



Dynamics of *Listeria monocytogenes* at suboptimal temperatures in/on fish-protein based model systems: Effect of (micro)structure and microbial distribution



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ARTICLE INFO

Article history:

Received 20 February 2016

Received in revised form

27 May 2016

Accepted 26 June 2016

Available online 29 June 2016

Keywords:

Listeria monocytogenes

Microbial growth

Food intrinsic factors

Model systems

Fish patés

ABSTRACT

Food (micro)structure is known to influence microbial dynamics to a large extent. However, the effect of this factor is difficult to isolate from physicochemical and compositional aspects of the food products. So far, studies focused on the investigation of the effect of food (micro)structure by comparing planktonic growth in liquid (microbiological) media with colonial growth in/on solid-like systems or on real food surfaces. In practice, however, foods are not only liquids or solids; they can also be emulsions or gelled emulsions with complex compositional and physicochemical characteristics.

In this study, *Listeria monocytogenes* growth dynamics were studied along the whole spectrum of food (micro)structure, in terms of food (model) systems with variable (micro)structural complexity, composition and physicochemical characteristics. The targeted (micro)structures were: liquids and emulsions which were homogeneously inoculated and aqueous gels and gelled emulsions which were surface inoculated. The composition of the gelled emulsion targeted fish patés. The other model systems were developed in order to exhibit characteristics of the targeted food (micro)structures and therefore varied in compositional aspects. The main compositional difference was due to the presence or absence of fat, and to a lesser extent the gelling agents (agar and guar gum) and emulsifiers. All systems were incubated at 4, 8 and 12 °C, at aerobic conditions. Physicochemical aspects (pH and water activity (a_w)) of the model systems were measured.

Results indicated that *L. monocytogenes* grows faster in colonies on solid surfaces and slower in liquid systems planktonically. At low temperatures (4 °C), emulsion systems (with fat presence) exhibited a cryoprotective effect and favoured *L. monocytogenes* growth as compared to liquids, where fat was absent. In contrast, at 8 and 12 °C, fat presence inhibited microbial growth, by replacing available space from the water phase which favours microbial growth. Therefore, a complex relation among temperature, compositional and (micro)structural aspects has been observed in this study, requiring further investigation.

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1. Introduction

Global fish consumption has significantly increased in recent years and is anticipated to increase considerably in the future (Claret, Guerrero, Gartzia, Garcia-Quiroga, & Ginés, 2016; FAO,

2012). Specifically, in the European Union, seafood is a major type of consumption product, e.g., 12.3 million ton in 2011 (12% of world consumption) corresponding to a market value of 52.2 billion euro. The European Union is the world's first importer of seafood products, absorbing 24% of the total world exchanges in value. Therefore, fish industry, being one of the most important food industries, aims at preventing and eradicating the presence of pathogenic species. Overall, food industry invests considerable amounts of money in ensuring food safety and quality, as well as in the extension of the shelf life of foods.

Predictive microbiology, being a discipline of food microbiology,

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is used as a tool for food safety assurance. Predictive models describe microbial dynamics, i.e., growth or inactivation as a function of environmental factors, or outline microbial growth/no growth regions, defined by intrinsic or extrinsic food factors. So far, this type of models has mostly been developed on the basis of experimental data obtained: i) in liquid microbiological media, supporting planktonic growth or ii) in and/or on real food products (challenge testing), where growth occurs as immersed or surface colonies on solid foods or planktonically in liquid foods. Both systems for experimental data collection exhibit advantages and disadvantages. Liquid microbiological media are reproducible, homogeneous and easy to prepare, but they do not include (micro) structural variations, naturally occurring in foods. In contrast, real foods include the effect of (micro)structure, but they are not as reproducible as the liquid microbiological media and the experimental work load is high and costly. Food products are not sterile, therefore, a pre-treatment applicable to challenge testing is required for the elimination of the background micro-flora, which affects the food (micro)structure and composition, e.g., dipping in boiling water (Baka, Noriega, Mertens, Van Derlinden, & Van Impe, 2014). Overall, validation studies have shown that predictions using models developed in liquid media are not always accurate when applied to real food environments exhibiting a more complex (micro)structure (Møller et al., 2013). Food model systems are an alternative for experimental data collection, where all physico-chemical and (micro)structural parameters can be controlled, reproducibility is achieved and results could be transferable to food products with similar properties.

Food (micro)structure is known as an influencing factor on microbial growth; the mechanisms of influence have been identified (Brocklehurst, Mitchell, & Smith, 1997; Robins & Wilson, 1994; Wilson et al., 2002). In principle, food microstructure and food structure are distinctly defined. Food microstructure has been defined as the layout of particles and spaces between the particles, while food structure as the shape and size of the material (Mebatsion, Verboven, Ho, Verlinden, & Nicolai, 2008). Food products have been classified in five categories, on the basis of their structural characteristics: (i) liquids, (ii) aqueous gels, (iii) emulsions, (iv) gelled emulsions and (v) food surfaces, with representative food examples proposed for each one of the categories (Wilson et al., 2002). Nevertheless, the influence of food (micro)structure has not been quantified, in order to incorporate it in predictive models. In literature, the effect of (micro)structure has been studied by adding gelling agents in liquid microbiological media (Antwi et al., 2006; Brocklehurst et al., 1997; Mertens et al., 2011; Theys et al., 2008), or by adding fatty/oily components combined with emulsifiers in liquid media (Brocklehurst, Parker, Gunning, Coleman, & Robins, 1995; Castro, Rojas, Campos, & Gerschenson, 2009; Parker, Brocklehurst, Gunning, Coleman, & Robins, 1995) or by developing food model systems simulating real food systems, e.g., liver pâté (Farber, McKellar, & Ross, 1995), cheese (Jeanson et al., 2011) and meat (Baka, Noriega, Van Langendonck, & Van Impe, 2016). The developed model systems do not cover the whole spectrum of (micro)structural complexity. Consequently, it is of utmost importance to develop model systems, which cover the whole spectrum of structural complexity. Thus, the influence of each level of (micro)structural complexity on microbial growth dynamics will be quantified for a target microorganism as well as for a target food product. In Baka et al., 2016, an attempt to study the influence of the whole spectrum of food (micro)structure on microbial dynamics was made by means of (food) model systems; the target food was Frankfurter sausages.

In the present study, the effect of food (micro)structure and *Listeria monocytogenes* cell distribution on its growth dynamics is investigated, by means of model systems mimicking fish patés. The

model systems exhibit various (micro)structures. As a consequence of the food (micro)structure diversity, compositional and physico-chemical variations of model systems will occur. In this work, *L. monocytogenes* was selected as the target microorganism, as it is often related to cases of fish contamination (Feldhusen, 2000; Huss, Reilly, & Karim Ben Embarek, 2000), which is the target group of food products. The growth dynamics of *L. monocytogenes* will be studied in/on four model systems (i) liquids and (ii) emulsions (homogeneous inoculation), (iii) aqueous gels and (iv) gelled emulsions (surface inoculation), at suboptimal temperatures, relevant for fish products storage 4 °C, and abuse storage temperature (8 and 12 °C).

2. Materials & methods

2.1. Model systems preparation

The composition of the model systems is provided in Table 1. For the preparation of the model systems, fish protein, NaCl, starch and pentasodium triphosphate were dissolved until homogenisation in water at 7 °C, to prevent lump formation of the starch (stage 1). Mixture was sterilised for 20 min at 121 °C in an autoclave (VAPOUR-Linelite, VWR International, Belgium). Then, it was stored at the appropriate temperature, e.g. 4, 8 and 12 °C incubator, prior to inoculation. This procedure refers to the **liquid systems**. Stage 1 was a common preparation stage for all model systems. For the **aqueous gels** preparation, the procedure as described in stage 1 was followed. Then, the developed mixture was continuously being stirred and heated up to 50 °C. Afterwards, guar gum and agar were added consecutively and slowly (stage 2). The mixture was further mixed till homogeneous for approximately 10 min. Then, the mixture was heat-sterilised (stage 3). After heat sterilisation the mixture was divided (7 mL/Petri dish) in small Petri dishes (diameter 50 mm, height 9 mm) and incubated at the appropriate storage temperature prior to inoculation. For the **gelled emulsions** preparation, the procedure is similar until stage 3. Yet, emulsifier is also added together with guar gum and agar during stage 2.

Fat was heat-treated separately for background flora elimination (2 h at 80 °C) and then added slowly to the mixture developed at stage 3 using a 5 mL pipet, which was continuously being mixed at 19,800 rpm for 6 min with an emulsifier (T25 digital ULTRA-TURRAX, IKA-Werke GmbH & Co, KG, Staufen, Germany with accompanying S 25N–18 G dispersing element) (stage 4). Breaks were made every 2 min in order to avoid overheating of the sample and the disperser. Small petri dishes were filled with 7 mL of the corresponding model system using a 10 mL syringe. The model system was then left in Petri dishes for 10 min, to solidify. The Petri dishes were stored in an incubator at the temperature used during the subsequent experiment, e.g. 4, 8 and 12 °C. For the **emulsion** systems, the procedure was similar as for the gelled emulsions, but at stage 3, instead of guar gum and agar, only emulsifier was added. After stage 4, the mixture was stored in the relevant incubator, e.g., 4, 8 and 12 °C. The protocol of the model system preparation was based on the protocol described by (Baka et al., 2016).

2.2. Model systems characterisation

The model systems were characterised by determining their pH and water activity (a_w) before cell inoculation. The pH was measured with a pH meter (DocuMeter, Sartorius, Germany), connected with two types of electrodes: (i) for liquid and emulsion systems, a pH electrode appropriate for homogeneous liquid systems (PY-P11, Sartorius, Germany) and (ii) for aqueous gel and gelled emulsion systems, a pH electrode (HI 1413B, Hanna Instruments) appropriate for pH measurement on food surfaces. The

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