Effects of Heating and Cream Addition on Fresh Raspberry Aroma Using a Retronasal Aroma Simulator and Gas Chromatography Olfactometry

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A flavor release method using a "retronasal aroma simulator" (RAS) combined with gas chromatography olfactometry (GCO) was used to study the aroma of raspberries, *Rubus idaeus* cv. Heritage. Dynamic headspace samples were generated with the RAS including synthetic saliva addition and shearing at 37 °C. A headspace dilution series was sampled from the RAS and analyzed by CharmAnalysis GCO to produce measures of odor potency called charm. The most potent flavor compounds in fresh raspberries were β-damascenone, diacetyl, sotolon, 1-hexen-3-one, 1-nonen-3-one, 1-octen-3-one, and (*Z*)-3-hexenal. Heating raspberries caused an increase in raspberry ketone and rated raspberry aroma. Likewise, β-damascenone, sotolon, vanillin, 1-nonen-3-one, and 1-octen-3-one showed at least 5-fold increases in charm upon heating. Cream addition markedly decreased aroma (GCO and sensory). Comparison of the fresh raspberries odor spectra between RAS−GCO and solvent extraction−GCO showed different profiles, with the former having greater odor potency values for diacetyl, 1-hexen-3-one, 1-octen-3-one, 1-nonen-3-one, and sotolon and the latter with a greater value for ethyl 2-methylbutyrate.

Keywords: *Aroma; GC; retronasal; raspberries*

INTRODUCTION

The release of volatile aroma compounds from food influences the perception of flavor. However, many food components, such as oil and thickeners, can affect this release. To conduct studies on basic release mechanisms, a device was constructed that simulates flavor release in the mouth, incorporating synthetic saliva addition, shearing at the shear rate in the mouth, air flow, and temperature regulation to 37 °C (Roberts and Acree, 1995b). This retronasal aroma simulator (RAS) was found to be a sensitive, reproducible device, with the potential for use with actual foods. Previously, the volatiles were trapped with a silica trap and desorbed with solvent prior to gas chromatography. The method described in this paper determines the odor-active compounds in an actual food by combining RAS with the gas chromatography−olfactometry (GCO) system called CharmAnalysis (Acree et al., 1984; Acree and Barnard, 1994).

The GCO methods of CharmAnalysis and a similar method, aroma extract dilution analysis (AEDA), (Ullrich and Grosch, 1987), have traditionally involved a serial dilution of solvent-extracted samples. It is only recently that the approach of using headspace samples has been investigated. The low volatile levels in headspace samples lead to difficulty in compound identification by methods such as GC/MS. However, the use of GCO offers a much higher sensitivity to odor-active compounds and is thus an appropriate method for headspace analysis (GCO-H).

In one of the first approaches of GCO-H, the aroma of coffee was investigated by sampling six static headspace volumes between 250 and 10 μL with GC sniffing port evaluations (Holscher and Steinhart, 1992). Highly volatile compounds that would normally coelute with the solvent peak were detected such as hydrogen sulfide, acetaldehyde, and methanethiol. In a study of tea, a comparison was made between a dilution analysis of static headspace samples of 2−40 mL (GCO-H) and solvent-extracted volatiles (AEDA) (Guth and Grosch, 1993). Several of the compounds with high volatility, such as 2-methylpropanal and diacetyl, were found with GCO-H but had reduced or no detection in AEDA, indicating that they may have been lost during the concentration method. Also, many compounds with high aroma values with AEDA, such as sotolon, β-damascenone, furaneol, and vanillin, were not detected by GCO-H. The next study by Guth and Grosch (1994b) used the results from AEDA and GCO-H to formulate a stewed beef juice flavor. Again, sotolon was detected in AEDA but not GCO-H, and several more volatile compounds (acetaldehyde, methanethiol, and diacetyl) were detected in GCO-H but not AEDA. Compounds uniquely identified from both analyses were included in the final stewed beef formulation, indicating that the methods were complementary. Other studies also indicated complementary AEDA and GCO-H studies for oatmeal (Guth and Grosch, 1994a), olive oil (Blekas et al., 1994), and meat-like flavorings (Blank et al., 1994) and determined the odor defects by GCO-H in cod and trout (Milo and Grosch, 1995).

Past studies of raspberry aroma have implicated raspberry ketone as the character impact compound and α-ionone, β-ionone, (*Z*)-3-hexenol, β-damascenone, linalool, and geraniol as important aroma compounds (Nursten, 1977; Larsen and Poll, 1990). Volatiles present in raspberry have been summarized (Guichard, 1982; Vincenzi et al., 1989). This research studied the influence of heating and cream addition on raspberry aroma and demonstrated the application of retronasal

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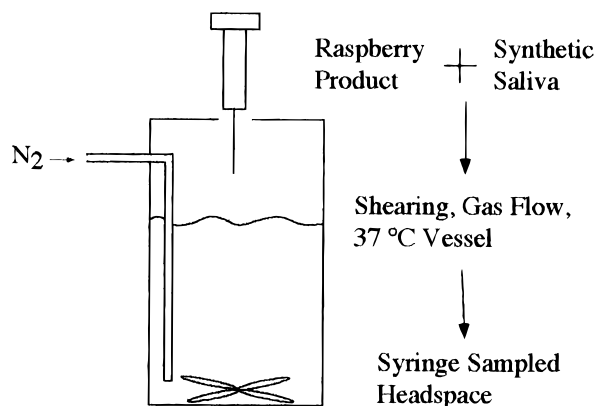
Figure 1. Syringe sampling protocol for the modified blender, retronasal aroma simulator.

headspace simulation combined with gas chromatography—olfactometry as a tool for determining the odor-active compounds that are released in food under simulated mouth conditions.

MATERIALS AND METHODS

Materials. Raspberry fruit (*Rubus idaeus* cv. Heritage) from Chile were purchased locally. Raspberries in a sealed flask were heated in boiling water for approximately 40 min (precisely 10 min after the berry temperature reached 80 °C), similar to jam production conditions (Larsen et al., 1991). The raspberries in cream sample consisted of heated raspberries plus 20% w/w heavy cream (0.34 g of fat/mL, purchased locally). Raspberry juice was made by shearing fresh raspberries in the RAS and then pressing through cheesecloth at 10 000 lb (Loomis Press, Caldwell, NJ). Authentic standards of diacetyl, furaneol [4-hydroxy-2,5-dimethyl-3(2*H*)-furanone], vanillin, raspberry ketone, ethyl butyrate, α-ionone, β-ionone, ethyl 2-methylbutyrate (Aldrich, Milwaukee, WI), (Z)-3-hexenal, octanal (Bedoukian, Danbury, CT), β-damascenone (Firmenich, Geneva, Switzerland), sotolon [3-hydroxy-4,5-dimethyl-2(5*H*)-furanone; IFF, Union Beach, NJ], 1-octen-3-one, 1-nonen-3-one, and 1-hexen-3-one (synthesized by chromic acid oxidation of the alcohols) (Brown and Garg, 1961) were used to determine mass spectral, retention index, and odor matches. Synthetic saliva (pH 7.0) consisted of 20 mmol/L NaHCO$_3$, 2.75 mmol/L K$_2$HPO$_4$, 12.2 mmol/L KH$_2$PO$_4$, and 15 mmol/L NaCl with 200 units/mL of porcine pancreas α-amylase (Sigma, St. Louis, MO).

RAS—GCO Method. The RAS—GCO method is described in Figure 1. Raspberry samples analyzed in duplicate were fresh raspberries, heated raspberries, and raspberries in cream. Synthetic saliva (112 mL = 1/4 volume) preheated to 37 °C and 450 g of raspberry sample (or 540 g for raspberry + cream) in the 37 °C RAS were sheared at 330 s^{-1}. As nitrogen gas flowed through the sample at 20 mL/s, a gastight syringe (Dynatech, Baton Rouge, LA), inserted into a hole in the lid, slowly (over 1–4 min) sampled the volatiles released with a final holding period in the RAS of 1–2 min.

The syringe contents were immediately and slowly injected (0.5 mL/min) into the CharmAnalysis gas chromatography—olfactometry (GCO) system which uses human sniffers to determine the potency of aroma compounds. Using the CharmWare program, the sniffer indicated the beginning and ending of each eluting aroma compound and selected a descriptor to describe the odor quality (i.e. raspberry, floral, grassy, etc). In addition to this procedure, the sniffer provided an intensity rating between 1 (very weak) and 7 (very strong) for each aroma compound. This method of gas chromatography—olfactometry is called perceived intensity quantitation. The method used here is similar to that of Dravnieks et al. (1979), in which panelists used an intensity scale to rate the perceived intensity. In another technique called OSME (da Silva et al., 1994), panelists produced time—intensity measures of intensity. These intensity data were not used in determin-

ing the charm values but only in a comparison between dilution analysis and perceived intensity quantitation. Each aroma compound's Kovats retention index, odor character, and charm were determined. The limit on zero (Lz) and least significant ratio (LSR) for the charm values were determined according to the method of Acree and Barnard (1994). The GC column was an OV101 fused silica capillary column (12 m × 0.32 mm) and also a Carbowax (Innowax) column (15 m × 0.32 mm) with the initial 20 cm loop immersed in liquid nitrogen. After injection, the loop was removed from liquid nitrogen and the temperature programming started: 3 min at 35 °C (50 °C for Innowax) and 6 °C/min to 225 ° C. Over a day, the volumes of gas were separately sampled with the syringes to give a dilution series of 10, 3.3, 1.1, 0.37, 0.123, 0.041, 0.014, and 0.004 mL.

Solvent Extraction. Raspberry juice (200 mL) was sequentially extracted with 133 mL of Freon, followed by 133 mL of ethyl acetate, which were both dried with magnesium sulfate. The solvent extracts were concentrated under reduced pressure and serially diluted by factors of 3 for analysis by GCO. Concentrated extracts were analyzed by a HP 5970 GC/MS system with an OV101 0.20 mm × 25 m fused silica capillary column, coated with 0.33 μm of methyl silicone. Duplication was performed through the whole process, starting with two different batches of raspberries to make juice. The duplicate samples were each analyzed by two sniffers, who were trained and tested for their olfactory ability using a set of standard aromas.

Sensory Test. The three raspberry samples analyzed by the RAS—GCO procedure were also analyzed in duplicate for their raspberry quality using a sensory test. Each raspberry sample (60 g) was placed in a 250 mL plastic squeeze bottle and completely wrapped in foil. The samples were presented to the panelists in a randomized order and labeled with three-digit codes. Fourteen panelists, 9 females and 5 males ages 21–31 and prescreened for a clear nasal passage on the test day, were instructed to evaluate the odor of each sample by squeezing the bottle in front of their nose with a moderate intensity as demonstrated by the panel administrator. In one test session, the panelists evaluated six samples for odor using a 9 box scale anchored at one end (box 1) with "weak raspberry" and at the other end (box 9) with "strong raspberry". After the test, panelists were asked to write down descriptors that characterized each sample. The results were analyzed by repeated measures ANOVA and the Tukey—Kramer test for all pairs.

RESULTS AND DISCUSSION

Raspberry aroma can be described as a combination of floral, buttery, fruity, raspberry, mushroom, maple syrup, vanilla, and green notes. With GCO—Charm-Analysis, the retention index of these notes and their importance to the aroma of food, as measured by the charm values, were determined. Results are presented in Tables 1 and 2; Table 1 shows the raw charm data for raspberries analyzed by the RAS and compound identification information, and Table 2 shows the odor spectrum values for comparison of the RAS and solvent extraction sampling methods.

Odor Spectra. An odor spectrum is similar to a mass spectrum in that it is a plot of fragments vs relative response. In mass spectrometry, the fragments are ions and their response is the relative population of these ions. A spectrum is generated by plotting relative ion populations against mass to charge ratios. In odor spectrometry, the fragments are the retention indices of the component odorants, and their response is their relative potency. In the odor spectra, charm values (or dilution values from AEDA) were modified by the Steven's law equation

$$\Psi = k\Phi^n$$

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Table 1. Raspberry Aroma Compounds Simulated To Be Released in the Mouth, As Determined by the Retronasal Aroma Simulator, GCO Analysis, and GC/MS Identification, with the Sensory Test Results for Raspberry Aroma

| compound	odor	$R_{CX 20 M}$	R_{OV101}	ID[a]	charm fresh[b]	heated	heated with 20% cream[b]
β-damascenone	damascenone	1816	1352	R, O, M	401	1922	34
diacetyl	buttery	968	566	R, O, M	94	57	121
sotolon	maple	2205	1063	R, O	72	348	27
1-hexen-3-one	leaves, metallic	1106	747	R, O	58	43	20
1-nonen-3-one	mushroom	1405	1052	R, O	18	157	9
1-octen-3-one	mushroom	1304	952	R, O	14	108	28
(Z)-3-hexenal	grassy	1145	770	R, O, M	9	34	8[c]
vanillin	vanilla		1341	R, O, M	8[c]	296	8[c]
raspberry ketone	raspberry		1498	R, O, M	8[c]	44	8[c]
unknown	leaves		1116		8[c]	27	57
unknown	paper		1127		8[c]	24	8[c]
furaneol	cotton candy	2043	1023	R, O	8[c]	19	8[c]
octanal	citrusy	1288	975	R, O	8[c]	18	10
ethyl 2-methylbutyrate	fruity	1050	837	R, O, M	8[c]	8[c]	8[c]
raspberry aroma[d]					3.8	5.9	3.5

[a] R, Retention index match with authentic standard on both columns; O, Odor match with authentic standard; M, mass spectrum match with authentic standard. [b] Compounds with values that were different by a ratio of 4 (LSR = 4.0) from the heated sample values had statistically significant differences from the heated sample [c] Charm values <8 were replaced by 8, the limit on zero for these data. [d] Sensory test results where 1 = weak raspberry aroma and 9 = strong raspberry aroma. ANOVA showed a significant sample effect but not panelist or replication. Heated raspberries were significantly different from both fresh raspberries and heated raspberries in cream at $\alpha = 0.05$.

Table 2. Odor Spectrum Comparison of Solvent-Extracted and RAS Headspace Sampled Fresh Raspberries

| compound[a]	odor	retention index OV101	odor potency extraction	RAS
β-damascenone	damascenone	1353	100	100
ethyl 2-methylbutyrate	fruity	836	40	10
ethyl butyrate[b]	fruity	785	13	<10
raspberry ketone	raspberry	1490	12	<10
vanillin	vanilla	1346	9	10
(Z)-3-hexenal	grassy	770	8	10
sotolon	maple syrup	1065	5	35
β-ionone	floral	1458	6	<10
2,6-nonadienal[c]	cucumber	1122	5	<10
unknown	burnt toast	842	5	<10
unknown	plastic	1645	5	<10
ethyl isobutyrate[c]	fruity	742	5	<10
unknown	tomato	863	5	<10
furaneol	cotton candy	1028	5	10
1-octen-3-one	mushroom	952	1.3	13
diacetyl	buttery	560	<1.3	35
1-hexen-3-one	leaves	747	<1.3	30
1-nonen-3-one	mushroom	1052	<1.3	15
unknown	leaves	1116	<1.3	10

[a] See Table 1 for identification method. [b] Identified by retention index, odor, and mass spectral match. [c] Tentatively identified by retention index and odor match

in which Ψ is equal to the perceived intensity of a stimulant, k is a constant, Φ equals stimulus level, and n is Steven's exponent. Steven's law exponent, n, of 0.6 for olfaction (Stevens, 1958, 1960) was used to convert all charm values to odor potency values. The use of Steven's law to compress GCO data was recently proposed by Ulrich Fischer when he used an exponent of 0.3 to transform AEDA data (Fischer and Berger, 1995). The compressed data have the added value of being normally distributed and amenable to statistical comparison (Acree and Barnard, 1994). This type of transformation is required when the data are to be used to study differences between samples of the same type. However, when the data are to be used to compare different types of samples, raspberries and grapes, for example, an odor spectrum could be more illuminating in the same way a mass spectrum eliminates most of the effects of instrumentation, methodology, and con-
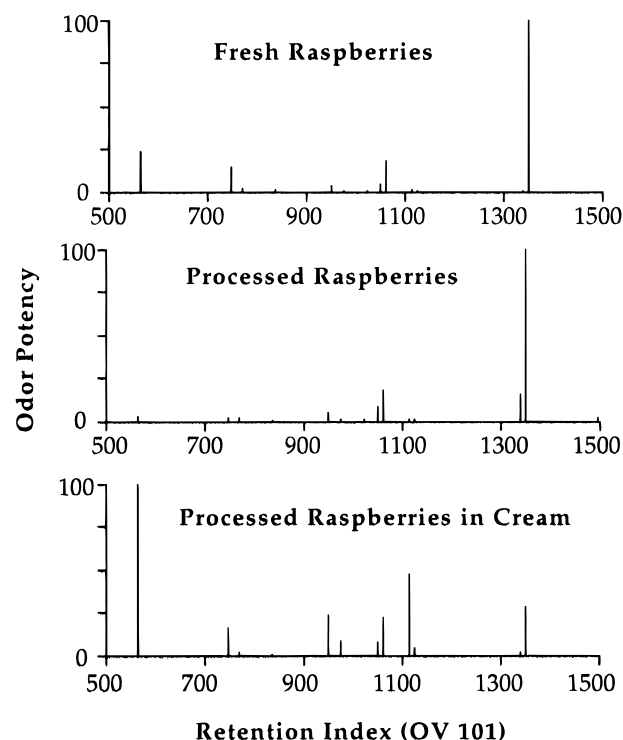
Figure 2. Odor spectra of raspberry samples analyzed by RAS–GCO.

centration from a comparison of two different molecules. To make an odor spectrum from transformed GCO data, the odor potency values are normalized to the odor potency of the compound with the highest potency as 100. In an odor spectrum charm values and flavor dilution values, which equate to stimulus level, are transformed into odor potency values, which relate to relative perceived intensity more accurately than charm values or dilution values do. Figures 2 and 3 show odor spectra made from GCO data.

The odor spectrum could be improved by using the actual Steven's law exponents, which can range between about 0.3 and 0.8, for each odorant in the chromatogram. This would result in a different transformation for each odorant and change the order of odor potency;

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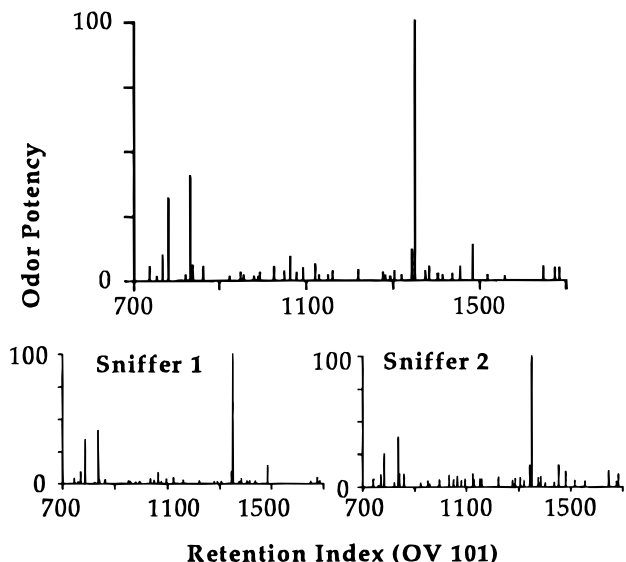
Figure 3. Odor spectra of fresh raspberries as sampled using Freon and ethyl acetate solvent extraction, showing the similarities between the two sniffers.

however, when the exponents are not known, using a median value of 0.5 or 0.6 would be a reasonable compromise.

Fresh Raspberries RAS–GCO. Each of the 12 compounds was identified by matching the odor and retention index on two columns with an authentic standard. However, only six were identified by mass spectrometry. The reported odor thresholds of furaneol, sotolon, octanal, 1-nonen-3-one, and 1-octen-3-one were 40, 1, 700, 0.008, and 5 ng/kg, respectively (Ott et al., 1996; Leffingwell and Leffingwell, 1991). Thus, in this case, they were perceived by the human nose but not by a HP 5970 GC/MS. β-Damascenone, with a characteristic floral aroma, had the largest odor potency. This compound was found using GCO to be a potent aroma compound in many natural products such as apples, grapes, loveage, and coffee (Braell, 1986; Cunningham et al., 1986; Blank et al., 1992; Blank and Schieberle, 1993). Diacetyl, abundant in many foods, contributes the buttery notes. Sotolon, the characterizing aroma compound of cane molasses (Tokitomo et al., 1984), sherry (Martin et al., 1992), fenugreek seeds, (Girardon et al., 1986), and lovage (Blank and Schieberle, 1993), caused the maple syrup aroma. The green notes were exhibited by (Z)-3-hexenal, 1-hexen-3-one, and an unknown compound at RI 1116. Likewise, the well-known mushroom compound, 1-octen-3-one (Fisher and Grosch, 1987), and the very potent 1-nonen-3-one provided the mushroom aromas. The series of three en-3-one compounds has also been reported in meat-like flavorings (Blank et al., 1994), and 1-nonen-3-one has been reported in yogurt (Ott et al., 1996). Furaneol, a minor odor contributor to raspberries, was found to be a major odor contributor to strawberries (Schieberle, 1994).

While raspberry aroma does change between varieties (Latrasse, 1991), this study only involved the analysis of one variety, Heritage. This may explain the absence of the ionones as important charm contributors. For example, the content of α- and β-ionones varies with the variety and harvest time (Hiirsalmi et al., 1974), both of which could result in this raspberry sample having a low ionone content.

Aroma Changes during Heating. To make comparisons with fresh raspberry, the Lz and the LSR were calculated (Acree and Barnard, 1994). The Lz corre-

sponds to the highest value that was not statistically significantly different from zero. The LSR was the least significant ratio for compounds to be statistically significantly different from each other. In Table 1, values below the Lz, 8, were replaced by 8 and values that differ in ratio by the LSR, 4, were significantly different. Compounds that significantly increased upon heating of raspberries were β-damascenone, sotolon, 1-nonen-3-one, 1-octen-3-one, vanillin, and raspberry ketone. The increase in these compounds caused an increase in the degree of raspberry aroma, as measured by the sensory panel (Table 1). The panelists indicated that the raspberry sample after heating was the most like their concept of "raspberry". They described it as "strong raspberry, ripe raspberry, candy, and preserves". By contrast, the fresh raspberry was described as "fruity, berry, green, mild raspberry, apple, grape, and floral". While the fresh raspberry was seen as fruity, raspberry was not in the descriptors as often as in the heated raspberry.

The compound with the most raspberry character of heated raspberries was raspberry ketone, (p-hydroxyphenyl)butan-2-one. The glucosidic precursor of raspberry ketone (Pabst et al., 1990) is probably converted to the free aroma compound during the heating step. This compound, with a candy-like raspberry aroma, has been shown to increase the raspberry character (Borejsza-Wysocki et al., 1992; Larsen et al., 1991).

The increase of β-damascenone's charm in heated berries lends strong evidence to the presence of glycosidic precursors in raspberries. The presence of β-damascenone precursors in apples, grapes, and tomatoes has been demonstrated (Williams et al., 1992; Buttery et al., 1990; Roberts et al., 1994). The β-damascenone precursor is very heat labile, and increases in β-damascenone with heating have been shown in apples and apple products (Schreier et al., 1978; Zhou et al., 1993). The main precursor to β-damascenone in apples was found to be the arabinoglucoside of the acetylenic diol, and its formation mechanism was postulated (Roberts and Acree, 1995a). Similarly, furaneol and vanillin in raspberries have been found in a glycosidic form (Pabst et al., 1991), and conversion to free aroma compounds upon heating may explain the increase in charm of furaneol and vanillin in the heated samples. However, thermal generation may also be responsible. Furaneol and 1-octen-3-one were shown to increase in strawberry during heating (Schieberle, 1994). Sotolon's increase in charm during heating indicates that it may be formed in a thermally generated reaction. Treatment of lovage and fenugreek extracts with α- and β-glucosidase did not result in an increase in sotolon, thus decreasing the possibility of a glycosidic form of sotolon (Blank et al., 1993). The slight decrease in charm that diacetyl exhibited during heating was also seen in heated strawberries (Schieberle, 1994). Although (Z)-3-hexenal exhibited an increase in charm upon heating, in previous work (Z)-3-hexenal was shown to decrease during heating (Schieberle, 1994).

Aroma Changes in Cream. The addition of 20% cream to the raspberry sample increased the fat content from 0 to 6%. This amount of fat resulted in an odor spectrum different from that of the heated berries, as seen in Figure 2. The raspberry aroma was also perceptibly decreased as seen by the lower sensory score (Table 1). Of the top nine heated raspberry aroma compounds in Table 1, all but two decreased 4-fold or more in cream. Diacetyl, a common constituent of milk

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and milk fat (Badings, 1991), increased with cream addition. The four most potent compounds in heated berries, β-damascenone, vanillin, sotolon, and 1-nonen-3-one, as well as the characterizing raspberry compound, raspberry ketone, all exhibited decreases in charm of 10-fold or more upon the cream addition.

The addition of 1% oil to nonoil systems has been shown to cause about an 80% decrease in volatility of nonpolar compounds such as heptanal, octanal, limonene, and ethyl heptanoate but no effect on more polar compounds such as diacetyl, propanol, and butanal (Buttery et al., 1973; Schirle-Keller et al., 1994). Thus, polar compounds were not expected to be affected by the cream addition to as large an extent as the nonpolar compounds. This occurred for nonpolar β-damascenone (98% decrease in charm) and more polar diacetyl (no significant change). However, most other compounds, nonpolar (β-damascenone and 1-octen-3-one) as well as polar (sotolon, furaneol, vanillin), decreased in volatility with the added cream. One other factor that might affect flavor release is the changing viscosity of the matrix when cream is added. In that case, mass transfer would affect nonpolar and polar compounds similarly.

Limitations to Method. The use of glass syringes for transferring aroma to a GC is not without drawbacks. High boiling point compounds were found to adsorb to glass surfaces (Buttery et al., 1969). It is recommended to silanize the glass to reduce adsorption (Chaintreau et al., 1995) and to replace syringes often to reduce leaks. In initial experiments with a silica trap, a meaty aroma note was observed, which was not trapped. This note, probably due to a highly volatile sulfur compound, was not detected as an important aroma with the syringe method but may be important to raspberry aroma.

The second point to note is that the dynamic release profile was not sampled in this experiment and the GCO chromatogram was an average of the aroma released. The first minutes of raspberry sample purging would not have contained the same aroma concentrations as the last minutes of purging. The initial headspace samples may have contained higher amounts of very volatile compounds than the last samples. A measurement of three highly volatile compounds from raspberries, α-pinene, α-phellandrene, and β-phellandrene, showed that their concentration decreased by 25% after purging with nitrogen for 45 min. That percentage decrease may not be perceptible because the human olfactory system, in general, is not able to perceive differences in odor concentration of <30% (Walker and Jennings, 1991).

Solvent Extraction and RAS Comparison. Figure 3 shows the odor spectrum of solvent-extracted fresh raspberries, with the differences between the sniffers. Both sniffers showed very similar relative sensitivities to the main aroma compounds. This similarity indicated that there was a low probability that the sniffers exhibited a specific anosmia to one of the main raspberry aroma compounds. Table 2 compares fresh raspberry odor between sampling with the RAS and solvent extraction. In odor potency units, the Lz for the extraction method was 1.3, while the RAS was 10. This shows the added sensitivity of the solvent extraction method to minor aroma-contributing compounds.

β-Damascenone was the most potent compound as measured by both methods. The esters, ethyl butyrate and ethyl 2-methylbutyrate, were emphasized with

1 very weak, not even sure its there
2 weak
3 moderately weak
4 moderate
5 moderately strong
6 strong
7 very strong - overwhelming
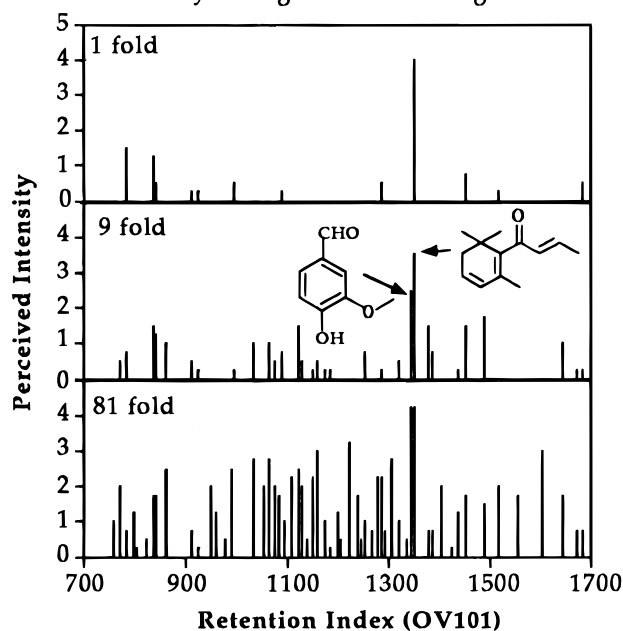
Figure 4. Effect of sample concentration (1-, 9-, and 81-fold) on perceived intensity quantitation for fresh raspberries. Average values for duplicate solvent extractions with one sniffer.

solvent extraction as opposed to RAS sample preparation. Similarly, a greater percentage of compounds as esters were recovered by Freon extraction as opposed to purge-and-trap (Guichard, 1984). Raspberry ketone showed a larger odor potency with solvent extraction as opposed to the RAS. The volatility under simulated mouth conditions may have been low. Several compounds had larger odor potency values in the RAS procedure than in solvent extraction: diacetyl, sotolon, 1-hexen-3-one, 1-octen-3-one, and 1-nonen-3-one. The solvent concentration procedure under reduced pressure resulted in the loss of some low-boiling aroma compounds, diacetyl in this study, which coeluted with the solvent in GCO. In opposition to past studies (Guth and Grosch, 1993, 1994b), furaneol, sotolon, and β-damascenone contributed equivalent or greater odor potency values in the RAS–GCO headspace method than solvent extraction. One possible reason is that the RAS's 37 °C temperature was higher than room temperature solvent extraction. The concentration of these three compounds was shown to increase upon heating. The larger odor potency of the three en-3-one compounds in the RAS procedure as compared to solvent extraction was also demonstrated in a study comparing AEDA and GCO-H of meat-like flavorings (Blank et al., 1994). In general, solvent extraction may have overemphasized compounds low in volatility, while the RAS method, incorporating shearing, air flow, and saliva addition, only sampled those volatiles that were released under mouth conditions. RAS–GCO gave a different aroma profile from solvent extraction, and the results from both may contribute to a complete picture of the aroma of food.

Comparison of Dilution Analysis with Other GCO Methods. The particular concentration of an

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extract or sampling amount has a large effect on the results using perceived intensity quantitation. Results from the solvent extraction of fresh raspberries were used to illustrate the difference between the perceived intensity chromatograms for three different concentrations (Figure 4). The low concentration sample in Figure 4 shows a pattern similar to the odor spectrum of fresh raspberries (Figure 3). If the sample chosen is more concentrated, minor aroma compounds that are not significant by GCO dilution analysis become very prominent. Upon concentration, compounds that are not detected in dilute samples will rise above their detection threshold. At a high concentration, these compounds could have perceived intensity ratings similar to those of the compounds with lower thresholds because of differences in their Steven's law exponents.

For example, β-damascenone had the highest intensity rating (about 4) for all concentrations. As noted in Figure 4, β-damascenone had a low Steven's law constant because its perceived intensity rose slowly with increasing concentration. On the contrary, vanillin's intensity increased with concentration. On the basis of this observation, vanillin would have a higher Steven's law constant, showing that its perceived intensity increased with increasing concentration at rate greater than that for β-damascenone. In the 81-fold concentration sample, vanillin and β-damascenone had similar perceived intensities, while at 1-fold, the perceived intensity of vanillin was lower than that of β-damascenone.

By its design, GCO dilution analysis minimizes random error due to the multiple runs that are made to form the chromatogram. Perceived intensity quantitation likewise must be run multiple times to distinguish noise from actual peaks. It is a technique with the potential to screen samples for an off-aroma and to provide odor-active information if the concentration of the sample is chosen to best match the flavor of the actual food.

Conclusion. Analyzing the RAS headspace volatiles from raspberry products with GCO dilution analysis resulted in high sensitivity to odor-active compounds released under mouth conditions, with a different odor profile from solvent extraction. Several tentatively identified aromas were newly found to contribute to the aroma of raspberry, with the en-3-one series providing an interesting future topic for formation mechanism research. The odor spectrum method for the presentation of GCO dilution analysis data transforms data with a Stevens' law constant and standardizes GCO data so that data from different laboratories can be compared.

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