Organoleptic Techniques in Chromatographic Food Flavor Analysis

Thomas H. Parliment* and Richard Scarpellino

The ultimate goal of basic flavor research is to establish the identity and importance of the volatile components in a flavor essence. This paper presents a scheme which has been developed for establishing the organoleptic importance of the individual components of a flavor in a systematic fashion. The procedure involves collecting portions of the gas chromatographic effluent in an appropriate medium and then evaluating the individual fractions and combinations thereof, by modified flavor profile techniques. The analysis of blueberry essence is used to illustrate the utility of these procedures and it is shown that linalool and *cis*-3-hexenol are essential to a characteristic flavor.

The ultimate goal of basic flavor research is to establish the identity and importance of the chemicals responsible for the characteristic aroma and flavor of foods. The achievement of these goals is complicated by a number of factors. First, substances which contribute to the flavor are typically minor constituents of the total volatile complex, frequently at parts per billion levels. Second, the substances which contribute to flavor and aroma comprise many different classes of organic compounds. Finally, a typical flavor essence has a wide boiling point range and many of the individual compounds are quite reactive species. In order to evaluate the important flavor constituents it is normally necessary to (1) isolate the aromatics, (2) separate the aromatics, (3) identify the individual compounds, and (4) determine the organoleptic importance. The purpose of this paper is to emphasize the latter step, which deals with the question: "How do you determine which of the many organic compounds in an aroma are critical to the aroma and which are superfluous?"

Various methods to this end have been utilized in flavor analysis. One of the first procedures followed in the early days of gas chromatography was to identify all compounds and recombine at the appropriate level. Occasionally, this was successful as in the case of blue cheese flavor where Anderson and Day (1966) used quantitative data to prepare a synthetic blue cheese flavor. Most times, however, this technique failed to give products with recognizable flavors.

The use of triangle testing methodology to evaluate the importance of fractions was practiced as early as 1960 when Jennings et al. (1960) studied the volatiles of pear aroma. In this study, the fractions were evaluated in either ice water or canned pear nectar, and it was concluded that several fractions contributed significantly to desirable pear aroma. A useful concept developed in their research was the evaluation of isolated components in a medium appropriate to the sample.

More recently, statistical techniques such as multiple regression analysis of panel sensory scores have been used to interpret the flavor significance of gas chromatographic data. For example, Persson et al. (1973) presented optimal correlation coefficients for certain odor qualities using combinations of four previously identified canned beef aroma chemicals.

In this paper, we present a scheme which permits the flavor chemist to determine the flavor compounds of major organoleptic importance in a systematic fashion. The procedure combines gas chromatographic separations and recombinations in conjunction with modified flavor profile techniques; the information thus generated is transmitted to the creative flavorists for use in compounding efforts. Our studies on blueberry flavor will be used as an example of the technique, although we have found the procedures amenable to coffee and meat flavor research when appropriate evaluating media are employed.

General Foods Technical Center, White Plains, New York 10625.

Table I. Evaluation of Blueberry Fractions

Step	Fraction	Aromatic character	Blueberry character
I	T	Very sharp, apple, green blueberry	+
II	A	Apple, green, fruity	–
	B	Green, apple, sharp	–
	C	Weak blueberry, green, burnt	+
	A + B	Apple, green leaves	–
	A + C	Fruity, estery, blueberry	+
	B + C	Strong green, fruity, sl. blueberry	+
III	D	Sharp green, green leaves, sl. vegetable	–
	E	Perfumy, citrusy, lemon	–
	F	Soapy, acrid, oxidized	–
	D + E	Good blueberry	+
	D + F	Green, acrid, soapy, burnt	–
	E + F	Sharp, green, fruity	–
IV	G	Nice fresh green, leaf alcohol-like	–
	H	Citrus-like, aldehydic, green	–
	J	Cucumber, vegetable	–
	G + H	Blueberry, citrus, berry	+
	G + J	Green vegetable	–
	H + J	Lemon, green, apple, vegetable	–
V	K	Green apple, fruity	–
	L	Nice fresh green, sharp green	–
	M	Lemony, part of blueberry	?
	K + L	Green apple	–
	K + M	Green berry, raw fruit	–
	L + M	Blueberry	+
VI	N	Sharp fresh green	–
	P	Apple, green	–
	Q	Lemon	–
	N + P	Sharp green	–
	N + Q	Blueberry, too green, citrus	+
	P + Q	Citrus, low blueberry	?
VII	R	Nice green, leaf alcohol-like	–
	S	Green, leafy, sharp	–
	T	Lemon, citrus, berry, lo berry	?
	R + S	Sour apple, sharp green	–
	R + T	Blueberry	+
	S + T	Sour sharp berry	+

EXPERIMENTAL SECTION

Preparation of Blueberry Essence. The blueberry essence was prepared by the atmospheric distillation of fresh blueberries as described previously (Parliment and Kolor, 1975).

Sample Preparation/Collection. Samples were separated in a Perkin-Elmer Model 900 gas chromatograph using a 1/8 in. × 8 ft column containing 10% SP-1000, a

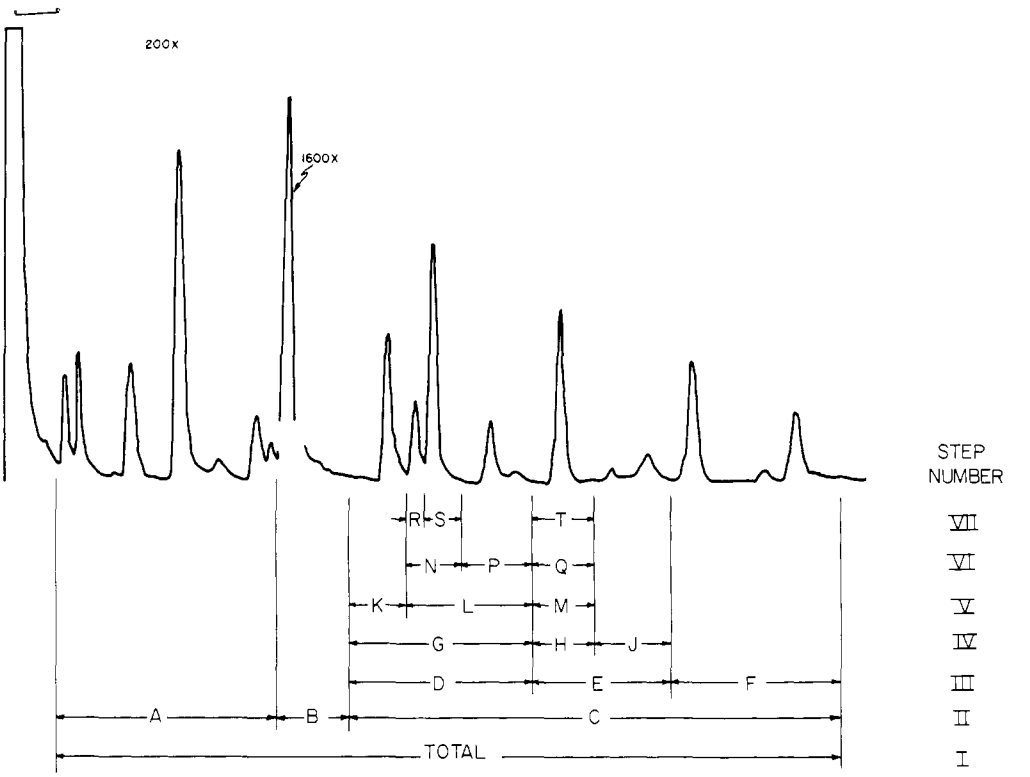

Figure 1. Relationship of gas chromatogram of blueberry volatiles to chromatographic regions evaluated.

modified Carbowax 20M, on 80–100 mesh Supelcoport. The column was temperature programmed from 80 to 240 °C at 6.5 °C/min. A typical gas chromatogram is presented in Figure 1.

Fractions were collected by diverting the gas chromatographic effluent through a glass tube (1 mm i.d.) into the bottom of a 10-ml graduate filled with a beverage base (Parliment, 1976). This base was prepared by dissolving 9 g of sucrose and 0.2 g of citric acid in 100 ml of spring water.

Sample Evaluation. Samples were evaluated directly as well as in various combinations according to the scheme developed below. These samples were evaluated by members of our flavor laboratory and by profile evaluation panel members who tasted the above solutions. Normally, three to five panel members were utilized for a given test. These panelists were asked to record on a ballot both the predominate aromatic character as well as the amount of blueberry quality perceived using a 14-point intensity scale. A composite profile for each sample was developed by averaging the individual panel member's intensity ratings.

RESULTS AND DISCUSSION

General Discussion. The general technique which we employ in our flavor research may be described briefly as follows. (1) Prepare aroma concentrate. (2) Separate aroma concentrate via gas chromatography. (3) Bubble the total gas chromatographic effluent into an appropriate medium and evaluate to ascertain whether the desired flavor has survived gas chromatography. (4) Develop modified profile evaluation panel ballot, listing desired flavor characteristics. (5) Separate sample via gas chromatography and trap several fractions, then recombine the gas chromatographic fractions in a systematic fashion. (6) Determine the organoleptic importance of the recombined fractions. (7) Repeat steps 5 and 6 until the flavor can be associated with individual peaks. (8) Identify these individual peaks. (9) Combine and assess the character of the identified compounds.

Evaluation Technique. The basic premise we employ is that if a flavor is present in a total gas chromatographic fraction, the aroma complex can be separated into several (typically three) individual fractions and the characteristics will be associated with either a single fraction or a combination thereof. Consider the chromatogram of Figure 1. If the region designated TOTAL has the desired character then either A, B, or C or some combinations thereof such as AB, AC, BC, or ABC must possess the character. Assume BC has the effect. Then BC is divided into three fractions and the same type of evaluation is performed. In this fashion, one is led directly to the components of greatest organoleptic importance.

Development of Ballot for Blueberry Flavor. Preliminary separation of the blueberry essence as well as aqueous distillates of fresh blueberries led to the development of the ballot described previously. Use of this ballot not only enables us to assess the level and quality of the flavor notes perceived, but also requires the evaluator to indicate any actual blueberry character. The latter information is especially useful when the desired note is present at a low level and masked by noncharacteristic flavors.

Blueberry Fractionation/Evaluation. Ten-microliter portions of the blueberry essence produced a gas chromatographic curve as shown in Figure 1. The total effluent (step I) was collected in the beverage base and the solution was evaluated by several experienced tasters using the ballot. Results for this and subsequent steps are compiled in Table I. The descriptors developed indicated the flavor was present at a high level and was blueberry in character, but with a quite sharp, green apple character. The fact that a blueberry note was present justified further efforts in the separation and evaluation of the essence.

Thereafter, the samples were collected in three portions (step II, Figure 1) and these were evaluated as A, B, C, AB, AC, BC. The latter three solutions were prepared by combining equal portions of the appropriate two solutions. In this case, fraction C (and those combinations containing

Table II. Evaluation of Characterized Blueberry Components

Flavor	Compound, ppm		
	Linalool	*cis*-3-Hexenol	*trans*-2-Hexenol
Lemon, woody	5		
Nice fresh green		5	
Green, sharp		10	
Spicy green apple			5
Green, olefinic			10
Blueberry	5	5	
Blueberry	5	10	
Blueberry-like, green apple	5		5
Green apple blueberry	5		10

it) possessed a blueberry character and only this fraction was studied further. In step III, an interesting phenomenon occurs. The desired flavor is not in any one fraction, but rather only in that combination of fractions which include both D and E. Step IV demonstrates that the component indicated by H is critical to blueberry flavor, but we do not know what other components are essential to the flavor. Step VI shows that the peak P is not critical to the flavor but that the two peaks encompassed in N are. In the final step, we observe that a blueberry character is developed by a combination of peaks containing fractions R plus T, but a sharper less desirable character results from a combination of S and T.

Standard identification techniques (GC/MS, GC/IR) were employed to characterize the components designated R, S, and T. These were found to be *cis*-3-hexenol, *trans*-2-hexenol, and linalool, respectively. The importance of linalool is not unexpected since Arctander (1969) indicates that linalool is used in imitation blueberry flavors.

Synthetic Blueberry Flavor. It cannot be expected that this approach will lead to a complete well-rounded flavor. On the other hand, the combination of the identified flavors in the correct proportion should produce a recognizable flavor. A number of mixtures were prepared in the sucrose/acid base and were evaluated (Table II). These results reinforced the importance of *cis*-3-hexenol and linalool as major characterizing flavorings of blueberry and demonstrate that a blueberry character, though sharper and greener, results from a combination of linalool and *trans*-2-hexenol.

CONCLUSION

A procedure has been developed which enables the analyst to systematically determine the important flavor notes of a food. This technique facilitates the recognition of the critical components in a complicated chromatographic pattern. The sample is chromatographically fractionated into successively smaller portions which are evaluated both alone and in combination by modified profile evaluation panel techniques. The procedure is viable providing the aromatic concentrate possesses the desired character and that it can survive gas chromatography. It is useful even if the flavor pursued is not complete, providing that flavor profile panelists can recognize the desired note with a high degree of consistency. This ability to focus on only the important compounds has enhanced the usefulness of flavor analysis in the creation of synthetic flavors.

LITERATURE CITED

Anderson, D. F., Day, E. A., *J. Agric. Food Chem.* **14**, 241 (1966).
Arctander, S., "Perfume and Flavor Chemicals", S. Arctander, Publisher, Montclair, N. J., 1969, Monograph 1803.
Jennings, W. G., Leonard, S., Pangborn, R. M., *Food Technol.* **14**, 587 (1960).
Parliment, T. H., *Chem. Ind. (London)* **9**, 418 (1976).
Parliment, T. H., Kolor, M. G., *J. Food Sci.* **40**, 762 (1975).
Persson, T., von Sydow, E., Akesson, C., *J. Food Sci.* **38**, 682 (1973).

Received for review July 27, 1976. Accepted October 18, 1976. Presented at the 172nd National Meeting of the American Chemical Society, San Francisco, Calif., Aug 1976, Abstract AGFD-80.

Volatile Components of Unprocessed Rice (*Oryza sativa* L.)

Roger W. Bullard* and Gilbert Holguin

Laboratory tests showed that the volatile flavor components of unprocessed rice are attractive to Philippine ricefield rats (*Rattus rattus mindanensis*). These results suggested that a synthetic bait attractant could be made if the important volatiles in rice were identified. To identify the compounds, ground rice was tumbled at 50 °C in a modified rotary evaporator while helium swept the volatiles into a liquid nitrogen trap coated with dibutyl phthalate. The dibutyl phthalate solution containing the volatiles was analyzed by combination capillary-column gas chromatography and mass spectrometry. Altogether, 73 compounds were identified, including alcohols, aldehydes, alkyl aromatics, furans, ketones, terpenes, and naphthalenes. The structural types of another 31 compounds were indicated. Of the 73 compounds identified, only 9 have been previously reported in unprocessed rice and 54 have never been reported in any unprocessed cereal grain.

Rice is the world's largest cereal crop. It is produced in all of the continents and is the staple food of nearly half of the world's population. Only about 3%, however, moves in world trade and the rest is internally consumed by the producing countries. Over 90% of the world crop is produced in East and Southeast Asia. Consequently, the developing countries in this area are highly dependent upon each rice crop to feed their people.

Because of dense rat populations, lack of effective control programs, and current farming practices, rice growers in the tropics are especially vulnerable to severe crop losses through rat damage. In the Philippines, wild ricefield rats, *Rattus rattus mindanensis*, damage rice in all growth stages—germinating seeds, growing seedlings, and

Denver Wildlife Research Center, U.S. Fish and Wildlife Service, Denver, Colorado 80225.