Phytochemistry, 1973, Vol. 12, pp. 487 to 492. Pergamon Press. Printed in England.

LIPIDS OF RIPENING TOMATO FRUIT AND ITS MITOCHONDRIAL FRACTION

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(*Revised Received* 4 *October* 1972. *Accepted* 15 *October* 1972)

Key Word Index—*Lycopersicon esculentum*; (Solanaceae); tomato fruit; ripening process; phospholipids; glycolipids; mitochondrial lipids.

Abstract—The lipid composition of tomato fruit and its mitochondrial fraction were examined at various stages of fruit ripeness. Phosphatidyl choline, phosphatidyl ethanolamine, monogalactosyl diglyceride, digalactosyl diglyceride and phosphatidyl inositol were found to be the major lipids of tomato pericarp at all stages of ripeness. Mitochondrial lipids resembled those of the parent tissue except for the absence of monogalactosyl diglyceride and a greater percentage of diphosphatidyl glycerol and phosphatidic acid. Changes in the lipid–protein ratio of mitochondria were noted with ripening.

INTRODUCTION

THE PHENOMENA of ripening and senescence in fruits have been the subject of numerous studies. Cocking and Gregory[1] noted a general reorganization of the cytoplasmic structures in fruit cells during the ripening process. Others[2,3] have observed a considerable increase in membrane permeability as the fruit ripened.

In order to understand the function of a biological system, the detailed molecular composition among other aspects of the tissue must be known. Like carbohydrates and proteins, lipids are important constituents which presumably undergo constant changes in ripening fruit. The importance of lipids is further reflected by their presence as major components of the membranous systems, and changes in membrane permeability are considered an early sign of the ripening process.[4] Recently,[5] it has been reported that changes in glycolipid and glycoprotein contents are the determining factors in drug resistance which is mediated by a change in permeability.

The information on lipid components of ripening fruit is rather scanty. Studies on fatty acid changes during the ripening process have been reported in banana pulp and peel[6] and in apple and pear.[7] Since the fatty acid composition of membrane lipids is known to change with external influences,[8] it may be necessary to study the lipid components in their entirety.

Galliard[9] studied the lipid contents of pre- and post-climacteric apple fruits, but in most fruits the climacteric period represents a relatively small part of the whole ripening process. Although such a study provides useful information, it is difficult to generalize the data for the entire ripening phenomenon in a fruit. The avocado, for example, with a high

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[3] R. F. M. VAN STEVENINCK and M. E. JACKMAN, *Austral. J. Biol. Sci.* **20**, 749 (1967).
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[5] H. B. BOSMANN, *Nature, Lond.* **233**, 566 (1971).
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[7] R. J. ROMANI, R. W. BREIDENBACH and J. G. VAN KOOY, *Plant Physiol.* **40**, 561 (1965).
[8] J. W. FARQUHAR and E. H. AHRENS, JR. *J. Clin. Invest.* **42**, 675 (1963).
[9] T. GALLIARD, *Phytochem.* **7**, 1915 (1968).

fat content, does not seem to utilize fat for respiration during the climacteric cycle.[10] The present study was undertaken to study lipid changes involved during the ripening process in tomato fruit and its mitochondrial fraction.

RESULTS AND DISCUSSION

Whole Tissue Lipids

Table 1 summarizes the data on lipid composition of tomato fruit tissue at three stages during the ripening process. Qualitatively the lipid composition of tomato fruit tissue is similar to that of potato[11] and apple.[9]

TABLE 1. PHOSPHOLIPID AND GLYCOLIPID COMPONENTS OF RIPENING TOMATO FRUITS

Lipid component	mg of lipid per 100 g of fresh tissue		
	Green	Breaker	'Breaker +6 days'
Phosphatidyl serine	1·3	0·9	0·5
Phosphatidyl inositol	5·0	2·5	4·2
Phosphatidyl choline	17·8	14·5	12·8
Phosphatidyl ethanolamine	10·2	8·5	6·1
Phosphatidyl glycerol	1·4	0·8	1·0
Phosphatidic acid	1·5	1·1	1·9
Diphosphatidyl glycerol	0·6	0·4	0·2
Total Phospholipid	37·8	28·7	26·7
Sulfolipid	0·8	1·3	1·0
Digalactosyl diglyceride	6·8	6·6	7·5
Cerebroside	3·6	1·9	2·8
Steryl glucoside	2·5	2·8	3·4
Monogalactosyl diglyceride	6·0	8·3	5·2
Total Glycolipid	19·7	20·9	19·9

Lipids of tomato fruit tissue were separated by two-dimensional TLC and the phospholipid and galactolipid spots were analyzed for phosphorus and galactose, respectively. The corresponding lipid components were calculated from the calculated molecular weights of the lipids. The data represent the mean for three samples of tomato fruits.

Among the phospholipids of mature green tomato pericarp, PC,* PE, PI, PA, PG, PS and DPG were found in decreasing order of abundance, with PC and PE forming the bulk (72–80%) of the phospholipid fraction. Phospholipids generally decreased with fruit ripening, with the change in DPG being the most dramatic. An increase in PI concentration, significant at the 5% level of confidence, was observed between breaker stage and the breaker +6 day stage. PA showed a similar trend, but with a lesser degree of confidence in

* Abbreviations used: PC, PE, PI, PG and PS = phosphatidyl-choline, -ethanolamine, -inositol, -glycerol and -serine, respectively; MGDG, DGDG and PGDG = mono-, di- and poly-galactosyl diglycerides, respectively; SG = steryl glucoside; SL = sulfolipid; CER = glucocerebroside; PA = phosphatidic acid; DPG = diphosphatidyl glycerol, ESG = esterified steryl glucoside.

[10] J. B. BIALE, *Science* **146**, 880 (1964).
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the data. MGDG and DGDG were the major glycolipids at the mature green stage, with CER, SG and SL being present in lesser amounts. ESG and PGDG were also detected but were not determined quantitatively. The level of total glycolipids remained relatively constant with fruit ripening.

It is difficult to ascribe the observed changes to specific subcellular organelles because, more or less, the whole tissue undergoes a change during the ripening process. Many studies[6,12] have implicated the lipid changes in ripening fruit to the degradation of chloroplast structures. But Sacher[13] noted increased permeability of all membranes during ripening and senescene in plant tissues. Since the cellular and subcellular membranes are lipoprotein complexes, changes in permeability may be accompanied by lipid changes.[5] The general decrease in lipid content may be due to failure of the mechanism for the replenishment of lipids. This was confirmed by a study of incorporation of acetate-^{14}C into lipids of tomato tissue during the ripening process.[14] A significant decrease in the synthesis of polar lipids was observed as the fruit ripened.

TABLE 2. COMPARISON OF LIPIDS OF TOMATO FRUIT TISSUE AND ITS MITOCHONDRIAL FRACTION

Lipid component*	Percentage of lipid components: Green		Breaker		Breaker + 6 days	
	Whole tissue	Mitochondria	Whole tissue	Mitochondria	Whole tissue	Mitochondria
PS	2·2	4·7	1·8	5·8	1·1	3·3
PI	8·7	8·3	5·0	8·0	9·1	6·2
PC	31·0	20·0	29·3	12·1	27·4	18·1
PE	17·7	13·9	17·1	9·7	13·0	15·0
PG	2·5	2·5	1·6	3·4	2·2	0·8
PA	2·6	4·3	2·3	6·7	4·1	16·1
DPG	0·9	4·8	0·7	6·3	0·4	5·2
Total Phospholipid	65·6	58·6	57·8	52·0	57·3	64·7
SL	1·5	1·9	2·6	3·3	2·2	1·4
DGDG	11·8	25·6	13·2	22·0	16·0	14·6
CER	6·3	5·8	3·9	12·3	6·1	11·9
SG	4·3	8·1	5·7	10·4	7·3	7·4
MGDG	10·5	(−)	16·8	(−)	11·1	(−)
Total Glycolipid	34·4	41·4	42·2	48·0	42·7	35·3

* Abbreviated in the same order as Table 1.
(−) Not detected.

Mitochondrial Lipids

The lipid compositions of the mitochondria obtained from tomato pericarp at several stages of ripeness is reported in Table 2. Percentage composition is reported since mitochondrial yield, which may vary with the stage of ripeness, was not determined. Relative lipid compositions of tomato pericarp tissue are presented for comparison.

[12] S. R. DRAPER, *Phytochem.* **8**, 1641 (1969).
[13] J. A. SACHER, *Plant Physiol.* **41**, 701 (1966).
[14] S. K. KALRA, Ph.D. Dissertation, West Virginia Univ., U.S.A. (1972).

The lipid composition of the tomato mitochondria qualitatively resembled that of the parent tissue. One noteworthy exception was the complete absence from the mitochondria of MGDG, which constituted 10–17% of the whole tissue lipids. DGDG, moreover, was present in higher concentration in mitochondrial lipids than in whole tissue lipids. MGDG and DGDG are generally considered to be predominantly associated with chloroplasts or plastids in plant cells, with Ongun *et al.*[15] having found 83% of the MGDG and 88% of the DGDG associated with chloroplasts in spinach leaf. The data[15] also indicate that MGDG and DGDG comprise a larger proportion of the total polar lipids of spinach leaf than the value obtained with tomato. Rosenburg and Gouaux[16] have indicated that in *Euglena* a portion of the MGDG and DGDG exist in membranes other than chloroplast membranes.

The relative percentages of PA, PS and DPG in mitochondrial lipids were 2–4, 2–3 and 5–13 times greater in mitochondrial lipids than in whole tissue lipids. Of these, DPG is the most interesting, since in animal systems DPG has been found only in the mitochondria.[17] Fleisher and Fleisher[18] observed that the major phospholipids of beef heart mitochondria, DPG, PC and PE, were present in the approximate ratio 1:4:4, which is very similar to the ratio 1:4:3 observed in green tomato mitochondria. The distribution of DPG in other plant cell subcellular fractions, however, is not well documented, although the overall concentration would appear to be less than in mitochondria.

The large amount of PA found in mitochondrial lipids strongly suggests the action of phospholipase D on endogenous phospholipids.[19] The increased PA content of ripening fruits might thus result from increased phospholipase D activity, but this point has not been investigated. PA constituted more than 4% of the lipids measured in tomato pericarp extracts where hot isopropanol was used as the first extractant. Hot isopropanol should inhibit enzyme activity, although some evidence to the contrary exists.[20] PA is reported as less than 1% of the total lipids extracted from potato tubers[11] and apple.[9] PA amounted to 2% of the total lipid extracted from spinach leaf homogenates,[15] but was absent from extracts of whole leaf tissue.

Protein-Lipid Ratio

Simultaneous protein determinations were carried out on the mitochondrial fractions isolated by sucrose density centrifugation. Total mitochondrial protein recovered at the breaker and breaker +3 days stages were slightly higher than at the mature green stage. Likewise the protein–lipid ratios at these two stages exceed that of the mature green stage.

The data in Table 3 might be interpreted in either of the two following ways. First, the process might be viewed as one of simple degradation, with loss of mitochondrial lipid beginning at breaker stage; loss of mitochondrial protein would be a subsequent step, beginning 4 or more days later. Alternately, a more complex picture might be assumed in which mitochondria are in constant turnover. In this picture mitochondria produced at the breaker stage would be lower in net lipid than the mitochondria at the mature green stage, but would revert to higher lipid form by breaker +6 days. Although the net mitochondrial protein recovered per g of tissue showed only a slight rise at the breaker and

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[16] A. ROSENBERG and J. GOUAUX, *J. Lipid Res.* **8**, 80 (1967).
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[18] S. FLEISCHER and B. FLEISCHER, in *Methods in Enzymology* (edited by R. W. ESTABROOK and M. E. PULLMAN), Vol. X, p. 406, Academic Press, New York (1967).
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[20] M. LEPAGE, *Lipids* **2**, 244 (1967).

breaker +3 days stages, acetate-^{14}C incorporation experiments exhibited a sharp peak of DPG synthesis at the breaker stage while the synthesis of other phospholipids declined.[14] Diphosphatidyl glycerol might be regarded as a mitochondrial lipid on the basis of previous discussion.[17] Increased synthesis of mitochondrial enzymes[21] in apples at the climacteric, which is coincident with the breaker stage in this study, has been reported by previous workers.

TABLE 3. PROTEIN AND LIPID CONTENTS OF THE MITOCHONDRIAL FRACTION

Description	μg per mitochondrial fraction			
	Green	Breaker	Breaker + 3 days	Breaker + 6 days
Protein†	918	1063	1158	601
Total lipid*	447	317	300	244
Protein–lipid ratio	2·1	3·4	3·9	2·5

* Sum of phospholipids and glycolipids.

† Protein content represents the mean of duplicate determinations of three samples of the mitochondrial preparation at each stage of fruit.

Korn[22] reviewed the protein and polar lipid contents of a host of membranes of biological systems and reported the protein–lipid ratio of the outer (1·2) and inner (3·6) mitochondrial membranes and the ratios for other membranes between 0·25 and 4·0. The ratios found in the tomato mitochondrial fraction in the present study were close to the values obtained on the animal mitochondrial systems. Again, this may be a reflection of similarity of structures of mitochondria from the two sources.

EXPERIMENTAL

Materials. Tomato fruits (*Lycopersicon esculentum* var. Manhattan) were grown under greenhouse conditions. Four stages of ripening fruit were selected to study lipid contents. *Green.* Mature green fruits were picked while they were turning from green to whitish color. *Breaker.* Fruits picked when they started turning pink at the distal end. *Breaker +3 days.* Fruits were picked at the breaker stage and stored for 3 days at 19° in plant growth chambers. *Breaker +6 days.* Fruits were picked at breaker stage and stored for 6 days at 19°. Only the outer pericarp of fruit was used for the present study.

Lipid composition of whole tissue. Lipid extraction. Tomato fruits were sliced and the locular material was discarded. 80 g of the slices were homogenized with 160 ml of boiling isoPrOH in a Waring blendor for 1 min. The homogenate was filtered (Whatman No. 1) and the residue rehomogenized 2× with 75 ml $CHCl_3$–MeOH (1:2) and refiltered and finally with 50 ml $CHCl_3$. The combined filtrate was diluted with 160 ml $CHCl_3$ + 160 ml H_2O. The $CHCl_3$ phase containing lipid was separated and the aqueous phase was reextracted with 20–25 ml of $CHCl_3$. Residual H_2O and H_2O-soluble impurities were removed with 15–20 g Na_2SO_4. The dry $CHCl_3$ was dried and the residue dissolved in $CHCl_3$ and stored at −20° under N_2. 2-D-TLC was performed according to the method of Nichols and James.[23] Phospholipid spots were identified with phosphomolybdate reagent[24] and cholesteryl esters with $FeCl_3$ spray.[25] Ninhydrin was used for lipid-containing amino groups. Glycolipids were detected with anthrone–H_2SO_4. Identification of lipids was made by a comparison to the TLC-patterns obtained with potato tuber lipids[11] and lettuce leaf lipids,[26] in conjunction with the indicated spray reagents. The MGDG and DGDG were further characterized by chromatographic identification of galactose after 2 hr of hydrolysis in 2 N HCl.[27]

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Determination of phospholipids. 2-D-TLC plates were developed and exposed to I_2. The detected phospholipid spots were scraped off and digested for phosphorus.[28] The wt of individual lipid components was calculated on the basis of their MWs.* The recovery for individual phospholipids was 80–85%.

Determination of glycolipids. The glycolipid spots were scraped off and weighed, and galactose determination was carried out by the method of Roughan and Batt.[29] The adjustments were made for the amount of silica gel and the recovery percentage (80%). The recovery percentage of 80% was assumed on the basis of similar experiments on phospholipids, due to unavailability of pure glycolipid samples.

Isolation of mitochondria. Tomato fruit slices (100 g) were homogenized in a Waring blendor with a medium (150 ml) containing 0·25 M mannitol, 0·25 M sucrose, 0·05 M sodium barbital, 5 mM EDTA, 4 mM cysteine–HCl (pH 7·8) and 1·5 mg of bovine serum albumin per ml of the medium, for 1–2 min at minimum speed. The homogenate was filtered through cheese cloth. The process was repeated with the residue and 50 ml of the medium. The pH was adjusted to 7·0–7·5 by 5 M KOH. From the combined filtrates (pH 7·5), mitochondrial pellet was obtained by differential centrifugation.[30] Only one mitochondrial pellet was collected as against heavy and light fractions mentioned in the original method. The crude mitochondrial suspension was purified on a sucrose density gradient. An 11-ml uniformly linear gradient (0·9–1·9 M sucrose) was prepared in thin-walled polypropylene tubes (IEC, poly-thin, 14·5 × 96 mm). The gradients were cooled for about 24 hr at 0·4° before use. 1·0 ml of the freshly prepared mitochondrial suspension was layered on top of each gradient and centrifuged at 24 000 rpm for 4 hr at 4°, using swinging bucket rotor. Each gradient was then fractionated on a density gradient fractionator. The mitochondrial band was identified by enzyme assays, viz. malate dehydrogenase, cytochrome-*c* oxidase, succinate dehydrogenase and NADH oxidase. The optimum activities of all the enzymes were observed in the same gradient fraction. Generally 3 gradient fractions having optimum enzyme activity were collected and designated the mitochondrial fraction.

Mitochondrial lipids. The mitochondrial fractions from all the gradient tubes were pooled and were diluted with 3–4 vol. of mixture of $CHCl_3$–MeOH (1:2). The mixture was shaken for 2 hr, filtered and then rinsed with $CHCl_3$. The filtrate was diluted with H_2O to make the final ratio of $CHCl_3$–MeOH–H_2O (2:2:1·8) The $CHCl_3$ phase was separated and the aqueous phase was re-extracted with small vol. of $CHCl_3$. The extract (Na_2SO_4) concentrated under N_2 and residue was taken up in $CHCl_3$. Lipids were analyzed as mentioned for the whole tissue.

Mitochondrial protein. An aliquot of the mitochondrial fraction was diluted and precipitated with 10% trichloroacetic acid. The precipitate was sedimented and dissolved in 0·1 N NaOH by the method of Anderson.[31] Protein determination was made by the method of Lowry *et al.*[32] using bovine serum albumin as a standard.

Acknowledgement—Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 1225.

* Calculated MWs of lipids: PS = 787; PI = 861; PC = 786; PE = 743; PG = 774; PA = 700; DPG = 1456; SL = 846; DGDG = 943; CER = 725; SG = 576; MGDG = 782.

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