Phytochemistry, Vol. 20, No. 9, pp. 2135–2138, 1981.
Printed in Great Britain.

0031-9422/81/092135-04 $02.00/0

METABOLISM OF PROPIONIC ACID BY GOLDEN DELICIOUS APPLES

HERMAN L. DE POOTER, PATRICK J. DIRINCK, GUIDO A. WILLAERT and NICEAS M. SCHAMP

Laboratory of Organic Chemistry, Faculty of Agricultural Sciences, State University of Gent, Coupure 533, B-9000 Gent, Belgium

(*Revised received* 26 *January* 1981)

Key Word Index—*Malus pumila*; Rosaceae; apple fruit; volatiles; biosynthesis; propanal; propyl esters; propionates; headspace analysis.

Abstract—The headspace of whole Golden Delicious apples treated with propionic acid vapour, was analysed by means of GC, after enrichment on Tenax GC, and its components were identified by GC/MS. The composition of the headspace seems to be dependent not only on the availability of individual substrates, but also on the nature of the substrates and on the relative amounts in which they are present. Comparison of the behaviour of fruits treated with propionic acid with that of butyric acid treated ones showed that the latter was much more smoothly esterified, and that its influence on the total ester formation was much less pronounced than when the former was added. No evidence for extensive β-oxidation was found.

INTRODUCTION

Carboxylic esters are some of the chief components of the volatiles emitted by mature fruits such as apples [1], pears [2], strawberries [3], melons [4], and contribute largely to their aroma. Until recently only minor attention was paid to the biosynthesis of these substances starting from their direct precursor carboxylic acids and alcohols (see reviews [5–9]). Yamashita *et al.* showed that intact strawberries were able to synthesise carboxylic esters on addition of alcohols and acids [10], aldehydes and acids [11] and pentanal [12]. Incorporation experiments with apples using carboxylic acids, alcohols and aldehydes as precursors have only been performed on simplified systems such as aged apple discs [13, 14] or cell suspensions [15]. Paillard found that discs of aged apple tissue in media containing carboxylic acids produced alcohols with the same number of carbons, or with shorter chains after β-oxidation, and esters by combination of the two substrates. The formation of the alcohols was supposed to proceed via an intermediate reduction of the acid into the corresponding aldehyde, although the latter was never found, neither in the headspace nor in the incubation medium [13, 14]. Ambid and Fallot found that addition of butyrate to a cell suspension of Golden Delicious apples resulted in an increase of ethyl acetate and ethanol production, and in the formation of isobutanol and ethyl butyrate [15].

Preliminary experiments in our laboratory (H. De Pooter, unpublished results) had shown that Golden Delicious apples (GD apples) absorb substances such as hydrocarbons (e.g. n-C_{11}) and esters (e.g. 3-methylbutyl acetate) fairly easily when kept in an atmosphere containing these additives. On separate analysis of the volatiles of the peel and of subcutaneous layers, both compounds proved to have been transported throughout the pulp and into the deeper tissues of the fruit. These results suggested the feasibility of studying the biosynthesis of carboxylic esters by treating intact GD apples with air carrying propionic acid. This substance was chosen as substrate, as changes in the content of propyl esters and propionates, which are either absent, or present in only low concentrations in these fruits, would be easily detected.

RESULTS AND DISCUSSION

The evolution of esters and aldehydes by full-grown, intact apples, which were first stored in a controlled atmosphere cell for 2 months after harvest, is shown in Table 1. The fruits were treated once with propionic acid at the start of the experiment.

Acetaldehyde is formed in larger amounts than in the blank, and reaches a maximum concentration after *ca* 7 days. The evolution of propanal starts immediately, becomes maximal after *ca* 3 days, and then declines rapidly, to disappear after 9 days. (In separate experiments, where the apple tissue was macerated prior to sampling, propanal was already detected in relatively large quantities after a 1-hr treatment of the intact apple with the acid.)

Meanwhile the content of individual propyl esters (of which only propyl acetate was found in GD apples) increases, to reach a maximum after 7–9 days, and then diminishes. The formation of butyl and hexyl propionate, which are produced by untreated apples also, increases markedly as compared to the blank. 3-Methylbutyl- and pentylpropionate are formed in small amounts, with a maximum concentration after 7 days.

The sum of the propionates in the treated apples shows a definite maximum after 7 days, while in the blank it increases steadily (Fig. 1). The sum of the propyl esters, on the other hand, follows a different pattern, in that it gives first a small maximum 3 days after treatment, and then a second, larger one after 9 days. In the blank, only propyl acetate is produced, and its curve shows a maximum after 9 days (the values for propyl hexanoate, which elutes together with butyl pentanoate and pentyl butyrate, and cannot be quantified, are not taken into account in Fig. 1).

The sum of the other esters of treated apples is clearly smaller than that of untreated ones (Fig. 2) up to the ninth day, after which the situation reverses.

Table 1. Volatiles in headspace of Golden Delicious apples treated with propionic acid

Volatile (μg/kg/15 min)	Treated						Blank					
	Days after treatment						Days after treatment					
	1	3	5	7	9	11	1	3	5	7	9	11
Acetaldehyde	0.43	0.70	0.95	1.38	0.12	0.49	0.21	0.38	0.52	0.53	0.44	0.59
Propanal	0.27	0.64	0.55	0.18	0.01	—	—	—	—	—	—	—
Propyl acetate	0.06	1.34	1.35	4.16	5.41	3.54	—	0.04	0.09	0.25	1.70	1.16
Isobutyl acetate	0.09	0.27	0.29	0.42	0.59	0.37	0.23	0.36	0.24	0.30	0.55	0.15
Propyl propionate	0.16	1.25	0.96	2.21	2.73	—	—	—	—	—	—	—
Butyl acetate	5.09	4.48	5.56	10.32	22.99	37.02	5.02	6.23	8.53	24.84	35.77	39.33
3-Me-butyl acetate	0.66	0.97	1.22	1.34	3.58	5.36	0.78	1.40	2.03	3.86	4.94	5.23
Propyl butyrate	0.03	0.44	0.45	0.83	1.05	0.77	—	—	—	—	—	—
Butyl propionate	3.14	2.57	3.03	3.31	3.57	3.32	0.09	0.27	0.73	1.23	2.58	2.78
Pentyl acetate	0.23	0.71	2.59	4.37	7.25	8.42	0.20	0.53	0.63	1.32	1.32	1.45
Propyl 2-Me-butyrate	—	0.20	0.29	0.62	0.15	0.11	—	—	—	—	—	—
3-Me-butyl propionate	0.06	0.14	0.21	0.26	0.03	0.03	—	—	—	—	—	—
Butyl butyrate	0.94	1.02	0.79	2.13	3.18	5.25	1.70	1.54	2.44	4.04	5.04	4.89
Pentyl propionate	0.11	0.45	0.92	1.33	—	—	—	—	—	—	—	—
Hexyl acetate	3.13	4.91	6.88	16.61	40.13	68.27	3.21	7.13	13.33	26.60	41.87	50.29
Butyl 2-Me-butyrate	0.05	0.44	0.52	1.26	0.86	1.26	0.07	0.35	0.98	2.55	1.42	1.31
Propyl hexanoate + butyl pentanoate + pentyl butyrate	0.11	0.53	0.67	0.85	1.13	1.53	0.05	0.23	0.27	0.34	0.31	0.36
Hexyl propionate	2.13	1.79	4.85	5.78	4.52	6.40	0.02	—	0.79	1.56	2.95	4.35
Butyl hexanoate + hexyl butyrate	1.21	2.18	3.45	5.65	10.63	17.53	1.44	4.04	6.65	11.02	15.08	15.53
Hexyl 2-Me-butyrate	0.06	0.78	1.25	2.27	4.82	8.84	0.08	1.26	2.38	5.47	7.42	8.25
Hexyl pentanoate	—	0.23	0.47	1.09	1.21	1.35	—	0.36	0.90	0.31	0.50	0.57
Hexyl hexanoate	0.03	0.77	1.34	2.33	4.37	5.87	—	1.73	1.44	4.82	5.84	6.16

Amounts expressed in μg volatile/kg apples, enriched on Tenax GC tubes in 15 min. As external standards for GC analysis, a mixture of 1 μg n-C_{11} + 1 μg n-C_{13} alkanes was used. The substances are given in order of elution from the GC column.

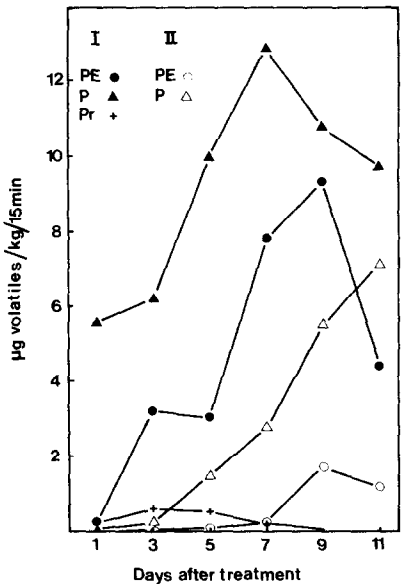

Fig. 1. Evolution of propanal (Pr) and of the sums of propyl esters (PE) and propionates (P) in the headspace of Golden Delicious apples after one treatment with propionic acid. Treated—I. Blank—II.

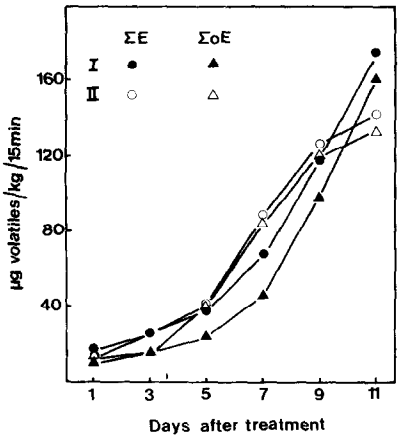

Fig. 2. Evolution of total esters (ΣE) and of esters not derived from propionic acid (ΣoE) in the headspace of Golden Delicious apples after one treatment with propionic acid. Treated—I. Blank—II.

GD apples, which had been stored in the controlled atmosphere cell for 8 months were still able to produce propionic acid derived esters and propanal after application of the acid (Table 2). The effect of the treatment was relatively short-lived, showing a maximum after 1–3 days and decreasing or disappearing after 5 days.

The results show that, in the circumstances used, propanal is formed as an intermediate in the transformation of propionic acid into propanol, prior to esterification. Comparison of the evolution of the sum of the propyl esters with that of propanal (Fig. 1) indicates their interdependence (both show a maximum after 3 days). Paillard already proposed such a sequence in apples and presented strong circumstantial evidence in favour of this synthesis of an aldehyde, but was not able to detect the substance as such in experiments with excised discs ([14] and references cited therein).

A second observation concerns the behaviour of the other esters, not derived from propionic acid, in treated apples. As long as the synthesis of propionates proceeds smoothly (7–9 days), the formation of the other esters diminishes. But when propionic acid is unavailable (as expressed by a drastic lowering in the production of propyl esters and propionates; see Fig. 1), the synthesis of, for example, hexyl acetate, butyl acetate, hexyl butyrate (or butyl hexanoate or both; the substances elute as a compound peak) increases strongly, and the total content of esters other than propionic acid derived in the headspace of treated fruits starts to exceed that of untreated ones (see Fig. 2). Only at the very start of the experiment, the total ester content of treated (unripe) apples surpasses that of untreated ones, mainly because of the supplementary production of propionates. This could indicate that a larger supply of ester synthesizing enzyme system is present in the early climacteric fruits, but that the availability of substrate is the limiting factor in the ester production (an analogous situation is encountered with 8-month-old apples; see Table 2). From the third day on, the ester-producing capacity of the apples increases, but it seems to be negatively influenced by propionic acid. In fact, while the total ester content increases both in treated and untreated fruits, it is always lower in the former. This phenomenon could be explained in terms of differences of reaction rates, whereby the esterification itself, and not so much the acceptance of the substrate by the enzyme system would be the limiting step. Such an antagonism was also noted by Paillard [14] when aged Calville apple discs were treated with a mixture of acetate, butyrate, ethanol and butanol.

GD apples were then treated with butyric acid, and in a parallel experiment with propionic acid. The results of Table 2 confirm the dependence of the esterification on the nature of the substrate. Indeed, much higher levels of volatiles are reached with butyric acid than with propionic acid. Moreover, if the respective amounts of hexyl acetate present may be taken as a measure, the effect on the other esters is much less pronounced with butyric acid than with propionic acid, indicating a smoother turnover of the former substrate.

In contrast to GD apple discs, which broke down butyric acid into acetic acid, and only formed acetates from added butyric acid [14], whole GD apples esterify butyric acid into butyrates. How much β-oxidation, if any, took place could not be determined, and how far this behaviour of GD apples is typical is unknown, as the treatment with butyric acid was only performed on 'old' apples.

It must be stated, however, that until now relatively large amounts of precursor were applied, and this may influence the extent of the observed phenomena, particularly with respect to the detection of propanal. On the other hand, as compared to experiments performed with aged apple discs [14] and apple cell suspensions [15], the enrichment technique yields more direct information, because most of the volatiles produced by the intact apple (detectable by GC) may be quantified in the course of their evolution.

EXPERIMENTAL

Fruits. GD apples from the 'Veiling Produco' (1977) and from the test-orchards at Meerdonk (1978, 1979) (Belgium) were stored in controlled atmosphere cells (1–2% CO_2, 0–5°) before use.

Concentration of headspace. Fruits (1.5–2 kg) were placed in desiccators (vol. ±8 l.; held at 17°), which were continuously

Table 2. Volatiles in headspace of Golden Delicious apples (8 months old, stored in a controlled atmosphere cell) treated with 100 μl butyric acid or propionic acid 1 day before sampling

Headspace components (μg/kg/15 min)	Blank	+ butyric acid	+ propionic acid
Acetaldehyde	0.15	2.43	0.98
Propanal + ethanol	—	—	1.00
Butanol	—	2.00	—
Ethyl propionate	—	—	0.06
Propyl acetate	—	—	0.55
Isobutyl acetate	0.86	1.36	1.17
Hexanal + ethyl butyrate	0.03	0.26	0.07
Propyl propionate	—	—	1.19
Butyl acetate	0.97	8.86	0.79
Propyl butyrate	—	0.12	—
Butyl propionate	—	0.30	1.04
Propyl 2-Me-butyrate	—	—	1.27
Butyl butyrate	0.29	20.41	0.58
Butyl 2-Me-butyrate	0.07	0.20	—
Propyl hexanoate	—	—	3.36
Hexyl propionate	—	—	1.91
Butyl hexanoate + hexyl butyrate	1.45	4.56	1.49
Hexyl acetate	2.97	2.07	0.97

flushed with air (150 ml/min). The apparatus is analogous to the one described in ref. [16]. Before sampling, the air flow was increased to 400 ml/min, and after 30 min a Tenax GC 60/80 mesh tube (length 11 cm; i.d. 12 mm; 1.8 g adsorbent) was attached to the outlet of the desiccator (sample time = 15 min), and the air flow readjusted to 400 ml/min. The tubes, when tightly closed and stored in a refrigerator, stay unchanged for long periods.

Treatment with additives. Between the air pressure flask and the desiccator, a 2-necked flask was installed containing 100 μl of the acid. The compounds were carried along as they evaporated at ambient temp.

GC analysis. Volatiles were desorbed from the Tenax GC tubes and analysed by GC as described in ref. [17]. Operating conditions: capillary column 250 m, i.d. 0.5 mm, coated with SE 52; FID, connected to an electronic integrator; temp programmed from 10 to 200° at 1°/min.

Interpretation of GC. Identification of compounds was made by means of GC/MS as described in ref. [17]. Structures were assigned by comparison of spectra with those of authentic samples.

Quantitative determinations were performed by co-injection of 1 μg of undecane and tridecane each as ext standards in the adsorption tubes, and by normalization of the values obtained from the integrator against that of the mean value for the alkanes. Consecutive samples showed a variation on the peak areas (or the amounts of substance present, expressed in μg/kg/15 min in tables) of max 10% (where the smaller area is taken as 100), from propyl acetate on, but a variation of up to 50% for acetaldehyde and propanal. Thus the values given in the tables for the latter substances represent only the general trend of the evolution, and give no information as to the exact quantities of these volatiles present in the headspace above the apples.

Acknowledgements—The investigations are supported by the Instituut tot Aanmoediging van het Wetenschappelijk Onderzoek in Nijverheid en Landbouw. The I.W.O.N.L., the Nationaal Fonds voor Wetenschappelijk Onderzoek and the Ministerie voor Wetenschapsbeleid are also thanked for financial aid.

REFERENCES

1. Nursten, H. E. (1970) in *The Biochemistry of Fruits and their Products* (Hulme, A. C., ed.) Vol. 1, p. 242. Academic Press, London.
2. Jennings, W. G. and Tressl, R. (1974) *Chem. Mikrobiol. Technol. Lebensm.* **3**, 52.
3. Pyysalo, T., Honkanen, E. and Hirvi, T. (1979) *J. Agric. Food Chem.* **27**, 19.
4. Yabumoto, K., Jennings, W. G. and Yamaguchi, M. (1977) *J. Food Sci.* **42**, 32.
5. Tressl, R. and Drawert, F. (1973) *J. Agric. Food Chem.* **21**, 560.
6. Tressl, R., Holzer, M. and Apetz, M. (1975) *Proc. Int. Symp. Aroma Res.*, p. 41. Zeist.
7. Tressl, R. and Renner, R. (1976) *Dtsch. Lebensm. Rundsch.* **72**, 37.
8. Salunkhe, D. K. and Do, J. Y. (1977) *Crit. Rev. Food Sci. Nutr.* **8**, 161.
9. Eriksson, C. E. (1979) in *Progress in Flavour Research* (Land, D. G. and Nursten, H. E., eds.) p. 159. Applied Science Publishers, Barking.
10. Yamashita, I., Nemoto, Y. and Yoshikawa, S. (1975) *Agric. Biol. Chem.* **39**, 2303.
11. Yamashita, I., Nemoto, Y. and Yoshikawa, S. (1976) *Phytochemistry* **15**, 1633.
12. Yamashita, I., Iino, K., Nemoto, Y. and Yoshikawa, S. (1977) *J. Agric. Food Chem.* **25**, 1165.
13. Paillard, N. (1978) *Int. Fed. Fruit Juice Producers*, Symposium, Bern, p. 25.
14. Paillard, N. (1979) *Phytochemistry* **18**, 1165.
15. Ambid, C. and Fallot, J. (1980) *Bull. Soc. Chim. Fr.* II, 104.
16. Williams, A. A., Tucknott, O. G. and Lewis, M. J. (1977) *J. Sci. Food Agric.* **28**, 185.
17. Dirinck, P., De Pooter, H., Willaert, G. and Schamp, N. (1981) *J. Agric. Food Chem.* **29**, 316.