Effects of aging and temperature on fatty acid composition of the flavedo tissue of Naruto (*Citrus medioglobosa*)¹

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Summary

Fatty acid composition of neutral, phospho- and glycolipids in the flavedo tissues of Naruto (*Citrus medioglobosa* hort. ex Tanaka) was determined in relation to the effects of aging and temperature.

The proportion of linoleic acid (18:2) in all lipid fractions increased, and that of linolenic acid (18:3) and the 18:3/18:2 ratio decreased during degreening period from October toward mid-winter in 1982 and 1984 trials. Thereafter, these changes were reversed as the fruit began to regreen, with the exception of glycolipids in the 1984 trial where no such reverse tendency was observed. The double bond index in fatty acids considerably decreased during winter in neutral lipids, but not in phospho- and glycolipids. Marked increases in the amounts of total fatty acids, particularly in neutral and phospholipids, were observed during winter.

During storage, the 18:2 proportion in all lipid fractions increased, and the 18:3 proportion and the 18:3/18:2 ratio decreased. GA treatment delayed a decline of the 18:3 proportion and the 18:3/18:2 ratio. Stored fruits at 5°C showed a higher 18:3 proportion and 18:3/18:2 ratio in all lipid fractions than did those at 20°C, and also larger amounts of phospho- and glycolipids.

Introduction

As air temperature decreases, citrus fruits change their rind color from green to orange and enter the maturation period. During this period the rind softens, and the soft rind makes the fruit susceptible to various physiological disorders that cause fruit drop, or reduce storage quality and market value. GA can be used to reduce the rind disorders that appear during maturation (1). 'Rind yellow spot' is a physiological rind disorder in Naruto (*Citrus medioglobosa* hort. ex Tanaka) that appears between late January and late March, and GA application is effective in preventing it (11). Although the period of occurrence seems to be associated with low temperature during winter (12), the basic cause is not clear.

Lipids and their associated fatty acids are

major constituents of biological membranes and have important functions in regulating membrane fluidity and the activity of membrane-bound proteins under stress conditions (20). Therefore, extensive studies have been made of roles of membrane lipids in chilling and freezing injuries (20), but little is known about their roles in physiological disorders of the fruit. The present study was undertaken to determine changes in fatty acid composition of neutral, phospho- and glycolipids in the rind of Naruto in relation to the effects of aging and temperature.

Materials and Methods

Plant materials and periodical sampling. Naruto fruits were obtained from an orchard at Tsuna in Awaji island. Ten fruits were picked from each of the two trees periodically between middle October and early April in 1982–1983, and between late October and

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early May in 1984–1985. One day after harvest, they were used for the extraction of lipids; results are presented as mean values of the two trees.

GA and temperature treatments. Seventy fruits were harvested on November 23, 1982. Ten fruits were used for the extraction of lipids one day after harvest. The remaining 60 fruits were divided into two groups. Fruits from one group were immersed in 100 ppm of GA solution for a moment, while the other group was untreated. Both groups were enclosed in polyethylene bags and stored at 15°C for three weeks. Ten fruits were removed from each treatment at one-week intervals and used for the extraction of lipids.

Sixty fruits were harvested on October 16, 1983, and divided into three groups of two 10-fruit lots each. Each group was enclosed in a polyethylene bag and held at 5, 10 or 20°C, respectively, for a month. A lot of 10 fruits was used for the extraction of lipids, and the results are presented as mean values of the two lots in each treatment.

During storage the fruits were frequently

wetted with water to prevent drying.

Extraction of lipids. Rinds were removed from the fruits and trimmed of albedo tissues with a razor blade. The remaining flavedo tissues (100 g) were blended with 200 ml of boiling isopropanol for 2 min in a Waring blender to avoid enzymatic lipid degradation (19). The homogenate was vacuum-filtered through filter paper, and the filter residue was blended again with 200 ml of chloroform : methanol (1 : 1, v/v) for 2 min. After filtration, the residue was washed twice with 200 ml of chloroform : methanol (1 : 1, v/v). The combined extracts were dried *in vacuo* and resolubilized in 120 ml of chloroform : methanol (1 : 1, v/v). The solvent extract was partitioned against 0.2 volumes of 1% (w/v) NaCl, then against 0.5 volumes of methanol : 1% NaCl (1 : 1, v/v) (19). The solution was dried *in vacuo* and the residue was redissolved in 50 ml chloroform. The final lipid extract was stored at -20°C under nitrogen in the dark.

Separation of lipids. The lipid extract was separated into neutral, glyco- and phospholipid fractions by silica gel chromato-

Fig. 1. Changes in fatty acid composition of neutral, phospho- and glycolipids in the flavedo tissues of Naruto fruits during the maturation period in 1982–1983 (circles) and 1984–1985 (triangles). Results are mean values of two trees. Vertical bars indicate L.S.D..

graphy(18). Columns (30 cm by 9 mm) were packed with 8 g silica gel (100–200 mesh, Wako Chemicals). Neutral lipids were eluted with 200 ml of 0.2% acetic acid in chloroform, and glycolipids with 200 ml of acetone. After elution of these two fractions, phospholipids were removed from the gel by batch-wise washing with five 50 ml aliquots of methanol. Each fraction was dried *in vacuo*, made up to 5 ml with chloroform or chloroform:methanol (1:1, v/v), and used for fatty acid analysis. Purity of each lipid fraction was tested through thin layer chromatography monitoring.

Fatty acid analysis. Preparation of methyl esters from each lipid fraction was followed by a slight modification of the method of Metcalfe and Schmitz(15). The lipid fraction was deacylated with 0.5 N sodium methylate in methanol at 80°C for 15 min. Fatty acids were methylated with 14% boron trifluoride in methanol at 80°C for 10 min, and analysed by a Shimadzu gas chromatograph equipped with a flame ionization detector. The chromatograph was operated under the following conditions: 200°C isothermal; 3 m by 3 mm column with 15% ethylene glycol succinate (EGS) on silanized Chromosob W,

60–80 mesh (Gasukuro Kogyo Co.); nitrogen flow 40 ml/min. Methylpentadecanoate was used as the internal standard. The double bond index was calculated from the sum of the products of the relative amounts of each fatty acid and its number of double bonds.

Results

Changes in fatty acid composition during maturation.

The major fatty acids in the flavedo tissues of Naruto were palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3) acids. The proportion of 18:0 was lowest (less than 3%). The predominant fatty acid was 18:2 in neutral and phospholipids, and 18:3 in glycolipids(Fig. 1).

As Fig.1 shows, the proportion of 18:2 in neutral and phospholipids increased and that of 18:3 decreased concomitantly from October until late January to early March, and thereafter the trends were reversed for both 1982 and 1984 trials. For glycolipids, the changing patterns were different with the two trials. In the 1982 trial the proportion of 18:2 increased from October to late January, then decreased. However, it con-

Fig. 2. Changes in double bond index and the 18:3/18:2 ratio of neutral, phospho- and glycolipids in the flavedo tissues of Naruto fruits during the maturation period in 1982–1983 (circles) and 1984–1985 (triangles). Results are mean values of two trees. Vertical bars indicate L. S. D..

tinued to increase throughout the period in the 1984 trial. In each trial, the change in the 18:3 proportion, although statistically insignificant, was opposite to that of the 18:2 proportion. The proportion of 18:1 in all lipid fractions decreased throughout the period, although a slight increase was observed shortly after October in neutral lipids. The proportion of 16:0+18:0 increased throughout the period in neutral lipids, while it decreased in phospho- and glycolipids, although there was a slight increase in the former after early March. The 18:3/18:2 ratio in all lipid fractions decreased at first sharply, then gradually from October toward late January to early March, and subsequently began to increase, with the exception of the ratio in glycolipids in the 1984 trial, which continued to decrease (Fig. 2). Double bond index decreased from October to early March, then increased slightly in neutral lipids, while it increased slightly in phospho- and glycolipids from October to late January or early March, then remained unchanged. Thus the double bond index varied in parallel with the 18:3/18:2 ratio in neutral lipids, but not in phospho- and glycolipids.

The amounts of total fatty acids in neutral

and phospholipids markedly increased during the period from December to early March, due largely to an increase in the amounts of 18:2 (Fig. 3). The amounts of total fatty acids in glycolipids, particularly those of 18:3, were high between October and late December in the 1982 trial, and between late

Fig. 4. Changes in fatty acid composition of neutral, phospho- and glycolipids in the flavedo tissues of GA-treated (closed) and untreated (open) Naruto fruits during storage at 15°C. Fruits were sampled on November 23, 1982.

Fig. 3. Changes in amounts of total (circles), 18:2 (triangles) and 18:3 (squares) acids of neutral, phospho- and glycolipids in the flavedo tissues of Naruto fruits during the maturation period in 1982-1983 (open) and 1984-1985 (closed). Results are mean values of two trees. Vertical bars indicate L.S.D.

Table 1. Fatty acid composition of neutral, phospho- and glycolipids in flavedo tissues of Naruto stored at different temperatures for a month.

Temperature	Percent of fatty acid				Ratio of 18:3/18:2	Double bond index	Total fatty acids (mg/g F. W.)
	16:0+18:0	18:1	18:2	18:3			
Neutral lipid							
5°C	12.5	10.0	42.4	34.8	0.83	1.99	1.07
10°C	10.5	14.4	42.5	32.5	0.77	1.97	0.97
20°C	12.1	12.9	46.6	28.5	0.61	1.92	1.16
L. S. D. (0.05)	N. S.	0.8	3.6	1.4	0.05	N. S.	N. S.
Phospholipid							
5°C	26.0	12.7	38.7	22.7	0.59	1.58	0.95
10°C	24.9	20.6	34.2	20.5	0.60	1.51	0.86
20°C	27.2	17.3	39.9	15.6	0.40	1.44	0.81
L. S. D. (0.05)	1.4	1.6	4.4	3.1	0.14	0.07	N. S.
Glycolipid							
5°C	16.3	7.4	11.7	64.9	5.62	2.25	0.54
10°C	13.6	10.4	12.5	63.4	5.07	2.26	0.27
20°C	16.4	10.0	15.6	58.2	3.93	2.16	0.31
L. S. D. (0.05)	1.4	N. S.	N. S.	6.4	N. S.	N. S.	0.14

Fruits were sampled on October 16, 1983.

Results are mean values of duplicate samples.

Fig. 5. Changes in double bond index and 18:3/18:2 ratio of neutral, phospho- and glycolipids in the flavedo tissues of GA-treated (closed) and untreated (open) Naruto fruits during storage at 15°C.

Fruits were sampled on November 23, 1982.

January and early March in the 1984 trial, and thereafter decreased in both trials.

Effect of GA on fatty acid composition.

During storage the proportion of 18:2 in-

creased and that of 18:3 decreased in neutral and phospholipids (Fig. 4). In glycolipids, after an initial decrease in the proportion of 18:2 and a concomitant increase in that of 18:3, a similar trend was observed. Changes in the 18:3/18:2 ratio during storage (Fig. 5) were similar to those in the 18:3 proportion. GA treatment delayed a decline in the 18:3 proportion in all lipid fractions (Fig. 4) as well as degreening, thus resulting in a 20-30% higher 18:3/18:2 ratio than the control (Fig. 5). Since the 18:1 proportion was lower in the GA treatment than in the control, an increase in the 18:3 proportion by GA treatment appeared to be mainly at the expense of the 18:1 proportion (Fig. 4). The double bond index did not change appreciably during storage, and was increased only slightly (about 5%) by GA treatment. The 18:3/18:2 ratio appeared a more sensitive indicator of the degree of senescence and aging than the double bond index.

During storage the amounts of total fatty acids increased by about 60% in phospholipids, decreased by about 30% in glycolipids, and remained unchanged in neutral lipids (data not shown). The amounts of total fatty acids in GA-treated fruits were slightly

Fig. 6. Changes in mean air temperature during the period from October to May in 1982-1983 (open) and 1984-1985 (closed) in Sumoto, Awaji island.

(12.5%) larger than those in untreated fruits.

Effect of temperature on fatty acid composition.

As the storage temperature decreased, the proportion of 18:3, the 18:3/18:2 ratio, and the double-bond index in all lipid fractions tended to increase, although some of their values were statistically not significant (Table 1). The 18:3/18:2 ratio and the double bond index were about 30% and 4-9% higher at 5°C than at 20°C, respectively, indicating that the temperature effect was more pronounced in the former.

The amounts of total fatty acids in phospho- and glycolipids tended to increase with a decrease in temperature, although there was no significance in the former. Those in glycolipids were about two-fold larger at 5°C than at 20°C. Fruits stored at lower temperatures showed delayed degreening.

Discussion

Figure 6 shows changes in mean air temperature from October to May in 1982-1983 and 1984-1985 in Sumoto, Awaji island. Naruto fruits usually begin to change from green to light yellow from late October through early November as air temperature decreases. The typical orange color develops toward February to March, and thereafter the fruit begins to regreen with a rise in air temperature. The proportion of 18:3 and the 18:3/18:2 ratio in the flavedo tis-

sues of Naruto decreased from October until late January to early March, then increased for all lipid fractions except for glycolipids in the 1984 trial. The opposite trend was observed in the 18:2 proportion. These changing patterns can be affected not only by changes in air temperature, but also by degreening (aging) and regreening (reversal of aging) of the rind.

The 18:3 acid is a major fatty acid of glycolipids, which are contained mainly in plastids(9). Its proportion or amount is generally known to decrease during degreening (16), and to increase with greening(16), regreening (23), or delayed senescence (3). Galliard(6) found a lower 18:3 proportion in apple pulp at post-climacteric stage than at pre-climacteric, particularly in total lipid fatty acid composition, due mainly to a reduction of glycolipids rich in 18:3. On the other hand, there is considerable evidence that low temperature causes a shift toward a greater proportion of unsaturated fatty acids: increases in 18:3(2, 4, 8, 10, 13, 21, 22) or 18:2(7, 17, 18, 24), depending upon the plant species or part.

In agreement with these findings by others, we also observed that the 18:3 proportion and the 18:3/18:2 ratio decreased during storage (senescence), and that GA treatment which delayed senescence kept them higher than the control. Furthermore, the fruit stored at 5°C showed higher values than that at 10 or 20°C, although the effect of storage temperature could not wholly be separated from that of senescence of the rind. These lines of evidence suggest that the changing patterns of the 18:3 proportion and the 18:3/18:2 ratio as seen for the fruit on the tree are correlated mainly with aging of the rind rather than with changes in air temperature. In contrast with the results of the 1982 trial, the 18:3 proportion and the 18:3/18:2 ratio in glycolipids did not increase during the regreening period in the 1984 trial; the regreening effect did not reflect their compositions. The reason for this difference between trials is not clear. A further trial in 1985-1986 showed a similar result to that of the 1984 trial (data not shown). The

mean air temperature in midwinter was considerably higher in the 1982 trial than in 1984 and 1985, indicating a possible involvement of weather conditions in this difference. Different from the 18 : 3/18 : 2 ratio, the double bond index of the fatty acids in phospho- and glycolipids did not change with aging of the rind, remaining slightly high in winter compared to October.

During winter, there was a marked increase in the amounts of fatty acids, especially 18 : 2 in neutral and phospholipids. In glycolipids, the amounts of fatty acids, especially 18 : 3, increased during the period of late January to early March in the 1984 trial, although they did not increase after late December in the 1982 trial, probably due to warm weather in mid-winter compared to the 1984 trial, as will be mentioned below. These increases may be a response to low temperature in winter, since fruits stored at 5°C showed more fatty acids than those at 10 or 20°C, although this was not true for neutral lipids. Low temperatures were reported to cause increased amounts of fatty acids in neutral(7, 17, 18, 21), phospho-(7, 8, 10, 14, 18, 21, 24), or glycolipids(14).

During storage, the amounts of fatty acids in glycolipids tended to decrease, but those in phospholipids did not. It is known that glycolipids begin to disappear at an earlier stage of senescence than phospholipids(5, 6).

In conclusion, the 18 : 3/18 : 2 ratio (or the 18 : 3 proportion) and the double bond index of fatty acids, particularly the former, seemed to be closely and inversely related both to the degree of senescence and aging of the rind, and to the temperature to which the fruit is exposed. A marked increase in the amounts of fatty acids, particularly in neutral and phospholipids during winter, may be a direct effect of low temperature.

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ナルトミカン果皮のフラベド組織の脂肪酸組成に 対する老化及び温度の影響

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摘 要

成熟期におけるナルトミカン (*Citrus medioglobosa* hort. ex. Tanaka) のフラベド組織の中性、リン及び糖脂質の脂肪酸組成と量の変化を調べた。また、貯蔵果を用いて、老化並びに温度との関係を検討した。

2年(1982年と1984年)とも、10月から冬季の中期までの着色期に各脂質の 18:2 (リノール酸) 組成が増加し、18:3 (リノレイン酸) 組成及び 18:3/18:2 比が減少した。以後、回青期に入ると、この傾向が逆転した。1984年の糖脂質では、このような逆転はみられなかった。脂肪酸の不飽和度は冬期に中性脂質では減少したが、糖脂質及びリン脂質は、減少がみられなかった。各脂質とくに中性及びリン脂質の脂肪酸量は、冬期に顕著に増加

した。

貯蔵中、18:2組成が日数に伴って増加し、18:3組成及び18:3/18:2比が減少した。GA処理によって、果皮の老化並びに18:3組成及び18:3/18:2比の減少が遅れた。5℃貯蔵では、各脂質の18:3組成及び18:3/18:2比が20℃より高い値を示した。また、リン脂質及び糖脂質の脂肪酸量は低温ほど多く、糖脂質では5℃は20℃の約2倍を示した。

以上の結果からナルトミカンのフラベド組織の脂肪酸の18:3組成及び18:3/18:2比は老化及び温度に伴って変化することがわかった。また、冬期の各脂質の脂肪酸量の著しい増加は低温によるものと考察した。