STUDIES IN THE NATURAL COATING OF APPLES

II. CHANGES IN THE FRACTIONS DURING STORAGE

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Summary

The oil fraction of the natural coating of Granny Smith apples increased during storage and reached a maximum at 3-4 times its original concentration. The increase was reduced by "gas" storage (in 5 per cent. CO_2, 16 per cent. O_2). Later pickings had a higher oil content. The iodine number of the oil increased with increasing concentration. Smaller increases occurred in the wax, ursolic acid, and "cutin" fractions after prolonged storage.

The fatty esters of the oil fraction were produced most rapidly at the beginning of storage. Subsequently the production of these non-volatile esters declined, while the rate of volatile ester production increased.

There was no definite correlation between the oil content and the resistance of the skin to gaseous diffusion, although both increased during storage.

I. Introduction

The preparation and properties of the major fractions of the natural coating of Granny Smith apples have already been described (Huelin and Gallop 1951). Changes have been found in every fraction during storage, and the data are presented in this paper. All results refer to the Granny Smith variety.

The changes in the oil fraction are likely to have the greatest effect on the resistance of the skin to gaseous diffusion and to water loss. These changes are relatively greater than the changes in the other fractions. Moreover, the oil is the only fraction that is liquid at room temperature. Its presence may increase resistance by filling the spaces between the solid particles. For this reason the oil fraction has been studied in some detail.

II. Methods of Analysis

The methods for determining the various fractions have already been described (Huelin and Gallop 1951). In 1945 whole apples were extracted with light petroleum and in 1947 with carbon tetrachloride. On account of the difficulties arising from extraction with carbon tetrachloride the results for 1947 are less accurate than for other years. In 1948 the separated skin was extracted first with light petroleum and then with ether. In 1949 whole apples were extracted with light petroleum for determination of "total fatty acids." The skin was subsequently separated and used for determination of insoluble "cutin."

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Iodine numbers were determined by the method of Hanus in 1945 and by the method of Wijs in 1947 and subsequent years. The method of Wijs gives considerably higher numbers.

The resistance of the skin to diffusion of oxygen was calculated by the method of Hicks from measurements of respiration and composition of internal atmosphere. The procedure is described by Trout *et al.* (1942).

All conclusions regarding changes during storage were examined for statistical significance.

III. Changes in Oil Content during Storage

Granny Smith apples were picked at Orange, New South Wales, on April 10, 1945, and stored at 1°C. Determinations of oil content were made after 0, 13, and 27 weeks at this temperature. Samples were also removed from 1°C. to 18°-20°C. for determination of oil content at fortnightly intervals. The iodine number of the oil and the concentration of "total fatty acids" were also determined. The results are given in Table 1 and Figure 1.

Table 1

CHANGES IN OIL CONTENT IN 1945

(Picked April 10, 1945 at Orange, N.S.W.)

Weeks at 1°C.	Weeks at 18°-20°C.	Oil (mg./sq. cm.)	Iodine No. of Oil (Hanus)	Total Fatty Acids (μE/sq. cm.)
0	0	0.106	50	0.265
	2	0.179	68	0.530
	4	0.247	74	0.694
	6	0.266	84	0.681
13	0	0.287	81	0.812
	2	0.277	78	0.765
	4	0.231	82	0.664
	6	0.242	81	0.726
27	0	0.302	73	0.956
	2	0.254	71	0.773
	4	0.233	72	0.654

The oil increased up to three times the original concentration at 1°C. The most rapid increase occurred at the beginning of storage. A similar, but more rapid increase occurred in apples held at 18°-20°C. after picking. In apples that had reached nearly maximum oil content at 1°C., removal to 18°-20°C. resulted in a decrease. Parallel changes occurred in the "total fatty acids."

The iodine number increased with increasing concentration of oil, indicating that the substances produced after picking are more unsaturated than those originally present. After prolonged storage the iodine number decreased.

In 1947 two pickings were made both at Orange, N.S.W., and Wantirna, Vic. The apples were stored at 0°C., and all determinations were made immediately after removal from this temperature. The results are given in Table 2.

TABLE 2

CHANGES IN OIL CONTENT IN 1947

District	Date of Picking	Weeks at 0°C.	Oil (mg./sq. cm.)	Iodine No. of Oil (Wijs)	Total Fatty Acids (μE/sq. cm.)
Orange, N.S.W.	26.iii.47	0	0.04	58	—
		10	0.07	104	0.31
		20	0.18	—	—
		30	0.18	112	0.60
	16.iv.47	0	0.07	94	—
		10	0.16	131	0.47
		20	0.18	138	0.66
		30	0.21	136	0.73
Wantirna, Vic.	9.iv.47	0	0.05	63	0.21
		10	0.14	129	0.46
		20	0.16	138	0.53
		30	0.22	127	0.71
	1.v.47	0	0.10	86	0.42
		10	0.19	145	0.52
		20	0.22	124	0.72
		30	0.28	134	1.06

In all samples the oil increased at 0°C. to 3-4 times the original concentration. The later pickings had a higher oil content throughout the storage period. Similar changes occurred in the "total fatty acids." The iodine number of the oil increased markedly during the first 10 weeks and changed very little subsequently.

TABLE 3

CHANGES IN "TOTAL FATTY ACIDS" IN 1949

(Picked April 12, 1949 at Orange, N.S.W.)

Weeks at 0°C.	Concentration (μE/sq. cm.) After Storage at 20°C. for			
	0 Weeks	2 Weeks	4 Weeks	6 Weeks
1	0.189	0.455	0.615	0.688
11	0.419	0.652	0.677	0.676
21	0.558	0.619	0.665	0.636
31	0.562	0.574	0.557	0.545

The data in Tables 1 and 2 indicate a significant correlation between oil and "total fatty acids" ($r = 0.931$, $P < 10^{-9}$). As the oil has a higher saponification number and increases much more than the wax during storage, most of the increase of "total fatty acids" is due to increase of oil. The mean ratio of oil content (as mg./sq. cm.) to "total fatty acids" (as μE/sq. cm.) is 0.32.

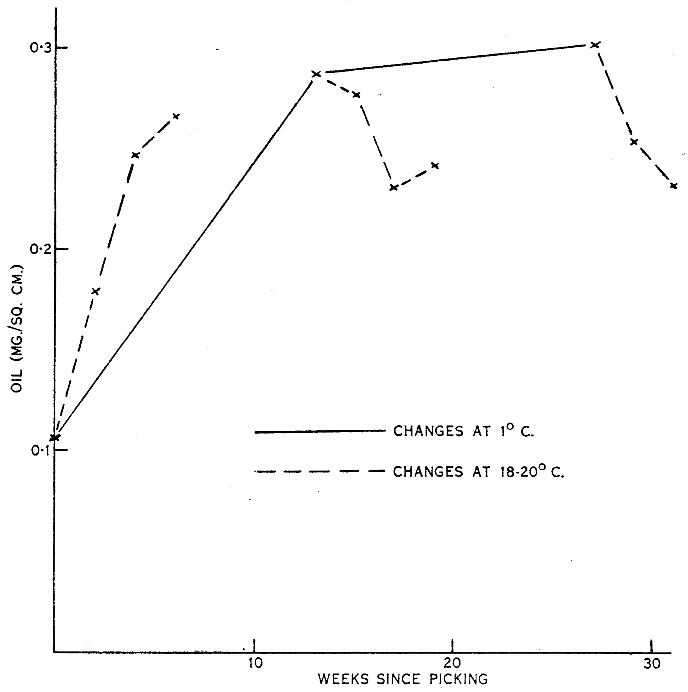

Fig. 1.—Changes in oil content in 1945 (picked April 10, 1945, at Orange, N.S.W.).

Further studies were made in 1949 with apples from Orange, N.S.W. The apples were stored at 0°C. and samples were removed to 20°C. at intervals. Determinations were made of "total fatty acids," which are an approximate index of the oil content. The results are given in Table 3 and Figure 2. "Total fatty acids" can be multiplied by 0.32 to give an approximate estimate of oil content.

Increases were obtained both at 0°C. and 20°C. similar to those obtained in 1945. The oil content appeared to reach a somewhat higher level at 20°C. Samples removed to 20°C. after 31 weeks at 0°C. did not change significantly, although the "total fatty acids" were still below the level attained previously at 20°C.

IV. Increase in Acids and Unsaponifiable Matter of Oil

The increase of oil during storage indicates a definite production of oily substances by the apple tissue. These substances may be produced by the epidermal cells and secreted into the cuticle; or they may be transported to the surface from other parts of the apple.

The production of oil was studied in more detail in 1950. The apples were picked at Orange on April 26. Samples were analysed immediately after picking and also after 6 weeks at 20°C. The oil was separated from the wax and weighed. It was then saponified, and the acids and unsaponifiable matter were separated and weighed. The acid fraction was separated with boiling light

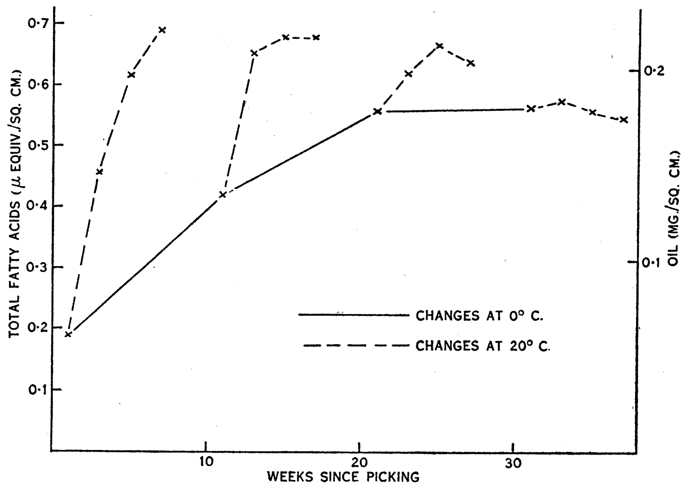

Fig. 2.—Changes in "total fatty acids" in 1949 (picked April 12, 1949, at Orange, N.S.W.).

petroleum (about 40 ml. per g.) into soluble and insoluble portions. Acid and hydroxyl numbers were determined on the acid fractions. Iodine numbers were determined by the Wijs method on the soluble acids and the unsaponifiable matter. The results are given in Table 4.

TABLE 4

CHANGES IN OIL SUBSTANCES AT 20°C.

Determination	Immediately after Picking	After 6 Weeks at 20°C.
Original oil (mg./sq. cm.)	0.099	0.226
Soluble acids (mg./sq. cm.)	0.062	0.135
Soluble acids (acid no.)	158	162
Soluble acids (hydroxyl no.)	64	39
Soluble acids (iodine no.)	100	102
Insoluble acids (mg./sq. cm.)	0.009	0.012
Insoluble acids (acid no.)	88	58
Insoluble acids (hydroxyl no.)	372	348
Unsaponifiable matter (mg./sq. cm.)	0.020	0.061
Unsaponifiable matter (iodine no.)	84	103

The increase in oil involved considerable increases both in acids and unsaponifiable matter. The iodine number of the soluble acids hardly changed during storage but the iodine number of the unsaponifiable matter increased considerably. The increase in iodine number of the oil appeared to be due mainly to increases in the iodine number and proportion of unsaponifiable matter.

The dark insoluble acids appeared to be mainly complex polyhydroxy acids. They may be products of oxidation of unsaturated acids. With a volume of light petroleum equivalent to 40 ml. per g. some hydroxy acids passed into the soluble portion, which had an appreciable hydroxyl number. However, the data are sufficient to show that the acids produced during storage are predominantly non-hydroxy. The increase of titratable acid in the soluble portion was 0.215 μE/sq. cm., while the increase of hydroxyl was only 0.023 μE/sq. cm. Hence there would not be more than one mole of hydroxy acid produced for every 10 moles of soluble acid.

V. Relation between Oil Content and Resistance of Skin to Gaseous Diffusion

During 1947, measurements were made of the resistance of the skin to diffusion of oxygen in parallel with the determinations of oil content. The measurements were made at 20°C. immediately after removal from 0°C. The results are given in Table 5.

Table 5

CHANGE IN RESISTANCE OF SKIN AT 0°C.

District and Picking	Weeks at 0°C.	Oil (mg./sq. cm.)	Resistance of Skin: Individual Apples	Mean
Orange, N.S.W. 26.iii.47	0	0.04	0.4, 0.6, 0.6, 0.8, 0.8, 0.8, 0.9, 0.9, 1.1, 1.3, 2.1, 2.5	1.1
	10	0.07	0.8, 0.9, 0.9, 1.1, 1.3	1.0
	20	0.18	1.0, 1.5, 1.6, 1.9, 2.2, 2.3	1.7
	30	0.18	—	—
Orange, N.S.W. 16.iv.47	0	0.07	1.2, 1.4, 1.5, 3.5	1.9
	10	0.16	1.3, 1.4, 1.5, 2.1, 2.7	1.8
	20	0.18	1.6, 1.6, 2.1, 3.2, 3.4, 5.4	2.9
	30	0.21	4.3, 4.6, 5.7, 5.9, 6.4, 6.5	5.6
Wantirna, Vic. 9.iv.47	0	0.05	1.3, 1.3, 1.7, 1.7, 1.8, 2.6	1.7
	10	0.14	1.6, 1.8, 2.1, 2.3, 2.5	2.1
	20	0.16	1.8, 2.3, 2.3, 2.7, 3.5, 4.8	2.9
	30	0.22	1.0, 1.4	1.2
Wantirna, Vic. 1.v.47	0	0.10	0.8, 1.9, 2.1, 2.8, 3.1	2.1
	10	0.19	1.0, 1.1, 1.6, 1.9, 2.0	1.5
	20	0.22	2.0, 2.2, 2.3, 2.8, 2.9, 3.2	2.6
	30	0.28	1.3, 2.1, 2.9	2.1

Measurements of resistance were usually made on 5-6 individual apples, but some of the apples in the last removal were lost through mould. In general, the resistance did not change significantly in the first 10 weeks but increased definitely in the second 10 weeks. In the last 10 weeks the resistance of the second picking from Orange continued to increase. The resistance of the apples from Wantirna appeared to decrease in the last 10 weeks, but the measurements were rather few. Similar trends were observed by Hackney (1943) who found that the resistance increased during several months at 1°C. and sometimes decreased after prolonged storage.

There was no definite correlation between resistance of skin and concentration of oil. The oil generally increased continuously during storage. The resistance of the skin is not determined solely by the concentration of oil but probably depends on other factors such as the distribution and physical state of the oil and other components of the natural coating.

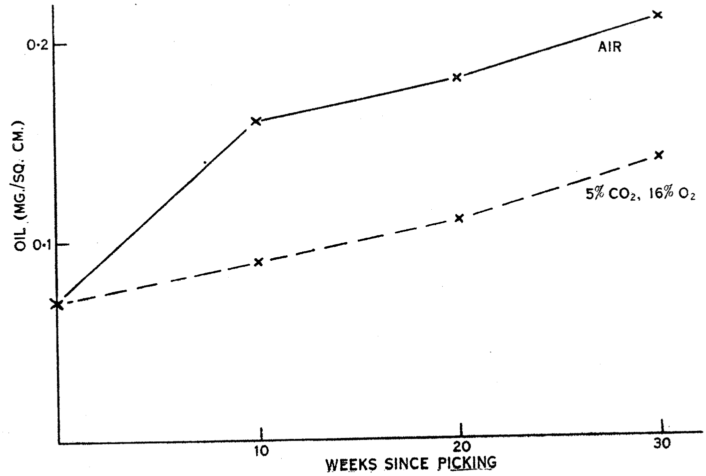

Fig. 3.—Changes in oil content at 0°C. in air and 5 per cent. CO_2, 16 per cent. O_2 (picked April 16, 1947, at Orange, N.S.W.).

VI. Effect of "Gas" Storage on Increase of Oil Content

Apples were picked at Orange on April 16, 1947 (second picking) and stored at 0°C. Samples were stored both in air and in an atmosphere containing 5 per cent. of carbon dioxide and 16 per cent. of oxygen and obtained by controlled ventilation. Storage in atmospheres containing more carbon dioxide and less oxygen than ordinary air is known as "gas" storage. Determinations of oil content were made on samples stored in air and in "gas." The results are given in Table 6 and Figure 3.

The increase in oil was definitely reduced by "gas" storage. It has been shown that other ripening changes, e.g. colouring, softening, hydrolysis of polysaccharides, and loss of acid are also retarded by "gas" storage.

VII. Changes in Wax Fraction during Storage

The results for 1945 are given in Table 7 and the results for 1947 and 1948 in Table 8.

Table 6
CHANGES IN OIL CONTENT IN AIR AND "GAS" STORAGE

Weeks at 0°C.	Oil (mg./sq. cm.) in Air	Oil (mg./sq. cm.) in 5% CO_2, 16% O_2
0	0.07	0.07
10	0.16	0.09
20	0.18	0.11
30	0.21	0.14

Increases during cold storage varied from negligible to about 40 per cent. of the initial concentration. The average increase was about 25 per cent. Most of this increase occurred in the last few weeks of storage.

Table 7
CHANGES IN WAX FRACTION IN 1945
(Picked April 10, 1945 at Orange, N.S.W.)

Weeks at 1°C.	Concentration (mg./sq. cm.) After Storage at 18°-20°C. for			
	0 Weeks	2 Weeks	4 Weeks	6 Weeks
0	0.253	0.258	0.250	0.220
13	0.232	0.244	0.242	0.265
27	0.282	0.286	0.264	—

Table 8
CHANGES IN WAX FRACTION IN 1947 AND 1948

District	Date of Picking	Concentration (mg./sq. cm.) After Storage at 0°C. for			
		0 Weeks	10 Weeks	20 Weeks	30 Weeks
Orange,	26.iii.47	0.23	0.28	0.25	0.26
N.S.W.	16.iv.47	0.24	0.26	0.33	0.29
	5.v.48	0.199	0.208	0.209	0.217
Wantirna,	9.iv.47	0.28	0.31	0.26	0.37
Vic.	1.v.47	0.31	0.29	0.33	0.44

VIII. Changes in Ursolic Acid Fraction during Storage

The changes during cold storage are given in Table 9. Increases up to 40 per cent. were also obtained in the ursolic acid fraction. Most of the increase usually occurred in the last 10 weeks at 0°C.

IX. Changes in "Cutin" Fraction during Storage

The changes in the acids of the "cutin" fraction are given in Table 10. A significant increase of about 20 per cent. occurred in the last 10 weeks at 0°C., but no other differences were significant. It seems that significant increases in the wax, ursolic acid, and "cutin" fractions usually appear after about 20 weeks at 0°C.

Table 9

CHANGES IN URSOLIC ACID FRACTION IN 1947 AND 1948

District	Date of Picking	Concentration (mg./sq. cm.) After Storage at 0°C. for			
		0 Weeks	10 Weeks	20 Weeks	30 Weeks
Orange,	26.iii.47	0.33	0.40	0.36	0.46
N.S.W.	16.iv.47	0.37	0.44	0.43	0.54
	5.v.48	0.373	0.367	0.439	0.425
Wantirna,	9.iv.47	0.44	0.50	0.42	0.60
Vic.	1.v.47	0.44	0.48	0.46	0.48

X. Discussion

The increase of oil during storage can be considered in relation to the lipoid metabolism of the apple. Evidence has been obtained that the apple cells produce fatty acids (mostly esterified) and other fatty substances. The production of fatty substances is only a small component of the total metabolism (although it may be a major component in certain cells), but its influence on other metabolic processes is probably quite considerable. The accumulation of oil may affect the resistance of the skin to diffusion of oxygen and hence the concentration of internal oxygen. The latter affect respiration and ripening changes. Changes in the resistance of the skin to water loss may also have effects on metabolism.

Table 10

CHANGES IN ACIDS OF "CUTIN" FRACTION IN 1949
(Picked April 12, 1949 at Orange, N.S.W.)

Weeks at 0°C.	Concentration (mg./sq. cm.) After Storage at 20°C. for			
	0 Weeks	2 Weeks	4 Weeks	6 Weeks
1	0.398	0.392	0.378	0.413
11	0.368	0.389	0.370	0.437
21	0.401	0.411	0.392	0.413
31	0.474	0.458	0.449	0.432

The production of oil was generally most rapid at the beginning of the storage period and then declined. In many cases the concentration of oil finally approached a maximum value. The apparent cessation of oil production may be due to (*a*) inactivation of the lipoid-producing systems, (*b*) exhaustion of lipoid precursors, or (*c*) the effect of increasing oil concentration on the lipoid metabolism. The first alternative is quite plausible, as there is a failure of other synthetic mechanisms during senescence.

The identification of the fatty acids of the oil is still being attempted, and preliminary results indicate a variation of chain length from 12 to 22 carbon atoms. The volatile esters of Granny Smith apples have been shown to be derived from lower acids and alcohols with a maximum chain length of six carbon atoms (Thompson 1951), and their production has recently been studied by Thompson and Huelin (1951). In 1949, determinations of volatile ester production and of the "total fatty acids" of the natural coating were made on corresponding samples from the same source. The production of volatile esters (Thompson and Huelin 1951) can be linked with the accumulation of non-volatile fatty acids shown in Table 3 and Figure 2. The production of volatile esters reached its maximum rate much later than the accumulation of the non-volatile fatty acids. This is demonstrated in Table 11, where the total increase of each group at 20°C. is given for fortnightly periods.

TABLE 11

TOTAL INCREASE OF VOLATILE ESTERS AND NON-VOLATILE ACIDS AT 20°C.

Weeks at 0°C.	Days at 20°C.	Volatile Esters (mM/kg.)	Non-Volatile Acids (mE/kg.)
1	0-14	0.04	0.28
	14-28	0.32	0.17
	28-42	0.90	0.08
11	0-14	0.15	0.24
	14-28	0.21	0.03
	28-42	2.52	0
21	0-14	1.09	0.06
	14-28	1.23	0.05
	28-42	0.06	—0.03
31	0-14	0.80	0.01
	14-28	0.74	—0.02
	28-42	0.60	—0.01

In the first series of measurements at 20°C., an increasing rate of volatile ester production was accompanied by a declining rate of non-volatile acid production. A similar result was obtained after 11 weeks at 0°C. In later removals, volatile ester production was comparatively high initially, while non-volatile acid production had already fallen to a low value. Thompson and Huelin (1951) have obtained evidence that the increasing rate of volatile ester production is associated with an accumulation of volatile alcohols.

The complex relation of oil production to other ripening changes is emphasized by the effect of "gas storage." This technique involves a decrease of oxygen and an increase of carbon dioxide concentration in the external atmosphere. The slower ripening which occurs in "gas storage" is probably due to similar changes in the internal atmosphere. By reducing oil accumulation "gas storage" could delay the increase of skin resistance and hence modify its more direct effect upon the internal atmosphere.

XI. Acknowledgments

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