POLYMERIZATION OF THE CUTIN ACIDS OF THE APPLE SKIN

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SUMMARY

Rapid in vitro polymerization of the cutin acids of "Golden Delicious" apple skin is possible at a temperature of 100°. The resulting polymers can be hydrolyzed so that the original constituents are obtained again. Not all the acids participate in the polymerization in the same way: after two days the concentration of monobasic acids in the polymer was low compared with the concentration of the hydroxy fatty acids. Pigmented material from the cuticle layers influences the elasticity of the polymer but has little influence on the polymer-air surface structure.

1. INTRODUCTION

Cutin is usually described as a polycondensate containing a high percentage of hydroxymonobasic acids and a low percentage of monobasic and dibasic acids which are inter-esterified. HEINEN (1963) proposed a possible scheme for cutin synthesis and a formula for the structure of cutin with peroxyde bridges beside ester bonds.

Since these polymers are amorphous we have asked the question whether accompanying molecules, probably phenolic in nature (RICHMOND & MARTIN 1959; BAKER c.s. 1964) could be responsible for the structure of the cuticles of "Golden Delicious" apples as seen in the electron microscope (DE VRIES 1968a, 1969).

The fact that it is never possible to extract a real cutin layer consisting of fatty acids using enzymic or chemical methods is still unexplored.

LEE (1925) mentioned the fact that cutin acids do not harden at room temperature, which represents the first reference to the polymerization of cutin acids. ROELOFSEN (1959) regards the possibility that the cuticle owes its origin to the polymerization of "monomers".

2. MATERIAL AND METHODS

The cuticles of the apple "Golden Delicious" were prepared by the method described previously (DE VRIES 1969). After refluxing with 3% KOH in methanol the soluble material was evaporated to dryness, acidified, extracted with ether (ether fraction) and subsequently with the same volume of butanol (butanol fraction). The ether fraction contains the cutin acids (DE VRIES 1970) and the

butanol fraction consists of a dark-brown material of unknown composition, probably phenolic in nature (RICHMOND & MARTIN 1959).

Polymerization experiments were carried out with the ether fraction and/or with the addition of the butanol fraction at room temperature (20°) in the dark, U.V. light (254 mμ, 35°), and in an oven at 100° in the dark.

Free fatty acids were determined colorimetrically according to HEINEN & DE VRIES (1966) and identified by gas liquid chromatography (GLC) as methyl-esters and trimethylsilyl (TMSi) ethers without previous separation by thin layer chromatography (TLC) (DE VRIES 1970).

The carbon-replica technique has been used for determining the polymer-air surface structure according to the method of JUNIPER & BRADLEY (1958) with the exception of the Formvar-layer, which has been omitted. The replicas were shadowed with platinum at an angle of 35–40°, and examined in a Philips EM 300 at 60 kV.

3. RESULTS

The polymerization of the cutin acids of smooth "Golden Delicious" apples has been studied by determining the decrease of the free fatty acids in the samples and by GLC analyses, both on the polymer material and on the residue which has not yet polymerized.

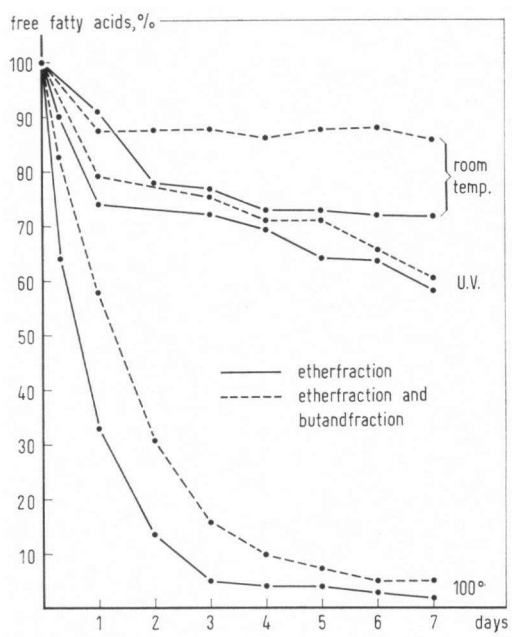


Fig. 1. The decrease of the free cutin acids in percentages at various times. The different conditions are mentioned in the text.

3.1. Polymerization of the free cutin acids under different conditions

The different conditions under which polymerization has been studied are: room temperature, no illumination; 35° with U.V. light; and 100° without illumination. Under all three circumstances the experiments were carried out with and without adding the butanol fraction.

The decrease of the free cutin acids is by far the greatest at a temperature of 100° as can be seen in *fig. 1*. Particularly the decrease during the first 48 hrs is considerable. During U.V. radiation the decrease of free acids is slight but steady, while there is hardly any decrease at room temperature except for the first 48 hrs. Adding of the butanol fraction has little influence on the polymerization process under all three conditions.

3.2. Characterization and analyses by GLC of the polymer

Samples of the ether fraction with or without the butanol fraction were placed at 100° for 48 hrs. The colour and the elasticity appear to depend on the rate ether fraction/butanol fraction as shown in *table 1*, in as much as the colour becomes browner and the elasticity becomes greater when more butanol fraction is added.

3.2.1. Analysis by GLC after hydrolyzing the polymerized material

After polymerization during 48 hrs at 100° the samples 1, 2 and 3 (see *table 1*) have become insoluble in the usual organic solvents. Therefore the complete samples were hydrolyzed with 3% KOH in methanol, from which the ether fraction was prepared, methylated, trimethylsilylated, and subsequently separated by GLC (see *fig. 2*). These chromatograms show that there is good agreement between sample 1 and the ether fraction which has not yet polymerized (DE VRIES 1970). Peaks a-g are sligthly lower in sample 2 and even somewhat lower in sample 3 compared with sample 1, while the peaks of the trihydroxymonobasic acids (m and n) have become higher. Obviously, the temperature of 100° has no influence on the cutin acids as such.

3.2.2. Analysis of the components of the polymer by means of GLC

Sample 1 (see *table 1*) was extracted in a Soxhlet apparatus after polymerization during 48 hrs at 100°. In this manner the ether soluble, not yet polymerized cutin acids (sample 1a) were separated from the polymerized material. The

Table 1. Samples of the ether fraction with or without the butanol fraction. The color and elasticity were described after polymerization at 100° for 48 hrs.

samples	fractions	colour	elasticity
nr. 1	ether	yellow	hard-brittle
nr. 2	ether-butanol (1:1, v/v)	light-brown	rather tough
nr. 3	ether-butanol (1:2, v/v)	brown	sirupy
nr. 4	butanol	brown	hard

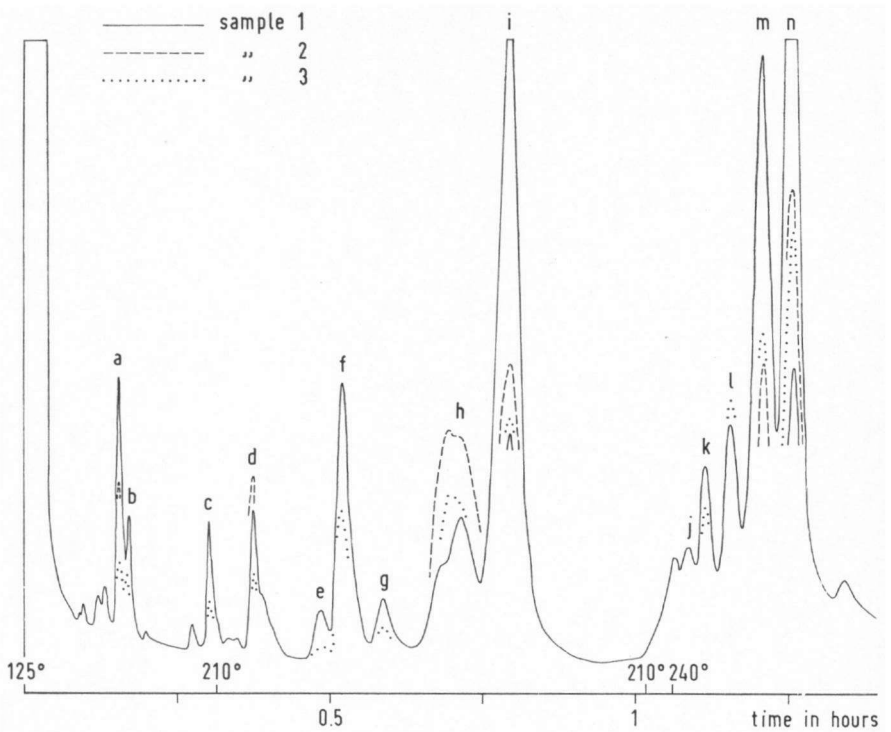


Fig. 2. Samples 1, 2 and 3 (see table 1) were hydrolyzed after 48 hrs at 100°. The chromatograms of the resulting acids are shown. Where the curves are superimposed, i.e., i, m and n, the smaller peaks are represented by $^1/_3$ their actual height.

polymerized material was hydrolyzed and the subsequent extraction procedure was carried out as described in 3.2.1. (sample 1b). The difference between the components of sample 1a and sample 1b, of which the chromatograms are shown in *fig. 3*, is mainly the concentration of the monobasic acids without any hydroxy groups. The concentration of these monobasic acids is low in sample 1b, the polymer, and high in sample 1a. This is also clearly shown in *table 2* in which the percentages of groups of peaks are shown. Consequently, the concentration of the trihydroxymonobasic acids is high in sample 1b and low in sample 1a.

3.2.3. Carbon-replicas of the polymer

The carbon-replicas were prepared from the polymer-air surface of the samples 1, 2, 3 and 4 (see *table 1*) after polymerization for 48 and 168 hrs at 100° in order to determine the structure. These replicas are shown in *figs. 4–11*. The replica of sample 1 shows ridge-like folds (*fig. 4*) after polymerization for 48 hrs;

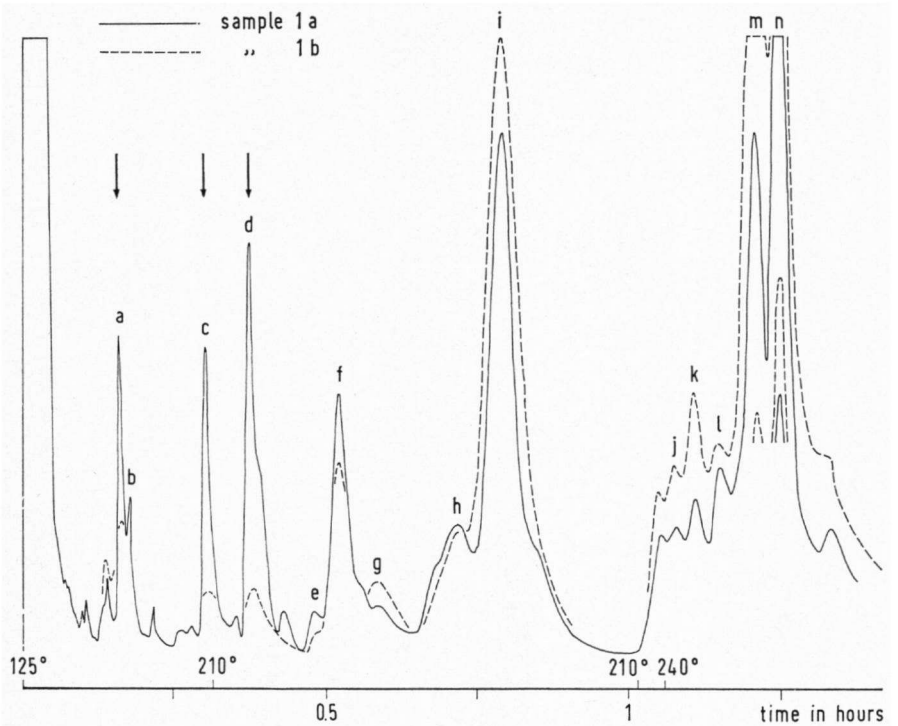


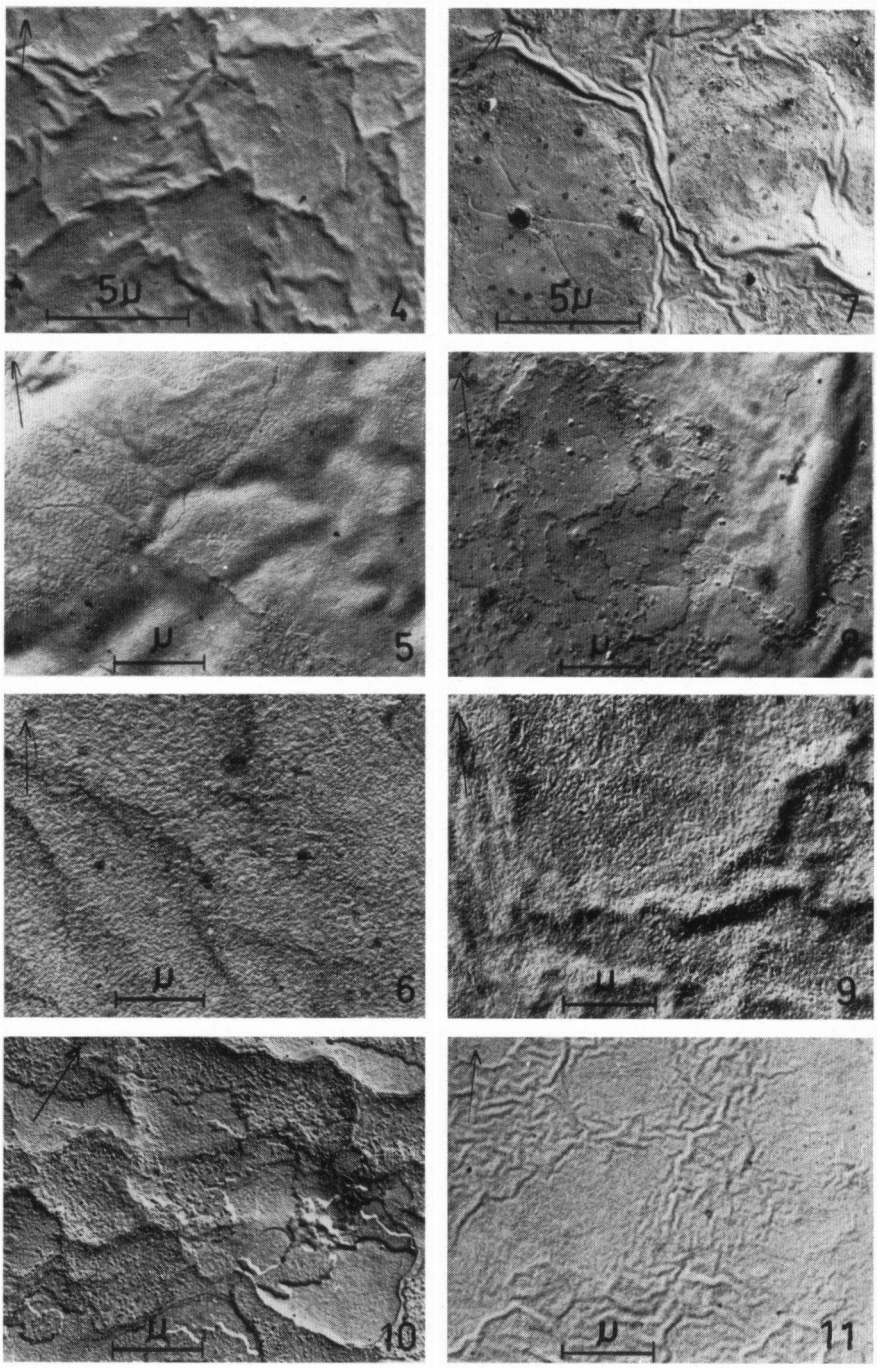
Fig. 3. Chromatograms of the samples 1a and 1b. The differences between both samples are indicated with arrows. Where the curves are superimposed, i.e., m and n, the smaller peaks are represented by $^1/_3$ their actual height.

Table 2. Percentages of the groups of peaks shown in *fig. 3*. ([1]) ether fraction which has not yet polymerized.

	peak a-d	peak e-i	peak j-n
ether fraction[1]	9%	35%	56%
sample 1a	17%	35%	48%
sample 1b	7%	33%	60%

higher magnification reveals agglomerates of irregularly shaped particles, arranged in layers (*fig. 5*). After 168 hrs of polymerization a regular arrangement of particles occurs besides some very thin ridges (*fig. 6*).

In sample 2 the ridges are more evident compared with sample 1 after 48 hrs of polymerization (*fig. 7*); higher magnification shows places with terrace formation (*fig. 8*). After 168 hrs of polymerization of sample 2 also particles are observed (*fig 9*); in this stage the ridges are just visible.


5μ
4
5μ
7
μ
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9
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10
μ
11

Fig. 10 shows clear terrace formation of very thin layers of sample 3 after 168 hrs polymerization. It seems that the rate of polymerization of this sample is retarded compared with that of sample 2.

Except for some ridges sample 4 shows even after 168 hrs polymerization at 100° an amorphous structure only (*fig. 11*).

4. DISCUSSION

Because the differences between the cutin acids of smooth and russeted "Golden Delicious" apple skins are quantitative only (DE VRIES 1969, 1970), we investigated the in vitro polymerization of the cutin acids of smooth apple skins. The experiments demonstrated clearly that at a temperature of 100°, which is considered to be rather low in Polymer Science, remarkable polymerization occurs. After hydrolysis of the polymer the same cutin acids were obtained as before polymerization, which is in agreement with the hypothesis mentioned by ROELOFSEN (1959).

When U.V. light was used, polymerization started very slowly and could be observed only after eight days, which is the reason why we have not looked into this further yet. Polymerization at room temperature in the dark is almost non-existent. However, it remains remarkable that in preliminary experiments polymerization did occur by chance at room temperature in sunlight, but the reproducibility turned out to be low, probably due to changing external conditions.

It is obvious that the temperature is a very important factor in the polymerization process. In nature rather high temperatures can occur also, as was demonstrated by ANSARI & LOOMIS (1959) who measured an increase of 20° in the temperature on the surface of a leaf compared with the air temperature. Addition of the pigmented material, probably phenolic in nature, obtained by the extraction with butanol, influences the elasticity of the polymer positively, but it has little influence on the rate of polymerization and on the participating cutin acids. Whether this pigmented material participates in the polymer and if so, in what way, is not known at this moment, neither is evidently the influence of this material on the structure of the polymer-air surface of the polymer.

The method developed seems to enable a new approach to the analysis of pathological deviation in the cuticle layer in a model system.

Fig. 4. Sample 1; replica of ether fraction after 48 hrs polymerization at 100°. In all figs. 4–11 the arrow (upper left) indicates the direction of the shadow.
Fig. 5. As *fig. 4;* higher magnification.
Fig. 6. As *fig. 5;* 168 hrs polymerization at 100°.
Fig. 7. Sample 2; replica of ether fraction and butanol fraction (1/1, v/v) after 48 hrs polymerization at 100°.
Fig. 8. As *fig. 7;* higher magnification.
Fig. 9. As *fig. 8;* 168 hrs polymerization at 100°.
Fig. 10. Sample 3; replica of ether fraction and butanol fraction (1/2, v/v) after 168 hrs polymerization at 100°; higher magnification.
Fig. 11. Sample 4; replica of butanol fraction after 168 hrs polymerization; higher magnification.

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