Effect of Temperature on Fatty Acid Composition of Mitochondrial Membrane in Tomato Fruits during Storage

Nagao OGURA, Yuko ABE, Hiroki NAKAGAWA
and Hidetaro TAKEHANA
Laboratory of Food Science and Technology

Abstract

Effect of temperature on fatty acid composition of mitochondrial membrane in tomato fruit during storage. Nagao OGURA, Yuko ABE, Hiroki NAKAGAWA and Hidetaro TAKEHANA Faculty of Horticulture, Chiba University, Matsudo, Japan. *Tech. Bull. Fac. Hort. Chiba Univ.*, No. 25 : 35—38, 1977.

Tomato fruits were harvested at mature green stage and stored at 4, 33°C and room temperature. The changes in the fatty acid composition of mitochondrial membrane of these fruits during storage were studied. The fatty acid in the mitochondrial membrane was rich in palmitic, linoleic, and linolenic acid, and they occupied about 90% of total fatty acid. The ratio of unsaturated fatty acid to total fatty acid (UFA/TFA ratio) was 66.2% in mature green stage of fruit. UFA/TFA ratio was progressively decreased during ripening at room temperature and this tendency was strengthened at 33°C. UFA/TFA ratio, however, was increased during storage at 4°C.

There has been known that many interesting physiological phenomena occur in post-harvest of fruits (SACHER 1973, SPENCER 1965). Changes in the phospholipids and their fatty acid composition in fruits are considered as an important subject concerned with ripening physiology.

In tomato fruits, it has been shown that saturated fatty acid in the cardiolipid fraction increased with progress of storage period, and that higher unsaturated fatty acid was metabolised rapidly during the first part of storage period (MINAMIDA et al. 1970). Additionally, it has been reported that the degree of unsaturation of fatty acids in the mitochondrial fraction was a higher value in a temperate-zone plant than in a tropical or subtropical plant to keep flexibility of the mitochondrial membrane (LYONS 1964).

In a previous paper on the effect of storage temperature of tomato fruits, we reported that mature green tomato fruits previously stored at 33°C for 10—20 days and afterward stored at room temperature were possible to extend the storage life about over 180 days(OGURA et al. 1975-a). Compared with fruits stored at room temperature, respiratory rate was repressed to low level in fruits stored at 33°C. In addition to this fact, changes of components of organic acids which are considered as respiratory substrate also made a difference at room temperature stored fruits and at 33°C (OGURA et al. 1975-b).

The present work was undertaken to examine the effect of storage temperature on the fatty acids composition of tomato mitochondrial membrane in relation to respiration and storage period.

Mature green tomato fruits (*Lycopersicon esculentum cv Hikari*) were harvested from field of Chiba University and stored at 4, 33°C and room temperature in the dark.

* Studies on the storage temperature of tomato fruit Part V. Abbreviations: BSA, bovine serum albumin.

Mitochondrial fraction was prepared by the method of Ku et al. (1968) with some modifications. The outer and radial wall tissues (200 g) were cut by hand into very thin slices with a stainless steel razor blade and homogenized for 1 min at the top speed in a Waring blendor with an equal amount (w/v) of grinding media(0.1 M Tris-HCl buffer, pH 7.6, containing 0.4 M sucrose, 2 mM $MgCl_2$, 8 mM EDTA, 4 mM cystein, 10 mM KCl, 0.5 mg/ml BSA). The homogenate was strained through 8 layers of gauze and centrifuged at 2,000×g for 20 min, and the sediment was resuspended in 60 ml washing media (10 mM Tris-HCl buffer pH 7.2, containing 0.4 M sucrose, 10 mM KCl, 0.5 mg/ml BSA, 1 mM $MgCl_2$, 10 mM KH_2PO_4). This operation was repeated once more. The preparation was carried out between 0 and 4 °C.

The mitochondrial membrane was prepared according to the method of Yamaki et al. (1972). The mitochondrial fraction was suspended in 0.1 M NaCl, then subjected to sonic treatment at 10 K cycle at 0°C for 60 sec. The mitochondrial membrane was immediately collected by centrifugation at 77,000×g for 60 min.

The method for the lipid extraction was that of Uritani et al. (1969). The mitochondrial membrane was homogenized with 10 ml of chloroform-methanol (2 : 1 v/v) in a Potter-Elvehjem homogenizer. The homogenate was heated at 50—60°C for 10 min, and after cooling to room temperature centrifuged at 3,000 rpm for 10 min. The supernatant was thoroughly shaken with 1 ml of 50 mM KCl solution. After the mixture was separated into two phases, the lower phase was washed twice with an aqueous solution in which 1 part of water had been shaken with 10 parts of chloroform-methanol (2 : 1 v/v), and taken as the lipid fraction.

Preparation of fatty acid methylester was as follows. The lipid fraction was dehydrated with anhydrous sodium sulfate and evaporated, and disolved in 3 ml of petroleum ether. About 3 ml of 0.3 N sodium methoxide solution was added, and petroleum ether was removed by warming on a water bath at about 50°C with shaking. To the reaction mixture, 3 N methanol solution of sulfuric acid was added until the solution was neutral to phenolphtalein. About 3 ml of petroleum ether and 1 ml of water was added, and then the mixture was shaken thoroughly. After standstill, the upper layer was taken. The solvent was removed by warming on a water bath at about 50°C in order to concentrate the solution.

The fatty acid composition were analyzed with gas-liquid chromatography. Gas chromatographic analysis of the methyl esters of fatty acids was made on a Hitachi gas chromatographic apparatus Model 023. The analytical column, 3 mm×2 m stainless steel tubing, was packed with 15% diethylene-

Table 1. Changes in the fatty acid composition of mitochondrial menbrane of tomato fruits stored at various temperatures.

temp / time (days) / fatty acid	room temp				33°C			4°C			33°C ↓ room temp
	0	3	6	9	3	6	9	3	9	37	
12 : 0	0.1	1.1	0.2	0.1	0.2	0.3	0.6	0.2	0.3	0.2	0.6
14 : 0	0.4	0.3	0.6	0.8	0.5	0.8	0.8	0.6	0.4	0.5	0.8
16 : 0	30.6	29.5	34.6	34.3	36.1	39.3	40.3	27.1	24.6	28.4	34.8
18 : 0	2.9	2.2	3.3	4.0	3.8	4.6	5.0	3.9	2.9	2.7	4.9
18 : 1	1.5	0.7	1.6	1.8	1.3	1.5	1.2	2.9	1.7	2.3	1.8
18 : 2	45.0	46.6	44.6	44.5	42.3	42.5	39.8	39.0	42.0	45.2	48.7
18 : 3	19.6	18.9	15.0	14.6	16.0	11.1	12.2	26.3	28.2	21.2	8.4
UFA/TFA ratio (%)	66.2	66.2	61.2	60.9	59.5	55.1	53.3	68.2	71.9	68.6	58.9

glycolsuccinate on 60—80 mesh Celite 545. The operating conditions were as follows: column temperature, 200°C, injection temperature, 280°C, and flow rate of helium as carrier gas, 40 ml/min. The amount of each separated compound was calculated by estimating the area under the recorded curve, and the identification was based on the retention time of the individual authentic fatty acids.

As shown in Table 1, the fatty acids in mitochondrial membrane from tomato fruits were rich in palmitic, linoleic, and linolenic acid and they were about over 90% total fatty acids.

With regard to room temperature-stored fruits, slight decrease of linolenic acid and an increase of palmitic acid were found. There were not distinguished changes in linoleic, stearic and myristic acid. In 33°C-stored fruits, a decrease of linolenic acid and an increase of palmitic acid were more remarkably than in room temperature-stored fruits. In contrast to the above examples, an increase of linolenic, oleic acid and a decrease of palmitic acid were found in 4°C-stored fruits. The ratio of lauric, myristic acid to total fatty acids was negligible in three temperature treatments. In fruits treated at 33°C for 10 days prior to room temperature storage, a considerable decrease of linolenic acid was found during the 164 day room temperature storage.

As a whole, the ratio of unsaturated fatty acid to total fatty acids(UFA/TFA ratio) in room temperature-stored fruits progressively decreased during ripening, and in 33°C-stored fruits UFA/TFA ratio decreased more rapidly than in room temperature-stored fruits. On the contrary, in 4°C-stored fruits UFA/TFA ratio increased during storage. It seemed that the changes of UFA/TFA ratio is due to changes of palmitic and linolenic acid.

From the fact as described above, it was considered that the temperature conditions after harvest had effects upon the changes of ratio of fatty acids composition in tomato mitochondrial membrane.

Reference

1) Ku, H. S., H. K. Pratt, A. R. Spurr and W. M. Harris (1968) : Plant Physiol. **43** : 883.
2) Lyons, J. M., T. A. Wheaton and H. K. Pratt (1964) : Plant Physiol. **39** : 262.
3) Minamide, T., Y. Ueda, K. Ogata and H. Kamata (1970) : Nippon Shokuhin Kogyo Gakkaishi 17 : 140.
4) Ogura, N., H. Nakagawa and H. Takehana (1975-a) : Nippon Nogeikagaku Kaishi **49** : 189.
5) Ogura, N., R. Kaneko, Y. Abe, H. Nakagawa and H. Takehana (1975-b) : Tech. Bull. Fac. Hort. **23** : 17.
6) Sacher, J. A., (1973) : Ann. Rew. Plant Physiol. **24** : 197.
7) Spencer, M. (1965) : Plant Biochemistry ed. by J. Bonner and J. E. Varner, Academic Press, New York. p. 793.
8) Uritani, I. and S. Yamaki (1969) : Agr. Biol. Chem. **33** : 480.
9) Yamaki, S. and I. Uritani (1972) : Plant & Cell Physiol. **13** : 67.

トマト果実のミトコンドリア膜の脂肪酸組成におよぼす貯蔵温度の影響*

小倉長雄・阿部雄幸・中川弘毅・竹花秀太郎
(農産製造学研究室)

摘　　要

緑白熟期のトマト果実を 4, 33°C および室温にそれぞれ貯蔵し，ミトコンドリア膜の脂肪酸組成の変化を検討した．主な脂肪酸は，パルミチン酸，リノール酸，およびリノレイン酸であった．室温貯蔵の果実では，追熟に伴い全脂肪酸に対する不飽和脂肪酸の割合は減少の傾向がみられ，33°C 貯蔵果実では，その減少傾向は室温貯蔵の果実より顕著であった．4°C 貯蔵果実では対照的に不飽和脂肪酸の割合が大きくなる傾向が認められた．

* トマト果実の貯蔵温度に関する研究．第 5 報．