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Sesquiterpene hydrocarbons in processed stored carrot sticks*

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The sesquiterpene hydrocarbon composition in processed, stored carrot sticks was studied as part of an investigation on the changes in secondary metabolites that occur during processing and storage of carrots. Carrot sticks were treated by infusion with antimicrobial compounds, antioxidants and cellular constituents and stored in plastic vacuum shrink bags at 2°C for up to three weeks. In each of the five treatments studied, the concentrations of the two major sesquiterpene hydrocarbons, caryophyllene and γ-bisabolene, increased markedly in the second week of storage while the concentrations of these two compounds in control samples decreased moderately during this same period. It appears that the infusion process must stimulate major changes in the sesquiterpene metabolism.

INTRODUCTION

Although cut carrot products are in demand, quality retention in storage is poor, relative to whole carrots (Bruemmer, 1987). Little is known about the effects of processing (such as slicing or storage in plastic bags) on the composition of secondary metabolites.

Compounds contributing to flavor or off-flavor in carrots have been summarized by Simon (1985). A pyrazine, 2-methoxy-3-*sec*-butylpyrazine, contributes a powerful basic carrot root-like aroma. Another important contributor is 6-methoxymellein (3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin) which adds a bitter off-flavor. Aside from these two compounds, various other substance classes such as terpenoids, aldehydes and ketones contribute less clearly defined elements to carrot flavor.

Processing methods may affect the levels of flavor and off-flavor compounds as well as terpenoids (Simon, 1985; Kaminski *et al.*, 1986). The latter group is particularly interesting, since it may contribute to pest resistance (Simon, 1982*b*) as well as flavor.

The composition of the terpenoid fraction has been determined (Heatherbell & Wrolstad, 1971; Simon *et al.*, 1980; Simon, 1982*a*; Senalik & Simon, 1987 and references therein). The major components of the sesquiterpene fraction are caryophyllene and γ-bisabolene (Buttery *et al.*, 1968; Seifert & Buttery, 1978): the former contributes a desirable 'perfumy' quality and the latter a harsh, 'terpeny' note to flavor. Various components of the monoterpene fraction also contribute similar perfumy or harsh attributes.

While there have been no reports on the effects of slicing or storage in plastic bags on sesquiterpenes, several groups have shown that this type of processing can produce significant changes in some of the other important constituents. A study of acetaldehyde and ethanol in carrots stored in plastic bags at low temperature demonstrated an increase in the concentrations of both with storage time. Although either senescence or anaerobic growth of carrot tissue could be responsible for the increase, the data could not distinguish between the two (Heatherbell & Wrolstad, 1971). Another study of proteins in stored carrots prepared by a processing and storage technique very similar to the method employed in this study showed significant changes in concentration and molecular weight distribution with storage time (Bruemmer, 1988).

The approach in this paper is based on an independent study of the processing and storage effects. Control samples of whole carrots or untreated sticks were prepared as well as five samples of sticks treated by vacuum infusion with various antimicrobials, antioxidants and cellular constituents. All of the samples were packed in oxygen transmissible bags and stored at

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2°C. Changes in the sesquiterpene hydrocarbon concentration were determined and correlations noted between these changes and processing or storage variables.

MATERIALS AND METHODS

General methods

Solutions were concentrated by evaporation in a rotary evaporator at 40–50°C at 40 torr. Storage and handling of concentrated solutions were carried out in a nitrogen atmosphere. All filtrations employed a coarse sintered glass filter. All solvents were HPLC grade. Methyl-*t*-butyl ether (MTBE) was HPLC grade, and was free of peroxides according to a KI test.

Sample preparation

'Imperator' carrots from Zellwood, Florida, were sanitized by immersion in sodium hypochlorite, peeled, cut into sticks, treated with various solutes by vacuum infusion, shrink packed in oxygen transmissible bags, and stored at 2°C as previously described (Bruemmer, 1987). The bags were Cryovac E (20 cm × 40 cm, 2 mm thick; O_2 transmission: 4000 cm^3 per m^2 at 20°C, 1 atm and 24 h). The following solutes were employed for the infusion treatments: 0·01% citric acid + 0·01% ascorbic acid, 0·002% $CaCl_2$, 0·7% glucose, 0·001% sodium pyruvate and 0·02% lecithin. The control samples consisted of whole carrots or carrot sticks that were prepared by the process described above, with omission of the infusion step.

Extraction and column chromatography

The extraction and preliminary column chromatographic separation procedure has been described previously (Lund & White, 1986, 1990; Lund & Bruemmer, 1991). Samples (400 g) were cut into approximately 1–2 cm pieces and extracted with MTBE containing 1 mg BHT/liter (800 ml). The MTBE extract was concentrated and separated into hexane and MTBE soluble fractions. The hexane soluble fraction was separated on a column of Cyanopropyl Sepralyte (Analytichem International, Harbor City, CA), a surface-modified silica gel containing covalently bonded cyanopropyl groups. The column was eluted with a mixture of hexane and MTBE. A linear concentration gradient was employed. The initial composition was 100% hexane for the hexane extract and 90% hexane–10% MTBE for the MTBE extract; the final value for both extracts was 20% hexane–80% MTBE. Eluting compounds were detected with a UV detector (211 nm). Each hexane soluble fraction was divided into two or three portions and the combined peaks collected from separate column runs on each portion were analysed by GC. Only the initial large double peak that contained the nonpolar hydrocarbon fraction was analyzed.

GC separation and analysis

Initial separation was carried out by injection of the crude column fraction on a stabilized DEGS column (modified diethylene glycol succinate, Analabs SLP 027, Analabs, Inc., New Haven, CT). The instrument was equipped with a modified injection port containing a removable Teflon-coated stainless steel liner. Fractions from the DEGS column were then separated on Carbowax columns: samples for further characterization were isolated from a packed column (liquid phase Carbowax HP; Chrompack, Middleburg, The Netherlands) and quantitative analyses were conducted on a DB Wax fused silica capillary column (J and W Scientific, Rancho Cordova, CA). The DEGS column was 3 m long and contained 18% liquid phase; the Carbowax packed column was 3 m long and contained 15% liquid phase. The capillary column was 30 m long and was coated with a 0·25 μm film. The two packed columns were ¼ in ID Teflon-lined stainless steel, and the collection port system was constructed of glass-lined steel. The capillary was fused silica, 0·32 mm ID. The stationary phase for the packed columns was 60/80 Gas-Pack FS (Chemical Research Services, Inc., Addison, IL). Conditions for the packed column separations were as follows: carrier gas flow rate (He) 140 ml/min; injection port *T*, 230°C; detector *T*, 240°C; column *T*, 80–220°C @ 2°C/min. Capillary column conditions were: carrier gas flow rate (H_2) 2 ml/min; injection port *T*, 220°C; detector *T*, 240°C; column *T*, 40–180°C @ 40°C/min. The capillary injection port was a Grob splitless type.

Compounds were characterized by comparison of retention times (RT), mass spectra (MS) and infrared spectra (IR) with those of known standards. Retention times and IR spectra were determined with commercial samples of caryophyllene and α-humulene (Nos 22075 and 53675, respectively; Fluka Chemical Co, Ronkonkoma, NY) and with samples of *t*-β-farnesene, β-bisabolene and γ (or α)-bisabolene synthesized from farnesol according to a published procedure (Moshonas & Shaw, 1980). The mass spectra were obtained by injection of individual DEGS column fractions on a GC–MS instrument equipped with a Carbowax capillary column (University of Florida, Gainesville, FL).

For quantitative analysis, an external standard of caryophyllene (Fluka 22075, approximately 99% pure by GC) was injected and the caryophyllene peak areas compared with those of individual compound peaks from both DEGS and Carbowax columns.

RESULTS AND DISCUSSION

Analysis of the sesquiterpene hydrocarbons in carrot sticks processed by five different infusion treatments and stored for one to three weeks showed that significant concentration changes occurred during storage.

Tables 1 and 2 show a list of the seven sesquiterpene hydrocarbons identified and their mass spectral data. Although monoterpene hydrocarbons were also present in this fraction, a large portion were lost because of their relative volatility. Due to the poor recovery, identification or quantitative analysis of monoterpenes was not attempted. The relative retention properties of the sesquiterpene hydrocarbons on DEGS and Carbowax are shown, and in the last column of Table 1 the method for identification is listed. The 'γ-bisabolene' peak was a large, single, symmetrical peak that eluted from the DEGS column near the end of the sesquiterpene area. Reinjection of this peak on the Carbowax column showed only the two α-curcumenes (A and B) together with a group of very small artifact peaks. Since the apparent instability of γ-bisabolene on the DEGS column prevented the comparison of IR or MS data, identification was based solely on the identity of retention times with those of the known γ-bisabolene sample. Attempts at isolation of the known bisabolene on DEGS also demonstrated that it was unstable on this column. It was concluded that the authors' sample, in common with all of the many previously reported carrot sesquiterpene hydrocarbon samples, contained γ-bisabolene as a major component, even though the authors were unable to confirm this by infrared or mass spectral comparison. The two compounds designated α-curcumene A and α-curcumene B had nearly identical mass spectra (Table 2) that closely matched the standard α-curcumene spectrum. Lack of sample prevented comparison of IR and retention time data. Although the difference between the structures of the two is not known, they must be very closely related.

Table 1. Sesquiterpene hydrocarbons in stored carrots

Compound	Retention time (min)		Identification method
	DEGS	Carbowax	
Caryophyllene	17·2	24·4	MS,IR,RT
t-β-Farnesene	19·0	27·6	MS,IR,RT
α-Humulene	20·7	28·0	MS,IR,RT
β-Bisabolene	21·5	31·4	MS,IR,RT
γ-Bisabolene[a]	23·2	32·8	RT
α-Curcumene (A)	23·2	32·8	MS
α-Curcumene (B)	23·2	33·9	MS

[a] Decomposed on DEGS column.

Table 2. Mass spectral data for sesquiterpene hydrocarbons

Compound	*m/z* (%)
Caryophyllene	39(17), 41(53); 53(17), 55(28); 67(27), 69(73); 91(61), 93(100); 105(48), 107(43); 119(52), 120(44); 133(84), 134(24); 147(32), 148(31); 161(42), 162(10); 175(13), 176(7); 189(23), 190(3); *204*(13)
t-β-Farnesene	41(49), 42(2); 53(8), 55(13); 67(18), 69(100), 79(18); 81(19); 91(14), 93(61); 107(8), 109(7); 119(10), 120(22), 133(33), 134(7); 147(3), 148(3); 161(20), 162(3); 175(2); 189(2); *204*(7)
α-Humulene	41(14), 43(7); 53(7), 55(8); 65(2), 67(10); 79(13), 80(33); 92(18), 93(100); 107(17), 109(11); 119(9), 121(7); 133(3), 136(3), 147(27), 148(5); 161(7), 162(2); 189(3); *204*(11)
β-Bisabolene	39(7), 41(52); 53(8), 55(12); 67(31), 69(100); 77(13), 79(30); 93(90), 94(37); 107(27), 109(33); 120(8), 121(18); 133(12), 135(12); 147(7), 148(5); 161(32), 162(5); 175(2), 189(9), 190(2); *204*(32)
α-Curcumene (A)	39(3), 41(17); 53(2), 55(14); 65(3), 69(10); 73(3), 77(7); 83(10); 91(18), 95(7); 105(41), 106(3); 119(83), 120(22); 131(29), 132(100); 145(27), 146(4); 159(5), 187(2); *202*(34)
α-Curcumene (B)	41(7), 43(7); 53(2), 55(7); 65(2), 68(1); 73(7), 77(2); 83(5); 84(1); 91(10), 92(3); 103(1), 105(14); 117(15), 119(25); 131(37), 132(100); 145(29), 146(6); 157(1), 159(3); 187(2); *202*(27)

While the initial concentrations of the two major sesquiterpene hydrocarbons, caryophyllene and γ-bisabolene, were not correlated with processing variables, there was a general increase in these two compounds in all of the infused samples during the second week of storage.

In Figs 1 and 2, the concentrations of caryophyllene and γ-bisabolene in the five infusion treated samples are plotted as a function of storage time. Since zero time values were not available, the initial time for these curves was one week. Experimental error for the concentration values is estimated at 15% of standard

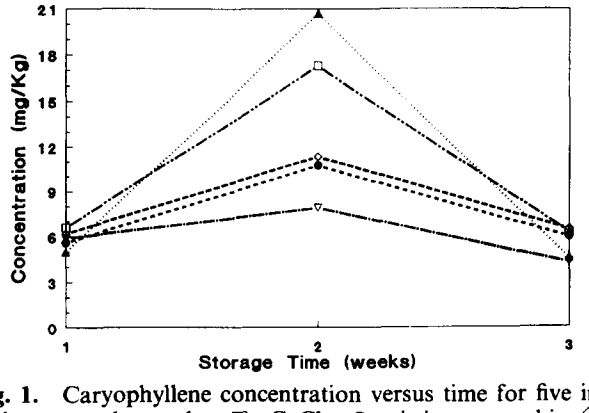

Fig. 1. Caryophyllene concentration versus time for five infusion treated samples. □, $CaCl_2$; ●, citric + ascorbic; ◇, dextrose; ▲, sodium pyruvate; ▽, lecithin.

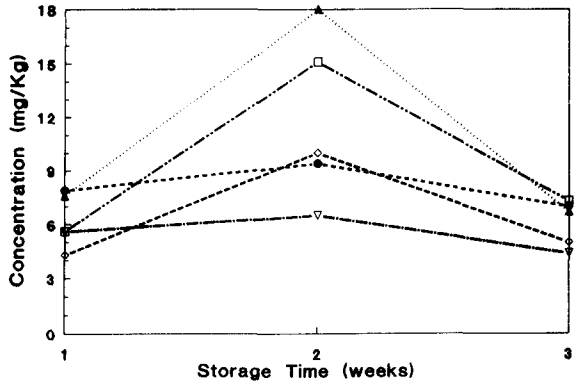

Fig. 2. 'γ-Bisabolene' concentration versus time for five infusion treated samples. □, $CaCl_2$; ●, citric + ascorbic; ◇, dextrose; ▲, sodium pyruvate; ▽, lecithin.

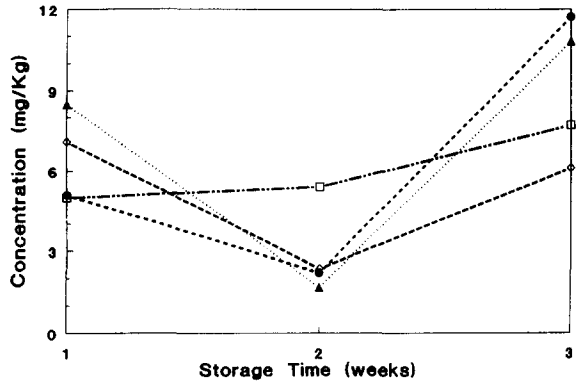

Fig. 3. Caryophyllene and 'γ-bisabolene' concentration versus time for two control samples. □, Caryophyllene, control sticks; ●, caryophyllene, whole treatment; ◇, γ-bisabolene, control sticks; ▲, γ-bisabolene, whole treatment.

deviation. In each of the five samples, there is a similar increase in concentration of both sesquiterpenes during the second week with the $CaCl_2$ and pyruvate-treated samples showing the greatest change. In contrast, most of the control samples showed a decline in the second week (Fig. 3). The treated samples apparently are all affected similarly, such that the increase in concentration overcame the decline that would normally occur.

Concentrations of the remaining five minor sesquiterpene hydrocarbons were not significantly correlated with processing treatment or storage time (Table 3). Since they are much less concentrated (maximum concentration 1·3 mg/kg), quantitative determination was more difficult and regularities could not be distinguished as readily.

In general, infusion treatment seems to alter the metabolic pathway leading to sesquiterpenes in the same way that chemical stress can stimulate the production of phytoalexins (Bailey, 1982). Since the solute penetrates mainly into the phloem area where the terpenoids are localized (Senalik & Simon, 1987), there is an optimum condition for chemical effects on metabolic intermediates. Although the more polar sesquiterpenes were not determined, it is possible that synthesis of biologically active compounds of the oxygenated

Table 3. Minor sesquiterpene hydrocarbons: concentration in stored carrots

Compound (time in weeks)	Concentration (mg/kg)						
	Control		Treated				
	Whole	Sticks	$CaCl_2$	Citric-ascorbic	Dextrose	Sodium pyruvate	Lecithin
t-β-Farnesene							
W1	0·480	0·120	0·143	0·330	0·299	0·131	0·238
W2	0·104	0·163	0·350	0·0157	0·079	0·076	0·057
W3	0·670	0·560	0·330	0·460	0·214	0·058	0
α-Humulene							
W1	1·060	0	0·840	0·620	0·400	0·0286	0·218
W2	0·293	0·069	0·690	0·055	0·470	0·232	0·106
W3	0·360	1·580	1·280	0·043	0·630	0·0195	0·207
β-Bisabolene							
W1	0·274	0	0·570	0·159	0·110	0·104	0·055
W2	0·138	0·082	0·189	0·043	0·071	0·128	0·113
W3	0·330	1·070	0·400	0·086	0·110	0·043	0·030
α-Curcumene (A)							
W1	0·032	0	0·091	0·183	0·043	0·0138	0·052
W2	0·0165	0·043	0·0244	0	0	0·0168	0
W3	0·051	0·179	0·035	0·183	0·103	0·0093	0·089
α-Curcumene (B)							
W1	0·0201	0·0189	0·046	0·0298	0·055	0·072	0·034
W2	0·045	0·063	0·071	0·048	0·056	0·082	0·025
W3	0·105	0·170	0·287	0·051	0·093	0·041	0·089

sesquiterpenoid type may accompany the greatly increased synthesis of the hydrocarbons (almost threefold for some treatments).

Since there were no significant flavor changes during the second week of storage (Bruemmer, 1987), any flavor improvement produced by the increase in caryophyllene (pleasant, aromatic odor) was probably offset by the negative effect of the bisabolene (unpleasant, harsh odor). Although the more volatile monoterpene hydrocarbons were not determined, changes in this group are also likely to affect the flavor.

It can be concluded that treatment of lightly processed carrot sticks by infusion of preservative chemicals followed by storage in plastic bags at reduced temperature produces a significant increase in the concentration of the two major sesquiterpene hydrocarbons during the second week of storage. The effect of this change in flavor is minimal, but there may be some alteration in the profile of stress-related protective components.

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