

Comparison of Mass Spectrometry-Based Electronic Nose and Solid Phase MicroExtraction Gas Chromatography—Mass Spectrometry Technique to Assess Infant Formula Oxidation

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Two headspace techniques based on mass spectrometry detection (MS), electronic nose, and solid phase microextraction coupled to gas chromatography—mass spectrometry (SPME-GC/MS) were evaluated for their ability to differentiate various infant formula powders based on changes of their volatiles upon storage. The electronic nose gave unresolved MS fingerprints of the samples gas phases that were further submitted to principal component analysis (PCA). Such direct MS recording combined to multivariate treatment enabled a rapid differentiation of the infant formulas over a 4 week storage test. Although MS-based electronic nose advantages are its easy-to-use aspect and its meaningful data interpretation obtained with a high throughput (100 samples per 24 h), its greatest disadvantage is that the present compounds could not be identified and quantified. For these reasons, a SPME-GC/MS measurement was also investigated. This technique allowed the identification of saturated aldehydes as the main volatiles present in the headspace of infant milk powders. An isotope dilution assay was further developed to quantitate hexanal as a potential indicator of infant milk powder oxidation. Thus, hexanal content was found to vary from roughly 500 and 3500 $\mu\text{g}/\text{kg}$ for relatively non-oxidized and oxidized infant formulas, respectively.

KEYWORDS: Infant formulas; volatiles; electronic nose; hexanal; solid phase microextraction; mass spectrometry; isotope dilution assay

INTRODUCTION

Considering milk powders, flavor defects are governed to a large extent by fat oxidation (1). Indeed, lipid oxidation is well-recognized as a major cause of quality deterioration during the processing or storage of lipid-rich foods (2). During peroxidation of unsaturated fatty acids, a complex mixture of secondary lipid oxidation products (alkanes, alkenes, aldehydes, ketones, etc.) is generated (2). These compounds impart off-flavors and loss of nutrients to food products such as milk powders and, thus, limit their shelf life stability (3, 4).

Because of its efficiency for chemical characterization, MS constitutes a frequently used technique for the analysis of volatiles in food products. Moreover, coupled to capillary GC, it is very useful for the determination/identification of chemicals responsible for off-flavors in foods and beverages. While MS is generally the preferred detection method in aroma analysis, various sampling techniques for aroma isolation and concentration have been reported, such as solid phase extraction (5), simultaneous steam distillation, liquid extraction (6, 7) but also headspace analysis. The increasing popularity of headspace sampling techniques is due to the minimal sample treatment, which reduces artifactual volatile formation. Different

approaches have been described such as static headspace (8), dynamic headspace (9), direct thermal desorption (10), and SPME (11–13). Moreover, direct coupling techniques of MS to SPME have also been described as rapidly and efficiently affording discriminative fingerprints of the food products analyzed (14–18). Another significant development for the study of flavors and off-flavors is the recent introduction of instruments based on sensor technologies using metal oxide sensors, gas sensitive field effect transistors, conducting polymers, and acoustic wave devices (19–23). Moreover, instruments using MS as the detector are also available and recognize complex headspaces when “coupled” to pattern analysis (24). The term “electronic nose” has been used to describe these instruments because the sensors detect volatiles in a sample and attempt to discriminate among samples based on their “aroma” profile. Nevertheless, no combination of sensors today available has the selective sensitivity or discriminating power of the human nose. Considering MS-based electronic noses, each ion, generated by the mass spectrometer, is considered as a virtual sensor and the whole system becomes the equivalent of an electronic nose with typically 100–300 “pseudo sensors” (25). These pseudo sensors have several advantages over other true sensors since they are highly reproducible and the exclusion of some of them enables the removal of water or alcohol (22, 23).

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The goal of this work was to evaluate the suitability and efficiency of headspace MS-based techniques for the early assessment of infant milk powders oxidation during storage. In the framework of this study, we have first evaluated a MS-based electronic nose without prior sample concentration or chromatographic separation. Such instrumentation has already been successfully applied to the discrimination of diesel fuels (24). In a second time, a technique using SPME coupled to GC-MS was also investigated to potentially characterize/identify markers of milk oxidation and further evaluated for quantitative purposes using isotope dilution technique.

MATERIALS AND METHODS

Chemicals. Hexanal (98% purity) was purchased from Fluka (Buchs, Switzerland). 5,6- d_2 -Hexanal (93% isotopic purity) was synthesized in-house (26). All other chemical reagents were of analytical grade.

Milk Powder Samples. Infant formula powder samples were produced at a pilot scale at Nestlé Product Technology Centre (Konolfingen, Switzerland) using the same basal composition and processing conditions. Except for the first sample (labeled A), the other samples ($n = 4$, noted from B to E) were produced with addition of antioxidants at different concentration levels (ranging from 0 to 100 mg/100 g milk).

The milk powder stability was studied by means of an accelerated storage test, which is commonly used to obtain reliable information on product stability. The latter was performed over a short period of time (4 weeks) in order to identify potential early oxidation markers. The milk powder sample A was stored ungasged in the dark at 37 °C as a "positive control" of off-flavors development. The other samples (from B to E) were also stored ungasged in the dark but at 37 °C whereas another sample (without addition of antioxidant) was stored in the dark at 20 °C flushed under nitrogen and used as reference (noted ref). Every week, an aliquot of each sample was withdrawn and stored at -80 °C under argon until analysis was performed.

Headspace Analysis Using an MS-Based Electronic Nose (Smart Nose). Smart Nose (Smart Nose, Marin-Epagnier, Switzerland) is an electronic nose, which allows the direct analysis by MS of volatile organic components from liquid and solid samples without separation of the headspace constituents.

Infant formula powder (5 g) was reconstituted in bidistilled water (50 mL final volume). An aliquot of this slurry (12 mL) was transferred into a 22 mL sealed glass vial and simultaneously heated and smoothly stirred (60 °C, 30 min) before analysis. A fraction of the headspace (2.5 mL) was transferred quantitatively without GC separation into the mass spectrometer via a 0.6 mL injection chamber, which was then flushed with nitrogen to avoid memory effects. The quadrupole mass spectrometer was used under EI ionization (70 eV) in full scan mode (m/z 40–100 at a rate of 2 scan/s). The TIC profile gave a global MS fingerprint of each sample headspace. The mass intensity lists thus generated were processed by a data pattern recognition software (Smart Nose software) to highlight the most discriminant ions between the different samples. After the ion intensities were normalized with m/z 40 (argon), a PCA treatment, based upon the six most discriminant ions, was performed on the different milk powder samples.

Headspace Analysis Using SPME-GC/MS. Infant formula powder (0.5 g) was solubilized in bidistilled water (5 mL final volume). The suspension was stirred during 10 min using a magnetic stirrer. An aliquot of this reconstituted milk (0.7 mL) was introduced in a 2 mL silanized amber vial (Supelco, Bellefonte, PA) and closed using crimp tops from Agilent Technologies (Palo Alto, CA) that were shown to give very little spectra contamination. The headspace of each sample was equilibrated at 25 °C for 1 h and then sampled by means of a Varian 8200 autosampler (Varian AG, Zug, Switzerland) thermostated at 25 °C. A PDMS/DVB fiber (film thickness 65 μ m, Supelco, Bellefonte, PA) was inserted into the headspace of the vial, and the volatiles in the headspace were sampled during 10 min at 25 °C. Analyses were realized on a Hewlett-Packard 6890 series II gas chromatograph system equipped with a splitless insert liner (0.75 mm i.d.) and interfaced with a 5973 series mass selective detector (interface

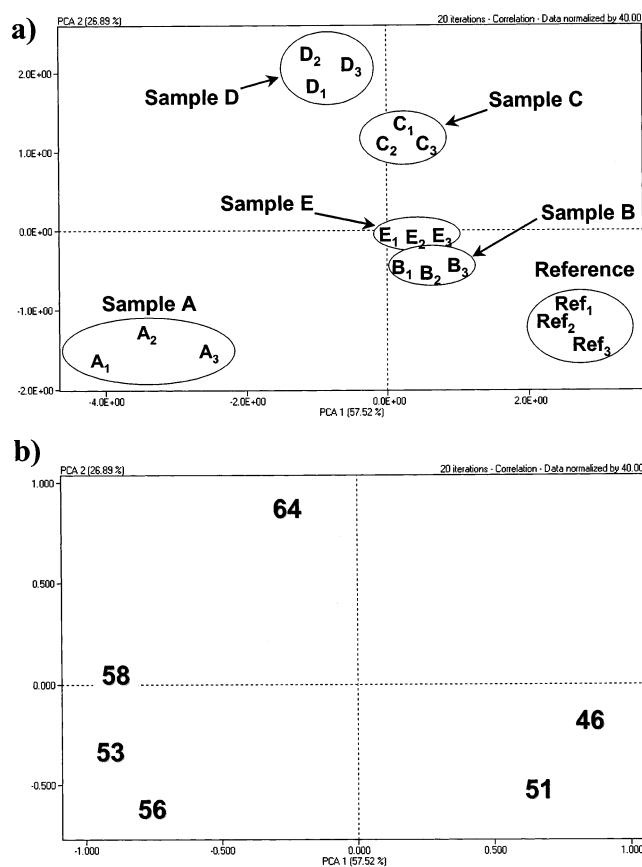


Figure 1. PCA of the infant formula after 3 weeks of storage data as obtained by the Smart Nose: (a) Scores plot and (b) loadings plot of the six major discriminant ions. Each sample has been analyzed in triplicates and numbered from 1 to 3.

temperature 230 °C). The volatiles were thermally desorbed from the fiber in the GC inlet (250 °C, 3 min), the split was then opened, and the fiber was baked out for further 2 min. The volatiles were separated on a J&W DB-WAX capillary column (0.25 mm i.d. \times 30 m, film thickness 0.25 μ m) operating at a constant flow of 1.0 mL/min. The GC temperature was initially 20 °C for 3 min, ramped at a rate of 6 °C/min up to 220 °C, and then held at 220 °C for 5 min. MS detection was performed on a single quadrupole mass spectrometer operating in full scan EI ionization mode (70 eV) by scanning a mass range of m/z 29–300 in 0.5 s. For quantitative analysis, 12 μ L of d_2 -hexanal (ethanolic solution, 5.3 ng/ μ L) was spiked into the different milk powders before the 10 min of stirring (900 μ g/kg final concentration). MS responses at m/z 56 and m/z 58 were integrated for hexanal and d_2 -hexanal, respectively. Milk powder slurries, for both qualitative and quantitative purposes, were always freshly prepared in order to avoid sample degradation after reconstitution in water. Data acquisition and processing were carried out with the Hewlett-Packard HP-Chem Station data software. The volatiles were identified using NIST MS library and further confirmed by analysis of pure standards. Hexanal was also quantified using an external calibration curve in water (after plotting the area ratio vs the amount ratio) in the concentration ranging from 100 to 4000 μ g/kg.

RESULTS AND DISCUSSION

MS-Based Electronic Nose. Figure 1a illustrates the PCA two-dimensional plot obtained for the analyses of the different milk powder samples after 3 weeks of storage. Clearly, the instrument enables the differentiation of the milk powders at different oxidation levels with an important distinction between the sample A (positive control sample) and the others. Moreover, samples B–D, differing by their respective amount of antioxidant, can be easily differentiated. It is worth noticing that the

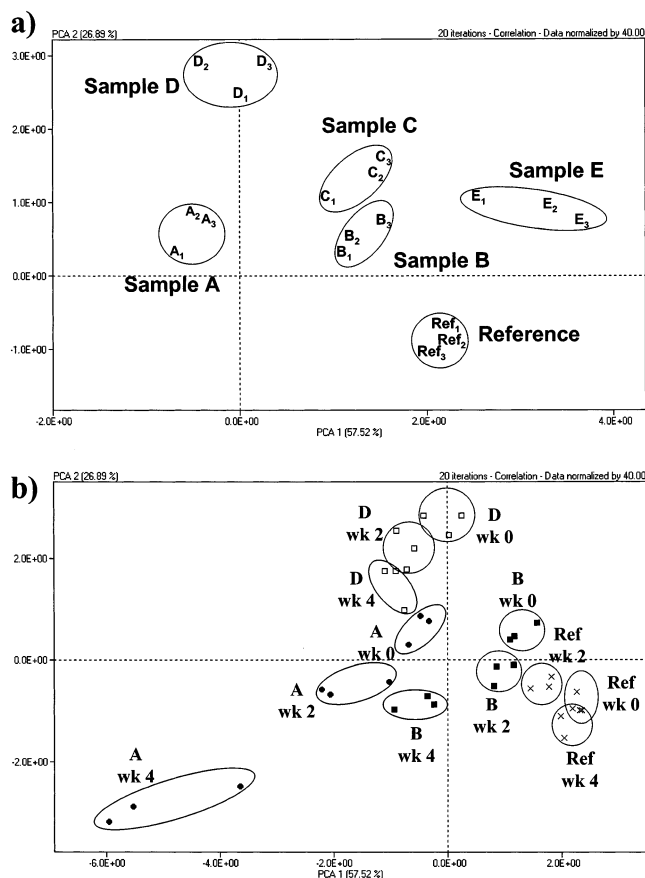


Figure 2. PCA, obtained using the SMart Nose, of the infant formula (a) at the initial storage time (week zero) and (b) kinetic data obtained from samples references A, B, and D at weeks 0, 2, and 4. Each sample has been analyzed in triplicates. For better comparison, the PCA analyses were done using the samples at week 3 and by further projecting on the same plot the data at week 0, 2, and 4. Week has been abbreviated by wk.

instrument, working in this mode, indicates that variants B and E are very similar (very close in the PCA plan). Indeed, these two samples contain very similar antioxidant addition. In conclusion, PC1 highlights the difference between oxidized (A) and non-oxidized (ref) samples, while PC2 highlights the difference due to the antioxidants used. Moreover, it should be noted that such samples discrimination was already observed to a lesser extent at the initial time of storage (Figure 2a). In addition, Figure 2b depicts the entire kinetic of selected infant formulas over the whole period of storage. The evolution of the product deterioration as a function of time could be clearly observed for these four samples. The SMart Nose allows a reproducible analysis with an average CV of 4% based on the most discriminant ions. This technique shows clear differences between milk samples even after a short period of storage, with a high throughput screening potential (around 100 samples analyzed per day).

Even though the weakest point of this technique is its failure in terms of compound identification, it is possible to visualize the most discriminant ions as illustrated in Figure 1b. Obviously, it is not possible to further relate these ions to a characteristic compound mainly because of the high ion fragmentation classically obtained in EI ionization mode. However, with some expertise acquired in the field of fragmentation patterns of volatiles, it is possible to suspect aroma compound classes known as representative of a considered foodstuff. Such a relationship could be established since the

global fragmentation of a complex aroma composition observed in direct inlet EI ionization is similar to the sum of individual compounds fragmentation after GC separation. Recombinations among ionic species were shown to be limited when complex volatiles mixtures were introduced directly into a mass spectrometer inlet instead of after a GC separation (17). For example, the ion observed at m/z 56 is commonly encountered in the fragmentation of saturated aldehydes, compounds already identified as good indicators of milk or milk-based products oxidation (1, 27–30). Because no volatile could be unequivocally identified with this technique, quantitative measurements were not assayed even though linear calibration curves were obtained for some volatile standard solutions (data not shown).

Thus, MS-based electronic nose appears to be a powerful tool to monitor changes in the composition of the gas phase of infant formulas. The advantages of such electronic nose instrument over classic headspace GC-MS instruments are its simpler use but, above all, the higher throughput screening potential (in terms of both data generation and interpretation). However, the greatest disadvantage of this technique is that due to the direct inlet EI ionization of the sample headspace, individual compounds cannot be identified using the system under its "native" configuration (i.e., without chromatographic separation or replacement of EI by chemical ionization). To further potentially characterize early indicators of infant formulas oxidation, a headspace SPME-GC/MS technique was investigated.

SPME-GC/MS. Sampling Optimization. A PDMS/DVB fiber was chosen for the analysis of volatiles originating from infant milk powder oxidation. In many cases, this fiber is preferred for aroma analysis since it shows a good sensitivity and linear behavior toward a wide range of compounds from different types of matrixes (12, 13). In a first set of experiments, headspace sampling optimization was performed by comparing milk powder headspace sampling with or without previous reconstitution in water. For this purpose, the same amount of the reference and an oxidized milk powder sample was analyzed either directly or after reconstitution in water (as described in the Materials and Methods). To maximize the MS response, the SPME fiber was exposed for 1 h to the headspace of the samples. The non-oxidized sample gave a rather low TIC with both preparation methods. The oxidized sample, however, showed an important enhancement in the MS response (more than 10-fold for all the volatiles) when the headspace above reconstituted milk was analyzed as compared to the powder. This indicates that the extraction of volatiles is much more efficient from the liquid state (data not shown). Much attention has to be taken to avoid artifactual oxidation occurring during sample preparation as a result of water reconstitution. Even at -20 °C, hexanal peak area was found to be artificially increased by a factor of two or three in reconstituted samples after several days. Therefore, it is mandatory to reconstitute milk powders just prior to analysis. The time for volatiles adsorption on the SPME fiber for all further experiments was set at 10 min, providing reproducible and sensitive enough results and by far avoiding fiber saturation even for the more oxidized milk powders. Improvement of volatiles extraction, by adding NaCl to the reconstituted samples, was not investigated because it induces compound-dependent extraction enhancements (14, 31) and probably leads to competition effects toward adsorption on fiber or to dramatic reconstituted milks modifications. The GC temperature and the appropriate desorption time were also optimized to ensure that volatiles were desorbed from the fiber in order to avoid carryover effects. Their optimal values were determined at 250 °C for 3 min, respectively. Carryover and

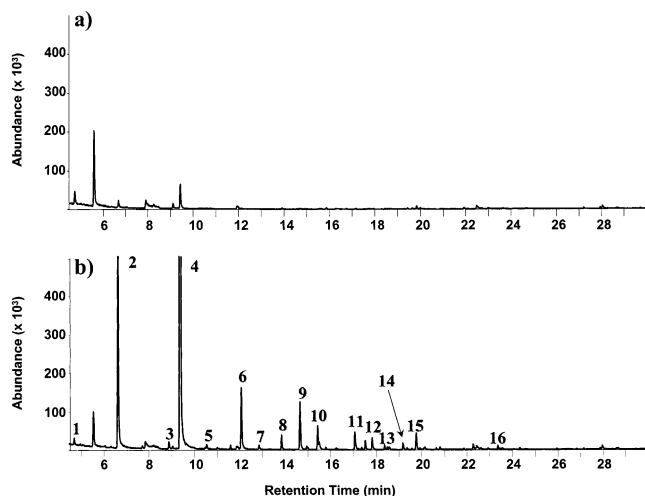


Figure 3. TIC chromatograms of the (a) reference (ref) and (b) A samples analyzed by headspace SPME-GC/MS after 3 weeks of storage. TIC of sample A has been amplified by ca. a factor of 10 to visualize volatiles of low abundance. Peaks numbered from 1 to 16 correspond to 1, 2-butanone; 2, pentanal; 3, 2,3-pentanedione; 4, hexanal; 5, 2-pentenal; 6, heptanal; 7, 2-hexenal; 8, 1-pentanol; 9, octanal; 10, 2-heptenal; 11, nonanal; 12, 2-octenal; 13, 1-octen-3-ol; 14, 2,4-heptadienal; 15, benzaldehyde; 16, 2,4-nonadienal. The volatiles were identified using the NIST MS library and further confirmed by analysis of pure standards.

Table 1. Volatile Compounds of Infant Formulas Detected by SPME-GC/MS

compd name	descriptors associated ^a	odor threshold in water ^b (μg/kg)
2-butanone	acetone, ethery	17 000
pentanal	woody, pungent, fruity	12
2,3-pentanedione	caramel, sweet, buttery	20
hexanal	green, grass, fatty	4.5
2-pentenal	fatty, green	1500
heptanal	fatty, woody	3
2-hexenal	fatty, green	17
1-pentanol	sweet, oil	4000
octanal	fatty, sweer, green, orange	0.7
2-heptenal	soapy, tallowy	13
nonanal	waxy, painly	1
2-octenal	fatty, green, raw nut	3
1-octen-3-ol	mushroom, musty	1
2,4-heptadienal	fatty, rancid	
benzaldehyde	bitter almond, aromatic, sweet	350
2,4-nonadienal	fatty, soapy	0.06

^a Adapted from literature (18, 37–39) and completed by personal observations.

^b Adapted from 32, 40–45.

peaks originating from the fiber (check of the fiber wear) were regularly assessed by running blank samples.

Infant Formula Powder Analyses. Figure 3 represents the TICs of samples ref and A after 3 weeks of storage. The reference sample exhibits a low global headspace response as compared to sample A. The main volatiles identified in sample A and their respective sensory attributes and perception levels (in water) are shown in Table 1. Thus, under the given analytical conditions, 16 principal volatile compounds were identified in stored milk powders. Among these volatiles, saturated aldehydes and hexanal followed by pentanal are the most abundant chemicals. Several authors have already identified this group of compounds in dairy products (1, 27, 30). A series of other lipid oxidation products were identified in the stored sample that may play important roles for the sensory perception of milk reconstituted from stored milk powder, like 2,4-nonadienal with

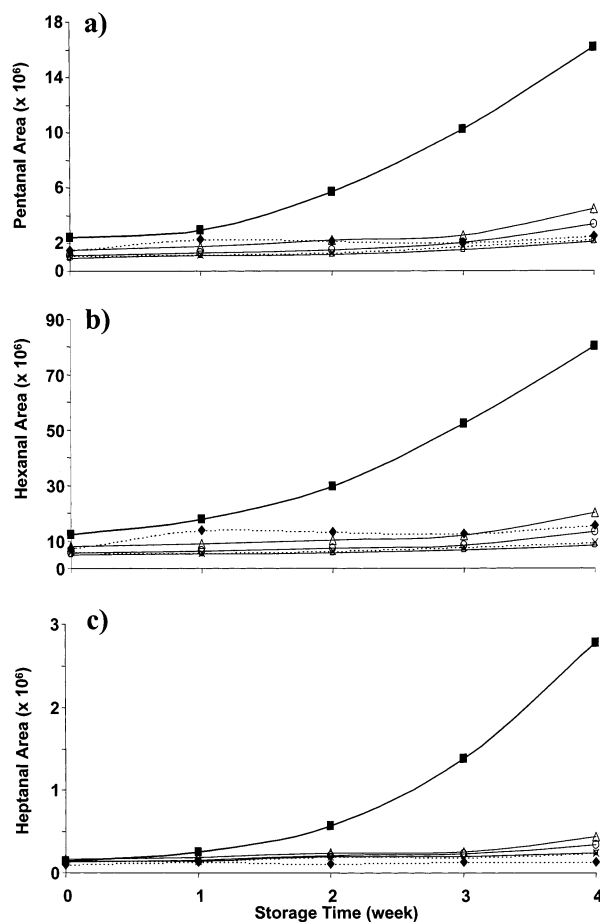


Figure 4. Kinetic formations of (a) pentanal, (b) hexanal, and (c) heptanal in the different infant formulas during the storage period (···◆···, ref sample; —■—, sample A; —△—, sample B; ···×···, sample C; —□—, sample D; and —○—, sample E).

a fatty green aroma and a very low odor threshold of 0.06 μg/kg in water. Even though odor thresholds in water do not reflect the perception thresholds in the food product itself, such values give useful information regarding the relative aroma activity of volatiles with similar physicochemical properties. For instance, pentanal and hexanal odor thresholds determined at 12 and 4.5 μg/kg in water (32) increase up to 130 and 50 μg/kg in 3.8% homogenized milk, respectively (33).

As saturated aldehydes were the most abundant volatiles, their ability to discriminate the different milk samples was further checked by following their formation kinetics through 4 weeks of storage. Thus, Figure 4 represents the kinetics of pentanal, hexanal, and heptanal for the different reconstituted milk samples. Every week, the volatile content of each sample was evaluated by headspace SPME-GC/MS in independent triplicates. As shown in Figure 4, the control sample A (stored ungasged at 37 °C) exhibits clear differences in term of the evolution of these three aldehydes as compared to the other samples. Regarding this sample, pentanal, hexanal, and heptanal contents increase from the first to the fourth week, to reach levels 7–20-fold higher as compared to the initial values. In contrast, these aldehydes increase only 2–3-fold in the other samples (B–E; stored ungasged at 37 °C), and merely 1.5-fold in the reference sample. On the basis of these results, pentanal, hexanal, or heptanal could be used to monitor changes in infant formulas oxidation during storage. Such methodology could be used to perform relative measurements as compared to a reference sample (if the sample preparation is standardized).

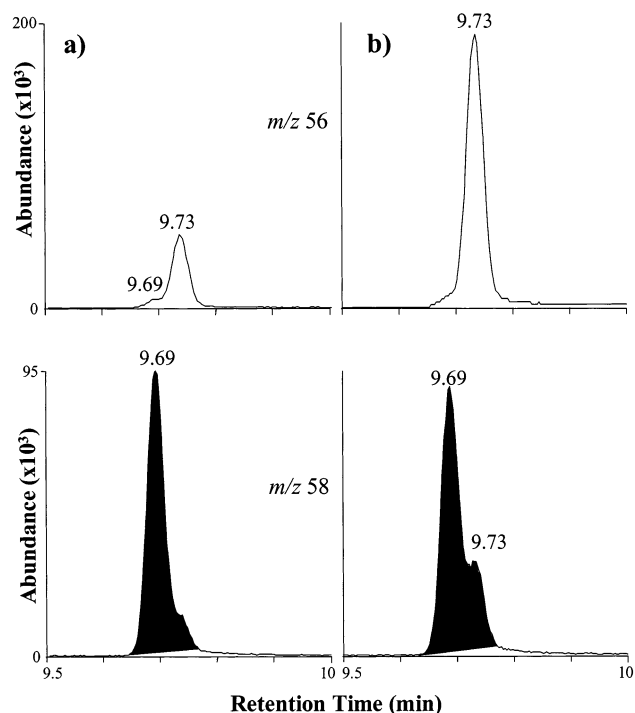


Figure 5. Extracted ions chromatograms of m/z 56 and m/z 58 (hexanal and d_2 -hexanal, respectively) from (a) sample A (week 0) and (b) sample A (week 3). Scales have been normalized according to the intensity of the higher peak.

Because of the high level of hexanal found in every sample and its already observed correlation with off-flavors development in milk products (1), this aldehyde was preferred as a potential chemical marker to evaluate infant formulas oxidation using SPME-GC/MS. Under our conditions, the CVs of pentanal, hexanal, and heptanal analyses were 12, 15, and 12%, respectively (considering more than 50 measurements), reflecting the range of fluctuation already described by Roberts et al. (12).

Because relative SPME-GC/MS measurements as compared to a reference are less precise and require strictly controlled sampling procedures, we evaluated also the potential of the headspace SPME-GC/MS for quantitative purposes, using d_2 -hexanal as an internal standard, to measure hexanal amounts in infant formulas.

Quantification of Hexanal by Headspace SPME-GC/MS Using Isotope Dilution. A d_2 -hexanal standard solution was prepared in ethanol and then spiked at 900 $\mu\text{g}/\text{kg}$ directly into the reconstituted milk slurries (final ethanol concentration 1.7%, v/v). With such solvent addition, we first checked if troublesome competition or fiber saturation effects were not induced. Such effects are dependent on both analytes and fiber natures. Indeed, it has been shown that ethanol can replace acetone and isoprene on a PDMS/DVB fiber (34) whereas ethanol in a model solution strongly decreased the amount of monoterpenes adsorbed on the fiber (35). Regarding hexanal extraction from a given milk sample, we were able to increase the ethanol volume up to 3.5% (v/v), relatively to the reconstituted milk volume in the injection vial, without modifying significantly the hexanal MS response. Thus, under our analytical conditions, ethanol brought via deuterated hexanal addition can be considered as not inducing biases in hexanal quantification.

Figure 5 represents the ions used for quantification (i.e., m/z 56 and m/z 58 for hexanal and d_2 -hexanal, respectively) from sample A stored for 0 and 3 weeks at 37 °C. These two ions

Table 2. Hexanal Amounts Found in Two Infant Milk Powder Samples Over an Accelerated Storage Test

milk sample		hexanal amount ($\mu\text{g}/\text{kg}$)
sample A ^a		
week 0		1181
week 1		1840
week 3		3430
sample B ^a		
week 0		466
week 2		617
week 4		1022

^a Sample stored ungasged at 37 °C.

correspond to the most intense fragment ions encountered under our analytical conditions and correspond to a loss of $\text{C}_2\text{H}_4\text{O}$. Thus, by comparing relatively non-oxidized and oxidized samples, a clear difference in hexanal amount is observed. Moreover, the use of a labeled compound as internal standard facilitates the unambiguous identification of the analyte. Using this isotope dilution procedure, the average CV was ca. 5% vs 15% without the internal standard. Furthermore, the labeled standard fully compensates potentially lower signal MS responses in the case of decreasing fiber performances. The ions chosen for the quantification, m/z 56 for hexanal and m/z 58 for d_2 -hexanal, are not highly specific, and we have to consider the contribution from one compound to the other and vice versa (12.0% contribution of d_2 -hexanal in m/z 56 response and 10.6% of hexanal in m/z 58). To compensate for these contributions, suitable correction factors were used. For the quantification of hexanal in our samples, the external calibration used showed a good linearity within the concentration range from 0 to 4000 $\mu\text{g}/\text{kg}$ ($Y = 0.979X + 0.001$, $r^2 = 0.999$).

In another set of experiments, for estimating the hexanal recovery, sample A (week 0) was spiked with hexanal at three fortification levels, i.e., ca. 50, 100, and 150% of the estimated initial hexanal amount. Thus, hexanal additions were performed on sample A week 0 at 570, 1140, and 1710 $\mu\text{g}/\text{kg}$ and led to recoveries above 76%. This IDA methodology has been further applied to the determination of hexanal in several milk powders from those previously analyzed without isotope dilution. This procedure gave hexanal amounts ranging from 466 $\mu\text{g}/\text{kg}$ (ppb), for a relatively non-oxidized sample, up to 3430 $\mu\text{g}/\text{kg}$ for an oxidized one (Table 2). Additionally, hexanal has also been quantitated in sample A (week 0) using the standard addition method. This led to a hexanal amount of 1316 $\mu\text{g}/\text{kg}$ vs 1181 $\mu\text{g}/\text{kg}$ using external calibration, i.e., a difference close to 11%, which is acceptable considering the variations of SPME-GC/MS. Even though hexanal amounts are very dependent on milk powder formulations and storage conditions, our calculated values are in the same order of magnitude than those already reported for infant formulas (1, 36). Moreover, it could be noted that the lowest hexanal amount found in our samples (466 $\mu\text{g}/\text{kg}$ in sample B week 0) is far above the hexanal perception thresholds (ca. 4.5 $\mu\text{g}/\text{kg}$ in water and 50 $\mu\text{g}/\text{kg}$ in 3.8% homogenized milk).

At the concentrations determined, hexanal is likely to contribute to the milk powders flavors and off-flavors (at higher concentrations), together with other compounds. Indeed, within the complex mixture of flavored compounds present in food products, synergic or antagonist effects can occur and contribute to a particular aroma.

All of these results demonstrate that SPME-GC/MS is an efficient technique to identify and further monitor (over a storage

test) individual volatiles of milk powder samples. Quantification in the ppm range has also been successfully demonstrated using IDA combined with SPME sampling. Despite its advantages in terms of characterization and quantification, this technique is more time-consuming than the electronic nose, since around 24 samples per day could be analyzed vs ca. 100 for the electronic nose.

In conclusion, MS-based electronic nose and SPME-GC/MS appeared to be reliable, reproducible, and sensitive enough techniques for detecting volatiles in infant formula powder samples. Indeed, these two headspace MS-based methods allow a good quality control of the different infant milk powder samples over 4 weeks of storage. The quasi-absence of sample preparation and the easy-to-use aspect of these techniques suggest potential applications in the food industry as a powerful quality control tool (using a sample as reference). The primary advantage of the electronic nose is its high throughput potential for screening purposes. However, the electronic nose is only a qualitative tool; it therefore becomes unsuitable when volatile(s) characterization is required. On the other hand, SPME-GC/MS is very suitable and efficient for such purposes. Short chain saturated aldehydes (C₅–C₉) were identified as the main volatiles present in the headspace fraction of the infant formula powders studied. Hexanal, previously identified as a potential marker of milk powder oxidation, has been fruitfully quantified in the ppm level using isotope dilution technique. Therefore, the two headspace techniques described are complementary. The SMart Nose technique can rapidly assess the milk quality as a qualitative tool whereas the SPME-GC/MS has the power to identify the volatile compounds formed and possibly to quantify them.

ABBREVIATIONS USED

CV, coefficient of variation; EI, electron impact; GC, gas chromatography; DVB, divinylbenzene; i.d., internal diameter; IDA, isotope dilution assay; MS, mass spectrometry; PCA, principal component analysis; PDMS, poly(dimethylsiloxane); SPME, solid phase microextraction; TIC, total ion current.

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LITERATURE CITED

- Ulberth, F.; Roubicek, D. Monitoring of oxidative deterioration of milk powder by headspace gas chromatography. *Int. Dairy J.* **1995**, *5*, 523–531.
- Gray, J. I.; Monahan, F. J. Measurement of lipid oxidation in meat and meat products. *Trends Food Sci. Technol.* **1992**, *3*, 315–319.
- Stapelfeldt, H.; Mortensen, G.; Skibsted, L. H. Early events in oxidation of whole milk powder detected by electron spin resonance spectrometry. Carry-over effects from butter oil used for instantization. *Milchwissenschaft* **1997**, *52*, 266–269.
- Esterbauer, H. Cytotoxicity and genotoxicity of lipid-oxidation products. *Am. J. Clin. Nutr.* **1993**, *57*, 779S–785S.
- Wang, Z. H.; Dou, J.; Macura, D.; Durance, T. D.; Nakai, S. Solid-phase extraction for GC analysis of beany flavours in soymilk. *Food Res. Int.* **1998**, *30*, 503–511.
- Pino, J. A.; Marbot, R.; Vazquez, C. Characterization of volatiles in strawberry guava (*Psidium cattleianum* Sabine) fruit. *J. Agric. Food Chem.* **2001**, *49*, 5883–5887.
- Vekiari, S. A.; Protopapadakis, E. E.; Papadopoulou, P.; Papanicolaou, D.; Panou, C.; Vamvakias, M. Composition and seasonal variation of the essential oil from leaves and peel of a Cretan lemon variety. *J. Agric. Food Chem.* **2002**, *50*, 147–153.
- Maetzu, L.; Sanz, C.; Andueza, S.; de Pena, M. P.; Bello, J.; Cid, C. Characterization of espresso coffee aroma by static headspace GC-MS and sensory flavor profile. *J. Agric. Food Chem.* **2001**, *49*, 5437–5444.
- Kjeldsen, F.; Christensen, L. P.; Edelenbos, M. Quantitative analysis of aroma compounds in carrot (*Daucus carota* L.) cultivars by capillary gas chromatography using large-volume injection technique. *J. Agric. Food Chem.* **2001**, *49*, 4342–4348.
- Marsili, R. T.; Miller, N.; Kilmer, G. J.; Simmons, R. E. Identification and quantitation of the primary chemicals responsible for the characteristic malodor of beet sugar by purge and trap GC-MS-OD techniques. *J. Chromatogr. Sci.* **1994**, *32*, 165–171.
- Arthur, C. L.; Pawliszyn, J. Solid-phase microextraction with thermal desorption using fused silica optical fibers. *Anal. Chem.* **1990**, *62*, 2145–2148.
- Roberts, D. D.; Pollien, P.; Milo, C. Solid-phase microextraction method development for headspace analysis of volatile flavor compounds. *J. Agric. Food Chem.* **2000**, *48*, 2430–2437.
- Fabre, M.; Aubry, V.; Guichard, E. Comparison of different methods: static and dynamic headspace and solid-phase microextraction for the measurement of interactions between milk proteins and flavor compounds with an application to emulsions. *J. Agric. Food Chem.* **2002**, *50*, 1497–1501.
- Yang, X.; Peppard, T. Solid-phase microextraction for flavor analysis. *J. Agric. Food Chem.* **1994**, *42*, 1925–1930.
- Marsili, R. T. SPME-MS-MVA as an electronic nose for the study of off-flavors in milk. *J. Agric. Food Chem.* **1999**, *47*, 648–654.
- Marsili, R. T. Shelf life prediction of processed milk by solid-phase microextraction, mass spectrometry, and multivariate analysis. *J. Agric. Food Chem.* **2000**, *48*, 3470–3475.
- Peres, C.; Viallon, C.; Berdague, J. L. Solid-phase microextraction-mass spectrometry: a new approach to the rapid characterization of cheeses. *Anal. Chem.* **2001**, *73*, 1030–1036.
- Peres, C.; Denoyer, C.; Tournayre, P.; Berdague, J. L. Fast characterization of cheeses by dynamic headspace-mass spectrometry. *Anal. Chem.* **2002**, *74*, 1386–1392.
- Mielle, P. “Electronic Noses”: Towards the objective instrumental characterization of food aroma. *Trends Food Sci. Technol.* **1996**, *7*, 432–438.
- Bartlett, P. N.; Elliott, J. M.; Gardner, J. W. Electronic Noses and their application in the food industry. *Food Technol.* **1997**, *51*, 44–48.
- Schaller, E.; Bosset, J. O.; Escher, F. “Electronic Noses” and their application to the food industry. *Lebens.-Wiss. Technol.* **1998**, *31*, 305–316.
- Strike, D. J.; Meijerink, M. G. H.; Koudelka-Hep, M. Electronic Noses – A mini-review. *Fresenius J. Anal. Chem.* **1999**, *364*, 499–505.
- Zubritsky, E. E-noses keep an eye on the future. *Anal. Chem.* **2000**, *72*, 421A–426A.
- Feldhoff, R.; Saby, C. A.; Bernadet, P. Discrimination of diesel fuels with chemical sensors and mass spectrometry based electronic noses. *Analyst* **1999**, *124*, 1167–1173.
- Visani, P. Why “electronic noses” in the food industry? *New Food* **2000**, *3*, 9–13.
- Lin, J.; Welti, D. H.; Vera, F. A.; Fay, L. B.; Blank, I. Synthesis of deuterated volatile lipid degradation products to be used as internal standards in isotope dilution assays. 1. Aldehydes. *J. Agric. Food Chem.* **1999**, *47*, 2813–2821.
- Hutchens, R. K.; Hansen, A. P. The effect of various UHT processing parameters and storage conditions on the saturated aldehydes in half-and-half cream. *J. Food Prot.* **1991**, *54*, 109–112.
- Andersson, K.; Lingnert, H. Influence of oxygen concentration on the flavour and chemical stability of cream powder. *Lebens.-Wiss. Technol.* **1998**, *31*, 245–251.

- (29) Christensen, T. C.; Holmer, G. GC/MS analysis of volatile aroma components in butter during storage in different catering packaging. *Milchwissenschaft* **1996**, *51*, 134–138.
- (30) Marsili, R. T. Comparison of solid-phase microextraction and dynamic headspace methods for the gas chromatographic-mass spectrometric analysis of light-induced lipid oxidation products in milk. *J. Chromatogr. Sci.* **1999**, *37*, 17–23.
- (31) Liu, T. T.; Yang, T. S. Optimization of solid-phase microextraction analysis for studying change of headspace flavor compounds of banana during ripening. *J. Agric. Food Chem.* **2002**, *50*, 653–657.
- (32) Buttery, R. G.; Turnbaugh, J. G.; Ling, L. C. Contribution of volatiles to rice aroma. *J. Agric. Food Chem.* **1988**, *36*, 1006–1009.
- (33) Day, E. A.; Lillard, D. A.; Montgomery, M. W. Autoxidation of milk lipids. III. Effect on flavor of the additive interactions of carbonyl compounds at subthreshold concentrations. *J. Dairy Sci.* **1963**, *46*, 191–194.
- (34) Grote, C.; Pawliszyn, J. Solid-phase microextraction for the analysis of human breath. *Anal. Chem.* **1997**, *69*, 587–596.
- (35) De La Calle Garcia, D.; Magnaghi, S.; Reichenbacher, M.; Danzer, K. Systematic optimization of the analysis of wine bouquet components by solid-phase microextraction. *J. High Resolut. Chromatogr.* **1996**, *19*, 257–262.
- (36) Giammarioli, S.; Lammardo, A. M.; Sanzini, E.; Bellomonte, G. Preliminary study on lipid oxidation in infant formulas during storage. *Riv. Sci. Aliment.* **1997**, *2*, 80–88.
- (37) Badings, H. T.; Neeter, R. Recent advances in the study of aroma compounds of milk and dairy products. *Neth. Milk Dairy J.* **1980**, *34*, 9–30.
- (38) Aparicio, R.; Rocha, S. M.; Delgadillo, I.; Morales, M. T. Detection of rancid defect in virgin olive oil by the electronic nose. *J. Agric. Food Chem.* **2000**, *48*, 853–860.
- (39) van Ruth, S. M.; Roozen, J. P.; Jansen, F. J. H. M. Aroma profiles of vegetable oils varying in fatty acid composition vs concentrations of primary and secondary lipid oxidation products. *Nahrung* **2000**, *44*, 318–322.
- (40) Schnabel, K. O.; Belitz, H. D.; von Ranson, C. Untersuchungen zur struktur-aktivitäts-beziehung bei geruchsstoffen. *Z. Lebensm. Unters. Forsch.* **1988**, *187*, 215–223.
- (41) Buttery, R. G. Importance of lipid derived volatiles to vegetable and fruit flavor. In *Flavor Chemistry of Lipid Foods*; Min, D. B., Smouse, T. H., Eds.; The American Oil Chemists' Society: Champaign, U.S.A., 1989; pp 156–165.
- (42) Buttery, R. G.; Teranishi, R.; Flath, R. A.; Ling, L. C. Fresh tomato volatiles: composition and sensory studies. In *Flavor Chemistry: Trends and Developments*; Teranishi, R., Buttery, R. G., Shahidi, F., Eds.; American Chemical Society: Washington, DC, 1989; pp 259–286.
- (43) Milo, C.; Grosch, W. Changes in the odorants of boiled trout (*Salmo fario*) as affected by the storage of the raw material. *J. Agric. Food Chem.* **1993**, *41*, 2076–2081.
- (44) Buttery, R. G.; Ling, L. C. Volatile flavor components of corn tortillas and related products. *J. Agric. Food Chem.* **1995**, *43*, 1878–1882.
- (45) Konopka, U. C.; Guth, H.; Grosch, W. Potent odorants formed by lipid peroxidation as indicators of the warmed-over flavour (WOF) of cooked meat. *Z. Lebensm. Unters. Forsch.* **1995**, *201*, 339–343.

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