Phytochemistry, Vol. 24, No. 12, pp. 2857–2859, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

LIPID METABOLISM OF RIPENING APPLES

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(*Received* 10 *April* 1985)

Key Word Index—*Malus domestica*; apple; fruit; steryl lipids; phospholipids; lipid synthesis.

Abstract—Little change was observed in the concentration of sitosterol, the principal free sterol of apple, during ripening of the fruit in air at 12°. Phospholipid increased by *ca* 10% during the first 15–18 days and thereafter showed little change. Phosphatidylcholine increased during ripening whilst phosphatidylethanolamine exhibited a transitory increase in the first 7–18 days. Incorporation of [^{14}C]acetate into free sterol by apple cortical discs showed little change during ripening but incorporation into phospholipids increased substantially between days 1 and 15 with a 27-fold increase in incorporation into phosphatidylcholine and an 8-fold increase into phosphatidylethanolamine and phosphatidylinositol.

INTRODUCTION

The ripening phase of fruits is a period of developmental change in which m-RNA codes for a number of a new enzymes [1, 2]; the synthesis of these enzymes together with changes in activity of the existing enzyme complement is thought to catalyse the ripening of the fruits [3]. Enhanced synthesis of ethylene may coordinate the changes observed [4]. Whilst much work has explored the role of enzymes in ripening, relatively little is known about changes which occur in membrane lipid composition and synthesis, and their role in ripening [5–8]. Analysis of the membrane lipid fraction prepared from apples at two dates (harvest and after storage in air at 12° for 34 days) showed that there was little change in the total phospholipid [principally phosphatidylcholine (PC) and phosphatidylethanolamine (PE)] but there was an increase of 46% in free sterol [5]. An increase in the sterol: phospholipid ratio is correlated with increased membrane viscosity and decreased membrane fluidity [9]; thus in apples, an increase in sterol concentration could affect the rate of ripening of the fruits by increasing membrane permeability [10]. This paper reports a study of the time course of the changes in membrane lipid composition and lipid synthesis in ripening apples.

RESULTS AND DISCUSSION

Apples picked in successive seasons from the same orchard ripened at similar rates during storage in air at 12°. Ethylene synthesis increased after 4 days and softening of the cortical tissue commenced after 7–8 days.

Free sterol was the major steryl lipid and in contrast to earlier observations [5] its concentration changed little during ripening (191 ± 4 nmol/g). Sitosterol was the principal sterol (187 ± 5 nmol/g) with a small amount of campesterol (11 ± 1 nmol/g); cholesterol was present at less than 1 nmol/g but stigmasterol was not detected. An unidentified sterol with an R_t of 3.74 relative to cholestane was present at 2–5 nmol/g.

Steryl ester, steryl glycoside and acylated steryl glycoside are also membrane components and these lipids may modulate membrane properties [11–13]. Acylated steryl glycoside was present at 11 nmol/g whilst steryl ester increased during ripening from 8 to 17 nmol/g (l.s.d. = 2; $P = 0.05$) and steryl glycoside changed from 27 to 13 nmol/g (l.s.d. = 8; $P = 0.05$).

The total phospholipid content of apples increased by *ca* 10% within 15–18 days and thereafter showed little change (Table 1). An increase in PC concentration accounted for most of the change in phospholipid; PE increased during the first 7–18 days but its concentration then returned to near the initial value. Phosphatidylinositol (PI), phosphatidic acid (PA) and diphosphatidylglycerol (DPG) were present at 15, 2 and 1 nmol/g, respectively. Of the lipids associated with plastids, only digalactosyl diacylglycerol (DGDG) changed during ripening (50–32 nmol/g; l.s.d. = 6, $P = 0.05$); phosphatidylglycerol (PG) and monogalactosyldiacylglycerol (MGDG) were present at 6 and 35 nmol/g, respectively.

An increase in sterol: phospholipid ratio during senescence of flowers is correlated with decreased membrane fluidity [9] and the concerted action of several neutral lipids, including sterol, appears to selectively rigidify the unsaturated acyl chains of phospholipids inducing gel phase formation in membranes and an increase in membrane permeability [13]. Changes in phospholipid headgroup composition [14] and in the degree of saturation of lipid fatty acids can also affect membrane fluidity. In apple, the unsaturated fatty acids of the polar lipids are present at much higher concentrations (3.6 fold) than the saturated acids and this relationship shows little change during ripening [15]. However, the sterol: phospholipid ratio of membrane lipids declined 10–13% during ripening whilst the ratio of PC: PE increased from 1.33 at day 8 to 1.56 at day 18 and thereafter, changes which indicate an increase in membrane fluidity. It seems unlikely therefore that there is a general increase in membrane permeability during ripening of apple [10] although there may be limited changes in the composition of particular membrane fractions which are not reflected in the analyses of bulk lipid. The plasma membrane and tonoplast of the fruit cells of ripening tomato show no changes in permeability [16].

Incorporation of [^{14}C]acetate into the neutral plus

Table 1. Concentrations of phospholipids in ripening apples in two storage seasons (1982, 1983)

Days at 12°	Phospholipids (nmol/g fr. wt)					
	Total		PC (PG)	PC	PE (PA)	PE
	1982	1983	1982	1983	1982	1983
1	371±5	381±4	209±3	189±1	141±1	144±1
7	408±18		219±9		155±5	
8		408±3		205±1		154±2
15	420±5		228±3		156±1	
18		422±2		225±1		145±1
21	425±10		239±2		143±1	
27		402±6		212±4		134±1
29	428±11		243±4		142±2	
39		415±8		219±4		138±1
L.s.d.	43	20	19	14	10	6

Results are expressed as mean ± s.d. ($n = 2$), l.s.d. determined at $P = 0.05$. In 1982, PC plus PG and PE plus PA were not separated prior to assay.

polar lipids of apple cortical discs increased 7-fold between day 1 and 15. Incorporation into free sterol changed little during ripening but incorporation into the phospholipids increased substantially, 27-fold for PC and 8-fold for PE and PI (Table 2). Lipid synthesis in preclimacteric fruit, picked 7 days before the main harvest, was similar to that assayed in apples after 1 day in store. Membrane phospholipids in plants are in a dynamic state; their concentration is governed by the rates of synthesis, transfer and degradation [17, 18]. Whilst the rates of synthesis of phospholipids increased substantially between days 1 and 15 the increase in phospholipid concentration was small; synthesis remained at a high level to 29 days. The data suggest that the rate of degradation of phospholipids also increases in apples during ripening. The precursors for the synthesis by the ripening fruits of low M_r alcohols and esters of carboxylic acids (aroma compounds) derive from the oxidation of fatty acids [19]; the increase in phospholipid degradation could supply these long chain acids.

EXPERIMENTAL

Source and storage of apples. Apples (*Malus domestica* Borkh. cv Cox's Orange Pippin) were harvested in September 1982 and 1983 from trees growing at East Malling Research Station. The apples were sorted into random 12, 16 or 20 fruit samples and stored in containers at 12° supplied with air at 5 l/hr. C_2H_4 in the headspace was determined by GC and firmness measured using an automated penetrometer with 8 mm plunger [20].

Table 2. Incorporation of [^{14}C]acetate into sterol and phospholipids by cortical discs from apple during ripening

Days at 12°	Sterol	PC	PE	PI
		(Bq × 10^{-1}/g fr. wt)		
Preclimacteric	8	167	47	13
1	12	160	52	15
15	8	4302	403	127
29	7	3985	288	108

Preclimacteric apples were picked 7 days before the main harvest.

Extraction of lipids. Duplicate plugs of apple, 1 cm diam., were cut from an equatorial slice of the fruit using a cork borer; the plugs were cut into discs and transferred to 50 ml *iso*-PrOH, butylated hydroxytoluene (BHT), 10 μg/ml. Six or nine apples were used for lipid preparation at each date and the duplicate samples of discs for analysis (25–30 g) were heated at 78° for 10 min [21], cooled and stored at −20° under N_2. Total lipids were extracted using the procedure of ref. [22] and stored in toluene–EtOH (4:1) at −20°.

Analysis of lipids. Steryl lipids were separated using TLC on silica gel G with a two step development system [23]. The plate was developed to 13 cm with toluene–Et_2O–EtOH–HOAc (50:40:2:0.1), dried and developed in the same direction using Et_2O–hexane (1:19) to 1 cm from the top of the plate. Steryl lipids were located by spraying standards in a marker lane with 2,7-dichlorofluorescein.

Phospholipids were separated on silica gel H developed with $CHCl_3$–MeOH–HOAc–H_2O (85:15:10:3). Lipids were located with I_2 vapour. Glycolipids were separated using silica gel G plates and the same solvent. The identity of individual lipids was confirmed using known lipids and specific spray reagents [5].

The acidic lipid fraction was separated from total lipid using columns (1.5 × 1 cm) of DEAE-cellulose (Whatman DE-23) [23, 24]. The phospholipids of this fraction were separated by TLC, as above.

Steryl lipids were extracted from silica gel with $CHCl_3$ (3 ml, × 2), centrifuging at 500 *g*, 5 min to ppt the gel. The supernatants were combined, evaporated to dryness under N_2 at 45° and dissolved in 0.2–0.4 ml $CHCl_3$. The lipids were assayed using *o*-phthalaldehyde [25]. Free sterols were also analysed as their TMSi derivatives using FID-GC [26]. Cholestane (40 μg) was used as int. standard. The column was 3% OV-17 on Chromosorb W/120 mesh, the carrier gas N_2 (flow rate 50 ml/min) and the operating conditions were column 250°, inj. temp. 250° and detector temp. 310°. Sterols were identified on the basis of RR_t compared to known standards and the rel. wt response of cholesterol to cholestane was used to determine the sterol concn.

Glycolipids were determined using the PhOH–H_2SO_4 method [24]. Phospholipids in the total lipid or phospholipids in the presence of silica gel H were determined by assay of Pi released following digestion with $HClO_4$ [27].

Experiments with apple cortical tissue. Discs, 1 mm thickness, were cut from plugs of cortical tissue taken from the equatorial region of five apples, pooled and 6 discs (*ca.* 1 g) incubated for 1 hr at 25° in 2.5 ml 0.5 M sucrose, 0.1 M K-Pi buffer, pH 4.5 containing 1.11×10^6 Bq [1-^{14}C]NaOAc (sp. act. 2.28 $\times 10^9$ Bq/mmol). The incubation buffer was removed and the discs washed twice with 5 ml buffer before addition of 20 ml *iso*-PrOH. The total lipid fraction was prepared, as above. [^{14}C]Lipids were assayed following separation by TLC, as before, except that phospholipids were fractionated on silica gel G and total lipids were co-chromatographed with standards to enable location of lipids with I_2 vapour. Lipids were displaced from silica gel by addition of 1 ml H_2O and 10 ml of Aquasol-2 (New England Nuclear) added per vial prior to determination of radioactivity by liquid scintillation spectrometry [28].

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