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Salmonella Typhimurium and *Staphylococcus aureus* dynamics in/on variable (micro)structures of fish-based model systems at suboptimal temperatures

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ABSTRACT

The limited knowledge concerning the influence of food (micro)structure on microbial dynamics decreases the accuracy of the developed predictive models, as most studies have mainly been based on experimental data obtained in liquid microbiological media or in/on real foods. The use of model systems has a great potential when studying this complex factor. Apart from the variability in (micro)structural properties, model systems vary in compositional aspects, as a consequence of their (micro)structural variation. In this study, different experimental food model systems, with compositional and physicochemical properties similar to fish patés, are developed to study the influence of food (micro)structure on microbial dynamics. The microbiological safety of fish products is of major importance given the numerous cases of salmonellosis and infections attributed to staphylococcus toxins. The model systems understudy represent food (micro)structures of liquids, aqueous gels, emulsions and gelled emulsions. The growth/inactivation dynamics and a modelling approach of combined growth and inactivation of *Salmonella* Typhimurium and *Staphylococcus aureus*, related to fish products, are investigated in/on these model systems at temperatures relevant to fish products' common storage (4 °C) and to abuse storage temperatures (8 and 12 °C). ComBase (http://www.combase.cc/) predictions compared with the maximum specific growth rate (μ_{max}) values estimated by the Baranyi and Roberts model in the current study indicated that the (micro)structure influences the microbial dynamics. Overall, ComBase overestimated microbial growth at the same pH, a_w and storage temperature. Finally, the storage temperature had also an influence on how much each model system affected the microbial dynamics.

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1. Introduction

Predictive microbiology is a discipline of food microbiology in which accurate and versatile mathematical models are developed in order to describe microbial dynamics as a function of environmental conditions (Van Impe et al., 2005). The use of cumulative databases is a substantial advantage of predictive microbiology, as new data are collected regularly (McDonald and Sun, 1999; Ross and McMeekin, 1994). Since well-developed predictive models allow a realistic estimation of microbial dynamics in foods, these are a positive asset to food safety (Koutsoumanis et al., 2004). However, the limited knowledge concerning the influence of food (micro)structure on microbial dynamics reduces the accuracy of predictive models, as most studies have mainly been based on experimental data obtained in liquid microbiological media (Mertens et al., 2011). Food model systems, which simulate the properties of real foods, are essential to study the influence of food (micro)structure on microbial dynamics. The possibility to alter different factors independently from one another, the absence of interfering background microflora, and simple transferability of findings to other food products are the main advantages of using food model systems instead of real food products for these studies (Baka et al., 2016).

Fish products are important consumption products in Europe: approximately 8.8 million tonnes in 2015 (EUMOFA, 2015). Fish is necessary to a healthy human diet, as it provides high-quality proteins and essential omega-3 polyunsaturated fatty acids, which have demonstrated to be protective against a plethora of diseases, e.g., coronary heart disease, diabetes, hypertension, cancer, autoimmune disorders (Simopoulos, 1996; Vaclavik and Christian, 2014). However, fish products are also a common cause of foodborne diseases. In 2014, fish products were the food vehicle in 6.8% of all foodborne outbreaks in Europe (EFSA and ECDC, 2015).

S. Typhimurium and *S. aureus* are two foodborne pathogens relevant to fish products (Mohamed Hatha, 1997; Rahimi et al., 2013; Saito et al.,

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2011). *S.* Typhimurium causes salmonellosis, with symptoms ranging from self-limiting gastroenteritis to life-threatening enteric fever (Cooke et al., 2007). *S. aureus* causes food poisoning due to the production of enterotoxins in food products (EFSA and ECDC, 2015; Saito et al., 2011). Between 1988 and 2007, 46.9% of all foodborne outbreaks were caused by *Salmonella*. In 1.7% of these *Salmonella*-related outbreaks, seafood products were the causative food vehicle. Similarly, 4.5% of all foodborne outbreaks were caused by *S. aureus*, with seafood products being the food vehicle in 3.3% of those outbreaks (Greig and Ravel, 2009).

In the present study, various experimental food model systems, simulating compositional and physicochemical properties of processed fish products (fish patés), are developed to study the influence of food (micro)structure on microbial dynamics. These model systems cover the entire range of possible food (micro)structures, i.e., liquids, aqueous gels, emulsions, gelled emulsions. Subsequently, the growth/inactivation dynamics of *S.* Typhimurium and *S. aureus* are investigated in and on these model systems at temperatures relevant for fish product storage (4 °C) and abuse storage temperatures, occurring during transportation or by malfunctioning refrigerators (8 and 12 °C).

2. Materials and methods

2.1. Model system development

For the gelled emulsion model system, the compositional and (micro)structural properties of fish patés were targeted. The composition of the gelled emulsion model system was established according to relevant literature (Aquerreta et al., 2002; Echarte et al., 2004; Gonçalves and Ribeiro Duarte, 2008; Tahergorabi et al., 2012). The other model systems (i.e., liquid, emulsion, aqueous gel), were developed based on the composition of the gelled emulsion, taking into account the specific characteristics of each (micro)structure (e.g., absence of fat and emulsifiers from the liquids and aqueous gels, absence of gelling agents from the liquids and emulsions). The model systems varied in physicochemical and compositional aspects, which is a consequence of achieving different (micro)structures. The composition of the model systems is provided in Table 1.

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 water activity of all model systems was measured with an a_w-Kryometer (AWK-40, NAGY) on 'liquid'-modus for liquid and emulsion systems and on 'solid'-modus for aqueous gels and gelled emulsions. The measured values are provided in Table 1.

2.3. Microorganisms and preculture conditions

S. aureus strain LMG 08195 was provided by the Belgian Coordinated Collection of Micro-organisms of the Laboratory of Microbiology of the University of Gent (BCCM/LMG, Ghent, Belgium). *Salmonella enterica* serovar Typhimurium strain SL1344 was provided by the Institute of Food Research (IFR, Norwich, UK).

Stock cultures were stored at −80 °C in Brain Heart Infusion (BHI, Oxoid, Hants, UK) supplemented with 20% (v/v) glycerol (Glycerol Bidistilled 99.5%, VWR International, Leuven, Belgium). Inocula were prepared by transferring a loopful (10 μL) of the stock culture into 20 mL of BHI. After incubating for 22 h at 37 °C under static conditions (Binder KB-series incubator; Binder Inc., NY, USA), 20 μL of the stationary-phase culture was inoculated into 20 mL of fresh BHI and incubated for 22 h under the same conditions. This resulted in stationary-phase cultures with an inoculum level of approximately 10^9 CFU/mL, as measured by plate counting. The preculture of each microorganism was prepared individually.

2.4. Challenge testing

The respective stationary phase preculture of *S.* Typhimurium and *S. aureus* was individually diluted to the appropriate cell level using peptone water, containing 1.8% w/v peptone (Peptone Bacteriological, Oxoid, Hants, UK) and 1.0% w/v NaCl. The different model systems were then inoculated to a final cell concentration of approximately 10^2 CFU/g. Each microorganism was grown individually in/on the model systems. Cell concentration is expressed in CFU/g for both types of inoculation (surface and homogeneous), in order to facilitate comparison (Baka et al., 2016). In liquid and emulsion model systems, a cell aliquot was inoculated (in the Schott bottles) and bottles were shaken to obtain a homogeneous dispersion of viable cells. For aqueous gel and gelled emulsion model systems, inocula were transferred onto the surface of each model system (in the petri plates) and spread using a disposable L-shaped cell spreader. Afterwards, the different inoculated model systems were incubated at temperatures of 4, 8 and 12 °C (Termaks Inc., Bergen, Norway). Samples were taken at different time intervals. For model systems inoculated with *S.* Typhimurium, the maximum sampling time was 1200 h (50 d), with 10–16 sampling points per model system for the experiments at storage temperature of 4 °C, and 600 h (25 d), with 11–20 sampling points for the experiments at temperatures of 8 and 12 °C. For model systems inoculated with *S. aureus*, the maximum sampling time was 500 h (≈21 d), with 7–10 sampling points for the experiments at temperatures of 4 and 8 °C, and 800 h (≈33 d), with 14–20 sampling points for the experiments at storage temperature of 12 °C. The duration of sampling varied among the model systems and storage temperatures. In most cases the target was to obtain the full growth curve (for growth experiments) or to observe microbial counts up to the detection limit, for inactivation or survival curves (10 CFU/mL) (Sutton, 2011). For the liquid and emulsion model systems, a sample of 400 μL was taken from the respective Schott

Table 1
Composition in (%w/w) and physicochemical characteristics of model systems.

Ingredients and physicochemical characteristics	Description of ingredients	Liquid	Emulsion	Aqueous gel	Gelled emulsion
Water	Distilled water	88.78	60.43	87.58	59.23
Protein	Fish protein, ProGoTM, Hofseth Biocare ASA, Alesund, Norway	7.66	7.66	7.66	7.66
Fat	Animal fat (local butcher)	0.00	26.85	0.00	26.85
Starch	Soluble Powder, Fisher Scientific, UK	2.00	2.00	2.00	2.00
Agar	Agar technical (No. 3), Oxoid, UK	0.00	0.00	0.70	0.70
Guar	Sigma Aldrich, Germany	0.00	0.00	0.50	0.50
Emulsifier	Citrem 3302, Paalsgaard, Denmark	0.00	1.50	0.00	1.50
Sodium chloride	Sigma Aldrich, Germany	1.06	1.06	1.06	1.06
Pentasodium triphosphate	Sigma Aldrich, Germany	0.50	0.50	0.50	0.50
pH	–	6.34	6.23	6.32	6.17
a_w	–	0.9852	0.9774	0.9853	0.9777

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