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FORMATION OF VOLATILE ALCOHOLS AND ESTERS FROM ALDEHYDES IN STRAWBERRIES*

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Key Word Index—*Fragaria ananassa*; Rosaceae; strawberry; flavour; formation of volatiles; aldehyde; alcohol; ester; flavor development.

Abstract—The aldehydes acetaldehyde, propanal, 2-methylpropanal, butanal, 3-methylbutanal, pentanal and hexanal, were reduced to their corresponding alcohols by incubation with strawberry fruit. The alcohols formed were then converted to their acetate, propionate, *n*-butyrate, isovalerate and *n*-caproate esters during the incubation with strawberry fruit. Simultaneous reaction of isobutyric acid, *n*-valeric acid and isocaproic acid with aldehyde and strawberry fruit resulted in the formation of esters of these acids. In all seven alcohols and 54 esters were produced by means of incubation of aldehydes and volatile fatty acids with strawberry fruit.

INTRODUCTION

Nursten has reviewed the volatile compounds in fruits [1]. Aldehydes, alcohols and esters often contribute to the aroma of fruit and vegetables, for example ethyl *trans*-2, *cis*-4-decadienoate to Bartlett pear aroma [2, 3], nona-*trans*-2, *cis*-6-dienal to cucumber aroma [4], ethyl 2-methylbutyrate to apple aroma [5], 3-methyl-1-butyl acetate to banana aroma [6], *trans*-2-hexenal, ethyl 3-methylbutyrate and ethyl 2-methylbutyrate to bilberry aroma [7].

Though strawberry aroma has no known character impact compound, Tressl *et al.* [8] identified 8 aldehydes, 40 alcohols and 91 esters in strawberry volatiles.

Our interest is focussed on the formation of aldehydes, alcohols and esters, and especially on the interconversion between them in strawberry.

The reciprocal interconversion of alcohols and aldehydes has been emphasized by Eriksson [9] in studies on alcohol:NAD oxidoreductase (ADH) from peas. ADH, which catalyses the conversion between aldehydes and alcohols, has been the subject of a review by Brändén *et al.* [10].

On the other hand Nordström [11, 12] has studied extensively the formation of volatile esters in fermentation with brewer's yeast. For ester formation in plants, Heinz *et al.* [3] reported the production of esters of *trans*-2, *cis*-4-decadienoic acid in ripening Bartlett pear; Myers *et al.* [13, 14] reported the formation of 3-methyl-1-butanol and 3-methyl-1-butyl acetate in banana fruit; Tressl *et al.* [15–19] and Drawert *et al.* [20–22] reported the interesting results concerning the formation of banana volatiles, especially formation of methyl-branched esters from leucine and valine; Tressl and Drawert [23] have reviewed the formation of banana volatiles and Ueda *et al.* [24] reported the esterification of 3-methyl-1-butanol in banana fruit.

* Part 2 in the series "Studies on Flavor Development in Strawberries" For Part 1, see reference [25].

In previous work we [25] reported that alcohols were converted to their corresponding acetate, propionate, *n*-butyrate, 3-methylbutyrate (isovalerate) and hexanoate (*n*-caproate) esters during incubation with strawberry fruit for 1 hr at 30°C.

Esters of 2-methylpropionic (isobutyric) acid and pentanoic (*n*-valeric) acid were also formed when these acids were incubated together with alcohol and strawberry fruit.

One of the possible precursors of an alcohol is the corresponding aldehyde, which is reduced by ADH. In this paper, some experiments are reported on the formation of alcohols, followed by their conversion to esters, from aldehydes that were incubated with whole strawberry fruit.

RESULTS AND DISCUSSION

Conversion of aldehyde to alcohol and esters in strawberry

Figure 1 shows the GLC profiles obtained using GLC system 1 of volatile compounds in the headspace flask in which butanal was allowed to react with strawberry fruit. Besides residual butanal, 1-butanol and a small quantity of 1-butyl acetate and 1-butyl *n*-butyrate were detected after a 1 hr incubation (Fig. 1a).

The concentration of 1-butanol and 1-butyl esters increased considerably between 1 hr and 2 hr incubations, the butanal concentration decreasing rapidly. 1-Butyl propionate, 1-butyl isovalerate and 1-butyl *n*-caproate were also detected in the 2 hr incubation (Fig. 1b). The concentration of 1-butanol, however, decreased after 2 hr incubation, while the concentration of 1-butyl esters increased continuously during the 4 hr incubation (Fig. 1c).

The time course of changes of butanal, 1-butanol and 1-butyl acetate concentration detected in the headspace volatiles are illustrated in Fig. 2. Though further studies are required it seems that butanal, when incubated with strawberry fruit, is reduced to 1-butanol and the 1-butanol produced is converted to 1-butyl acetate. This

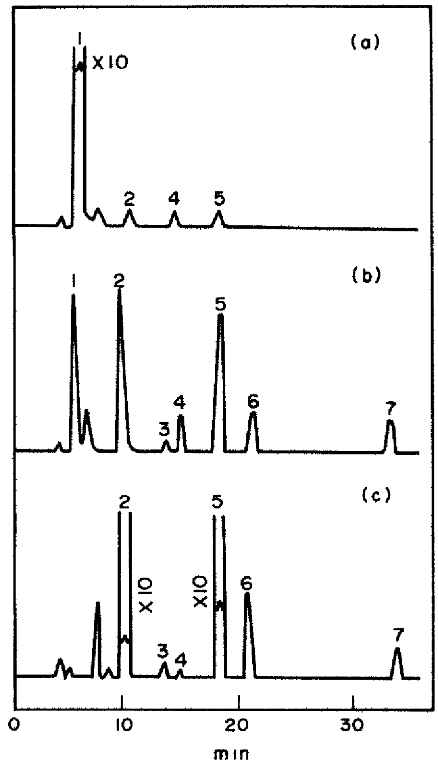

Fig. 1. Gas chromatographic profiles of volatile compounds produced by incubation of strawberry fruit with butanal. A strawberry fruit was incubated with butanal at 30° for (a) 1 hr, (b) 2 hr, (c) 4 hr. GLC system 1 was employed; peaks are 1: Butanal; 2: 1-butyl acetate; 3: 1-butyl propionate; 4: 1-butanol; 5: 1-butyl butyrate; 6: 1-butyl isovalerate; 7: 1-butyl caproate.

hypothesis was supported by the following experiments in which other substrates were used instead of butanal. Ethanol and ethyl esters were formed from acetaldehyde; 1-propanol and 1-propyl esters were formed from propanal; 2-methylpropanol and 2-methylpropyl esters were formed from 2-methylpropanal; 3-methyl-1-butanol and 3-methyl-1-butyl esters were produced from 3-methylbutanal; 1-pentanol and 1-pentyl esters were formed from pentanal; 1-hexanol and 1-hexyl esters were formed from hexanal (Table 1). Acid moieties of esters formed were acetic, propionic, *n*-butyric, isovaleric and *n*-caproic acids (Table 1).

On the other hand, neither alcohols nor esters were produced when strawberry fruit which had been heated to inactivate enzymes was used.

These results show that there are enzymes required for the formation of these volatile compounds from aldehydes.

The conversion of alcohols to the corresponding esters in strawberry fruit was discussed in the previous paper [25].

The reduction of aldehydes to alcohol is presumably catalysed by ADH, which is known to exist in the tissues of many animals, higher plants and microorganisms.

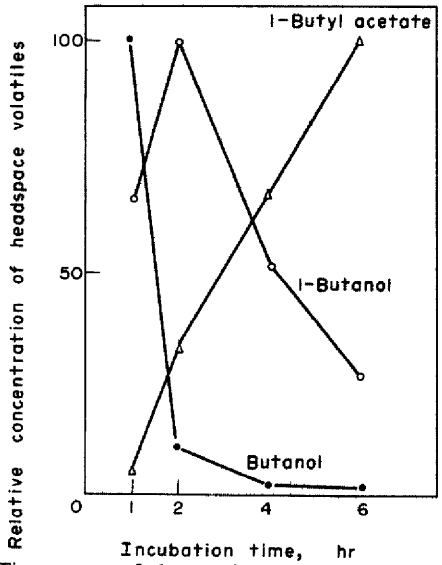

Fig. 2. Time course of changes in concentration of butanal, 1-butanol and 1-butyl acetate produced by incubation of strawberry fruit with butanal.

Table 1. Alcohols and esters formed from aldehydes and volatile fatty acids by strawberry fruit

Aldehydes and fatty acids incubated with strawberry fruit	Alcohols and esters formed
Acetaldehyde*, propionic acid, isobutyric acid, *n*-valeric acid and isocaproic acid	Ethanol*
	Ethyl acetate*
	Ethyl propionate*
	Ethyl isobutyrate*
	Ethyl *n*-butyrate*
	Ethyl isovalerate*
	Ethyl *n*-valerate
	Ethyl isocaproate
	Ethyl *n*-caproate*
Propanal*, propionic acid, isobutyric acid, *n*-valeric acid and isocaproic acid	1-Propanol*
	1-Propyl acetate*
	1-Propyl propionate
	1-Propyl isobutyrate
	1-Propyl *n*-butyrate*
	1-Propyl isovalerate
	1-Propyl *n*-valerate
	1-Propyl isocaproate
	1-Propyl *n*-caproate
2-Methylpropanal, propionic acid, isobutyric acid, *n*-valeric acid and isocaproic acid	2-Methyl-1-propanol*
	2-Methyl-1-propyl acetate*
	2-Methyl-1-propyl propionate
	2-Methyl-1-propyl isobutyrate
	2-Methyl-1-propyl *n*-butyrate*
	2-Methyl-1-propyl isovalerate
	2-Methyl-1-propyl *n*-valerate
	2-Methyl-1-propyl isocaproate
	2-Methyl-1-propyl *n*-caproate
Butanal*, propionic acid, isobutyric acid, *n*-valeric acid and isocaproic acid	1-Butanol*
	1-Butyl acetate*
	1-Butyl propionate
	1-Butyl isobutyrate
	1-Butyl *n*-butyrate*
	1-Butyl isovalerate
	1-Butyl *n*-valerate
	1-Butyl isocaproate
	1-Butyl *n*-caproate

Table 1.—*continued*

Aldehydes and fatty acids incubated with strawberry fruit	Alcohols and esters formed
3-Methylbutanal, propionic acid, isobutyric acid, *n*-valeric acid and isocaproic acid	3-Methyl-1-butanol*
	3-Methyl-1-butyl acetate*
	3-Methyl-1-butyl propionate
	3-Methyl-1-butyl isobutyrate
	3-Methyl-1-butyl *n*-butyrate*
	3-Methyl-1-butyl isovalerate
	3-Methyl-1-butyl *n*-valerate
	3-Methyl-1-butyl isocaproate
	3-Methyl-1-butyl *n*-caproate*
Pentanal, propionic acid, isobutyric acid, *n*-valeric acid and isocaproic acid	1-Pentanol*
	1-Pentyl acetate*
	1-Pentyl isobutyrate
	1-Pentyl *n*-butyrate*
	1-Pentyl isovalerate
	1-Pentyl *n*-valerate
	1-Pentyl isocaproate
	1-Pentyl *n*-caproate*
Hexanal*, propionic acid, isobutyric acid, *n*-valeric acid and isocaproic acid	1-Hexanol*
	1-Hexyl acetate*
	1-Hexyl propionate
	1-Hexyl isobutyrate
	1-Hexyl *n*-butyrate*
	1-Hexyl isovalerate
	1-Hexyl *n*-valerate†

* These compounds are known strawberry oil components [8]. † 1-Hexyl isocaproate and 1-hexyl *n*-caproate were not detected. In the majority of cases the products were identified by both GLC Systems 1 and 2 and by GC–MS.

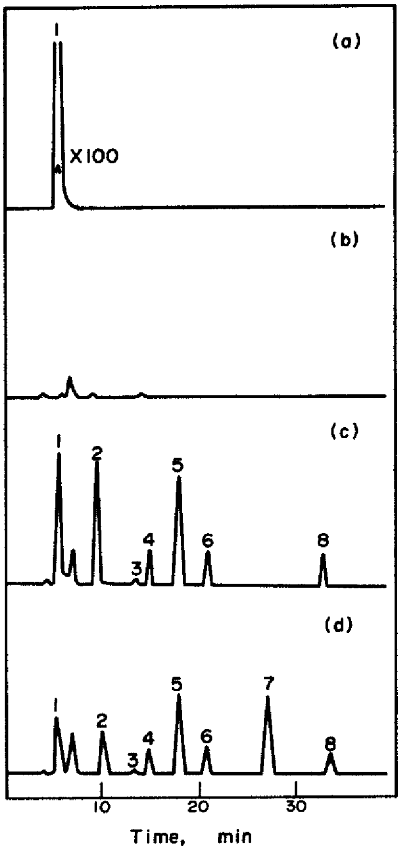

Fig. 3. Gas chromatographic profiles of volatile compounds produced by incubation of strawberry fruit with butanal and n-valeric acid. (a) Butanal and volatile fatty acids (acetic, propionic, isobutyric, *n*-butyric, isovaleric, *n*-valeric and *n*-caproic acids) were incubated without strawberry fruit at 30° for 2 hr. (b) A strawberry fruit alone was incubated at 30° for 2 hr. (c) A strawberry fruit was incubated with butanal at 30° for 2 hr. (d) A strawberry fruit was incubated with butanal and *n*-valeric acid at 30° for 2 hr. GLC system 1 was employed; peaks are 1: Butanal; 2: 1-butyl acetate; 3: 1-butyl propionate; 4: 1-butanol; 5: 1-butyl *n*-butyrate; 6: 1-butyl isovalerate; 7: 1-butyl *n*-valerate; 8: 1-butyl *n*-caproate.

Simultaneous reaction of aldehyde and volatile fatty acid with strawberry

The analysis of volatiles produced in the headspace by GLC system 1 showed that the simultaneous incubation of butanal and *n*-valeric acid in the headspace flask containing strawberry fruit resulted in the formation of 1-butyl *n*-valerate with depression of the formation of 1-butyl acetate, 1-butyl propionate, 1-butyl *n*-butyrate, 1-butyl isovalerate and 1-butyl caproate (Fig. 3c, d). Neither 1-butanol formation nor 1-butyl ester formation was observed when strawberry fruit alone was incubated (Fig. 3b) or butanal and volatile fatty acids were incubated without strawberry fruit (Fig. 3a). As retention times of added volatile fatty acids were very long, they are omitted from Fig. 3a.

The most appropriate interpretation of these results is that strawberry fruit itself does not produce enough alcohol or ester to be detected under the condition employed. Since aldehyde does not react with volatile fatty acids in the absence of strawberries it seems that the fruit needs a certain amount of aldehyde to produce enough alcohol and esters to be detected, though strawberry fruit does contain volatile fatty acids (unpublished results). The results obtained from Figs. 3c and d also show that exogenous acids are esterified as well as endogenous acids.

For a further understanding for the formation of alcohols and esters from aldehydes in strawberry fruit, the following experiments were performed. (1) Aldehydes, e.g., acetaldehyde, propanal, 2-methylpropanal, butanal, 3-methylbutanal, pentanal and hexanal, were incubated with volatile fatty acids for 2 hr at 30°, and then the headspace volatiles were analysed by GLC system 2 to examine the non-enzymic reaction of aldehyde with volatile fatty acids. The volatile fatty acids used were propionic acid, isobutyric acid, *n*-valeric acid and 4-methylpentanoic (isocaproic) acid. (2) Alcohols, e.g., ethanol, 1-propanol, 2-methylpropanol, 1-butanol, 3-methylbutanol, 1-pentanol and 1-hexanol, were incubated with volatile fatty acids under the same conditions as (1) and the headspace volatiles were analysed by GLC system 2 to examine the non-enzymic reaction of alcohol with volatile fatty acids. (3) Strawberry fruit alone was incubated and the headspace volatiles were analysed by GLC system 2 to examine if strawberry fruit itself is able to produce alcohols and esters without addition of aldehyde. (4) Aldehydes were incubated with strawberry fruit and the headspace volatiles analysed by GLC system 2 to check the formation of alcohols from aldehydes in strawberry fruit. (5) Aldehydes were incubated with the volatile fatty acids mentioned above together with strawberry fruit, and the headspace volatiles were analysed by GLC System 2 to test the reduction of aldehydes to alcohols and the esterification of alcohols with volatile fatty acids.

The analytical results obtained, taking butanal, with volatile fatty acids as an example, are shown in Fig. 4. Butanal did not react with volatile fatty acids without strawberry fruit (Fig. 4a). The same result is shown in Fig. 3a using GLC System 1. The incubation of 1-butanol with volatile fatty acids did not result in the formation of esters under the conditions employed (Fig. 4b). No volatile compounds were found when strawberry fruit was incubated alone (Fig. 4c). This result is supported by Fig. 3b and the previous work [25].

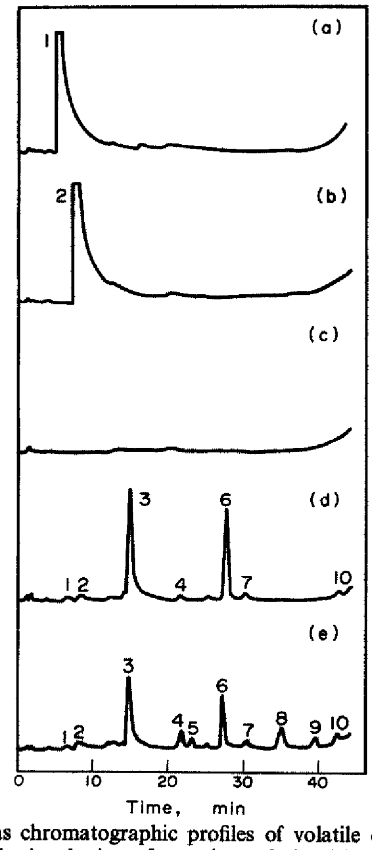

Fig. 4. Gas chromatographic profiles of volatile compounds produced by incubation of strawberry fruit with butanal and volatile fatty acids. (a) Butanal and volatile fatty acids (acetic, propionic, isobutyric, *n*-butyric, isovaleric, *n*-valeric, isocaproic and *n*-caproic acids) were incubated without strawberry fruit at 30° for 2 hr. (b) 1-Butanol and the volatile fatty acids listed in (a) were incubated without strawberry fruit at 30° for 2 hr. (c) A strawberry fruit was incubated alone at 30° for 2 hr. (d) A strawberry fruit was incubated with butanal at 30° for 2 hr. (e) A strawberry fruit was incubated with butanal, propionic acid, isobutyric acid, *n*-valeric acid and isocaproic acid at 30° for 2 hr. GLC system 2 was used, peaks are 1: 1-Butanol; 2: 1-butanol; 3: 1-butyl acetate; 4: 1-butyl propionate; 5: 1-butyl isobutyrate; 6: 1-butyl *n*-butyrate; 7: 1-butyl isovalerate; 8: 1-butyl *n*-valerate; 9: 1-butyl isocaproate; 10: 1-butyl *n*-caproate.

Formation of 1-butanol and 1-butyl esters was observed when butanal was incubated with strawberry fruit. The esters detected were 1-butyl acetate, 1-butyl propionate, 1-butyl *n*-butyrate, 1-butyl isovalerate and 1-butyl *n*-caproate (Fig. 4d). 1-Butyl isobutyrate, 1-butyl *n*-valerate and 1-butyl isocaproate were observed in addition when the corresponding acids were present (Fig. 4e). Parallel results were obtained with other aldehydes and acids (Table 1). Thus the seven different aldehydes tested were all reduced to the corresponding alcohols and the alcohols produced were converted to their esters. Many of these aldehydes, alcohols and esters have been found in strawberry oil [8].

Strawberry fruit treated with 3-methylbutanal is noteworthy. 3-Methylbutanal was converted to 3-methyl-1-butyl acetate, the dominant ester produced. As a result, the strawberry fruit incubated with 3-methylbutanal for 2 hr emanates the aroma of bananas, because 3-methyl-1-butyl acetate gives the flavour typical of bananas [6, 15–22].

EXPERIMENTAL

Material. Strawberry fruit (*F. ananassa* Duchesne) was obtained from a local market.

Headspace flask. A 100 ml wide-mouthed (50 mm) Erlenmeyer flask was modified for headspace analysis. A glass stopper with a small central hole (5 mm) was made, and a small silicone rubber cap (a GLC injection port septum), provided a gas-tight seal. As it is known that a rubber cap can absorb volatiles and subsequently release them [26], a new cap was used for each experiment.

Reaction. A whole strawberry fruit was placed in a headspace flask and 3 μl of aldehyde with or without 1 μl of each volatile fatty acid was added onto the wall of the flask. The flask was sealed and kept at 30° for 2 hr in a water bath in the dark.

Analysis of the volatile compounds in the headspace. A 2 μl vapour sample was taken through the silicone rubber cap by means of a gas-tight syringe and injected into the gas chromatograph equipped with a flame ionization detector. GLC system 1 used a glass column (4 m × 3 mm i.d.) of 25% PEG 1000 on Chromosorb WAW (60–80 mesh) with N_2 as carrier gas (20 ml/min). The hydrogen flow rate was 20 ml/min; air 800 ml/min. The column oven temp was programmed as follows: 60–110° at 1°/min for acetaldehyde, ethanol and ethyl esters; 70–120° at 1°/min for propanal, 1-propanol and 1-propyl esters; 90–120° at 2°/min for butanal, 1-butanol, 1-butyl esters, 2-methylpropanal, 2-methyl-1-propanol and 2-methyl-1-propyl esters; 100–120° at 1°/min for 3-methylbutanal, 3-methyl-1-butanol, 3-methyl-1-butyl esters, pentanal, 1-pentanol and 1-pentyl esters, 130° isothermal for hexanal, 1-hexanol and 1-hexyl esters. Injection and detector temperature were 150°. GLC system 2 employed a glass column (4 m × 3 mm i.d.) of 30% silicone DC 550 on Chromosorb WAW (60–80 mesh) with the same N_2, hydrogen and air flow rates as for System 1. The column oven temp was programmed as follows: 60–160° at 2°/min for acetaldehyde, ethanol and ethyl esters; 70–170° at 2°/min for propanal, 1-propanol and 1-propyl esters; 90–190° at 2°/min for 2-methylpropanal, 2-methyl-1-propanol, 2-methyl-1-propyl esters, butanal, 1-butanol, 1-butyl esters, 3-methylbutanal, 3-methyl-1-butanol, 3-methyl-1-butyl esters, pentanal, 1-pentanal, 1-pentyl esters, hexanal, 1-hexanol and 1-hexyl esters. Injection and detector temperatures were 220°. GLC System 1 gave good chromatograms without tailing, but was not able to separate the alcohol from its esters with propionic acid and isobutyric acids. On the other hand, System 2 permitted perfect separation of aldehyde, alcohol and esters, but unfortunately gave tailing for ethanol, 1-propanol and 1-butanol. However the use of these two systems permitted satisfactory analysis of the volatile compounds. The relative concentrations of volatile compounds were determined by a digital integrator. The volatile compounds were analysed by GC–MS with the GLC System 1. MS were recorded at 70 eV. The compounds were identified by comparison of the MS with those obtained from known compounds.

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