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Influence of Composition (CO_2 and Sugar) on Aroma Release and Perception of Mint-Flavored Carbonated Beverages

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The aim of the present work was to identify and quantify physical mechanisms responsible for in-nose aroma release during the consumption of mint-flavored carbonated beverages in order to better understand how they are perceived. The effect of two composition factors (sugar and CO_2) was investigated on both the sensory and physicochemical properties of drinks by studying in vitro and in vivo aroma release. Sensory results revealed that the presence of CO_2 increased aroma perception regardless of the sugar content. In agreement with volatility parameters, in vivo measurements showed that carbonated drinks released a greater quantity of aroma compounds in the nose space than non-carbonated ones. CO_2 seemed thus to induce large modifications of the physicochemical mechanisms responsible for the aroma release and flavor perception of soft drinks. Moreover, sugar content seemed to have an impact (increase) on aroma perception only in the case of non-carbonated beverages. Sensory interactions were thus observed, in particular, between sweet and aroma perceptions. For carbonated beverages, sugar content had an impact only on aroma release, but not on their perception.

KEYWORDS: Soft drink; flavor compounds; sensory analysis; release; nose space

INTRODUCTION

The main sensory properties of soft drinks induced by carbonation are sparkle and effervescence and are responsible for flavor enhancement and refreshing sensation. These properties largely contribute to consumer choices and preferences. A better understanding of the phenomena involved represents thus a real challenge for the food industry. The oral sensations produced by carbonated beverages could either be of chemogenic origin (formation of carbonic acid) or mechanical origin (bursting of CO_2 bubbles that stimulates tongue mechanoreceptors) (*1*, *2*). Some recent evidence supports the first hypothesis (*3*). However, the composition of the aqueous phase (natural or artificial sugars, hydrocolloids, etc.) may also contribute to enhancing the effects of carbonation due, in particular, to an increase in the surface-active charges of the liquid. Consequences on bubble number and size, bubble growth rate, and ascending bubble velocity due to modification of the CO_2 environment can affect the sensory properties of these beverages (*4*). These phenomena have been studied in hydroalcoholic media such as champagne wines and beers, in particular.

The presence of carbon dioxide in beverages can modify their taste and flavor perception (*5*). Concerning the influence of CO_2 on taste perception, the few publications that exist reported opposite results (*6*). On the one hand, some studies showed that carbonation had no effect on sweetness perception (*7*,*8*), whereas, on the other hand, Passe et al. reported sweetness suppression by carbonation (*9*). With regard to sourness, the effects of citric acid and phosphoric acid at low concentrations on taste were enhanced by carbonation (*8*). In addition, concerning the effect of CO_2 on aroma perception, results are also controversial in the literature. In blueberry-flavored milk, overall aroma intensity was enhanced by carbonation (*10*). In contrast, in flavored milk beverages, Lederer et al. found that carbonation suppressed cooked milk notes and flavor in the mouth (*11*). All of these studies described an effect of CO_2 on flavor perception but, to the best of our knowledge, it has not yet been investigated how carbonation of a beverage influences aroma perception (biological, physicochemical, or sensory origins).

In addition to the understanding of the effects of carbonation, insight into the interaction between sugar and CO_2 is a challenging issue in relation to the development of new products. Focusing on commercial beverages, some authors have suggested reducing sugar content in carbonated soft drinks within a context of nutritional recommendations (*12*, *13*).

Despite short residence time in the mouth, food beverages undergo changes during consumption (mixing with saliva, temperature increase in the mouth, biochemical reactions) (*14*).

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To better understand retronasal aroma perception, the dynamic aspects of drinking processes have to be taken into account. Several approaches, both instrumental and sensory, exist and can be combined to investigate the dynamics of stimulus release at the origin of aroma perception. These include (i) online breath analysis via proton transfer reaction−mass spectrometry (PTR-MS) or atmospheric pressure chemical ionization-mass spectrometry (APCI-MS); (ii) sensory temporal analysis; and (iii) in some specific cases, medical monitoring techniques (radiological and nuclear imaging techniques) (*15*). The complementarity between these methods is interesting because it enables a better focus on relationships that can exist between formulation parameters and physicochemical and sensory properties during consumption. Online breath analysis is characterized by short response times and relatively high sensitivity with detection thresholds in the low ppt range (*16*, *17*). Concerning food perception, dynamic aspects can be investigated by different methods: time−intensity method (*18*), discontinuous temporal profile (evaluation of overall aroma perception at the main steps of consumption, i.e., at product introduction in mouth, at swallowing and at persistence time) (*19*), or temporal dominance of sensations (*20*). These dynamic methods allow us to monitor the intensity of one or several descriptors over time. However, the problem of inter-individual variations on notation was revealed, particularly due to physiological differences between panelists (*21*) and to the phenomenon of "subject signature" (similar perception profiles for the different products for one subject but different perception profiles between subjects) (*22*).

The present study aims to identify and quantify the mechanisms of aroma release from flavored carbonated beverages in oral and nasal cavities during consumption that are responsible for their perceptions. This study is original because of the use of an integrated approach combining physicochemical and sensory methodologies to investigate the effects of sucrose and CO_2 (the major constituents of carbonated soft drinks).

MATERIAL AND METHODS

Products. Four flavored beverages varying in the presence or not of sucrose (1% w/w) and carbon dioxide (5 g/L) (CO_2) (Aligal 2, Air Liquide, Les Loges-en-Josas, France) were manufactured (**Table 1**). The beverage products were flavored to 0.1% (w/w) with a mint flavor containing three aroma compounds mixed with propylene glycol (Aldrich, Paris, France). *Z*-Hex-3-en-1-ol, menthol, and menthone were provided by Aldrich. These molecules differed in their physicochemical properties and in their final concentrations in the product (from 2.6 to 30 mg/kg of product, **Table 2**). Carbonation was performed by Air Liquide, on a mixture of all the ingredients (sucrose, water, flavor) with a carbonator (Table Top Carbonator CF121, OMVE, The Netherlands). After preliminary tests, CO_2 concentration in beverages was fixed at 5 g/L to simulate commercial beverages.

Starting from the same batch, the beverages were immediately placed in 500 mL polyethylene terephtalate bottles with impermeable caps. They were then stored at 4 °C for a maximum of 10 days for physicochemical and sensory analyses. Beverage pH was measured at 4 °C with a pH-metric probe (Mettler Toledo, France). The values were 5.5 (±0.1) and 7.5 (±0.2) for carbonated and non-carbonated beverages, respectively.

The pH and CO_2 quantity were considered as manufacturing checkpoints, to avoid a replication problem between samples.

Physicochemical Methods. *Determination of Gas-to-Product Partition Coefficients by Phase Ratio Variation and Liquid External Calibration Methods*. The gas-to-product partition coefficient is defined as the ratio of aroma compound equilibrium concentrations in the gas phase and in the product. For non-carbonated products, the phase ratio variation (PRV) method was used to determine the gas-to-product partition coefficient. One of the main advantages of this method is that no external calibration is required (*23*). Vials (22.4 mL, Chromacol, France) were filled with different quantities of matrices: 0.05, 0.5, 1, and 2 mL. The corresponding volume ratios (β) were 448.0, 43.8, 21.4, and 10.2. Measurements were carried out at 10 and 25 °C, which corresponded to the range of temperature consumption. All experiments were performed in triplicate to validate the repeatability of the measurements. When the thermodynamic equilibrium state was reached (after 12 h at 10 or 25 °C), vials were placed on a thermostated support at 10 or 25 °C. An aliquot of 2 mL of headspace gas was sampled with a syringe and injected with an automatic headspace sampler CombiPal (CTC Analytics, Switzerland) into a gas chromatograph with a flame ionization detector (GC-FID 6890, Agilent Technologies, Germany) and heated at 230 °C. Aroma compounds were transferred to a semicapillary column of 30 m in length, with an internal diameter of 0.53 mm and a film thickness of 1 μm (BP20 Carbowax, Interchim, France). The carrier gas was helium at a flow rate of 8.4 mL/min corresponding to a 50.5 cm/s average velocity. For the FID detector, H_2 and air flow rates were 40 and 450 mL/min, respectively. The oven program was 10.7 min long, starting at 70 °C, 12 °C/min ramp to 130 °C, 15 °C/min ramp to 140 °C, 2 min at 140 °C, 5 °C/min ramp to 150 °C, and 1 min at 150 °C.

Table 1. Premix Composition of Beverages

beverage[a]	water (Evian, France) (mL QSP)	sucrose (Daddy, SucrUnion, France) (g/kg)	CO_2 (Aligal2, Air Liquide, France) (g)
W	1000		
WS	1000	10	
WG	1000		5
WGS	1000	10	5

[a] W, water; S, sugar; G, CO_2 gas.

The PRV method could not be used for carbonated products because of the pressure difference induced by the variable amounts of CO_2 in each vial. Gas-to-product partition coefficients were thus determined by the liquid external calibration method. For liquid external calibration, five concentrations of each aroma compound were analyzed in triplicate. For measurements, vials (22.4 mL) were filled with 2 mL of beverage. When the thermodynamic equilibrium state was reached (after 12 h at measurement temperature), vials were placed on a thermostated support at 10 or 25 °C. An aliquot of 2 mL of headspace gas was sampled and injected as previously described for the PRV method. All experiments were performed in triplicate to validate the repeatability of the measurements. For the calculation of gas-to-product partition coefficients, air phase aroma concentrations (w/w) were divided by the initial aroma concentrations in the product phase (w/w).

Characterization of in Vitro and in Vivo Aroma Release by PTR-MS. The influence of beverage composition on the dynamic release of aroma compounds was studied using a proton transfer reaction mass spectrometer (Ionicon, Innsbruck, Austria) (in vitro and in vivo online measurements).

The PTR-MS inlet was connected to samples via a 1/16 in PEEK tube maintained at 60 °C. The PTR-MS instrument drift tube was thermally controlled (Tdrift = 60 °C) and operated at Pdrift = 200 Pa with a voltage set of Udrift = 600 V. Measurements were performed with the multiple ion detection mode on 13 specific masses with a dwell time of 0.1 s per mass. From the fragmentation patterns of the individual compounds, the studied compounds were monitored with m/z 83 (*Z*-hex-3-en-1-ol and menthol), 139 (menthol), and 155 (menthone) (**Table 2**). The signal-to-noise ratio varied from 5 to 100 depending on the measured m/z, meaning that the responses for the three compounds sufficiently exceeded the baseline. In addition, masses m/z 21 (signal for H_3O^+) and m/z 37 (signal for water clusters $H_2O-H_3O^+$) were monitored to check the instrument performances and cluster ion formation. For in vivo experiments, m/z 21 intensity was $(1.52 \pm 0.05) \times 10^4$ cps (with intrameasurement and day-to-day variations lower than 5.0 and 3.8%, respectively). The ratio of intensities of m/z 37 and 21 was 2.16 ± 0.31%. Concerning in vitro experiments, m/z 21 intensity was $(1.43 \pm 0.06) \times 10^4$ cps (with intrameasurement variations lower than 5.0%).The ratio of intensities of m/z 37 and 21 varied between 0.8 and 5.6 depending on the temperature and the tested products. We assumed that these differences were not sufficient to modify PTR-MS performances. Transmission factors for the measured m/z were considered as specified by Ionicon for the PTR-MS used in this study (transmission factors of 0.982, 0.353, and 0.287 for m/z 83, 139, and 155, respectively).

Table 2. Physicochemical Properties, Fragmentation Patterns, and Final Concentrations of Aroma Compounds

aroma compound	formula	mol wt (g/mol)	beverage concentration (mg/kg)	log P[a]	solubility at 25 °C (mg/L)	sensory aroma attribute	fragmentation pattern m/z (percentage related to main fragment)
menthol	$C_{10}H_{20}O$	156.27	30	3.4[b]	450[b]	mint leaf	81 (100); 55 (62); 83 (42); 137 (22); 139 (4)
menthone	$C_{10}H_{18}O$	154.25	30	3.05[b]	500−688[b]	fresh and polar mint	81 (100); 137 (30); 155 (10)
Z-hex-3-en-1-ol	$C_6H_{12}O$	100.16	2.6	1.61[c]	1600[c]	green note	55 (100); 83 (34); 91 (10); 81 (12)

[a] Log P = logarithm of the ratio of the compound concentration in octanol and in water (EPI, 2000, estimation Programs Interface V3,10: database). [b] Experimental value. [c] Estimated value (KowWin v1.67 estimate).

Specific Conditions for in Vitro Measurements. An aliquot of 20 mL of beverage was poured into 100 mL glass vials. Vials were hermetically sealed and stored for 24 h at 10 or 25 °C before analysis. The headspace mixture was continuously extracted for 10 min with a constant air flow (from 10 to 30 mL/min depending on the experiment). The evacuated volume was replaced by outdoor air through a second orifice present on the container. PTR-MS operating conditions were as described in the previous paragraph. Measurements were performed in triplicate. Areas under curve (AUC) were extracted from release curves measured by PTR-MS (extraction of signal from 1 min after the beginning of recording to 10 min) and investigated by a one-way analysis of variance (product) for each ion to compare products.

Specific Conditions for in Vivo Measurements. Four panelists were recruited for the study. They were instructed not to smoke, eat, drink, or use any persistent-flavored product for at least 1 h before the session.

Eight replicates of each beverage sample were analyzed per subjects. Four sessions of 60 min were organized for each subject. During a session, subjects drank eight beverage samples at 10 °C according to a defined procedure, in order to reduce the interindividual variability: after positioning a homemade nosepiece in the two nostrils (linked to the PTR-MS instrument with a 1/16 in. PEEK tube), panelists were asked to breathe regularly for 30 s. Fifteen milliliters of beverage was then sipped with a straw. Panelists had to keep the beverage in their mouths for 6 s. At the end of this 6 s period, they were asked to swallow as they did normally, mouth closed, and to breathe into the nosepiece. Between each sample, panelists were asked to clean their mouth with bread and water at 35 °C to avoid desensitization of the oral receptors. In addition to the previously monitored ions, ion m/z 59 (acetone) was monitored as an indicator of breathing patterns of subjects. For data treatment, release curves were divided into three main periods: (i) the phase before sipping the product; (ii) the oral phase of consumption, when the beverage is kept in the mouth; and (iii) the phase after swallowing. For each ion, the mean intensity of the signal before swallowing was subtracted from the ion intensity after sipping. Using software developed in the laboratory, breath parameters were extracted from each individual release curve for the two phases of the drinking protocol designated by subscripts to the parameters as phase 1 (before swallowing) and phase 2 (after swallowing), for example, maximum concentrations reached in each phase (I_{max1} and I_{max2}), times at which I_{max} occurred (t_{max1} and t_{max2}), and area under the curve (AUC_1 and AUC_2). At the end of the second phase, the area under curve from 50 to 60 s after swallowing (time window of 10 s) was also calculated (A_{50-60}), as was the intensity at 60 s after swallowing in order to determine persistence parameters (I_{60}).

Sensory Methods. To reduce possible biases due to successive evaluations of the same product and to compile a global and rapid response, the discontinuous dynamic sensory procedure appeared to be the most appropriate methodology. This method permits one global attribute to be monitored several times during the consumption of a single sample.

Subjects and Testing Conditions. The same four trained panelists participated in this study. They all had previously participated in sensory panels of mint beverages and were specifically trained to perform sensory analyses in parallel to in vivo measurements. Samples were coded with three-digit random numbers, and 40 mL of sample was served at 10 °C in a 70 mL hermetically sealed cup. Data were collected on a computer screen with FIZZ software (Couternon, France).

Sensory Procedure. During nose-space aroma release measurement, subjects scored the perceived overall aroma intensity at three main consumption times: (i) upon introduction of the beverage into the mouth; (ii) when swallowing (6 s after introduction of sample into the mouth); and (iii) 60 s after introduction of the beverage into the mouth (persistence). For each sample, a 10 cm unstructured scale anchored with the terms "not intense" and "very intense" was used. Each sample was evaluated eight times by each subject. Samples were randomized over subjects according to a balanced experimental design.

Statistical Analysis. Statistical analysis of variance (ANOVA) and Student's t test were carried out on physicochemical and sensory data, using the GLM (general linear model) module and the t test module of the SAS software package. A Student−Newman−Keuls (SNK) full comparison test was also carried out to screen for significant differences between individual instances of sample type and timing.

RESULTS

Influence of CO_2 and Sucrose on Physicochemical Properties (in Vitro Studies). *Aroma Compound Gas-to-Product Partition Coefficients*. **Table 3** summarizes the results obtained for product W at 25 °C for the three aroma compounds. Our experimental results were compared with experimental data from the literature or with calculated data (*24*, *25*) when no experimental data were available in the literature. The experimental gas-to-product partition coefficient of menthone was in accordance with the experimental value found in the literature (*24*). For *Z*-hex-3-en-1-ol and menthol, the differences observed were probably due to the comparison between calculated and experimental values. Among the three molecules, menthone appeared to be the most volatile compound because its gas-to-product partition coefficient at 25 °C was 3.5−5 times higher than those observed for menthol and *Z*-hex-3-en-1-ol ($p < 0.001$).

To compare values obtained for the different studied beverages, relative gas-to-product partition coefficients were calculated, considering the value of the gas-to-product partition coefficient for product W as a reference. A one-way analysis of variance was conducted for each temperature to determine the effects of sugar and gas on aroma compounds volatility. Results are illustrated in **Figure 1**. The expected effect of the temperature was highlighted because relative gas-to-product partition coefficients at 25 °C were higher than those calculated at 10 °C ($p < 0.001$).

The presence of CO_2 in beverages significantly increased gas-to-product partition coefficients, regardless of the aroma compound or temperature considered. For both temperatures, this effect was much more pronounced for (*Z*)-hex-3-en-1-ol (5−9-fold increases) than for menthol and menthone (1.5−2.5- and 1.5-fold increases, respectively), independent of the presence of sucrose.

Concerning the effect of sucrose on gas-to-product partition coefficients, results were similar regardless of the temperature ($p < 0.05$); sucrose addition only increased the volatilities of menthol and menthone in carbonated beverages (1.1-fold increase), and this effect was not preponderant in comparison with the effect of CO_2 addition.

Dynamic Measurements of in Vitro Aroma Release by PTR-MS. An example of release kinetics of menthone (m/z 155) at 25 °C is illustrated in **Figure 2** for the four beverages. Initial concentrations and kinetics for the two non-carbonated

Table 3. Comparison of Aroma Compound Gas-to-Product Partition Coefficients ($K_{gas/product}$) for Water Product at 25 °C with Data Available in the Literature (Calculated and Experimental Values)[a]

	$K_{gas/product}$($\times 10^{-3}$), 25°C		
compd	exptl value (IC 95%[b]) (product W)	lit. value	ref
(*Z*)-hex-3-en-1-ol	2.25 (1.25−3.26)	0.63 (calcd value)	EPI suite[c]
menthol	3.14 (2.36−4.17)	0.62 (calcd value)	EPI suite[c]
menthone	11.21 (9.53−12.90)	6.49 (calcd value)	EPI suite[c]
		7.10 (exptl value)	Marin et al., 1999

[a] The experimental values of this study were determined using the PRV method. [b] IC 95%, confidence interval at 95%. [c] EPI, 2000, estimation Programs Interface V3,10: database.

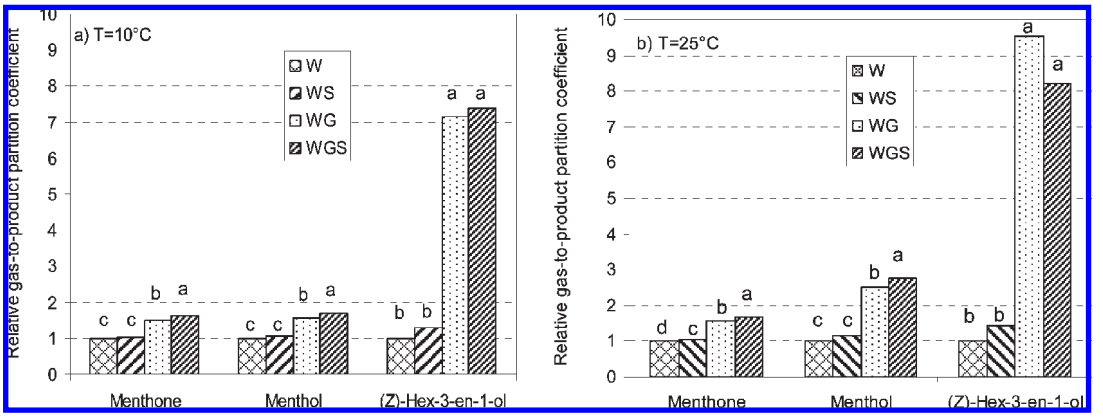

Figure 1. Relative gas-to-product partition coefficients of the three aroma compounds at 10 °C (**a**) and at 25 °C (**b**) from water beverage (W), from water and sweet beverage (WS), from water and gaseous beverage (WG), and from water, sweet, and gaseous beverage (WSG) (calculated using the gas-to-product partition coefficient of product W as a reference). Letters a−c indicate means that significantly differ at $p < 0.05$ (SNK).

beverages (W and WS) are effectively similar, assuming similar partition properties. Initial concentrations for carbonated products were higher than the ones of non-carbonated products, illustrating higher partition coefficients. These observations are in agreement with results presented in **Figure 1b**. Similar trends were obtained for the two other *m*/*z* values (data not shown). At 25 °C, regardless of the sugar content considered, the AUC parameter of all ion signals was significantly higher for carbonated drinks than for non-carbonated drinks ($p < 0.005$), that is, 1.6−3.0-fold increase depending on the aroma compound (**Figure 3**). Similar results were obtained at 10 °C (data not shown). Concerning the effect of sucrose at 25 °C, AUC parameters were significantly higher (1.1-fold increase) for sweetened products than for unsweetened ones, but only in the presence of gas ($p < 0.05$) (**Figure 3**). The effect of sucrose addition on AUC parameter values at 10 °C was not significant.

Influence of CO_2 and Sucrose on in Vivo Aroma Release. Nose-space analysis by PTR-MS made it possible to study the effect of product formulation on aroma release under real consumption conditions. Examples of release kinetics of menthone (ion *m*/*z* 155) obtained with panelist 1 for the four products are illustrated in **Figure 4**.

For all subjects, we observed that the in-nose aroma release quantity was lower before compared to after swallowing (**Figure 4**). Moreover, when beverages were in the mouth, no significant difference between products was observed on the parameter extracted from curves (I_{max1} and AUC_1), regardless of the ion considered.

Just after swallowing (5 s after the introduction of the product into the mouth), a boost of aroma delivery occurred and led to the maximal value of the PTR-MS signal. It is interesting to observe that despite a protocol imposed for swallowing, the time during which the PTR-MS signal was maximal was significantly different between the three aroma compounds. Results of a two-way ANOVA performed on t_{max} indicated that t_{max2} ranged from

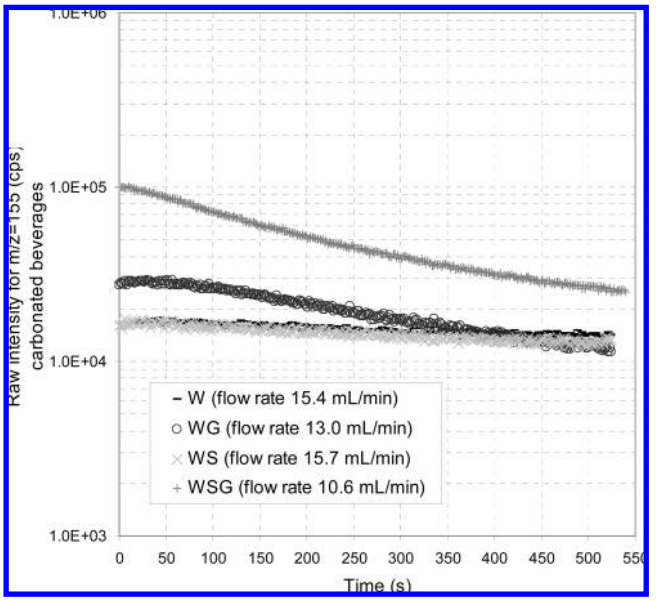

Figure 2. In vitro release kinetics obtained for menthone (*m*/*z* 155) at 25 °C for the four beverages [from water beverage (W), from water and sweet beverage (WS), from water and gaseous beverage (WG), and from water, sweet, and gaseous beverage (WSG)]. Measurements were performed using PTR-MS (Tdrift = 60 °C; Pdrift = 200 Pa; Udrift = 600 V; dwell time = 0.1 s).

8 s for ion *m*/*z* 155 (menthone) to 34 s for ion *m*/*z* 83 (menthol and *Z*-3-hexenol) (**Figure 5**), regardless of the composition of the product considered. Menthone seemed to be released at the highest speed and was thus susceptible to be the first to bind with the olfactory receptors (in the phase after swallowing).

Concerning the effect of product composition, CO_2 addition seemed to have a preponderant effect with regard to sucrose

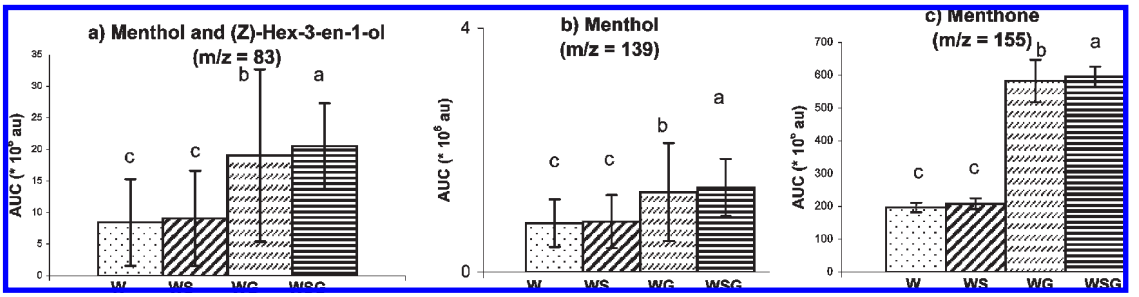

Figure 3. Means and confidence intervals of area under curve (AUC) extracted from in vitro aroma release kinetics determined by PTR-MS measurements at 25 °C for water beverage (W, cross-hatched bars), water and sweet beverage (WS, bold slashed bars), water and gaseous beverage (WG, dotted bars), and water, sweet, and gaseous beverage (WSG, slashed bars). Letters a−c indicate values that significantly differ at $p < 0.05$ (SNK).

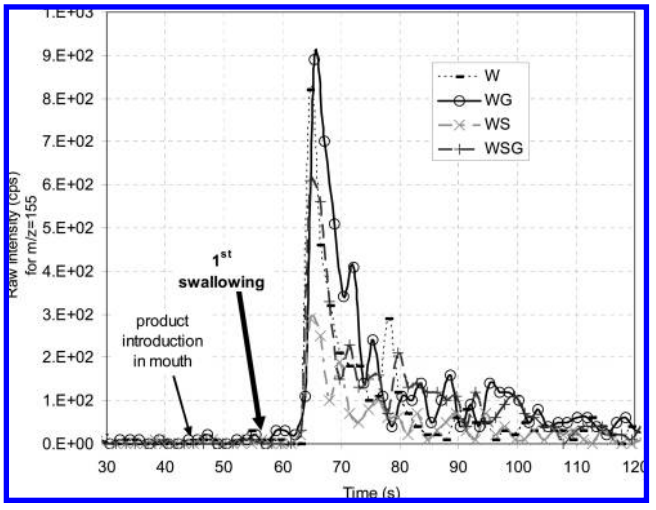

Figure 4. Example of in vivo release kinetics of menthone (ion *m*/*z* 155) for panelist 1 for the four beverages [water beverage (W), water and sweet beverage (WS), water and gaseous beverage (WG), and water, sweet, and gaseous beverage (WSG)]. Measurements were performed using PTR-MS (Tdrift = 60 °C; Pdrift = 200 Pa; Udrift = 600 V; dwell time = 0.1 s).

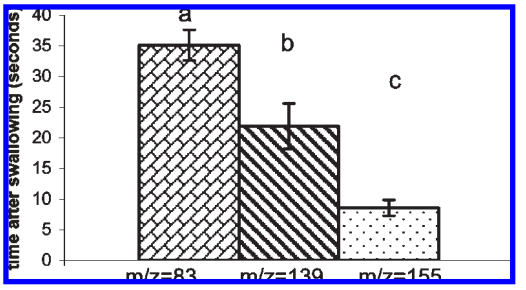

Figure 5. Comparison of the values of the t_{max2} parameter for the three ions for product W. Results were extracted from in vivo measurements (four subjects and eight replicates to evaluate each beverage). Letters a−c indicate values that significantly differ at $p < 0.05$ (SNK).

addition on in-nose aroma release after swallowing: the carbonated beverages had significantly ($p < 0.05$) higher values for AUC_2 than the non-carbonated beverages for the three ions (ions with *m*/*z* 83, 139, and 155) (**Figure 6**). A 1.4−1.5-fold increase in the AUC_2 parameter was observed for beverages with CO_2 compared to beverages without CO_2. This increase in the quantity of aroma compound released in the nasal cavity when CO_2 was added occurred regardless of the sugar content considered. Sucrose addition tended only to increase in vivo aroma release for the three ions for carbonated and non-carbonated beverages, but results were not significant ($p < 0.1$).

Concerning the in-nose aroma release at persistence, the parameters A_{50-60} and I_{60} did not significantly discriminate the products with regard to sucrose or CO_2 addition.

Influence of CO_2 and Sucrose on Temporal Sensory Properties. Dynamic sensory properties were characterized at the major consumption times in parallel with PTR-MS measurements. Regardless of the beverage composition, the overall aroma intensity was significantly higher at swallowing time than when the beverage was introduced into the mouth (2.8-fold increase) as well as at persistence (3.2-fold increase) ($p < 0.001$).

With regard to the effect of CO_2 addition on aroma perception at introduction into the mouth and at swallowing, a higher intensity for the carbonated beverages was observed compared to the non-carbonated beverages ($p < 0.05$). The aroma intensity was increased 1.4-fold for beverages without sucrose and 1.7-fold for beverages with sucrose. At persistence time, a significant effect of CO_2 addition on overall mint aroma intensity was observed only for beverages with sucrose ($p < 0.05$). Moreover, sucrose had an impact on aroma perception only during consumption for non-carbonated beverages. A 1.2-fold increase at swallowing and a 1.3-fold increase at persistence in aroma perception were observed when sucrose was added to non-carbonated beverages.

DISCUSSION

The delay in the t_{max2} value between aroma compounds illustrated the temporal competition to reach the olfactory receptor. Thus, it is likely that the note induced by menthone (fresh and polar mint) must be the first perceived by panelists. It might be followed by a green note and then by a mint leaf note. However, the sensory discontinuous profile chosen for this study did not make it possible to confirm this assumption because it indicated only an overall mint note intensity.

Effect of CO_2 on Aroma Release and Perception. CO_2 addition seemed to have a high impact on aroma release, under both in vitro and in vivo conditions (only after the first swallow), and on aroma perception. When the beverage was in the mouth, the presence of CO_2 had no effect on the amount of aroma available in the nasal cavities of the subjects. However, the in vivo results (no effect between carbonated and non-carbonated beverages) were not in agreement with in vitro measurements and with aroma perception when the beverage was in the mouth (higher gas-to-product partition coefficient and higher aroma perception for carbonated beverages than for non-carbonated beverages). Some studies in the literature highlighted the effect of CO_2 on PTR-MS performances, notably with regard to fragmentation patterns or ion mobilities (*26*). Even if this effect on ionization cannot be completely rejected, gas chromatography measurements confirmed the physical impact of CO_2 on aroma physicochemical properties and release. Another possible explanation is

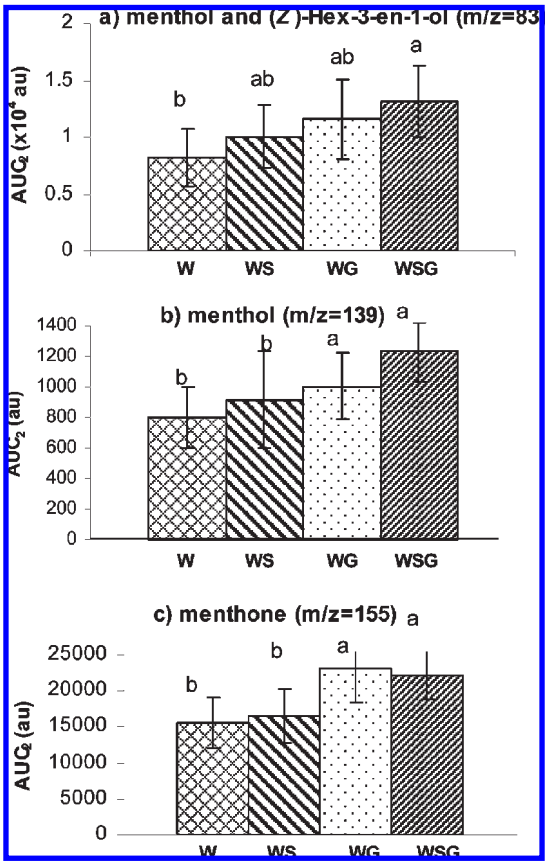

Figure 6. Significant effect of CO_2 and sucrose on the area under the curve after swallowing (AUC_2) (mean value across subjects and standard deviations) for the three ions and the four beverages (ANOVA) [water beverage (W, cross-hatched bars); water and sweet beverage (WS, bold slashed bars); water and gaseous beverage (WG, dotted bars); and water, sweet, and gaseous beverage (WSG, slashed bars)]. Results are extracted from in vivo measurements (four subjects and eight replicates to evaluate each beverage). Letters a and b indicate means that significantly differ at $p < 0.05$ (SNK test).

that differences in volatility properties of aroma compounds between beverages were not sufficient to induce significant differences of aroma quantity in the nasal cavity. The results corroborated the findings of Trelea et al. (*27*), who showed that equilibrium air/product partition coefficient was not the only influential parameter involved in in vivo aroma release and that some physiological parameters are also largely involved. Moreover, the small quantity of aroma compounds in the nasal cavities before the first swallow suggested an "almost perfect" closure between the oral and nasal cavities, limiting the transfer of aroma molecules between the two cavities and explaining the absence of significant differences between products (*28*). Another assumption to explain aroma sensory differences when the drink was in the mouth could be that, because carbonated beverages are more complex products than non-carbonated beverages, panelists might report these more complex sensations on the aroma perception scale (higher aroma perception when the drink was in the mouth). Moreover, it is well-known that in the mouth, CO_2 is converted into HCO_3^- at a very high rate by anhydrase carbonic activity. This conversion phenomenon is responsible for the tingling perception due to the presence of CO_2 and is in accordance with the very short time that the product is present in the mouth (*3*). We do not know the interaction of this phenomenon with the release and sensory perception of flavor in carbonated beverages, but it cannot be ignored in our case.

After swallowing, sensory and physicochemical results were in agreement, suggesting that physicochemical mechanisms could be at the origin of aroma perception. In vitro measurements, either under static or dynamic conditions, showed higher aroma compound release above carbonated products than above non-carbonated products, regardless of the sugar content. This physicochemical mechanism could explain in vivo measurements showing that CO_2 significantly increased the amount of aroma compounds in the nasal cavities of subjects, despite the between-subject variability. A greater amount of aroma compounds was likely to reach the olfactory receptors when carbonated beverages were consumed than when non-carbonated beverages were consumed. This result was in accordance with aroma perception evaluated by subjects at swallowing: the panel perceived the carbonated products to be about 1.5-fold more intense than the non-carbonated products. These results were in agreement with those of Yau et al. (*10*) on blueberry-flavored milk. Physicochemical mechanisms induced by CO_2 addition could thus explain aroma perception. Aroma stripping and convection phenomena induced by ascendant gas bubble movement could explain the effect of CO_2 on aroma release in beverages and also in aroma perception.

At persistence, no effect was observed on in vivo aroma parameters (I_{60}, A_{50-60}) whereas aroma perception was higher 50 s after swallowing for carbonated drinks than for non-carbonated drinks. This result suggested that subjects used their short-term memory to evaluate and discriminate between products, with or without CO_2.

Nevertheless, even if these physicochemical mechanisms have been clearly identified to explain aroma perception in beverages, sensory interactions could contribute to global perception.

Effect of Sugar on Aroma Release and Perception. With regard to the effect of sugar on aroma release and perception, non-carbonated and carbonated beverages must be considered separately, due to differences in their results.

First, in the absence of CO_2, in vitro measurements under static and dynamic conditions and at 10 and 25 °C showed that sugar had no effect on aroma compound release. In vivo measurements showed only a tendency of the presence of sugar to increase the amount of aroma compounds released into the nasal cavity. Concerning the sensory characterization of products, sugar addition significantly increased aroma perception by a factor of 1.2 in the absence of gas. Thus, the presence of sugar had an effect on the aroma perception of non-carbonated beverages, but no physicochemical mechanism was revealed that could explain this phenomenon. To explain perception, sensory interactions between sugar and mint aroma may occur in the absence of CO_2. A congruence phenomenon could be presumed. Mint and sweetness are strongly associated in food (candy, ice cream, etc.). As a consequence, judges perceived mint flavor in the beverages more intensely in the presence of sugar. These data corroborated the results of Frank and Byram and Jaime et al. (*29*, *30*) obtained between strawberry and sweetness.

Moreover, in regard to the results of the literature, some sweetness−flavor physicochemical interactions in soft drink model systems have been carried out under static conditions in previous studies (*31*, *32*), but it must be mentioned that the difference in sucrose contents between samples was higher than in ours (*33*). In these studies, an increase in the concentration of sucrose (from 20 to 60% w/w) was shown to significantly increase the release of several aroma compounds, particularly L-menthone, in the gas phase in soft drinks (*33*, *34*). For these authors, it was probably due to a "salting-out" effect of sucrose whereby sucrose interacts with water, increasing the concentration of flavor compounds in the remaining "free" water.

Second, concerning carbonated products, no agreement between in vitro aroma release and in vivo and aroma perception was observed. In vitro measurements showed a significant effect of sugar on the release of aroma compounds in the presence of CO_2. The gas-to-product partition coefficients of the three aroma compounds were higher in the carbonated product with sugar than in the carbonated product without sugar. Moreover, it was shown that the aroma quantity released under dynamic conditions was significantly higher (by a factor of 1.2−1.3) in the presence of sugar. However, in vivo measurements did not make it possible to significantly discriminate between the products, either with or without sugar (only a trend), during consumption. In agreement with in vivo aroma release, no effect of sugar on aroma perception was revealed. All of these results allowed us to conclude that sugar addition increased in vitro aroma release, but this increase was not sufficient to induce differences in aroma release in the nasal cavity of subjects and thus in aroma perception. Several assumptions could explain these results: (i) differences in physicochemical parameters would be too small to induce differences in aroma perception; (ii) trigeminal perception due to CO_2 could mask aroma perception (phenomenon of "numbness" of olfactory receptors); or (iii) a carbonated product is more complex than a non-carbonated product, inducing more difficulties for the panelists to assess the product. This result was in agreement with the work of Weel et al. (*35*), who showed that sucrose has no significant effect on the in vivo aroma release of lemon-lime beverages. For the authors, sucrose might influence the aroma release, but only at concentrations far beyond the relevance of application in beverages (above 50% w/v).

Moreover, as mentioned above, the influence of carbonic anhydrase on CO_2 conversion and then on aroma release and perception cannot be ignored.

This study contributes to a better understanding of the impact of composition (CO_2 and sucrose contents) on the physicochemical and sensory properties of drink matrices. The main effects that we observed were that CO_2 played a major role in aroma release. Flavor stripping played a preponderant role in aroma release in the nasal cavity of subjects and, consequently, in aroma perception. Nevertheless, sensory interactions, in particular with sugar, must be taken into account in the global aroma perception of soft drinks. Moreover, the results on temporal perception highlighted the dynamic evolution of aroma quality. Further experiments are in progress to validate our hypotheses.

Finally, it might be worthwhile to consider the influence of some biological parameters and in particular the effect of carbonic anhydrase, in our system, during in vitro as well as in vivo measurements.

ABBREVIATIONS USED

PTR-MS, proton transfer reaction−mass spectrometer; I_{max1}, maximum intensity of PTR-MS signal during phase 1 of the release profile; I_{max2}, maximum intensity of PTR-MS signal during phase 2 of the release profile; t_{max1}, time at which I_{max1} occurred; t_{max2}, time at which I_{max2} occurred; AUC_1, area under curve during phase 1 of the release profile; AUC_2, area under curve during phase 2 of the release profile; A_{50-60}, area under the last 10 s of the curve; I_{60}, intensity of signal at 60 s; ANOVA, analysis of variance; SNK, Student−Newman−Keuls test.

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