

# Flavor Release from Mixtures of Sodium Cyclamate, Sucrose, and an Orange Aroma

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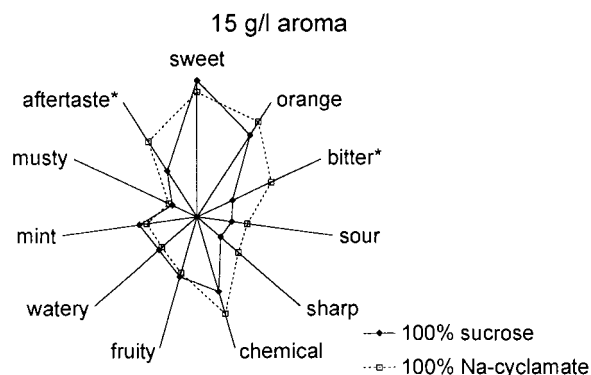
Mixtures of sucrose and sodium cyclamate were studied to evaluate possible interactive effects between these sweeteners and orange aroma compounds. Equisweet mixtures (10% sucrose equivalent value) of the sweeteners were prepared by taking into account the observed synergistic effects between sucrose and sodium cyclamate. The release of volatile compounds from the solutions containing these mixtures and a water soluble orange aroma was studied. The volatile compounds were quantified and identified by gas chromatography combined with flame ionization detection (GC/FID) and mass spectrometry (GC/MS). The presence of sucrose significantly changes the release of 15 selected volatile compounds from a solution containing the aroma compared to the aqueous control. Increasing sucrose concentrations in the solutions (0–60 w/v %) caused an increased release of the volatile compounds with short GC/FID retention times and a decreased release of the compounds with longer retention times. Increasing sodium cyclamate concentrations (0–2.658 w/v %) did not change the release of volatile compounds.

**Keywords:** Flavor release; sodium cyclamate; sucrose; orange aroma; interactions

## INTRODUCTION

Sweetness–flavor interactions in models for soft drinks can be studied by sensory and instrumental analysis. In a previous sensory experiment equisweet mixtures of sucrose and sodium cyclamate were evaluated (Nahon et al., 1998). As the mixtures of sucrose and sodium cyclamate showed positive synergy, solutions containing these mixtures and a water soluble orange aroma were chosen to study sensory-perceived properties by quantitative descriptive analysis (QDA). Comparison of a sucrose and a sodium cyclamate solution at 10% sucrose equivalent value (SEV) (a sweetness equivalent to a 10 w/v % sucrose solution) revealed significant differences for the attributes bitter and aftertaste (Figure 1). As 15 g/L of orange aroma was present in the solutions, differences for some other attributes were found as well. Interactive effects between the sweeteners and volatile compounds of the orange aroma could be present in these solutions.

The possible interactive effects would change the release of volatile compounds from solutions made up from sweeteners and an aroma. An instrumental analysis by gas chromatography provides information on this release. Von Sydow et al. (1974) noted that adding sucrose to blueberry and cranberry juices gave only little change in the headspace composition and did not significantly alter the profile determined from “sniffing”. According to them, sucrose appears to enhance aroma on a psychological level, rather than acting



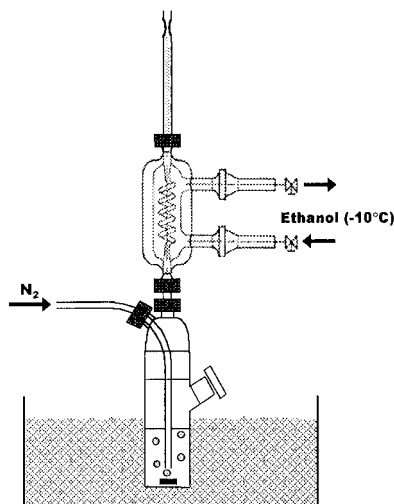
**Figure 1.** Spider web diagram representing the mean scores for sensory attributes of a sucrose and a sodium cyclamate solution at 10% SEV (in the presence of 15 g/L of a water soluble orange aroma); \* = significant differences ( $P < 0.05$ ).

upon chemical constituents and thus modifying the vapor composition. Their semiquantitative data showed that an increasing sucrose level in the juices increases the peak areas for the very low-boiling constituents in the gas phase. However, they reported that these compounds are not important for the desirable aroma of a fruit juice. Wiseman and McDaniel (1991) found that sucrose-sweetened solutions did not significantly affect the perception of orange or strawberry fruitiness. Le Quéré et al. (1994) observed chemical effects of intense sweeteners on the flavor of diet orange soft drinks. They mainly followed tendencies upon aging of the soft drinks. Johnson and Vora (1983) and Ohta et al. (1992) recognized the oxygen-containing components, including aldehydes such as octanal and alcohols, as the major contributors to the fresh and pleasant flavor of orange essences because of their low threshold and their olfactory characteristics. According to Moshonas and

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**Figure 2.** Mouth model used for the purge and trap method. Nitrogen gas passes through the stirred solution to trap the volatile compounds in Tenax TA.

Shaw (1984), citrus juice volatiles concentrated in the aqueous fraction reflect both quantitatively and qualitatively the flavor and aroma of the parent juice. The aqueous fraction is thus a desirable flavoring material.

Mixtures of sucrose and sodium cyclamate equisweet at 10% SEV were prepared, and interactive effects between both sweeteners and a water soluble orange aroma were studied by instrumental analysis. The effect of sucrose on the flavor release was compared with the effect of sodium cyclamate by studying the release of the volatile compounds in solutions containing increasing concentrations of sucrose or sodium cyclamate.

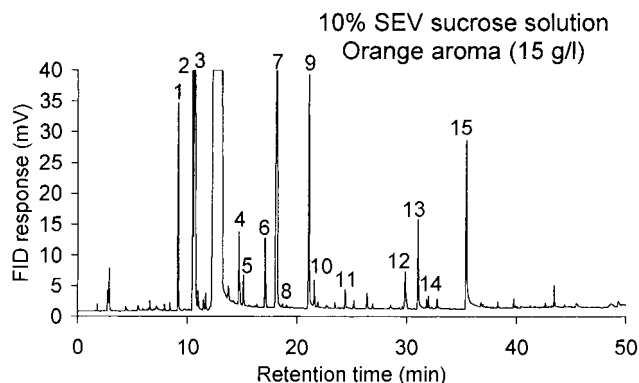
## MATERIALS AND METHODS

The release of volatile compounds from the solutions was studied by gas chromatography.

**Stimuli.** Stimuli were solutions of sucrose (CSM Suiker BV, Amsterdam, The Netherlands) and sodium cyclamate (Flevo Chemie BV, Harderwijk, The Netherlands) and mixtures of these two substances in demineralized water. Nine sucrose/sodium cyclamate ratios (100/0, 90/10, 75/25, 60/40, 50/50, 40/60, 25/75, 10/90, and 0/100) were chosen to study possible interactive effects between (mixtures of) sweeteners and aroma compounds. The overall sweetness of these mixtures met with a constant perceived taste intensity of 10% SEV (Nahon et al., 1998). The orange aroma added was a sample of the watery vapor phase of stripped orange juice (Cargill Juice Division, Amsterdam, The Netherlands). It was used in a constant concentration of 15 g/L. Increasing concentrations of sucrose (0, 5, 10, 20, 40, and 60 w/v %) and sodium cyclamate (0, 0.222, 0.443, 0.886, 1.772, and 2.658 w/v %) were chosen to study the effect of adding sweeteners on the release of volatile compounds from a solution containing the aroma.

All solutions were prepared at least 24 h before evaluation and stored at 4 °C overnight. In all evaluations, a sample consisted of 15 mL of solution. The samples were brought to room temperature (22 °C) before further analysis.

**Isolation of Volatile Compounds.** Samples consisting of 15 mL of the different mixtures mentioned (identical to the stimuli) were transferred in a so-called mouth model (Figure 2), consisting of a sample flask (50 mL) at a temperature of 37 °C (water bath), to which 4 mL of diluted artificial saliva containing salts, mucine, and  $\alpha$ -amylase in water was added (Van Ruth et al., 1994). A purified nitrogen gas flow (20 mL/min) passed through the stirred solution for 10 min to trap the volatile compounds in 0.10 g of Tenax TA [poly(2,6-diphenyl-*p*-phenylene oxide,  $\varnothing$  0.25–0.42 mm, Alltech Nederland b.v., Zwijndrecht, The Netherlands), positioned in a glass



**Figure 3.** Gas chromatogram obtained by purging a 10% SEV sucrose solution, containing 15 g/L of a water soluble orange aroma. Peak numbers refer to the volatile compounds listed in Table 1.

tube, 100 mm long and 3 mm i.d.. One measurement consists of four repetitions of this method.

**Gas Chromatography (GC).** The volatile compounds were desorbed from Tenax by a thermal desorption/cold trap device (Carlo Erba TDAS 5000, Interscience b.v., Breda, The Netherlands). The compounds were analyzed by GC on a Carlo Erba HRGC 5300 (Interscience b.v.), equipped with a Supelcowax 10 capillary column (60 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m) and a flame ionization detector (FID) at 275 °C. The oven temperature was 40 °C for 4 min and then programmed to 92 °C at a rate of 2 °C/min and then to 272 °C at 6 °C/min.

The volatile compounds were identified with the help of a gas chromatograph/mass spectrometer (GC/MS; Varian 3400/Finnigan MAT 95, Bremen, Germany), equipped with a Chrompack 16200 thermal desorption/cold trap unit (Chrompack, Middelburg, The Netherlands). The capillary column and the temperature program were the same as described above. The mass spectrometer was operated in the 70 eV EI ionization mode and scanned from mass 24 to 300 with a cycle time of 1.0 s.

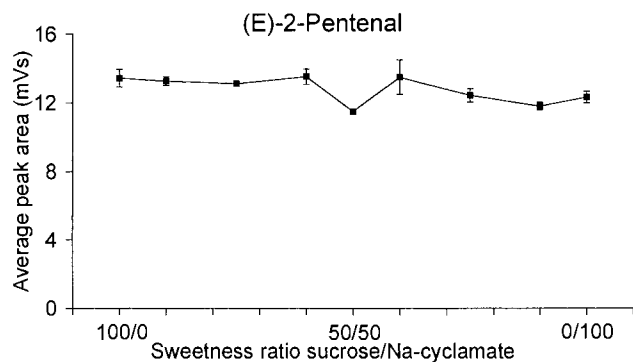
## RESULTS

Flavor release from nine equisweet sucrose/sodium cyclamate mixtures was studied. Figure 3 shows an example of the gas chromatograms obtained by purging 10% SEV sucrose solutions, containing 15 g/L of orange aroma. With the help of GC/FID for quantity evaluation and GC/MS for identity evaluation, 15 volatile compounds were selected (Table 1). To observe possible interactive effects between sweeteners and aroma compounds, graphs of the average peak area versus the sweetness ratio were plotted for each volatile compound. In Figure 4 the results obtained for (*E*)-2-pentenal are given, which are representative for results obtained for the other volatile compounds. In Table 1, the average peak areas for the extremes, that is, sucrose (100/0 mixture) and sodium cyclamate (0/100 mixture) are compared. For six volatile compounds (peaks 1–6, 6–13, and 14–15). Log *P* represents the hydrophobicity of the volatile compound; a negative value implies hydrophilicity. In Figure 5A the relative sum of the peak areas presented was obtained by summing the average peak

**Table 1. Volatile Compounds of an Orange Aroma, Released from Solutions Containing Sucrose or Sodium Cyclamate at 10% SEV, Their Peak Numbers, Retention Times (RT), Hydrophobicity Constants  $\log P$  As Calculated (Rekker, 1977), Average Areas, and Standard Deviations (SD;  $n = 4$ )<sup>a</sup>**

peak	RT (min)	volatile compound	$\log P$	av areas and SDs (mVs)	
				sucrose	sodium cyclamate
1	9.1	1-ethoxy-1-methoxy-ethane	-0.291	103 ± 11	85 ± 7* <sup>b</sup>
2	10.4	ethyl acetate	0.642	460 ± 31	396 ± 22*
3	10.6	1,1-diethoxyethane	0.239	867 ± 86	724 ± 70*
4	14.7	2-pentanone	1.033	56 ± 6	39 ± 4*
5	15.0	methyl butanoate	1.172	20 ± 2	17 ± 1*
6	17.0	1-penten-3-one	0.736	51 ± 4	42 ± 2*
7	18.1	ethyl butanoate	1.702	519 ± 32	473 ± 25
8	18.1	2-methyl-3-buten-2-ol	1.123	106 ± 7	97 ± 5
9	20.9	hexanal	1.476	220 ± 18	204 ± 11
10	21.4	2-methyl-1-propanol	0.678	14 ± 1	13 ± 1
11	24.3	( <i>E</i> )-2-pentenal	0.356	14 ± 2	12 ± 1
12	29.8	3-methyl-1-butanol	1.208	29 ± 10	27 ± 8
13	30.8	( <i>E</i> )-2-hexenal	0.886	67 ± 11	64 ± 6
14	31.8	ethyl hexanoate	2.762	9 ± 2	10 ± 0
15	35.3	octanal	2.536	206 ± 37	238 ± 9
		CV <sup>c</sup> (%)		11.9	8.2

<sup>a</sup> Short (<15 min), medium (15–31 min), and long (>31 min) retention time groups are distinguished. <sup>b</sup> \*, significant differences ( $P < 0.05$ ). <sup>c</sup> CV (%), overall coefficient of variance.

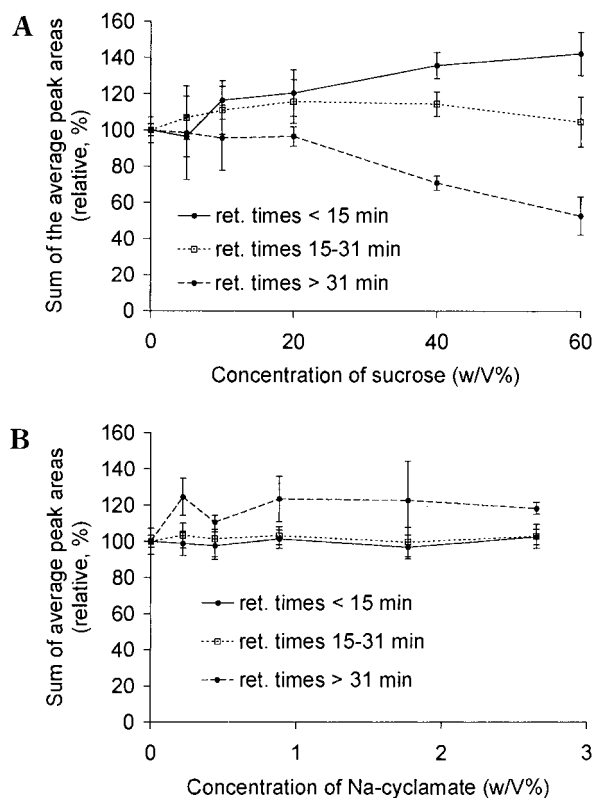


**Figure 4.** Average peak areas and standard deviations ( $n = 4$ ) for (*E*)-2-pentenal, released from solutions containing mixtures of sucrose and sodium cyclamate, for different sweetness ratios, all equaling to a total sweetness of 10% SEV.

areas within one retention time group and then relating to the 0 w/v % solution, which is the control. Increasing the concentration of sucrose enlarged the release of the volatile compounds with smaller RT; it did not change the release of the volatile compounds with medium RT and decreased the release of the volatile compounds with larger RT. Increasing the concentration of sodium cyclamate did not reveal any differences among the three retention time groups (Figure 5B).

#### DISCUSSION

The release of volatile compounds from solutions containing a water soluble orange aroma and sucrose and/or sodium cyclamate was studied; 15 volatile compounds were selected for the instrumental analysis (Table 1). Although ethanol is the volatile compound present in the largest quantity in fresh orange juice (Shaw, 1977, 1991), this compound was not selected for the analysis. Ethanol is mainly used as a solvent and might enhance the fruity character of the aroma without contributing to a distinct aroma by itself (Williams and Rosser, 1981; Shaw, 1991). Moshonas and Shaw (1984,



**Figure 5.** Volatile compounds of an orange aroma, released from solutions with increasing concentrations of sucrose (A) or sodium cyclamate (B). Sum of average peak areas and mean coefficients of variance, both related to a control solution of orange aroma in water, for volatile compounds with short (<15 min), medium (15–31 min), and long (>31 min) retention times (Table 1).

1987, 1989, 1994) and Lin et al. (1993) showed typical gas chromatograms of various orange aromas with peak identifications. For some volatile compounds the retention times were different in this study, which could be ascribed to the use of a stationary phase made up of 5% phenyl–95% methyl silicone in their GC analysis. The chromatograms and compounds shown by Moshonas et al. (1972), Ohta et al. (1992), and Tønder et al. (1998) gave the same sequence and similar retention times as shown in Figure 3; they all used poly(ethylene glycol) as a stationary phase in the GC analysis. All volatile compounds found in this study were reported by other authors as well (Table 2). In fresh juice octanal, ethyl butanoate, and hexanal were among the most important volatile compounds. Ethyl butanoate, octanal, and 2-pentanone were the most odor active compounds (Tønder et al., 1998). Of the 15 volatile compounds selected, methyl butanoate, ethyl butanoate, and octanal give strong fruity or orange flavors (Ahmed et al., 1978; Shaw, 1991). Other volatile compounds present in the orange aroma might contribute indirectly to the orange flavor through additive or synergistic effects with other components. Ethyl acetate is one of the major esters in fresh orange juice, but it is mostly present at less than its flavor threshold value in water and probably does not make a direct contribution to orange flavor. In some perfumes ethyl acetate shows an azeotropic effect to citrus notes (Shaw, 1991).

Six volatile compounds with short retention times give significantly higher peak areas when sucrose is present in the solution; they were significantly more released in solutions containing sucrose compared to sodium

**Table 2. Volatile Compounds Present in Orange Aroma/Juice As Reported by Several Authors<sup>a</sup>**

volatile compound	1	2	3	4	5	6	7	8	9	10	11	12
1-ethoxy-1-methoxyethane							×					×
ethyl acetate			×	×	×	×	×	×	×	×	×	×
1,1-diethoxyethane	×	×	×				×		×			×
2-pentanone											×	×
methyl butanoate	×		×	×	×	×	×	×	×	×		×
1-penten-3-one			×	×	×							×
ethyl butanoate	×	×	×	×	×	×	×	×	×	×	×	×
2-methyl-3-buten-2-ol						×	×		×		×	×
hexanal	×	×	×				×		×	×	×	×
2-methyl-1-propanol			×	×	×				×			×
( <i>E</i> )-2-pentenal		×				×						×
3-methyl-1-butanol				×	×		×		×			×
( <i>E</i> )-2-hexenal	×	×	×	×					×	×		×
ethyl hexanoate					×			×				×
octanal	×	×	×	×	×	×		×	×	×	×	×
(ethanol)		×	×	×	×	×	×	×	×		×	×

<sup>a</sup> 1, Moshonas et al. (1972), volatile compounds in orange; 2, Moshonas and Shaw (1984), aqueous orange essence; 3, Moshonas and Shaw (1987), fresh orange juice; 4, Moshonas and Shaw (1989), aseptically packaged orange juice; 5, Moshonas and Shaw (1994), fresh-squeezed unpasteurized orange juice; 6, Shaw (1991), volatiles important to orange flavor, overview; 7, Johnson and Vora (1983), major components of orange aroma; 8, Ohta et al. (1992), orange essences; 9, Lin et al. (1993), typical natural orange aroma; 10, Le Quéré et al. (1994), diet orange soft drinks; 11, Tønder et al. (1998), orange juice; 12, Shaw (1977), aqueous essences, overview article.

cyclamate (Table 1). Purging simple solutions containing sweeteners but no orange aroma did not release any volatile compounds. Apparently the presence of sweeteners, and especially sucrose, influences the release of volatile compounds from an orange aroma. To fully understand the effect of the addition of sweeteners, their concentrations were increased.

Parts A and B of Figure 5 show the changes in peak area with increasing concentrations of sucrose and sodium cyclamate, respectively. Volatile compounds with short, medium, and long RT are distinguished. The influence of sugars on solute volatility varies with the specific sugar and specific volatile as well as with their concentrations (Ebeler et al., 1988). When the sucrose concentration increases, the peak areas of the volatile compounds with short RT increase significantly. Increases in the concentration of sucrose enhance the release of volatile compounds with short retention times. The hydrophobicity constants,  $\log P$ , for these compounds (Table 1) indicate that these are rather hydrophilic and thus better soluble in water (Espinosa-Díaz et al., 1996). De Roos and Wolswinkel (1994) explained that the addition of sucrose increases the hydrophobic character of the solution. The quality of the solvent then changes. Wientjes (1968) reported an increase in peak height for ethyl butanoate upon addition of rather high concentrations of invert sugar (73.1 w/w %) and fructose (79.1 w/w %). The headspace concentration of acetone increased by addition of sucrose to solutions (Nawar, 1971; Voilley et al., 1977). For one of the volatile compounds with short retention times, ethyl acetate, Voilley and Bosset (1986) determined the partition and activity coefficients in pure water compared with a 50 w/w % glucose solution. Both partition and activity coefficients were larger in the 50 w/w % glucose solution. Chandrasekaran and King (1972) measured the activity coefficients of ethyl acetate and hexanal as a function of sucrose concentration. The experimentally determined activity coefficients of the

volatile compounds increased with increasing sucrose concentration. Kieckbusch and King (1979) found the partition coefficients for C<sub>1</sub>–C<sub>5</sub> acetates between air and solutions of sucrose to increase sharply with increasing sucrose contents. This effect could be attributed qualitatively to a loss of free water due to hydration of sugar molecules. Only the free water is available as a solvent for the acetates. Several other authors (Nawar, 1971; Darling et al., 1986) described this effect as "salting-out". According to Nawar (1971), the effects on the headspace concentration of volatiles are related to the interactions of the solids with water. Changes in headspace concentrations of volatiles upon addition of sucrose to aqueous volatile solutions do not involve a direct interaction between sugar and volatile but mostly occur via interaction of the sugar with the water molecules.

The peak areas of the volatile compounds with medium RT remain constant, and those of the volatile compounds with long RT decrease significantly. This decrease in the release of the volatile compounds with longer retention times might be due to an effect of an increasing viscosity of the solution. According to Fick's law, the release of volatile compounds is linearly related to their diffusion coefficients, which are inversely proportional to the viscosities. The diffusion of volatile compounds then decreases with increasing viscosity of the solution (Roberts et al., 1996). As we used a purge and trap method to analyze the release of volatile compounds, the viscosity of the solutions is probably less important. Nawar (1971) reported viscosities for 0, 20, 40, and 60 w/w % sucrose solutions to be 0.8, 1.5, 4.3, and 33.8 cP, respectively. As we composed sucrose solutions of 0 to 60 w/w %, which is 0 to 48.75 w/w %, the viscosities obtained will increase less. In the experiments of Nawar (1971) the headspace concentrations of heptanone and heptanal decreased with increasing sucrose concentrations in the solutions. Contradictory findings by Wientjes (1968) show an increase in peak height for ethyl hexanoate upon addition of invert sugar or fructose, both in rather high concentrations (73.1 and 79.1 w/w %, respectively).

De Roos and Wolswinkel (1994) reported a retention of hydrophobic volatile compounds, such as  $\alpha$ -ionone and naphthalene, upon addition of sucrose. The volatility did not change for esters and carbonyl compounds, such as methyl butanoate, 2-hexanone, 1-hexanol, methylbenzoate, and methylcinnamate. Bakker et al. (1996) found a detectable decrease in release of rather large volatiles with high volatility when increasing viscosity and thus sucrose concentrations. For the less volatile flavors no detectable effect was found by them. According to Roberts et al. (1996), the volatility of a flavor molecule may be affected by the reduction of diffusion of flavor molecules, by the formation of barriers occurring in high-viscosity matrices, or by specific binding interactions with the solute/thickening agent. Highly volatile compounds are most affected by a change in viscosity. Less volatile flavors do not show a significant decrease in volatility. Roberts et al. (1996) suggest also that inclusion complexes may be present in the sucrose solutions for hydrophobic molecules. They determined whether any decreases in aroma release were due to mass transfer alone or to odorant binding by the thickening agents. From a comparison between the thickening agents sucrose, carboxymethylcellulose, and guar gum they concluded that binding interactions are

probably present, giving a different flavor release for the various thickening agents. As sucrose solutions are concentrated, they become more glassy or crystalline in state. Trapping of volatiles in these solutions could occur due to partial crystalline formation. Hydrophobic complexes will be formed, which results in a greater depression of volatility by sucrose. Pangborn and Szczesniak (1974) investigated the effect of hydrocolloids and viscosity on flavor and odor intensities of flavor compounds and also found effects that were specific for the gum/odorant combination. The addition of hydrocolloids generally decreased both odor and flavor intensities.

Increasing concentrations of sodium cyclamate do not significantly change the peak areas for either of the three groups of volatile compounds (Figure 5B). Beck (1956) determined the increase of viscosity of a solution with an increasing concentration of sodium cyclamate. The properties found are radically different from those of a sucrose solution of comparable sweetness. The relative viscosity  $\eta/\eta_0$  at 25 °C for a 10% SEV sodium cyclamate solution (0.44 g/L) is indistinguishable from 1. The viscosity of this solution is thus equal to the viscosity of water. Beck (1956) also reported sodium cyclamate to have the properties of a strong electrolyte salt and to act as NaCl would. Therefore, at higher concentrations, sodium cyclamate could have the same effect as salts and salt-out volatile compounds. Bopp and Price (1991) reported cyclamate to enhance fruit aromas. In the results obtained in the GC analysis (Figure 5B) no specific changes could be observed by increasing the concentration of sodium cyclamate.

Apparently the significant differences observed within the instrumental analysis cannot be confirmed by the panelists in the quantitative descriptive analysis (Nahon et al., 1998), as the sensory analysis did not show significant differences for any of the aroma-related attributes when a water soluble orange aroma was present in the solutions (Figure 1). Ebeler et al. (1988) found that an addition of up to 40 w/v % sucrose did increase the headspace concentrations but did not significantly affect the perceived aroma intensity of either menthone or isoamyl acetate. Their GC analysis also showed that the GC was much more sensitive to small changes in the headspace than the sensory panel was. As expected from Fechner's law, the perceived aroma intensity is logarithmically related to the concentration of the volatile compound. Godshall (1995) concluded that the aroma of a beverage could, theoretically, be intensified by increasing the volatility of its trace aroma constituents by increasing the sucrose content. The results obtained in the described experiment, however, show that increasing the sucrose content will influence the release of the volatiles differently, depending on the properties of the volatile compound itself. In contrast to the desired effect, a retention of volatile compounds might result. Besides, threshold values for odor and flavor may differ in such a way that a volatile compound might be detected as a flavor (e.g., mouthfeel) but not as an odor. In the study of Ahmed et al. (1978), octanal was found to have a significantly higher odor threshold when compared to the corresponding flavor threshold. Their panelists found octanal to possess an orange-like flavor and aroma and a slightly bitter taste. Shaw and Wilson (1980) observed such significant differences between aroma and taste thresholds for octanal, citral, nonanal, and (*E*)-2-hex-

enal. Apparently, the flavor of an aroma can be more important to the overall perception than the odor.

In conclusion, it was shown that increasing concentrations of sucrose significantly alter the release of volatile compounds but that these changes are probably not noticeable at the sweetness level normally found in soft drinks (i.e., 10% SEV). Increasing concentrations of sodium cyclamate have no influence on the release of volatile compounds from a water soluble orange aroma. Flavor differences observed among various sweeteners and aroma combinations can probably be ascribed to differences in flavor among the sweeteners.

#### ABBREVIATIONS USED

QDA, quantitative descriptive analysis; SEV, sucrose equivalent value; GC, gas chromatography; FID, flame ionization detection; MS, mass spectrometry; RT, retention times.

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