Gas Chromatography–Olfactometry of Orange Juice To Assess the Effects of Plastic Polymers on Aroma Character[†]

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Changes in orange juice composition in a model system simulating Brik-Pak aseptic packaging composed of two polymers, LDPE and Surlyn, were measured. GC-FID was used to measure the amount of d-limonene in the juice, while gas chromatography–olfactometry (GCO) was used to measure the odor-active volatiles in orange juice. More than 70% of the limonene was absorbed by the polymers in 24 h at 25 °C. However, results from the GCO analysis indicated that limonene had only trace odor activity. Furthermore, the plastic polymers tested did not substantially alter the odor-active components detected in orange juice. GCO was also used to compare the aroma components of pasteurized, mechanically squeezed orange juice (MS), unexposed to plastics, and fresh orange juice hand squeezed (HS) from oranges of the same lot. Although the overall aroma intensity of both juices was the same, large changes in odor activity were observed for several key aroma constituents.

INTRODUCTION

Single-strength, "not from concentrate", orange juice packed in glass containers offers better product quality than frozen concentrated juice but at higher container and shipping costs (Varsel, 1980). Since there has been a growing demand for this type of orange juice, producers have turned to lighter weight, less expensive packaging systems to meet public demand.

A popular alternative to glass packaging for juice is the Brik-Pak: a six or seven layer laminate pouch of polyolefins, paper, paperboard, and aluminum foil. Although this packing system offers many economic advantages over glass, absorption of flavor by the polymeric packaging materials has been identified as a potential problem (Mannheim et al., 1985; Shimoda et al., 1984). For example, Shimoda et al. (1984) reported the loss of volatiles from mandarin orange juice held in plastic pouches. They found that nonpolar constituents were absorbed more than polar ones. Mannheim et al. (1985) documented changes in aseptically packaged citrus juices by sensory as well as chemical analysis. They related loss of orange juice flavor to the absorption of d-limonene, a major orange juice volatile, into the polyolefin packaging. Kwapong (1986) examined the rates of absorption of limonene and other volatile compounds from pure standards and from orange oil into plastic polymers. Kwapong and Hotchkiss (1987) confirmed Shimoda's findings that nonpolar compounds, such as limonene, are most readily absorbed. Researchers in Japan (Dainippon Printing Co., Ltd., 1984) reported good orange juice flavor retention when juice was stored in a special polyolefin laminate of a thermoplastic film

(CPP) with a gas barrier (Al) and a LDPE film containing 1% limonene as the inner layer. Although the rate of limonene absorption by polymers can be suppressed by components in the juice such as orange oil (Kwapong, 1986; Kwapong and Hotchkiss, 1987), juice pulp (Shimoda, 1984), cocoa butter, or coconut oil (Fustier and Dale, 1985), it is not clear what role absorption plays in aroma retention. However, in a comparison of the sensory quality of orange juice stored in glass with juice stored in polyethylene-lined paperboard cartons at different temperatures, Duerr et al. (1981b) reported temperature to be the main variable affecting flavor. They proposed that limonene absorption was an advantage since limonene was known to be a precursor to off-flavor compounds. Furthermore, they reported that desirable aroma volatiles were practically unabsorbed.

Although it is well documented that certain orange juice constituents are absorbed by polymeric packaging materials (Imai et al., 1990) and that flavor changes take place in packages composed of these materials (Mannheim et al., 1985), it is not clear that these changes are caused by absorption of odor-active orange juice constituents. With this in mind, we exposed orange juice to plastic packaging materials in a model system similar to that used by Kwapong (1986) and measured the changes in the limonene content using GC-FID and the components with the most aroma using GCO. In addition, we used GCO to compare the aroma compositions of pasteurized, mechanically squeezed (MS) orange juice, unexposed to plastics, and fresh orange juice (HS) hand squeezed from oranges of the same lot used for the MS juice.

MATERIALS AND METHODS

Orange Juice. Valencia oranges harvested at the Florida Department of Citrus were extracted in an FMC extractor, pasteurized at 92 °C in an APV plate-type heat exchanger 1 day after harvest, and sent to Geneva, NY, by overnight express along with fresh oranges from the same lot. Upon arrival, the fresh oranges were sliced in half, hand squeezed into juice (HS) using a domestic juicer (KitchenAid Model K45), and immediately extracted serially with two solvents, Freon 113 (1,1,2-trichloro-1,2,2-trifluoroethane) Precision cleaning agent (E. I. du Pont, Inc., Buffalo Solvent and Chemical Corp., Buffalo NY) and ethyl

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acetate (Burdick & Jackson, spectrograde), following the method of Braell (1986). The pasteurized mechanically squeezed orange juice (MS) was extracted similarly. Other samples of pasteurized MS orange juice were stored in polymeric extraction cells in a shaking incubator maintained at 25 °C and 150 rpm for 24 h and extracted after exposure to the plastics.

Polymeric Extraction Cells. Extraction cells designed to model juice exposure to plastic packaging in a Brik-Pak container were prepared using the design of Kwapong (1986). Two commercial food grade resins were tested: (1) LDPE 4516 resin beads from the Gulf Oil Co., Houston, TX; (2) Surlyn 1652 resin beads from the Du Pont Co., Wilmington, DE. Resin beads were stored at room temperature in glass jars sealed with foil-lined screw caps until pressed into disks. Plastic disks were prepared by placing 12 g of resin beads between two clean sheets of Mylar in a hot Loomis press at 119 °C at 22 000–25 000 psi pressure for 2 min. Molton sheets were immediately quenched by immersion in ice water. Then a number of small uniformly thick disks were cut with a cork borer from the larger sheets. These disks were 3.5 cm in diameter and 0.65 mm thick. Each disk has a surface area of 19.2 cm^2 and weighs approximately 0.3 g. The disks were rinsed with acetone, dried to ensure they were free of contaminating odors, and handled with forceps to minimize contamination. The thickness of all disks was determined and the combined weight of eight disks recorded.

Eight disks were then placed on an 8.6 cm long × 1.6 mm stainless steel rod at 0.5-cm intervals. The disk and rod assemblies were placed in a 125-mL screw-top glass jar, and 125 mL of the processed juice brought to 25 °C was added to each sample cell. Sample cells were closed with a Teflon-lined screw up. Control cells were identically prepared but without plastic disks. Samples were immediately placed in a shaking incubator maintained at 25 °C and 150 rpm. Control and treatment samples were removed from the shaker for analysis at 3-h intervals for the first 12 h and then at 12-h intervals up to 64 h for GC-FID and gas chromatography–mass spectrometry (GC–MS) analysis.

Extraction of Orange Juice Volatiles. The extraction-concentration method used for all juice samples for GCO and GC-FID analyses was developed for the analysis of grape juice by Braell (1986) and is similar to that used by Schreier (1981) to examine the volatile constituents of fresh and processed orange juice. Each orange juice sample (125 mL) was combined with 12.5 mL of methanol (Burdick & Jackson, spectrograde) and extracted with 82.5 mL of freon. This was followed by extraction with 82.5 mL of ethyl acetate. After extraction, the freon fraction was brought to a constant volume of 85 mL and divided into two portions: 5 mL was spiked with an internal standard for 110 μg of *n*-tridecane for the limonene quantification by GC-FID, and the remaining 80 mL was used for GCO. The ethyl acetate extract was dried by filtration through a Whatman 2V filter paper containing 2 g of MgSO$_4$ and then brought to a volume of 37 mL and analyzed by GCO.

GC-FID. Gas chromatography was performed using either a Hewlett-Packard 5840 or 5890 gas chromatograph equipped with a 0.32 mm i.d. × 12 m fused silica capillary column coated with methyl silicon (OV-101). Both GCs were equipped with dual flame ionization detectors, one of which was modified for GCO. They were operated with the injection port at 250 °C, the detector at 250 °C, and the column heated isothermally at 35 °C for 3 min and then programmed at 6 °C/min to 185 °C. Limonene concentration was determined in the freon extracts of treatment and control samples by comparison of their FID peak areas with that of the internal standard.

GCO. Gas chromatography–olfactometry was conducted using the method called CharmAnalysis (Acree et al., 1984; Cunningham et al., 1986). The GC was adapted for use with either an FID or an olfactometer at its output. The FID mode was used to determine the retention times of the *n*-paraffins used to index the retention times measured in the olfactometry runs. Normal hydrocarbons from heptane to octadecane were chromatographed daily with the GC in the GC-FID mode and their retention times used to convert GCO retention values to retention indices (Kováts, 1958). To preserve chromatographic resolution in the olfactometer mode, helium carrier gas flowing at 25 cm/s from the column was diluted in two stages, first with 60 mL/min helium and then with humidified air at 20 L/min (Acree et al., 1984). In
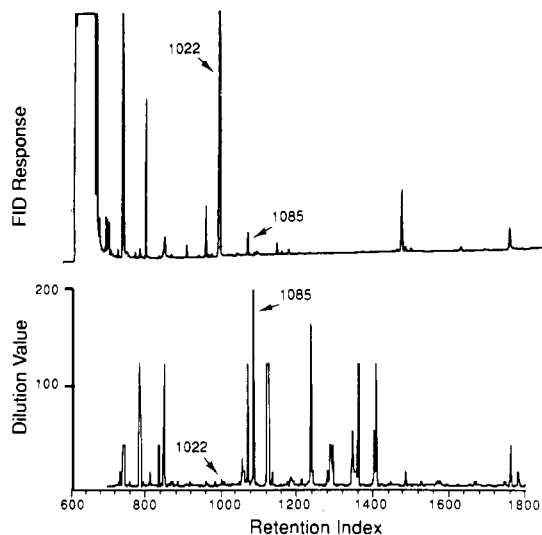

Figure 1. Comparison of GC-FID and GCO response to combined extracts of mechanically squeezed, processed orange juice unexposed to plastics.
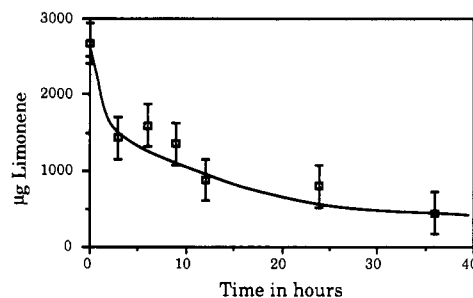

Figure 2. Limonene concentration (milligrams) in mechanically squeezed, processed orange juice exposed to Surlyn in a model system vs time of exposure in hours.

this way, the linear velocity at each stage was equal to or greater than the carrier gas velocity.

Data produced by CharmAnalysis was displayed as a charm chromatogram with retention index (RI) on the horizontal axis and dilution values (Schieberle and Grosch, 1987) on the vertical axis. Dilution values can be thought of as odorant titer or the number of dilutions to threshold for the subject monitoring the odors from the GCO. Dilution values were determined in the following way. Volatiles eluted from the GCO were monitored for odor activity at the olfactometer output over the retention time of heptane to that of octadecane. Responses to odors were recorded on an Apple Macintosh using a Charmware program that eventually combined data from seven GCO runs of 3-fold serial dilutions of each orange juice extract to produce a charm chromatogram. All GCO samples were analyzed by a single subject selected for olfactory sensitivity and trained for GCO analysis with a procedure and standard compound mixture specifically designed for GCO subject selection (Marin et al., 1988). Composite charm chromatograms of the freon and ethyl acetate extracts were produced for each juice sample and the peaks for each odor were integrated to give charm values. Charm values are the areas under the peaks in the charm chromatogram and are proportional to the amount of odor-active compound in the most concentrated sample and inversely proportional to the subject's threshold for that compound. Thus, charm values obtained for each odor from the different charm chromatograms for each orange juice were then compared.

GC-MS. Mass spectra were obtained on a Hewlett-Packard 5885 equipped with Helwett-Packard 5840 GC containing the same type of column used in the GCO studies but which was twice as long (25 m) and operated at 35 °C isothermally for 6 min and then programed at 4 °C/min to 225 °C. Spectra were associated with retention indices linearly interpolated between pairs of normal paraffins from heptane to octadecane. The presence of limonene at retention index 1022 was determined by

Table I. Charm Values for 15 Major Aroma Components of Mechanically Squeezed, Processed Orange Juice Unexposed (Control) or Exposed to Plastics (LDPE, Surlyn) for 24 h in a Model System[a]

	charm			charm ratio		MDR(n)		
RI	control	LDPE	Surlyn	LDPE/control	Sur/control	LDPE/control	Sur/control	compd
1237	1103	266	212	0.24	0.19	b	0.19	citral
1085	554	465	250	0.84	0.45	b	b	linalool
1282	439	282	364	0.64	0.83	b	b	
1350	393	86	271	0.22	0.69	b	b	vanillin
1344	391	71	49	0.18	0.13	0.18	0.13	
1289	150	74	198	0.49	1.32	b	b	
1402	139	66	91	0.47	0.65	b	b	
1095	139	101	197	0.73	1.42	b	b	
1358	127	194	52	1.53	0.41	b	b	
837	108	133	79	1.23	0.73	b	b	ethyl 2-methylbutyrate
1124	78	96	165	1.23	2.12	b	b	
785	77	57	188	0.74	2.44	b	b	ethyl butyrate
1072	43	77	77	1.79	1.79	b	b	
1408	41	316	350	7.71	8.54	7.71	8.54	
1062	19	104	106	5.47	5.58	5.47	5.58	
1022	7	2	1	1				limonene
total charm	3808	2390	2650	0.63	0.70	b	b	

[a] Comparisons are between treated and control samples assessed by their most divergent ratio [MDR(n)]. MDR(n) is a charm ratio $>n$ or $<1/n$. $n = 5$. [b] MDR(n) <5 or $>1/5$.

GC-MS on a HP 5985 GC-MS equipped with the same column used for GC-FID and GCO analysis.

RESULTS

Aroma Volatiles of MS Orange Juice. Figure 1 compares a chromatogram produced by GC-FID with a composite charm chromatogram produced by GCO of the freon and ethyl acetate extracts for the MS orange juice unexposed to plastics. The horizontal axis, retention index (RI), is the same in both chromatograms, but the vertical axes are different. FID response is a measure of relative mass, and FID chromatogram peak areas indicate the amount of volatile component present. In GCO, the dilution value is the number of dilutions a sample extract must receive to reach a subject's threshold and peak areas in a charm chromatogram are charm values which are equal to the mass of the odorant in the extract divided by the odor detection threshold for the odor and the subject.

The charm chromatogram shows that the most intense aroma in the MS juice was at RI 1085 and was due to linalool. An authentic standard of linalool produced the same retention index and odor during GCO. The presence of linalool in the juice was confirmed by comparison of its mass spectrum with that of the standard. Similarly, the FID response for the compound at RI 1022, limonene, is large since it is the most abundant volatile in the orange juice extract. The presence of limonene in the juice was also confirmed by comparison of its retention index and mass spectrum with that of a standard. However, limonene produced almost no charm response, indicating it has trace impact on orange juice aroma.

Limonene Absorption by Plastics. Figure 2 shows a plot of limonene concentration in orange juice exposed to Surlyn vs time. A first-order equilibrium kinetic model was fitted to the limonene data, and equilibrium was approximated at 24 h with 70% of the limonene absorbed by the Surlyn. Similar results were obtained for LDPE, with 80% of the limonene absorbed by the polymer at 24 h.

Effects of Plastic Exposure on MS Orange Juice Aroma. The effects of plastic exposure on the MS juice aroma were determined by comparing the charm response chromatograms for the 24-h control samples with those of the 24-h treated samples. Results are summarized in Table I, which gives the charm values for the 15 most odor-active compounds of the control and treated MS orange

juice samples. The results in Table I are presented in order of decreasing charm response in the control juice. Comparisons between the treated and control samples were assessed by computing their most divergent ratio MDR-(n). MDR(n) was defined here as a charm ratio [charm (treatment)/charm (control)] $>n$ or $<1/n$. If Weber's law to discriminate perceived odor intensity is applied to selecting the value for n for the MDR(n), then an approximate 3-fold change, or $n = 3$, is necessary for a just noticeable difference (JND) for odors (Engen, 1982). For these comparisons, using a value of $n = 5$, a value conservative relative to the Weber fraction, demonstrated few changes in the orange juice aroma as a result of exposure to the plastics. Decrease in orange juice aroma due to the plastic exposure was shown only for two compounds: the odor at RI 1237 for Surlyn and the odor at RI 1344 for both plastics. An increase in aroma due to the plastic exposure was shown for two compounds as well for both plastics: the odors at RI 1408 and 1062. Note that the odors at these retention indices have very low charm values relative to other components of the juice and so might be considered as having little impact on the overall juice aroma. The phenomenon of aroma increase at RI 1408 and 1062 may be due to migration of contaminating odor components from the plastic to the juice. This possibility could be tested by studying the qualititative changes in the juice to determine if the odorants at the same retention index in the control and treated juices were described as the same odor and due to the same compound.

The overall change in the odor intensity of the juice due to plastic exposure was assessed by computing the MDR-(n) for the sum of the 15 charm values, or total charm ratio. The MDR(n) values for both plastics show that the decrease in overall aroma intensity would be low: 37% loss for LDPE and 30% for Surlyn. When compared with the Weber fraction of a 30% change for a JND, the loss in orange juice aroma intensity due to plastic exposure would be barely noticeable.

Comparison of Fresh Hand Extracted and Pasteurized Mechanically Extracted Orange Juice Aroma. Table II gives the charm values for the 15 most odor-active compounds of the MS and fresh HS juice presented in order of decreasing charm response in the HS juice. Comparisons between the HS and MS samples were assessed by computing their most divergent ratio

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Table II. Charm Values for 15 Major Aroma Components of Mechanically Squeezed, Processed Orange Juice (MS) and Hand Squeezed Orange Juice (HS)[a]

| | charm | | charm ratio, | MDR(n), | |
RI	MS	HS	HS/MS	HS/MS	compd
1124	202	421	2.08	b	
1344	38	419	11.0	11.03	
1057	24	396	16.5	16.50	
1282	70	334	4.77	b	
1289	128	189	1.48	b	
837	92	184	2.00	b	ethyl 2-methyl-butyrate
1350	161	178	1.11	b	vanillin
1062	97	169	1.74	b	
1072	88	166	1.89	b	
785	332	152	0.46	b	ethyl butyrate
1403	101	129	1.28	b	
1237	408	104	0.25	b	citral
1408	143	43	0.30	b	
1358	298	8	0.03	0.03	
1085	792	1	0.00	0.00	linalool
1022	1	1	1.00	b	limonene
total charm	2976	2895	1.01	b	

[a] Comparison is between samples assessed by their most divergent ratio [MDR(n)]. MDR(n) is a charm ratio $>n$ or $<1/n$. $n = 5$. [b] MDR(n) <5 or $>1/5$.

MDR(n) as previously described. The compounds at RI 1057 and 1344, which are among the most odor-active components in the HS juice, seem to be destroyed or diminished by pasteurization or the mechanical extraction process, as is evident by their large MDR(n) values. The compounds at RI 1085 and 1358 are not present in the HS juice but appear as a result of processing. This is not surprising since the introduction of peel oil constituents such as linalool, citral, and limonene during juice manufacture is a well-documented phenomenon (Nisperos-Carriedo and Shaw, 1990). The odor-active compound at RI 1085 was identified as linalool by comparison of its odor using GCO and spectrum and retention index using GC-MS with that of an authentic standard. The compound at RI 1350 [m/z = 151 (100%), 152 (88%), 81 (56%), 109 (25%), 123 (23%), 65 (19%), 95 (12%), 137 (6%)] was similarly identified for the first time in orange juice as vanillin. Note that the MDR(n) values for the compounds at RI 1085, 1358, 1344, and 1057 are not only greater than the conservative estimate of the Weber fraction, $n = 5$, but indicate greater than a 10-fold change in odor activity. Figure 3 illustrates the divergence in charm values for the HS and MS juice for the four most prominent odors in the HS and the four most prominent odors in the MS juice. Also, the trace odor response to limonene in both samples is shown.

The overall odor intensity of the HS juice as compared to the MS juice was assessed by computing the MDR(n) for the sum of the 15 charm values, or total charm ratio. The total charm values and the MDR(n) value show that the overall aroma intensity is the same for both HS and MS juice.

Conclusions. Although analysis by GC-FID showed that 70–80% of the limonene in orange juice was absorbed by LDPE and Surlyn in a model system simulating plastic exposure for a Brik-Pak container, analysis by GCO showed that limonene produced only trace odor activity. This finding agrees with a similar report by Sizer et al. (1988) regarding the flavor assessment of limonene in orange oil: "The major components of orange oil—the hydrocarbons, such as d-limonene—are not terribly important for the orange flavor, and, on the other hand, are precursors of the off-flavor component α-terpineol". Therefore, ab-
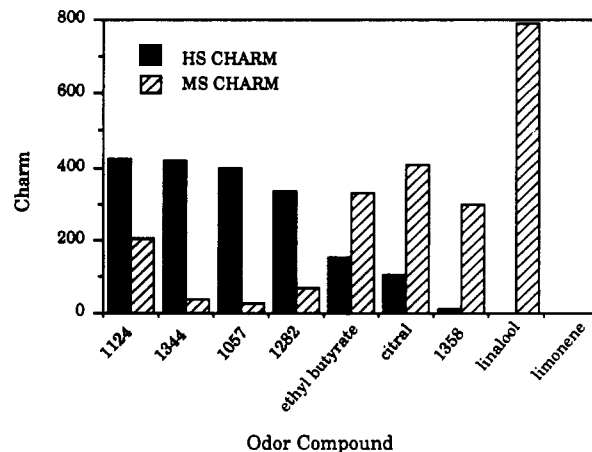

Figure 3. Comparison of charm values for selected aroma components in hand squeezed (HS) and mechanically squeezed (MS) orange juice.

sorption of limonene by container plastics is unlikely to impact the aroma of orange juice directly. Indeed, its loss may minimize the formation of off-aromas as proposed by Duerr et al. (1981a). Furthermore, results obtained from GCO showed that exposure of LDPE or Surlyn did not change the odor activity of the aroma components of MS orange juice after 24 h or perceptibly decrease the overall aroma intensity. However, even though the same overall odor intensity was observed for HS and MS orange juice, large changes in odor activity were observed for several key aroma constituents. Compounds with potent aromas (high charm response) at RI 1057 and 1344 in HS juice were absent or diminished in the MS juice, while the component linalool at RI 1085 and an unknown odor at RI 1358 were absent or barely detected in the HS juice but important to the aroma of the MS juice. Nisperos-Carriedo and Shaw (1990) also demonstrated a similar divergence between MS and HS juice levels of limonene and linalool.

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