Effect of Food Matrix and pH on the Volatilization of Bases (TVB) in Packed North Atlantic Gray Shrimp (Crangon crangon): Volatile Bases in MAP Fishery Products

8 AUTHORS, INCLUDING:

Bert Noseda
Ghent University
21 PUBLICATIONS **173** CITATIONS

SEE PROFILE

Jo Dewulf
Ghent University
273 PUBLICATIONS **5,083** CITATIONS

SEE PROFILE

Danny Pauwels
Ghent University
2 PUBLICATIONS **24** CITATIONS

SEE PROFILE

Herman Van Langenhove
Ghent University
339 PUBLICATIONS **7,098** CITATIONS

SEE PROFILE

Effect of Food Matrix and pH on the Volatilization of Bases (TVB) in Packed North Atlantic Gray Shrimp (*Crangon crangon*): Volatile Bases in MAP Fishery Products

BERT NOSEDA,[†] JO DEWULF,[‡] JOKE GOETHALS,[†] PETER RAGAERT,[†] ILSE VAN BREE,[†] DANNY PAUWELS,[†] HERMAN VAN LANGENHOVE,[‡] AND FRANK DEVLIEGHERE*,[†]

[†]Laboratory of Food Microbiology and Food Preservation, Member of Food2know, Department of Food Safety and Food Quality and [‡]Laboratory of Environmental Organic Chemistry, Department of Organic Chemistry, Ghent University, Coupure Links 653, 9000 Ghent, Belgium

The total volatile basic nitrogen fraction (TVB-N) is often used as a quality parameter in the fish industry to assess spoilage. This parameter often leads to discussions between producers and retailers when it comes to defining clear limits of acceptability for modified atmosphere (MA) packed fish and fishery products. Suggested product limits (mg N/100 g fish) do not always correlate with the presence of off-odors. Gray shrimp are an economic valuable, very perishable niche product, where the TVB-N fraction plays an important role considering its shelf life. This research focuses on the effect of a shrimp matrix and its pH on the volatilization of these formed bases, revealing the relationship between concentrations in the fishery product and the concentrations of these bases present in the headspace of the packed product. Especially, the pH of the product, which is lowered when fishery products are packed under a carbon dioxide enriched atmosphere, appeared to have an immense effect on the volatilization of these bases. The effect of the fish matrix itself is established by means of calculated equilibration constants (dimensionless) being $2.13 \times 10^{-4} \pm 0.38 \times 10^{-4}$ for trimethylamine, $6.34 \times 10^{-5} \pm 1.71 \times 10^{-5}$ for dimethylamine, and $2.58 \times 10^{-5} \pm 0.49 \times 10^{-5}$ for ammonia. Comparison of these constants with the equilibration constants of an aqueous solution indicated the retention of these bases in the product. This article provides not only the important insights for the interpretation of TVB-N values in modified atmosphere packaged gray shrimp but also the methodology to extend these findings to other fish and fishery products.

KEYWORDS: Total volatile bases; TVB-N; TMA-N; MAP; gray shrimp (*Crangon crangon*); pH

INTRODUCTION

The formation of trimethylamine (TMA), dimethylamine (DMA), and ammonia (NH_3), which constitute the volatile basic nitrogen fraction (TVB-N), is a well described spoilage phenomenon for marine fishery products stored in ice. In the last decades, studies on the effect of modified atmosphere (MA) packaging on fishery products are prevalent. Modification of the atmosphere within a package significantly prolongs the shelf life of fishery products, which results in an extensive use of this technique in practice. Nevertheless, in the use of this preservation technique, volatile bases still cause in many cases a deflecting odor in marine fishery products (*1*). Consequently, TVB-N and the trimethylamine–nitrogen fraction (TMA-N) are still generally used in the fish industry as quality parameters for spoilage assessment. Limits (mg N/100 g fish) of acceptability have been established for fish stored in ice, by means of classical methodologies (*2*). These limits, however, appeared to be ambiguous for fishery products packed under a modified atmosphere. Possible reasons for this lack of clarity are the following. On the one hand, a modification

of the atmosphere will cause a change in the composition (*1*) and growth (*3*, *4*) of the spoilage microflora but also an alteration in the spoilage metabolism (*5*, *6*). Whether or not amines will be produced in MAP fishery products will thus depend on these conditions. The chemical composition of the food matrix on the other hand also plays an important role in the volatilization of formed metabolites because of conceivable interactions (*7*, *8*). In this matter, pH is an important parameter considering the volatile bases (*2*). Carbon dioxide, dissolving in the aqueous phase of the food matrix, will cause a modification of this food matrix by lowering the pH (*9*, *10*). Whether the volatile bases present in MAP fishery products will be perceived is therefore also highly dependent on the chemical composition of the food matrix (proteins, fat, and salt content) and its pH.

When considering a base in an aqueous solution, inside a closed two-phase gas–liquid system, the Henderson–Hasselbalch equation describes the relationship between pH and the dissociation of the base (B^{liquid}) and its conjugated acid (BH^+). In addition, the Henry coefficient (H) describes the volatilization of the base from a liquid (B^{liquid}) to the headspace (B^{gas}) at a certain temperature T (*11*). **Figure 1** schematically depicts the equilibration reaction for bases in a closed gas–liquid system. The coinciding

*Corresponding author. Tel: 0032-(0)9 264 61 64. Fax: 0032-(0)9 264 55 10. E-mail: Frank.Devlieghere@Ugent.be.
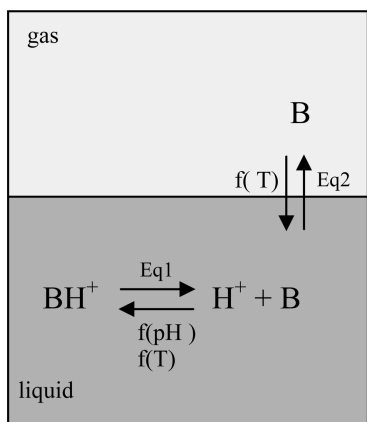
Article

J. Agric. Food Chem., Vol. 58, No. 22, 2010   **11865**

Figure 1. Theoretical representation of the equilibrium reactions of a base in a closed two-phase gas—liquid system.

Henderson—Hasselbalch equation and Henry's law are given in eqs 1 and 2, respectively (*12*).

$$pOH = pK_b + \log\left(\frac{[BH^+]}{[B^{\text{liquid}}]}\right) \tag{1}$$

$$H = \frac{[B^{gas}]}{[B^{\text{liquid}}]} \tag{2}$$

Yet, some restrictions are allied to these laws. Both laws are approximations and are only valid in aqueous solutions, in case the base is sufficiently dissolved in the solvent and when the base does not react in any way with this solvent. Moreover, Henry's law is valid at atmospheric conditions. Notwithstanding the universality of these laws for a gas—liquid system and taking into account the mentioned limitations, one could assume that a similar equilibrium exists for these amines in a food matrix, but then again under defined and standardized conditions. Starting from eq 1, the concentration of B^{liquid} (%) in this reaction, based on the pH and the pK_a of the conjugated acids, can be calculated according to eq 3. Plotting these calculated percentages as a function of the pH results in a sigmoidal curve (as shown in **Figure 2**) (*13*). As can be seen in this Figure, the pK_a values of trimethylamine, dimethylamine, and ammonia are, respectively, 9.81, 10.73, and 9.25 at a standardized temperature of 25.0 °C.

$$\frac{10^{(\text{pH}-pK_a)}}{10^{(\text{pH}-pK_a)}+1} \cdot 100 = \frac{[B^{\text{liquid}}]}{[B^{\text{liquid}}]+[BH^+]} \cdot 100 \tag{3}$$

To calculate the pK_a at another temperature, the van 't Hoff equation can be used (eq4) (*12*), where ΔH^0 is the standard enthalpy of the reaction, R is the gas constant, K_{aT1} the thermodynamic ionization constant at 25.0 °C (T_1 = 298.15 K), and K_{aT2} is the ionization constant at the experimental temperature T_2. According to the NIST webdatabase, values for ΔH^0 for TMA, DMA, and NH_3 are found to be −45.73, −43.9 and, −80.8 kJ/mol, respectively (*14*).

$$\ln\left(\frac{K_{aT_2}}{K_{aT_1}}\right) = \frac{-\Delta H^0}{R}\left(\frac{T_1-T_2}{T_1 T_2}\right) \tag{4}$$

A final equation valid for the two-phase gas—liquid system (**Figure 1**) is the principle of mass conservation (eq 5), stating that the mass of a component in a closed system will stay constant over time.

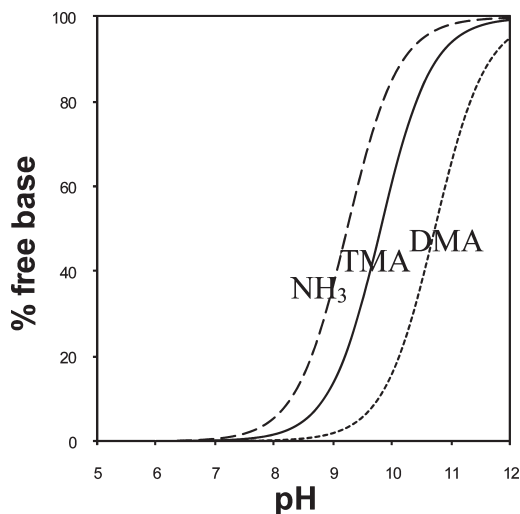$$M^{\text{tot}} = M(B)^{gas} + M(B)^{matrix} + M(BH^+)^{matrix} \tag{5}$$

Figure 2. Theoretical calculation of the percentage free base (B^{liquid}) (%) for TMA, DMA, and NH_3 based on the Henderson—Hasselbalch equation, given the pK_a and varying the pH.

Whether one can adopt these equilibriums from a two-phase gas—liquid system to a packed fishery product is the subject of this investigation. The choice of gray shrimp (*Crangon crangon*) as the investigated fishery product relies on the characteristic high free amino acid content, together with a typical high concentration of nonprotein nitrogenous substances such as ammonia, trimethylamineoxide (TMAO), and betaine (*15*). The initial natural pH of fresh crustaceans is generally higher than that of other fish species. One of the causes is the presence of a higher content of nonprotein nitrogenous compounds. Furthermore, the carbohydrate content in crustaceans is negligible, limiting a pH depression associated with the production of lactic acid during rigor mortis (*16*). Moreover, the formation of volatile bases in crustacea stored in ice or packed with MA, has already been observed in the literature (*17*). The aim of this work is to investigate the effect of the pH and the food matrix on the volatilization of TMA, DMA, and NH_3 in packed gray shrimp (*Crangon crangon*).

MATERIALS AND METHODS

Raw Material. Gray shrimp (*Crangon crangon*) caught at the Belgian coast side in October, 2009, were cooked during 3 min on board in salted water (±90 °C), stored overnight at 2.0 °C, and brought ashore at the Ostend harbor (Belgium), where they were mechanically sorted in size (6.0—8.0 cm equivalent to approximately 800 unpeeled shrimp/kg) and peeled at a local shrimp processing company. The time elapsed between capture and transport to the Laboratory of Food Microbiology and Food Preservation (LFMFP) in Ghent (Belgium) was approximately 12 h. During these manipulations, no preservatives or salts were added, and the temperature (2.0 °C) was respected. Once in the laboratory, the shrimp were portioned in bags of 500.0 g and stored in a freezer (−24.0 °C) until needed for experimental purposes. After 12 h of thawing at 4.0 ± 0.5 °C, the samples were mixed to homogenize this food matrix and portioned according to the experimental setup. The initial pH of the peeled shrimp product was found to be 8.2 ± 0.1.

pH Measurements. pH was measured with a pH-electrode (InLab SolidsPro, Mettler Toledo GmbH, Schwerzenbach, Switzerland) connected with a pH meter (SevenEasy, Mettler Toledo GmbH).

Effect of Carbon Dioxide on the Matrix pH. Gray shrimp (200 g) samples were packed in high barrier PA/PE/EVOH/PE bags (Euralpack, Schoten, Belgium) with an oxygen transmission rate of 2.0 cm³/m²·d·bar (23.0 °C, 85% R.H.). Different concentrations of carbon dioxide were applied with nitrogen gas used as filling gas. The gas/product ratio was fixed at 2/1. The modified atmosphere gases were measured after packing and after 24 h of storage at 4.0 °C using a Checkmate 9900

11866 *J. Agric. Food Chem.,* Vol. 58, No. 22, 2010

Noseda et al.

(PBI Dansensor, Ringsted, Denmark) O_2/CO_2 headspace gas analyzer. The concentration of dissolved carbon dioxide was calculated according to Sivertsvik et al. (*18*). The pH of the packed gray shrimp samples was measured immediately after opening the package. Further pH evolutions were monitored storing samples at 4.0 °C and at 20.0 °C.

Effect of Matrix pH on the Volatilization of the TVB-N Content. Thirty portions of 50.0 ± 0.5 g mixed shrimp were spiked with trimethyl-amine hydrochloride with a concentration of 5.0 mg N/50.0 g shrimp. The pH of this mixture was subsequently adjusted by adding small volumes of HCl (2 N) or KOH (1 N) aiming at a pH varying from 6.0 until 9.0. The samples were packed in high barrier film bags (Euralpack, Schoten, Belgium) filled with 950.0 ± 5.0 mL of inert N_2 gas using a Multivac A300/42 packaging unit (Hagenmüller, Wolfert-schwenden, Germany). Samples were stored 60 min at 4.0 ± 0.5 °C before analyzing with the selective ion flow tube mass spectrometer to allow the liquid and gas phase to equilibrate. After analyzing, sampling bags were opened, and the pH was immediately measured. The product temperature of the measured samples was 4.0 ± 2.0 °C.

This procedure was repeated for dimethylamine hydrochloride and ammonium chloride spiked on mixed gray shrimp. This experiment was also performed with distilled water as the matrix instead of shrimp.

SIFT-MS Analysis. The Selected Ion Flow Tube Mass spectrometer (Voice 200, Syft Technologies) sampled the headspace through a septum on the sampling bag during a time period of 120 s with a flow of 77.3 Pa L s^{-1}. Volatile organic compounds (VOCs) of interest were introduced through the heated inlet into the flow tube, where reactions with precursor ions H_3O^+, NO^+, and O_2^+ resulted in ionized masses, which were monitored by a mass spectrometer, located at the downstream end of the flow tube. Quantification of the VOCs occurred by using the reaction rate coefficients (k) and the branching ratios (b) of the reaction between the precursor ions and the VOCs. The reaction rate coefficient is a concentration independent measure of the reaction speed in units of molecules cm^{-3} s^{-1}. The branching ratio reflects the proportion (%) of a specific ion product which is produced, resulting in the reaction with one precursor. The methodology behind the determination of these parameters can be found in the literature (*19, 20*). The ionized masses used for quantification were $C_3H_8N^+$ (m/z 58; $k = 2.0E^{-9}$; $b = 10\%$), $(CH_3)_3N \cdot H^+$ (m/z 60; $k = 2.0 E^{-9}$; $b = 90\%$), $(CH_3)_2NH \cdot H^+$ (m/z 46; $k = 2.1 E^{-9}$; $b = 100\%$), NH_4^+ (m/z 18; $k = 2.5 E^{-9}$; $b = 100\%$), and NH_3^+ (m/z 17; $k = 2.7 E^{-9}$; $b = 100\%$). The LOQ values for TMA, DMA, and NH_3 for this method were 10 μg/m^3, 1 μg/m^3, and 2 μg/m^3, respectively.

Statistical Analysis. The obtained equilibration constants (H') were subjected to Student's t-test to assess significant ($p < 0.05$) differences between H' values for water and for the shrimp matrix. The software used to explore statistical significances on the obtained results was S-Plus 8.0 for Windows.

RESULTS AND DISCUSSION

Carbon dioxide dissolved in a fishery product may cause a pH drop of the fishery product because of the formation of carbonic acid in the aqueous phase of the product (*10*). This is illustrated in **Figure 3**, indicating a pH decrease of gray shrimp, which is consistent with an increase of the concentration of carbon dioxide dissolved in the matrix. This pH decrease is dependent on the solubility of carbon dioxide in the product, the initial product pH, the pH buffering capacity of the fishery product, and the storage temperature. When opening the package, the dissolved carbon dioxide diffuses from the product into the atmosphere causing a subsequent increase of the product pH. This diffusion rate of carbon dioxide is temperature dependent. The pH evolution in gray shrimp after opening the MA packed samples, was monitored at an environment temperature of 4.0 °C and of 20 °C (results not shown). For samples in which 600 ppm carbon dioxide was dissolved, it took 2 to 6 h, depending on the temperature, until the initial product pH was reached.

The values of the headspace measurements of TMA, DMA, and NH3 for water and for the mixed shrimp as food matrix, obtained with SIFT-MS, are shown respectively in **Figure 4A, B**, and **C**. They depict the importance of pH on the volatilization of
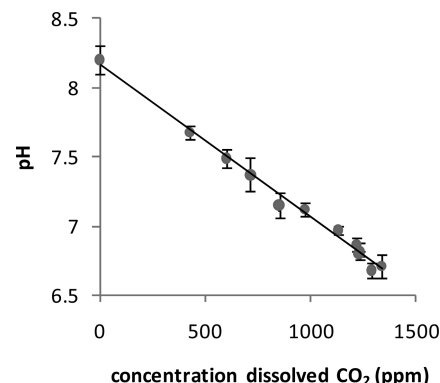
Figure 3. Effect of carbon dioxide dissolving in gray shrimp on the pH of gray shrimp.

TVB-N components. The higher the pH, the more the free bases are released and the more base is found in the headspace of the closed system. The measured pH ranges from approximately pH 6.0 to 9.0. An exponential increase was observed for the headspace concentrations with pH increasing. The introduced 5.0 mg TMA-N/50.0 g shrimp corresponds with a headspace TMA concentration of < LOQ at pH 6.1 and with 8126 μg/m^3 at pH 8.9. DMA headspace concentrations range from < LOQ at pH 6.3 to 111 μg/m^3 at pH 8.7, while NH_3 headspace concentrations range from < LOQ at pH 6.3 to 1142 μg/m^3 at pH 8.9. The natural content of the amines in the shrimp matrix was evaluated by headspace measurements of unspiked shrimp samples. This initial concentration was found to be < LOQ for TMA, < LOQ for DMA, and 11 μg/m^3 for NH_3 at a pH of 8.2. This initial concentration amounts < 0.51%, < 1.17%, and 3.06%, respectively, of the concentration of the amines in the spiked samples measured at the same pH. The data were fitted to exponential equations and the corresponding equation parameters are shown in **Table 1**. Since this relationship is described with an exponential function, one can state that small pH deviations may result in major headspace concentration deviations. Considering the effect of carbon dioxide, which is used as an antimicrobial agent in modified atmosphere packaging, on the product pH, large headspace concentration differences of the volatile bases can be expected inside the packaging, depending on the concentration of carbon dioxide dissolved in the product. Furthermore, after opening the package, the pH will increase again, and more bases will tend to volatilize.

Shrimp products are commonly preserved with sodium sulfites or potassium sulfites. But an often used alternative preservation technique for gray shrimps is the use of benzoic acid and sorbic acid in combination with citric acid, where pH is lowered to approximately 6.0. Also here, potential amines present in shrimp will most probably not engender off-odors due to the lowered pH.

The pK_a values for the bases at 4.0 °C were calculated using eq 4. Hereby, the temperature dependent deviations of the pK_a values appeared to be negligible. **Figure 5A, B**, and **C** describes the relationship between the measured headspace TMA, DMA, and NH_3 concentrations and the theoretical percentage free base B^{liquid} (%) calculated by means of eq 3 and resulted in a good linear correlation. The obtained correlation coefficients for TMA, DMA, and NH_3 were found to be 0.93, 0.98, and 0.97, respectively, for water as matrix and 0.96, 0.86, and 0.97, respectively, for mixed shrimp as food matrix. This indicates that the Henderson–Hasselbalch equation is also valid inside a matrix such as mixed shrimp or at least at the surface of that matrix. Volatile bases are mainly engendered due to microbiological activity on the surface of shrimp; therefore, one could assume that this simulation with mixed shrimp is valid for real shrimp as a
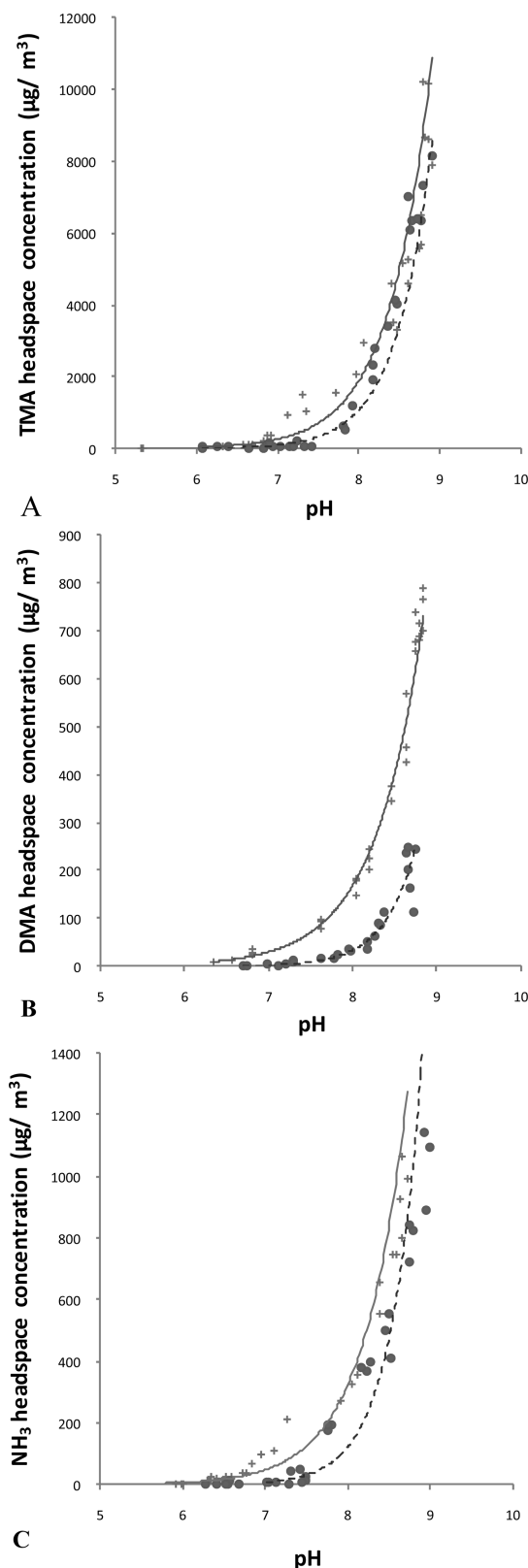
Figure 4. TMA (**A**), DMA (**B**), and NH$_3$ (**C**) headspace concentrations (μg/m^3) of 5.0 mg spiked N in 50.0 g water ($+$) and in 50.0 g mixed shrimp ($\bullet$), as a function of the pH.

Table 1. Exponential Equations with Corresponding Correlation Coefficients (R^2) Describing the pH Dependent Headspace Concentrations (μg/m^3) of TMA, DMA, and NH$_3$ after Spiking the Matrixes, Water and Gray Shrimp, with 5.0 mg N of the Respective Bases

	$[B^{\mathrm{gas}}]$ (water)	$[B^{\mathrm{gas}}]$ (*C. crangon*)
TMA	$3.0E^{-4}e^{1.9424\mathrm{pH}}$ ($R^2 = 0.97$)	$1.0E^{-5}e^{2.2941\mathrm{pH}}$ ($R^2 = 0.90$)
DMA	$2.0E^{-4}e^{1.7339\mathrm{pH}}$ ($R^2 = 0.99$)	$8.0E^{-8}e^{2.6717\mathrm{pH}}$ ($R^2 = 0.95$)
NH$_3$	$8.0E^{-5}e^{1.9039\mathrm{pH}}$ ($R^2 = 0.97$)	$5.0E^{-8}e^{2.69957\mathrm{pH}}$ ($R^2 = 0.89$)

On the basis of the principle of mass conservation, a mass balance can be considered in eq 5 for the closed system suggested in **Figure 1**, taking into consideration that for shrimps the liquid phase in **Figure 1** has been replaced by a food matrix. Implementing eqs 1 and 2 into this mass balance resulted in eq 6, describing the equilibrium between headspace concentrations of the bases and concentrations of bases retained by the matrix, with V^{gas} and V^{matrix} the headspace and the matrix volume, respectively, and with C a correction factor for B^{gas}. This correction factor C describes the ratio of the natural headspace concentration of the base present in the unspiked sample to the measured headspace concentration of the respective base in the spiked sample.

$$M^{\mathrm{tot}} = (1-C)[B^{\mathrm{gas}}] \cdot V^{\mathrm{gas}} + \frac{(1-C)[B^{\mathrm{gas}}]}{H'} \cdot V^{\mathrm{matrix}}$$
$$+ \frac{(1-C)[B^{\mathrm{gas}}]}{10^{(\mathrm{pH}-\mathrm{p}K_a)} \cdot H'} \cdot V^{\mathrm{matrix}} \qquad (6)$$

Here, H' should be considered as an equilibration coefficient of a volatile component in the food matrix, similar to the Henry coefficient, which is theoretically describing the solubility of volatile components in aqueous solutions. H' can be described by the following equation, derived from the latter equation (eq 6).

$$H' = \frac{V^{\mathrm{matrix}}\left(1 + \frac{1}{10^{(\mathrm{pH}-\mathrm{p}K_a)}}\right)}{\dfrac{M^{\mathrm{tot}}}{(1-C)[B^{\mathrm{gas}}]} - V^{\mathrm{gas}}} \qquad (7)$$

The H' values for TMA, DMA, and NH$_3$ were determined by means of eq 7 using the headspace measurements at a given pH and a temperature of 4.0 °C. The average H' was estimated on the basis of the headspace measurements, taking into account the limit of quantification of the SIFT-MS, which resulted in the dimensionless equilibration constants given in **Table 2**. The H' values appeared to be significantly higher for water as matrix than for shrimp.

The differences in slopes between the regressions for water or shrimp as matrix (**Figure 5A**, **B**, and **C**) can be attributed to a difference in equilibration constants H'. This effect is due to a difference in composition. According to Nubel, the Belgian food product nutrient webdatabase (www.internubel.be), gray shrimp (*Crangon crangon*) generally exists out of 79% water, 18.5% proteins, and 1.5% fat. The proteins and/or the fats most probably interact with the bases resulting in a lower availability of free bases (7, 8). The higher the H', the more free bases tend to volatilize, the lower H', the more the bases are restrained by the matrix. This H' is considered to be a constant at a fixed temperature. A temperature increase would result in a higher H'.

The slightly higher standard deviations of the average H' coefficients for TMA, DMA, and ammonia in the shrimp matrix are highly likely caused by product variability and the fact that this matrix is not completely homogeneous. The calculated H'
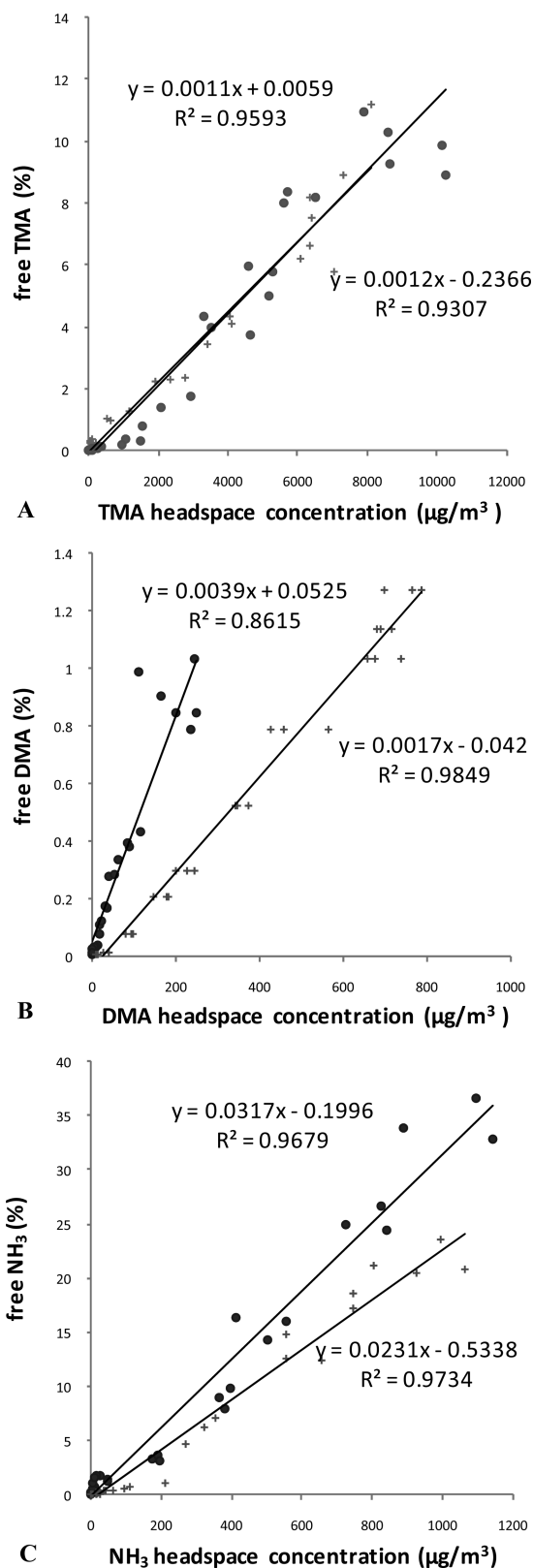
fishery product. As can be seen in **Figure 5A**, the correlation of TMA in water appears to be coinciding with the correlation in the shrimp matrix, though for DMA and NH$_3$ the slopes of the linear regressions for water were found to be different from the shrimp matrix (**Figure 5B** and **C**).

11868 *J. Agric. Food Chem.,* Vol. 58, No. 22, 2010

Noseda et al.

A

B

C

Figure 5. Obtained correlation between the measured headspace TMA (**A**), DMA (**B**), and NH$_3$ (**C**) concentrations measured in water (+) and in mixed shrimp (●) and the theoretical percentage free base (%) calculated by means of Eq3 (**Figure 2**).

value for TMA, DMA, and NH$_3$ in water are theoretically considered to be an estimation of the Henry coefficients of these amines at 4.0 °C which are found in literature. Sander reports Henry coefficients (4.0 °C) for DMA of 2.59×10^{-4} and

Table 2. Average Equilibration Constants H′ (Dimensionless) at a Temperature of 4.0 °C with Respective Standard Deviations for TMA, DMA, and NH$_3$ in Water and Mixed Shrimp As Food Matrix

	H′ (water)	H′ (C. crangon)
TMA	$3.83 \times 10^{-4} \pm 0.32 \times 10^{-4}$	$2.13 \times 10^{-4} \pm 0.38 \times 10^{-4}$
DMA	$1.99 \times 10^{-4} \pm 0.27 \times 10^{-4}$	$6.34 \times 10^{-5} \pm 1.71 \times 10^{-5}$
NH$_3$	$3.52 \times 10^{-5} \pm 0.24 \times 10^{-5}$	$2.58 \times 10^{-5} \pm 0.49 \times 10^{-5}$

4.77×10^{-4} and for ammonia of 2.26×10^{-4} and 8.88×10^{-4} (11). For TMA, no temperature correction factor was suggested. These coefficients confirm that the calculated H′ value for DMA is a good estimation of the Henry coefficient. For NH$_3$, the observed values are found to be lower. A possible reason for a part of this aberration is the interaction of the compounds with the packaging material. Roland and Hochkiss referred to this absorption phenomenon of the packaging material, which involves the process of partitioning and diffusion, as scalping (21,22). Sajilata et al. mentioned that polyethylene is a top scalper. The affinity of a volatile compound being absorbed by the packaging material is dependent on many factors such as molecular size, polarity, boiling point, surface area of the packaging material, temperature, pH product, polymer density, and polymer crystallinity (22).

The equilibration of the bases in a closed system explains the difficulties of determining limits (in mg N/100 g fish) for MAP fishery products. By looking at the effect of the fish matrix and the importance of the product pH, the complexity of the volatilization of the TVB-N fraction in spoiled fishery products becomes more clear. The Henderson−Hasselbalch equation and the obtained equilibration coefficients might help establishing new limits of acceptability in gray shrimp. The obtained results allow us to calculate the headspace concentration of the volatile bases inside a package, given the pH and the temperature of 4.0 °C, starting from the quantity of amines present in the product (mg N/100 g shrimp), and vice versa. According to Devos et al. (23), human olfactory thresholds for TMA, DMA, and NH$_3$ are found to be, respectively, $6 \mu g/m^3$, $151 \mu g/m^3$, and $4073 \mu g/m^3$. But these thresholds are determined by observers sniffing at a continuous airflow containing the concerning compound and not by opening a package with a certain concentration of this volatile compound. Moreover, these thresholds are developed for single compounds in an aqueous solution. These thresholds will be different in a mixture with other volatile compounds present, such as in a food product. Furthermore, there might be a difference in being olfactorily observed and causing off-odor, indicating that to cause an off-odor, higher concentrations are needed. More research with respect to determining sensorial odor thresholds for these volatile bases is needed. However, previous studies showed that 15 mg of TMA-N/100 g cod corresponds with a concentration of approximately $2.28 mg/m^3$ TMA in the headspace at a pH of 6.8 ± 0.2 and a temperature of 4.0 °C (2). Here, one could assume, to demonstrate the contribution of this research in this matter, that the headspace concentrations of these volatile bases is correlated to the odor of the product. The same headspace TMA concentration of $2.28 mg/m^3$ in the packed gray shrimp would correspond with 15.7 mg of TMA-N/100 g at pH 8.0, 49.4 mg of TMA-N/100 g at pH 7.5, and 155.5 mg of TMA-N/100 g at pH 7, on the basis of eq 6. These numbers illustrate the importance of pH again. Nevertheless, to establish limits of acceptability, next to odor, also other sensorial parameters such as taste, color, and texture should be taken into consideration, which certainly needs further investigation.

To conclude, this knowledge should be considered as a tool for the interpretation of TVB-N values in modified atmosphere packaged gray shrimp (*Crangon crangon*). The methodology and

the working approach allows the expansion of data on the same matter for other fishery products.

LITERATURE CITED

(1) Sivertsvik, M.; Jeksrud, W. K.; Rosnes, J. T. A review of modified atmosphere packaging of fish and fishery products: significance of microbial growth, activities and safety. *Int. J. Food Sci. Technol.* **2002**, *37*, 107−127.

(2) Noseda, B.; Ragaert, P.; Pauwels, D.; Anthierens, T.; Van Langenhove, H.; Dewulf, J.; Devlieghere, F. Validation of selective ion flow tube mass spectrometry for fast quantification of volatile bases produced on atlantic cod (*Gadus morhua*). *J. Agric. Food Chem.* **2010**, *58*, 5213−5219.

(3) Devlieghere, F.; Debevere, J.; Van Impe, J. Concentration of carbon dioxide in the water-phase as a parameter to model the effect of a modified atmosphere on microorganisms. *Int. J. Food Microbiol.* **1998**, *43*, 105−113.

(4) Devlieghere, F.; Debevere, J.; Van Impe, J. Effect of dissolved carbon dioxide and temperature on the growth of *Lactobacillus sake* in modified atmospheres. *Int. J. Food Microbiol.* **1998**, *41*, 231−238.

(5) Boskou, G.; Debevere, J. Reduction of trimethylamine oxide by *Shewanella* spp. under modified atmospheres in vitro. *Food Microbiol.* **1997**, *14*, 543−553.

(6) Pin, C.; de Fernando, G. D. G.; Ordonez, J. A. Effect of modified atmosphere composition on the metabolism of glucose by *Brochothrix thermosphacta*. *Appl. Environ. Microbiol.* **2002**, *68*, 4441−4447.

(7) Guichard, E. Interactions between flavor compounds and food ingredients and their influence on flavor perception. *Food Rev. Int.* **2002**, *18*, 49−70.

(8) Perez-Juan, M.; Flores, M.; Toldra, F. Effect of pork meat proteins on the binding of volatile compounds. *Food Chem.* **2008**, *108*, 1226−1233.

(9) Ho, C. S.; Shanahan, J. F. Carbon-dioxide transfer in bioreactors. *Crit. Rev. Biotechnol.* **1986**, *4*, 185−252.

(10) Sivertsvik, M.; Rosnes, J. T.; Jeksrud, W. K. Solubility and absorption rate of carbon dioxide into non-respiring foods. Part 2: Raw fish fillets. *J. Food Eng.* **2004**, *63*, 451−458.

(11) Sander, R. *Compilation of Henry's Law Constants for Inorganic and Organic Species of Potential Importance in Environmental Chemistry*; Max-Planck Institute of Chemistry: Mainz, Germany, 1999.

(12) Butler, J. N. *Ionic Equilibrium: Solubility and pH Calculations*; Wiley-Interscience: New York, 1998.

(13) Korner, S.; Das, S. K.; Veenstra, S.; Vermaat, J. E. The effect of pH variation at the ammonium/ammonia equilibrium in wastewater and its toxicity to *Lemna gibba. Aquat. Bot.* **2001**, *71*, 71−78.

(14) Issoire, J.; Vanlong, C. Etude de la thermodynamique chimique de la reaction de formation des methylamines. *Bull. Soc. Chim. Fr.* **1960**, 2004−2012.

(15) Zeng, Q. Z.; Thorarinsdottir, K. A.; Olafsdottir, G. Quality changes of shrimp (*Pandalus borealis*) stored under different cooling conditions. *J. Food Sci.* **2005**, *70*, S459−S466.

(16) ICMSF *Micro-organisms in Food 6*; Kluwer Academic/Plenum Publishers: New York, 2005.

(17) Goncalves, A. C.; Lopez-Caballero, M. E.; Nunes, M. L. Quality changes of deepwater pink shrimp (*Parapenaeus longirostris*) packed in modified atmosphere. *J. Food Sci.* **2003**, *68*, 2586−2590.

(18) Sivertsvik, M.; Jeksrud, W. K.; Vagane, A.; Rosnes, J. T. Solubility and absorption rate of carbon dioxide into non-respiring foods - Part 1: Development and validation of experimental apparatus using a manometric method. *J. Food Eng.* **2004**, *61*, 449−458.

(19) Böhme, D. K. Experimental studies with positive ion chemistry with flow-tube mass spectrometry:birth, evolution and achievements in the 20th century. *Int. J. Mass Spectrom.* **2000**, *200*, 97−136.

(20) Smith, D.; Spanel, P. Selected ion flow tube mass spectrometry (SIFT-MS) for on-line trace gas analysis. *Mass Spectrom. Rev.* **2005**, *24*, 661−700.

(21) Roland, A. M.; Hotchkiss, J. H. Determination of flavor polymer interactions by vacuum-microgravimetric method. *ACS Symp. Ser.* **1991**, *473*, 149−160.

(22) Sajilata, M. G.; Savitha, K.; Singhal, R. S.; Kanetkar, V. R. Scalping of flavors in packaged foods. *Compr. Rev. Food Sci. Food Saf.* **2007**, *6*, 17−35.

(23) Devos, M.; Patte, F.; Rouault, J.; Laffort, P.; Van Gemert, L. J. *Standardized Human Olfactory Thresholds*; Oxford University Press: New York, 1990.