, triacylglycerols (TG) and diacylglycerols (DG), which decreased dramatically early in development, increased 100 days AFB. The DG content was always higher than that of TG. FFA, undetectable at the first stage, were present in small amounts thereafter. Free sterol content and the FS/PL molar ratio showed a peak at 100 days AFB, decreasing at the last sampling date (Table 1).

Table 2 shows data for the four desmethyl sterols present in the peach mesocarp. During fruit development, sitosterol, the main free sterol, and campesterol

Table 1. Lipid evolution (μmol 100 g^{-1} dry wt) in mesocarp of peach (cv. Redhaven) fruits during development and ripening

Days AFB	PL	GL	TG	DG	FFA	FS	Total	FS/PL
49	1952.9d	722.2d	64.4c	75.3c	ND*	509.1c	3323.9d	0.26
65	573.5c	333.3c	5.9a	15.3b	1.2a	130.8a	1060.0c	0.23
100	487.7b	222.2b	5.5a	10.9a	1.1a	184.9b	912.3b	0.38
113	442.3a	166.7a	7.8b	15.7b	0.8a	123.0a	756.3a	0.28

Results are means of three replicates. For comparisons among means, analysis of variance was used. Means in columns followed by different letters are significantly different at $P \leq 0.01$.

*ND, not detectable.

AFB: after full bloom; PL: phospholipids; GL: galactolipids; TG: triacylglycerols; DG: diacylglycerols; FFA: free fatty acids; FS: free sterols.

Table 2. Free sterol composition (mol%) of mesocarp of peach (cv. Redhaven) fruits during development and ripening

Days AFB	Cholesterol	Campesterol	Stigmasterol	Sitosterol
49	0.2a	6.2c	0.6a	93.0c
65	0.3a	5.0b	1.5b	93.2c
100	0.5b	4.2a	6.7c	88.6b
113	0.5b	4.1a	10.4d	85.0a

Results are means of three replicates. For statistical analysis see Table 1.

decreased by 12 and 24%, respectively. On the contrary, stigmasterol and cholesterol continuously increased. The sitosterol to stigmasterol molar ratio showed a marked decrease during maturation, mostly due to a remarkable increase in stigmasterol.

The galactolipids consistently found at all the harvest dates were MGDG and DGDG (Table 3). The MGDG contents were always higher than that of DGDG and their proportion remained constant until 65 days AFB. Between 65 and 100 days AFB, while the MGDG decreased by 5%, the DGDG increased by 18%. From 100 to 113 days AFB, MGDG increased by 14% and the DGDG decreased by 40%. These changes resulted in an irregular trend in the MGDG/DGDG molar ratio (Table 3), which decreased by 20% between 65 and 100 days AFB and increased by 47% between 100 and 113 days AFB. The fatty acids palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3) were found in all lipid classes. Arachidic acid (20:0) was detected only in total polar lipids (TPL). In the TPL class (Fig. 1), 16:0 was the most abundant, followed by 18:2 and 18:1. From 65 days AFB, unsaturated C_{18} fatty acids increased significantly, whereas all the saturated acids decreased, the major changes occurring between 65 and 100 days AFB. In the DG and TG (Fig. 1), 18:2 acid was always the predominant fatty acid. As a consequence the DBI, relatively low in TPL and higher in TG and DG, showed a general increase during maturation. 16:0 and 18:2 were the major fatty acids of GL at all stages, whereas 18:3 was the main fatty acid in the DGDG only at 100 days AFB.

Table 3. Galactolipid composition (mol %) of mesocarp of peach (cv. Redhaven) fruits during development and ripening

Days AFB	MGDG	DGDG
49	77.5b	22.5b
65	77.9b	22.1b
100	74.0a	26.0c
113	84.3c	15.7a

Results are means of three replicates. For statistical analysis see Table 1.

MGDG: monogalactosyldiacylglycerol; DGDG: digalactosyldiacylglycerol.

The marked decrease in 18:3 observed in the DGDG from 100 to 113 days AFB is noteworthy. The unsaturation level of the individual GL significantly increased from 65 to 100 days AFB (Fig. 2). From 100 days AFB, the unsaturation level of the MGDG showed a further increase, whereas that of the DGDG decreased by 48% from 100 to 113 days AFB.

DISCUSSION

At 49 days AFB, fruits were at the end of the first period of exponential growth (SI), during which fruit development is characterized by an intense cell prolifer-

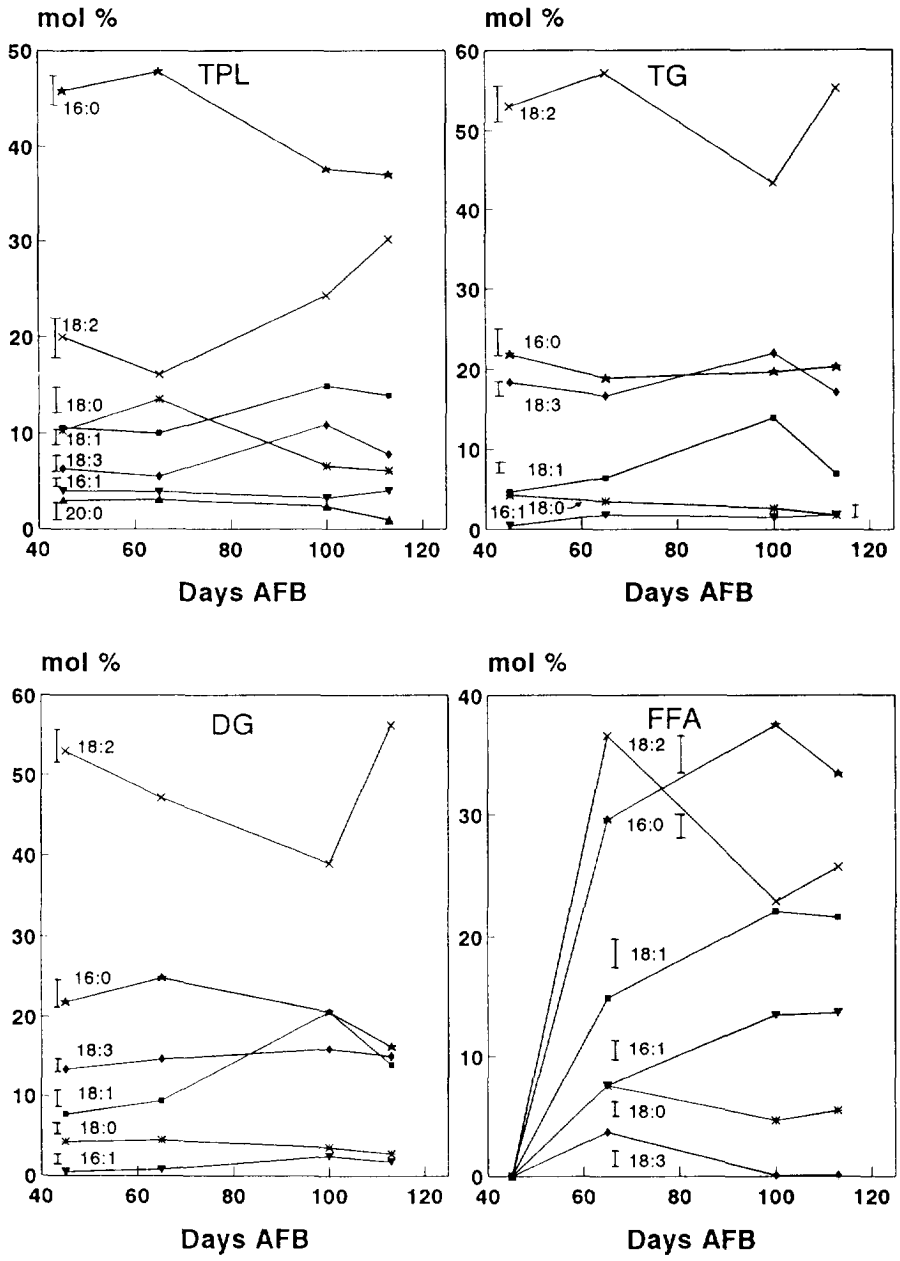

Fig. 1. Fatty acid composition of TPL, TG, DG and FFA in the mesocarp of peach (cv. Redhaven) fruits during development and ripening. Data are means of three replicates (LSD $\leqslant 0.01$).

ation. At 65 days AFB (SII phase) the fruits grew slowly, while in the following stage (100 days AFB) the fruits were at the end of the second exponential growth phase (SIII). This phase is characterized by rapid cell expansion, with a consequent increase of the cell volume in the mesocarp and formation of large intercellular spaces [12]. During this period peach fruit increases greatly in size, with a continuous accumulation of dry matter. At 113 days AFB, the exponential growth is completed and the fruits were in the SIV growth phase. In this stage, fruits no longer accumulate dry matter, showing final appearance and composition that make them ready for consumption.

In the first exponential growth stage (49 days AFB), the presence of high quantities of lipids, including storage lipids, such as DG and TG, indicates intense metabolic activity and a rapid rate of lipid synthesis in mesocarp cells. The decrease in total lipid content observed in the following stages (Table 1) is expected, because during maturation peaches accumulate carbohydrates rather than lipids. A decrease in polar lipids during the ripening process has been previously found in tomato fruits and it was explained by a rapid decline of lipid replenishment [9].

The increase in the FS/PL molar ratio (Table 1) and in the DBI of TPL (Fig. 3) observed at the third stage of

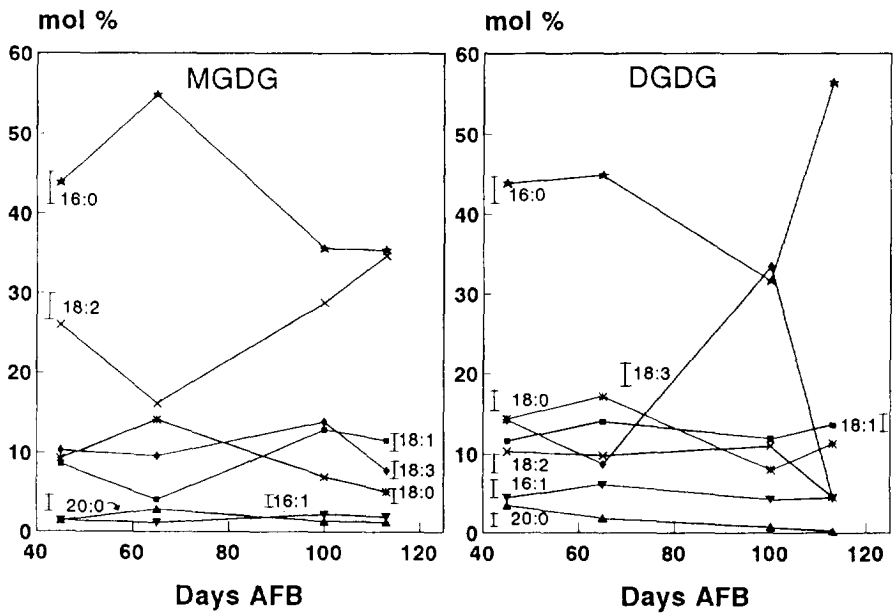

Fig. 2. Fatty acid composition of MGDG and DGDG in the mesocarp of peach (cv. Redhaven) fruits during development and ripening. Data are means of three replicates (LSD ≤ 0.01).

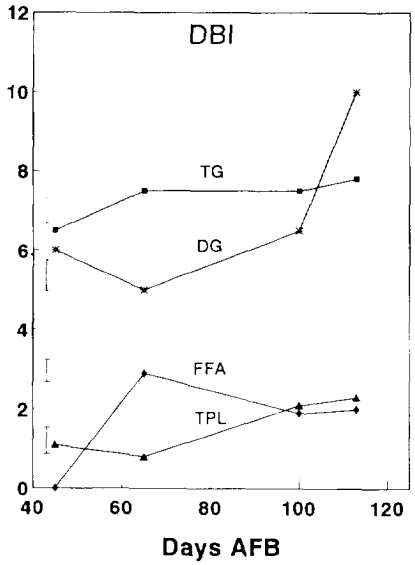

Fig. 3. Double bond index of lipid classes in the mesocarp of peach (cv. Redhaven) fruits during development and ripening. Data are means of three replicates (LSD ≤ 0.01).

growth (100 days AFB) has been associated with physical changes in biological membranes [13]. During ripening, senescence and abiotic stresses, an increase in the FS/PL molar ratio has been related to a decrease in membrane fluidity [3, 13–16].

Free sterols also have specific structural properties in regulating membrane stability, reducing the mobility of the phospholipid acyl chains and limiting the loss of structural integrity. They maintain the correct distance between lipid molecules in the bilayer when present, as in our experiment, in amounts of less than 33% of TPL [17] and reduce the rate of permeation by water [18]. Besides the level of FS, their composition also alters membrane status [5, 20]. The decrease in sitosterol and increase in stigmasterol during peach fruit growth (Table 2) causes a continuous decrease in their molar ratio, which is usually considered as an index of metabolic disorder and cell disorganization [19–21]. A decrease in this ratio has been observed previously in pericarp tissues during maturation of tomato fruit [22, 23] and it was found to be coincident with a reduction in membrane lipid fluidity [14]. The highest percent reduction in this ratio in peach fruit occurred at 100 days AFB. At this growth stage, it corresponded to an increase in the FS/PL molar ratio and in the DBI of TPL and a decrease in the more planar (cholesterol + campesterol) to less planar (stigmasterol + sitosterol) sterol ratio. All these variations lead to changes in the structural organization of membranes.

The significant increase in the DBI (Fig. 3) found in all lipid classes could be due to an increased activity of desaturase enzymes, which determined, in TPL in particular, a progressive increase of C_{18} polyunsaturated fatty acids (Fig. 1). Microsomal desaturases appear to be activated when bilayer fluidity decreases [16]. The decrease in fluidity during senescence is likely to maintain the desaturases in the active state and thereby facilitate the formation of polyunsaturated molecular species prone to lipolytic hydrolysis [24].

MGDG and DGDG are the major lipids associated with plastid structure. During peach fruit development all the plastids of the mesocarp are converted to chromo-

plasts with an electron dense stroma rich in plastoglobules [12]. These changes could be expected to be reflected in alterations in lipids of the plastid envelope and thylakoid membranes. Thus, variation in the MGDG/DGDG molar ratio (Table 3) could in part be the result of changes which occur at the plastid membrane level during the ripening of peach fruit. In addition, an increased MGDG/DGDG molar ratio was found in plastids of pepper fruit during their transformation into chromoplasts [25]. The marked decrease of 18:3 in DGDG between 100 and 113 days AFB (Fig. 3) might be due to the degradation of this lipid component (Table 3), as a consequence of the changes which occur during plastid transformations in the mesocarp of peach fruit. In the mesocarp of apple fruit during ripening, the decrease in 18:3 acid has been correlated with a breakdown of plastid membranes [10]. The changes in lipid composition observed in the mesocarp of peach fruit support the idea that lipids may have a role to play in ripening of peach fruit. In particular, the data reported here show that the greatest changes occur at 100 days AFB. This stage, at the end of SIII growth phase, corresponds to the onset of the climacteric period in peach fruit [26]. Our data indicate that the onset of the climacteric in peach fruit is accompanied by significant changes in lipids. Previously it has been observed that the greatest changes in membrane properties in apple [3] and in avocado [2] fruits occurred as the fruits reached the climacteric. These changes were also correlated with variations in the lipid composition of the membranes [3]. The disorganization of membranes following lipid changes may favour decompartmentation of cell constituents and intensify the catabolic processes leading to fruit senescence. Moreover, some of these changes may be adaptative reactions of cells of fruit occurring to reduce the disorganization of membranes and the consequent increase in permeability. Biophysical studies have indicated that there are changes in the molecular organization of lipids in microsomal and plasma membranes of senescing tissues with consequent destabilization of the bilayer [14]. This leads to the belief that these are possible responses common to all plant tissues at the end of their growth or maturation, or in unbalanced physiological conditions.

EXPERIMENTAL

Plant material. Fruit of *Prunus persica* (L). Batsch (cv. Redhaven) were harvested from 25 6-year-old trees grown at the Legnaro Horticultural Farm of the University of Padova (Italy). Fruits were sampled at 49, 65, 100 and 113 days AFB, corresponding to the SI, SII, SIII, and SIV stages of the double-sigmoid growth curve [12]. At each harvest date, 60 fruits were selected for uniform size and colour. Fresh wt was recorded and samples taken for dry wt determinations.

Lipid extraction and fractionation. Fruits were peeled and the mesocarp, cut into small pieces, was boiled in *iso*PrOH for 5 min. Lipids were extracted with $CHCl_3$–MeOH (2:1) containing butylated hydroxytoluene (50 $\mu g\,ml^{-1}$) at 4° for 3 hr, and re-extracted for 1 hr with additional solvent in a sealed flask under N_2. The combined extracts were centrifuged at 35000 *g* for 15 min and the supernatants collected. The organic phases were washed according to ref. [27] and fractionated into DG, FFA, FS, TG and TPL by TLC on activated silica gel using *n*-hexane–Et_2O–HOAc (70: 30:1) [5]. The band containing the TPL was then fractionated by 2D-TLC, using $CHCl_3$–Me_2CO–MeOH–HOAc–H_2O (10:4: 2:2:1) and $CHCl_3$–Me_2CO–MeOH–HOAc–H_2O (6:8: 2:2:1), respectively. Each component was identified by cochromatography with authentic standards under UV light after spraying with 0.1% Rhodamine 6G in EtOH. Known aliquots from TPL were used to determine PL and GL through their phosphorus and galactose contents, respectively [5]. TPL, individual GL, FFA, DG and TG were analysed for their fatty acid content. 17:0 was used as int. standard and the Me esters, obtained by transmethylation in 2 N MeOH, were analysed by GC [5]. DG, FFA and TG assayed for their fatty acid content were quantified using the conversion factors for each lipid class [28]. M_rs used were calculated on the basis of the average percent fatty acid composition of each lipid class (DG, TG, FFA) at each harvest date. The DBI was calculated as described in ref. [29]. Individual sterols were identified by GC, comparing RR_ts with those of authentic sterols. Cholestane was used as int. standard and, for their quantitation, corrections were made for differences in detector response [19].

Statistical analysis. A completely random experimental design was run in triplicate. Data from each experimental design determined in triplicate were analysed by analysis of variance. The significance of differences was determined according to Tukey's test. P values $\leqslant 0.01$ are considered to be significant.

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