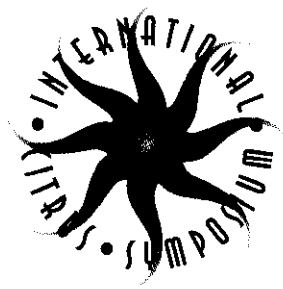

CharmAnalysis of Two *Citrus sinensis* Peel Oil Volatiles

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Essential orange oil is expressed from orange peel during orange juice processing and is a basic flavoring ingredient in most citrus products. The pressed oil from the *Citrus sinensis* variety is the most abundant and extensively used in the flavoring industry. Brazil and Florida produce the largest volumes of cold-pressed orange oil. The Florida late season Valencia and the Brazilian Pera are the two commercially available cold-pressed orange oil sources. Many of the volatile constituents of the natural product oils undergo separation based on their solubility. Then they are grouped by families according to their flavor impact for use in flavoring systems.

Therefore a major task in flavor chemistry is to distinguish the strongly odor-active compounds from the less odor-active ones. Vital to this flavor characterization and classification has been the application of sensory techniques capable of associating flavor intensity or flavor activity with each chemical constituent. Such a technique is CharmAnalysis,** a procedure described elsewhere that uses gas chromatography, olfactometry and computer software to quantitate the odor significance of individual volatile constituents.[1]

In this paper, we will examine the CharmAnalysis of the Valencia and Pera cold-pressed oils to understand how interchangeable the oils can be in beverage systems. We will also identify compounds of odor significance that can be used in flavor formulations, and target compounds for improved QC monitoring.

Experimental

Two commercially important *Citrus sinensis* peel oils were sourced: the late season Florida Valencia and the Brazilian Pera, which is composed mainly of Pera Rio with lesser amounts of Natal and Valencia. Each oil from the 1993-1994 and 1994-1995 crop years was sourced from three different suppliers. Composite samples of Valencia and Pera were prepared by blending 20 grams of oil from each supplier and each crop year to produce a master batch representative of an average oil quality. Serial dilutions at a factor of three were prepared (1:3, 1:9, 1:27 and 1:81) from the original concentration of 5% oil solution in an ethyl acetate base.

Charm, a bioassay for flavor analysis, combines sniffing of the gas chromatographic effluent with the measurement of ethyl ester standard retention indices.[1] The technique measures the odor intensity of separated volatiles of natural compounds in units of Charm over a range of retention indices. Charm is the ratio of the amount of an odor-active compound to its detection threshold in air. For this study, a Hewlett Packard 5890 gas chromatograph was modified in such a way that the effluent was mixed with a stream of humidified air (50-75% relative humidity).[2] Duration, not perceived magnitude, of odor stimulus was recorded. Magnitude was computed from responses collected from the serial diluted samples. The diluted samples were chromatographed on a 25 m by 0.32 mm fused silica column coated with cross-linked methyl silicone. Data was collected between 400 and 1,800 N-ethyl ester standard retention indices. We counted the number of times an odor was detected at a specific retention time. Then we calculated the Charm value according to the following formula:

$$C = d^{n-1}$$

* This article was adapted from a speech presented by Brendan Gaffney at the International Citrus Symposium in Orlando, Florida, on January 30, 1996.
** CharmAnalysis is the trade name of Datu, Inc., Geneva, NY

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where C is the Charm value (a percentage), d is the dilution factor of the dilution series and n is the number of times an odor was detected at a given retention time.

We plotted Charm value against retention time. The resulting graphs, called Charm response chromatograms, gave a profile of the odor activity of the sample. Charm values, themselves, are measures of the odor intensities of the individual volatile compounds.

GC/MS analysis was carried out on an HP 5890 gas chromatograph directly interfaced to a Finnigan MAT INCOS 50 Quadrupole Mass Spectrometer. A 50 m x 0.32 mm I.D. DB1 capillary column containing a 0.25 υm film thickness was employed, using helium as a carrier gas. The oven temperature program was 40°C for 4 minutes, followed by an increase of 6° per minute over 15 minutes to 250°C.

GC/MS was used to identify the odor-active constituents detected by CharmAnalysis. GC-FID (flame ionization detection) retention indices of reference standards were determined under the same chromatographic conditions as for CharmAnalysis. Any compound whose retention indices and mass spectra could not be matched with those of reference standards was designated tentative or unknown.

Results and Discussion

Figures 1 and 2 compare a chromatogram produced by GC-FID with a composite Charm chromatogram produced by GC-O of the dilution sample series for the Valencia and Pera orange oil, respectively. In each figure, the horizontal axis—retention index (RI)—is the same in both chromatograms, but the vertical axis is different. FID response is a measure of the relative mass; FID chromatogram peak areas indicate the amount of volatile component present. In GC-O, the dilution value is the number of dilutions a sample must undergo to reach a subject's threshold; peak areas are Charm values (proportional to odor activity units in air) that are equal to the mass of the odorants in the sample divided by the odor detection threshold for the odor and the subject.

The FID chromatogram for each oil is dominated by peaks in the monoterpenes (limonene in the 620-680 RI range and myrcene in the 580-600 RI range) and sesquiterpenes (in the 950-1,250 RI range). The terpene hydrocarbons make up 96% of the orange oil composition and contribute least to the odor-active volatile composition. This is confirmed by their absence or the relatively low number of peaks found in the same positions on the Charm chromatogram with the exception of an odor-active peak under limonene.

The Charm chromatogram is rich in odor-active polar compounds. The compounds that gave similar peak areas on both the FID and Charm chromatogram are octanal (RI 599) and linalool (RI 712) found at levels of 0.5% and 0.4%, respectively. A region of odor-active compounds after decanal (RI 806) is made up of the alcohol citronellol (RI 834) and

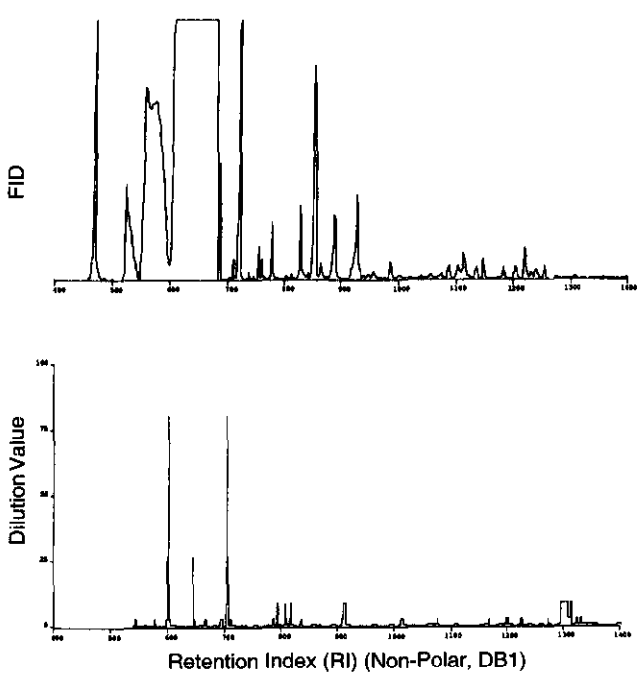

Figure 1. FID chromatogram and Charm response chromatogram of the Valencia orange oil

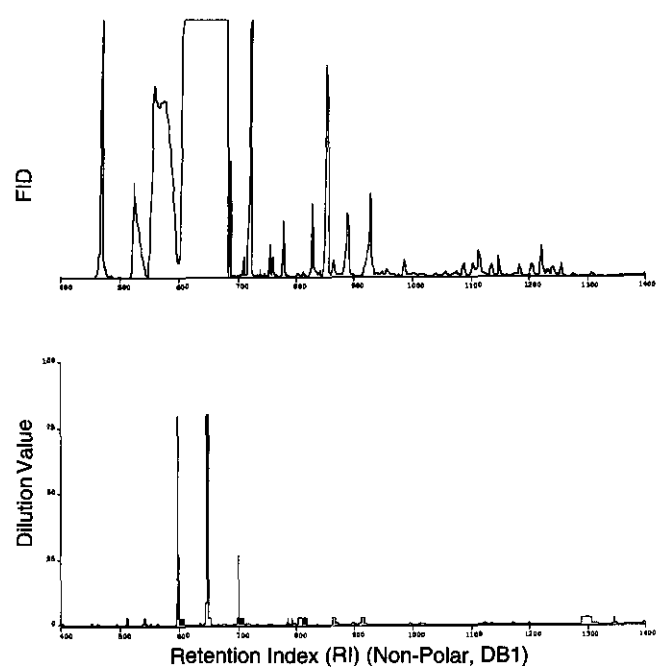

Figure 2. FID chromatogram and Charm response chromatogram of the Pera orange oil

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an unsaturated alcohol (RI 865). These compounds, which were determined to have high levels of odor activity, are common to both orange oil profiles. Hidden among the sesquiterpenes (in the 1,000–1,250 RI range) are other odor-active aldehydes and alcohols. Two prominent peaks near the end of the Charm chromatogram for both oils are the sesquiterpene aldehydes β-sinensal (RI 1,295) and α-sinensal (RI 1,350) found at levels of less than 0.01% in the oil. Specific to Pera in the beginning of the Charm chromatogram is a tiny peak identified as hexanal (RI 400).

The primary odorants of the Valencia orange oil are shown in Table I. These are the compounds for which the % Charm value was at least 1. The identified compounds matched the retention indices, odor character and mass spectra of the reference standards. Five out of the nine compounds were identified as aldehydes, accounting for slightly more than 75% of the total Charm sample. Octanal (No. 1) accounted for nearly half of the total Charm sample. Nonanal (No. 4), which coeluted with linalool (No. 3) on the DB1 column, was separated on a Carbowax column. The % Charm for nonanal was estimated to be 4.2%. The aldehyde compounds are believed to be one of the most important contributors to the flavor of orange oil, and the total aldehyde content is used as one measure of oil quality.[3] β-Sinensal, the sesquiterpene aldehyde, makes a significant contribution to the total % Charm with a Charm value of 14%. The only identified alcohol was linalool, whose fruitloop odor is considered an important aroma in orange oil flavor.

The primary odorants of the Pera oil, shown in Table II, were the same as those of the Valencia oil with the addition of geranial, which was found at slightly higher % Charm in Pera than in Valencia. Geranial (No. 8), the trans isomer of citral, was determined to have a 2% Charm value.

The six most odor-active compounds—octanal, unknown licorice compound, linalool, β-sinensal, nonanal and decanal—are common to both oils and account for 90% of the total Charm, although in different proportions. The findings are significant to their interchangeable features in beverage systems. Sensory evaluations conducted by a trained panel found both oils to taste like orange in a beverage matrix. Minor attribute differences of sweetness and aroma existed between the two oils. GC/MS confirmed the remaining unknown compounds of the Valencia and Pera oils to be at their correct retention indices, however those same compounds could not be verified based on odor description. This leads to the conclusion that the compounds exist at low concentrations under the peaks of known compounds.

An important feature of Charm is its ability to lead chemical identification into new regions of odor activity. A particular region of new odor activity that underwent further investigation was the odor impact of the unknown compound under the limonene peak. This compound (at RI 640) had the largest Charm value in the Pera and the third largest in the Valencia. Never cited in any of the extensive research conducted on orange oil, this licorice odor compound may contribute to limonene's significance in orange oil flavor as a carrier and precursor of other flavor compounds.[4] One of the first in a series of approaches was to try to isolate the licorice odor compound in synthetic d- and l-limonene; however, none of the unknown licorice odor was found there. A chiral column did not find any increased chiral activity over d-limonene standard. The use of various column polarities (DB1, DB5 and Carbowax) failed to separate the compound from the limonene peak. A slow isothermal program (with initial time 40 minutes at initial temperature 40°C) eluted the compound, but separation from the limonene was incomplete. The use of column chromatography found the odor collected in 100% pentane fraction only. A final attempt using Multi Dimensional Gas Chromatography (MDGC [Siemens, Sichromat II]), tried to heart-cut the odor volatile away from the limonene chromatographed on a Megabore HP-1 30 m x 0.53 mm x 1.5 υm. This was done by cryotrapping the odor on tenax multiple times at the interphase of the double column oven system. The odor underwent subsequent thermal desorption into the second column Carbowax 30 m x 0.32 mm x 0.5 υm directly interfaced with the mass spectra. The initial trials with the MDGC failed to separate the unknown licorice odor compound from limonene. Although this series of experiments fell short of isolating and identifying the compound, the information gathered revealed the com-

Table I. Primary odor-active volatiles of Valencia orange oil

No.	Charm (RI)	Compound	Descriptor	% Charm
1	599	octanal	citrus/aldehydic	45.0
2	640	unknown	licorice	16.0
3	712	linalool	fruitloops	39.7
4	712	nonanal	fatty-floral	4.2
5	794	unknown	sweet/fresh/candy	1.6
6	806	decanal	sweet/citrus/orange	5.5
7	816	unknown	nutty/spice/licorice	2.1
8	909	undecanal	citrus/peely/orange	6.4
9	1,298	β-sinensal	fishy/herbal	14.0

Table II. Primary odor-active volatiles of Pera orange oil

No.	Charm (RI)	Compound	Descriptor	% Charm
1	599	octanal	citrus/aldehydic	35.0
2	640	unknown	licorice	38.0
3	712	linalool	fruitloops	12.8
4	712	nonanal	fatty-floral	4.2
5	794	unknown	sweet/fresh/candy	2.1
6	806	decanal	sweet/citrus/orange	4.0
7	816	unknown	nutty/spice/licorice	1.5
8	866	geranial	lemon/lime/citrus	2.0
9	909	undecanal	citrus/peely/orange	3.5
10	1,298	β-sinensal	fishy/herbal	8.2

pound to be confined to natural orange limonene of trace concentration with uncharacteristic terpene odor.

Summary

CharmAnalysis was used to measure the odor intensities and qualities of odor-active components of two commercial sweet orange oils: Florida Valencia and Brazilian Pera. Charm chromatogram results of the Valencia and Pera oils indicated that the most odor-active constituents are associated with the polar fraction compounds, which do not correspond to the major chemical compounds (terpenes) in orange oil. The major Charm responses of the Valencia and Pera Charm chromatograms were the straight chain aldehydes (C_8-C_{14}), β-sinensal and the alcohol linalool. These compounds were later judged to be key to future flavor formulations and improved QC monitoring of the oil. An unknown compound of licorice odor character, which coeluted with limonene, demonstrated considerable aroma activity and was determined to be the most odor active compound in the Pera oil. A series of employed chromatographic techniques (including chiral, column polarity and MDGC) failed to separate the non-polar compound from limonene, while further CharmAnalysis confirmed the compound to be present only in natural d-limonene. The higher dilutions of both oils produced Charm responses from the same five compounds, supporting interchangeable odor features of the Valencia and Pera oils. A comparsion of the taste profiles of Valencia and Pera oils by a panel of trained flavorists found the oils to have interchangeable flavor functionality in beverage systems.

Apart from showing close odor behavior of the two oils and identifying key components as a target for flavor formulation and QC monitoring, the Charm technology has demonstrated itself to be a powerful research tool in discovering new regions of odor activity. As can be seen in this application, CharmAnalysis presents a new opportunity for further research and understanding of the volatiles of orange oil flavor.

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