Maturity and Its Regulation in Satsuma Mandarin Fruit

By HIROSHI DAITO

Research Planning and Coordination Division, Fruit Tree Research Station
(Yatabe, Ibaraki, 305 Japan)

The recent renewed interest in the maturity or ripeness of satsuma mandarins (*Citrus unshiu* Marc.) is largely due to the increased production of citrus fruits, because the increased production has brought about corresponding lowering of fruit quality. There is a need, therefore, for a means of determining and maintaining high quality.

In this paper, studies on fruit components as a basis of getting objective indices of maturity and quality are presented. Furthermore, some regulating means for quality of satsuma mandarin fruit are introduced. These studies were done when the author belonged to Okitsu Branch, Fruit Tree Research Station, and Shikoku National Agricultural Experiment Station.

Changes during fruit development and maturation

1) *Respiratory rate and ethylene evolution*

It has long been known that respiratory rates of citrus fruits continue decreasing during their development and maturation.[1,2,3,15] However, it is only in recent years that the respiratory rate of satsuma mandarin fruit was found to vary as follows during maturation.[11]

The respiratory rate of 'Okitsu wase' fruit, an early maturing cultivar, decreased slightly up to early October, and thereafter increased until January or March of the following year. On the other hand, the respiratory rate of 'Silverhill' fruit, a common cultivar, increased slightly until mid October or early November and continued to increase steadily in the following year (Fig. 1).

Daito et al.[11] reported that satsuma mandarin fruit produced a very small amount of ethylene during development and maturation. Within a narrow range, the rate of ethylene production decreased until October, remained constant at a low level up to early January of the next year, and then increased. This increase may be caused by fruit damage by a cold wind in winter season. Vines et al.[20] also found that oranges, tangerines and grapefruits suffering from frost injury on trees or suffering from mild mechanical injury produced some ethylene, and they concluded that ethylene production by citrus fruits is a stress symptom and not a normal metabolic process.

It is clear that unlike many fruits, citrus fruits do not give off carbon dioxide and ethylene at maturity, when in a sound condition on trees, under normal conditions. Therefore, the author concluded that changes in the respiratory rate and ethylene evolution are not suitable to be used as a maturity or ripeness index of satsuma mandarin fruit.

2) *Ethanol and acetaldehyde*

Concentration of ethanol and acetaldehyde in fruit juice and peel of both the cultivars increased gradually during maturation.[12] Davis[13] suggested that ethanol serves as an additional measure of maturity of citrus fruits, as the range and degree of its increase was greater than changes in solids-acid ratio.

As given in Fig. 2, however, their low concentrations and narrow range of changes seem to preclude these components as useful indicators of maturity. The rapid increase in Brix-acid and total sugar-acid ratio during the season provides a more sensitive indication of maturity in satsuma mandarins.

3) *Volatile flavor components*

In the peel of 'Sugiyama' fruit, a common cul-

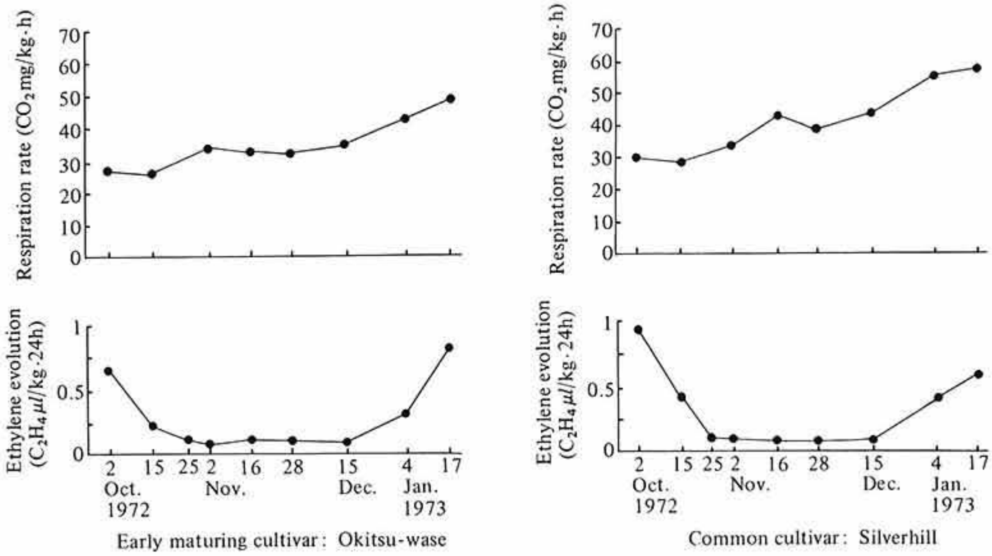

Fig. 1. Seasonal changes in respiratory rate and ethylene evolution of satsuma mandarin fruit of 2 cultivars

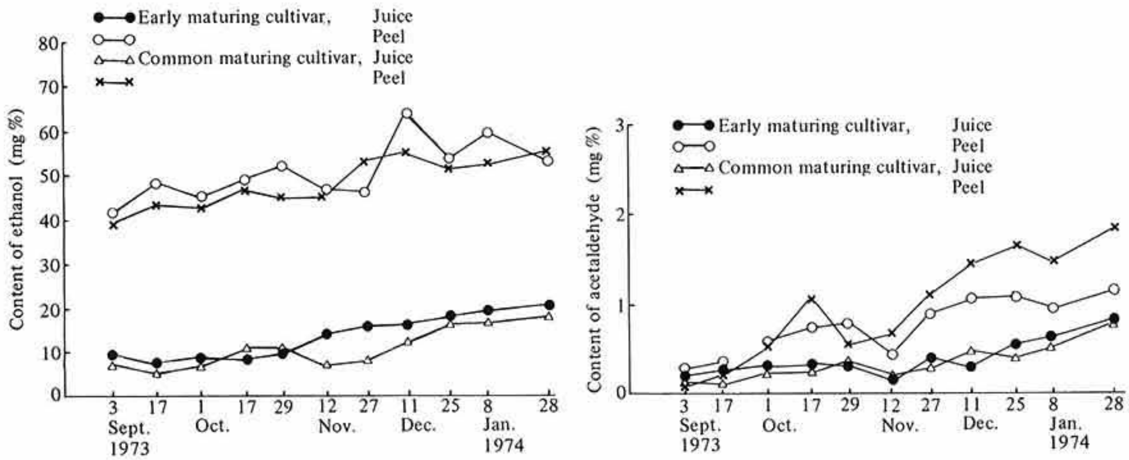

Fig. 2. Seasonal changes in ethanol contents and acetaldehyde contents in juice and peel of satsuma mandarin fruit

tivar, the most pronounced percentage variations were found in *d*-limonene and terpinene. *d*-Limonene increased gradually, while terpinene decreased rapidly till early October, and thereafter they remained relatively constant.[9] α-Pinene, nonyl aldehyde, trans-sabinene, linalool, β-caryophylene, α-terpineol, *p*-cymene, β-copaene and geranyl acetate decreased, while myrcene increased (Table 1).

In the juice, in early November, *d*-limonene was most abundant, followed by terpinene, myrcene, *p*-cymene, β-caryophylene, geranyl acetate, δ-elemene, α-pinene, β-pinene, α-terpineol, transsabinene, β-copaene, γ-elemene and linalool. After one month, *d*-limonene, decreased markedly, and neither geranyl acetate nor transsabinene was detected, whereas the other components increased considerably. The increase was especially significant for nonyl-

Table 1. Percentage variations in volatile flavor components from satsuma mandarin fruit

Component	Peel							Juice	
	June 22	July 10	Aug. 4	Sept. 12	Oct. 9	Nov. 11	Dec. 11	Nov. 11	Dec. 11
Air	—	—	+	0.01	0.002	—	—	—	— (%)
Unknown	+	+	+	0.002	0.001	—	—	—	—
Acetaldehyde, ethylether	+	+	+	+	+	+	+	—	—
Unknown	+	+	+	+	+	+	+	—	—
Unknown	+	+	+	0.02	+	+	+	—	—
Methanol, butylaldehyde	+	+	+	0.02	+	+	+	—	—
α-Pinene	0.67	0.79	0.09	0.12	0.54	0.42	0.55	0.17	2.07
Camphene	0.01	0.01	—	0.01	0.01	0.02	0.01	—	—
β-Pinene	0.91	0.61	0.15	0.14	0.25	0.20	0.28	0.14	0.54
Myrcene	0.21	1.01	0.68	0.80	1.04	0.94	1.24	0.91	1.48
d-Limonene	64.15	82.65	87.11	91.73	94.08	94.57	93.20	92.47	69.68
Terpinene	23.49	10.96	7.82	4.65	2.84	2.57	3.19	3.06	6.68
p-Cymene	1.13	0.42	0.42	0.29	0.13	0.14	0.08	0.37	1.61
Unknown	0.53	0.31	0.11	0.10	0.11	0.15	0.20	0.57	0.81
Undnown	0.01	0.02	0.02	0.001	0.004	0.001	0.01	—	—
Unknown	0.02	0.05	0.02	0.03	0.09	0.03	0.01	—	—
Nonyl aldehyde	0.02	0.02	0.02	0.02	0.01	0.01	0.01	—	1.16
Unknown	0.06	0.04	0.07	0.02	0.01	0.01	0.01	—	—
Trans-sabinene	0.28	0.14	0.09	0.05	0.02	0.07	0.11	0.08	—
δ-Elemene	0.04	0.02	0.04	0.04	0.02	0.04	0.08	0.21	1.16
Decyl aldehyde	0.13	0.04	0.16	0.04	0.01	0.02	0.03	—	—
Linalool	3.01	0.90	0.60	0.25	0.09	0.10	0.16	0.009	2.64
Unknown	0.08	0.12	0.09	0.08	0.04	0.04	0.02	—	—
Unknown	0.18	0.07	0.10	0.05	0.02	0.02	0.03	—	—
Unknown	1.42	0.43	0.38	0.03	0.01	0.01	0.01	—	—
β-Caryophylene	0.35	0.10	0.22	0.31	0.16	0.12	0.12	0.31	0.94
Unknown	0.02	0.02	0.01	0.004	0.009	0.02	0.02	—	—
β-Farnesene	0.01	0.01	0.07	0.86	0.02	0.01	0.02	—	—
α-Terpineol	0.81	0.25	0.34	0.18	0.08	0.06	0.08	0.08	2.15
Unknown	—	—	0.07	0.10	0.04	0.02	0.03	0.07	1.08
β-Copaene	0.53	0.27	0.33	0.25	0.10	0.10	0.17	0.07	1.48
Geranyl acetate	1.72	0.63	0.60	0.36	0.16	0.24	0.23	0.22	—
Unknown	0.05	0.02	0.05	0.03	0.01	0.03	0.04	0.10	—
Perillyl aldehyde	0.07	0.02	0.05	0.03	0.01	0.03	0.04	—	—
Unknown	0.02	0.01	0.04	0.01	0.004	0.01	0.001	—	—
γ-Elemene	0.01	0.004	0.07	0.07	0.03	0.004	0.001	0.02	1.83
Unknown	0.01	0.01	0.04	0.004	0.004	0.004	0.001	—	—
Unknown	0.02	0.01	0.05	0.02	0.01	0.004	0.002	—	—

aldehyde, which was not present at all in the previous month.

4) Sugars

The predominant sugars of satsuma mandarin fruit are sucrose, fructose and glucose.[14] The most pronounced variation was found with sucrose, which increased approximately from 1.00 to 7.00 mg per 100 m*l* of juice in the early and common cultivars, respectively. Fructose also increased from 0.80 to 2.00 mg per 100 m*l* of juice (Table 2).

Glucose remained relatively constant throughout all stages in both cultivars. The total sugars in the early and common cultivars increased approximately 2.8 to 11.0 mg and 4.4 to 10.0 mg per 100 m*l* of juice, respectively.

5) Organic acids

The predominant organic acids of satsuma mandarin fruit are glucronic, lactic, acetic, pyruvic, malic, citric, succinic and iso-citric acid[14] (Table 3).

In an early stage of maturation, citric and malic acids were most abundant, followed by iso-citric acid.

The most pronounced variation was observed with citric acid, which decreased from 2,000 to 800 or 900 mg per 100 m*l* of juice in the early and common cultivars, respectively.

Table. 2. Changes in sugar constituents in juice of satsuma mandarin

	1973									1974				
	Sept.		Oct.			Nov.		Dec.		Jan.			Feb.	Mar.
	3	17	1	17	29	12	27	11	25	8	18	28	18	4
Early satsuma														(%)
Sucrose	1.08	1.98	2.74	3.38	4.32	5.49	5.85	6.87	7.53	7.39	6.85	7.24	6.84	7.18
Fructose	0.81	0.97	0.99	1.35	1.42	1.69	1.80	2.03	2.06	1.99	1.80	1.65	1.16	1.11
Glucose	0.92	0.82	0.90	0.97	1.18	1.02	1.12	1.01	1.30	1.26	1.32	1.11	0.78	0.57
Total	2.81	3.77	4.63	5.70	6.92	8.20	8.86	9.91	10.89	10.64	9.97	10.00	8.78	8.86
Common satsuma														
Sucrose	1.23	1.45	2.24	2.73	3.17	3.51	4.30	5.10	6.19	6.39	6.42	6.61	6.90	5.91
Fructose	1.31	0.75	1.02	1.20	1.25	1.16	1.30	1.38	1.60	1.64	1.84	1.90	1.63	1.21
Glucose	1.87	0.97	0.89	0.98	1.03	1.49	1.21	1.03	1.18	1.22	1.29	1.46	1.31	1.00
Total	4.41	3.17	4.15	4.91	5.45	6.16	6.81	7.51	8.97	9.25	9.55	9.97	9.84	8.12

During the maturation, malic acid also decreased to one third and one half, and iso-citric acid decreased to one forth and one half, respectively, in the juice of the early and common cultivars. Glucronic acid decreased with maturation. Lactic, acetic, and succinic acid remained relatively constant throughout all stages in both cultivars. The total organic acid content in the early and common cultivars decreased from 2,000 to 800 mg and from 2,000 to 900 mg per 100 m*l* of juice, respectively.

6) *Amino acids*

The predominant amino acids of satsuma mandarin fruit are aspartic acid, glutamic acid, asparagine, threonine, serine, glutamine, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, γ-aminobutyric acid, ornitine, lysine, arginine, histidine and ammonia, in both cultivars[4] (Tables 4 and 5).

In an early stage of maturation, aspartic acid and asparagine are most abundant, followed by glutamic acid, serine and alanine in each cultivar. The most pronounced variation was observed with proline, which increased approximately from 1.0 to 70.0 mg and 3.0 to 65.0 mg per 100 m*l* of juice of early and common cultivars, respectively.

During the maturation of both cultivars, arginine increased to 8- or 9-fold and γ-aminobutyric acid increased to approximately 5 times. Aspartic acid decreased with maturation. Ammonia decreased slowly with maturity, reaching a low point in fruit pick in mid December, and a much higher level in fruit pick in mid January. Glutamic acid, asparagine, threonine, glutamine, valine, isoleucine and leucine remained relatively constant throughout all stages in both cultivars.

Content of α-ketoglutaric acid in the TCA cycle began to decrease to its low point during mid September.[16] Thereafter, arginine increased markedly during fruit maturation. γ-Aminobutyric acid increased slowly during a maturation period, but sharply during the late season. The progressive changes in arginine and γ-aminobutyric acid appear to be related to biochemical mechanisms involved in the maturation of the oranges during maturation.

Rockland[17] suggested that amino acid variations during maturation of the California 'Valencia' orange may provide a basis for developing an improved objective index of fruit maturity. It was reported that the ratio of γ-aminobutyric acid to total acid increased at a more rapid rate than the Brix-acid ratio during maturation.

Wedding et al.[21] showed changes in the ratio of soluble solids to acid, of γ-aminobutyric acid to acid and of cloud to acid in 'Valencia' oranges during fruit maturation. That experiment indicated that only the soluble solids-acid showed a relatively smooth rate of increase during fruit maturation. While the value of both the cloud-acid ratio and (to a lesser degree) the γ-aminobutyric acid–acid ratio increased, the variation from sample to sample was usually as large as the overall change during fruit development. This resulted in Wedding et al.[21] concluding that the Brix-acid ratio was a satisfactory index of orange maturity.

Table 3. Changes in organic acids in juice of satsuma mandarin

	1974 (mg/100 ml)								
	Sept.			Oct.		Nov.	Dec.		
	5	18	29	15	29	12	5	17	24
Early satsuma									
Glucuronic	6.41	4.01	3.50	2.59	1.08	1.53	1.15	1.19	+
Lactic	0.97	0.95	0.86	0.85	0.76	0.63	0.54	0.46	0.51
Acetic	0.54	0.48	0.50	0.52	0.55	0.54	0.51	0.93	1.63
Pyruvic	4.59	4.33	3.81	2.59	1.62	1.60	1.58	1.54	1.55
Malic	122.43	118.62	92.09	76.05	46.96	45.18	51.57	48.01	45.39
Citric	2022.91	1729.32	1331.85	1188.53	911.74	830.74	804.15	817.91	815.46
Succinic	3.59	2.97	1.74	1.51	0.94	1.15	1.66	1.14	0.95
Isocitric	18.61	17.00	12.74	12.32	11.34	9.57	8.46	8.05	6.45
α-Ketoglutaric	+	+	+	+	+	+	+	+	+
Total	2180.05	1877.68	1447.09	1284.96	975.80	890.94	869.62	879.23	871.94
Common satsuma									
Glucuronic	7.36	5.81	4.83	3.68	1.31	1.03	0.97	0.85	0.75
Lactic	0.59	1.33	1.61	1.80	2.56	1.59	0.58	0.63	0.68
Acetic	0.68	0.60	0.54	0.58	0.61	0.55	0.54	0.53	0.56
Pyruvic	3.64	3.61	3.58	3.56	3.57	2.84	1.58	2.99	5.57
Malic	85.83	84.99	60.11	63.58	66.59	59.34	58.71	55.15	41.04
Citric	2148.94	2099.46	2090.54	1588.46	1020.81	936.80	904.56	885.05	838.83
Succinic	1.56	1.87	2.71	1.32	0.91	0.88	0.85	1.78	2.59
Iso citric	13.47	12.71	11.94	11.90	11.83	13.96	14.56	15.03	20.56
α-Ketoglutaric	+	+	3.48	2.12	+	+	+	+	—
Total	2262.07	2210.38	2179.34	1677.00	1108.19	1016.99	982.35	962.01	910.58

Table 4. Changes in amino acids content in juice of early satsuma mandarin fruit during fruit maturation

	mg per 100 ml juice				
	Sept. 17 1969	Oct. 29	Nov. 28	Dec. 22	Jan. 20 1970
Aspartic acid	31.637	32.072	24.364	19.947	18.764
Glutamic acid	16.159	20.176	20.225	15.802	12.593
Asparagine	18.798	29.646	30.004	50.236	37.897
Threonine	1.618	1.707	9.849	2.210	2.626
Serine	9.172	11.614	10.115	16.768	13.563
Glutamine	1.155	6.856	7.354	13.720	11.141
Proline	1.300	9.418	17.789	41.440	73.161
Glycine	0.311	0.496	0.308	0.394	0.902
Alanine	4.950	8.977	9.411	11.210	17.020
Cystine	—	0.709	0.470	0.783	0.879
Valine	0.829	0.835	0.823	0.936	1.620
Methionine	0.336	0.471	0.334	0.340	0.895
Isoleucine	0.275	0.335	0.362	0.259	0.488
Leucine	0.297	0.278	0.352	0.298	0.504
Tyrosine	0.605	0.684	0.655	0.618	1.539
Phenylalanine	0.399	1.695	1.858	1.656	2.399
γ-Aminobutyric acid	2.501	5.005	5.132	8.392	13.096
Ornithine	0.765	0.613	0.625	2.057	3.108
Lysine	1.001	0.620	—	1.804	—
Ammonia	5.930	0.829	3.043	1.685	4.156
Histidine	0.201	2.815	0.182	0.480	0.403
Arginine	5.379	27.678	33.473	44.964	41.602
Total	103.620	163.461	176.728	236.061	258.355
Total nitrogen	29.8	28.6	40.8	53.4	
Protein nitrogen	2.86	3.24	5.16	9.36	

(Table 3 Continued)

1975 (mg/100 m*l*)					
Jan.		Feb.		Mar.	
7	21	4	19	4	17
+	+	+	+	+	+
0.57	0.55	0.52	0.56	0.54	0.51
1.12	0.57	0.52	0.51	0.56	0.59
0.97	0.85	1.02	1.73	1.45	1.02
46.71	57.46	45.11	44.94	40.08	39.97
842.02	881.39	858.44	818.85	841.50	802.47
0.92	0.81	1.54	3.82	2.95	3.55
5.90	4.46	4.57	5.01	5.32	5.40
—	—	—	—	—	—
897.85	946.09	911.72	875.42	892.40	1010.64
0.98	1.04	0.99	0.95	0.90	0.97
0.57	0.55	0.51	0.56	0.54	0.58
0.61	0.63	0.90	1.58	1.12	0.91
2.01	0.84	1.07	2.83	2.77	2.76
38.31	36.95	37.74	46.81	43.23	37.91
857.90	892.55	890.03	891.58	891.21	896.60
2.91	3.87	3.64	3.54	3.50	2.59
19.51	5.46	5.00	5.39	7.65	8.84
—	—	—	—	—	—
922.80	941.89	948.88	953.24	950.92	951.16

Rockland et al.[18] reported that the arginine-acid ratio increased more rapidly than either the Brix-acid or γ-aminobutyric acid-acid ratios and that it appeared to be more closely related to the organoleptic quality of the fruit juice. Rockland[17] also introduced the Bar-acid ratio (Brix-acid $\times$ arginine). Bar-acid is a simple product of the Brix-acid ratio (generally used to determine commercial maturity) and arginine content reported in mg per 100 m*l* juice.

It was suggested that the Bar-acid ratio might be a better index of orange quality and maturity than the recognized Brix-acid ratio.

Maturation had a very distinct effect on the amount of arginine and γ-aminobutyric acid in both cultivars. The author initiated a work to determine the utility of the Bar-acid ratio as an index of satsuma mandarin maturity.

7) *Pectic substances*

Three pectic fractions in peel, pulp and segment wall[10] of satsuma mandarin fruit were examined during development and maturation (Fig. 3).

Table 5. Changes in amino acids content in juice of common satsuma mandarin fruit

	mg per 100 m*l* juice			
	Sept. 17 1969	Oct. 29	Nov. 28	Dec. 22
Aspartic acid	37.895	26.526	24.041	22.224
Glutamic acid	17.699	16.505	17.079	16.313
Asparagine	42.760	37.982	33.991	33.611
Threonine	1.890	1.643	1.438	2.099
Serine	15.348	11.921	11.798	16.906
Glutamine	6.033	5.025	9.566	9.612
Proline	3.189	14.500	25.026	47.324
Glycine	0.393	0.278	0.616	0.477
Alanine	7.454	5.787	10.042	13.682
Cystine	—	—	0.715	0.832
Valine	0.985	0.804	0.938	1.148
Methionine	0.895	0.783	0.589	0.457
Isoleucine	0.471	0.254	0.343	0.499
Leucine	0.367	0.260	0.297	0.397
Tyrosine	0.849	0.478	0.859	1.373
Phenylalanine	0.776	0.799	1.469	2.405
γ-Aminobutyric acid	2.681	3.758	4.876	6.863
Ornithine	0.334	0.491	1.434	2.499
Lysine	0.869	—	—	—
Ammonia	6.781	3.802	3.741	1.703
Histidine	0.183	0.134	0.182	0.411
Arginine	3.946	16.782	30.926	40.091
Total	187.315	151.115	179.956	220.926
Total nitrogen	23.8	22.0	37.4	40.2
Protein nitrogen	2.35	2.66	4.35	7.93

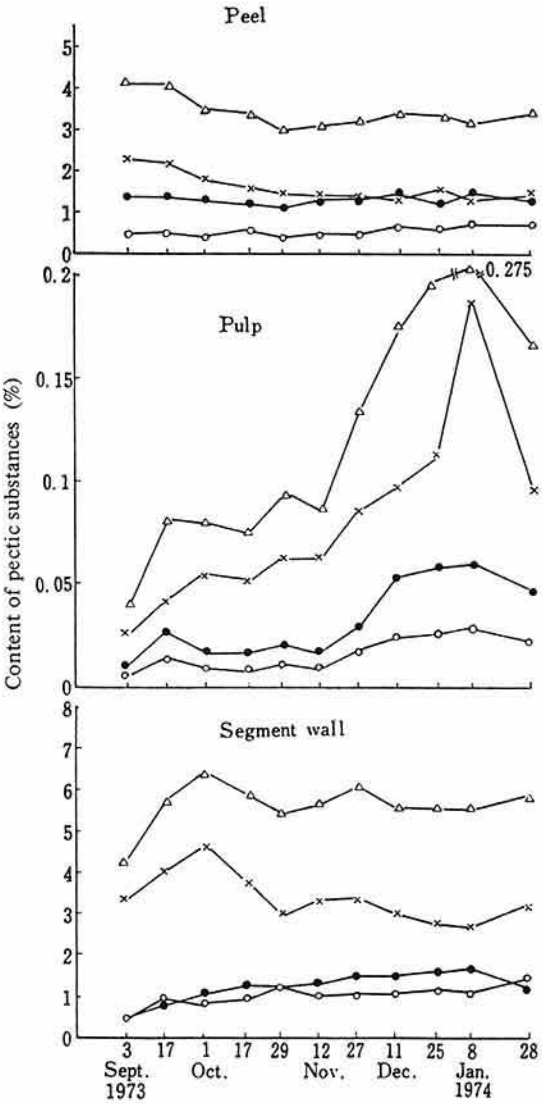

Fig. 3. Changes in pectic fractions in the peel, pulp and segment wall of early satsuma mandarin fruit

△—△ Total-pectic substances
○—○ Water-soluble
●—● Sodium hexametaphosphate-soluble
×—× Sodium hydroxide-soluble

Contents of water- and sodium hexametaphosphate-soluble pectine in the peel of an early cultivar increased, but that of the sodium hydroxide-soluble fraction decreased from early September to early December. Thereafter all of the fractions increased slightly.

Water- and sodium hexametaphosphate-soluble pectins in the pulp increased rapidly during late December, and thereafter decreased gradually. Sodium hydroxide-soluble pectin in the pulp increased rapidly from early September to early January of the following year, and then decreased.

In the segment wall, water- and sodium hexametaphosphate-soluble pectins increased gradually from early September to early January of the following year. The decrease was slower in sodium hydroxide-soluble pectins which continued to decrease until January.

8) *Carotenoids*

Rind color and the carotenoid groups of pigments in peel and pulp of satsuma mandarin fruit were investigated in relation to maturation.[7]

The early cultivar fully colored during the period from late November to mid December, and the common cultivar also fully colored from mid to late December.

In both cultivars, the decrease in chlorophyll a content was rapid from mid September to early November and became more gradual thereafter.

In both cultivars, chlorophyll b content generally tended to decrease regularly from early September to October. After early and mid October, chlorophyll b levelled off in the early and common cultivar, respectively.

Thin layer chromatography emerged as an invaluable technique in the qualitative analysis and identification of carotenoids. All carotenoids were divided into 9 groups according to the number of hydroxy and epoxide. The groups in peel of both varieties were hydrocarbon, monol, monol monoepoxide, diol, diol monoeposide 1 and 2, diol diepoxide 1 and 2, and polyol (Tables 6 and 7).

In an early stage of both cultivars, diol and hydrocarbon were most abundant, followed by polyol, diol diepoxide, diol monoepoxide 1, and monol. In both cultivars, monol monoepoxide, diol monoepoxide 2 and diol diepoxide 2 were not present in mid September, and monol monoepoxide was not contained until mid October.

The most pronounced quantitative variation was found in the monol group, which increased from 0.1 to 6.7 mg% and 0.1 to 8.2 mg% in the peel in early and common cultivars, respectively. Similarly, diol

Table 6. Changes in the total carotenoid content and various fractions in pulp of early satsuma mandarin fruit during fruit maturation

Carotenoid group	Content of each fraction in relation to total carotenoid (mg %)				
	Sept. 18 1972	Oct. 15	Nov. 16	Dec. 15	Jan. 17 1973
Hydrocarbon	0.016	0.028	0.043	0.063	0.095
Monol (Cis-) 1	0.005	0.050	0.080	0.130	0.106
Monol 2	0.126	0.409	0.544	0.914	1.151
Diol	0.042	0.046	0.053	0.066	0.103
Diol monoepoxide 1	—	0.014	0.028	0.038	0.065
Diol diepoxide 1	0.052	0.032	0.027	0.034	0.062
Diol monoepoxide 2	0.030	0.023	0.043	0.070	0.088
Diol diepoxide 2	0.039	0.040	0.061	0.094	0.129
Polyol	0.038	0.046	0.040	0.056	0.067
Total	0.349	0.688	0.918	1.465	1.866

Table 7. Changes in the total carotenoid content and various fractions in pulp of common satsuma mandarin fruit during fruit maturation

Carotenoid group	Content of each fraction in relation to total carotenoid (mg %)				
	Sept. 18 1972	Oct. 15	Nov. 16	Dec. 15	Jan. 17 1973
Hydrocarbon	0.004	0.028	0.074	0.078	0.076
Monol (Cis-) 1	0.014	0.082	0.113	0.172	0.155
Monol 2	0.059	0.583	1.188	1.385	1.376
Diol	0.004	0.041	0.087	0.095	0.114
Diol monoepoxide 1	—	—	0.046	0.039	0.058
Diol diepoxide 1	0.016	0.073	0.086	0.059	0.069
Diol monoepoxide 2	0.007	0.052	0.071	0.048	0.094
Diol diepoxide 2	0.009	0.082	0.117	0.128	0.159
Polyol	0.005	0.036	0.076	0.096	0.139
Total	0.118	0.977	1.856	2.100	2.240

diepoxide 2 increased from 0.2 to 3.0 mg% and 0.1 to 2.2 mg%, and diol diepoxide 1, from 0.6 to 2.7 mg% and 0.7 to 2.8 mg% in the peel.

Monol monoepoxide, diol monoepoxide 1, and diol monoepoxide 2 groups increased gradually during fruit maturation, especially, the diol monoepoxide 2 group, increased rapidly as the fruit approached maturity.

The level of the polyol group in the early variety decreased, whereas the hydrocarbon group varied irregularly during fruit maturation.

The carotenoid groups in pulp of both cultivars were similar to that of peel except monol 2 instead of monol monoepoxide in peel. The most pronounced quantitative variation was found in the monol 2 group, which increased from 0.12 to 1.15 mg% and 0.05 to 1.37 mg% in pulp in the early and common cultivars during the 4-month period from mid September to mid January of the following year. Similarly, diol diepoxide 2 increased by 3 and 18 times, and monol 1 to 20 and tenfold in pulp. In the early cultivar, hydrocarbon, diol, diol monoepoxide 1, diol monoepoxide 2, and polyol groups all increased gradually, but diol diepoxide 1 moves irregularly, and diol monoepoxide 1 of the common cultivar was not present until mid October.

In both cultivars, chlorophyll content of the peel decreased rapidly from mid September to early November, and levelled off thereafter.

It is suggested the orange-red color development may have resulted from increasing monol, diol diepoxide 1 and 2, diol monoepoxide 1 and 2 in peel, and monol 2 in pulp of both cultivars, respectively.

Regulation of fruit maturity and quality

1) *Effects of ethychlozate (ethyl 5-chloro-1H-3-indazolylacetate) on fruit quality of satsuma mandarin*

Experiment I (1977)

Ethychlozate was sprayed at 100 and 200 ppm on the whole tree of 'Sugiyama'. Applications were made 2 times (July 7 and Aug. 11, 1977) with a high pressure sprayer, using a hand gun. The leaves and fruit were thoroughly wetted.

Experiment II (1978)

Entire tree sprays of 100 ppm ethychlozate were made to 'Sugiyama' as follows: A) 60, 70 and 80 days after full bloom (July 21, 31, and August 10, respectively), B) 60 and 80 days, C) 60 and 70 days, D) 70 and 80 days, E) nonsprayed control.

The results obtained in Experiments I and II are shown in Tables 8, 9 and 10. Ethychlozate accelerated degreening of satsuma mandarin fruit, and especially increased a color index and 'a' value of color difference meter of fruit peel. Brix and sugar content of fruit juice were slightly increased by ethychlozate spray, and acidity of fruit was obviously decreased. The reduction of acidity was mainly due to a reduction in citric acid. During an early mature stage, ethychlozate treatment increased total amount of free amino acids in fruit juice. Fruit weight, and Brix and acidity of fruit juice were high in the fruit treated with 200 ppm ethychlozate, while an average color index and 'a' value

Table 8. Effects of ethychlozate on sugar constituents of satsuma mandarin fruit on Nov. 20, 1978

Treatment	Fructose	Glucose	Sucrose	Total (g/100 ml)
A	2.22	1.38	5.19	8.80
B	2.38	1.42	4.58	8.38
C	2.38	1.41	5.11	8.90
D	2.40	1.40	5.36	9.16
Control	2.22	1.28	4.39	7.89

Table 9. Effects of ethychlozate on organic acid constituents of satsuma mandarin fruit on Nov. 20, 1978

Treatment	Gluc.	Pyro-Glu.	Lact.	Acet.	Pyr.	Mal.	Cit.	Succ.	Iso-Cit.	Total (mg/100 ml)
A	0.5	0.3	0.2	0.1	1.3	90.0	1,460.5	0.3	11.7	1,564.9
B	0.3	0.4	0.1	0.2	0.8	89.0	1,499.0	0.2	14.1	1,604.1
C	0.3	0.2	0.1	0.2	1.1	83.0	1,611.5	0.2	13.5	1,710.1
D	0.3	0.3	0.1	0.1	0.9	86.0	1,650.0	1.8	14.1	1,753.6
Control	0.6	0.3	0.1	0.2	1.1	87.0	1,555.0	0.3	12.3	1,656.9

Abbreviations: Gluc.=Glucuronic, Pyro-Glu.=Pyro-Glutamic, Lact.=Lactic, Acet.=Acetic, Pyr.=Pyruvic, Mal.=Malic, Cit.=Citric, Succ.=Succinic, Iso-Cit.=Iso-Citric.

Table 10. Effects of ethychlozate on amino acid constituents of satsuma mandarin fruit on Nov. 20, 1978

Treatment	Asn.	Gln.	Asp.	Thr.	Ser.	Glu.	Pro.	Gly.	Ala.	Cys.	Val.
A	69.5	13.1	32.9	2.6	13.7	15.7	23.7	0.6	15.0	0.6	2.6
B	35.2	4.8	41.0	2.0	11.0	20.0	26.8	0.7	15.9	0.3	1.7
C	30.0	2.8	34.2	3.0	12.0	24.4	34.4	0.9	23.0	0.5	2.4
D	35.0	4.8	44.4	2.9	11.2	20.0	34.5	0.9	24.6	0.4	2.2
Control	23.9	3.9	35.6	2.5	10.8	23.2	34.6	0.8	20.1	0.3	2.0

Treatment	Met.	ILeu.	Leu.	Tyr.	Phe.	γ-ABA.	Lys.	His.	Arg.	Total (mg/100 ml)
A	0.7	0.8	1.0	0.6	1.4	9.6	3.5	0.4	70.6	278.6
B	0.5	0.5	0.6	0.5	1.0	10.8	2.5	0.3	51.8	227.9
C	0.7	0.6	0.7	0.8	1.4	8.2	2.7	0.3	51.6	234.6
D	0.6	0.6	0.8	0.8	1.6	10.0	2.6	0.4	48.9	242.2
Control	0.5	0.6	0.8	0.9	1.8	6.1	2.4	0.3	42.5	213.6

of color difference meter of fruit peel were high in the fruit treated with 100 ppm ethychlozate. Effects of ethychlozate on color development and acid reduction were most markedly manifested by 3 sprays on 60th, 70th and 80th day after full bloom and by 2 sprays on 60th and 80th day, followed by 2 sprays on 60th and 70th day. Two sprays on 70th and 80th day had no effect. The ethychlozate is used in 5,000 to 7,000 ha of groves per year (1981 to '83) as a maturity accelerating chemical.

2) *Effects of $CaCO_3$ on quality and storage of fruit of satsuma mandarin*

One month before harvesting, $CaCO_3$ solution was sprayed at concentration of 50 times to the whole trees of 'Aoshima'.[5]

Acidity of the treated fruit slightly decreased, and hardness of the fruit also somewhat increased.

As rind of the treated fruit on trees shrank due to excess evaporation caused by $CaCO_3$ spray, the fruit could directly be transferred into the storage house for long period storage with pretreatment like rind shrinking.

$CaCO_3$ was sprayed in 26,000 ha of satsuma mandarin groves in 1983.

3) *The effects of CPTA (2-(4-chlorophenylthio)-triethylamine hydrochloride) on color development and lycopene accumulation in satsuma mandarin fruit*

Effect of CPTA on the acceleration of coloring of satsuma mandarin fruit was investigated. CPTA aqueous solution was sprayed at the concentrations of 1,250 ppm, 2,500 ppm and 5,000 ppm on the whole trees of 'Silverhill'.[6]

All of CPTA-treated ordinary fruit developed bright red color and their appearance turned as 'Dobashibeni'. Determination of carotenoid groups of CPTA-treated fruit peel by thin layer chromatography (TLC) showed the remarkable increase of hydrocarbon carotenoid group (H group) content (Table 11). The maximum content of H group which was resulted from CPTA-treatment of 2,500 ppm was 9 times as high as non-treated ones. The

Table 11. Changes of carotenoid pattern in the peel of satsuma mandarin treated with CPTA
(10 days after treatment)

Carotenoid group	Carotenoid									
	%	mg%	%	mg%	%	mg%	%	mg%	%	mg%
	CPTA (ppm)								Dobashibeni	
	0		1250		2500		5000			
Hydrocarbon	2.0	0.31	9.6	1.70	13.9	2.61	14.7	2.63	2.5	0.39
Monol	43.5	6.83	40.0	7.08	36.6	6.88	32.3	5.78	30.1	4.73
Monol monoepoxide	4.4	0.69	3.7	0.66	3.1	0.58	3.3	0.59	6.9	1.08
Diol	5.6	0.88	7.1	1.26	6.5	1.22	7.1	1.27	8.2	1.29
Keto-carotenoid									20.1	3.16
Diol monoepoxide 1	6.1	0.96	5.7	1.01	5.7	1.07	6.1	1.09	10.7	1.68
Diol diepoxide-1	14.8	2.32	13.0	2.30	13.3	2.44	13.2	2.36	4.9	0.77
Diol monoepoxide 2	11.5	1.81	6.0	1.06	7.4	1.39	6.8	1.22	7.2	1.13
Diol diepoxide-2	9.5	1.49	12.6	2.23	10.1	1.90	12.8	2.29	7.5	1.18
Polyol	2.5	0.39	2.6	0.46	3.4	0.64	3.6	0.64	1.9	0.30
Total carotenoid		15.7		17.7		18.8		17.9		15.7

Table 12. Effects of soil managements on sugar constituents of satsuma mandarin fruit

Treatment	Brix		Sugar constituents on Dec. 2, 1977 (g/100 ml)			
	Dec. 10 1976	Dec. 2 1977	Fructose	Glucose	Sucrose	Total
Clean	11.9	11.1	2.43	2.04	5.92	10.39
Sod	10.9	9.8	1.92	1.25	5.53	8.70
Mulching	10.3	10.2	2.60	1.67	5.06	9.33

Table 13. Effects of soil managements on organic acids constituents of satsuma mandarin fruit

Treatment	Titratable acidity (%)		Organic acids constituents on Dec. 2, 1977 (mg/100 ml)												
	Dec. 10, 1976	Dec. 2, 1977	Glu. etc	Gluc.	Pyro-Glu.	Lact.	Acet.	Pyru.	Mal.	Cit.	Suc.	Iso-Cit.	α-Ket Glu.	Total
Clean	0.84	0.93	76.9	0.7	0.3	0.1	0.2	1.1	71.9	1,232.4	0.7	12.9	—	1,397.2
Sod	0.91	1.09	79.5	0.5	0.4	0.6	0.2	1.5	65.5	1,452.0	1.2	10.3	—	1,611.7
Mulching	0.96	1.13	79.5	0.8	0.2	0.2	0.2	1.3	76.5	1,434.8	0.5	12.3	—	1,606.3

Table 14. Effects of soil managements on amino acids constituents of satsuma mandarin fruit on Dec. 2, 1977

Treatment	Asn.	Gln.	Asp.	Thr.	Ser.	Glu.	Pro.	Gly.	Ala.	Val.
Clean	7.6	2.1	0.8	0.1	1.8	0.4	4.6	0.1	0.9	0.3
Sod	5.4	2.6	0.4	0.1	1.7	0.2	2.3	0.2	0.5	0.1
Mulching	5.3	1.8	1.3	0.1	1.6	0.7	5.5	0.2	1.4	0.4
Treatment	**Met.**	**ILeu.**	**Leu.**	**Tyr.**	**Phe.**	**γ-ABA**	**Lys. etc.**	**Arg.**	**Ammo.**	**Total (g/100 ml)**
Clean	0.2	0.3	+++	+++	+++	0.2	3.7	1.0	0.1	24.2
Sod	0.1	0.4	+++	+++	+++	0.3	2.4	0.7	0.1	17.5
Mulching	0.1	0.2	+++	+++	+++	0.6	3.0	1.3	0.1	23.6

content of the other carotenoid groups showed no significant difference between CPTA-treated samples and non-treated ones.

H group of CPTA-treated fruit peel was subdivided into 6 fractions by alumina-TLC with petroleum ether as solvent. These fractions were identified and relative content was determined: F_1: β-carotene + ζ-carotene (6%), F_2: ζ-carotene (4%), F_3: γ-carotene (7%), F_4: γ-carotene (8%), F_5: Cis-lycopene (15%) and F_6: lycopene (60%). In non-treated samples, only F_1 and F_2 fractions were contained. The color development of fruit peel by CPTA treatment seemed to be caused by the activation of lycopene biosynthesis pathway and lycopene was accumulated to about 12% of the total carotenoids.

4) Effects of the short term soil management on yield and quality of satsuma mandarin fruit on the sloping citrus grove

Effects of 3 kinds of soil management, i.e., clean, sod, and straw-mulch, on yield and quality of satsuma mandarin fruit were investigated on a sloping grove[8] (Tables 12, 13 and 14).

In the clean culture, yield and mean fruit weight decreased, but the index of fruit color increased, in which 'a' value of color difference meter of fruit peel increased markedly. Sugar content of fruit also increased, but fruit acidity decreased. The increase of sugar content of juice was due to increased glucose and sucrose, and the decrease of fruit acidity was due to decreased citric acid. Free amino acids such as asparagine, proline and lysine increased under the clean culture.

There was no difference in yield, degreening of fruit, sugar content and acidity of fruit juice between sod culture and straw mulching. However, the total amount of free amino acids in the fruit juice was higher under the straw-mulching than under the sod culture.

As mentioned above, it is evident that, though treated in 3 years, fruit maturity of the clean culture is earlier than the sod and straw-mulch culture.

References

1) Aharoni, Y: Respiration of oranges and grapefruits harvested at different stages of development. *Plant Physiol.*, **43**, 99–102 (1968).
2) Bain, J. M.: Morphological, anatomical and phygiological changes in the developing fruit of the Valencia orange *Citrus sinensis* (L) Osbeck. *Aust. J. Bot.* **6**,

1-24 (1958).
3) Biale, B. J.: The post-harvest biochemistry of tropical and subtropical fruits, *Advances in Food Research*, **10**, 293-354 (1960).
4) Daito, H. & Hirose, K.: Studies on maturity of citrus fruit. I. Changes in the amino acid content of satsuma mandarin juice during maturation. *Bull. Hort. Res. Sta., Ser.* B, **11**, 19-32 (1971).
5) Daito, H. & Hirose, K.: Effects of $CaCO_3$ on the quality and pretreatment for fruit storage in satsuma mandarins. *Ann. Rev. Fruit Tree Res. Sta. Okitsu Branch*, **1**, 71-72 (1974) [In Japanese].
6) Daito, H. & Hirose, K.: Effects of CPTA on the color development and the lycopene accumulation in satsuma mandarin fruits. *Ann. Rev. Fruit Tree Res. Sta. Okitsu Branch*, **2**, 42-45 (1975) [In Japanese].
7) Daito, H. et al.: Studies on maturity of citrus fruit. II. Changes in the color and carotenoid group of pigments of satsuma mandarin fruit during maturation. *Bull. Fruit Tree Res. Sta., Ser.* B, **2**, 53-74 (1975).
8) Daito, H., Tominaga, S. & Morinaga, K.: Effects of the short term soil managements on yield and quality of satsuma mandarin fruit on the sloping citrus grove. *Bull. Shikoku Agr. Exp. Sta.*, No. 37, 87-94 (1981) [In Japanese with English summary].
9) Daito, H. & Morinaga, K.: Changes in the volatile flavor components of satsuma mandarin fruit during maturation. *J. Jpn. Soc. Hort. Sci.*, **53**, 141-149 (1984) [In Japanese with English summary].
10) Daito, H. & Sato, Y.: Changes in the content of pectic substances in satsuma mandarin fruit during maturation. *J. Jpn. Soc. Hort. Sci.*, **53**, 150-156 (1984) [In Japanese with English summary].
11) Daito, H. & Sato, Y.: Changes of respiratory and ethylene evolution rate in maturing fruit of satsuma mandarins. *J. Jpn. Soc. Hort. Sci.*, **53**, 405-411 (1985) [In Japanese with English summary].
12) Daito, H. & Sato, Y.: Changes in the ethanol and acetaldehyde contents of satsuma mandarin fruit during maturation. *J. Jpn. Soc. Hort. Sci.*, **54**, 33-38 (1985) [In Japanese with English summary].
13) Davis, P. L.: Relation of ethanol content of citrus fruits to maturity and to storage conditions. *The citrus industry*, March 5-10 (1971).
14) Daito, H. & Sato, Y.: Changes in the sugar and organic acid components of satsuma mandarin fruit during maturation. *J. Jpn. Hort. Sci.*, **54**, 155-162 (1985) [In Japanese with English summary].
15) Eaks, I. L.: Respiratory response, ethylene production, and response to ethylene of citrus fruit during entogeny. *Plant Physiol.*, **45**, 334-338 (1970).
16) Kakiuchi, N., Iba, Y. & Ito, S.: Fundamental studies on citrus juice. I. Seasonal changes of organic acids and sugars of satsuma mandarin. *Bull. Hort. Res. Sta. Ser.*, B, **10**, 149-162 (1970) [In Japanese with English summary].
17) Rockland, L. B.: Nitrogenous constituents. *In* The Organge. ed. Sinclair, W. B., Univ. California, Div. Arg. Sci., California, 230-264 (1961).
18) Rockland, L. B. & Underwood, J. C.: Rapid procedure for estimation of seven free amino acids in orange juice. *Anal. Chem.*, **28**, 1679-1684 (1956).
19) Tominaga, S. & Daito, H.: Effects of ethychlozate (ethyl 5-chloro-1H 3-indazolyl-acetate) on fruit quality of satsuma mandarin (*Citrus unshiu* Marc.) *Proc. Int. Soc. Citriculture*, 223-236 (1981).
20) Vines, et al.: Ethylene. *In* The biochemistry of fruits and their products. **2**, ed. Hulme, A. C., Academic Press, London and New York, 153 (1971).
21) Wedding, R. T. & Horspool, R. P.: Compositional changes in juice of Valencia and Wasington navel oranges during fruit development. *Citrus leaves*, **35** (2), 12-13 (1955).

(Received for publication, October 9, 1985)