Journal of Chromatography, 106 (1975) 73–81

CHROM. 7990

COMPUTER EVALUATION OF GAS CHROMATOGRAPHIC PROFILES FOR THE CORRELATION OF QUALITY DIFFERENCES IN COLD PRESSED ORANGE OILS

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(Received October 7th, 1974)

SUMMARY

Cold pressed orange-peel oils were evaluated by essential-oil experts and analyzed by gas–liquid chromatography. The chromatographic data were correlated using a computer program that determined mathematical combinations of peak areas that served to distinguish between the samples. A set of sample component ratios was ascertained which, when calculated for each sample, yielded values that ranked the samples in the same order as the experts.

INTRODUCTION

In the past, the selection of essential oils for use in flavorings and fragrances has been dependent upon the olfactory talents of persons trained in evaluation of these types of materials. Of particular interest are the essential oils derived from vesicles in the flavedo of *Citrus sinensis*, the so-called cold pressed sweet orange-peel oils. Much of the early work concerning the chemistry of orange oils employed classical techniques which led to the specifications established by the Essential Oil Association of the U.S.A.[1] and the United States Pharmacopoeia[2]. With the advent of more sophisticated methods of analysis, particularly gas chromatography–mass spectrometry (GC–MS) and gas chromatography– infrared spectrometry (GC–IR), chromatograms containing over 340 peaks have been obtained[3] and more than 120 compounds were identified[4] by a multitude of workers in the field[5–15].

Although much of the chemistry of orange oil components has been published[16], there did not exist an objective method for measuring the "quality" of these oils as determined by the flavor expert. A basic problem was the choice of an analytical technique which would be sensitive to the chemical changes detected by the experts as changes in quality. Intuitively, the place to start was with a definition of what the technique should accomplish.

Historically, the expert's evaluation of essential oils has depended upon his olfactory sense. He discerns aromas essentially based on two criteria. The first is the

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vapor pressure of the substance or substances at ambient temperature, and the second is the discrimination for various molecules inherent in his olfactory sense.

After first impressions, a portion of oil is allowed to "dry"; that is, the compounds with high vapor pressure are allowed to evaporate, leaving behind generally higher-molecular-weight compounds of lower vapor pressure. These compounds may be in any concentration relative to those of higher vapor pressure; however, they are often present in relatively low concentrations and often are the source of characteristic so-called "off-flavors", or odors.

The analytical technique in question must be capable of separating and distinguishing between a large number of different molecules whose chemical and physical properties may vary over a wide range. It should be sensitive to differences in vapor pressure of these molecules at various temperatures and to other chemical properties, in particular, functional groups (alcohols, aldehydes, esters, amines, mercaptans, etc.) to which the human olfactory sense responds. In addition, it should be capable of detecting relatively low concentrations of substances with lower vapor pressures.

Employing temperature-programmed, gas–liquid chromatography with high-resolution capillary columns and specific detectors, it is possible to design a technique that fits the definition almost exactly. As with any chromatographic procedure, the basis for sample discrimination is the component separation provided by the analytical column. Often the column material influences stationary-phase parameters, such as film thickness, uniformity, etc., in capillary columns and thus affects the separation qualities of the column. With packed columns, the uncovered wall may enter directly into the chromatographic separation. For these reasons, several column materials, including stainless steel, nickel, copper and glass, were evaluated. Properties of the stationary phase, such as temperature stability, relative polarity, ease (or difficulty) in coating, prior uses and results, as well as the separation obtainable for a particular type of sample, all influence the choice of the phase or phases to be employed. Emulphor ON-870, Triton X-305, Carbowax 20M, and polypropylene glycol were investigated in this study.

Sampling procedures and inlet systems have proven to be sources of difficulty in the analysis of cold pressed orange oils in the past. The blame for these problems has generally been attributed to the so-called "waxes". These compounds have been characterized as C_{20} to C_{30} paraffins, are relatively non-volatile, and tend to precipitate slowly over a period of months at subambient temperatures. In addition, the fact that one compound, limonene, is generally present in concentrations over 90% has often made detection of minor components difficult. Several sample-preparation and introduction methods, including the use of microsyringes, sample splitting, adsorption of headspace volatiles and solvent extraction, were evaluated in anticipation of problems from these sources.

After evaluation of the chromatographic parameters (required to produce chromatograms sufficiently detailed for differences to be ascertained between sets of samples ranging from very high quality to unusable, as rated by flavor experts) the data were fed into a sophisticated computer program. The program was developed to find correlations among groups of analytical data that are representative of sample differences[17,18]. Once the discriminating combinations were determined, a second computer program was written to provide only these necessary calculations and to assign each oil a proper quality value. Although it is possible to use only the one best

discriminating combination for this assignment, a representative group of differentiating combinations including peaks from all areas of the chromatogram is preferred for statistical reasons.

EXPERIMENTAL

Instrumentation

A Perkin-Elmer 900 gas chromatograph (Perkin-Elmer, Norwalk, Conn., U.S.A.) equipped with a flame ionization detector was the basic instrument employed for this work. The chromatograms were recorded on a Leeds and Northrup, Speedomax G recorder (Leeds & Northrup, Philadelphia, Pa., U.S.A.) and the peak areas determined using a Hewlett-Packard Model 3370A digital integrator (Hewlett-Packard, Avondale, Pa., U.S.A.). The correlations were developed using an IBM 360 computer system.

Materials

The preparation of the nickel capillary columns used in this work was that developed by Bertsch *et al.*[19]. The material of choice for these columns was 0.5 mm I.D., 0.8 mm O.D. Nickel 200 tubing, 100 m in length (Handy and Harman Tube Co., Norristown, Pa., U.S.A.). Basically, preparation of the columns consisted of fitting the tubing with nuts for attachment to the chromatograph, cleaning the metal with suitable solvents and etching the inner tubing wall to provide a suitable surface for the stationary phase. All columns were coated by the dynamic plug method. Carbowax 20M (Analabs, North Haven, Conn., U.S.A.) was the stationary phase of choice. A 5% by weight solution in spectrograde chloroform (Mallinckrodt, St. Louis, Mo., U.S.A.) gave the best results. Three percent Silanox (Grade 101, particle size 6–10 μm, Cabot Corporation, Boston, Mass., U.S.A.), a hydrophobic, fumed silicon dioxide powder, was added on a volume to volume basis (*e.g.* 3 ml Silanox per 100 ml of phase solution). These columns contained in excess of 100,000 theoretical plates thus yielding HETP values of less than one millimeter. The chromatographic parameters employed are shown in Table I.

TABLE I

CHROMATOGRAPHIC PARAMETERS

Column	0.5 mm × 100 m nickel
Phase	Carbowax 20M
Sample	0.1 μl —direct, whole-oil injection
Injection-port temperature	180°
Amplifier range	10°
Chart speed	0.6 cm/min
Detector	FID; hydrogen —1.2 atm; air —3.4 atm
Temperature program	
0–60 min	80–200° at a rate of 2°/min; inlet carrier pressure, 0.8 atm; carrier flow-rate, 5.6–4.0 ml/min
60–120 min	Isothermal at 200°; inlet carrier pressure 0.8 atm; carrier flow-rate, 4.0 ml/min

RESULTS AND DISCUSSION

Based upon earlier unpublished work, the assumption was made that quality changes, as discerned by essential-oil experts, result from oxidation and/or hydrolysis of particular acyclic, monocyclic and bicyclic monoterpenes. For this reason, two samples of cold pressed orange oil were hydrolyzed and oxidized to varying degrees by a combination of sunlight, atmospheric oxygen and water vapor. The experts rated these as very poor quality. If, as suspected, the experts determined quality based upon changes due to oxidation and hydrolysis, these samples would serve as the low end of a quality scale. Fig. 1 shows the adequacy of the analytical method for distinguishing between samples of high quality and very poor quality. Obvious differences, some peak areas changing by orders of magnitude, may be seen. Other less obvious, but extremely significant, changes were detected by the computer evaluation.

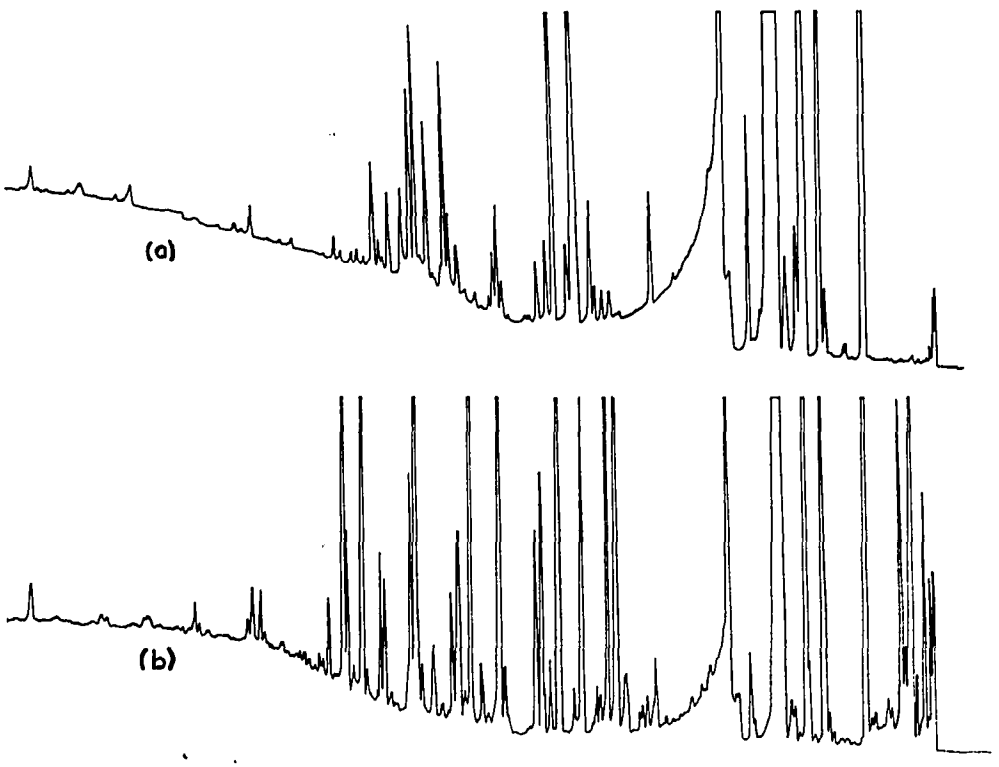

Fig. 1. Comparison of high-quality (a) and very-poor-quality (b) orange oils.

When searching for specific correlations among a set of samples, the results will be more valid if the set can be subdivided into more than two classes and these can be ordered to give a data base that shows a continuous change from one extreme to the other. If only two classes are considered, then all differences between the groups correlate, some relationships being due to factors other than the one of interest. When several sample classes of known order are considered and only those correlations ac-

cepted that rank the samples in that order, then the assumption that the desired variable is the object of the correlations should have greater validity. Care must be taken not to restrict the ordering beyond the limits or ability of the basis for distinction, in this case, the flavor experts. To do so would force inaccurate restraints upon the system and possibly obscure desired correlations.

The samples that served for the computer data set are shown in Table II. These samples were divided into three quality classes upon which all of the experts were in agreement. Fig. 2–4 are chromatograms from each of the three quality classes. There was an obvious quality distinction between class II and class III while the difference between class I and class II was not so great. The experts did not agree on the relative quality ranking of the samples within the classes. The order shown in Table II was based upon an average of their evaluations. For this reason, no constraints were placed on the sample ordering within each class. However, no overlap was allowed between the three classes in developing the correlations because all experts agreed on these divisions.

TABLE II

SAMPLES FOR COMPUTER CORRELATIONS

Class no.	*Relative quality rank*	*Sample quality description*	*No. of replicate analyses*
I	1	Very high quality Fully acceptable	4
	2	Very high quality Fully acceptable	3
	3	High quality Fully acceptable	3
	4	High quality Fully acceptable	3
	5	High quality Fully acceptable	5
II	6	Average quality Usable	3
	7	Below average quality Usable	3
III	8	Very poor quality Unfit for use	4
	9	Very poor quality Unfit for use	3

A basic understanding of the computer correlation program used for this study should clarify the results. The program is of a general nature and will accept data from different analytical techniques. For chromatographic data, any mathematical combination of up to four peaks may be considered. Thus for two peaks, A and B, from a chromatogram, combinations such as A + B, A−B, A/B, etc. may be calculated for these same two peaks in all chromatograms. The results are then checked to determine if this combination does correlate with the quality classes. Certain statistical parameters are calculated to determine the differentiating efficiency of each combination

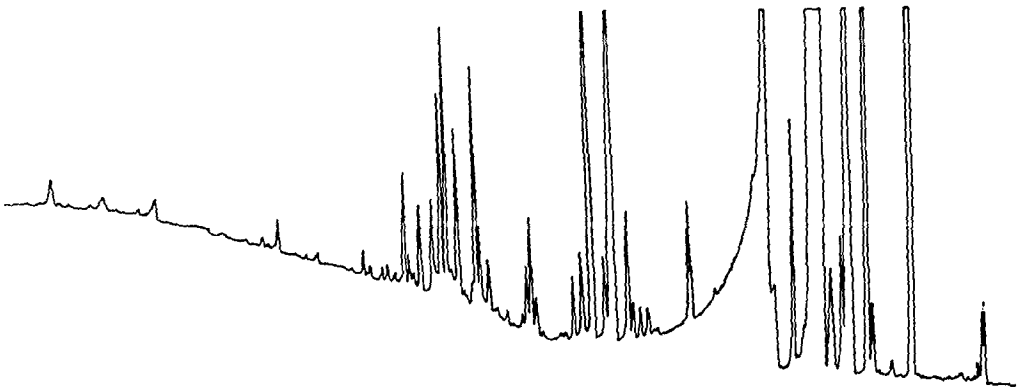

Fig. 2. High-quality Valencia orange oil.

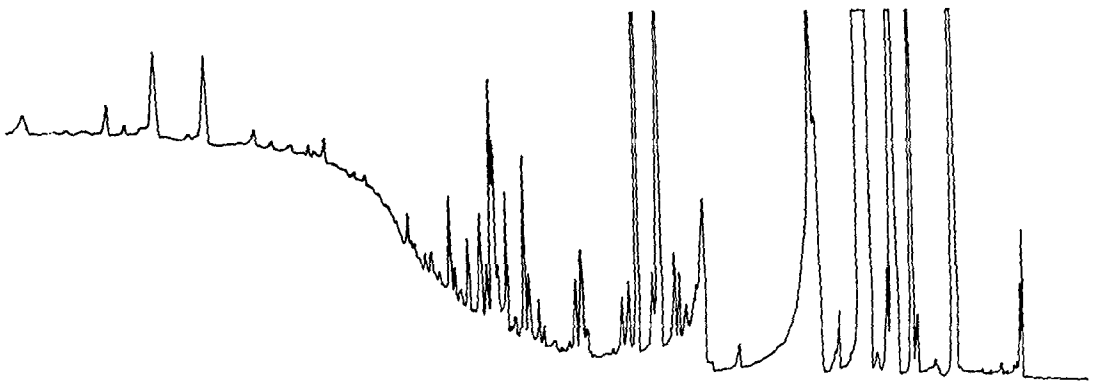

Fig. 3. Acceptable-quality Valencia orange oil.

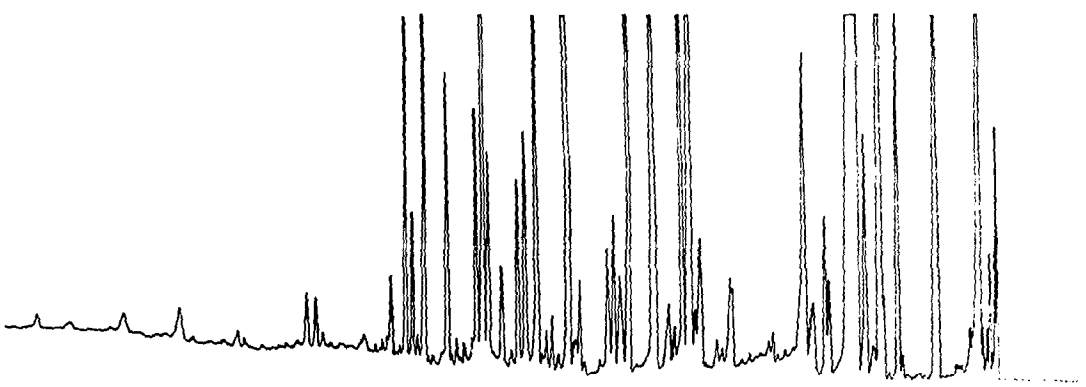

Fig. 4. Very-poor-quality Valencia orange oil.

and the combinations printed in order of their discriminating ability. Often sums and differences of certain peaks are found to correlate well; however, these combinations are subject to many technique and operational errors, such as varying sample size. For this reason, the use of ratios is often preferred because errors of this sort divide out.

The tendency of a peak to be either in the numerator or denominator of a ratio indicates whether it is a "good" component or a "bad" component from a quality standpoint. The program is written in such a manner as to pick combinations that yield larger values for the better-quality samples. Thus, if a compound is found in larger quantity in a high-quality sample, it should tend to be in the numerator, while a compound found in larger quantity in a poor-quality sample would tend to be placed in the denominator.

For this work, it was not necessary to identify the compounds represented by each peak because the things of interest were the differences in the peak areas (or the chromatogram profiles) from sample to sample. Earlier work in the field, particularly GC–MS and GC–IR studies with Carbowax 20M capillary columns, has allowed the tentative identification of many peaks. The tentative names for particular peaks of interest allowed some indication of the validity for the correlations discerned. For example, based upon comparisons of absolute peak areas for high-quality *versus* very poor-quality oils, there appeared to be a decrease of approximately 30% for limonene and 75% for α-pinene with corresponding increases in the oxidation, hydrolytic and other degradation products.

In this study, 155 peak areas were determined from each chromatogram and transferred to computer punch cards. These cards contained the absolute peak areas as measured by the integrator from each chromatogram. No normalization, scaling, etc., was used to correct for sampling, methodology, or other errors. If any peak was absent or immeasurably small in any chromatogram, it was assigned a value of approximately one part in ten million of the total area of the chromatogram. This was done in order to prevent the computer program from skipping the peak in all chromatograms, which it would do to avoid division by zero. Results of replicate analyses were averaged for each sample by the computer before the correlations were attempted. Initially, a computer run was made in which only ratios of two peaks (A/B) were considered for all peaks in each averaged chromatogram. Five hundred and eighty-four combinations resulted that ordered the three quality classes correctly with no overlap. When ratio combinations of three peaks (*e.g.*, A/(B + C), 1/(A + B + C), (A + B)/C, etc.) were considered, 10,000 combinations were found after only considering combinations of the first 27 peaks with all others, and the run aborted. The number of peaks was reduced to 68, which appeared most frequently in the first two runs, and a third run with ratio combinations of three peaks was made. Over 50,000 ratios were computed and 6056 were accepted as differentiating between the three quality classes with no overlap and placing the classes in the correct quality order (class I better than class II better than class III).

After other considerations such as discriminating ability, ordering of samples within each quality class and the particular compounds in combination, the number of ratios was reduced to 120. These 120 ratios incorporated 63 of the 68 peaks allowed for consideration. Of these, the peaks representing α-pinene and linalool occurred most frequently. Either or both appeared in 70 of the 120 ratios. Without exception, α-pinene appeared in the numerator, either alone or summed with another peak, and linalool appeared in the denominator, either alone or summed with other peaks. This indicated that a large amount of α-pinene was found in the higher-quality oils with less in the poorer, with the reverse being true for linalool.

A small computer program was written that took the areas for the 63 peaks,

calculated the values for the 120 ratios, and scaled each by an appropriate factor such that the sum of the weighted ratio values should equal 500 for an average oil. By giving all ratio values equal weight (1/120 of 500), the requirement for making apriori decisions about the significance of each ratio to the total quality was removed.

Instrumental precision and technique error

Five replicate analyses were made of an oil under standard conditions over a period of two days to evaluate overall instrumental precision, method and technique error, as well as sampling procedure. It was possible to overlay two chromatograms with no observable differences in peak shape or retention times indicating reproducible instrument operating parameters and stable column conditions. Some differences in sample size were apparent and were attributed to the microsyringe used to make the injections. This problem was corrected and the discrepancies in sample size essentially disappeared. To preclude, as much as possible, that sample-size differences and other errors would lead to unwanted correlations, all runs for each sample were averaged.

The overall error in the final quality-rating value for the five standard runs was reduced by employing the following considerations: (1) only ratios (not sums, differences, etc.) were used in the quality rating program to eliminate sample-size error; (2) only those ratios with strong discriminating ability were chosen; (3) combinations of three peaks (instead of two) were chosen to give more ratios with better discriminating ability; and (4) the ratios were scaled such that each contributed equally and represented only 4% of the final rating value. Thus, five replicate analyses of the same oil over a two-day period yielded quality rating values that had a total standard deviation from the mean of ±2%. If the data for the five runs were averaged prior to entering it into the quality-rating program, a value was obtained that differed from the mean by slightly more than 1%. Thus, the uncertainty in the quality-rating number may be lowered by using the average data from several runs to evaluate the quality of an individual sample.

Table III shows computer quality-rating values for the nine oils used to develop the correlations. In addition, the average and standard deviation for each oil are given. Note that the range of standard deviations for samples five and six or samples seven and eight do not overlap. Also, the samples are arranged in order of the combined

TABLE III

QUALITY RATING OF COLD PRESSED ORANGE OILS

	Quality class I Very high–high quality Fully acceptable					*Quality class II Average–below quality; Usable*		*Quality class III Oxidized Unfit for use*	
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Computer rating number	860	719	748	756	717	586	465	121	107
	781	736	707	666	691	612	529	115	106
	781	730	684	657	676	526	498	120	99
					681				
					698				
Average	807	728	713	693	693	575	497	119	104
Standard deviation	±37	±7	±26	±44	±14	±36	±26	±3	±4

expert's quality rating, with the best sample being first, the next second, etc. The average computer quality-rating number placed the nine samples in the same order. A second set of samples, which contained oils from several varieties of fruit as well as from different seasons of the year, was evaluated by both the experts and the computer. Again, they were in direct agreement.

CONCLUSIONS

An analytical technique has been developed that allows an objective quality evaluation of cold pressed orange oils. The technique has been shown to parallel the essential-oil expert's opinion of sample quality and should prove to be a useful tool in further studies of orange-oil quality. With the chromatographic procedure well in hand, the extension of this work to include other essential oils should follow directly.

ACKNOWLEDGEMENTS

This research was supported by The Coca-Cola Company, Foods Division, Houston, Texas, U.S.A. The authors wish to thank Dr. R. E. Biggers of The Coca-Cola Company for providing the computer correlation program.

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