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Influence of food intrinsic complexity on *Listeria monocytogenes* growth in/on vacuum-packed model systems at suboptimal temperatures

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ABSTRACT

Food intrinsic factors e.g., food (micro)structure, compositional and physicochemical aspects, which are mutually dependent, influence microbial growth. While the effect of composition and physicochemical properties on microbial growth has been thoroughly assessed and characterised, the role of food (micro)structure still remains unravelled. Most studies on food (micro)structure focus on comparing planktonic growth in liquid (microbiological) media with colonial growth in/on solid-like systems or on real food surfaces. However, foods are not only liquids or solids; they can also be emulsions or gelled emulsions and have complex compositions. In this study, *Listeria monocytogenes* growth was studied on the whole spectrum of (micro)structure, in terms of food (model) systems. The model systems varied not only in (micro)structure, which was the target of the study, but also in compositional and physicochemical characteristics, which was an inevitable consequence of the (micro)structural variability. The compositional and physicochemical differences were mainly due to the presence or absence of fat and gelling agents. The targeted (micro)structures were: i) liquids, ii) aqueous gels, iii) emulsions and iv) gelled emulsions. Furthermore, the microbial dynamics were studied and compared in/on all these model systems, as well as on a compositionally predefined canned meat, developed in order to have equal compositional level to the gelled emulsion model system and represent a real food system. Frankfurter sausages were the targeted real foods, selected as a case study, to which the canned meat had similar compositional characteristics. All systems were vacuum packed and incubated at 4, 8 and 12 °C. The most appropriate protocol for the preparation of the model systems was developed. The pH, water activity and resistance to penetration of the model systems were characterised. Results indicated that low temperature contributes to growth variations among the model systems. Additionally, the firmer the solid system, the faster *L. monocytogenes* grew on it. Finally, it was found that *L. monocytogenes* grows faster on canned meat and real Frankfurters, as found in a previous study, followed by liquids, aqueous gels, emulsions and gelled emulsions. This observation indicates that all model systems, developed in this study, underestimated *L. monocytogenes* growth. Despite some limitations, model systems are overall advantageous and therefore, their validation is always recommended prior to further use.

1. Introduction

Predictive microbiology describes microbial dynamics, i.e., growth or inactivation as a function of environmental factors, by means of mathematical models. Most predictive models have been developed on the basis of experimental data obtained: i) in liquid microbiological media (planktonic growth) or ii) in and/or on real food products (homogeneous or surface inoculation, respectively). On the one hand, food products are not always liquid and often exhibit a more complex structure, which is not considered in the models based on liquid media. Therefore, these models describe well microbial growth in liquid food products, but not necessarily in/on real, or more complex, food systems. Additionally, other food intrinsic factors, e.g., compositional and some physicochemical characteristics, e.g., resistance to penetration are not taken into account when working with liquid microbiological media. On the other hand, challenge tests, where the growth of target microorganisms is directly monitored in/on real food products, and therefore, the effect of food (micro)structure, compositional and physicochemical characteristics is intrinsically present, are expensive and

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time-consuming (Wareing and Komitopoulou, 2013). Moreover, food products exhibit high batch to batch variability and a pre-treatment is required for the elimination of their background micro-flora, which affects their food (micro)structure and composition, such as dipping in boiling water (Baka et al., 2014), applicable to challenge testing. For the preparation of model systems of various (micro)structures is crucial to ensure reproducibility and exclude the background microflora effect, when this is necessary. Additionally, it is of high importance to develop such systems at which the isolated effect of (micro)structure, composition and physicochemical variability can be studied.

Food (micro)structure has been defined as the layout of particles and spaces between the particles, whilst structure as the shape and size of the material (Mebatsion et al., 2008). Food structure has been classified in five categories (liquids, aqueous gels, emulsions, gelled emulsions and food surfaces), where representative examples of food products have been proposed for each one of the categories (Wilson et al., 2002) and examples are provided in Fig. 1. Therefore, liquids and gelled emulsions are two experimental systems which represent the edges of a wide spectrum of food matrix complexity. The food (micro)structure has been acknowledged to influence microbial growth and the mechanisms of influence have been identified (Brocklehurst et al., 1997; Robins and Wilson, 1994; Wilson et al., 2002). Cells grow planktonically in liquid and oil-in-water emulsion systems, with fat level lower than 83% (Brocklehurst and Wilson, 2000). Nevertheless, in oil-in-water emulsions, the available physical space for growth is limited, in comparison to the liquid systems. In solid-like systems, the mechanisms of nutrients and oxygen transportation are different than in liquids. It has been reported that in solid-like systems, constraints in oxygen availability, and limited water, nutrients, preservatives and metabolites distribution take place (Koutsoumanis et al., 2004; Noriega et al., 2008a, 2008b, 2009; Wilson et al., 2002; Wimpenny and Coombs, 1983). Additionally, solid-like foods confine cells and force them to form colonies. As a consequence, stress is induced to the immobilised cells. Stress is reflected with changes in microbial metabolism, where alterations in growth rate, cell morphology, membrane permeability, superficial tension and osmotic pressure have been observed (Dervakos and Webb, 1991; Meldrum et al., 2003; Wilson et al., 2002). Immobilised cells exhibit substrate and product concentration gradients around the colony, as molecular diffusion is much slower than convection occurring in liquid systems (Brocklehurst et al., 1997; Malakar et al., 2000, 2003; Walker et al., 1997; Wimpenny et al., 1995). Nevertheless, contradictions exist in literature. Solid-like systems have also been reported to enhance survival or even microbial growth (Antwi et al., 2007; Mertens et al., 2011; Wilson et al., 2002).

Numerous studies have been carried out, studying the effect of environmental factors or the concentration of gelling agents, fat and emulsifiers on model systems of various (micro)structures. These studies include: i) liquids that have been solidified by the addition of gelling agents (Antwi et al., 2006; Brocklehurst et al., 1997; Mertens et al., 2011; Theys et al., 2008), or emulsified by the addition of fat/oil and emulsifiers (Brocklehurst et al., 1993, 1995; Castro et al., 2009; Parker et al., 1995), and ii) food model systems similar to real food systems, such as liver pâté (Farber et al., 1995), minced chicken breast (Noriega et al., 2010a) and cheese (Jeanson et al., 2011). Furthermore, there are studies where microbial growth dynamics have been studied under the same environmental conditions, in liquid microbiological media compared to solidified microbiological media (Boons et al., 2013a, 2013b, 2014; Koutsoumanis et al., 2004; Lebert et al., 2004; Mertens et al., 2011). Significant differences on the growth dynamics of the two types of model systems were found. Consequently, it is of utmost importance to develop model systems, which cover the whole spectrum of structural complexity. Additionally, the microbial dynamics among all the model systems and the target food product need to be studied and compared. Thus, the influence of each level of structural complexity

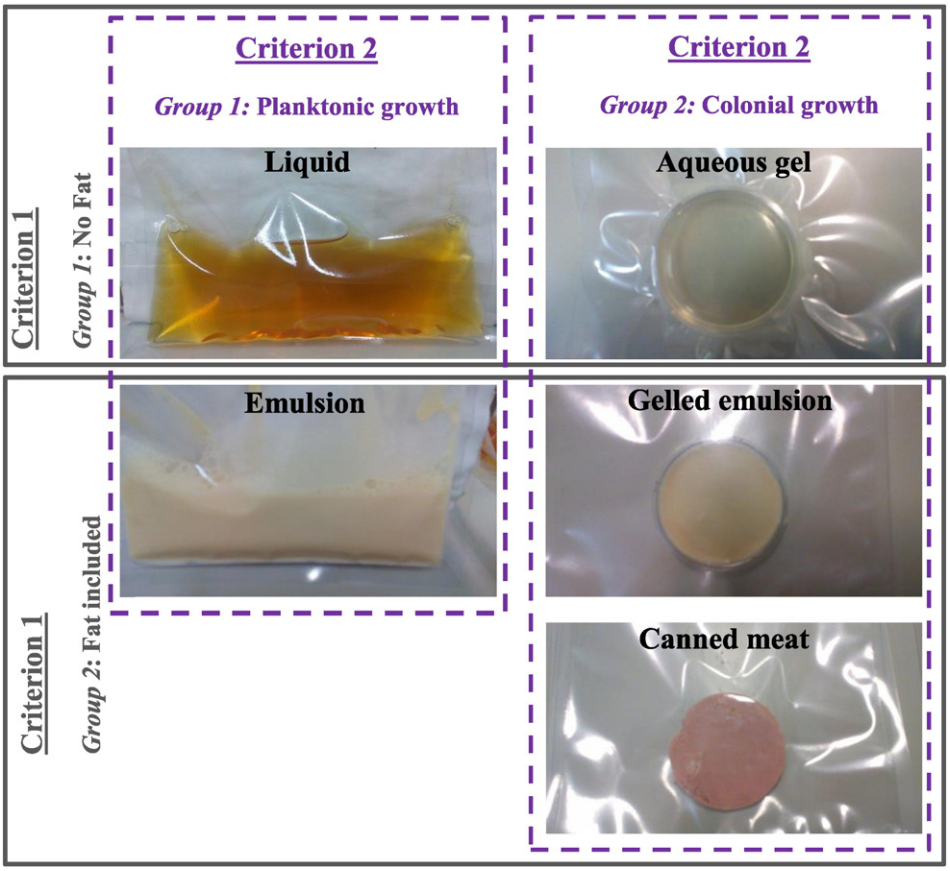

Fig. 1. Demonstration of the model systems and schematic representation of the different criteria influencing microbial growth.