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Differential Effects of Tomato (*Lycopersicon esculentum* Mill) Matrix on the Volatility of Important Aroma Compounds†

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Significant tomato matrix effects on the volatility of certain fresh tomato odorants were found. The concentrations of odorants such as (*E*,*E*)-2,4-decadienal, β-damascenone, and β-ionone, in crushed fresh tomato fruit obtained by solid-phase microextraction (SPME), resulting from a tomato matrix calibration curve were 5.5-, 2-, and 12-fold higher, respectively, than those calculated by calibration based on buffer solutions. Static headspace analyses indicated that, in most cases, the tomato matrix significantly retains the odorants relative to the buffer solution. Thus, the concentration of odorants in the headspace of tomato is lower than expected compared to a simple matrix such as buffer. $CaCl_2$, although needed in crushed fruit tissue to block enzymatic activity, was found to interact specifically with 2-isobutylthiazole, reducing its content in the headspace by at least 6-fold. If a matrix effect is found, analysis of the odorant molecule contents in the headspace rather than in the food is recommended in order to better evaluate their access to the olfactory receptors.

KEYWORDS: Matrix effect, tomato (*Lycopersicon esculentum* Mill), odorant, SPME, static headspace, $CaCl_2$, $ZnSO_4$, $MgCl_2$

INTRODUCTION

Analysis of aroma components in foods involves the isolation of volatiles from the food or its headspace. This may be difficult with fresh fruits and vegetables due to the unstable nature of some of the most important flavor compounds. Many of the important flavor compounds are formed enzymatically in appreciable amounts only when the tissue is disrupted by slicing, chewing, or blending and may also be degraded due to enzyme action (*1*−*3*). Saturated calcium or sodium chloride solutions are often used to block enzymatic activity in disrupted fruit tissue when the identification and quantification of key volatiles are desired (*2*).

Recent studies indicated that the nature of the food matrix affects the concentration of various odorants in the headspace. It had become evident that components in the food matrix may interact selectively with certain odorants, thus affecting their volatility. Interactions with odorants may result from matrix components such as proteins, e.g., β-lactoglobulin, which is widely used in dairy products. In a number of studies, this protein was shown to interact with various classes of flavor compounds such as ionones (*4*), hydrocarbons (*5*), and aldehydes and ketones (*6*). Indeed, recent studies (*7*, *8*) have demonstrated a decrease in aroma concentration in the headspace of model solutions containing β-lactoglobulin. Maillard reaction products such as melanoidins and a class of pyrazinium ions have also been shown to interact with aroma compounds, such as thiols in coffee, and thus modify the perceived aroma (*9*, *10*). Further, structural properties of the food matrix, such as emulsions and gels, have been shown to modify the air/liquid partitioning of aroma compounds. Modifications in the hydrophobic−hydrophilic environments of emulsions can increase or decrease the retention of aroma compounds in the food matrix (*11*). Additionally, aroma compound concentration decreased in the headspace of pectin−citric acid-based gels, mainly due to physical entrapment of the volatile molecules (*12*). The effect was shown to increase with increasing gel firmness. Thus, it appears that the physicochemical properties of the aroma compounds in combination with the chemical nature of the food matrix ingredients and the formed matrix structure can modify the concentration of the aroma compounds in the headspace. As a result, odor activity values based on odorants' quantities in the food (e.g., by the standard addition or any other recovery method) and odor thresholds in water may not always be

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indicative of the actual odor potency, which is determined by the odorant concentration in the headspace.

The objective of this study was to evaluate the effect of crushed fresh tomato matrix on the release of important known odorants to the headspace, by use of solid-phase microextraction (SPME) and static headspace methodologies.

MATERIALS AND METHODS

Materials. Fresh tomatoes (*Lycopersicon esculentum* Mill, cvs. R-144 and FA-624, HaZera Genetics, Brurim, Israel), grown under controlled conditions in a phytotron at 28/22 °C, day/night temperatures at the Rehovot campus of the Hebrew University of Jerusalem, were obtained in October 2001 and March 2002.

Chemicals. 3-Methylbutanal, (*E*,*E*)-2,4-decadienal and *R*-limonene were purchased from Fluka (Steinheim, Germany); 1-penten-3-one, 2-isobutylthiazole, and ethyl antioxidant 330 [1,3,5-trimethyl-2,4,6-tris-(3,5-di-*tert*-butyl-4-hydroxybenzyl)benzene] were from Aldrich (Milwaukee, WI). 2-Phenylethanol, β-ionone, gallic acid, and a 1 M standard solution of magnesium chloride were from Sigma (St. Louis, MO); β-damascenone was from a stock of the USDA (WRRC, Albany, CA); (*Z*)-3-hexenal was from Bedoukian Reaserch Inc. (Danbury, CT); and 2-octanone was from Riedel-de Haen (Steinheim, Germany). Citric acid and calcium chloride were purchased from Merck (Darmstadt, Germany); potassium citrate, potassium chloride, and zinc sulfate were from BDH (Poole, England). Sodium chloride was purchased from Frutarom (Haifa, Israel), and diethyl ether and methanol were from J. T. Baker (Deventer, Holland). Deionized water was used throughout the study.

Fruit Samples. Sample preparation followed essentially the procedure of Buttery et al. (*2*) with some modifications. Each sample consisted of at least eight red-ripe tomato (cv. FA-624) fruits picked from two plants of the same cultivar. The fruits were crushed in a kitchen blender for 35 s, and then after 3 min, 1 g of the blend was transferred to a 4-mL amber vial (15 × 45 mm) and mixed with 1 mL of saturated $CaCl_2$ solution to deactivate enzymes in the fresh extracts and also as a means of salting out volatiles. The vials were sealed with a screw-cap Teflon septum (National Scientific Co.). The crushed tomato matrix pH was 4.05, and refractometer reading of the serum showed a 7.2° Brix. Samples were then immediately subjected to SPME extraction.

Calibration Mixtures for SPME Quantification. Stock solutions (10 000 mg/L) of each of the aforelisted odorants were prepared by dissolving the commercial compounds in methanol. For identification and quantification of odorants in tomato samples, five calibration mixtures were prepared by serial dilution of the stock solutions in citrate buffer (0.05 M, pH 4.0, containing 3 mM gallic acid as antioxidant) to the target concentrations, to a final volume of 50 mL for each mixture. The calibration range for each odorant was 5.5−550 μg/L for 3-methylbutanal, 8.3−830 μg/L for 1-penten-3-one, 200−20 000 μg/L for (*Z*)-3-hexenal, 1.0−280 μg/L for 2-isobutylthiazole, 0.8−80 μg/L for (*E*,*E*)-2,4-decadienal, 0.4−40 μg/L for both β-damascenone and β-ionone, and 150−760 μg/L for 2-phenylethanol.

Preparation of matrix for use as a comparable calibration medium was as follows: 100 g of mature green tomatoes (cv. R-144, pH 4.1, 4.5° Brix) were halved and immediately blended with 100 mL of saturated $CaCl_2$ solution for 40 s in a kitchen blender. This matrix was used as the base for five calibration mixtures with identical odorant composition and concentration as for the buffer mixtures described above. An unfortified portion of this matrix was used as a reference representing the background concentration of the relevant odorants in mature green tomatoes. This reference was close to the original matrix of fresh ripe tomatoes but with no significant interference by endogenous amounts of the analyzed odorants.

SPME Procedure. Each vial containing tomato sample or calibration mixture (1 mL + 1 mL of saturated $CaCl_2$ solution) was continuously agitated in a water bath at 35 ± 0.3 °C. Samples were allowed to equilibrate for 5 min prior to SPME and maintained at 35 °C throughout the 30-min assay. SPME of the sample's volatiles was conducted by inserting a 2-cm stable flex fiber coated with 50/30 μm DVB/Carboxen/PDMS (Supelco, Bellefonte, PA). The full length of the coated fiber was exposed to the headspace for 25 min. The fiber was then removed from the headspace and immediately inserted into the GC injector. Tomato samples and each calibration mixture were analyzed in triplicate (three different batches of the same cultivar).

Table 1. Selected Odorants Used for Static and SPME Headspace Experiments and Their Concentrations in Both Mature Green Tomato Matrix and Buffer Media

odorant	static headspace (mg/400 mL mixture)	SPME (μg/mL sample)
3-methylbutanal	0.4	0.55
1-penten-3-one	0.4	0.30
(*Z*)-3-hexenal	0.8	2.00
2-isobutylthiazole	0.4	0.05
(*E*,*E*)-2,4-decadienal	0.2	0.02
β-damascenone	0.2	0.038
2-phenylethanol	0.4	0.76
β-ionone	0.2	0.036

Interaction of Divalent Cations with 2-Isobutylthiazole. A stock solution consisting of 5 mg/L 2-isobutylthiazole in citrate buffer was the basis for this experiment. Five salt solutions containing KCl, NaCl, $MgCl_2$, $CaCl_2$, and $ZnSO_4$ (1 M each) were prepared in deionized water. For each analysis, 1 mL of the 2-isobutylthiazole solution was mixed with 1 mL of one of the salt solutions in a 4-mL amber vial sealed with a Teflon screw-cap. Sampling was conducted by SPME by the above procedure.

Sample Preparation for Headspace Analysis Experiments. For static headspace analyses, 400 g of mature green tomatoes (pH 4.05 ± 0.05, 4.6° ± 0.3° Brix) were cut and immediately crushed in the presence of 400 mL of saturated $CaCl_2$ solution. Then a mixture of odorants used for the quantification of tomatoes was added to the resulting matrix. A $CaCl_2$-free version of the same matrix was prepared by heating the intact tomatoes for 4 min in a kitchen microwave oven (500 W) in order to inactivate enzymes, followed by blending of the tomatoes and fortification with the odorant mixture. Unfortified samples of each of these two media were used as the baseline concentration of the relevant odorants in the mature green tomatoes. Similarly, 400 mL of citrate buffer containing the same odorant composition was made with and without the addition of 400 mL of saturated $CaCl_2$. Analogously, 50 g of above-mentioned mature green tomato matrix and 50 mL of citrate buffer solution with addition of $CaCl_2$ were prepared and fortified with the artificial odorant mixture for a comparable set of experiments. The composition and concentration of the mixtures used are listed in **Table 1**. Since static headspace has relatively inferior extraction efficiency, higher concentrations of the odorants were required in the mixture.

Static-Headspace Analysis. Analyses were done in four replicates in 1-L borosilicate glass bottles (Pyrex, Germany), each sealed with a screw-cap Teflon-lined septum (Corning, New York). Four of these bottles were used for each replicate. The solutions for analysis (4 × 200 mL including saturated $CaCl_2$ solution, or 4 × 100 mL in the absence of $CaCl_2$) were allowed to equilibrate in bottles in a water bath set to 35 °C for 30 min (no stirring). Next, 4 × 100 mL of headspace volumes were pooled from each of the four bottles by use of a 100-mL borosilicate glass gastight syringe (SGE International, Ringwood, Australia). Each 100-mL headspace aliquot was transferred from the syringe into a high-efficiency liquid nitrogen cold trap (Aldrich), connected to a vacuum pump (KNF, Freiburg, Germany). The whole procedure was repeated three times at 30-min intervals. At the end of the 90-min sampling process, the accumulated total 4800-mL volume of headspace was loaded on the cold trap. Atmospheric pressure was maintained in the bottles throughout the sampling period by insertion of a thin needle through the septum. The cold trap was washed three times with freshly distilled ether (containing 0.01% ethyl antioxidant 330) in order to extract the trapped volatiles. The combined ether extracts were dried over anhydrous sodium sulfate and then filtered. At this stage, 0.5 mg of 2-octanone was added as an internal standard. Next, the solvent was distilled off in two steps by use of a micro-Vigreux column to a final volume of 50 μL, and finally 0.1 mg

of *R*-limonene was added as an internal standard. A 1-μL aliquot of this aroma extract was used for GC analysis.

High-Resolution Gas Chromatography (HRGC). Analyses were performed with an HP-4890 (Agilent Technologies) gas chromatograph equipped with a flame ionization detector (FID) and DB-WAX capillary column (60 m × 0.32 mm i.d., df = 0.25 μm) (J&W Scientific, Folsom, CA). Following SPME, volatiles were desorbed in the injector (240 °C) for 3 min, in splitless mode, or 1 μL of the etheral aroma extract was injected (injector 180 °C, split $^1/_{10}$ for 1 min). Operating conditions were as follows: column held at 30 °C for 4 min, then increased 3 °C/min to 200 °C, and then held for 20 min. Helium was used as the carrier gas with a linear velocity of 31 cm/s. A 0.75-mm i.d. SPME injector liner was used when necessary.

HRGC−Mass Spectrometry. All odorants quantified in the fresh tomato samples were identified by use of a Saturn 2000 mass spectrometer (Varian, Walnut Creek, CA) connected to a Varian 3800 gas chromatograph, equipped with a DB-WAX capillary column (60 m × 0.32 mm i.d., df = 0.25 μm). Following the aforementioned SPME procedure, samples were inserted into the injector (held at 250 °C) for 3 min, in splitless mode. The oven program was as detailed for GC-FID. Helium was used as the carrier gas with a linear velocity of 32 cm/s. A 0.75-mm i.d. SPME injector liner was used. The transfer line was held at 300 °C and the source at 170 °C. Mass spectra in the electron impact (EI) mode were generated at 70 eV. Chromatograms and spectra were recorded with Saturn GC/MS Workstation software, version 5.41 (Varian).

Quantitative Data. Quantification was performed with the GC−FID system. The concentration of odorants in fresh tomato samples was calculated from the calibration curves produced from either buffer or mature green tomato matrix mixtures by the SPME technique. Linear coefficients of the calibration curves ranged from 0.987 to 0.999 with respect to the different odorants. For static headspace analyses, concentrations and response factors were calculated by comparing the FID peak area of the analyzed odorants with that of the internal standards. For this purpose, known amounts of the odorants and standards were mixed and directly injected into the GC−FID. The extracted amounts from samples were calculated by first comparing the peak area of the odorants with that of 2-octanone and then correcting according to the peak area of *R*-limonene. Response factors were calculated relative to *R*-limonene. Amounts of odorants sorbed on the SPME fiber were determined by comparing their peak area with that of reference odorants injected directly.

Data Processing. Data from the GC were processed with 3390 Chemstation software (Agilent Technologies). Statistical analysis of the resulting data was performed with JMP statistical software (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

SPME Analysis. Quantitative determination of key tomato odorants in fresh tomato fruit by SPME−headspace methodology revealed significant differences between the amount of certain odorants quantified by a calibration curve derived from reference markers in buffer solutions and that obtained by the same markers calibrated in mature green tomato matrix (**Figure 1**). For example, the concentrations of (*E*,*E*)-2,4-decadienal, β-damascenone, and β-ionone resulting from the tomato matrix calibration curve were 5.5-, 2-, and 12-fold higher, respectively, than those calculated by calibration against buffer solutions (**Figure 1B**). Although the concentrations of odorants may differ significantly among tomato cultivars (*3*), the results based on the tomato matrix are compatible with those previously reported. On the other hand, the SPME results indicated that the concentrations of 1-penten-3-one, 2-isobutylthiazole, and 2-phenylethanol are significantly lower in the matrix-based results than those based on the buffer calibration curve (**Figure 1A**). These major differences in odorant contents in the matrix vs buffer could suggest the following possibilities: first, the matrix may retain one odorant but not the other, which may give a relative advantage to the second odorant in the competition for SPME binding sites, leading to artifacts in quantitative odorant determinations (*13*). Second, the matrix may either retain certain odorants more than others compared with the buffer solutions, or vice versa, leading to the above differences. Note that SPME experiments involving these types of calibrations provide the amount of odorants in the tested food system but they do not reveal the content of the volatiles in the headspace or the real concentration (i.e., the real odor potency) that the olfactory receptors may sense.

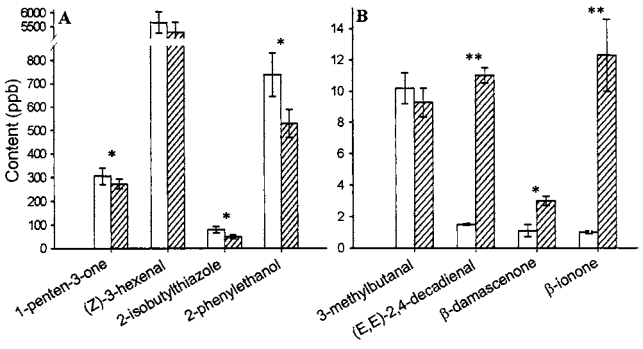

Figure 1. Concentration of odorants in disrupted fresh tomatoes (cv. FA624) determined by SPME headspace and calibrated by buffer solutions (open bars) or by mature green tomato matrix (hatched bars). (**A**) High range (above 80 ppb) of odorant concentrations; (**B**) low range (below 80 ppb) of odorant concentrations. Exposure to SPME was 25 min at 35 °C. Values are the mean and SEM of three replicates for each data point. Single asterisks indicate significant difference ($p < 0.05$) in paired *t*-test between the content of each odorant derived from calibration with the buffer solution vs that derived from calibration with the mature green tomato matrix. Double asterisks indicate significant difference at $p < 0.01$.

Static Headspace. Are the differences in odorant contents due to the SPME limits, or do the results represent the actual partitioning of each odorant between the liquid and vapor phases? To test the above hypotheses, we next performed static headspace experiments, in which we determined the concentrations of the same odorants directly in the headspace of (i) mature green tomato matrix and (ii) the buffer (**Figure 2**). In most cases, the tomato matrix significantly retained the odorants compared with the buffer solution. This means that the actual concentration of odorants in the headspace of tomato's matrix is lower than expected compared to a simple matrix such as buffer. This may be relevant to all cases where odor thresholds have been determined in water or buffer solutions but not in the matrix (*14*). Certainly, estimated odor activity values, the profile of odorants in the headspace (a critical parameter for food acceptance), and the concluded odor potency may be affected.

The above static headspace analysis method, although not suitable for a determination of air/liquid partition coefficients due to the headspace dilution with air (as a consequence of the large volumes of pooled headspace) (*15*), is useful for a comparative study of different matrix effects, as it is a direct method that is capable of extracting low odorant concentrations without the use of a sorbent. A comparison of the headspace analysis (**Figure 2A**) with that of the corresponding SPME (**Figure 3**) suggested, in general, a similar pattern of matrix effects with both methodologies. The results described in the two figures represent experiments in which synthetic mixtures of odorants were used. Calculations of the absolute amount of the extracted odorants were made independently of the calibration curves used to quantify the total amount of odorants in the food (e.g., **Figure 1A**). In both experimental systems, there was higher retention of aroma by the matrix phase compared with

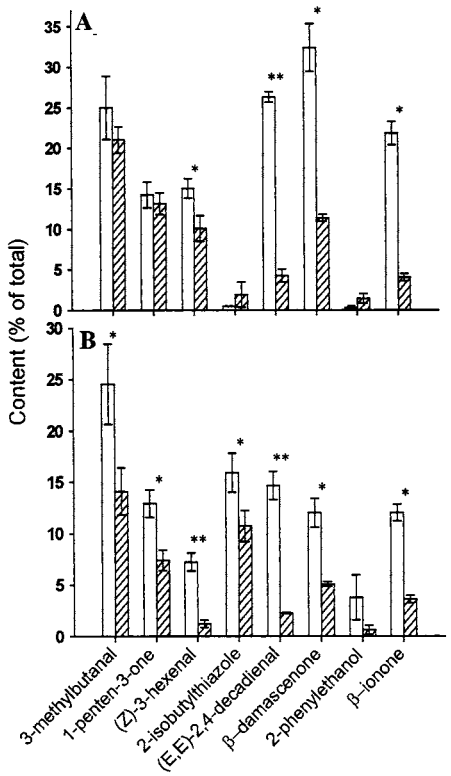

Figure 2. Static headspace: Contents of odorants in the headspace expressed as a fraction of the initial amount added into the buffer solution (open bars) or into the mature green tomato matrix (cv. R144, hatched bars). (**A**) In the presence of saturated $CaCl_2$ solution; (**B**) without $CaCl_2$. Values are the mean and SEM of four replicates for each data point. Single asterisks indicate significant difference ($p < 0.05$) in paired *t*-test between the content of each odorant derived from the buffer solution vs that derived from the tomato matrix. Double asterisks indicate difference at $p < 0.01$.

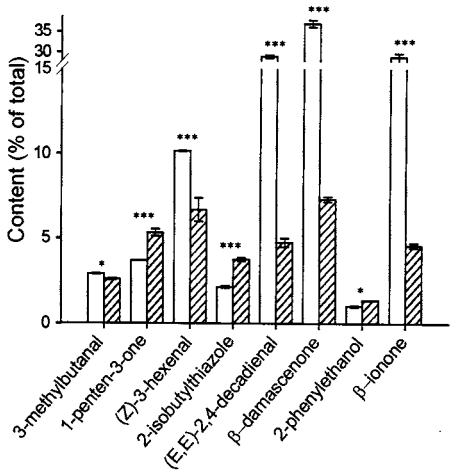

Figure 3. SPME headspace: Contents of odorants sorbed on the SPME fiber following exposure of 25 min at 35 °C to the headspace of buffer solution (open bars) or mature green tomato matrix (cv. R144, hatched bars) in the presence of saturated $CaCl_2$ solution. Results are expressed as a fraction of the initial amount administered to the liquid mixture. Values are the mean and SEM of three replicates for each data point. Single asterisks indicate significant difference ($p < 0.05$) in paired *t*-test between the content of each odorant derived from the buffer solution vs that derived from the tomato matrix. Double asterisks indicate significant difference at $p < 0.01$; triple asterisks, at $p < 0.001$.

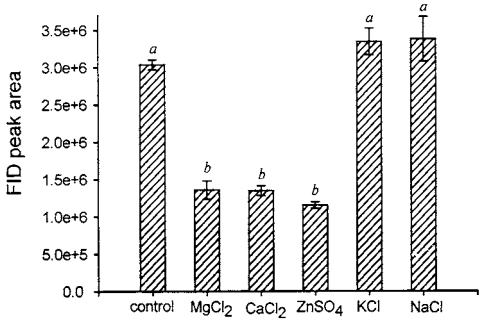

Figure 4. Influence of various cations on the headspace content of 2-isobutylthiazole as determined by SPME sampling for 25 min at 35 °C. Initial odorant concentration was 5 ppm in all cases. All solutions contained potassium citrate buffer. Control samples did not contain any of the salts added to other samples. Values are the mean and SEM of three replicates for each data point. Values not sharing the same superscript letter differ at $p < 0.01$.

that of the buffer, with minor differences between the SPME and the static headspace analyses. For example, in the SPME analysis (**Figure 3**), the extraction efficiency for 1-penten-3-one from the matrix phase was higher than that from the buffer phase. On the other hand, no difference was observed between the two phases when static headspace analyses were performed (**Figure 2A**).

Effects of $CaCl_2$. Saturated salt solutions are often added to disrupted fruit tissue, mainly to block enzymatic activity if a quantitative flavor analysis is to be achieved (*2*). At the same time, due to a salting-out effect, an increased concentration of volatiles in the headspace is expected as a result of odorant partition coefficient modifications between the liquid and the vapor phases. Thus, such treatments would not be suitable for a quantitative measurement of air/liquid partition coefficients in the food matrix. Nevertheless, there is no better procedure than this salting-out effect to block enzymatic activity leading to the formation and degradation of volatiles in fresh fruits and vegetables. To verify that the $CaCl_2$ salt does not interact specifically with one or more of the tested odorants, enzymatic activity was blocked (or significantly reduced) by heat treatment (**Figure 2B**). Volatile concentrations in both matrix and buffer media were generally lower in the absence (**Figure 2B**) than in the presence (**Figure 2A**) of $CaCl_2$. The ratio between the volatile contents in the matrix and buffer systems was essentially maintained for the two experiments except for (*Z*)-3-hexenal and 2-isobutylthiazole. When $CaCl_2$ was not present (**Figure 2B**), the content of (*Z*)-3-hexenal was about 5-fold lower in the matrix than in the buffer medium, compared with the case in which $CaCl_2$ was present, where this ratio was only 1.5-fold (**Figure 2A**). GC analysis indicated that the reason for this change is isomerization to (*E*)-2-hexenal in the matrix but not the buffer system. Such isomerization (*1*) is likely to occur due to residual enzymatic activity present in the matrix, which can be inhibited by $CaCl_2$ (*3*) (of course, no enzymes in the buffer), thus explaining the differences.

The presence of $CaCl_2$ dramatically reduced (6- and 32-fold) the content of 2-isobutylthiazole in the headspace of both the matrix and the buffer medium, respectively (**Figure 2**). This appeared to be a specific effect and led us to investigate whether other cations may interact specifically with 2-isobutylthiazole. SPME experiments in a citrate buffer (pH 4.0, 5 mg/L 2-isobutylthiazole) revealed that the content of this odorant decreased significantly when divalent cation salts are present but that monovalent cation salts had no effect (**Figure 4**). Therefore, 2-isobutylthiazole specifically interacts with divalent

cation salts. The nature of such interactions remains to be elucidated. Although it is known that thiazole compounds can form complexes with transition metals such as Pd and Pt (*16*), and in one example a thiazole-based reagent (e.g., thiazole yellow reagent) has been used to bind divalent cations such as magnesium (*17*), it is premature to conclude that such binding is related to the 2-isobutylthiazole odorant.

Taking into account the above effects, it seems that the relative flavor release from the tomato matrix is best represented in **Figure 2B** [with the exception of (*Z*)-3-hexenal], where $CaCl_2$ effects and the possibility of SPME-related interference are excluded. From this figure it can be seen that, except for 2-phenylethanol, there is a significant reduction in the release of odorants from the mature green tomato matrix as compared with the buffer medium. Again, the most consistent and significant aroma retention caused by the effect of the mature green tomato matrix, regardless of the headspace sampling technique, was for the odorants (*E*,*E*)-2,4-decadienal, β-damascenone, and β-ionone, with content of 6.5-, 2.3-, and 3.3-fold lower, respectively, than in the buffer medium (**Figure 2B**). The proportional content (pattern) in the headspace may also be modified between these two different media [e.g., the proportional content of (*E*,*E*)-2,4-decadienal is higher in the buffer medium compared with that in mature green matrix]. This observation suggests that the differential pattern of various odorants between the matrix and the buffer media cannot be simply explained by inferior mass transfer from the green tomato matrix. Rather, it is the selected interactions of each odorant with the matrix components that determine volatility. However, the complexity of the tomato matrix makes it hard to relate a specific interaction to an odorant. Nevertheless, one may speculate that, in the case of (*E*,*E*)-2,4-decadienal, hydrophobic interactions within the matrix are important, and with regard to β-damascenone and β-ionone, binding to proteins or peptides might account for the observed differences (*4*, *6*).

In conclusion, this study indicates significant effects of tomato matrix on the concentration of certain odorants as revealed by SPME headspace and static headspace analyses. Key aroma compounds such as (*E*,*E*)-2,4-decadienal, β-damascenone, and β-ionone were particularly retained by the tomato matrix and therefore, their concentrations in the headspace and hence their odor potency are significantly reduced. This study is in line with other recent studies (*8*, *10*−*12*, *18*) indicating the significance of the food matrix in the overall profile of odorants in the headspace. It is evident that the aroma perception will be different if a mixture of certain odorants is included in a buffer solution or if the same mixture is present in the food matrix. When a matrix effect is found, analysis of the odorant molecule contents in the headspace is required in order to determine their access to the olfactory receptors. Under such circumstances, perhaps the best methodology is to measure the odor threshold in air, e.g., by GC−O (*14*, *19*), and use this to calculate odor activity based on odorant concentrations in the headspace.

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