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Reprinted from **AGRICULTURAL AND FOOD CHEMISTRY**, Vol. 23, No. 2, Page 229, Mar./Apr. 1975
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Carrot-Root Oil Components and Their Dimensional Characterization of Aroma

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Cold hexane–acetone extraction of fresh carrots produced an oil in which 28 components, in decreasing concentration, were tentatively identified as isoprene, β-caryophyllene, linalool, acetaldehyde, *p*-cymene, terpinolene, dipentene, ethanol, camphene, bisabolene, β-ionone, 2-nonenal, nonanal, α-pinene, γ-terpinene, β-pinene, α-terpineol, α-ionone, dodecanal, α-terpinene, nopol, borneol, bornyl acetate, 4-terpineol, biphenyl, myrcene, carotol, and ionene. Multidimensional scaling of the odor of these components against 52 descriptive terms produced a two-dimensional relationship in which distances between points in the geometry correspond to the appropriateness of the terms for the chemical odors.

Compared to fruit, there has been relatively little attention given to the flavor and aroma of vegetables. These are usually tenuous and difficult to describe, and often, in spite of botanical and other differences, difficult to easily distinguish. Strong and distinctive vegetables, like onion or cabbage, or those which are economically significant, such as potatoes, are exceptions. The delicate flavor and aroma of fresh carrots fall in the elusive and more difficult to define category.

The flavor and aroma of cooked carrots have been studied by several authors. Otsuka and Take (1969) attribute the taste of a carrot soup to the presence of three carbohydrates, glutamic acid, and the buffer action of various other amino acids. Isolation by steam distillation produced a carrot-root oil that possessed an aroma similar to that of cooked carrots (Buttery *et al.*, 1968). The nature of the compounds responsible for the aroma remains undefined, however, and the results of Heatherbell and Wrolstad (1971) illustrate the complexity of the problem. Degradation of terpenoid substances is largely responsible for loss of carrot acceptability (Ayers *et al.*, 1964; Farine *et al.*, 1965; Heatherbell *et al.*, 1971) and the flavor and aroma of carrots, whether raw or cooked, probably result from a complex interaction of several of these compounds and the nonvolatile constituents (Alabran and Mabrouk, 1973).

Recent approaches in sensory measurement have employed a new type of data analysis called "multidimensional scaling" (Schiffman, 1974). The aim of this class of procedures is to construct a "map" in a geometrical space. Odors that are similar to each other are located near each other in this geometry, whereas those that are dissimilar are placed far away from each other. To achieve the map, subjective differences between pairs of odors (or between odors and words) are obtained through experimental procedures. Computer programs treat these differences as distances between points in the geometry (Woskow, 1968; Kruskal and Carmone, 1969). Programming procedures have been described in sufficient detail for developing a program on automatic computers, and such a program is available from J. B. Kruskal (Kruskal, 1964). These procedures have been used to study the geometrical configuration of odors (Berglund *et al.*, 1972; Yoshida, 1972).

The first dimension to be uncovered in the analysis is the hedonic dimension of pleasantness–unpleasantness (Yoshida, 1972). Other dimensions can vary, depending upon the selection of starting odors, and the dimensions are named according to the odors that lie at either extreme. The procedures do not provide names to the dimensions, but only the projection of points on each axis.

The present study modified the procedure of multidimensional scaling by attempting to place both stimuli and descriptors in the same geometrical space. The space contains sufficient information to determine what labels best correspond to its dimensions. Also, since both odorants and descriptors are placed in the same space, subtle variation of aroma description becomes evident. This investigation obtains information on a collection of odors of essential oil components of raw carrots and tests a method of quality representation of carrot aroma. Gas chromatography was used to identify and quantify the oil components. Heating during preparation of the oil was avoided to prevent artifact formation, and organic solvents were used to assure complete extraction of all oil components.

EXPERIMENTAL SECTION

Imperator carrot variety was purchased in California and shipped air freight to Natick Laboratories. Upon arrival the carrots were stored at 5° for 2–4 days.

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Table I. Odor Descriptors

Fragrant	Vegetables, cooked	Warm
Soft	Sweet	Metallic
Almond	Fishy	Cool
Burnt	Spicy	Fruity—not citrus
Green (grass)	Heavy	Musky
Etherish	Painty	Garlic–onion
Sour, vinegar	Rancid	Vanilla
Dry, powder	Minty	Dung-like
Disinfectant	Piney	Floral
Familiar	Woody	Bright
Animal	Resinous	Memorable
Objectlike	Aromatic	Musty
Oily, fatty	Meaty, cooked	Simple
Oily, waxy	Moldy	Mixture
Mothballs	Sharp	Carroty
Citrus-orange	Light	Cabbage
Citrus-lemon		Kerosene
Solvent		Pungent

Extraction of the Essential Oil. Sound carrots were washed by hand in running water and dried by blotting with paper towels. The crowns and tips were discarded. About 450 g at a time was weighed, diced, and placed in a heavy duty Waring Blendor with approximately 1 l. of hexane–acetone (3:2, v/v) and powdered Dry Ice to minimize oxidation of unsaturated components with a CO_2 atmosphere and keep the contents cool during blending. The contents were blended for 2 min and the liquid decanted onto a fritted glass funnel. After a second chilled blending with fresh hexane–acetone, the solids and additional liquid were transferred to the funnel and the slurry filtered. The combined solids were stirred by hand with fresh hexane–acetone and filtered three more times. The total filtrate was dried with anhydrous sodium sulfate, filtered, and concentrated to a thick orange oil on a rotary evaporator at less than 40° with water aspirator vacuum. The oil was stored under N_2 at −20°, protected from light.

Gas Chromatography. A Beckman GC-4 gas chromatograph equipped with a dual hydrogen-flame detector was used with the following condition: (1) 50 ft × 0.020 in. i.d. Carbowax 20M SCOT column, injection port 180°, inlet lines 225°, column 120°, detector lines 225°, detector 250°, helium carrier flow 3 cm^3/min; (2) 50 ft × 0.020 in. i.d. SE-30 SCOT column, injection port 200°, inlet lines 200°, column 90–200° at 4.5°/min and held to end of analysis, detector lines 235°, detector 250°, helium carrier flow 3 cm^3/min. After establishing an effective resolution of the oil, tentative peak identification was performed by retention-time comparison and co-injection with known standards. The concentration of individual components was established from the straight line correlation between the responses of an Infotronics digital integrator Model CRS-11 HSPB (Infotronics, Houston, Tex.) and known concentrations of standards.

Chemical Standards. Ionene was synthesized (Bogert and Fourman, 1933), and carotol was obtained by fractional distillation (Sorm and Urbanek, 1948) of carrot-seed oil (Fritzche, Dodge and Olcott, Inc., New York, N.Y.). Isoprene was obtained from the Chemical Procurement Laboratories, College Point, N.Y., α-terpineol from Analabs, Inc., North Haven, Conn., 4-terpineol from Pfaltz & Bauer, Inc., Flushing, N.Y., α-terpinene, γ-terpinene, and terpinolene from the Glidden Co., Jacksonville, Fla., and the remainder from the Aldrich Chemical Co., Cedar Knolls, N.Y., and K&K Laboratories, Inc., Plainview, N.Y.

Odor Evaluation of Identified Components. Twenty-two female and three male subjects were provided the list of 52 descriptive terms shown in Table I. These terms were used to evaluate 31 stimuli, 28 of which were the identified essential oil components, one a synthetic mixture of these components, one the naturally occurring oil, and one simply the word "carrot." Five standards, menthol, vanillin, ether, lemon extract, and vinegar, were also provided as a check on consistency and descriptive ability. Each subject had previous experience in odor evaluation and rating procedures but none with these odors. Each descriptor was rated for each odorant on a scale of six levels: 100, 80, 60, 40, 20, and 0% appropriate.

Table II. Essential-Oil Components of Fresh Carrots

Compound	ppm fresh carrot
Isoprene	84.10
β-Caryophyllene[a-c]	47.10
Linalool[a]	37.60
Acetaldehyde[c]	21.70
p-Cymene[a-c]	21.60
Terpinolene[b,c]	21.10
Dipentene[a-c]	15.20
Ethanol[c]	11.70
Camphene[a-c]	9.31
Bisabolene[a-c]	9.25
β-Ionone	8.96
2-Nonenal[b]	6.61
Nonanal[b]	6.20
α-Pinene[a-c]	6.10
γ-Terpinene[a-c]	5.95
β-Pinene[a-c]	4.06
α-Terpineol[a,b]	2.60
α-Ionone	2.54
Dodecanal[b]	2.36
α-Terpinene[a,b]	2.24
Nopol	1.90
Borneol	1.12
Bornyl acetate[a-b]	1.10
4-Terpineol[b,c]	1.00
Biphenyl[b]	1.00
Myrcene[a-c]	0.65
Carotol[a-c]	0.30
Ionene[a]	0.16
Total	333.51

[a] Reported by Seifert *et al.* (1968) in carrot-seed oil. [b] Reported by Buttery *et al.* (1968) in cooked carrot-root oil. [c] Reported by Heatherbell *et al.* (1971) in raw carrots.

Stimuli Preparation. The chemical stimuli were carrot oil component emulsions in deionized, redistilled-in-glass water, deoxygenated with N_2. The emulsions were prepared by ultrasonication (Mabrouk and Dugan, 1960) at 20 kHz, 45 W for about 90 sec with magnetic stirring in an ice–water bath (Ultrasonics, Inc., Plainview, N.Y.). Component-droplet size in the emulsion was determined by oil-immersion microscopy. Concentrations were at the level previously determined by gas chromatography except where this was below threshold, in which case the concentration was adjusted until a characteristic odor was obtained.

Dimensional Analysis. The mean ratings were computed for the judgments, and then submitted to the computer program MDSCAL 5M (Kruskal and Carmone, 1969). The program was instructed to treat high ratings as similarities and low ratings as dissimilarities. One of the options of the program used in the present analysis was to determine the best fitting placement of 83 points (52 descriptors, 31 odorants) in a geometrical space of two dimensions. The nonmetric option of MDSCAL 5M was used to obtain placement of points so that the rank order of distances between points corresponded as closely as possible to the rank order of dissimilarities judgments (or

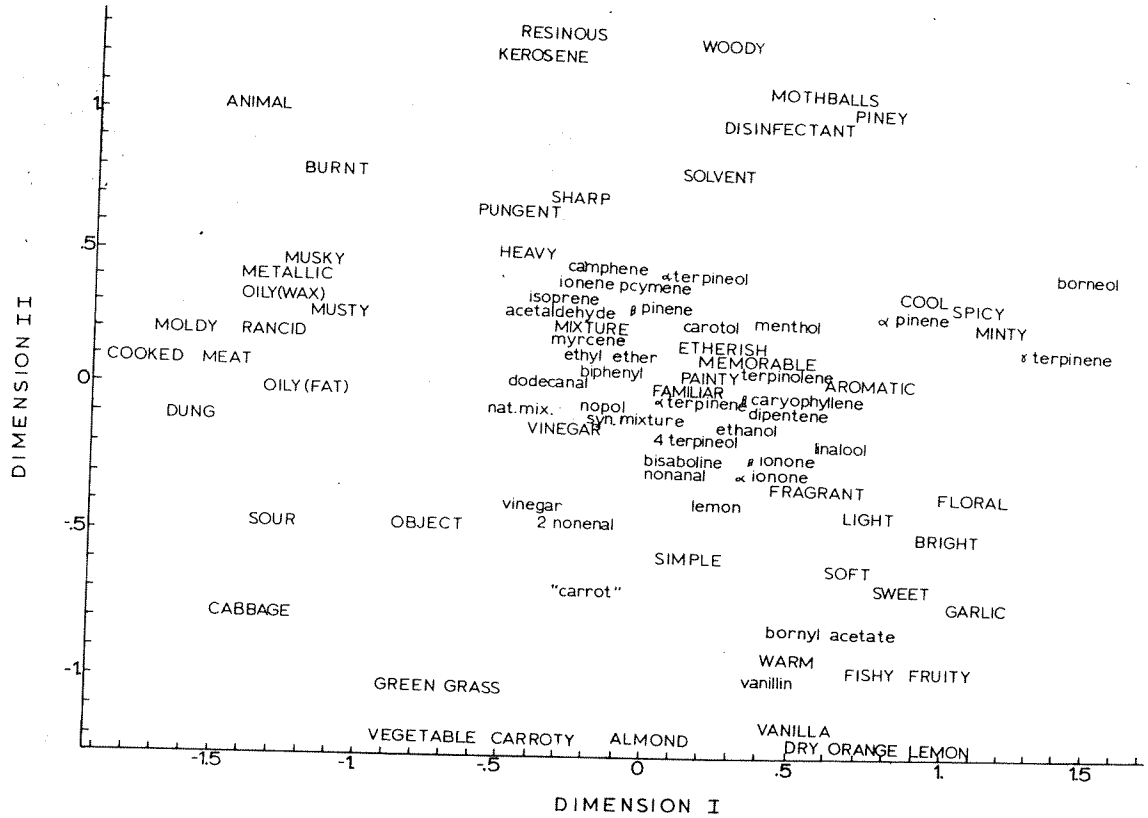

Figure 1. Geometrical relationship of descriptive terms and the carrot oil components.

corresponded as closely as possible to the inverse rank order of appropriateness judgments). Goodness-of-fit was assessed by the "stress value," corresponding to the unexplained sum-of-squares.

RESULTS AND DISCUSSION

Upon extraction of 1331.72 g of fresh carrots with cold hexane–acetone, 7.85 g of a dark orange oil representing 0.59% of fresh carrots was obtained. The results of gas chromatographic analysis of the essential oil are listed in Table II.

Extraction of Imperator carrot essential oil with hexane–acetone gave a higher yield (333.51 ppm) than that reported by Buttery *et al.* (1968) and Heatherbell *et al.* (1971). The efficiency of the extraction procedure was revealed in the production of odorless and colorless carrot residues. The variation in total and individual component yield could be attributed to climatic, maturity, and storage conditions as well as the method of preparing the oil.

Seifert *et al.* (1968) report *p*-thymol, geraniol, geranyl acetate, citronellol, citral, β-selinene, coumarin, and α-curcumene in carrot-seed oil. Buttery *et al.* (1968) report myristicin, octanal, sabinene, and heptanal, and Heatherbell *et al.* (1971), in addition to myristicin, octanal, and sabinene, report propanal and α-phellandrene in their carrot-root samples. These compounds were not identified in our oil. We identified acetone, as did Heatherbell *et al.* (1971), and hexane, in small amounts, but since these were our extraction solvents, they are not reported in Table II. Isoprene, linalool, β-ionone, α-ionone, nopol, borneol, and ionene, or compounds with the same retention times, were tentatively identified in our carrot sample. Although isoprene, at least, is an unlikely natural component (Bonner, 1965) these compounds were included in the subsequent multidimensional scaling.

Droplet size of the chemical emulsions was approximately 2 μ and was stable for periods of up to 1 week, but the emulsions were reestablished about every 3 days. Essential oil composition is the result of products formed in many metabolic processes taking place in the plant and for a variety of purposes, *i.e.*, pollination attractants, depredation protection, synthesis intermediates and moderators, waste products, etc. Secretion appears in different cell groups, and some of the cells or intercellular spaces in the tissue are filled with oil droplets (Guenther, 1948). There is little information on oil droplet size in carrots, but starch grains and chromatophore crystals are on the order of 2–4 μ (Winton and Winton, 1935). Therefore, since carrots are as much as 85% water (Alabran and Mabrouk, 1973), a water emulsion at 2 μ was felt to approximate the natural system.

Figure 1 presents the two-dimensional solution for the placement of points in the geometrical space. Note that the points in the space correspond both to the descriptive terms and to the chemicals (including "carrot" as a word). The points closest together are those that are most similar to each other qualitatively, whereas those farthest apart are least similar. The analysis of the data in this fashion allows the representation of the judgments of applicability of descriptive terms to chemicals, and the inference that the odors of some chemicals are more similar to each other than to others. This type of multidimensional scaling, called *multidimensional unfolding,* is a powerful technique for analyzing profile ratings of many different chemicals into distances between descriptors and chemicals, as well as distances between one chemical and others, provided that the chemicals were rated with the same terms.

In general, the stimuli tend to be located closer to the center of the space than descriptors. This implies that there is greater similarity among the chemicals tested in this experiment than among the descriptors. Such results are reasonable, since a typical odor contains many different attributes (which makes a profiling procedure possible—otherwise only one single attribute and its intensity rating would be needed for any particular odor) (Moskowitz and Gerbers, 1975).

The geometrical space can be analyzed by two tech-

niques. One is to search for fundamental dimensions underlying the arrangement of points. Each point is considered to be a projection of each of two axes. lt least one axis here is the axis of pleasantness–unpleasantness. Points located to one extreme on this axis are pleasant (*e.g.*, spicey, minty), whereas points located at the other extreme (*e.g.*, moldy, oily) are not. The second axis is harder to name. At one extreme are chemical names and at the other are food names. Perhaps this dimension might be called edible–inedible. In a similar series, but with direct judgments of odor similarity (Woskow, 1968), the two dimensions uncovered were pleasantness–unpleasantness, and woodyness. The selection of stimuli critically influences the final dimensions. All pleasant odors would force the development of other dimensions besides the hedonic one along which subjects make estimates of similarity.

Note that the results distribute themselves in the shape of an ellipse. The negative end of dimension I ("moldy, dung-like, cooked meat") may be deformed so that distances seem greater than expected. Also, distances between points at the upper right-hand corner ("piney, mothballs, disinfectant") are exaggerated. Perhaps the two-dimensional figure is a projection and flattening of a higher structure shaped more or less like a football.

The second way of analyzing these configurations is to assume that the methods provide nothing more than a convenient method to locate chemicals and descriptors in a single space at specific points. These points may (or may not) lie in compact clusters, and may not be distributed evenly. Cluster analysis (Cormack, 1971) has been used extensively to find regions in geometrical spaces that are closely packed with points. Clusters in this experiment are: (1) floral and fragrant odors that are soft, sweet, and light; (2) a chemical set (isoprene, myrcene, pinene); (3) a cluster of vegetable-like odors; (4) a circular cluster of oily, waxy, musty odors; (5) a diffuse cluster of solvent-like chemical or disinfectant odors.

Since distances reflect similarities, one can determine relations between chemicals and their descriptor word. Because carrots are of interest, the concept "carrot" was included as a dummy odor. The concept of a carrot odor differs from "carroty" as a descriptor and actual carrot odor. Those descriptors for the carrot concept and carroty chemical stimuli significantly preferred by the panelists were "aromatic, light, fragrant, sweet, soft, green, warm" and 2-nonenal, the synthetic mixture, terpinolene, β-caryophyllene, myrcene, 4-terpineol, bisabolene, and the natural mixture, in that order. These points establish a triangular shaped cluster from myrcene at the top, "sweet" to the right, and "green grass" at the lower left. With the exception of acetaldehyde, which occurs close to myrcene, this cluster includes the descriptors and chemicals mentioned by others (Buttery *et al.*, 1968; Heatherbell *et al.*, 1971).

The results of the present experiment are important for attempts at synthesizing flavors as well as for analyses of subjective odor perception. By appropriate use of words as "odor stimuli" (here the word "carrot") along with synthetic and natural mixtures, the flavor scientist can determine how closely his mixtures approximate what the typical panelist conceives as being the desired flavor. Since the representation of flavor or odor varies along several dimensions, the disagreemgnt between a formulation and the "ideal" flavor can be resolved in terms of one of several directions of differences. The approach, therefore, simultaneously provides a tool for synthesis and capacity for analysis.

LITERATURE CITED

Alabran, D. M., Mabrouk, A. F., *J. Agr. Food Chem.* **21**, 205 (1973).
Ayers, J. E., Fishwick, M. J., Land, D. G., Swain, T., *Nature (London)* **203**, 81 (1964).
Berglund, B., Berglund, U., Engen, T., Ekman, G., "Reports of the Psychological Laboratories," No. 345, University of Stockholm, 1972.
Bogert, M. T., Fourman, V. G., *J. Amer. Chem. Soc.* **55**, 4670 (1933).
Bonner, J., "Plant Biochemistry," Bonner, J., Varner, J. E., Ed., Academic Press, New York, N.Y., 1965, p 666.
Buttery, R. G., Seifert, R. M., Guadagni, D. G., Black, D. R., Ling, L. C., *J. Agr. Food Chem.* **16**, 1009 (1968).
Cormack, R. M., *J. Roy. Stat. Soc., Ser. A. (Gen.)* **134**, 321 (1971).
Farine, G., Wuhrmann, J. J., Patron, A., Vuatax, L., "Food Science and Technology," Leitch, J. M., Ed., Vol. III, Gordon and Breach, New York, N.Y., 1965, pp 603–613.
Guenther, E., "The Essential Oils," Vol. I, D. Van Nostrand Co., New York, N.Y., 1948, pp 17–83.
Heatherbell, D. A., Wrolstad, R. E., *J. Agr. Food Chem.* **19**, 281 (1971).
Heatherbell, D. A., Wrolstad, R. E., Libbey, L. M., *J. Food Sci.* **36**, 219 (1971).
Kruskal, J. B., *Psychometrika* **29**, 1, 115 (1964).
Kruskal, J. B., Carmone, P., unpublished manuscript, Bell Laboratories, Murray Hill, N.J., 1969.
Mabrouk, A. F., Dugan, L. R., Jr., *J. Amer. Oil Chem. Soc.* **37**, 486 (1960).
Moskowitz, H. R., Gerbers, C., *Ann. N.Y. Acad. Sci.*, in press (1975).
Otsuka, H., Take, T., *J. Food Sci.* **34**, 392 (1969).
Schiffman, S. S., *Science* **185**, 112 (1974).
Seifert, R. M., Buttery, R. G., Ling, L., *J. Sci. Food Agr.* **19**, 383 (1968).
Sorm, F., Urbanek, L., *Collect. Czech. Chem. Commun.* **13**, 49 (1948).
Winton, A. L., Winton, J. B., "The Structure and Composition of Foods," Vol. II, Wiley, New York, N.Y., 1935, p 95.
Woskow, M. H., "Theories of Odor and Odor Measurement," Tanyolac, N., Ed., Robert College, Bebec, Turkey, 1968, pp 147–188.
Yoshida, M., *Jap. Psychol. Res.* **14**, 70 (1972).

Received for review April 25, 1974. Accepted November 8, 1974. Research was undertaken at the U.S. Army Natick Laboratories and has been assigned No. TP-1517 in the series of papers approved for publication. The findings in this report are not to be construed as an official Department of the Army position. Mention of a trade product, equipment, or company does not imply its endorsement by U.S. Army Natick Laboratories over similar products of companies not mentioned.