# Effect of Nonvolatile Orange Juice Components, Acid, Sugar, and Pectin on the Flavor Threshold of *d*-Limonene in Water

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Flavor thresholds of *d*-limonene, the major volatile organic constituent of orange juice, were determined in aqueous solutions of nonvolatile citrus juice constituents, acid, sugar, pectin, and combinations thereof. Effects of the nonvolatile constituents on precision among flavor panelists were examined also. The presence of acid caused a significant increase in the threshold of *d*-limonene in water. Sugar tended to increase in the threshold of *d*-limonene in water, while pectin tended to have the opposite effect. Precision among panelists was less when solutions contained acid or pectin than when they contained sugar. These studies show that nonvolatile constituents of citrus juices can influence the flavor threshold of volatile organics present in the juice.

It is widely known that volatile and nonvolatile constituents of foods interact and modify flavor effects of other constituents present. The variety of methods used to study interactions between food components in flavorful solutions has resulted in conflicting results that are difficult to apply to particular foodstuffs (Pangborn, 1961). To date, no systematic study has been carried out to measure effects of the major nonvolatile constituents of citrus juices on volatile flavor constituents. Such studies are needed to help correlate odor and flavor thresholds of individual flavor components in water with their contribution to the overall flavor of citrus juices (Ahmed et al., 1978).

Flavor studies have been carried out to measure the effect of one volatile food component on other volatile components (Guadagni et al., 1963; Keith and Powers, 1968) or of one nonvolatile component on other nonvolatile components (Pangborn, 1961; Kamen et al., 1961), but the interaction of nonvolatile and volatile components with regard to flavor effects has received less study. Berg et al. (1955) determined threshold of sucrose in ethanol–water solutions and found that ethanol enhanced the sweetness of sucrose solutions by decreasing the amount of sucrose needed for an equivalent level of sweetness. Hinreiner et al. (1955a), studying the interactions of sucrose, organic acids, and ethanol, found that sucrose increased the threshold for ethanol while organic acids diminished the effect of sucrose on ethanol; however, organic acids alone increased the threshold for ethanol. They also found the threshold for organic acids was increased by ethanol but was unaffected by sucrose. In other studies, Hinreiner et al. (1955b) found that minimum concentrations for detectable differences were higher in wine than in water for sucrose, ethanol, tannin, ethyl acetate, acetaldehyde, and tartaric acid. Siek et al. (1969) found thresholds of butter volatiles in oil to be higher than in water and thresholds in butter media to be very close to those in oil.

This study reports the effects of organic acids, sugars, and pectin at the concentrations normally present in orange juice on the flavor threshold of *d*-limonene in water as well as flavor effects involving interactions between these nonvolatile components. From these data, effects of the nonvolatile constituents on precision among flavor panelists were studied.

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## EXPERIMENTAL SECTION

Threshold is defined as that concentration of a substance at which panelists can detect a difference from a specified standard 50% of the time (Patton and Josephson, 1957). Flavor is defined as the total sensory response by a panelist to the sample taken into the mouth.

**Sample Preparation.** Reagents used were: analytical reagent grade sucrose, glucose, fructose, and citric acid from Mallinckrodt Chemical Works, St. Louis, Mo.; practical grade malic acid from Nutritional Biochemical Corp., Cleveland, Ohio; and 180 grade pectin from Sunkist Growers, Corona, Calif.

Stock solutions of *d*-limonene (purity 96.5%) in doubly distilled water were prepared within 24 h of panel testing using quantitative procedures described previously (Ahmed et al., 1978). The concentrations of *d*-limonene tested throughout the study were 100, 300, 500, and 1000 ppb. The percentage of each nonvolatile component used was approximately equal to that often found in orange juice: 0.04% pectin (Rouse, 1971), 0.8% acid (Florida Citrus Mutual, 1969), and 9.8% sugar (Florida Citrus Mutual, 1969). The sugar solution contained 4.9% sucrose, 2.4% glucose, and 2.6% fructose, and the acid solution contained 0.1% malic acid and 0.7% citric acid (Ting and Attaway, 1971). Solutions with combinations of these compounds contained the same concentration of each compound as was used in the individual solutions.

**Testing Procedure.** A group of 12 screened and trained panelists was chosen from the untrained panel used previously (Ahmed et al., 1978) on the basis of their consistency and reliability. The panel consisted of five males and seven females and ranged in age from 22 to 47 years with an average of 30.6 years. The same panel members were used throughout the study and they participated in each session. Flavor threshold values for *d*-limonene were determined using the single stimulus difference test described previously (Ahmed et al., 1978). The method involved presenting the panel members with five samples along with a standard for reference and asking them to compare each sample to the standard and to judge if there was a difference (Siek et al., 1969; Langler and Day, 1964). One of the five samples was a blank identical with the standard. The water blanks were placed between samples so that the blank appeared before each concentration once. Sample volume presented to the panelists was approximately 50 cm$^3$. Panelists were allowed to retest any sample, if needed. Panelists were requested to express their detection of any flavor difference between samples

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Table I. Flavor Thresholds for *d*-Limonene in Aqueous Solutions Containing Pectin, Acid, and Sugar

| Solutions | Threshold concn, ppm | 95% confidence limits, ppm | Correlation[a] coefficient ($r$) |
|---|---|---|---|
| Water only | 0.21 | 0.05–0.79 | 0.82 |
| Acid | 0.41 | 0.17–0.99 | 0.91 |
| Pectin | 0.22 | 0.07–0.61 | 0.88 |
| Sugar | 0.35 | 0.12–0.96 | 0.89 |
| Pectin and acid | 0.31 | 0.08–1.20 | 0.83 |
| Pectin and sugar | 0.23 | 0.08–0.64 | 0.89 |
| Acid and sugar | 0.38 | 0.14–1.10 | 0.88 |
| Pectin, acid, and sugar | 0.36 | 0.13–1.00 | 0.89 |

[a] All correlation coefficients were significant at the 0.01 level.

and reference by a yes/no response. A different medium was tested each week during an 8-week period including: water; pectin and sugar; sugar and acid; and pectin, sugar, and acid. The panel was conducted 5 days a week from 2:30 to 3:30 p.m.

**Statistical Analysis.** The statistical analysis for determining threshold values was similar to the one used previously (Ahmed et al., 1978). In this analysis, there were four $X$ values (concentrations), which were the same for all the thresholds determined, and five $Y$ values (percent positive responses) corresponding to each $X$ value, one for each day of the week. Each $Y$ value consisted of 12 judgements, for a total of 60 judgements corresponding to each $X$ value. From these data the threshold value, correlation coefficient, and confidence limits were calculated for *d*-limonene in each medium.

The threshold values for *d*-limonene were arranged in a $2 \times 3$ factorial design, three components (acid, pectin, and sugar) at two concentrations (0 and the concentration present in orange juice). There were three main effects (acid, pectin, and sugar), three two-factor interactions (acid × pectin, acid × sugar, and sugar × pectin), and one three-factor interaction (acid × sugar × pectin). These values were treated by an analysis of variance to determine the effect of each medium on the flavor threshold of *d*-limonene. If no significant interactions were present, a $t$ test was used to indicate the presence of a significant difference for main effects.

Chi square analysis was used to determine variations present in panel results. Judges variations in ability to detect low concentrations were tested by comparing positive responses to concentration differences. Precision of judges' responses was tested by comparing correct responses to water blanks.

## RESULTS AND DISCUSSION

Three of the major nonvolatile constituents present in orange juice were evaluated by a trained flavor panel for their effects on the flavor threshold of *d*-limonene in water. Table I contains the flavor threshold values for *d*-limonene in various solutions of acid, pectin, sugar, and their combinations. The 95% confidence limits are included to estimate variation, and the significant simple correlation coefficients ($r$) are listed to show linearity. The threshold for *d*-limonene in water (Table I) is very close to that established for *d*-limonene by an untrained panel (0.21 ppm with 95% confidence limits of 0.14–0.33 ppm) almost a year earlier (Ahmed et al., 1978), indicating good reproducibility. The two methods were identical except that the composition of the panel varied. According to analysis of variance, the individual or combinations of nonvolatile components did not vary significantly in their effect on the threshold of *d*-limonene.

Table II. Average Flavor Threshold of *d*-Limonene in Aqueous Solutions with and without Pectin, Acid and Sugar

| Solution | Average thresholds, ppm | Significance level |
|---|---|---|
| With acid | 0.36 | 0.05 |
| Without acid | 0.25[a] | |
| With sugar | 0.33 | ns[b] |
| Without sugar | 0.28 | |
| With pectin | 0.28 | ns |
| Without pectin | 0.34 | |

[a] Significant difference at the 0.05 level. [b] ns = not significant.

Since the judges responded similarly to each nonvolatile component in different solutions, the average effect of each component was evaluated using a $t$ test and the results are contained in Table II. The average for flavor threshold values in solutions containing acid was significantly higher than that in solutions not containing acid. The $t$ test indicated no significant changes in the average threshold values when sugar or pectin was added. However, the results in Table II show a tendency for threshold values to be lower in solutions containing pectin than those without, and higher in solutions containing sugar than those without, although neither difference was significant.

*d*-Limonene may not be representative of all volatile flavor components present in orange juice, but these results indicate that acid may play an important role in masking the effect of at least some characteristic volatile flavor components. This conclusion is in agreement with the results of Pangborn (1960) who reported that in fruit nectar, the greater the acidity, the greater the depressing effect on the intensity of the compound added. The same depressing effect from acid on glycerol flavor was found by Bennett et al. (1965).

The observation that sugar had a tendency (although not significant) to raise the threshold of *d*-limonene agreed with the findings of Valdes et al. (1956) that sweetness beyond that imparted by 15% sugar interferred with flavor perception, and the results of Hinreiner et al. (1955a) that sucrose increased the threshold for ethanol.

Pectin appeared to depress the effect of acid and sugar on *d*-limonene even though it did not significantly decrease the threshold of *d*-limonene. Bennett et al. (1965) stated that the fat in cream could have decreased its apparent acid taste, thus allowing diacetyl to be detected more easily. Since pectin is generally regarded as a thickening agent, its effect on the threshold of *d*-limonene may be due to textural properties.

Some generalizations can be made about variables encountered in running a sensory panel from the results obtained with this trained panel. The variables were evaluated using two sources of information: the number of positive responses to the concentrations, which could reflect ability to detect low concentrations, and the number of correct responses to the blanks, which could measure precision in panelist sampling.

Results of a chi square analysis to determine significant variation in panel responses are presented in Table III. This table shows a highly significant variation (99% confidence level) in ability of panelists to detect concentration, which agrees with previous findings that individuals vary in their ability to detect small differences in concentrations (McNamara and Danker, 1968). However, the variation among panelists on the number of correct responses to blanks was significant only at the 95%

**Table III. Chi Square Analysis of Variation of Flavor Panels in Detecting Concentrations and Correct Responses to Water Blanks**

| Source | Concentrations | Blanks |
|---|---|---|
| Panelists | 34.19[b] | 19.53[a] |
| Days | 5.14 | 4.58 |
| Positions | | 17.53[b] |
| Solutions | | 34.18[b] |

[a] Significant difference at 0.05 level. [b] Significant difference at 0.01 level.

**Table IV. Percentage of Correct Responses to Blanks in Different Solutions**

| Solution | Percentages |
|---|---|
| Water | 90.0 |
| Pectin | 72.9 |
| Acid | 66.7 |
| Sugar | 94.4 |
| Pectin × acid | 68.7 |
| Pectin × sugar | 76.7 |
| Acid × sugar | 73.3 |
| Pectin × acid × sugar | 80.0 |

confidence level, indicating panelists do not vary as much in precision as they do in ability to detect low concentrations. This table also shows a lack of significant variation in positive responses to concentrations and in correct responses to blanks among days of the week, indicating that judges generally do not vary during the week in their abilities to detect minimum concentrations or in their precision.

Table III reveals a significant difference in the correct responses to blanks placed in different positions. Since the responses to blanks were progressively less precise as they were placed after the higher concentrations, the loss in precision could have been from a carry-over effect which would leave an "after taste" from the previous sample in the mouth, making the following blank appear to have a flavor.

Finally, a large variation in correct responses to blanks was found among the different solutions in which the threshold of *d*-limonene was determined (Table IV). These results indicate that the various solutions affected the precision with which a panelist could detect small differences. Precision appeared high in solutions of water alone and in the aqueous solution of sugar alone. Solutions containing acid showed a definite decrease in precision of response, probably because the acid taste was strong enough to adversely affect the ability of panel members to detect small differences. Bennett et al. (1965) reported a similar finding that acetic acid had such a strong effect on the threshold of diacetyl in sour cream that it could not be precisely determined in that medium.

The presence of acid not only significantly raised the threshold in water of the major volatile organic constituent of orange juice, *d*-limonene (Table II), but it also caused decreased precision among panelists in choosing blank solutions arbitrarily placed between solutions containing *d*-limonene (Table IV). Addition of sugars or pectin at the levels normally found in orange juice tended to raise the apparent threshold of *d*-limonene with sugar present and lower its apparent threshold with pectin present (Table II).

## LITERATURE CITED


Ahmed, E. M., Dennison, R. A., Dougherty, R. H., Shaw, P. E., *J. Agric. Food Chem.*, preceding paper in this issue (1978).

Bennett, G., Liska, B. J., Hempenius, W. L., *J. Food Sci.* **30**, 35 (1965).

Berg, H. W., Filipello, F., Hinreiner, E., Webb, A. D., *Food Technol.* **9**, 138 (1955).

Florida Citrus Mutual, Annual Statistical Report, 1968–69 season, Lakeland, Fla., 1969.

Guadagni, D. G., Buttery, R. G., Okano, S., Burr, H. K., *Nature (London)* **200**, 1288 (1963).

Hinreiner, E., Filipello, F., Webb, A. D., Berg, H. W., *Food Technol.* **9**, 351 (1955a).

Hinreiner, E., Filipello, F., Berg, H. W., Webb, A. D., *Food Technol.* **9**, 489 (1955b).

Kamen, J. M., Pilgrim, F. J., Gutman, N. J., Kroll, B. J., *J. Exp. Psychol.* **62**, 348 (1961).

Keith, E. S., Powers, J. J., *J. Food Sci.* **33**, 213 (1968).

Langler, J. E., Day, E. A., *J. Dairy Sci.* **47**, 1291 (1964).

McNamara, B. P., Danker, W. H., in "Basic Principles of Sensory Evaluation", American Society for Testing and Materials, Philadelphia, Pa., 1968.

Pangborn, R. M., *Food Res.* **25**, 245 (1960).

Pangborn, R. M., *J. Food Sci.* **26**, 648 (1961).

Patton, S., Josephson, D. V., *Food Res.* **22**, 316 (1957).

Rouse, A. H., Agricultural Research and Education Center, Lake Alfred, Fla., personal communication, 1971.

Siek, T. J., Albin, I. A., Sather, L. A., Lindsay, R. C., *J. Food Sci.* **34**, 265 (1969).

Ting, S. V., Attaway, J. A., in "The Biochemistry of Fruits and Their Products", Vol. 2, Hulms, A. C., Ed., Academic Press, New York, N.Y., 1971.

Valdes, R. M., Hinreiner, E., Simone, M. J., *Food Technol.* **10**, 282 (1956).


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