

Identification of Sulfur Volatiles in Canned Orange Juices Lacking Orange Flavor

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The purpose of this study was to understand why some canned orange juices are not perceived as orange juice. Sensory flavor profile data indicated that the primary odor (orthonasal) attributes were tropical fruit/grapefruit, cooked/caramel, musty, and medicine. By comparison fresh-squeezed juice lacked these odor attributes. GC-O analysis found 43 odor-active components in canned juices. Eight of these aroma volatiles were sulfur based. Four of the 12 most intense aroma peaks were sulfur compounds that included methanethiol, 1-*p*-menth-1-ene-8-thiol, 2-methyl-3-furanthiol, and dimethyl trisulfide. The other most intense odorants included 7-methyl-3-methylene-1,6-octadiene (myrcene), octanal, 2-methoxyphenol (guaiacol), 2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone (homofuraneol), (*E*)-non-2-enal, (*E,E*)-deca-2,4-dienal, 4-hydroxy-3-methoxybenzaldehyde (vanillin), and α -sinensal. Odorants probably responsible for the undesirable sensory attributes included grapefruit (1-*p*-menth-1-ene-8-thiol), cooked [2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (Furaneol), and 3-(methylthio)propanal (methional)], musty [7-methyl-3-methylene-1,6-octadiene and (*E*)-non-2-enal], and medicine (2-methoxyphenol). The canned juices also lacked several aldehydes and esters normally found in fresh orange juice.

KEYWORDS: Citrus; pulsed flame photometric detection; GC-O; GC-MS; thermally generated odors

INTRODUCTION

Numerous studies have focused on defining high-quality, fresh-squeezed orange juice in terms of quantitative composition or aroma-active component identification (1–9). It is generally accepted that the characteristic odor of the fresh, hand-squeezed orange juice is due to a balance of many volatile compounds (esters, aldehydes, alcohols, hydrocarbons, and ketones) combined in specific proportions (10). However, fresh-squeezed orange juice aroma can be adversely modified by thermal processing and unfavorable storage conditions. Aroma quality changes may be considered the result of both losses of fresh juice aroma volatiles (mainly aldehydes and esters) and the development of off-odors foreign to fresh orange juice (11).

Canned orange juices are typically reconstituted from concentrate, which means they have been heated twice. The first heating occurs during the concentration process, when most of the water and aroma volatiles are removed using steam. A mixture of flavor volatiles is added back to the concentrate before it is shipped to its final destination, where water is added

and the reconstituted juice is heated again to destroy possible spoilage microorganisms. Finally, the hot juice is filled into a can to sterilize the container. Canned juices are typically warehoused, shipped, and displayed without benefit of refrigeration. These thermal treatments induce chemical changes that degrade the original fresh orange juice volatiles and produce new volatiles. Kirchner and Miller observed that the major changes in the canned orange juice were conversion of hydrocarbons to alcohols and loss in esters, aldehydes, and terpene aliphatic alcohols (12). In 1975, Tatum and co-workers identified 4-vinyl-2-methoxyphenol, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone [4-hydroxy-2,5-dimethyl-3(2*H*)-furanone], and α -terpineol among the off-odors found in canned orange juice stored at 35 °C (13). Few studies have used GC–olfactometry (GC-O) to directly examine the wide range of off-odors that can occur in orange juice heated multiple times. This study attempts to examine many of the odor defects observed in orange juice using GC-O and for the first time specifically examines the role that sulfur compounds play in perceived off-odors.

MATERIALS AND METHODS

Juice Samples. Five canned orange juices [1.36 L (46 oz) tin-coated steel cans] were purchased locally in supermarkets over a period of 2 months. According to their labels, they had been reconstituted from

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concentrate using juices from Florida and Mexico. As a point of comparison, late Valencia oranges were obtained from the Citrus Research and Education Center groves and were juiced the same day they were harvested. Juice was extracted using a Black & Decker reamer style juice extractor, just before each sensory session. Juices from various processing technologies [not from concentrate (NFC), reconstituted from concentrate (RFC), frozen concentrated, and canned juices) and cultivars (Valencia late, Temple, Navel, Hamlin, and Amber sweet) were used as training samples for sensory analysis.

Chemicals. Dimethyl trisulfide, 1-methene-8-thiol, and geraniol were purchased from Acros Organics (Geel, Belgium). Hexanal, 3-mercapto-2-butanone, ethyl butanoate, 7-methyl-3-methylene-1,6-octadiene (myrcene), 2-methyl-3-furanthiol, octanal, 1-octen-3-one, nonanal, 3-(methylthio)propanal (methional), (*E*)-2-nonenal, (*Z*)-2-nonenal, linalool, octanol, (*E,E*)-2,4-decadienal, decanal, nerol, 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone (Furaneol), 2-ethyl-4-hydroxy-5-methyl-3(*2H*)-furanone, 4-hydroxy-3-methoxybenzaldehyde (vanillin), 4-vinyl-2-methoxyphenol (4-vinylguaiacol), eugenol, carveol, 2-methoxyphenol, *p*-cymene, and β -ionone were obtained from Aldrich Chemical Co. (Milwaukee, WI). Neryl acetate, 1,8-cineole, α -pinene, α -sinensal, β -sinensal, terpinene-4-ol, and *l*-carvone were gifts from SunPure (Lakeland, FL), and β -damascenone was from Danisco (Lakeland, FL). (*Z*)- β -Ocimene and methanethiol were obtained from Fluka (Buchs, Switzerland). Acetic acid was purchased from Fisher and 4-mercapto-4-methyl-2-pentanone from Oxford Chemicals (Harlow Essex, U.K.).

CAS Registry Numbers for identified compounds: acetic acid, 64-19-7; methanethiol, 74-93-1; dimethyl sulfide, 75-18-3; ethyl butanoate, 105-54-4; hexanal, 66-25-1; 3-mercapto-2-butanone, 40789-98-8; 2-methyl-3-furanthiol, 28588-74-1; 3-methylthiopropional (methional), 3268-49-3; 4-mercapto-4-methyl-2-pentanone, 19872-52-7; dimethyl trisulfide, 3658-80-8; 1-octen-3-one, 4312-99-6; 7-methyl-3-methylene-1,6-octadiene (myrcene), 123-35-3; octanal, 124-13-0; *p*-cymene, 99-87-6; 1,8-cineole, 470-82-6; 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone (Furaneol), 3658-77-3; (*Z*)- β -ocimene, 3338-55-4; 1-octanol, 111-87-5; 2-ethyl-4-hydroxy-5-methyl-3(*2H*)-furanone (homofuraneol), 27538-09-6; 2-methoxyphenol (guaiacol), 90-05-1; linalool, 78-70-6; nonanal, 124-19-6; (*Z*)-non-2-enal, 60784-31-8; (*E*)-non-2-enal, 18829-56-6; terpinene-4-ol, 562-74-3; decanal, 112-31-2; *trans*-carveol, 1197-07-5; nerol, 106-25-2; *l*-carvone, 6485-40-1; geraniol, 106-24-1, 1-*p*-menth-1-ene-8-thiol, 71159-90-5; (*E,E*)-deca-2,4-dienal, 25152-84-5; 2-methoxy-4-vinylphenol (4-vinylguaiacol), 7786-61-0; eugenol, 97-53-0, 4,5-epoxy-(*E*)-dec-2-enal, 134454-31-2; β -damascenone, 23726-93-4; 4-hydroxy-3-methoxybenzaldehyde (vanillin), 121-33-5; undecanoic acid, 112-37-8; 3a,4,5,7a-tetrahydro-3,6-dimethyl-2(*3H*)-benzofuranone (wine lactone), 182699-78-1, β -ionone, 14901-07-6; β -sinensal, 60066-88-8; α -sinensal, 17909-77-2.

Headspace Sampling. Static headspace sampling was used to collect and concentrate the volatiles. Ten milliliters of canned orange juice was placed into a 40 mL glass vial containing a micro stirring bar. The vial headspace was purged with nitrogen before sealing with a Teflon-coated septum. The sample was equilibrated at 35 °C for 15 min in a water bath, and a 2 cm 50/30 μ m divinylbenzene/Carboxen/polydimethylsiloxane (DVB/Carboxen/PDMS) Stable Flex fiber (Supelco, Bellefonte, PA) was manually inserted into the vial and exposed for 45 min. The fiber was then transferred to the injector of the GC and desorbed for 5 min at 220 °C.

Gas Chromatography—FID/Olfactometry. Chromatography was performed using an HP-5890A GC (Hewlett-Packard Inc., Palo Alto, CA) equipped with a sniffing port. Samples were run separately on a polar (DB-wax, J&W Scientific, Folsom, CA; 30 m \times 0.32 mm. i.d. \times 0.5 μ m film thickness) and a nonpolar column, 5% phenyl, 95% dimethylpolysiloxane (Zebron ZB-5, Phenomenex, Torrance, CA). The ZB-5 column oven temperature was programmed from 40 to 265 °C and from 40 to 240 °C for DB-wax at 7 °C/min, with a 5 min hold at the maximum temperature. Helium was used as carrier gas at flow rate of 1.5 mL/min. Injector and detector temperatures were 220 and 290 °C, respectively. A 0.75 mm injector liner was employed to improve peak shape and chromatographic efficiency. Injections were splitless. The column effluent was split: one-third of the flow was conducted to the FID and the other two-thirds to the olfactory port for sniffing, previously mixed with warm humid air. Two assessors, trained in a

Table 1. Masses (*m/z*) Used and Resulting LRI Peak Values Observed from the SIM GC-MS Experiments

odor-active compound	selected ions	LRI DB-5
ethyl butanoate	71, 88	801
1-octen-3-one	70, 83, 97	983
1,8-cineole	111, 139, 154	1051
4-hydroxy-2,5-dimethyl-3(<i>2H</i>)-furanone	128	1073
2-methoxyphenol	109, 124	1106
linalool	93, 121	1107
(<i>E</i>)-2-nonenal	70, 83, 96	1169
terpinen-4-ol	111, 154	1203
nerol	93, 121, 154	1239
geraniol	111, 123, 154	1263
β -damascenone	175, 190	1383
<i>trans</i> -4,5-epoxy-(<i>E</i>)-2-decenal	169	1390
4-hydroxy-3-methoxybenzaldehyde (vanillin)	151, 152	1422
α -ionone	149, 177, 192	1456
wine lactone	167	1459
β -ionone	177	1518

similar way to Dreher and co-workers (14), evaluated each sample in triplicate on both ZB-5 and DB-wax columns. Odor descriptors and retention times were recorded for every sample. Assessors rated odor intensity continuously throughout the chromatographic separation process using a linear potentiometer as previously described (2). Intensities of all odor-active compounds of each GC-O run were normalized so the highest intensity from each assessor was given a score of 10. The normalized intensities of all the runs were then averaged. A peak was considered to be odor-active only if at least half of the panel responses found a similar odor quality at the same retention time. Olfactory assessor and FID responses were separately recorded and integrated using two channels and ChromPerfect software version 5.0 (Justin Innovations, Inc., Palo Alto, CA).

Mass Spectrometry. Identities of odor-active compounds were confirmed using gas chromatography–mass spectroscopy (GC-MS). Analyses were performed with a Perkin-Elmer Clarus 500 quadrupole mass spectrometer equipped with Turbo Mass software (Perkin-Elmer, Shelton, CT) and an RTX-5 capillary column (Restek; column length = 60 m, inner diameter = 0.25 mm, film thickness = 0.50 μ m). Helium was used as the carrier gas in the constant flow mode of 2 mL/min. The source was kept at 200 °C, and the transfer line and injector were maintained at 260 °C. The oven temperature program consisted of a linear gradient from 40 to 260 °C at 7 °C/min. Electron impact ionization in the positive ion mode was used (70 eV), either scanning a mass range from *m/z* 25 to 300 or acquiring data in the selected ion mode (see Table 1 for the selected ions used of the specific compounds). Mass spectra matches were made by comparison of NIST 2005 version 2.0 standard spectra (NIST, Gaithersburg, MD). Only those compounds with spectral fit values \geq 800 were considered positive identifications. Authentic standards were used to confirm identifications.

Gas Chromatography—PFPD. Sulfur compounds were analyzed using a pulsed flame photometric detector (PFPD) (model 5380, OI Analytical Co., College Station, TX) coupled to a HP-5890 series II GC. Separation was accomplished using two different capillary columns, DB-5 and DB-wax (30 m \times 0.32 mm. i.d. \times 0.5 μ m film thickness; J&W Scientific, Folsom, CA). The oven temperature program used was the same as employed for the GC-O. The injector temperature was 220 °C, and the detector temperature was set at 250 °C. Odor-active sulfur-containing compounds were confirmed by comparison with authentic standards on both columns and LRI values matching.

Sensory Analysis. Twelve highly trained flavor assessors (6 females, 6 males) from the University of Florida's Citrus Research and Education Center (Lake Alfred, FL), aged 25–60 years, participated in this study. Assessors were selected and trained following international standards (ISO). The panel had completed a 50 h training course in all aspects of sensory analysis (recognition, description, ranking, discrimination, and descriptive tests) and was specifically trained in orange juices. In training sessions, the panelists generated the lexicon following ISO 11035:1994, assessed different solutions containing guaiacol (medicine),

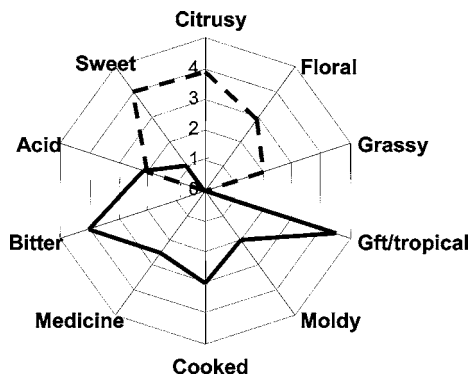


Figure 1. Sensory panel descriptive analysis average scores for canned orange juice (solid line) and fresh, hand-squeezed Valencia orange juice (dashed line).

trichloroanisole (moldy), geosmin (earthy), 2,6-dichlorophenol (medicine, antiseptic), vinegar (pungent), and caffeine (bitter) as orange juice off-flavors, and profiled different juices. Next, seven odor (orthonasal smell), six aroma (retronasal smell), and three basic taste attributes were scored from one (very slight perception) to five (very intense) in either canned and fresh hand-squeezed orange juices. Sensory analyses were carried out in triplicate following ISO standards and a flavor methodology developed for orange juices in a previous project (15).

Identification Procedures. Identification of odor-active volatiles was determined from matching sensory descriptors with the linear retention index values on both polar (wax) and nonpolar (ZB-5) columns. All odor-active compounds were confirmed by comparison of target compound retention behavior, sensory descriptor, MS fragmentation pattern, and PFPD response on both columns with authentic standards. Compounds for which identification could not be confirmed using MS data were indicated as tentatively identified.

RESULTS AND DISCUSSION

Sensory Analysis. Sensory profiles for canned and fresh-squeezed orange juices are compared in **Figure 1**. The differences between the two juice types are pronounced. Seven different odor (orthonasal) attributes were employed to describe these juices. Canned juices were described almost exclusively by the attributes of tropical fruit/grapefruit, cooked/caramel, medicine, and moldy. Fresh-squeezed juice completely lacked these odor attributes. Tropical/grapefruit was the single most intense attribute of canned orange juice and, in the absence of visual clues, was a major reason why these juices were not recognized as orange juices. Cooked, moldy, and medicine sensory attributes were found only in the canned juices together, which produced an overall sensory impression that was not associated with orange juice. A final reason canned juices were not recognized as orange juice is they completely lacked the expected citrusy, grassy, or floral odor attributes of fresh-squeezed juice.

Juices were also scored for three basic tastes (sweet, acid, and bitter). The intense sweet attribute in the fresh-squeezed Valencia juice is probably associated with the fact that oranges were harvested late in the season, when sugar content is highest and bitterness is absent. Canned orange juice had a much lower sweetness score and was also judged to be moderately bitter. Bitterness was not observed in the late-season fresh-squeezed Valencia juice.

These sensory results are in general agreement with previous studies that used descriptive analysis with a variety of orange juices (15, 16). They found that floral, green, and citrus were the most intense odor descriptors for fresh-squeezed juices, whereas canned juices generally lacked these attributes. These

same authors also indicated that canned orange juices were characterized by non-orange citrus, metallic, pungent, and bitter attributes.

Odor-Active Compounds. Forty-three odor active compounds were observed in the canned juice and are listed in **Table 2** in terms of increasing elution times on a nonpolar (5% phenyl, ZB-5) column. Linear retention index (LRI) values on a polar column (wax), chemical identifications, odor descriptors (OD), relative odor intensity, and MS and GC-PFPD responses are also presented. Volatiles that could not be confirmed by GC-MS are denoted with an asterisk, and these identifications should be considered tentative.

Approximately 16 odorants were present at such low levels that a clean full-spectrum MS fragmentation pattern could not be obtained for confirmation purposes. As the detection limit of the human nose is considerably lower than full-scan MS detection limits for many potent odorants, selected ion monitoring (SIM) was employed to increase both instrumental sensitivity and selectivity. The ion masses (m/z) used for each of these 16 trace odorants are listed in **Table 1**. Some of the ions chosen were the same as in an earlier isotopic dilution MS study of orange juice volatiles (4). However, in this study two or more masses were employed whenever possible to allow mass ratios to be employed for additional confirming evidence. If a single unique (or at least uncommon) mass can be found, the peak area of this peak can be used for quantitation purposes if it also occurs at the expected retention time. However, there is no way to confirm the identity of this peak as only ions from a single mass are collected. If two or more ions are chosen, it then becomes possible to compare the ratio of the two ion masses of the unknown with the same ratio of an authentic standard. If both retention time and mass ratio are the same as the standard, it can be used for identification purposes with a high degree of probability. However, a few potent odorants such as 1-octen-3-one, 4,5-epoxy-(*E*)-dec-2-enal, β -damascenone, and most of the sulfur volatiles could not be confirmed even with SIM MS.

Intensities of all odor-active compounds were averaged and normalized so the highest intensity was given a score of 10 (**Table 2**). It is worth noting that 4 of the 12 most intense aroma peaks (normalized intensities 9.8–10) were sulfur compounds and included methanethiol, 1-*p*-menth-1-ene-8-thiol, 2-methyl-3-furanthiol, and dimethyl trisulfide. Initial sources for these sulfur volatiles are probably tasteless, nonvolatile sulfur-containing amino acids such as cysteine and methionine, which are subsequently thermally degraded into odor-active sulfur compounds (17, 18). Some of the potent non-sulfur odors observed are from well-known Maillard thermal degradation pathways and include 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone and 2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone (19). Other highly potent odorants include 2-methoxyphenol, octanal, β -7-methyl-3-methylene-1,6-octadiene (myrcene), (*E*)-non-2-enal, (*E,E*)-deca-2,4-dienal, 4-hydroxy-3-methoxybenzaldehyde (vanillin), and α -sinensal. Several of the latter compounds are found in higher concentration in peel oil as peel oil is usually added to juice concentrate to compensate for the volatiles lost during the concentration process. 4-Hydroxy-3-methoxybenzaldehyde (vanillin) is a thermally induced decomposition product of ferulic acid (20).

Comparison of Canned Orange Juice GC-O and Sensory Profiling Results. In GC-O, odor-active volatiles are evaluated individually and not in the context of the sample matrix. In food products, odor compounds partition and otherwise interact with the food matrix, changing the resulting odor profile. Therefore, the sensory impression from the combined volatiles in the

Table 2. Characterization and Identification of Odor-Active Volatiles in Canned Orange Juice Lacking Orange Flavor^a

LRI ZB5	LRI wax	odor descriptor	identification	confirmation	relative intensity
	1460	sour	acetic acid	LRI, OD, MS	7.9
666		cabbage-like	methanethiol*	LRI, OD, PFPD	10
703		sulfur	dimethyl sulfide	LRI, OD, MS, PFPD	6.1
803	1041	fruity	ethyl butanoate	LRI, OD, MS	8.7
803	1090	green	hexanal	LRI, OD, MS	8.7
820		burning tire, sulfur	3-mercapto-2-butanone*	LRI, OD, PFPD	7.1
866		vitamin B, meaty	2-methyl-3-furanthiol*	LRI, OD, PFPD	9.9
908	1469	cooked potato	3-(methylthio)propanal (methional)*	LRI, OD, PFPD	9.3
944		sulfur, tropical	4-mercapto-4-methyl-2-pentanone*	LRI, OD, PFPD	7.5
970	1385	cabbage-like	dimethyl trisulfide	LRI, OD, MS, PFPD	9.8
978	1314	mushroom-like	1-octen-3-one*	LRI, OD	8.9
992	1159	green leaf, metallic, musty	7-methyl-3-methylene-1,6-octadiene (myrcene)	LRI, OD, MS	9.9
1003	1301	orange peel	octanal	LRI, OD, MS	10.0
1026	1283	minty, sweet, solventy	<i>p</i> -cymene	LRI, OD, MS	8.1
1033	1204	minty	1,8-cineole	LRI, OD, MS	8.8
	2050	burnt sugar, caramel	4-Hydroxy-2,5-dimethyl-3(2 <i>H</i>)-furanone (Furaneol)	LRI, OD, MS	9.0
	1249	sweet floral	(<i>Z</i>)- β -ocimene	LRI, OD, MS	6.6
1082	1573	musty, rancid	1-octanol	LRI, OD, MS	9.0
1082	2104	caramel	2-ethyl-4-hydroxy-5-methyl-3(2 <i>H</i>)-furanone (homofuraneol)*	LRI, OD	9.9
	1871	medicine, disinfectant	2-methoxyphenol (guaiaicol)	LRI, OD, MS	10.0
1103	1559	floral sweet	linalool	LRI, OD, MS	8.5
1111	1401	soapy/solventy	nonanal	LRI, OD, MS	7.9
	1707	roasted nut	unknown		9.6
1150	1517	metallic, fatty	(<i>Z</i>)-non-2-enal	LRI, OD, MS	9.1
1164	1549	green, metallic	(<i>E</i>)-non-2-enal	LRI, OD, MS	9.8
1176	1606	solventy, musty	terpinen-4-ol	LRI, OD, MS	5.8
1206	1516	green, metallic	decanal	LRI, OD, MS	9.2
	1825	fresh air	<i>trans</i> -carveol	LRI, OD, MS	5.6
1228	1803	fruity sweet, cashew	nerol	LRI, OD, MS	8.9
1238	1752	minty	<i>l</i> -carvone	LRI, OD, MS	9.2
1257	1861	floral, green	geraniol	LRI, OD, MS	7.8
1287	1598	passion fruit, grapefruit	1- <i>p</i> -menth-1-ene-8-thiol*	LRI, OD, PFPD	10.0
	1828	fried	(<i>E,E</i>)-deca-2,4-dienal*	LRI, OD	10.0
1321	2210	spice, woody, stale	2-methoxy-4-vinylphenol	LRI, OD, MS	9.0
1368	2182	clove-like	eugenol	LRI, OD, MS	9.4
1385	2021	fatty, solventy	4,5-epoxy-(<i>E</i>)-dec-2-enal*	LRI, OD	6.3
1390	1837	honey, sweet floral	β -damascenone*	LRI, OD	7.6
1419	>2500	vanilla-like, sweet	4-hydroxy-3-methoxybenzaldehyde (vanillin)	LRI, OD, MS	10.0
	2398	sweet, fatty, cooked	undecanoic acid	LRI, OD, MS	8.0
1459		floral, sweet, metallic	3,4,5,7a-tetrahydro-3,6-dimethyl-2(3 <i>H</i>)-benzofuranone (wine lactone)*	LRI, OD	8.4
1494	1958	raspberry, floral	β -ionone	LRI, OD, MS	8.4
1693	2250	sweet citrusy, aquarium	β -sinensal	LRI, OD, MS	9.1
	2363	sweet floral	α -sinensal*	LRI, OD	10.0

^a Observed odor activity indicated by retention values in the first two columns. Average odor intensity and techniques used to confirm peak identification are presented in separate columns. The identification of compounds followed by an asterisk should be considered tentative.

presence of the food matrix will usually be different from that of the combined odorants in the absence of the matrix. It may be further argued that synergic and antagonism effects among the juice odorants will alter the overall odor perception, making it difficult to evaluate the contribution of individual odorants. However, in this study, some individual odorants have odors which so closely match the overall sensory attribute that it would be logical to associate them together. The most intense sensory descriptors for canned orange juices were tropical fruit/grapefruit, cooked/caramel, medicine, and moldy/musty.

Tropical Fruit/Grapefruit. The prominent tropical fruit/grapefruit sensory attribute is due primarily to 1-*p*-menth-1-ene-8-thiol. Its prominent aroma peak can be seen in **Figure 2** as peak 23. 4-Mercapto-4-methyl-2-pentanone is the other potent sulfur volatile that contributes to this non-orange aroma (see **Table 2**). They are the major aroma impact compounds in grapefruit juice (21) and are primarily responsible for the tropical fruit/grapefruit sensory attribute in canned orange juice.

Cooked/Caramel. The cooked sensory attribute is probably due to a more diverse group of odorants. Some of the most prominent would include 2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone with their

burnt sugar/caramel odors along with the meaty odor of 2-methyl-3-furanthiol and the cooked potato odor of 3-(methylthio)propanal (methional). Two of these five odorants are sulfur compounds; the other two are Maillard sugar-amine reaction products, which should not be unexpected from the high levels of sugars and thermal history of canned orange juice.

Medicine. The medicine aroma attribute is perhaps easiest to associate with individual odorants, as it is an untypical citrus odor and few odorants in **Table 2** were described using this term. 2-Methoxyphenol was one of the 12 most intense odorants found in canned orange juice and characterized in fruit juices as medicine. It is probably due to microbial contamination of the thermoacidophilic, nonpathogenic bacteria *Alicyclobacillus acidoterrestis* (22, 23). This bacteria has become problematic in the citrus industry because it is not controlled with heat as are most other bacteria. Eugenol and 2-methoxy-4-vinylphenol (4-vinyl-2-methoxyphenol) are probably secondary contributors to the medicine off-odor.

Moldy/Musty. This odor note is probably the result of several odorants. The most likely major contributor would be β -7-methyl-3-methylene-1,6-octadiene (myrcene) (green leaf, metallic, musty). (*E*)-2-Nonenal, with its green/metallic odor, is also

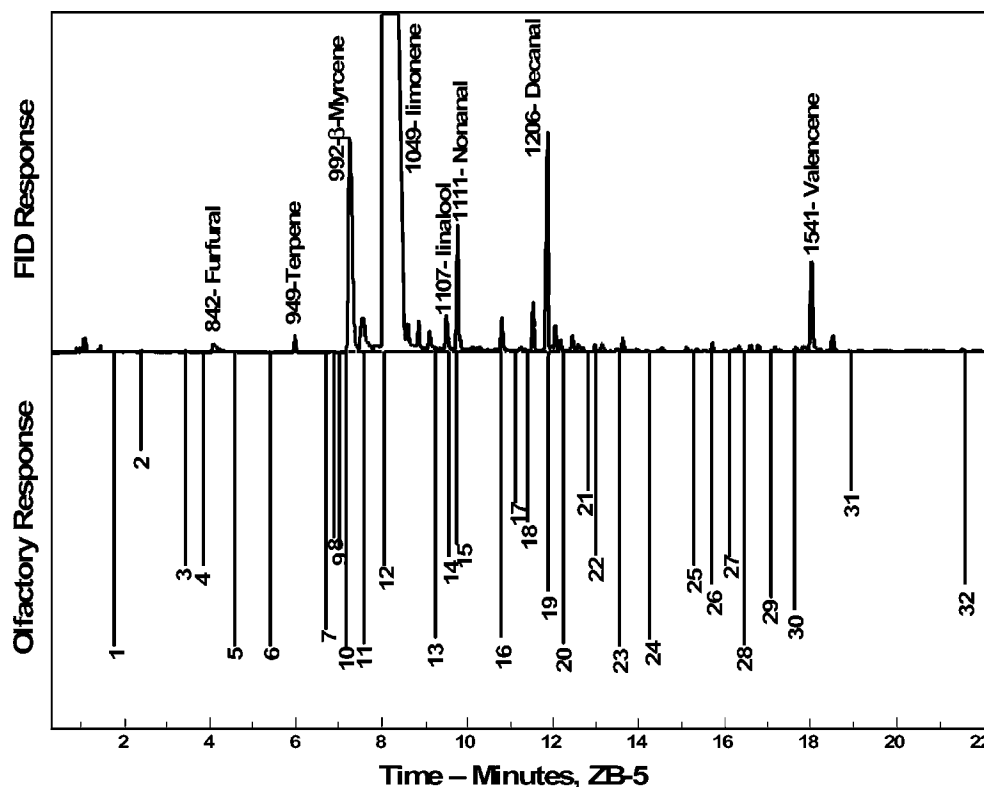


Figure 2. Comparison of FID and olfactory responses from a single analysis of canned orange juice. Olfactory descriptors: 1, cabbage (methanethiol); 2, sulfur (dimethyl sulfide); 3, fruity, green (ethyl butanoate/hexanal); 4, burning tire, sulfur (3-mercapto-2-butanone); 5, cooked meat (2-methyl-3-furanthiol); 6, cooked potato (methional); 7, cabbage-like (dimethyl trisulfide); 8, mushroom (1-octen-3-one); 9, metallic, green leaf (myrcene); 10, green leaf; 11, orange peel (octanal); 12, minty (1,8-cineole); 13, caramel (homofuraneol); 14, floral (linalool); 15, solventy (nonanal); 16, green, metallic [(*E*)-non-2-enal]; 17, solventy, musty (terpinene-4-ol); 18, earthy; 19, green, metallic (decanal); 20, minty (*l*-carvone); 21, floral, green (geraniol); 22, floral sweet; 23, grapefruit, tropical fruit (1-*p*-menth-1-ene-8-thiol); 24, spice, woody (4-vinylguaiaicol); 25, clove-like (eugenol); 26, sweet floral, honey (β -damascenone); 27, mushroom; 28, vanilla-like, sweet (vanillin); 29, floral sweet (wine lactone); 30, raspberry, sweet floral (β -ionone); 31, fatty; 32, citrus, sweet (β -sinensal).

a probable contributor to the overall moldy/musty sensory attribute along with other aldehydes with their green/rancid/fatty notes.

Sulfur-Containing Volatiles. Eight of the 43 identified odor-active volatiles identified in canned orange juice were sulfur compounds (Table 2). Of these, four were identified for the first time in processed orange juice (methanethiol, 3-mercapto-2-butanone, 4-mercapto-4-methyl-2-pentanone, and dimethyl trisulfide). Shaw and co-workers reported finding hydrogen sulfide and occasional peaks thought to be due to dimethyl sulfide, methanethiol, sulfur dioxide, and carbonyl sulfide in citrus juices (24) but could not confirm the identifications of either methanethiol or dimethyl disulfide. Kirchner and co-workers found hydrogen sulfide in both fresh (25) and canned orange juices (12). Analytical studies quantified hydrogen sulfide and dimethyl sulfide in the headspace of citrus juices and occasionally observed peaks at the same retention times as methanethiol and dimethyl disulfide but could not quantify or confirm them with authentic standards (26). More recent works have reported finding 3-(methylthio)propanal (methional), 2-methyl-3-furanthiol, and *p*-meth-1-ene-8-thiol in both fresh (4, 6, 18) and processed (18, 27) orange juices.

Although sulfur-containing compounds can be formed in natural products as a result of biochemical and enzymatic pathways, many important sulfur aroma compounds are derived from reactions occurring during the thermal processing of food (28). As canned orange juices are typically reconstituted from concentrate, which means they have been heated twice, it is possible that the majority of the sulfur compounds identified in

the present study are derived from these thermal processes and increased by ambient temperature storage.

A canned orange juice sulfur chromatogram is shown in Figure 3. Because the sulfur-specific PFPD was employed, all peaks were due to sulfur volatiles. Although many sulfur peaks shown in Figure 3 were aroma active, not all of them produced aroma activity at the same retention time. Two of the largest sulfur peaks (peaks 3 and 11) were not associated with odor activity. In spite of the fact that two different GC-MS techniques were used to confirm volatiles, just two sulfur odor-active sulfur compounds could be identified using mass spectroscopy (dimethyl sulfide and dimethyl trisulfide). However, the response from the highly sensitive sulfur detector (PFPD) shown in Figure 3 was able to detect eight sulfur volatiles (methanethiol, dimethyl sulfide, dimethyl disulfide, 3-mercapto-2-butanone, 2-methyl-3-furanthiol, methional, dimethyl trisulfide, and 1-*p*-menth-1-ene-8-thiol).

1-*p*-Methene-8-thiol is shown in the sulfur chromatogram (Figure 3) as peak 12 at 14.4 min. It is also one of the 12 most intense GC-O odorants listed in Table 2 (LRI 1287) and shown in Figure 2 as aroma peak 23. Its odor was described as passion fruit/grapefruit, which was almost identical with the most intense sensory attribute for canned orange juice (tropical fruit/grapefruit). Because its odor threshold is so low (0.0001 $\mu\text{g}/\text{kg}$ in water), even trace quantities of this volatile can produce a significant flavor impact (28). However, its PFPD retention time matched that of standards perfectly on both polar and nonpolar columns, and its observed aroma peak LRI values (1287

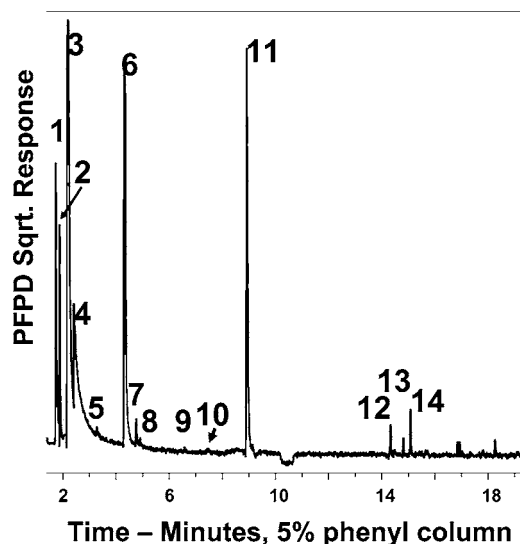


Figure 3. Sulfur volatiles detected in a canned orange juice using PFPD in the square root mode: 1, methanethiol; 2, dimethyl sulfide; 3, unknown; 4, dimethyl disulfide; 5, 3-mercapto-2-butanone; 6, 2-methyl-3-furanthiol; 7, 3-(methylthio)propanal (methional); 8, unknown; 9, dimethyl trisulfide; 10, unknown; 11, unknown; 12, 1-*p*-menth-1-ene-8-thiol; 13 and 14, unknown.

nonpolar and 1598 polar) matched well with published values for this compound (1283 nonpolar and 1598 polar) (29).

1-*p*-Methene-8-thiol is a key flavor aroma compound of grapefruit (30) and has been previously identified as a minor odorant in fresh-squeezed orange juice (4). Thus, the thermal processes experienced by canned orange juices are probably responsible for the elevated intensity of this odorant. Elevated levels of 1-*p*-Methene-8-thiol were also observed in grapefruit juices that had been heated (31). Furthermore, Tatum and co-workers (13) reported a grapefruit-like aroma in many canned orange juices that had been thermally abused. Although they could not identify the aroma-active compound responsible for this odor note, they concluded that it was not due to nootkatone and further speculated it was due to some unknown trace component.

Two volatile peaks described with a cabbage smell also produced sulfur responses. They were identified as methanethiol (peak 1) and dimethyl trisulfide (peak 9), respectively. Two other odor-active sulfur peaks were characterized with cooked odors (meaty and cooked potato) and were later identified as 3-(methylthio)propanal (methional) (peak 7) and 2-methyl-3-furanthiol (peak 6). During thermal processing, 3-(methylthio)propanal (methional) could be formed from methionine by Strecker degradation, which releases methanethiol by β -elimination (17, 18, 32), whereas 2-methyl-3-furanthiol can be produced either in the Maillard reaction (33, 34) or from the degradation of thiamin (14, 35). Dimethyl sulfide (peak 4) and 3-mercapto-2-butanone (peak 5) were described as possessing a sulfur odor and were confirmed by comparing GC-PFPD retention times of unknowns with standards. Dimethyl sulfide is likely formed by the heating process. It is well-known that methanethiol oxidizes easily to dimethyl disulfide, which can disproportionate to dimethyl sulfide and dimethyl trisulfide (32). 3-Mercapto-2-butanone (peak 5) may be derived from the Maillard reaction as well (34). 4-Mercapto-4-methyl-2-pentanone was described as possessing a sulfury and tropical fruit odor and probably contributed to the pronounced tropical fruit/grapefruit sensory attribute as it is one of the major aroma impact compounds in grapefruit juice (21).

Missing Fresh Juice Odor Components. It is also worth mentioning that many typical fresh juice aroma components are missing from Table 2. Noteworthy absences include the characteristic aldehydes [acetaldehyde, (*Z*)-hex-3-enal, (*E*)-hex-2-enal, neral, geranial, or citronellal] and esters (methyl butanoate, ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, ethyl hexanoate, or ethyl 3-hydroxyhexanoate) typically found in fresh orange juices (1, 4–6, 10, 36). Because esters contribute to the fruity odor note and aldehydes to the green and citrus odor notes observed in squeezed orange juices, their lower quantities in canned juices could be the reason that canned juices lacked fruity and green sensory attributes (see Figure 1). These results are in agreement with Kirchner and Miller, who observed that canned orange juice had lower concentrations of esters, aldehydes, and aliphatic terpenes than fresh juices (12).

Less Desirable Odors. Even though some aldehydes were not present in canned juices, others such as alk-2,4-dienals and aliphatic saturated aldehydes (hexanal, octanal, and decanal) with a characteristic rancid/fatty odor were identified (Table 2). These compounds appear in higher concentrations in heated juices than in fresh juices (2, 7, 9, 27). Degradation of free, long-chain unsaturated fatty acids during thermal processing and subsequent storage of juices could be the reason for their increase (11). Hexanal, octanal, and decanal along with α -pinene, β -7-methyl-3-methylene-1,6-octadiene (myrcene), β -ocimene, α -sinensal, β -sinensal, and linalool are also typical constituents of the peel oil. Their levels in commercial orange juices depend on the peel oil content of the juice (10). As previously mentioned, processed juices made from concentrate are commonly fortified with peel oil to partially restore lost volatiles, so their increased presence in canned juices should be expected. Moreover, under acidic conditions linalool degrades primarily to α -terpineol (which was not odor-active) but also to 1,8-cineole, geraniol, nerol (37), and terpinen-4-ol (38) by acid-catalyzed hydration reactions. (+)-Limonene can also yield carvone via an oxidative formation pathway (39).

ABBREVIATIONS USED

LRI, linear retention indices; GC-O, gas chromatography–olfactometry; GC-PFPD, gas chromatography–pulsed flame photometric detection; SIM, selected ion monitoring; MS, mass spectrometry.

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LITERATURE CITED

- (1) Arena, E.; Guarrera, N.; Campisi, S.; Asmundo, C. N. Comparison of odour active compounds detected by gas-chromatography–olfactometry between hand-squeezed juices from different orange varieties. *Food Chem.* **2006**, *98* (1), 59–63.
- (2) Bazemore, R.; Goodner, K.; Rouseff, R. Volatiles from unpasteurized and excessively heated orange juice analyzed with solid phase microextraction and GC–olfactometry. *J. Food Sci.* **1999**, *64* (5), 800–803.
- (3) Brat, P.; Rega, B.; Alter, P.; Reynes, M.; Brillouett, J. M. Distribution of volatile compounds in the pulp, cloud, and serum of freshly squeezed orange juice. *J. Agric. Food Chem.* **2003**, *51*, 3442–3447.
- (4) Buettner, A.; Schieberle, P. Evaluation of aroma differences between hand-squeezed juices from valencia late and navel oranges by quantitation of key odorants and flavor reconstitution experiments. *J. Agric. Food Chem.* **2001**, *49*, 2387–2394.

- (5) Hinterholzer, A.; Schieberle, P. Identification of the most odour-active volatiles in fresh, hand-extracted juice of Valencia late oranges by odour dilution techniques. *Flavour Fragrance J.* **1998**, *13* (1), 49–55.
- (6) Mahattanatawee, K.; Rouseff, R.; Valim, M. F.; Naim, M. Identification and aroma impact of norisoprenoids in orange juice. *J. Agric. Food Chem.* **2005**, *53*, 393–397.
- (7) Nisperos-Carriedo, M. O.; Shaw, P. E. Comparison of volatile flavor components in fresh and processed orange juices. *J. Agric. Food Chem.* **1990**, *38*, 1048–1052.
- (8) Rega, B.; Fournier, N.; Guichard, E. Solid phase microextraction (SPME) of orange juice flavor: odor representativeness by direct gas chromatography olfactometry (D-GC-O). *J. Agric. Food Chem.* **2003**, *51*, 7092–7099.
- (9) Rouseff, R.; Bazemore, R.; Goodner, K.; Naim, M. GC-olfactometry with solid phase microextraction of aroma volatiles from heated and unheated orange juice. In *Headspace Analysis of Foods and Flavors*; Kluwer Publishing: New York, 2001; Vol. 488, pp 101–112.
- (10) Shaw, P. E. Fruits II. In *Volatile Compounds in Foods and Beverages*; Maarse, H., Ed.; Dekker: New York, 1991; pp 305–327.
- (11) Nagy, S.; Rouseff, R. L.; Lee, H. S. *Thermally Degraded Flavors in Citrus Juice Products*; American Chemical Society: Washington, DC, 1989; Vol. 196, p 173-AGFD.
- (12) Kirchner, J. G.; Miller, J. M. Canning and storage effects—volatile water-soluble and oil constituents of Valencia orange juice. *J. Agric. Food Chem.* **1957**, *5*, 283–291.
- (13) Tatum, J. H.; Nagy, S.; Berry, R. E. Degradation products formed in canned single-strength orange juice during storage. *J. Food Sci.* **1975**, *40* (4), 707–709.
- (14) Dreher, J. G.; Rouseff, R.; Naim, M. GC-olfactometric characterization of aroma volatiles from the thermal degradation of thiamin in model orange juice. *J. Agric. Food Chem.* **2003**, *51*, 3097–3102.
- (15) Ruiz Perez-Cacho, P.; Soldevilla, H. G.; Mahatanatawee, K.; Smoot, J.; Rouseff, R. Development of a flavor descriptive analysis procedure for orange juices by two trained panels in Spain and U.S.A. *Chem. Senses* **2006**, *31* (8), E72–E73.
- (16) Lotong, V.; Chambers, E.; Chambers, D. H. Categorization of commercial orange juices based on flavor characteristics. *J. Food Sci.* **2003**, *68* (2), 722–725.
- (17) Mottram, D. S.; Mottram, H. R. An overview of the contribution of sulfur-containing compounds to the aroma in heated foods. In *Heteroatomic Aroma Compounds*; Reineccius, G. A., Reineccius, T. A., Eds.; American Chemical Society: Washington, DC, 2002; Vol. 826, pp 73–92.
- (18) Bezman, Y.; Rouseff, R.; Naim, M. 2-Methyl-3-furanthiol and 3-(methylthio)-propanal (methional) are possible off-flavors in stored orange juice: aroma-similarity, NIF/SNIF GC-O, and GC analyses. *J. Agric. Food Chem.* **2001**, *49*, 5425–5432.
- (19) Lee, H. S.; Nagy, S. Relationship of sugar degradation to detrimental changes in citrus juice quality. *Food Technol.* **1988**, *42* (11), 91.
- (20) Peleg, H.; Naim, M.; Zehavi, U.; Rouseff, R. L.; Nagy, S. Pathways of 4-vinyl-2-methoxyphenol formation from ferulic acid in model solutions of orange juice. *J. Agric. Food Chem.* **1992**, *40*, 764–767.
- (21) Buettner, A.; Schieberle, P. Evaluation of key aroma compounds in hand-squeezed grapefruit juice (*Citrus paradisi* Macfayden) by quantitation and flavor reconstitution experiments. *J. Agric. Food Chem.* **2001**, *49*, 1358–1363.
- (22) Gocmen, D.; Elston, A.; Willians, T.; Parish, M.; Rouseff, R. L. Identification of medicinal off-flavors generated by *Alicyclobacillus* species in orange juice using GC-olfactometry and GC-MS. *Lett. Appl. Microbiol.* **2005**, *40*, 172–177.
- (23) Orr, R. V.; Shewfelt, R. L.; Huang, C. J.; Tefera, S.; Beuchat, L. R. Detection of 2-methoxyphenol produced by *Alicyclobacillus acidoterrestris* in apple juice by sensory and chromatographic analyses, and comparison with spore and vegetative cell populations. *J. Food Prot.* **2000**, *63* (11), 1517–1522.
- (24) Shaw, P. E.; Ammons, J. M.; Braman, R. S. Volatile sulfur-compounds in fresh orange and grapefruit juices—identification, quantitation, and possible importance to juice flavor. *J. Agric. Food Chem.* **1980**, *28*, 778–781.
- (25) Kirchner, J. G.; Rice, R. G.; Miller, J. M.; Keller, G. J. The presence of hydrogen sulfide in citrus juices. *Arch. Biochem.* **1950**, *25* (1), 231–232.
- (26) Shaw, P. E.; Wilson, C. W. Volatile sulfides in headspace gases of fresh and processed citrus juices. *J. Agric. Food Chem.* **1982**, *30*, 685–688.
- (27) Buettner, A.; Schieberle, P. Application of a comparative aroma extract dilution analysis to monitor changes in orange juice aroma compounds during processing. In *Gas Chromatography—Olfactometry: The State of the Art*; Leland, J., Schieberle, P., Buettner, A., Acree, T. E., Eds.; American Chemical Society: Washington, DC, 2001; Vol. 782, pp 33–45.
- (28) Blank, I. Sensory relevance of volatile organic sulfur compounds in food. In *Heteroatomic Aroma Compounds*; American Chemical Society: Washington, DC, 2002; Vol. 826, pp 25–53.
- (29) Buettner, A.; Schieberle, P. Characterization of the most odor-active volatiles in fresh, hand-squeezed juice of grapefruit (*Citrus paradisi* Macfayden). *J. Agric. Food Chem.* **1999**, *47*, 5189–5193.
- (30) Demole, E.; Enggist, P.; Ohloff, G. 1-*p*-Menthene-8-thiol: a powerful flavor impact constituent of grapefruit juice (*Citrus paradisi* Macfayden). *Helv. Chim. Acta* **1982**, *65* (6), 1785–1794.
- (31) Lin, J. M.; Rouseff, R. L.; Barros, S.; Haim, M. Aroma composition changes in early season grapefruit juice produced from thermal concentration. *J. Agric. Food Chem.* **2002**, *50*, 813–819.
- (32) Belitz, H.-D.; Grosch, W.; Schieberle, P. *Food Chemistry*, 3rd rev. ed.; Springer: Berlin, Germany, 2004.
- (33) Mottram, D. S. Flavour formation in meat and meat products: a review. *Food Chem.* **1998**, *62* (4), 415–424.
- (34) Hofmann, T.; Schieberle, P. Evaluation of the key odorants in a thermally treated solution of ribose and cysteine by aroma extract dilution techniques. *J. Agric. Food Chem.* **1995**, *43*, 2187–2194.
- (35) Werkhoff, P.; Bruning, J.; Emberger, R.; Guntert, M.; Kopsel, M.; Kuhn, W.; Surburg, H. Isolation and characterization of volatile sulfur-containing meat flavor components in model systems. *J. Agric. Food Chem.* **1990**, *38*, 777–791.
- (36) Moshonas, M. G.; Shaw, P. E. Quantitative determination of 46 volatile constituents in fresh, unpasteurized orange juices using dynamic headspace gas chromatography. *J. Agric. Food Chem.* **1994**, *42*, 1525–1528.
- (37) Askar, A.; Bielig, H. J.; Treptow, H. Aroma modification of orange juice. III. Model test of the decrease of linalool and limonene with respect to the storage of orange juice in bottles and cans. *Dtsch. Lebensm.-Rundsch.* **1973**, *69* (10), 360–364.
- (38) Haleva-Toledo, E.; Naim, M.; Zehavi, U.; Rouseff, R. L. Formation of α -terpineol in citrus juices, model and buffer solutions. *J. Food Sci.* **1999**, *64* (5), 838–841.
- (39) Marcotte, M.; Stewart, B.; Fustier, P. Abused thermal treatment impact on degradation products of chilled pasteurized orange juice. *J. Agric. Food Chem.* **1998**, *46*, 1991–1996.

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