Diffusion of Aroma Compounds in Stirred Yogurts with Different Complex Viscosities

ISABELLE DÉLÉRIS,* CLÉMENTINE LAUVERJAT, IOAN CRISTIAN TRÉLÉA, and ISABELLE SOUCHON

UMR 782 Génie et Microbiologie des Procédés Alimentaires, INRA-AgroParisTech BP 1, 1 avenue Lucien Brétiognières, 78850 Thiverval-Grignon, France

INTRODUCTION

During food consumption, flavor release from food matrices conditions the aroma compound availability in the oral and nasal cavities and participates in aroma perception. Flavor release and perception are complex processes in which physicochemical (interaction between the aroma compounds and the food components, partitioning, diffusion, interfacial mass transport), physiological (breathing, swallowing, salivation, and mastication), and perceptual phenomena may be involved (1). Since both thermodynamic and kinetic mechanisms control the release of stimuli, these two approaches are needed to obtain a complete overview of involved phenomena. The thermodynamic factor determines the partition of the volatile compounds between the food and the air phase under equilibrium conditions. The kinetic factor influences the rate at which the equilibrium is achieved and can be affected by resistances to mass transport (limitation of the diffusion within the food matrix and/or the release from the matrix to the gaseous phase, depending on the equilibrium properties). An improved understanding of the behavior of aroma compounds in complex multiphase media, in relation to the nature of the volatile compounds and the composition and the structure of the food product, is of great interest; beyond the scientific relevance, the management of food flavoring could be improved, notably for the development of new food products (lower fat or lower sugar formulations).

Recent studies dealing with strawberry aroma emphasized the role of product structure on aroma compound release and perception. In the case of dairy gels, products with the highest complex viscosity presented a lower amount of released aroma and were perceived as being less intense than products with the lowest complex viscosity (2, 3). When pectin or gelatin gels were considered, the firmest gels presented the highest amount of released aroma but were surprisingly perceived as being the least intense, which was justified by a lower release rate (4). Several hypotheses (physicochemical, sensory, and/or mechanical) were suggested, but the lack of some physicochemical properties such as diffusion coefficients limited the understanding of the origins of the observed differences in aroma release and perception.

To better understand aroma release in relation to yogurt structure and perception, the apparent diffusivity of aroma compounds within complex dairy gels was determined using an experimental diffusion cell. Apparent diffusion coefficients of four aroma compounds (diacetyl, ethyl acetate, ethyl hexanoate, and linalool) at 7 °C in yogurts (varying in composition and structure) ranged from 0.07 × 10⁻¹⁰ to 8.91 × 10⁻¹⁰ m² s⁻¹, depending on aroma compounds and on product structure. The strong effect of yogurt fat content on the apparent diffusivity of hydrophobic compounds was revealed (15-fold and 50-fold decreases in the apparent diffusion coefficient of linalool and ethyl hexanoate, respectively). Protein composition seemed to have a greater effect than that of mechanical treatment. However, variations in the apparent diffusion coefficient for the considered products remained limited and cannot completely explain differences in flavor release and in perception that were previously observed.

KEYWORDS: Yogurt; aroma release; diffusion; rheological properties; matrix structure; modeling
resonance, or fluorescence spectroscopy) (14), but they are often not adapted to characterizing aroma diffusivity in gelled matrices such as yogurts because of the product complexity in terms of composition and structure. A diffusion cell especially adapted to characterize aroma diffusivity in gelled matrices (0.05, 0.20, 0.50, and 2.00 mL). Matrices were flavored with compounds between the gaseous phase and the food product. It was defined as the ratio of the equilibrium concentrations of the aroma compounds in mixture were added under stirring conditions (2 min, at 2 °C). The milks were inoculated with L. delbrueckii subsp. bulgaricus (LB18 incorporated in 0.005% in milk) and Streptococcus thermophilus (ST17 and ST143 in 0.01%) provided by Chr. Hansen (Arpajon, France). Fermentation was stopped when the pH reached 4.6, and yogurts were pumped from the fermenter through a pipe (length: 1.5 m; diameter: 6 mm) and immediately stored at 4 °C (low level of mechanical treatment, MT−). The additional mechanical treatment (MT+) was performed the day after the fermentation by pumping the yogurts at 4 °C through the same type of pipe but ending with a conical tip (diameter: 0.8 mm; angle: 6°) at 4 °C. All details are specified by Saint-Eve et al. (2). Rheological properties, using a controlled-stress rheometer (Rheostress RS1, HAACKE, Germany), and pH were measured and used as controls to check the reproducibility of yogurt production (2). Measurements of complex viscosities were performed 7 and 15 days after yogurt production and demonstrated that the product structure was not modified over the diffusion measurement period (data not shown).

Since the fat content in food matrices is known to influence aroma retention and release (17), a commercial fat-free yogurt (Taillefine, Danone) was studied in comparison with yogurts containing 4.0% fat. Its composition, given by the producer, was 4.4 g of proteins, 5.0 g of carbohydrates, and 0.06 g of lipids (for 100 g of product). The complex viscosity at low shear stress (0.1 Pa) of this yogurt, determined experimentally at 10 °C, was 19.2 Pa·s.

Experimental Determination of Product/Headspace Partition Coefficients KPH. The product/headspace partition coefficient KPH was defined as the ratio of the equilibrium concentrations of the aroma compounds between the gaseous phase and the food product. It was determined using the phase ratio variation method (PRV) (18) with previously described operating conditions (2). Glass vials (22.4 mL, Chromacol, France) were filled with different volumes of flavored matrices (0.05, 0.20, 0.50, and 2.00 mL). Matrices wereavored with the four aroma compounds at 0.1% (w/w) each. For agar gels, aroma compounds in mixture were added under stirring conditions (2 min, at 50 °C), and glass vials were filled just before gelation. For yogurts, the flavoring step was performed with a food processor (Kenwood) under controlled conditions as described by Saint-Eve et al. (2).

Table 1. Main Physicochemical Characteristics of the Studied Aroma Compounds

<table>
<thead>
<tr>
<th>compound</th>
<th>molecular mass (g mol⁻¹)</th>
<th>hydrophobicity constant log P*a</th>
<th>air/water partition coefficient Kwater × 10⁻³ at infinite dilution (dimensionless, 25 °C)</th>
<th>saturated vapor pressure Psat, 25 °C (Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>diacetyl</td>
<td>86.09</td>
<td>-1.34</td>
<td>0.547b</td>
<td>7718.5</td>
</tr>
<tr>
<td>ethyl acetate</td>
<td>88.05</td>
<td>0.73</td>
<td>5.48b</td>
<td>12108.1</td>
</tr>
<tr>
<td>ethyl hexanoate</td>
<td>144.2</td>
<td>2.83</td>
<td>28.5e</td>
<td>225.1</td>
</tr>
<tr>
<td>linalool</td>
<td>154.2</td>
<td>2.97</td>
<td>0.879d</td>
<td>27.27</td>
</tr>
</tbody>
</table>

*a log P = logarithm of the ratio of the compound concentration in octanol and in water, calculated value (EPI, 2000, estimation Programs Interface V3.10; database).
*b Reference 31.
*c Reference 32.
*d Calculated on the basis of the Antoine equation.

Table 2. Characteristics of the Unflavored Stirred Yogurts Used in This Study (2) in Terms of Composition of the Protein Fraction, Level of Mechanical Treatment (−: Low; +: High) and Complex Viscosity η* Determined at Low Shear Stress of 0.1 Pa and at 10 °C; Six Yogurts Had the Same Total Composition (Dry Matter: 22.5%; Total Protein Content: 5.4%; Fat Content: 4.0%)

<table>
<thead>
<tr>
<th>composition (g /L of water)</th>
<th>matrices</th>
<th>milk powderb</th>
<th>soy bean caseinatesb</th>
<th>whey proteinsb</th>
<th>lactoseb</th>
<th>fatb</th>
<th>ducrose (Daddy)</th>
<th>level of mechanical treatment</th>
<th>complex viscosity η* (Pa·s) at 0.1 Pa</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAS− yogurt</td>
<td>100</td>
<td>14</td>
<td></td>
<td></td>
<td>43.2</td>
<td>58.9</td>
<td>−</td>
<td>−</td>
<td>100.4</td>
</tr>
<tr>
<td>CAS+ yogurt</td>
<td>100</td>
<td>14</td>
<td></td>
<td>21</td>
<td>43.2</td>
<td>58.9</td>
<td>+</td>
<td>39.1</td>
<td></td>
</tr>
<tr>
<td>MPO− yogurt</td>
<td>135</td>
<td></td>
<td></td>
<td></td>
<td>43.2</td>
<td>58.9</td>
<td>−</td>
<td>73.2</td>
<td></td>
</tr>
<tr>
<td>MPO+ yogurt</td>
<td>135</td>
<td></td>
<td></td>
<td></td>
<td>43.2</td>
<td>58.9</td>
<td>+</td>
<td>24.6</td>
<td></td>
</tr>
<tr>
<td>WP− yogurt</td>
<td>100</td>
<td>14</td>
<td></td>
<td>21</td>
<td>43.2</td>
<td>58.9</td>
<td>−</td>
<td>30.7</td>
<td></td>
</tr>
<tr>
<td>WP+ yogurt</td>
<td>100</td>
<td></td>
<td></td>
<td>21</td>
<td>43.2</td>
<td>58.9</td>
<td>+</td>
<td>19.5</td>
<td></td>
</tr>
</tbody>
</table>

*a Purchased by Ingredia, France. b Purchased by Lactalis, France.

**MATERIALS AND METHODS**

**Aroma Compounds.** Diacetyl, ethyl acetate, ethyl hexanoate, and linalool were provided by Aldrich (Germany). These four aroma compounds were chosen for their high contribution to strawberry aroma (2) and their reliable quantification by gas chromatography. As shown in Table 1, they presented a wide range of physicochemical properties, particularly in terms of volatility (Kwater) and hydrophobicity (log P).

**Gels.** As a reference experiment, diffusion measurements were first performed in an aqueous gel (water (Volvic, Danone, France), addition of 1% agar w/w (Merck, Germany). The use of the gelling material made it possible to avoid convection phenomena without inducing any diffusivity change in the entrapped solution (16).

Six unflavored stirred yogurts (dry matter: 22.5%; total protein content: 5.4%; fat content: 4.0%) presenting different complex viscosities η* were prepared by varying the milk protein composition (caseinate-enriched yogurt (CAS), milk powder-enriched yogurt (MPO), and whey protein-enriched yogurt (WP)) and/or the intensity of the mechanical treatment applied after fermentation (Table 2) (2). After the reconstitution of the milk base, the first step of yogurt manufacture was a two-stage homogenization (homogenizer APV1000, APV, France). A thermal treatment (92 °C for 5 min) was then applied. The fermentation was carried out in a 7 L fermenter (SGI, Toulouse, France), maintained at a constant temperature of 44 °C. The milks were inoculated with Lactobacillus delbrueckii ssp. bulgaricus (LB18 incorporated in 0.005% in milk) and Streptococcus thermophilus (ST17 and ST143 in 0.01%) provided by Chr. Hansen (Arpajon, France). Fermentation was stopped when the pH reached 4.6, and yogurts were made it possible to avoid convection phenomena without inducing any diffusivity changes in the entrapped solution.
measured using Hewlett-Packard Chemstation integration software. A nonlinear regression was applied in order to accurately determine the partition coefficients (19). All experiments were performed in triplicate to validate the repeatability of the measurements. Results, summarized in Table 3, are in accordance with data available in the literature (2).

Diffusion Cell. The system was composed of two main gaseous compartments, separated by the food product being studied (15). The bottom compartment ($V_b = 0.78$ L) constituted the “aroma tank”; ~10 mL of liquid aroma compounds (mixture of pure aroma compounds) ensured a constant gaseous concentration $C_{bi}$ throughout the whole experiment. The food product was supported by a thin hydrophobic porous membrane (polypropylene, porosity: 55%; thickness: 25 μm). The upper gaseous compartment corresponded to the sampling zone (headspace volume $V_h = 0.90$ L).

The diffusion cell was closed and placed in a temperature-controlled vessel after a known weight of product was deposited on the membrane. The product height $h_p$ was $5 \times 10^{-3}$ m for dairy gels and $2 \times 10^{-2}$ m when aqueous gels were studied. The experiment started with the introduction of aroma compounds in the bottom part of the apparatus with a 50 mL syringe ($t_0$) and lasted about 300 h. Aroma compounds moved from the gaseous phase of the lower compartment diffused through the food product and were finally released in the gaseous phase of the sampling compartment. These release kinetics were monitored by a daily sampling of 2.0 mL (Hamilton gastight syringe, type 10025L, 2.5 mL) and gas chromatography analysis. (Analysis conditions were the same as the ones used for the PRV method and described in the previous section.) At least two replicate experiments were performed for each product. The bottom gaseous phase was also sampled to check the rapid establishment of the gaseous concentration $C_{bi}$ (equilibrium value was reached less than 1 h after the beginning of the assay) and its constant value throughout the experiment (data not shown).

**Determination of the Apparent Diffusion Coefficient.** Similarly to the mechanistic approach used by Juteau et al. (20), a mass transfer analysis was performed within each compartment of the experimental system, as described by Deléris et al. (15). The main assumption was a limiting diffusive mass transfer of aroma compounds within the product layer. The gaseous phases were considered as uniform, and a convective mass transfer was assumed. Transport was considered as one-dimensional along the vertical axis and uniform on the cross section $A$. Assuming local thermodynamic equilibrium at the interfaces and mass flux conservation through the interfaces at any time, mass balances on each phase were performed, leading to a mass transfer model. A summary of the main model equations used to describe mass transfer in the diffusion cell (diffusion into the product and mass transfer toward the upper gaseous phase) is presented in the Appendix. The apparent diffusion coefficient $D_a$ was determined by numerically fitting the mechanistic model to the experimental release data using the Levenberg–Marquardt algorithm (least-squares curve fitting). Numeric calculations were performed using MatLab 7 software (The Mathworks, MA) and the associated statistical toolbox. Confidence intervals were determined to evaluate the accuracy of the estimated diffusion coefficients.

### RESULTS

**Diffusion in Aqueous Agar Gels.** Diffusion coefficients within 1% agar gel at 7 °C were $6.10 \times 10^{-10}$, $7.24 \times 10^{-10}$, $3.60 \times 10^{-10}$, and $2.84 \times 10^{-10}$ m$^2$ s$^{-1}$ for diacetyl, ethyl acetate, ethyl hexanoate, and linalool, respectively (Table 4). The suitability of our system to accurately determine diffusion parameters was confirmed by comparing the experimental values with the ones calculated from the Wilke and Chang equation (21). Although it is empirical, this equation was assumed to give a correct estimation of the diffusion properties of molecules (22, 23).

The impact of the physicochemical characteristics of the aroma compounds on their diffusivity properties was revealed: diacetyl and ethyl acetate presented diffusion coefficient values that were twice as high as the ones of ethyl hexanoate and linalool (Table 4). As expressed in the Wilke and Chang equation, the size and the molecular weight of the molecules contribute to these differences. But other phenomena, as specific interaction between aroma compounds and the other constituents of the product, might also contribute to apparent diffusivity.

**Influence of the Matrix Composition.** Fat Content. Concerning the diffusivity of aroma compounds in fat-free yogurt, apparent diffusion coefficients ranged from $1.03 \times 10^{-10}$ to $8.91 \times 10^{-10}$ m$^2$ s$^{-1}$, depending on the nature of the molecule (Figure 1). It is interesting to observe that apparent diffusion coefficients in fat-free yogurt were quite close to those obtained in the 1% agar gel at the same temperature, regardless of the aroma compound. The presence of others constituents in these complex food matrices (proteins, lactose, etc.) had an effect on the product/headspace partition coefficients (Table 3), as already reported in the literature (24–26) but not on the apparent diffusivity of the aroma compounds.

The presence of 4% fat in yogurt affects the apparent diffusion coefficients of the four aroma compounds (Figure 1, $D_a$ ranging from $0.071 \times 10^{-10}$ to $5.17 \times 10^{-10}$ m$^2$ s$^{-1}$). Depending on the aroma compounds, this effect was more or less pronounced and varied from a 2-fold decrease for the most hydrophilic and smallest molecules (diacetyl and ethyl acetate) to a 15-fold decrease for linalool and to about a 50-fold decrease for ethyl hexanoate.

Release kinetics of ethyl hexanoate and diacetyl from fat-free yogurt or 4% fat yogurt are illustrated in Figure 2. The normalized concentrations (ratio between the gaseous concentrations in the upper and in the bottom compartments) were represented to facilitate the comparison between the assays (concentration gradient dependent on the amount of liquid aroma compounds and proper to each experiment).

In the case of diacetyl, a 2-fold reduction in the value of the apparent diffusion coefficient can be observed when fat is added.
(\(D_{\text{PF}} = 7.12 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}\) in fat-free yogurt and \(D_{\text{PF}} = 1.51 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}\) in WP+ yogurt) (**Figure 2a**). However, no significant difference was observed on the release kinetics, either on the initial rate or on the time necessary to reach thermodynamic equilibrium (90 h), due to the chosen representation mode (relative concentrations). Similar results were obtained for ethyl acetate, with a 3-fold decrease in the apparent diffusion coefficient when fat was present without any modification of the initial release rate (data not shown). This result clearly revealed the interest of the modeling approach to correctly evaluate the effect of product modifications on diffusion properties.

On the contrary, for ethyl hexanoate (**Figure 2b**), fat addition resulted in a 40-fold decrease in the apparent diffusion coefficient of ethyl hexanoate, from 3.46 \(\times 10^{-10} \text{ m}^2 \text{ s}^{-1}\) to 0.085 \(\times 10^{-10} \text{ m}^2 \text{ s}^{-1}\) (for CAS+ yogurt). Similar behavior was obtained for linalool, even if the impact of fat addition on the apparent diffusion coefficient of this molecule was less pronounced (12-fold decrease). The presence of fat induced a considerable slowdown of the initial part of the release curve and delayed equilibrium from being reached, emphasizing the retention effect of fat (**Figure 2b**). Longer experiment times were not conceivable because of yogurt postacidification, which modified the complex viscosity after 15 days. We made sure that an accurate determination of the diffusion coefficient was not prevented in the event that equilibrium was not reached. Since hydrophobic compounds are preferentially located in the lipid phase, these results suggested that 4.0% fat was sufficient to act as a reservoir for these molecules. This mechanism could explain the observed differences in the time lag and in the release kinetics of aroma compounds from products with different fat contents in the mouth and notably the prolonged release of lipophilic compounds in high-fat yogurts (27) or high-fat emulsions (28). Vitrac and Hayert applied the principles of statistical physics to improve the understanding of diffusion mechanisms in biphasic systems (29); in simulated or digitized emulsions, they evaluated the impact of local physicochemical properties (partition coefficient between a continuous and a dispersed phase, local diffusion coefficients in each phase) on the diffusion path of small molecules and on the effective diffusion coefficient. The possible confinement of molecules within fat globules in relation to their physicochemical properties was revealed. The diffusivity of small molecules at macroscopic scale in multiphase products depends on their local diffusion properties (at microscopic scale) in relation to food composition and structure and to their physicochemical properties (17).

**Protein Content.** The protein effect on aroma diffusivity was evaluated by comparing caseinate-enriched yogurt (CAS), milk powder-enriched yogurt (MPO), and whey protein-enriched yogurt (WP) for a similar mechanical treatment (**Figure 1**). For both a low (TM−) or a high (TM+) level of mechanical treatment, apparent diffusion coefficients for all aroma compounds were higher in the MPO yogurt in comparison with the two others products (from a 20% to 43% difference, depending...
on the aroma compounds) (Figure 1). CAS yogurt and WP yogurt presented similar diffusion properties. We can notice that these diffusion results were not correlated with complex viscosities determined at 0.1 Pa (Table 2). Microstructure study (by scanning electron microscopy (2)) highlighted differences in the organization of the gel network between the three products: the CAS yogurt presented a heterogeneous structure with large pores, the WP yogurt a more uniform distribution of pore size in the gel, and the MPO yogurt an intermediate structure. However, even if the protein content had an impact on the rheological properties of the product and on its structure, the effect on aroma diffusion remained globally limited.

Influence of the Mechanical Treatment. The variation of the intensity of the applied mechanical treatment was a way to evaluate structure effects independently from the composition (comparison between MT− and MT+ yogurts for a similar composition, Figure 1). Concerning the CAS yogurt, the level of the mechanical treatment did not significantly modify the values of the apparent diffusion coefficients except in the case of ethyl hexanoate, for which a higher mechanical treatment induced a 15% decrease in the diffusion properties. The impact of the mechanical treatment on aroma diffusivity also remained limited in the case of MPO yogurt, except for linalool (17% decrease), despite a high breakdown of the product structure (3-fold decrease in the complex viscosity at 0.1 Pa, Table 2). For WP yogurt, apparent diffusion coefficients of all aroma compounds were always higher for a low mechanical treatment (variations between 7% and 30%, depending on the aroma compounds). Despite rheological modifications induced by the mechanical treatment (Table 2), no direct correlation between product structure and aroma diffusion was observed. Moreover, when an effect was measured, it was the opposite of what could be expected in the first place: higher diffusion properties were obtained in the most structured gel (i.e., MT− yogurts). Although they are surprising, these results were in agreement with data found in the literature (30), which investigated aroma compound self-diffusion by NMR measurements in carrageenan gels with different structures. The 15% increase in the diffusion coefficients with highest gel strength was explained by a decrease of the obstruction effect with a better-structured product.

RELATION TO SENSORY STUDIES

Previous studies in our laboratory highlighted the influence of yogurt structure on both in vivo aroma release and perception during consumption; for the same matrix composition, aroma release and intensity of olfactory perception were higher with less viscous yogurts than with more viscous yogurts (3). The impact of the mechanical treatment was higher than the composition effect (2). Nevertheless, CAS yogurt presented higher aroma retention under static conditions and was perceived as being less intense for a majority of olfactory notes than the other yogurts. The authors proposed several hypotheses to better explain the impact of the product microstructure on the release of aroma compounds: independently from sensory interactions that could occur, such as texture–aroma interaction, some modifications of physicochemical parameters were assumed. In these studies, experimental investigations enabled the effects of product structure on partition properties between yogurt and the gaseous phase or on local mass transfer properties in the product to be refuted. But the effect of the product structure on aroma diffusivity could not be verified without an appropriate experimental system. In the present study, the apparent diffusivity of aroma compounds within these dairy gels could be characterized thanks to the diffusion cell: even if some differences were observed between the six yogurts, the impact of the product structure on aroma diffusivity was found to be too weak to explain the differences in aroma release and perception between products. Simulations performed with the mechanistic model demonstrated that a ±20% variation in the contact surface between the yogurt and the gaseous phase had twice as much impact on the release kinetics as a ±20% variation in the diffusion coefficients. All these results indicated the preponderant role that the air/product contact area generated in the mouth could have on mass transfer, as already suggested by several authors (2, 4, 33).

APPENDIX

Mass Transfer Modeling in the Product. Molecular diffusive transport was assumed within the food product (eq 1), characterized by an apparent diffusion coefficient of the aroma compound in the product ($D_P$) on the basis of Fick’s second law:

$$ D_P = \frac{D}{1 + \frac{D_{\text{gas}}}{D}} $$
At the product/headspace interface, mass conservation was written as

\[
AD_p \frac{\partial C_p(x,t)}{\partial x} = \frac{\partial^2 C_p(x,t)}{\partial x^2} \quad (1)
\]

Partition at the Product/Headspace Interface. The interfacial balance was characterized by the product/headspace partition coefficient \( K_{PH} \), defined as the ratio between the aroma concentrations on either side of the interface (eq 3):

\[
K_{PH} = \frac{C_{PH}^g(t)}{C_{PH}^m(t)} \quad (3)
\]

To solve the partial differential equations, the product was split into \( n \) layers (discretization using the finite volume method).

Mass Transfer Modeling in the Sampling Compartment. Convective mass transport, characterized by a mass transfer coefficient \( k_h \), was assumed in the sampling compartment (eq 4):

\[
V_h \frac{dC_h^m(t)}{dt} = Ak_{PH} \left[ C_{PH}^m(t) - C_{PH}^g(t) \right] \quad (4)
\]

The whole model was obtained by establishing similar equations to describe mass transfer in the other compartments (the bottom gaseous compartment and the membrane) and through interfaces. The assumption that mass transfer within the membrane was not a limiting step was checked.

LIST OF SYMBOLS

- \( A \) : gas–product contact area (m²);
- \( C_{PH}^g(t) \) : volatile concentration in the upper gaseous compartment (kg•m⁻³);
- \( C_{PH}^m(t) \) : volatile concentration in the upper gaseous compartment at the product/headspace interface (kg•m⁻³);
- \( C_p(x,t) \) : volatile concentration in the product (kg•m⁻³);
- \( D_p \) : apparent diffusivity of aroma compound in the product (m²•s⁻¹);
- \( k_h \) : gas mass transfer coefficient (m•s⁻¹);
- \( h_n \) : membrane height (m);
- \( h_v \) : volume of the upper gaseous compartment (m³);
- \( V_h \) : volume of the upper gaseous compartment (m³);
- \( x \) : vertical position (m).

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