This document contains the **post-print pdf-version** of the referred paper:

“Development of fish-based model systems with various microstructures”

by Davy Verheyen, Maria Baka, Seline Glorieux, Barbara Duquenne, Ilse Fraeye, Torstein Skåra, and Jan F. Van Impe

which has been archived on the university repository Lirias (<https://lirias.kuleuven.be/>) of the Katholieke Universiteit Leuven.

The content is identical to the content of the published paper, but without the final typesetting by the publisher.

When referring to this work, please cite the full bibliographic info:

Verheyen, D., Baka, M., Glorieux, S., Duquenne, B., Fraeye, I., Skåra, T., Van Impe, J. F. (2017). Development of fish-based model systems with various microstructures. Food Research International, <https://doi.org/10.1016/j.foodres.2017.12.047>

The journal and the original published paper can be found at:

<https://www.sciencedirect.com/science/article/pii/S096399691730902X?via%3Dihub>

The corresponding author can be contacted for additional info.

Conditions for open access are available at:

<http://www.sherpa.ac.uk/romeo/>

Development of fish-based model systems with various microstructures

Davy Verheyen^{a,b,c}, Maria Baka^{a,b,c}, Seline Glorieux^d, Barbara Duquenne^e, Ilse Fraeye^d,

Torstein Skåra^f, Jan F. Van Impe^{a,b,c}

^aBioTeC+ - Chemical and Biochemical Process Technology and Control, KU Leuven,

Gebroeders de Smetstraat 1, 9000 Gent, Belgium

^bOPTEC, Optimization in Engineering Center-of-Excellence, KU Leuven, Belgium,

^cCPMF², Flemish Cluster Predictive Microbiology in Foods - www.cpmf2.be

^dLeuven Food Science and Nutrition Research Centre (LFoRCe), Research Group for
Technology and Quality of Animal Products, KU Leuven, Gebroeders de Smetstraat 1, 9000
Gent, Belgium

^eFlanders Research Institute for Agriculture, Fisheries and Food (ILVO), Technology and

Food Science Unit, Brusselsesteenweg 370, 9090 Melle, Belgium

^fNofima, P.O. Box 8034, 4068 Stavanger, Norway

davy.verheyen@kuleuven.be, maria.baka@kuleuven.be, seline.glorieux@kuleuven.be,
barbara.duquenne@ilvo.vlaanderen.be, ilse.fraeye@kuleuven.be, torstein.skara@nofima.no,
jan.vanimpe@kuleuven.be

Correspondence to:

Prof. J. F. Van Impe

Chemical and Biochemical Process Technology and Control (BioTeC+)

Department of Chemical Engineering, KU Leuven

Gebroeders de Smetstraat 1, B-9000 Gent (Belgium)

jan.vanimpe@kuleuven.be

Tel: +32-16-32.14.66

1 **ABSTRACT**

2 The effectiveness of predictive microbiology is limited by the lack of knowledge
3 concerning the influence of food microstructure on microbial dynamics. Therefore,
4 future modelling attempts should be based on experiments in structured food model
5 systems as well as liquid systems. In this study, fish-based model systems with
6 various microstructures were developed, i.e., two liquid systems (with and without
7 xanthan gum), an emulsion, an aqueous gel, and a gelled emulsion. The
8 microstructural effect was isolated by minimising compositional and physico-
9 chemical changes among the different model systems. The systems were suitable for
10 common growth and mild thermal inactivation experiments involving both
11 homogeneous and surface inoculation. Average pH of the model systems was $6.36 \pm$
12 0.03 and average a_w was 0.988 ± 0.002 . The liquid system without xanthan gum
13 behaved like a Newtonian fluid, while the emulsion and the liquid containing xanthan
14 gum exhibited (non-Newtonian) pseudo-plastic behaviour. Both the aqueous gel and
15 gelled emulsion were classified as strong gels, with a hardness of 1.35 ± 0.07 N and
16 1.25 ± 0.05 N, respectively. Fat droplet size of the emulsion and gelled emulsion
17 model systems was evenly distributed around $1 \mu\text{m}$. In general, the set of model
18 systems was proven to be suitable to study the influence of important aspects of food
19 microstructure on microbial dynamics.

20
21 **Keywords:** Predictive microbiology; Model systems; Food microstructure.

22

1 INTRODUCTION

Predictive microbiology is a sub-discipline of food microbiology in which accurate and versatile models are developed that describe microbial evolution in foods as a function of environmental conditions (McMeekin et al., 2002; Van Impe et al., 2005). However, the effectiveness of predictive microbiology is restricted by the lack of knowledge concerning the influence of food microstructure on microbial dynamics (Mertens et al., 2011). Food microstructure is defined as the spatial arrangement of elements (e.g., water and oil droplets, gas cells, fat crystals, strands, granules, micelles and interfaces) in a food and their interactions (Heertje, 1993). Therefore, investigating the microstructure of a food product requires the consideration of its elements (i.e., solid, liquid and gaseous) and the resulting architecture in a dynamic and interrelated condition (Aguilera, 2009). In this regard, all food products can be classified into six categories of food architectures: (i) liquids (e.g., soups), (ii) aqueous gels (e.g., jellies), (iii) oil-in-water emulsions (e.g., milk), (iv) water-in-oil emulsions (e.g., butter), (v) gelled emulsions (e.g., sausages), and (vi) surfaces (e.g., vegetable tissue) (Wilson et al., 2002). The food microstructural influence on microbial dynamics is well-documented in literature (Brocklehurst et al., 1997; Robins & Wilson, 1994; Wilson et al. 2002), but has so far not been sufficiently quantified in order to enable its incorporation into predictive models (Baka et al., 2017a; Velliou et al., 2013). Since most predictive models are based on studies in liquid model systems, the influence of the specific microstructure of non-liquid foods is not taken into account (Velliou et al., 2013; Wilson et al., 2002). Therefore, experimental model systems with various microstructures are more suitable to study the microstructural influence on microbial dynamics. Advantages of employing experimental model systems compared to real food products include the possibility of

independent alteration of different factors influencing microbial dynamics, the elimination of background microflora, and the increased transferability of findings to other food products with similar properties (Baka et al., 2017b). In this regard, Baka et al. (2016; 2017a; b) already used model systems with various microstructures to study the influence of food microstructure on microbial dynamics. These studies confirmed the potential of the concept, although improvements are still needed in order to use the model systems for the development of predictive models that incorporate the food microstructural influence.

Listeria monocytogenes is one of the most heat tolerant non-spore forming foodborne pathogens and is also able to grow at refrigeration temperatures (Hudson & Mott, 1993). Therefore, the bacterium is a large threat for ready-to-eat food products designed to have a short shelf life under refrigeration temperatures, as mild heat treatments are generally applied to those products (Ates et al., 2014). More specifically, fish products constitute a food category which is especially susceptible to *Listeria* (Ben Embarek, 1994). In 2015, *L. monocytogenes* was detected in 3.5% of sampled fish products at processing plants in Europe (EFSA & ECDC, 2016). This number is troublesome as fish products are major type of consumption products in Europe (i.e., total consumption of 11 million tons in 2014) (EUMOFA, 2016).

In this study, different food model systems with various microstructures (i.e., liquid, aqueous gel, emulsion, gelled emulsion) were developed. The compositional aspects were based on processed fish products (e.g., fish soup, surimi, fish paté), while the microstructure was simulated by including the most important food microstructural aspects that occur in such products (e.g., a visco-elastic matrix, fat droplets). Being

73 one of the most stress-resistant non-spore forming bacteria and a common pathogen in
74 fish products, *L. monocytogenes* served as a target microorganism during the
75 development process. Physico-chemical, structural and rheological properties, and
76 (where applicable) fat droplet size distribution of the model systems were
77 characterised. The main difference in comparison with previous studies was the
78 isolation of the microstructural effect, achieved by minimising compositional and
79 physico-chemical changes among the different model systems. This novel concept
80 enables researchers to use the model systems more systematically in order to get more
81 insight into the sole effect of food microstructure on microbial dynamics, in turn
82 leading to more accurate predictive models. The systems were designed to be usable
83 in a wide variety of common growth and mild thermal inactivation studies, both
84 involving homogeneous (simulating contamination during food processing) and
85 surface inoculation (simulating post-process contamination of food products).
86

2 MATERIALS AND METHODS

2.1 Model system development

The different model systems were classified in four categories, with respect to their microstructural characteristics: liquids, aqueous gels, oil-in-water emulsions, and gelled emulsions. Oil-in-water emulsions, henceforth described as emulsions, were chosen over water-in-oil emulsions, since they are more representative for commercial fish products.

2.1.1 Format

Model systems were placed in small vials (4mL, 45 x 14.7 mm, BGB Analytik Benelux B.V., Harderwijk, the Netherlands) when homogeneous inoculation was necessary (for planktonic cells and submerged colonies) and in round Petri dishes (55 x 12 mm, Anicrin S.R.L., Scorzè, Italy) when surface inoculation was necessary (for surface colonies).

2.1.2 Components

Since the model systems needed to be usable in mild thermal inactivation studies, hydrolysed fish protein powder served as a protein source. Mild heating of non-hydrolysed proteins would result in aggregation or gelation, in turn changing the microstructural properties of the model systems (Çakir-Fuller, 2015). In order to form the gelled model systems, alginate was chosen as a gelling agent, since it forms heat stable gels in the presence of Ca^{2+} -ions (Tavassoli-Kafrani et al., 2016). Breaking down the structural integrity of alginate gels, i.e., for sampling purposes, is possible using the protocol of Hamoud-Agha et al. (2013). For the emulsion and gelled emulsion system, sunflower oil was chosen as a fat source, since vegetable oils are

commonly used as an ingredient in processed fish products, e.g., fish paté (Aquerreta et al., 2002; Echarte et al., 2004). A hydrophilic (i.e., Tween) and a hydrophobic (i.e., Span) emulsifier were combined to stabilise the emulsion. Since emulsifiers from the 80-class are most suitable to stabilise emulsions containing unsaturated oils (e.g., plant oils) (Mollet & Grubenmann, 2001), Tween 80 and Span 80 were used as emulsifiers in this study.

2.1.3 Preparation protocols

To prepare the **liquid** system, two different aqueous solutions were prepared. The first solution (A) contained hydrolysed fish protein powder (ProGo™, Hofseth Biocare ASA, Ålesund, Norway) and NaCl (Sigma-Aldrich, MO, USA) and was autoclaved. The second solution (B) contained sodium alginate (Sigma-Aldrich, MO, USA) and was filter-sterilised with a pore size membrane of 0.22 µm (TPP, Trasadingen, Switzerland), since depolymerisation of the alginate due to extensive heating during autoclaving would lead to a reduced gel strength (Draget et al., 2006). Afterwards, solution A and B were poured together in aseptic conditions and mixed to retrieve a sterile model system.

For the preparation of the **aqueous gel**, the protocol of Gowney Kalaf et al. (2016) was used. Applied to this study, the protocol involved the addition of calcium carbonate (CaCO₃, Sigma-Aldrich, MO, USA) to solution A before autoclaving. Then, D-(+)-gluconic acid δ-lactone (GDL, Sigma-Aldrich, MO, USA) was dissolved in a small amount (e.g., 5-10 mL) of distilled water. The resulting solution was mixed at 400 rpm for 5 min and filter-sterilised. After solution A and B were mixed together, the GDL solution was added to the mixture while continuously mixing at 400 rpm for

30 min in order to initiate gelation. Afterwards, the model system was distributed to small vials or Petri dishes, using a 1 mL viscous pipet (Microman, Gilson, France). This gelation mechanism allows effective homogeneous inoculation in microbiological experiments, as the systems can be inoculated before the GDL addition.

The **emulsion** model system was prepared based on the combined protocols of Baka et al. (2016) and Ong et al. (2015). Tween 80 (Sigma-Aldrich, MO, USA) was added to solution A before autoclaving. Span 80 (Sigma-Aldrich, MO, USA) was added to the sunflower oil (Eldorado, local supermarket, Stavanger, Norway). In order to remove background flora, the resulting mixture was pasteurised by heating for 2 h at 80°C while being continuously stirred at 400 rpm. The pasteurised mixture was slowly added to the aqueous solution and stirred at 10 000 rpm for 6 min to prepare a crude emulsion. The crude emulsion was further homogenised (Ultra-Turrax, S 18 N – 19 G, IKA-Werke GmbH & Co, Staufen, Germany) at 22 000 rpm for 6 min, while being placed in an ice bath.

To prepare the **gelled emulsion**, the protocols of the other model systems were combined. First, the emulsion was prepared with CaCO₃ present in solution A. Then, GDL was added to the emulsion to initiate gelation.

2.1.4 Model system design procedure

For the **liquid** model system, a protein content of 10.0 - 15.0% (w/w) and a NaCl content of 1.00 - 1.50% (w/v) were targeted as an initial benchmark (Aquerreta et al., 2002; Echarte et al. 2004; Manthey-Karl et al., 2014; Raju et al., 2003). Aqueous

solutions with various protein concentrations, ranging from 1.00 to 15.00%, were prepared and checked over time to monitor the protein solubility. The initial NaCl concentration of 1.00% (w/v) was amended once the maximum protein concentration was determined, since NaCl concentration is known to influence protein solubility due to salting-in and salting-out phenomena (Vaclavik & Christian, 2014). Sodium alginate was used at the same concentration as determined to be feasible for the aqueous gel.

The **aqueous gel** needed to be suitable for both homogeneous and surface inoculation. Hence, gels with alginate concentrations ranging from 0.50% to 5.00% were tested for their possibility of surface inoculation. Concerning the CaCO_3 and GDL concentrations, Growney Kalaf et al. (2016) concluded that final concentrations of 30 mM and 60 mM, respectively, were optimal for the formation of a homogeneous gel. However, since the CaCO_3 -GDL ratio influences the pH of the final system (Growney Kalaf et al., 2016), the appropriate CaCO_3 concentration in this study depended on the pH of the other model systems. Therefore, the pH of gels with CaCO_3 concentrations ranging from 30 to 60 mM and a constant GDL concentration of 60 mM was measured.

For the **emulsion**, a fat content of 1.00% was used in order to minimise compositional and physico-chemical differences with the other model systems. As structural stability was required, the emulsifier concentration was determined using the hydrophilic-lipophilic balance (HLB) system (Griffin, 1949). Briefly, emulsions were prepared with emulsifier blends of Tween 80 and Span 80 with different HLB values to determine the appropriate emulsifier ratio. Since sunflower oil in most cases requires

an HLB value of 8 (Kruglyakov, 2000), emulsifier blends with HLB values around 8 were tested for their stabilising effect on the emulsion, stored at 4°C. The initial total (i.e., sum of Tween 80 and Span 80) emulsifier concentration was chosen to be 30.00% of the fat content. After the most suitable HLB value was determined, the total emulsifier concentration was altered to find the concentration that resulted in a stable emulsion.

The **gelled emulsion** was prepared using the CaCO₃, GDL, oil, and emulsifier concentrations as determined for the other systems. If surface inoculation was not possible using these concentrations, the aforementioned concentrations were varied.

2.1.5 Evaluation of design requirements

The model systems had to meet five design requirements: (i) isolation of the microstructural effect among all model systems, (ii) structural stability over the course of common growth experiments and mild thermal treatments, (iii) minimal concentration of additives, (iv) possibility of homogeneous inoculation, and (v) possibility of surface inoculation (for gelled systems). The structural stability of the model systems was investigated visually after the relevant time periods and at the different storage temperatures for relevant long-lasting growth experiments of *L. monocytogenes* in fish-based model systems, i.e., 35 days at 4°C and 20 days at 10°C (Baka et al., 2017a). The thermal stability was also visually inspected, after heating 1 mL in a water bath at 95°C for 30 min, covering the range of mild thermal treatments applied to fish products (Rosnes et al., 2011). The suitability for surface inoculation of the gelled systems was tested by moving an L-shaped spreader over the surface of the model system in a small Petri dish (3 mL of structured medium). Surface inoculation

was possible if the movement did not visually disrupt the surface. The remaining three design requirements (i.e., isolation of the microstructural effect, possibility of homogeneous inoculation, and minimal concentration of additives) were taken into account during the design procedure of each specific model system.

2.2 Model system characterisation

In the following sections, the characterisation of the model systems is described. Every experiment was independently performed in triplicate, using three samples per replicate.

2.2.1 Physico-chemical characterisation

pH was measured using a pH meter (FiveEasy Plus FEP20, Mettler Toledo, Greifensee, Switzerland), connected with two types of electrodes. Viscous model systems were analysed using a pH electrode for homogeneous liquid systems (pH Electrode LE438, Mettler Toledo, Greifensee, Switzerland). Gelled model systems were analysed using an electrode for measurements on surfaces (InLab Surface, Mettler Toledo, Greifensee, Switzerland).

a_w was measured using an a_w meter (AquaLab 3TE, Decagon Devices Inc., WA, USA) suitable for viscous and gelled systems.

2.2.2 Rheological and structural characterisation

Rheological characterisation of the different model systems was performed using an AR2000ex stress controlled rheometer (TA instruments, New Castle, USA) equipped as described by Glorieux et al. (2017). The gap size between the plates was 500 and

1000 μm for viscous and gelled model systems, respectively. Experiments were conducted at 20°C. Prior to the actual measurement, a delay of 2 minutes was set after sample loading, allowing relaxation of stresses induced during loading and temperature calibration. Model systems were prepared 24 h before measurement and stored at 4°C.

Viscous systems were characterised by means of their steady-state rheological behaviour. The shear rate on 1.6 mL of viscous system was increased linearly from 0.01 to 50.00 1/s and the corresponding shear stress after the sample reached the steady-state was evaluated. The Power law model (Reiner, 1926), $\tau = K \cdot \dot{\gamma}^n$, was used to describe the steady-state relationship between shear stress and shear rate, with τ [Pa], the shear stress; $\dot{\gamma}$ [1/s], the shear rate; K [Pa/s], the consistency index; and n [-], the flow behaviour index that signifies the extent of deviation from Newtonian behaviour. Model parameters were estimated by fitting the experimental data to a power function using Microsoft Excel (Microsoft Corporation, WA, USA).

Rheological properties of the gelled systems were characterised by means of stress and frequency sweeps. Stress sweeps were run between 1 and 10 000 Pa at a fixed frequency of 1 Hz until structure breakdown. The storage modulus (G' , a measure of elastic properties) and loss modulus (G'' , a measure of viscous properties) were obtained from the rheometer software (Rheology Advantage, TA version 5.7). The critical shear stress σ_c was calculated as described in Glorieux et al. (2017). Frequency sweeps were conducted at a constant oscillation stress of 20 Pa in a frequency range of 0.1 to 10.0 Hz.

The texture of gelled systems was evaluated using a Lloyd Texture Analyzer (Model LF plus, Lloyd Instruments, Hampshire, UK). Gelled systems (± 30 mm sample height) were axially penetrated to a depth of 10 mm using a load cell of 100 N and a conical probe (diameter 20 mm) at a speed of 10 mm/min. Hardness was directly obtained from the recorded force-distance curves and described as the maximum force (N) to penetrate the sample.

2.2.3 Emulsion characterisation

Size distribution of oil droplets in emulsion systems was determined by laser diffraction using a Mastersizer instrument (Model 2000, Malvern Instruments Ltd., Worcestershire, UK) with a Hydro 2000G sample unit. The refractive indices were set at 1.49 for the oil droplet particles and 1.33 for the dispersant medium. The absorbance value of the oil droplet particles was set at 0.001. Droplet size distribution was measured at time intervals of 1, 9, 18, 28, and 36 days.

Emulsion stability over time was analysed by measuring variations in light intensity of samples from bottom to top using the Turbiscan LAB (Formulaction, L'union, France), as described by Kips et al. (2016). Results were expressed as Turbiscan Stability Index (TSI) at days 9, 18, 28, and 36, using the measurement at day 1 as a reference.

2.3 Statistical analysis

For the statistical analysis, significant differences were determined using analysis of variance (ANOVA, single variance) test at a 95.0% confidence level ($\alpha = 0.05$). Fisher's Least Significant Difference (LSD) test was used to distinguish which means

287 were significantly different from others. The analyses were performed using the
288 anova1 routine of the Statistical Toolbox of Matlab version R2016b (The Mathworks,
289 Inc., MA, USA). Test statistics were regarded as significant when $P \leq 0.05$.

290

291

3 RESULTS AND DISCUSSION

3.1 Model system development

3.1.1 Model system design procedure

In order to isolate the microstructural effect among the model systems, possible differences in compositional and physicochemical aspects (i.e., pH, a_w) had to be minimised. Therefore, several iterations were needed to reach an appropriate set of model systems meeting all design requirements described in section 2.1.5.

Starting with the **liquid** model system, the protein solubility test demonstrated that the maximum solubility of the protein powder was 4.00%. In solutions with higher protein concentrations, precipitation was visible after a few days. Increasing the NaCl concentrations to 1.06% (w/v) also stabilised the aqueous solution with 5.00% protein for at least 35 days. Therefore, protein and NaCl concentrations of 5.00% and 1.06% were used, respectively. The protein concentration was considerably lower than the initial benchmark. However, since other studies involving fish-based model systems used lower protein concentrations which still allowed growth of *L. monocytogenes* (dos Reis et al., 2011; Rabiey et al., 2013; 2014), the protein content of 5.00% was no limiting factor for cell growth in this study. Furthermore, the nutrients from hydrolysed protein are readily available for microorganisms, resulting in an efficient uptake of the available proteins (Ummadi & Curic-Bawden, 2010).

Aqueous gels with various sodium alginate concentrations with the previously determined protein and NaCl concentrations were prepared and checked for their possibility of surface inoculation. The minimum sodium alginate concentration that still allowed surface inoculation was 3.00%. Since sodium alginate also needed to be

added to the liquid model system, the solubility test was repeated with this sodium alginate concentration, showing no loss of protein solubility. Consequently, the sodium alginate concentration of 3.00% was also suitable for the liquid system. In order to determine the necessary CaCO_3 concentration for the aqueous gel, pH and a_w of the liquid system with 3% sodium alginate were measured and demonstrated to be equal to 6.36 ± 0.16 and 0.989 ± 0.001 , respectively. To get similar pH values for the aqueous gel, gels with different CaCO_3 concentrations were prepared. Figure 1 illustrates the pH values of the different gels. A CaCO_3 concentration of 45 mM resulted in a pH value most similar to the value of the liquid system.

For the **emulsion** model system, it can be observed in Table 1 that an emulsifier blend with an HLB value of 8 resulted in the most stable emulsion, being stable for approximately 7 days at 4°C. After 7 days, phase separation of the aqueous serum and the emulsion was visible. Increasing the emulsifier concentration did not visually increase the emulsion stability. Therefore, xanthan gum (Sigma Aldrich, MO, USA) was added in small concentrations, ranging from 0.05 to 1.00%, in order to stabilise the emulsion. The emulsion with a xanthan gum concentration of 0.50% was visually stable for 35 days at 4°C, sufficing for the duration of growth experiments with *L. monocytogenes* at this temperature. Ideally, xanthan gum would then also be added to the other model systems in order to minimise compositional differences. While this posed no problems for the liquid model system, addition of xanthan gum to the aqueous gel led to irreversible bubble formation during stirring. Since the presence of air bubbles in the gel would influence microbial dynamics, it was not recommended to add xanthan gum to the aqueous gel. The question then rose whether it was also suitable to add the xanthan gum to the liquid model system. In order to investigate

possible influences of the increased viscosity due to the xanthan gum on the growth morphology of *L. monocytogenes*, a growth experiment was conducted in the liquid system with and without the small concentration of xanthan gum. Briefly, both model systems were inoculated with a cocktail of three *L. monocytogenes* strains (i.e., LMG23773, LMG23774, and LMG26484, acquired from the BCCM/LMG bacteria collection of Ghent University in Belgium) to a cell density of 10^2 CFU/mL. Both systems were incubated at 10°C under static conditions and visually inspected over time. Figure 2 illustrates the result of the experiment after 10 days. Colonies of *L. monocytogenes* were visible in the system containing xanthan gum, while no colonies were visible in the system without xanthan gum. Since the addition of xanthan gum to the initial liquid system had a clear influence on microbial growth morphology, a second liquid model system was developed. This system, henceforth addressed as “xanthan”, consisted of the original liquid model system with an additional concentration of 0.50% xanthan gum.

Finally, the **gelled emulsion** was prepared using the protein, NaCl, sodium alginate, CaCO₃, GDL, sunflower oil, and emulsifier concentrations from the other model systems. Similarly to the aqueous gel, no xanthan gum was added to this model system. It was observed that the gelled emulsion, prepared using the aforementioned component concentrations, was suitable for surface inoculation.

Table 2 illustrates the pH and a_w values of the model systems. There were no significant differences in pH and a_w values among the different model systems. Consequently, a set of five model systems (i.e., liquid, xanthan, emulsion, aqueous

gel, gelled emulsion) was developed and the model system design procedure was terminated.

3.1.2 Evaluation of design requirements

After the design procedure, it was checked whether the model systems met the five design requirements as discussed in section 2.1.5. The microstructural effect needed to be isolated, by means of minimal variation in compositional and physico-chemical factors among the different model systems. In this regard, the final composition of the model systems is illustrated in Table 3. Protein and sodium alginate concentrations were constant among the different model systems. The same applied to the NaCl concentration, although it was kept constant in the water fraction of the model systems (i.e., the relevant concentration regarding microbial dynamics), which is not shown in Table 3, as weight percentages in relation to the total model system volume are given. Similarly, CaCO_3 and GDL concentrations in the water fraction were constant in the two gelled systems. The two systems with fat (i.e., emulsion and gelled emulsion) only contained small concentrations of sunflower oil and emulsifiers, minimising compositional differences with the other model systems. This was the most important improvement in comparison to other studies using model systems with various microstructures (Baka et al., 2016; 2017a; b). In these studies, fat levels of more than 18% were utilised, resulting in significant compositional and physico-chemical differences between model systems with and without fat. As already mentioned in section 3.1.1 and illustrated in Table 2, there were no significant differences in pH and a_w among the different model systems in the current study. Therefore, the microstructural effect was effectively isolated among the five different model systems. It was also demonstrated that all systems met the four remaining

design requirements. More specifically, all model systems were structurally stable at 4°C for at least 35 days and at 95°C for at least 30 min. Due to the method followed in the model system optimisation procedure, the lowest possible concentration of additives was also used for all model systems. In addition, all systems were suitable for homogeneous inoculation, while the gelled systems were also suitable for surface inoculation. As a consequence, the developed model systems met all five predetermined design requirements.

3.2 Model system characterisation

3.2.1 Physico-chemical characterisation

pH and a_w values of the different model systems are summarised in Table 2. The average pH value of the different model systems was 6.36 ± 0.03 , being representative for fish-based food products as their pH mostly ranges from 5.5 to 6.8 (Jay et al., 2005). The average a_w value of the model systems was 0.988 ± 0.002 , also being representative for fish products which mostly have an $a_w > 0.938$ (Schmidt & Fontana, 2007). Furthermore, fish-based model systems used in other studies had similar pH and a_w values (Baka et al., 2017a; b).

3.2.2 Rheological and structural characterisation

Parameters of the viscous systems, according to the Power law model, are summarised in Table 4. For the liquid model system, n was approximately equal to 1, indicating Newtonian behaviour. For the xanthan and emulsion model system, n was lower than 1, indicating (non-Newtonian) pseudo-plastic behaviour (Chu et al., 1954). n was significantly higher for the emulsion system than for the xanthan system, but the difference was rather small as compared to the liquid system. For the parameter K ,

no significant differences were observed between the xanthan and emulsion system and K of the liquid system was significantly lower compared to the other two viscous systems. Consequently, rheological properties of the xanthan and emulsion system were very similar, indicating that the presence of fat droplets had no severe effect on these properties. This way, the two aforementioned systems are suitable to study the influence of the presence of fat droplets on microbial dynamics, as this is the only distinction between the two systems. The occurrence of colony growth in the xanthan system, as indicated in section 3.1.1, was probably caused by its difference in viscosity compared to the liquid. Since compositional and rheological differences between the emulsion and xanthan system were very small, the emulsion model system would most likely (the emulsion was non-transparent so this could not be observed) also support colony growth of *L. monocytogenes* under static conditions. Therefore, the xanthan system can be considered as an intermediate step between the liquid and the emulsion system in order to study the separate influence of colony growth (liquid vs. xanthan) and the presence of fat droplets (xanthan vs. emulsion). Growth morphology is known to be an important influencing factor on microbial dynamics (Smet et al., 2015; Velliou et al., 2013).

Rheological and structural properties of the gelled systems are summarised in Table 5. When the aqueous gel and the gelled emulsion are compared, no significant differences are observed. Therefore, the presence of fat droplets was the only factor that distinguished the gelled emulsion from the aqueous gel, indicating that the two gelled systems are also suitable to study the influence of the presence of fat droplets on microbial dynamics. The influence of the frequency on the dynamic moduli G' and G'' is illustrated in Figure 3. For both the aqueous gel and the gelled emulsion, G' was

significantly higher than G'' , and also independent of the frequency. These observations indicate strong gel behaviour of both gels (Clark, 1992; Clark & Ross-Murphy, 1987; Ross-Murphy, 1984). As mentioned in section 3.1.2, there are no significant differences in compositional and physico-chemical factors between the gelled systems and their corresponding viscous systems (i.e., aqueous gel vs. xanthan and gelled emulsion vs. emulsion) and all those systems are able to support submerged colony growth. Therefore, provided that alterations according to the specific target are made, the systems can be used in future studies to investigate the sole effect of a gellified matrix on microbial dynamics, which is known to be an important influencing factor (Antwi et al., 2006; Wilson et al., 2002). Furthermore, the influence of microorganism growth morphology on microbial dynamics can be further investigated using the gelled systems by comparing dynamics in a gelled system which is homogeneously inoculated (submerged colonies) with dynamics in a corresponding gelled system which is surface inoculated (surface colonies).

3.2.3 Emulsion characterisation

Preliminary experiments with the Mastersizer equipment demonstrated that a fat content of 1.00% was not suitable for accurate measurements, since this fat level was too low to reach adequate obscuration levels. Therefore, emulsions with a higher fat level (i.e., enabling more accurate measurements) of 5.00, 10.00 and 20.00% were prepared. The composition of these model systems was similar to the composition of the emulsion containing 1.00% fat, using a lower water content as compared to the original emulsion to compensate for the higher fat levels. In order to ensure emulsion stability at these higher fat levels, the total emulsifier concentration was increased from 0.30 to 1.00% in a similar fashion. Figure 4 illustrates the evolution of droplet

size distribution over time for the different emulsions. All emulsions exhibited two distinct peaks around 0.1 and 1 μm . Being similar for the three emulsions and having a particle size typical of dissolved protein powder (McCarthy et al., 2014), the peak at 0.1 μm represented the fish protein. Therefore, the peak at 1 μm was due to the fat droplets in the emulsions. As the average droplet size of the fat was constant among the emulsions with 5.00, 10.00, and 20.00% fat content, it can be assumed that a similar distribution was present in the emulsion with 1.00% fat content.

Turbiscan measurements were conducted for the emulsions containing 1.00, 5.00, 10.00, and 20.00% fat, as shown in Figure 5. Emulsion stability decreased over time (TSI increased during storage) and delta backscattering profiles (example profile shown in Figure 6) suggest this to be caused by a small amount of creaming (Chauvierre et al., 2004). This creaming, which was not observed visually, was probably due to depletion flocculation caused by the presence of xanthan gum in the emulsions (Zhang & Liu, 2011). Since TSI and fat droplet size distribution of all emulsions remained approximately constant over time, it can be assumed that this small amount of creaming would not result in a significant emulsion instability over the course of common growth experiments. Consequently, emulsions containing 5.00, 10.00, and 20.00% of fat, although not complying with the requirement of the isolated microstructural effect, can also be used in future studies aiming to investigate the influence of fat content on microbial dynamics.

In order to determine the fat droplet size of the gelled emulsion, emulsions were also prepared without the addition of 0.50% xanthan gum and droplet size distribution was measured after 1 day, as shown in Figure 7. For the emulsion with 5.00% fat content,

491 a small decrease in droplet size was observed as compared to the situation where
492 xanthan gum was present, suggesting that the presence of xanthan gum was an
493 influencing factor on droplet formation during the emulsification process. Small
494 droplet size increase for emulsions with 10.00 and 20.00% fat content was attributed
495 to droplet coalescence due to the absence of the stabilising effect of the xanthan gum.
496 In general, however, differences in droplet size distribution between emulsions with
497 and without xanthan gum were rather small. Consequently, fat droplet size in all
498 gelled emulsions was also approximately equal to 1 μm . Similar to the viscous
499 emulsion systems, these gelled systems can also be used in future studies to
500 investigate the influence of fat content on microbial dynamics.

501

4 CONCLUSIONS

In this study, fish-based model systems with various microstructures were developed, i.e., two liquid systems (with and without xanthan gum), an emulsion, an aqueous gel, and a gelled emulsion. Among the different model systems, the microstructural effect was isolated by means of minimal variation in compositional and physicochemical factors. The systems were designed to be suitable for a wide variety of microbiological studies, i.e., common growth and thermal inactivation essays involving both homogeneous and surface inoculation of various microorganisms. The model systems were characterised for their physico-chemical, structural and rheological properties and fat droplet size distribution (if applicable). These actions are a first step in the determination of suitable microstructural parameters for predictive models that incorporate the influence of food microstructure. In future studies, the developed set of model systems can be used to study the influence of three important aspects of food microstructure on microbial dynamics: i.e., (i) the effect of microorganism growth morphology, (ii) the effect of a gelled matrix, and (iii) the effect of fat droplets. In addition, the emulsion and gelled emulsion model systems can easily be adapted to study the influence of the fat content of foods on microbial dynamics.

522 **5 ACKNOWLEDGEMENTS**

523 This work was supported by the Norconserv Foundation, the KU Leuven Research
524 Fund (Center of Excellence OPTEC-Optimization in Engineering, project
525 PFV/10/002), the Fund for Scientific Research-Flanders (project G.0930.13), and the
526 Belgian Program on Interuniversity Poles of Attraction, initiated by the Belgian Federal
527 Science Policy Office (IAP Phase VII/19 DYSCO). Author Maria Baka was supported
528 by the research council of KU Leuven for post-doctoral researchers (PDM/16/125).

529

530

6 REFERENCES

- Aguilera, J. M. (2009). Food microstructure. In G. V. Barbosa-Cánovas (Ed.), *Food Engineering: Volume 1* (pp. 223–239). Oxford (UK): Eolss Publishers Co. Ltd.
- Antwi, M., Geeraerd, A. H., Vereecken, K. M., Jenné, R., Bernaerts, K., & Van Impe, J. F. (2006). Influence of a gel microstructure as modified by gelatin concentration on *Listeria innocua* growth. *Innovative Food Science and Emerging Technologies*, 7, 124-131.
- Ates, M. B., Skipnes, D., Rode, T. M., & Lekang, O. -I. (2014). Comparison of bacterial inactivation with novel agitating retort and static retort after mild heat treatments. *Food Control*, 43, 150-154.
- Aquerreta, Y., Astiasaran, I., Mohino, A., & Bello, J. (2002). Composition of pâtés elaborated with mackerel flesh (*Scomber scombrus*) and tuna liver (*Thunnus thynnus*): Comparison with commercial fish pâtés. *Food Chemistry*, 77, 47–153.
- Baka, M., Noriega, E., Van Langendonck, K., & Van Impe, J. F. (2016). Influence of food intrinsic complexity on *Listeria monocytogenes* growth in/on vacuum-packed model systems at suboptimal temperatures. *International Journal of food microbiology*, 235, 17–27.
- Baka, M., Vercruyssen, S., Cornette, N., & Van Impe, J. F. (2017a). Dynamics of *Listeria monocytogenes* at suboptimal temperatures in/on fish-protein based model systems: Effect of (micro)structure and microbial distribution. *Food Control*, 73, 43-50.
- Baka, M., Verheyen, D., Cornette, N., Vercruyssen, S., & Van Impe, J. F. (2017b). *Salmonella* Typhimurium and *Staphylococcus aureus* dynamics in/on variable (micro)structures of fish-based model systems at suboptimal temperatures. *International Journal of Food Microbiology*, 240, 32-39.

- 556 Ben Embarek, P. K. (1994). Presence, detection and growth of *Listeria monocytogenes*
 557 in seafoods: a review. *International Journal of Food Microbiology*, 23, 17-34.
- 558 Brocklehurst, T. F., Mitchell, G. A., & Smith, A. C. (1997). A model experimental gel-
 559 surface for the growth of bacteria on foods. *Food Microbiology*, 14(4), 303–311.
- 560 Çakir-Fuller, E. (2015). Enhanced heat stability of high protein emulsion systems
 561 provided by microparticulated whey proteins. *Food Hydrocolloids*, 47, 41–50.
- 562 Chauvierre, C., Labarre, D., Couvreur, P., & Vauthier, C. (2004). A new approach for
 563 the characterization of insoluble amphiphilic copolymers based on their
 564 emulsifying properties. *Colloid and Polymer Science*, 282(10), 1097-1104.
- 565 Chu, J. C., Burridge, K. C., & Brown, F. (1954). The viscosity of pseudo-plastic fluids.
 566 *Chemical Engineering Science*, 8(6), 229-247.
- 567 Clark, A. H. (1992). Gels and gelling. In H. G. Schwartzberg, & R. W. Hartel (Eds.),
 568 *Physical chemistry of foods* (pp. 263–305). New York: Marcel Dekker Inc.
- 569 Clark, A. H., & Ross-Murphy, S. B. (1987). Structural and mechanical properties of
 570 biopolymer gels. In A. H. Clark, K. Kamide, S. B. Ross-Murphy, & M. Saito
 571 (Eds.), *Advances in Polymer Science* (pp. 57-192). Heidelberg: Springer-Verlag.
- 572 Dos Reis, F. B., de Souza, V. M., Thomaz, M. R. S., Fernandez, L. P., de Oliveira, W.
 573 P., & De Martinis, E. C. P. (2011). Use of *Carnobacterium maltaromaticum*
 574 cultures and hydroalcoholic extract of *Lippia sidoides* Cham. against *Listeria*
 575 *monocytogenes* in fish model systems. *International Journal of Food*
 576 *Microbiology*, 146, 228-234.
- 577 Draget, K. I., Moe, S. T., Skjåk-Bræk, G., & Smidsrød, O. (2006). Alginates. In A. M.
 578 Stephen, G. O. Phillips, & P. A. Williams (Eds.), *Food Polysaccharides and Their*
 579 *Applications* (pp. 289–334). Florida: CRC Press.
- 580 Echarte, M., Conchillo, A., Ansorena, D., & Astiasaran, I., (2004). Evaluation of the

- 581 nutritional aspects and cholesterol oxidation products of pork liver and fish patés.
582 *Food Chemistry*, 86, 47–53.
- 583 EFSA (European Food Safety Authority), & ECDC (European Centre for Disease
584 Prevention and Control) (2016). The European Union Summary Report on trends
585 and sources of zoonoses, zoonotic agents and food-borne Outbreaks in 2015. *EFSA*
586 *Journal* 2016, 14(12), 4634.
- 587 EUMOFA (European Market Observatory for Fisheries and Aquaculture products).
588 The EU fish market. (2016). <http://www.eumofa.eu/home/> Accessed 13.07.17.
- 589 Glorieux, S., Goemaere, O., Steen, L., & Fraeye, I. (2017). Phosphate Reduction in
590 Emulsified Meat Products: Impact of Phosphate Type and Dosage on Quality
591 Characteristics. *Food Technology and Biotechnology*, 55(3), in press.
- 592 Griffin, W.C. (1949). Classification of surface active-agents by HLB. *Journal of*
593 *Society of Cosmetic Chemists*, 1, 311-326.
- 594 Growney Kalaf, E. A., Flores, R., Bledsoe, J. G., & Sell, S. A. (2016). Characterization
595 of slow-gelling alginate hydrogels for intervertebral disc tissue-engineering
596 applications. *Materials Science and Engineering C*, 63, 198–210.
- 597 Hamoud-Agha, M. M., Curet, S., Simonin, H., & Boillereaux, L. (2013). Microwave
598 inactivation of *Escherichia coli* K12 CIP 54.117 in a gel medium: Experimental
599 and numerical study. *Journal of Food Engineering*, 116, 315-323.
- 600 Heertje, I. (1993). Structure and function of food products. *Food Structure*, 12, 343-
601 364.
- 602
- 603 Hudson, J. A., & Mott, S. J. (1993). Growth of *Listeria monocytogenes*, *Aeromonas*
604 *hydrophila* and *Yersinia enterocolitica* in pate and a comparison with predictive
605 models. *International Journal of Food Microbiology*, 20, 1-11.

- 606 Jay, J. M., Loessner, M. J., & Golden, D. A. (2005). *Modern Food Microbiology*. (7th
607 ed.). New York: Springer, (Chapter 3).
- 608 Kips, L., De Paepe, D., Bernaert, N., Van Weyenberg, S., Van Pamel, E., De Loose,
609 M., Raes, K., & Van Droogenbroeck, B. (2016). Using a novel spiral-filter press
610 technology to biorefine horticultural by-products: The case of tomato. Part II:
611 Evaluation of the process impact on the physical tomato juice quality. *Innovative
612 Food Science and Emerging Technologies*, 38, 213-220.
- 613 Kruglyakov, P. M. (2000). *Hydrophile-Lipophile Balance of Surfactants and Solid
614 Particles*. (1st ed.). The Netherlands: Elsevier Science, (Chapter 3).
- 615 Manthey-Karl, M., Ostermeyer, U., Altinelataman, C., Çelik, U., & Oehlenschläger, J.
616 (2014). Chemical Composition, Cholesterol, Trace Metals and Amino Acid
617 Composition of Different Canned Fish Products Produced and Sold in Turkey.
618 *Journal of Fisheries Sciences.com*, 8(1), 17–26.
- 619 McCarthy, N. A., Kelly, P. M., Maher, P. G., & Fenelon, M. A. (2014). Dissolution of
620 milk protein concentrate (MPC) powders by ultrasonication. *Journal of Food
621 Engineering*, 126, 142-148.
- 622 McMeekin, T. A., Olley, J., Ratkowsky, D. A., & Ross, T. (2002). Predictive
623 microbiology: Towards the interface and beyond. *International Journal of Food
624 Microbiology*, 73(2–3), 395–407.
- 625 Mertens, L., Van Derlinden, E., Dang, T. D. T., Cappuyns, A. M., Vermeulen, A.,
626 Debevere, J., Moldenaers, P., Devlieghere, F., Geeraerd, A. H., & Van Impe, J. F.
627 (2011). On the critical evaluation of growth/no growth assessment of
628 *Zygosaccharomyces bailii* with optical density measurements: liquid versus
629 structured media. *Food microbiology*, 28(4), 736–45.
- 630 Mollet, H., & Grubenmann, A. (2001). *Formulation Technology: Emulsions*,

- 631 *Suspensions, Solid Forms*. Germany: Wiley-VCH.
- 632 Ong, W. D., Tey, B. –T., Quek, S. Y., Tang, S. –Y., & Chan E. –S. (2015). Alginate-
- 633 Based Emulsion Template Containing High Oil Loading Stabilized by Nonionic
- 634 Surfactants. *Journal of Food Science*, 80(1), E93–E100.
- 635 Rabiey, S., Hosseini, H., & Rezaei, M. (2013). The hurdle effect of *Bunium persicum*
- 636 essential oil, smoke and NaCl for controlling the *Listeria monocytogenes* growth in
- 637 fish model systems. *Journal of Food Safety*, 33, 137-144.
- 638 Rabiey, S., Hosseini, H., & Rezaei, M. (2014). Use *Carum copticum* essential oil for
- 639 controlling the *Listeria monocytogenes* growth in fish model system. *Brazilian*
- 640 *Journal of Microbiology*, 45, 89-96.
- 641 Raju, C. V., Shamasundar, B. A., & Udupa, K.S. (2003). The use of nisin as a
- 642 preservative in fish sausage stored at ambient (28 ± 2 °C) and refrigerated (6 ± 2
- 643 °C) temperatures. *International Journal of Food Science and Technology*, 38,
- 644 171–185.
- 645 Reiner, M. (1926). Über die Strömung einer elastischen Flüssigkeit durch eine Kapillare.
- 646 *Kolloid Zeitschrift*, 39, 80-87.
- 647 Robins, M. M., & Wilson, P. D. G. (1994). Food structure and microbial growth.
- 648 *Trends in Food Science & Technology*, 5(9), 289–293.
- 649 Rosnes, J. T., Skåra, T., & Skipnes, D. (2011). Recent Advances in Minimal Heat
- 650 Processing of Fish: Effects on Microbiological Activity and Safety. *Food and*
- 651 *Bioprocess Technology*, 4(6), 833–848.
- 652 Ross-Murphy, S. B. (1984). Rheological methods. In H. W. S. Chan (Ed.), *Biophysical*
- 653 *methods in food research (Critical reports on applied chemistry), volume 5*.
- 654 Oxford: Blackwell Scientific Publications.
- 655 Schmidt, S. J., & Fontana, A. J. (2007). Appendix E: Water Activity Values of Select

- 656 Food Ingredients and Products. In G. V. Barbosa-Cánovas, A. J. Fontana, S. J.
 657 Schmidt & T. P. Labuza (Eds.), *Water Activity in Foods: Fundamentals and*
 658 *Applications* (pp. 407–420). New Jersey: Blackwell Publishing.
- 659 Smet, C., Noriega, E., Van Mierlo, J., Valdramidis, V. P., & Van Impe, J. F. (2015).
 660 Influence of the growth morphology on the behavior of *Salmonella Typhimurium*
 661 and *Listeria monocytogenes* under osmotic stress. *Food Research International*,
 662 77, 515-526.
- 663 Tavassoli-Kafrani, E., Shekarchizadeh, H., & Masoudpour-Behabadi, M. (2016).
 664 Development of edible films and coatings from alginates and carrageenans.
 665 *Carbohydrate Polymers*, 137, 360–374.
- 666 Ummadi, M. S., & Curic-Bawden, M. (2010). Use of Protein Hydrolysates in Industrial
 667 Starter Culture Fermentations. In V. K. Pasupuleti, & A. L. Demain (Eds.), *Protein*
 668 *Hydrolysates in Biotechnology* (pp. 91–114). New York: Springer
 669 Science+Business Media B.V.
- 670 Vaclavik, V. A., & Christian, E. W. (2014). *Essentials of Food Science*. (4th ed.). New
 671 York: Springer, (Chapter 8).
- 672 Van Impe, J. F., Poschet, F., Geeraerd, A. H., & Vereecken, K. M. (2005). Towards a
 673 novel class of predictive microbial growth models. *International Journal of Food*
 674 *Microbiology*, 100(1–3), 97–105.
- 675 Velliou, E. G., Noriega, E., Van Derlinden, E., Mertens, L., Boons, K., Geeraerd, A.
 676 H., Devlieghere, F., & Van Impe, J. F. (2013). The effect of colony formation on
 677 the heat inactivation dynamics of *Escherichia coli* K12 and *Salmonella*
 678 *typhimurium*. *Food Research International*, 54(2), 1746–1752.
- 679 Wilson, P. D. G., Brocklehurst, T. F., Arino, S., Thuault, D., Jakobsen, M., Lange, M.,
 680 Farkas, J., Wimpenny, J. W. T., & Van Impe, J. F. (2002). Modelling microbial

681 growth in structured foods: towards a unified approach. *International Journal of*
682 *Food Microbiology*, 73, 275-289.

683 Zhang, X., & Liu, J. (2011). Effect of Arabic Gum and Xanthan Gum on the Stability
684 of Pesticide in Water Emulsion. *Journal of Agricultural and Food Chemistry*, 59,
685 1308-1315.

686

687

688 TABLES

689 **Table 1: Overview of emulsion stability in function of the tested HLB values of**
 690 **emulsifier blends for the development of the emulsion model systems and their**
 691 **respective concentrations of Tween 80 and Span 80, according to the HLB-**
 692 **system of Griffin (1949). Emulsion stability is expressed as the numbers of**
 693 **storage days at 4°C during which no phase separation is observed.**

HLB value	Tween 80 (% w/w)	Span 80 (% w/w)	Stability duration (days)
6	0.16	0.84	3
7	0.25	0.75	6
8	0.35	0.65	7
9	0.44	0.56	5
10	0.53	0.47	2

694

695

Table 2: pH and a_w of the model systems and their standard deviation. Per column, the statistical analysis demonstrated that there were no statistical differences among the different model systems ($P > 0.05$).

Model system	pH (-)	a_w (-)
Liquid	6.36 ± 0.16^a	0.989 ± 0.001^a
Xanthan	6.34 ± 0.01^a	0.987 ± 0.002^a
Emulsion	6.35 ± 0.01^a	0.988 ± 0.001^a
Aqueous gel	6.43 ± 0.18^a	0.985 ± 0.001^a
Gelled emulsion	6.34 ± 0.11^a	0.985 ± 0.003^a

Table 3: Final composition of the five model systems, i.e., liquid, xanthan, aqueous gel, emulsion, and gelled emulsion.

Ingredients (% w/w)	Liquid	Xanthan	Aq. Gel	Emulsion	Gelled Emulsion
Protein	5.00	5.00	5.00	5.00	5.00
Alginate	3.00	3.00	3.00	3.00	3.00
NaCl	0.96	0.96	0.95	0.95	0.94
CaCO ₃	/	/	0.40	/	0.40
GDL	/	/	0.96	/	0.95
Sunflower oil	/	/	/	1.00	1.00
Tween 80	/	/	/	0.10	0.10
Span 80	/	/	/	0.20	0.20
Xanthan gum	/	0.50	/	0.50	/
H ₂ O	91.04	90.54	89.69	89.25	88.41

Table 4: Rheological parameters and their standard deviation of the viscous systems, according to the Power law model (Reiner, 1926), i.e., K and n . Per column, values bearing different lowercase letters are significantly different ($P \leq 0.05$).

Model system	K (Pa/s)	n (-)
Liquid	0.104 ± 0.017^a	0.995 ± 0.013^c
Xanthan	1.006 ± 0.119^b	0.658 ± 0.014^a
Emulsion	1.041 ± 0.098^b	0.700 ± 0.012^b

Table 5: Rheological and structural properties and their standard deviation of the gelled systems, i.e., σ_c , G' (1 Hz), and G'' (1 Hz). Per column, the statistical analysis demonstrated that there were no statistical differences among the different model systems ($P > 0.05$).

Model system	σ_c (Pa)	G' (Pa, 1 Hz)	G'' (Pa, 1 Hz)	Hardness (N)
Aqueous gel	595 ± 203^a	1274 ± 142^a	40.91 ± 2.61^a	1.35 ± 0.07^a
Gelled emulsion	890 ± 241^a	1328 ± 142^a	44.33 ± 7.43^a	1.25 ± 0.05^a

FIGURE CAPTIONS

Figure 1: pH values for aqueous gel model systems with different CaCO₃ concentrations, compared with the pH of the liquid model system. Bars bearing different lowercase letters are significantly different ($P \leq 0.05$).

Figure 2: Picture of the liquid model system inoculated with *L. monocytogenes*, without (A) and with (B) the addition of 0.50% of xanthan gum. The picture is taken after 10 days of incubation at 10°C under static conditions.

Figure 3: Frequency dependence of the storage modulus (G') and loss modulus (G'') of the aqueous gel and gelled emulsion.

Figure 4: Emulsion droplet size distribution. A) Distribution at day 1 in the emulsions with 5.00, 10.00, and 20.00% fat content; B) Evolution over time of the droplet size distribution in the emulsion with 5.00% fat; C) Evolution over time of the droplet size distribution in the emulsion with 10.00% fat; D) Evolution over time of the droplet size distribution in the emulsion with 20.00% fat.

Figure 5: Turbiscan stability index (TSI) in function of time for the emulsions with 1.00, 5.00, 10.00, and 20.00% fat content. For the same emulsion at different days, bars bearing different uppercase letters are significantly different ($P \leq 0.05$).

Figure 6: Example delta backscattering profile from the Turbiscan measurements. Profile derived from a sample from the emulsion with 20.00% fat content.

Figure 7: Droplet size distribution at day 1 in the gelled emulsions with 5.00, 10.00, and 20.00% fat content. Results are compared for emulsions with and without xanthan gum.

Delta Backscattering - Measurement 003