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Validation of a predictive model coupling gas transfer and microbial growth in fresh food packed under modified atmosphere

V. Guillard [a, *], O. Couvert [b], V. Stahl [c], A. Hanin [d], C. Denis [d], V. Huchet [e], E. Chaix [a], C. Loriot [f], T. Vincelot [f], D. Thuault [e]

[a] UMR IATE University of Montpellier, 2 place Pierre Viala, F-34060, Montpellier Cedex, France
[b] LUBEM, 2 Rue de l'Université, F-29334, Quimper, France
[c] Aérial, 250 rue Laurent Fries, F67412, Illkirch, France
[d] ACTALIA, Bd du 13 juin 1944 – BP2, 14310, Villers-bocage, France
[e] ADRIA Développement, Z.A. Creac'h Gwen, F29196, Quimper Cedex, France
[f] LNE, 1, rue Gaston Boissier, 75724, Paris Cedex 15, France

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ABSTRACT

Predicting microbial safety of fresh products in modified atmosphere packaging implies to take into account the dynamic of O_2, CO_2 and N_2 exchanges in the system and its effect on microbial growth. In this paper a mechanistic model coupling gas transfer and predictive microbiology was validated using dedicated challenge-tests performed on poultry meat, fresh salmon and processed cheese, inoculated with either *Listeria monocytogenes* or *Pseudomonas fluorescens* and packed in commercially used packaging materials (tray + lid films). The model succeeded in predicting the relative variation of O_2, CO_2 and N_2 partial pressure in headspace and the growth of the studied microorganisms without any parameter identification. This work highlighted that the respiration of the targeted microorganism itself and/or that of the naturally present microflora could not be neglected in most of the cases, and could, in the particular case of aerobic microbes contribute to limit the growth by removing all residual O_2 in the package. This work also confirmed the low sensitivity of *L. monocytogenes* toward CO_2 while that of *P. fluorescens* permitted to efficiently prevent its growth by choosing the right combination of packaging gas permeability value and initial % of CO_2 initially flushed in the pack.

1. Introduction

Packaging protects foodstuffs from contact with the external environment and from air-borne contamination. Combined with modified atmosphere, packaging also helps to prolong the shelf life of a food product and can also contribute to improve food safety. The gaseous environment in a package can slow down or totally inhibit microbial development and can also modify the microbial ecology of the product (Chaix et al., 2015b; Gill and Ahvenainen, 2003; Mcmillin, 2008). Modified Atmosphere Packaging (MAP) of fresh product relies on the interplay of two mechanisms: (1) modification of the atmosphere inside the package in order to reduce degradation reaction (microbial growth, oxidation, etc.) and (2) mass transfer of gases through the packaging (Floros and Matsos, 2005). The main objective of the packaging material is thus to maintain this atmosphere during food storage and therefore high barrier films are usually used by default (precautionary principle) to ensure this objective without knowing *a priori*, the specific needs of the product as regard O_2 and/or CO_2 headspace composition and its tolerance towards variations in this gas composition (Šcetar et al., 2010). As a consequence, it may be possible that, for many foods, such high barrier properties are oversized, sometimes unnecessary or even inappropriate (i.e. the material is too powerful compare to the needs of the product). Barrier films are often multilayered and consequently expensive materials that are currently not recyclable. MAP thus represents high direct costs for the food industry.

In this context there is an increasing demand for modelling tools that would permit to identify in advance the headspace gas composition and associate material suitable for ensuring microbial safety and/or anticipate microbial degradation of a given application. Such modelling tools rely on the modelling of mass transfer in

* Corresponding author.
E-mail address: valerie.guillard@umontpellier.fr (V. Guillard).

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Symbols, acronyms

A	Surface m^2
C	Mass concentration (within gas or condensed phase) kg m^{-3}
D	Apparent diffusivity m^2 s^{-1}
E_a	Activation energy in Arrhenius law for calculation of k (Eq. (22)) J $mole^{-1}$
e	Thickness m
k	General symbol to designate indifferently solubility coefficient k_H, apparent diffusivity D or gas permeability Pe (Eq. (22))
k_H	Solubility coefficient (according to Henry's law) mol Pa^{-1} m^{-3}
K_m	Mickaëlis–Menten constant in Eq. (4) Pa
lag	Lag time for microbial growth h or s
m	Mass of food g or kg
M	Molar mass kg mol^{-1}
N	Microorganism population CFU g^{-1}
$\overline{N}_t$	Average microorganism population at time t in Eq. (4) CFU g^{-1}
p	Partial pressure Pa
P_T	Total pressure in the headspace (assumed constant) Pa
Pe	Permeability of a gas species through the lid film mol m^{-1} s^{-1} Pa^{-1}
$r_{O_2\ max}$	Maximum respiration rate in Eq. (4) kg s^{-1} CFU^{-1}
R	Ideal gas constant = 8.314 J mol^{-1} K^{-1}
S	Net mass production rate of gas species by microbial activity kg s^{-1}
t	Time s
T	Temperature of the food/packaging system K
T_{ref}	Reference temperature in Arrhenius law for calculation of k (Eq. (22)) K
V	Volume m^3
x	Spatial coordinate m
$\%_{CO_2\ max}$	Maximal % of CO_2 in headspace for growth %
$\%_{O_2\ min}$	Minimal % of O_2 in headspace for growth %

Greek letters

γ	Adimensional weighing function representing the influence of environmental parameters over microbial growth rate -
μ	Growth rate for microbial growth s^{-1} or h^{-1}
ξ	Adimensional weighing function (?) representing the influence of the interactions between environmental parameters over microbial growth rate -
φ	Mass flow kg s^{-1}
ϕ	Interaction terms representing the influence of environmental parameters over ξ parameter -

Subscripts, superscripts

∞	Relative to the surrounding atmosphere
F	Food sample within the food/packaging system
HS	Headspace volume within the food/packaging system
I	Interface between headspace volume and food or in the headspace at the immediate vicinity of the food surface (for equilibrium partial pressure calculation)
j	Gas species: O_2, CO_2 or N_2
L	Lid film
min	Minimal
max	Maximum
opt	Optimal
W	Environmental parameters influencing microbial growth rate namely temperature, water activity, pH and O_2 and CO_2 concentration

the food/packaging system and their coupling with models of predictive microbiology (Chaix et al., 2015b). Such semi-mechanistic model would permit to take into account in addition to constant environmental factors such as temperature, pH, aw, acids, the impact of dynamic of O_2/CO_2 exchanges in the system on the microbial growth. In spite of their interest, very few modelling tools combined mass transfer with predictive microbiology. One may cite the works of Devlieghere et al. (2001, 1998) and that of Mejlholm and Dalgaard (2007) that took into account the impact of CO_2 on bacteria growth but this concentration was considered constant. Only Simpson et al. (2003) modelled the permeation of gas through the packaging material and its impact on headspace partial pressure on bacteria growth and more recently Chaix et al. (2015a) considered in their mathematical model, in addition to permeation, the dissolution/diffusion of O_2/CO_2 into the food itself and its impact on local bacterial growth. From a practical point of view, such numerical tools would decrease the number of time consuming experiments necessary for experimentally quantify and assess the evolution of a given microorganism in terms of changes in O_2 and CO_2 headspace partial pressures and corresponding dissolved content in the food.

CO_2 has an inhibitory effect and it is the most important component in the choice of a gas mixture. Its effect on several bacteria species is well known and has been evaluated, at least quantitatively by several groups of co-workers. For example, Gram-negative microorganisms such as *Pseudomonas, Shewanella* and *Aeromonas* are very sensitive to CO_2 (Boskou and Debevere, 1997; Debevere et al., 2001; Molin, 1983). Gram-positive bacteria show less sensitivity and lactic acid bacteria are resistant to or even stimulated by CO_2 (Arsène-Ploetze et al., 2006). Pathogens such as *Clostridium perfringens*, *C. botulinum* and *Listeria monocytogenes* are minimally affected by CO_2: the Minimal Inhibitory Concentration – for *L. monocytogenes* even exceeds 100% expressed in equivalent headspace partial pressure (Augustin et al., 2005), that means that more than 1 bar of CO_2 pressure would be requested in the pack to inhibit *Listeria* growth which is not possible to achieve in practice. In predictive microbiology, secondary models have been developed to describe the effect of CO_2. They were listed and discussed in Chaix et al. (2015b). We can cite for example the work of Koutsoumanis et al. (2000) that proposed a model for taking into account the combined effect of temperature and CO_2 on spoilage microflora growth, the work of Alfaro et al. (2013a) that proposed a Cardinal Type Model (CM) to predict the effect of both environmental gases O_2 and CO_2 on the growth of a mixture of spoilage bacteria or the work of Emborg and Dalgaard (2008) that modelled effect of CO_2 on *Morganella psychrotolerans* growth by using a CM. In all these studies, except in the work of Alfaro et al. (2013a), the concentration of CO_2 was considered constant during storage.

Contrary to CO_2, O_2 is an activating agent for aerobes growth. The role of O_2 on microbial growth has been scarcely considered and modelled in predictive microbiology. Only 4 modelling attempts considered the impact of O_2 on microbial growth (Alfaro et al., 2013a; Farber et al., 1996; Geysen et al., 2006; Pin et al., 2000). The impact of O_2 is nevertheless indispensable to consider for modelling the growth of aerobes such as *Pseudomonas*.

The objective of this paper was to validate in real conditions