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On the selection of relevant environmental factors to predict microbial dynamics in solidified media

T.E. Theys [a], A.H. Geeraerd [b], F. Devlieghere [c], J.F. Van Impe [a,*]

[a] Chemical and Biochemical Process Technology and Control Section (BioTeC), Department of Chemical Engineering, Katholieke Universiteit Leuven, Belgium
[b] Division of Mechatronics, Biostatistics and Sensors (MeBioS), Department of Biosystems (BIOSYST), Katholieke Universiteit Leuven, Belgium
[c] Laboratory of Food Microbiology and Food Preservation, Department of Food Safety and Food Quality, Ghent University, Belgium

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ABSTRACT

Several studies have shown that food structure causes slower growth rates and narrower growth boundaries of bacteria compared to laboratory media. In predictive microbiology, both a_w or corresponding solute concentration (mainly NaCl) have been used as a growth influencing factor for kinetic models or growth/no growth interface models. The majority of these models have been based on data generated in liquid broth media with NaCl as the predominant a_w influencing solute. However, in complex food systems, other a_w influencing components might be present, next to NaCl. In this study, the growth rate of *Salmonella typhimurium* was studied in the growth region and the growth/no growth response was tested in Tryptic Soy Broth at 20 °C at varying gelatin concentration (0, 10, 50 g L^{-1} gelatin), *pH* (3.25–5.5) and water activity (a_w) (0.929–0.996). From the viewpoint of water activity, the results suggest that NaCl is the main a_w affecting compound. However, gelatin seemed to have an effect on medium a_w too. Moreover, there is also an interaction effect between NaCl and gelatin. From the microbial viewpoint, the results confirmed that the a_w decreasing effect of gelatin is less harmful to cells than the effect of Na^+ ions. The unexpected shift of the growth/no growth interface to more severe conditions when going from a liquid medium to a medium with 10 g L^{-1} gelatin is more pronounced when formulating the models in terms of a_w than in terms of NaCl concentrations. At 50 g L^{-1} gelatin, the model factored with NaCl concentration shifts to milder conditions (concordant to literature results) while the model with a_w indicates a further shift to more severe conditions, which is due to the water activity lowering effect of gelatin and the interaction between gelatin and NaCl. The results suggest that solute concentration should be used instead of a_w, both for kinetic models in the growth region and for growth/no growth interface models, if the transferability of models to solid foods is to be increased.

1. Introduction

Water activity (a_w) is a commonly used factor in food preservation and is a measure for the availability of water (for microorganisms). However, water activity can be changed in different ways. A first – often used – method in food engineering to decrease a_w is by adding solutes. Ionic solutes like NaCl or KCl seem to be more inhibitory than non-ionic solutes like sugars and glycerol (Van den Berg and Bruin, 1981). Buchanan and Bagi (1997) tested the effect of water activity and humectant identity of mannitol, sorbitol and sucrose and compared the results with a response surface model developed for NaCl. At higher a_w values, reasonable estimates for all four humectants were provided by the model. However, at values close to the minimum a_w differences among the solutes were observed and the model seemed to be less accurate. The different solutes have different effects on the physical characteristics of the environment (e.g., viscosity, oxygen solubility) on the one hand. On the other hand, the compatibility of the solute with the microorganism's metabolic systems (i.e., the ability of the cell's enzymes to continue to function at high solute concentrations) can differ.

A second method to decrease a_w is by lowering the moisture content of the food product. Lebert et al. (2004) studied differences in effect on the growth of *Listeria innocua* of decreasing a_w (*i*) by adding solutes (NaCl, KCl, glucose, sucrose and glycerol) to broth, (*ii*) by adding NaCl to gelatin, or (*iii*) by removal of water from the gelatin system. In broth all solutes seemed to have the same inhibitory effect on the growth rate, except for glycerol where a faster growth rate was observed. When broth and gelatin systems were compared, they concluded that for an a_w of 0.95, growth rate was faster in broth with NaCl, followed by gelatin with NaCl and

* Corresponding author.
E-mail address: Jan.Vanimpe@cit.kuleuven.be (J.F. Van Impe).

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finally growth rate seemed to be much slower on gelatin where a_w was adjusted by removing water. So, removal of water seemed to be more stressful to *L. innocua* than adding NaCl.

Predictive models developed in the past used a_w or the corresponding solute concentration as the primary factor affecting microbial growth. Examples of secondary models based on solute concentration (mainly NaCl concentration) are Sutherland and Bayliss (1994), McClure et al. (1997), Membré et al. (1999), Olmez and Aran (2005) or Park et al. (2005) and models based on a_w are described in Augustin and Carlier (2000), Devlieghere et al. (2000), Salter et al. (2000), Wijtzes et al. (2001) or Ross et al. (2003). Also for probabilistic models, solute concentration (López et al., 2007) or a_w (Presser et al., 1998; Salter et al., 2000; Tienungoon et al., 2000; Lanciotti et al., 2001; Koutsoumanis et al., 2004b) have been used. In some studies (Augustin and Carlier, 2000; Augustin et al., 2005), a mathematical formula was used to recalculate NaCl concentrations to a_w values.

Despite the solute-specific differences observed, a_w seems to be used more commonly than solute concentration in predictive models, mainly because the minimum a_w allowing microbial growth is independent of product specifications. So, the way the product a_w is adjusted can differ and in some cases, like with some solutes, no growth will be possible even though the a_w is higher than the minimum a_w allowing growth. In most studies, NaCl is chosen as the solute to adjust a_w and for most food products, this is indeed the most important a_w influencing component (Buchanan and Bagi, 1997). However, the authors claimed that users should be aware of the additional toxic effect of sodium ions besides the a_w decreasing effect when they use this solute to adjust the a_w.

In liquids, microorganisms grow planktonically. However, many foods are not liquid but constitute an environment that is heterogenous in physical structure and chemical composition (Fleet, 1999). The structured matrix causes that microorganisms are immobilized and colonies are formed. This constrained colony growth causes an additional stress resulting in lower growth rates and has been reported to result in narrower boundaries of the habitat domain (Brocklehurst et al., 1997; Wilson et al., 2002). In microbiology, gelatin and agar are the most important agents to induce structure. Agar is a polysaccharide which is obtained from seaweed, while gelatin is a fibrous protein derived from skin or bone collagen (Imeson, 1997). Both gelling agents have advantages and disadvantages. Gelatin has a low melting point (between 25 and 37 °C) which enables inoculation and sampling in a molten medium at relative low temperatures, but which makes studies at different temperatures difficult because gel strength will not be constant. Agar, on the other hand, solidifies at 45 °C and melts at 80 °C which are temperatures that can be stressful for bacteria, but its gel strength is less influenced by temperature. Koutsoumanis et al. (2004a) studied the growth limits of *Listeria monocytogenes* as influenced by temperature, *pH* and a_w while growing planktonically in broth or on a solid agar surface and concluded that the growth boundary of *L. monocytogenes* shifted to less stressful conditions on agar compared to liquid broth. Also Meldrum et al. (2003) came to the same conclusions that under certain *pH*, a_w (a_w ajusted with sucrose) combinations no growth of *L. monocytogenes* was possible in broth supplemented with gelatin while growth occurred when no gelatin was added.

In previous research we studied the effect of *pH* and a_w on the growth kinetics of *S. typhimurium* in TSB at 0, 10 and 50 g L^{-1} gelatin at 20 °C (Theys et al., 2008). We observed that lowering *pH* or a_w caused both in broth and in gelatinized medium a decrease of growth rate. In TSB with gelatin a reduction of growth rate was observed compared to broth at all conditions. However, the effects of decreasing *pH* and a_w were less pronounced in TSB with gelatin. Because only growth conditions were tested, no definitive conclusions could be made about a possible shift of the growth boundary in TSB with 50 g L^{-1} gelatin compared to liquid TSB. In this study, the growth boundaries of *S. typhimurium* in TSB with 0, 10 and 50 g L^{-1} gelatin was investigated as a function of *pH* and a_w, which were adjusted by HCl and NaCl respectively. The overall objective of this study was to reflect on the formulation of predictive microbial models based on liquid experiments considering their transferability to solidified media.

2. Materials and methods

2.1. Preliminary experiment: correlating NaCl concentration to a_w for different gelatin concentrations

In a first, preliminary experiment Tryptic Soy Broth (TSB without dextrose, Becton, Dickinson and Company, France) solutions were prepared with different gelatin concentrations (approx. 225 bloom from bovine skin, Sigma–Aldrich, Germany) (0, 10 and 50 g L^{-1}) and NaCl (Acros organics, USA) (between 0.5 and 8.5% (w/v) NaCl) concentrations. *pH* was adjusted with 1 M HCl to *pH* 5.5 and 3.25, the extremes of the *pH* range tested. The a_w of these solutions were measured with an aw-Kryometer Type AWK-20 (NAGY Messysteme GmbH) and an aw meter (Aw Sprint, Axair, Zurich, Switzerland). Both measurements gave similar results. No significant differences were observed between samples with different *pH* at equal NaCl concentrations. a_w seemed to decrease linearly with increasing NaCl concentration. Linear curves, with values of a_w at 0% (w/v) NaCl fixed at value 1.0, were fitted through the data and compared with the Equation of Resnik and Chirife (1988), which correlates NaCl concentration and a_w.

$$a_w = 1 - 0.0052471WPS - 0.00012206WPS^2 \quad (1)$$

with WPS the water phase salt, in %, i.e., $WPS = 100 \times \%NaCl/(\%moisture + \%NaCl)$ with *NaCl* the NaCl concentration (w/v).

2.2. Microorganism and inoculum preparation

A stationary phase culture of *S. typhimurium* SL1344 was stored as a freezing culture at –80 °C in Luria Bertani Broth (LB) supplemented with 25% (v/v) glycerol (Merck). Yearly, a stock culture was prepared from this freezing culture by inoculating a Tryptone Soy Agar plate (Tryptone Soy Broth, Oxoid, England, with Agar technical no. 3, Oxoid, England) and incubating it for 24 h at 37 °C which was subsequently stored at 4 °C. Every month this stock culture was refreshed. Preculture I was prepared from this stock culture by suspending one colony in 10 mL fresh Tryptone Soy Broth (TSB) and incubating it for 24 h at 25 °C. Subsequently, 10 μL of this stationary phase culture were transferred to 10 mL fresh TSB and incubated for 24 h at 20 °C, called Preculture II. This protocol was used to minimize the adaptation phase at the start of the experiment.

2.3. Kinetic experiments

2.3.1. Proposed model structures

In a previous study (Theys et al., 2008) the influence of *pH* (5 values between *pH* 4.50 and 5.50), a_w (5 values between a_w 0.970 and 0.992) and gelatin concentration (w/v) (3 conditions: 0 g L^{-1}, 10 g L^{-1} and 50 g L^{-1}) on the growth kinetics of *S. typhimurium* at 20 °C was studied. A new secondary model, based on the model of Ross et al. (2003), was proposed to describe the effect of gelatin concentration and is extensively evaluated in Theys et al. (2009).