Metabolism of Natural Volatile Compounds by Strawberry Fruit

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As part of experiments to evaluate naturally occurring volatile compounds as fumigants to reduce microbial populations, especially pathogenic fungi on strawberry fruit, several test compounds were found to be metabolized by the fruit to yield additional volatile compounds. The natural products used as fumigants comprised alcohols, aldehydes, ketones, esters, and hydrocarbons and were tested with fruit in closed containers at refrigeration (2−4 °C) temperatures. The three principal types of volatile metabolites formed by strawberry fruit as determined by GC and GC−MS analyses of headspace vapor samples were esters produced from aliphatic alcohols [e.g., (*Z*)-3-hexenyl acetate from (*Z*)-3-hexen-1-ol], alcohols formed by the reduction of aliphatic aldehydes (e.g., 1-hexanol from hexanal), and saturated products from reduction of carbon−carbon double bonds conjugated with an aldehyde or ketone carbonyl moiety [e.g., 2-nonanone from (*E*)-3-nonen-2-one]. Metabolic pathways leading to the formation of the volatile products and applications for the metabolites are discussed.

Keywords: *Strawberry; volatile compounds; flavor; antimicrobial compounds; Botrytis*

INTRODUCTION

It is well-known that volatile compounds impart the aroma component of flavor to foods, including those derived from plants. Numerous investigations have shown that several plant-emitted volatile compounds, including aldehydes, ketones, alcohols, and other classes of natural products, exhibit antimicrobial properties against pathogenic fungi such as *Aspergillus* (Gueldner et al., 1985; Zeringue and McCormick, 1989), *Fusarium* (Nandi, 1977), *Penicillium* (Kurita et al., 1981), and *Botrytis* (Wilson et al., 1987; Vaughn et al., 1993; Andersen et al., 1994) and bacteria (Deng et al., 1993; Kubo et al., 1993).

Studies were undertaken on the use of natural volatile compounds to control populations of deleterious microorganisms in food, including reducing the incidence of *Botrytis* mold on strawberries. During the course of these studies, fruit were exposed to varying concentrations of numerous volatile compounds using conditions that partially simulate commercial handling practices. Sampling and analysis of the headspace within the test containers revealed that the fruit metabolized vapors of the compounds and produced an array of new compounds which were released into the vapor phase. Among the major compounds formed, at refrigeration temperatures, were volatile products derived from esterification of alcohols, carbonyl reduction, and reduction of carbon−carbon double bonds in aliphatic chains.

MATERIALS AND METHODS

Exposure of Strawberry Fruit to Test Compounds. Strawberry (*Fragaria annanasa*) cultivars were grown, using conventional cultural practices, in a greenhouse (cv. Tribute) and at the University of Kentucky Experiment Station South Farm (cv. Chandler) in Lexington. Fruit were harvested and placed individually in 250 mL jars. (Initially, fruit were inoculated with a *Botrytis cinerea* spore suspension for the pathological component of the experiment; however, subsequent tests showed that the same compounds were formed by the fruit in the absence of inoculum.) Each jar contained a wire screen shaped to suspend an individual fruit above 1 mL of water which was added to maintain high humidity. A sample of a test compound was added to a glass vial which was also placed in the jar. The lowest levels of compounds were chosen on the basis of quantities that yielded measurable GC signals from vapor phase samples; larger quantities of the less volatile compounds were required. A lid with a Teflon liner and a center hole fitted with a rubber septum, to allow sampling of the vapors within the system, was used to seal the jar. Controls, without fruit but including test compounds, were set up in the same manner as the treatments. The jars were then placed in a refrigerator at 2−4 °C.

Test Compounds. The following compounds used in the experiments were purchased from Aldrich Chemical Co., Milwaukee, WI, or were gifts from Bedoukian Chemical Co., Danbury, CT, and were the highest purity available from these sources: 1-hexanol, (*E*)-2-hexen-1-ol, (*Z*)-3-hexen-1-ol, hexyl acetate, hexanal, (*E*)-2-hexenal, (*Z*)-3-hexenal (50% in triacetin), (*E*,*E*)-2,4-hexadienal, 3-hexanone, (*E*)-4-hexen-3-one, nonanal, (*E*)-2-nonenal, (*Z*)-6-nonenal, (*E*,*E*)-2,4-nonadienal, (*E*,*Z*)-2,6-nonadienal, 2-nonanone, (*E*)-3-nonen-2-one, Δ-2-carene, D-limonene, methyl benzoate, and methyl salicylate.

Sampling Volatile Compounds in Jars. At 2−3 days (2−4 °C) after setup of an experiment, 25−400 μL headspace vapor samples were removed from the jars with a gas-tight syringe. The samples were analyzed by direct injection into a Varian 3700 gas chromatograph fitted with a 30 m × 0.53 mm DB wax (polyethylene glycol) column (J&W Scientific, Folsom, CA). The injector and FID detector temperatures were 220 and 240 °C, respectively, and He was used as the carrier gas at a flow rate of 6 mL/min. The GC area units for a given test compound and its identified metabolites were summed, and the yield of each individual compound is expressed as a percentage of the total. The same procedure was also used for 2-nonanone which had two distinct metabolites which were not identified.

To obtain samples for mass spectral analyses, treatments were set up as described above except that each lid contained two holes fitted with rubber septa. After an incubation period

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(2 days at 2 °C), each jar was placed on a lab bench and inlet and outlet air lines consisting of Teflon tubing were attached. The inlet line was connected to a charcoal filter, and the outlet was attached to a porous polymer trap consisting of Porapak Q (Altech, Deerfield, IL) packed in a glass tube. A vacuum source was used to draw the headspace sample into the trap at a flow rate of 100 mL/min for 15 min. The trap was then removed and eluted with 300 μL of hexane/CH_2CI_2 (80:20, v/v). Sample aliquots were analyzed by GC−MS using a Hewlett-Packard 5895 instrument operated in the EI mode (40−300 amu) with a 30 m × 0.32 mm DB-5 (phenylmethylsilicone) column (J&W Scientific).

The O_2 content of the jars was determined 3 days after the setup of the experiment. A 5 mL aliquot of the headspace was withdrawn from each sample jar and analyzed using an O_2/CO_2 Headspace Analyzer ZR 892 HS (Illinois Instruments, McHenry, IL).

RESULTS AND DISCUSSION

The metabolic products which resulted from exposure of strawberry fruit to volatile compounds being tested for inhibition of pathogen development are presented in Table 1. The first group of compounds studied was six-carbon alcohols. These compounds are produced by the lipoxygenase−hydroperoxide lyase metabolic pathway and are apparently ubiquitous in plant species (Hatanaka, 1993). The three commonly naturally occurring alcohols, 1-hexanol, (*E*)-2-hexen-1-ol, and (*Z*)-3-hexen-1-ol, were all metabolized to their corresponding acetate esters to a significant extent (40% or greater in all treatments). Subsequent work with Porapak used to trap the volatile components revealed the production of additional compounds such as small amounts of butyrates in the headspace samples. The esterification reaction was the first evidence we observed showing that strawberry fruit metabolized volatile compounds in the test system. The jars remained aerobic during the experiments since O_2 levels did not fall below 19% in the headspace. With no fruit present in the jar, there was virtually no conversion of the alcohol to its ester (data not shown). Similarly, unless noted otherwise, none of the other compounds studied (Table 1) yielded measurable amounts of volatile reaction products in controls without added fruit. In addition, strawberry fruit placed in the jars without added chemicals did not yield measurable amounts of volatile compounds using the direct headspace sampling method.

The esterification reaction with the six-carbon lipoxygenase−lyase alcohols was observed at all three test quantities with Tribute fruit. The results were also confirmed with the cultivar Chandler using the three alcohols at a midlevel quantity. Overall, the results demonstrate that strawberry fruit readily convert six-carbon alcohols to their corresponding volatile acetate esters. In contrast to the results with alcohols, tests with a six-carbon ester, hexyl acetate, yielded relatively low amounts of the hydrolysis product 1-hexanol.

The other major group of lipoxygenase−lyase alcohols, the nine-carbon alcohols, was not tested. These compounds have relatively high boiling points and low vapor pressures and are difficult to analyze using the direct sampling method for GC quantitation.

The six-carbon aldehydes, which are found naturally via lipoxygenase−lyase oxidation of linoleic and linolenic acid, were also metabolized by the fruit. Hexanal was readily converted to its corresponding alcohol, 1-hexanol, and ester, hexyl acetate. The yield of products was less at the highest level of hexanal. This was observed with several other compounds and may indicate the occurrence of greater compound phytotoxicity at higher levels of test compounds. (*E*)-2-Hexenal also yielded its corresponding alcohol, (*E*)-2-hexen-1-ol, and ester, (*E*)-2-hexenyl acetate, in all treatment conditions. In addition, smaller amounts of 1-hexanol and hexyl acetate were detected in the headspace of jars containing (*E*)-2-hexenal. These results indicated the capacity of strawberry fruit to reduce a carbon−carbon double bond. Results from (*Z*)-3-hexenal, which is not readily available in pure form but also contains (*E*)-2-hexenal, yielded the esters (*Z*)-3-hexenyl acetate, (*E*)-2-hexenyl acetate, and hexyl acetate as volatile metabolites in all but the lowest test level. There was GC retention time evidence to indicate the presence of the corresponding alcohols. The alcohols were relatively minor components in the headspace and like other minor compounds encountered in the experiments were not quantitated. The control for (*Z*)-3-hexenal which contained no fruit showed the presence of 2.4% (*E*)-2-hexenal which arises from the spontaneous rearrangement of (*Z*)-3-hexenal.

The diunsaturated six-carbon aldehyde, (*E*,*E*)-2,4-hexadienal, was also tested, and two major volatile metabolites were detected and identified as 2,4-hexadien-1-ol and 2,4-hexadienyl acetate. The starting aldehyde in this experiment was relatively unstable chemically, and several small peaks were observed which were not identified in the GC trace for this compound and its products. The results with the six-carbon aldehydes demonstrate that strawberry fruit has the capacity to convert these compounds to their corresponding alcohols and acetate esters which are released into the atmosphere of the bioassay system.

Several nine-carbon aldehydes, including some compounds derived from the lipoxygenase−lyase pathway, were studied. These compounds had much lower volatility than the six-carbon aldehydes, and their products exhibited even lower volatility; thus, the metabolites from this group were more difficult to detect than products from lower-molecular weight substrates. However, most of the nine-carbon compounds, including nonanal, (*E*)-2-nonenal, (*E*,*E*)-2,4-nonadienal, and (*E*,*Z*)-2,6-nonadienal, showed at least one well-separated product peak which could be quantitated by GC. In all cases, except nonanal which yielded nonanol, the product detected was the acetate ester corresponding to the test aldehyde added to the atmosphere. No well-separated GC peaks were obtained with (*Z*)-6-nonenal which may indicate that the fruit could not metabolize this aldehyde or that the products were too unstable to yield well-resolved peaks. The experiments with the nine-carbon aldehydes indicated that the fruit metabolized most of these compounds to yield esters which are rarely reported as plant components.

Studies with the six-carbon ketones showed that the saturated compound, 3-hexanone, was not metabolized by the fruit to yield volatile products detected by our methods. This contrasted with the results with the six-carbon aldehyde where the carbonyl was reduced to form an alcohol. However, the α,β-unsaturated ketone, (*E*)-4-hexen-3-one, underwent carbon−carbon double bond reduction under all test conditions to yield the saturated ketone 3-hexanone. Similar results were obtained with the nine-carbon α,β-unsaturated ketone, (*E*)-3-nonen-2-one, which also underwent carbon−carbon double bond reduction to yield the saturated nine-carbon ketone 2-nonanone. Similar carbon−carbon double bond reduction could account for the production of saturated metabolites which were observed in tests with (*E*)-2-hexenal as described earlier.

There were small peaks produced by the fruit from 2-nonanone, but these compounds could not be identified from their spectral patterns. These results show that

Table 1. Volatile Compound Formation by Strawberry Fruit Exposed to Several Classes of Natural Compounds with Antimicrobial Properties[a]

test compound[b]	metabolite[b]	liquid, μL[c]	Tribute vapor, %[d]	liquid, μL[c]	Tribute vapor, %[d]	Chandler vapor, %[d]	liquid, μL[c]	Tribute vapor, %[d]
			C_6 Aldehydes					
hexanal		2		10	1.3	4.6	100	80.5
	1-hexanol		10.8		50.3	28.6		9.4
	hexyl acetate		89.2		48.4	66.9		10.1
(*E*)-2-hexenal		2	4.5	10	27.3	5.8	100	72.7
	(*E*)-2-hexen-1-ol		10.6		15.4	24.9		12.1
	(*E*)-2-hexenyl acetate		69.1		45.4	37.9		11.4
	1-hexanol		3.7		6.6	17.8		2.1
	hexyl acetate		12.1		5.3	13.5		1.6
(*Z*)-3-hexenal[e]		4		20	4.0	6.1	200	14.8
	(*E*)-2-hexenal				10.0	10.7		24.6
	(*Z*)-3-hexenyl acetate				17.3	22.6		8.3
	(*E*)-2-hexenyl acetate		100		53.4	46.0		42.2
	hexyl acetate				15.3	14.5		10.2
(*E*,*E*)-2,4-hexadienal		2	9.4	10	13.8	17.7	100	84.5
	(*E*,*E*)-2,4-hexadien-1-ol		12.5		20.4			7.2
	(*E*,*E*)-2,4-hexadienyl acetate		78.1		65.7	82.3		8.3
			C_6 Alcohols and Ester					
1-hexanol		12	5.3	60	17.6	53.3	600	10.6
	hexyl acetate		94.7		82.4	46.7		89.4
(*E*)-2-hexen-1-ol		12	45.2	60	40.6	40.1	600	56.1
	(*E*)-2-hexenyl acetate		54.8		59.4	59.9		43.9
(*Z*)-3-hexen-1-ol		12	49.2	60	37.5	59.9	600	54.2
	(*Z*)-3-hexenyl acetate		50.8		62.5	40.1		45.8
hexyl acetate		2	91.7	10	93.2	93.4	100	88.9
	1-hexanol		8.3		6.8	6.6		11.1
			C_6 Ketones					
3-hexanone		2	100	10	100	100	100	100
(*E*)-4-hexen-3-one		2	43.0	10	8.3	10.0	100	51.2
	3-hexanone		57.0		91.7	90.0		48.8
			C_9 Aldehydes					
nonanal		6	86.0	30	63.9	42.8	300	79.0
	1-nonanol		14.0		36.1	57.2		21.0
(*E*)-2-nonenal		6	19.0	30	19.3	77.1	300	23.4
	(*E*)-2-nonenyl acetate		81.0		80.7	22.9		76.6
(*Z*)-6-nonenal		6	100	30	100	100	300	100
(*E*,*E*)-2,4-nonadienal		6	68.0	30	30.2	37.1	300	55.5
	(*E*,*E*)-2,4-nonadienyl acetate		32.0		69.8	62.9		44.5
(*E*),(*Z*)-2,6-nonadienal		6	52.6	30	64.0	48.2	300	31.9
	(*E*,*Z*)-2,6-nonadienyl acetate		47.4		36.0	51.8		68.1
			C_9 Ketones					
2-nonanone		6	93.8	30	92.0	84.9	300	92.8
	peak 2		6.2		5.2	10.9		4.3
	peak 3		0.0		2.7	4.1		2.9
(*E*)-3-nonen-2-one		6	25.4	30	34.0	22.9	300	37.4
	2-nonanone		74.7		66.0	77.4		62.6
			Miscellaneous					
2-carene		2	100	10	100	100	100	100
D-limonene		2	100	10	100	100	100	100
methyl benzoate		12	100	60	100	100	600	100
methyl salicylate		12	100	60	100	100	600	100

[a] None of the metabolic products (above 1%) was observed in the vapor phase of control jars without strawberry fruit except the (*Z*)-3-hexenal treatment which yielded 2.4% (*E*)-2-hexenal and hexyl acetate which contained 1.7% hexanol. Controls contained the midlevel quantities of the test compounds. [b] Identification based on MS and GC comparison with authentic standard except 2-nonenyl acetate and 2,4-nonadienyl acetate which were based on MS interpretation since no standard was available. [c] Amount of liquid test compound added to vial within jar at start of experiment. [d] Percent of test compound and metabolites in the vapor phase of a jar containing strawberry fruit after 2−3 days at 2−4 °C. [e] Fifty percent solution in triacetin.

a methyl ketone carbonyl was metabolized by strawberry fruit; however, there was no evidence that the ethyl ketone, 3-hexanone, yielded volatile products perhaps due to greater steric hindrance of the latter substrate. The results with the ketones demonstrate that strawberry fruit can readily reduce unsaturated carbon−carbon bonds adjacent to carbonyl groups. Also, the metabolism of ketone carbonyls was not as facile as was observed for aldehyde carbonyl groups, especially the six-carbon aldehydes studied.

The remaining compounds studied, such as the monoterpenes, Δ-2-carene, and D-limonene, did not yield major GC product peaks, indicating that these compounds were not metabolized by the strawberry fruit at the levels studied in the assay system. Likewise, the aromatic natural products, methyl benzoate and methyl salicylate, exhibited no discreet peaks representing volatile metabolic products.

Among the unusual yield results observed with the various test compounds was the greater extent of metabolite formation from the midrange levels than from the low or high levels of (*E*)-4-hexen-3-one and (*E*,*E*)-2,4-nonadienal. This suggests that the midlevel tested was closer to the optimum quantity for the given biochemical conversion than was either the low or high quantity tested. Also, there were apparent differences in metabolite yields for the cultivars Chandler and Tribute when 1-hexanol, (*E*,*E*)-2,4-hexadienal, and (*E*)-2-nonenal were tested. This might indicate different capacities for either transport or metabolism of volatile compounds.

Investigations by Yamashita et al. (1976) and Pesis and Avissar (1990) showed that shorter chain aldehydes, including acetaldehyde, and intermediate chain length saturated compounds, including hexanal, were reduced to their corresponding alcohols which were then esterified. Similar reactions occurred with apples exposed to atmospheres containing volatile compounds (DePooter et al., 1983; Bartley et al., 1985). The aldehydes were also oxidized to corresponding acids by apple fruit. The present studies show that strawberry fruit also has the capacity to metabolize long chain (nine-carbon) volatile compounds and unsaturated six-carbon and nine-carbon compounds, including compounds containing carbon−carbon double bonds adjacent to aldehyde and ketone carbonyls. These findings indicate that it might be possible to add volatile precursors of desirable aroma components of strawberry flavor to the atmosphere of strawberries in cold storage to enhance the flavor quality of the fruit prior to marketing.

The pathways active in the fruit resulting in the metabolites observed appear to involve three systems. The conversion of alcohols to the acetate products observed is due to an esterification system present in fruit that produces the various volatile esters necessary for the development of the characteristic aroma associated with fruit (Perez et al., 1993). The reduction of aliphatic aldehydes to their corresponding alcohols is likely accomplished through the action of alcohol dehydrogenase (Bartley and Hindley, 1980). This enzyme system produces ethanol from acetaldehyde when plant tissue is placed under anaerobic conditions. The third system resulting in the reduction of carbon−carbon double bonds adjacent to carbonyl groups is not apparent; however, the closest analogy seems to be the reduction of unsaturated acyl moieties during the chain elongation steps of fatty acid synthesis. Determination of the specific enzymes involved in the observed reactions will require further studies.

In summary, the present studies demonstrate that strawberry fruit has the capacity to remove volatile compounds from the atmosphere, metabolize these compounds, and subsequently return the volatile metabolites to the vapor phase. These processes are observed during refrigeration storage (2−4 °C). The three principal types of reactions observed with the fruit were esterification of alcohols, reduction of aliphatic aldehydes, including unsaturated compounds, to alcohols, and reduction of carbon−carbon double bonds adjacent to the carbonyl moiety in aldehydes and ketones. These reactions on exogenous volatile compounds may provide a method for augmenting the organoleptic quality of strawberry fruit.

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