The proportions of disteryl ethers in lipids from organs and tissues as well as feces of mice that had been fed diets containing dicholesteryl ether and disitosteryl ether, respectively, were determined by capillary GLC. In the lipids of tissues of the gastrointestinal tract, minor proportions (1–27 μg/g of tissue, fresh weight) of these unmetabolized ether lipids are found, whereas the lipids of other organs and tissues do not contain any disteryl ethers at all. In feces, however, major proportions (up to 3.4 mg/g of feces, dry weight) of dicholesteryl ether and disitosteryl ether are detected. These findings are in good agreement with the results that had been obtained with radioactive disteryl ethers (Figure 1).

To summarize, it was found that both di([4-^{14}C]-cholesteryl) ether and di([4-^{14}C]sitosteryl) ether are virtually not absorbed in the gastrointestinal tract of mice. Radioactivity from the two substrates is detected neither in organs and tissues outside the alimentary canal nor in urine or in carbon dioxide of the expired air. Large proportions of both unmetabolized substrates, however, are excreted with the feces. In addition, remarkable proportions of radioactive metabolites of both substrates, caused most probably by bacterial degradation, are found in digesta of cecum and colon as well as in feces.

Both dicholesteryl ether and disitosteryl ether, fed to mice at a level of 400 mg/kg of body weight for 4 weeks, do not show results different from those of the control group with respect to feed intake, weight gain, and organ weights. No ill effects are observed during this time, and no abnormalities are detected in feces (color, blood, consistency) or urine (color, blood).

These results are in good agreement with earlier findings that dicholesteryl ether neither has deleterious effects on the growth of chick heart explants in vitro (Biswas et al., 1964) nor does it induce the formation of tumors in mice and rats (Kirby, 1943; Larsen and Barrett, 1944). The influence of bile acids on the absorption of disteryl ethers will be the subject of further investigations.

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Influence of Dispersion Medium on Aroma Intensity and Headspace Concentration of Menthone and Isoamyl Acetate

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Trained judges evaluated the aroma intensities of samples containing six concentrations of methone or isoamyl acetate dispersed in distilled water, soy oil, 2, 8, and 15% NaCl, 5, 20, and 40% sucrose, or 12, 25, and 50% egg albumin. The concentration of volatile compound in the headspace was quantified by gas chromatography (GC) using a direct sampling procedure. Relative to water, oil eliminated, albumin decreased, NaCl increased, and sucrose had little effect on perceived intensity and headspace concentrations. However, differences in magnitudes of the sensory and GC responses were observed for the two compounds due to their different physical behaviors. For example, increasing concentrations of NaCl significantly increased both perceived aroma and headspace concentrations of methone, with only small increases for isoamyl acetate. Results of the sensory and GC methods were highly correlated ($r > 0.90$), but the GC was more sensitive to small changes in volatility than were the human sensors.

Foods contain many ingredients such as proteins, lipids, carbohydrates, and salts, yet little is known of the effects of these ingredients on the intensity and quality of the main flavoring. Because most food systems contain a high percentage of water, the behavior of dissolved solutes such as flavor compounds in aqueous systems is important. In dilute water solutions, flavor volatiles usually follow Henry's law (Burnett, 1963; Buttery et al., 1969, 1971; Land and Reynolds, 1981)

$$p_B = kX_B$$

where p_B is the vapor pressure of the solute, B, X_B is the

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mole fraction of B, and k is an experimentally determined constant that depends on the interactions between solute and solvent. Under these very dilute conditions, all solute molecules are surrounded by solvent molecules, making solute–solute interactions unimportant; however, the molecules of solute and solvent may be physically different (Eisenberg and Crothers, 1979). Nawar (1966) showed that the volatilities of ethanol, a water-soluble compound, and of 2-heptanone, which is only slightly water soluble, were decreased in water solution. Headspace concentrations of aqueous solutions of heptane, a water-immiscible hydrocarbon, were nearly identical with those of heptane alone.

In simple lipid–water systems, most volatiles will partition themselves between the fat and the aqueous phases according to the physical law of partitioning (Solms et al., 1973; King, 1978). Buttery et al. (1973) showed that the vapor-phase concentration of the C_4–C_8 aliphatic aldehydes was decreased by the presence of as little as 1% vegetable oil in a water solution.

The influence of sugars on solute volatility varies with the specific saccharide and specific volatile as well as with their concentrations (Land and Reynolds, 1981; Nawar, 1971; Maier, 1970; Wientjes, 1968; Ahmed et al., 1978). Polysaccharides such as starches and gums generally decrease the volatility of flavor compounds (Solms et al., 1973; Maier, 1975; Ahmed et al., 1978).

In general, salts will increase the headspace concentration of volatile compounds. Jennings (1965) and Land and Reynolds (1981) reported that increasing the NaCl concentration increased the headspace concentration of ethyl acetate and diacetyl, respectively. In apple juice, the effect of NaCl addition on volatility varied; esters were unaffected whereas headspace concentrations of aldehydes and alcohols increased (Poll and Flink, 1984).

There is some evidence that flavor compounds interact with proteins, resulting in a decrease in perceived taste and aroma (Land and Reynolds, 1981; Nawar, 1971; Maier, 1970). According to King (1978), adsorption of butanol, benzaldehyde, and benzyl alcohol was directly proportional to the amount of protein present and did not depend on the temperature, pH, and ionic strength of the medium. Beyeler and Solms (1974) showed that binding increased as the concentration of the free ligand increased and was affected by the presence of various functional groups on the ligand molecule. They found that aldehydes, ketones, and alcohols, respectively, had the greatest binding affinity to soy protein and bovine serum albumin. Damodaran and Kinsella (1980, 1981a,b, 1983) also showed that binding affinity was influenced by the chain length and structure of the flavor compound and by the structural state of the protein.

Studies of Tunaley et al. (1985) indicated that the aroma intensities of ethyl benzoate and anisole decreased significantly with increasing additions of caffeine. Headspace concentrations for these odorants in 0.02 M solutions of caffeine at 20 °C were decreased by 19% for anisole and 39% for ethyl benzoate. Corresponding decreases in perceived odor intensity from caffeine-free to 0.02 M caffeine solutions were 43% for anisole and 32% for ethyl benzoate.

The present study was undertaken to measure the effects of various dispersion media (water, oil, and solutions of sucrose, NaCl, and egg albumin) on the volatility of menthone and isoamyl acetate by GC and to compare the data with sensory aroma intensities.

MATERIALS AND METHODS

Experimental Samples. Flavor-grade menthone and isoamyl acetate were obtained from International Flavors and Fragrances (Union Beach, NJ). Distilled, deionized water was used for all solutions. Solutions of reagent-grade sucrose (5, 20, 40%, w/v) and NaCl (2, 8 15%, w/v) were prepared approximately 12 h prior to testing. Undiluted, partially hydrogenated soybean oil (Albertson's brand) was used as an oil dispersion medium.

Hen eggs (UCD Avian Science Department) were separated and the whites homogenized to a consistent viscosity in a Waring blender. The homogenized albumin was frozen at −20 °C until 24 h prior to testing. The thawed albumin was diluted with water to obtain 12, 25, and 50% (v/v) albumin solutions. The solutions were centrifuged (6000 rpm, for 10 min) at 5 °C to remove aggregated proteins that precipitated upon dilution and were stored at 4 °C until testing. Actual protein concentrations, determined by the Biuret method (Gornall et al., 1949), were 17.7 ± 2.0, 34.2 ± 3.0, and 58.2 ± 1.0 mg/mL. [The concentrations of ingredients in these model systems were not necessarily related to concentrations occurring in natural or processed foods.]

Experimental samples were prepared approximately 2 h prior to testing. The aroma compound was weighed, dispersed in the selected media, and stored in capped vials for later GC or sensory testing. A concentration series of 0, 1.25, 2.5, 5, 10, and 20 ppm of each compound was chosen by eight experienced laboratory personnel to provide a range from low to high aroma intensity.

Sensory Procedures. Seventeen students were selected for each experiment on the basis of availability and interest. Of the 17 judges 15 evaluated both compounds. Judges were trained in three test sessions prior to each experiment.

Samples of 15 mL were presented at room temperature (21 ± 2 °C) in coded 60-mL brown, screw-top bottles. Within a set, samples consisted of duplicates of the six concentrations plus four corresponding references within a concentration of the dispersion medium. Resniffing was allowed, but judges were asked to use the bottles alternately to allow time for the vapor to reequilibrate after opening. Judges were asked not to swirl the containers prior to sniffing in order to maintain equilibrium conditions within the bottle.

Judges sniffed the reference sample (R) first and indicated the aroma intensity of each experimental sample in terms of deviation from R, which was 5 ppm of the aroma compound dispersed in water. R also was included as a blind sample to test for judge reproducibility. The perceived intensity of each sample was recorded on an unstructured 10-cm line scale marked with "Less than R" or "More than R" at the left and right ends, respectively, and "Same as R" in the center. Responses were decoded for statistical analysis by converting the mark on the line scale to centimeters using a Hewlett-Packard digitizer.

All evaluations were conducted in well-ventilated individual booths at 20 ± 2 °C, under white illumination. Each series was replicated twice, except for water which was replicated three times—at the beginning, middle, and end of each experiment—to test for learning effects throughout the test.

Gas Chromatographic Procedures. A direct headspace sampling technique similar to that used by Takeoka and Jennings (1984) was employed to quantify the amount of menthone or isoamyl acetate in the headspace above the solutions. Of the solution 3-mL portions were held in 8-mL glass vials fitted with a stopcock in a water bath at 20 ± 1 °C for at least 15 min. A 500-μL headspace sample was withdrawn with a 500-μL syringe with a fused silica capillary needle. Of the vapor 450 μL was injected slowly

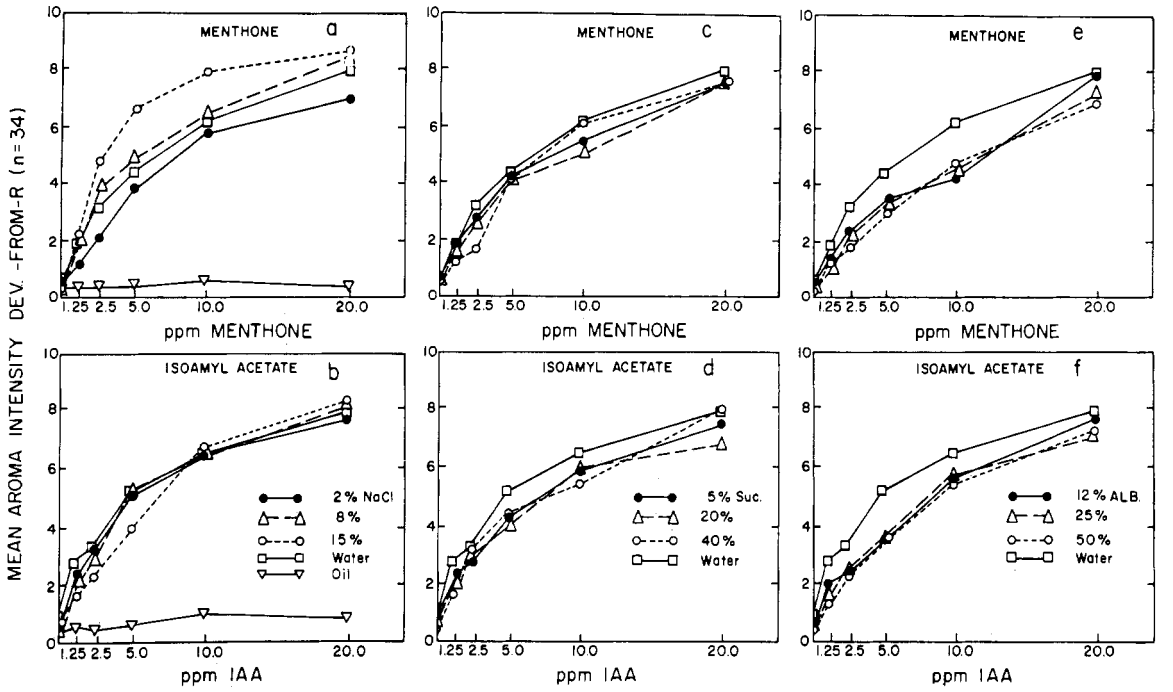

Figure 1. Mean aroma intensities of menthone and isoamyl acetate: a and b, dispersed in water, soybean oil, and in 2, 8, and 15% NaCl; c and d, dispersed in 5, 20, and 40% sucrose; e and f, dispersed in 12, 25, and 50% egg albumin.

Table I. Gas Chromatographic Conditions for Headspace Analysis of Volatiles

gas chromatograph: Hewlett-Packard 5880	
column: DB-5;[a] 15 m × 0.32 mm; 0.25-μm film thickness	
on-column injection	
sample size: 450 μL	
carrier gas: hydrogen	
carrier gas flow rate: 40 cm/min	
program conditions: menthone, T_i = 40 °C; rate = 5 °C/min to 120 °C; isoamyl acetate, T_i = 40 °C; isothermal	
attenuation: 2^2	
chart speed: 1 cm/min	

[a] J & W Scientific, Inc., Folsom, CA.

over a period of approximately 30 s onto the GC column via an on-column injector (J&W Scientific, Inc., Folsom, CA) fitted to a Hewlett-Packard 5880 GC. A single loop of the GC column (approximately 25 cm) was immersed in a Dewar flask of liquid nitrogen, which was removed 35–40 s after injection and temperature programming were initiated. GC conditions are given in Table I. The area of the menthone or isoamyl acetate peak was calculated by a Hewlett-Packard 5880 integrator attached to the GC.

Each day, GC analysis was done on all samples of one media in randomized order. Two replications for menthone were completed. Because of the large variability associated with the headspace analysis of isoamyl acetate, three replications were completed. During each replication the headspace concentration of each solution was determined in duplicate. A solution corresponding to the sensory reference containing 5 ppm of the aroma compound dispersed in water was also run each day.

Relative peak areas were calculated by subtracting the peak area of R, which contained 5 ppm menthone or isoamyl acetate in water, from the peak area of each experimental sample. This resulted in greater interday reproducibility as indicated by analysis of variance.

Statistical Analyses. Data analyses were performed with the Statistical Analysis System (SAS Institute Inc., Cary, NC) software packages using analysis of variance (anova) and general linear models programs. Means were compared at $p < 0.05$ by Fisher's least significant difference test. Intensity and GC peak area values for the blind reference were not included in the anova in order to maintained a balanced statistical design. The reference was compared to solutions containing 5 ppm menthone or 5 ppm isoamyl acetate with use of the Student's t statistic program of a Hewlett-Packard 9817A computer. Correlation and regression analyses were done with Minitab (University of Delaware).

RESULTS

Perceived Aroma Intensity. As anticipated, mean perceived aroma intensity responses gave a parabolic or logistic function with concentration in all media (Figure 1).

Water. Dispersion of 1.25–20 ppm menthone and isoamyl acetate (IAA) in water resulted in mean aroma intensities ranging from 1.92 to 8.04 and from 2.79 to 7.93, respectively (Figure 1a,b). For both compounds, the intensity ascribed to the blind reference (R) was very close to the prescribed value of 5 (4.5 for menthone and 5.2 for IAA). Analysis of variance showed no significant difference across the three replications for either menthone or IAA in water, demonstrating panel reproducibility. For uniformity, only data from the second and third replications were utilized in subsequent data analyses. Interactions of judge X replication, media X replication, and the three-way interactions involving replication were significant for both odorants, indicating that some judges modified their responses across sessions.

Soybean Oil. Oil essentially eliminated the aroma intensities of both menthone and isoamyl acetate (Figure 1a,b).

Sodium Chloride. Increasing NaCl from 2 to 15% increased the perceived aroma intensity at all menthone concentrations (Figure 1a). Solutions with 8 and 15% NaCl had the highest perceived intensities for all menthone concentrations, but neither the 1.25 nor the 20 ppm levels differed significantly from each other or from water. Compared to water, 15% NaCl significantly increased intensity of 2.5, 5, and 10 ppm menthone. Comparison of R with solutions containing 5 ppm menthone showed no difference in 2% NaCl, but there were significant differ-

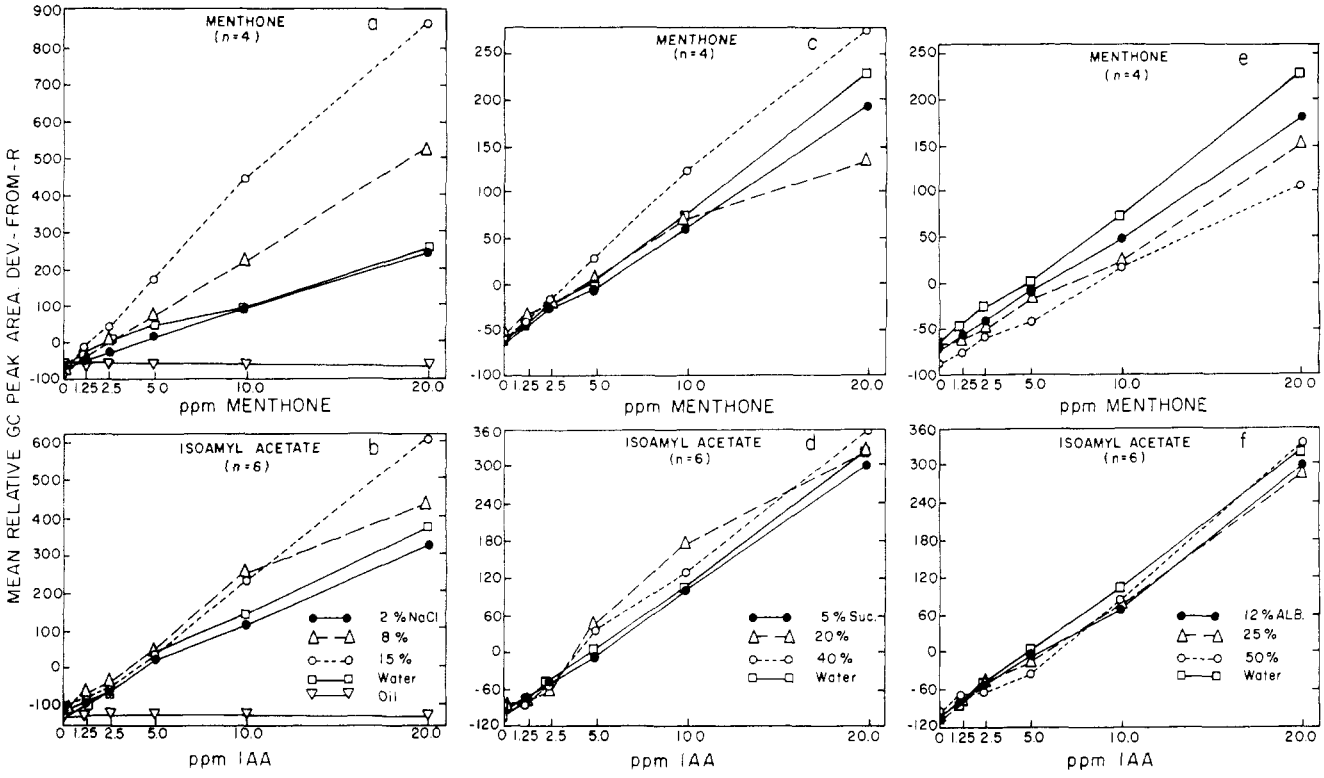

Figure 2. Mean relative GC peak areas for menthone and isoamyl acetate: a and b, dispersed in water, soybean oil, and in 2, 8, and 15% NaCl; c and d, dispersed in 5, 20, and 40% sucrose; e and f, dispersed in 12, 25, and 50% egg albumin.

ences in the 8% ($p < 0.01$) and 15% ($p < 0.001$) NaCl solutions. For isoamyl acetate, 15% NaCl slightly increased perceived intensity of the 10 and 20 ppm solutions (Figure 1b). There was no difference in intensity between R and the 5 ppm isoamyl acetate solution at the lower concentrations of NaCl, but in the 15% NaCl solution, intensity was significantly lower than R ($p < 0.01$).

Sucrose. Solutions of 5–40% sucrose had no significant effect on the perceived aroma intensity of either menthone or isoamyl acetate (Figure 2a,b). The two sets of curves were slightly lower than the corresponding distributions in water. There was no significant difference between the reference and the solutions containing 5 ppm menthone at each of the three sucrose concentrations.

Egg Albumin. Compared to water, the perceived aroma intensity of most concentrations of menthone decreased when dispersed in egg albumin (Figure 1e). At 5 ppm, menthone was perceived as significantly ($p < 0.01$) lower than R for all three concentrations of albumin. All albumin concentrations also significantly decreased the perceived aroma intensity of isoamyl acetate, and R differed significantly ($p < 0.001$) from the 5 ppm solution at all albumin concentrations.

GC Headspace Concentration (HC). Figure 2 displays the plots of the relative peak areas for menthone and isoamyl acetate dispersed in five media. Most HC distributions were linear with concentration. Analysis of variance indicated significant variation attributable to replications for the isoamyl acetate measurements, indicating more variability than with menthone.

Soybean Oil. Oil completely eliminated the contribution of menthone and isoamyl acetate to headspace compositions (Figure 2a,b). For both compounds, the peak area for the 5 ppm reference in water was significantly ($p < 0.001$) greater than that concentration in the oil media.

Sodium Chloride. There was a highly significant increase in HC with increasing concentrations of NaCl, particularly for menthone (Figure 2a). Dispersion of 20 ppm menthone in 15% NaCl resulted in an almost 4-fold increase in HC compared to dispersion in 2% NaCl or in water. At 2.5, 5, 10, and 20 ppm menthone, the HC of solutions with 8 and 15% NaCl differed significantly from each other and was greater than that of water. For isoamyl acetate, increasing NaCl from 2 to 15% increased the headspace concentration of only the two highest levels of isoamyl acetate (Figure 2b).

Sucrose. HC of menthone was significantly increased by the addition of 40% sucrose compared to the 5 and 20% sucrose levels and to water (Figure 2c). There was an unexpected deceleration of the HC of 20 ppm menthone in the 20% sucrose solution. The HC of R differed significantly from the 5 ppm sample in 40%, but not in 5 and 20% sucrose solutions. Sucrose had no significant effect on the HC of the three lowest levels of isoamyl acetate, but 20 and 40% sucrose produced elevated HC values for solutions with 10 and 20 ppm (Figure 2d). A significant difference was observed between the peak areas of R and the 5 ppm sample dispersed in 20 and 40% sucrose.

Albumin. Compared to water, the HC of menthone solutions was decreased by increasing amounts of albumin (Figure 2e). There was no significant difference between R and the 5 ppm sample dispersed in 12%, but large differences were observed in the 25 and 50% albumin solutions. Albumin produced only minor changes in the HC of isoamyl acetate solutions (Figure 2f). There was no significant difference between R and the 5 ppm sample dispersed in any of the three albumin solutions.

Correlation of Sensory and GC Results. Sensory aroma intensity and HC results were highly related ($r = 0.90$ or greater). The relationship was best described by a parabolic equation. No correlations were calculated for the oil medium as there was little or no change in the perceived aroma intensity and GC peak area across odorant concentrations. For both compounds, the regression line for water solutions intercepted the perceived intensity axis very close to an intensity score of 5.00, the value of

R. The NaCl media had the lowest intercept for menthone, and the sucrose media was lowest for isoamyl acetate. The slope of the linear portion of the curve increased most rapidly when both flavor compounds were dispersed in NaCl. The curves for menthone dispersed in water and in sucrose were nearly identical. For isoamyl acetate, the shapes of the regression lines were similar when water, albumin, and sucrose were the dispersants.

DISCUSSION

Influence of Dispersion Media. As expected, perceived intensity and HC increased significantly ($p < 0.05$) with increasing concentrations of menthone and isoamyl acetate (except in oil). The relationship between aroma intensity and odorant concentration was best described by parabolic or logistic curves. Intensity is expected to increase as a logarithmic function of concentration as predicted by Fechner's law ($I = k \log c$, where I is perceived intensity, k is a constant, and c is the stimulus concentration). Ignoring the 0 ppm sample, a closer logarithmic function is obtained, but some curvature of the line persists. The psychophysical function used to describe sensory responses depends on the stimulus tested, the concentration range tested, the psychophysical method employed, and variations in scale usage by the judges (Trant et al., 1981; Giovanni and Pangborn, 1983). In dilute water solutions, a linear relationship between HC and actual concentrations of menthone and isoamyl acetate was observed, as predicted by Henry's law.

Soybean oil completely eliminated the perceived intensity of both menthone and isoamyl acetate. The soybean oil had a slight, potentially distracting odor of its own that may have masked the aroma of the two compounds. However, there was a complete elimination of the GC headspace of both compounds due to their greater solubility in oil, a nonpolar medium, than in water, a relatively polar medium. These observations confirm earlier reports that aroma, taste, and volatility of flavor compounds were diminished even in small amounts of lipids (Mackey and Valassi, 1956; van Eijk, 1971; Solms et al., 1973; Buttery et al., 1973; Grab et al., 1977; King, 1978).

The effect of sucrose on the perceived aroma intensity of isoamyl acetate over the concentration range tested was minimal. The increased HC on menthone in 40% sucrose may be related to a decrease solubility, but the effect was not proportional to sugar concentration. The HC for isoamyl acetate also was increased slightly when dispersed in 40% sucrose, but the effect was not as great, possibly because isoamyl acetate is slightly more polar than menthone and is initially more soluble in water. The presence of other polar molecules (e.g., sucrose) does not affect solubility of isoamyl acetate as greatly. The mechanism by which sucrose interacts with flavor compounds is not well understood, but the manner in which the solutions are prepared may be important. Nawar (1971) observed differences in the HC of 2-heptanone and 2-pentanone depending on whether sucrose was added to a solution of the flavor compound or vice versa. He attributed the effect to interaction of sugar with water molecules rather than between sugar and the volatile compounds.

NaCl increased both aroma intensity and HC of menthone at levels greater than 2% NaCl. The highest level slightly depressed the intensity of lower concentrations of isoamyl acetate with a slight increase in intensity at the 20 ppm level. NaCl increased the HC of both isoamyl acetate and menthone, but the effect was more pronounced for the latter. Salts are thought to increase flavor volatility by increasing vapor pressures as expressed by

$$p_i = x_i y_i P_{io}$$

where p_i is the partial pressure of the solute i, x_i is the concentration of i, and P_{io} is the vapor pressure of the pure compound (Jennings and Rapp, 1983). The activity coefficient, y_i, is a measure of the deviation of a real solution from the ideal Henry's law solution. Solutions may differ from Henry's law when the solution is not dilute and/or when solute–solute interactions are present. The activity coefficient is 1 when the solution follows Henry's law. When salts are added to solutions of dissolved flavor compounds, the activity coefficient and the HC are greater than predicted by Henry's law due to mutual interactions of the solutes and solvents. The amount of increase in volatility in the presence of salts is dependent on the flavor compound (Nawar, 1966; Poll and Flink, 1984).

Egg albumin decreased aroma intensity of menthone and isoamyl acetate, but HC was decreased only for menthone. Although few albumin concentration effects were noted, there was a decrease in the HC of menthone with increasing albumin concentration. The slight aroma of the albumin solution may have interfered with the aroma judgments. Alternatively, the judges may have been unable to discriminate between differences that could be detected by the GC. Because binding is mainly a result of hydrophobic interactions (Franzen and Kinsella, 1974; Maier, 1975; King, 1978; King and Solms, 1979), menthone, a relatively less polar compound than isoamyl acetate, may have interacted with the hydrophobic regions of the albumin to a greater extent. As a result, the solubility of menthone may have increased, opening up previously buried hydrophobic zones of the protein that become exposed for further binding, further decreasing the HC (Solms et al., 1973).

The manner in which solutions are prepared can influence results. Several researchers have used long equilibration periods with constant shaking to study binding of flavor compounds to proteins (Beyeler and Solms, 1974; King, 1978; Damodaran and Kinsella, 1980, 1981a,b, 1983). A shorter equilibration time was used by Franzen and Kinsella (1974) who agitated solutions for 1 min after which they were allowed to equilibrate for 20 min. In the present study, the protein and odorant were agitated gently for 1–2 min and allowed to equilibrate for at least 30 min before testing. This should have allowed for complete adsorption because the HC did not change over a period of 5–6 h. Altering the quaternary structure can greatly alter the affinity of flavor compounds to proteins. Denatured proteins generally adsorb more volatiles than do native proteins (King, 1978; Damodaran and Kinsella, 1980, 1981a,b, 1983).

Correlation of Sensory and GC Results. GC and sensory results were highly correlated for all media except oil; however, the relationship was not linear but parabolic. It is important to test a large concentration range of flavor compounds to establish correlations between instrumental and sensory methods (Noble, 1975; Trant et al., 1981). Although perceived intensity and the HC were highly correlated for the sucrose, NaCl, and albumin media, the individual data points indicated that the GC was much more sensitive than the sensory panel to small changes in HC. For example, the increased HC of menthone in 40% sucrose was not perceived by the sensory panel. Slight differences in the regression curves were observed between menthone and isoamyl acetate; e.g., in NaCl solutions, the slope of the line increased more rapidly and reached a higher maximum value for menthone than for isoamyl acetate. Albumin did not affect the relationship between perceived intensity and GC peak area of isoamyl acetate as much as that of menthone. The results suggest that it

is possible to predict the perceived intensity of a flavor compound from HC measurements. However, knowledge of the effects of each dispersant on each volatile species over a wide concentration range must be known before accurate correlations can be obtained.

CONCLUSIONS

With sensory and GC headspace techniques, dispersion media differentially influenced the volatility of menthone and isoamyl acetate. In general, oil eliminated, albumin decreased, NaCl increased, and sucrose had little effect on the perceived intensity or HC as compared to water. However, differences in magnitudes of the responses were observed between compounds due to their different physical properties. Over the concentration ranges studied, aroma intensity increased as a parabolic or logistic function of actual odorant concentration, while HC increased as a linear function. Both the deviation from reference and the GC headspace analysis procedures proved to be reliable for measuring the headspace concentration of these compounds, especially menthone. However, better training of the judges in scale usage and utilization of a larger concentration range during training may have improved the sensory reproducibility. The precision of both methods, especially the GC procedure, could have been improved by better control of temperature during analysis. Modification in the headspace sampling syringe is necessary to make it air-tight and easier to use. Only intensity of aroma was measured herein. Information is needed on the effects of dispersion media on flavor by mouth and taste intensities of these volatiles.

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Registry No. Menthone, 89-80-5; isoamyl acetate, 123-92-2; sodium chloride, 7647-14-5; sucrose, 57-50-1.

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