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Prevalence and challenge tests of *Listeria monocytogenes* in Belgian produced and retailed mayonnaise-based deli-salads, cooked meat products and smoked fish between 2005 and 2007

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ABSTRACT

Processed ready-to-eat (RTE) foods with a prolonged shelf-life under refrigeration are at risk products for listeriosis. This manuscript provides an overview of prevalence data ($n=1974$) and challenge tests ($n=299$) related to *Listeria monocytogenes* for three categories of RTE food i) mayonnaise-based deli-salads (1187 presence/absence tests and 182 challenge tests), ii) cooked meat products (639 presence/absence tests and 92 challenge tests), and iii) smoked fish (90 presence/absence tests and 25 challenge tests), based on data records obtained from various food business operators in Belgium in the frame of the validation and verification of their HACCP plans over the period 2005–2007. Overall, the prevalence of *L. monocytogenes* in these RTE foods in the present study was lower compared to former studies in Belgium. For mayonnaise-based deli-salads, in 80 out of 1187 samples (6.7%) the pathogen was detected in 25 g. *L. monocytogenes* positive samples were often associated with smoked fish deli-salads. Cooked meat products showed a 1.1% ($n=639$) prevalence of the pathogen. For both food categories, numbers per gram never exceeded 100 CFU. *L. monocytogenes* was detected in 27.8% (25/90) smoked fish samples, while 4/25 positive samples failed to comply to the 100 CFU/g limit set out in EU Regulation 2073/2005. Challenge testing showed growth potential in 18/182 (9.9%) deli-salads and 61/92 (66%) cooked meat products. Nevertheless, both for deli-salads and cooked meat products, appropriate product formulation and storage conditions based upon hurdle technology could guarantee no growth of *L. monocytogenes* throughout the shelf-life as specified by the food business operator. Challenge testing of smoked fish showed growth of *L. monocytogenes* in 12/25 samples stored for 3–4 weeks at 4 °C. Of 45 (non-inoculated) smoked fish samples (13 of which were initially positive in 25 g) which were subjected to shelf-life testing, numbers exceeded 100 CFU/g in only one sample after storage until the end of shelf-life. Predictive models, dedicated to and validated for a particular food category, taking into account the inhibitory effect of various factors in hurdle technology, provided predictions of growth potential of *L. monocytogenes* corresponding to observed growth in challenge testing. Based on the combined prevalence data and growth potential, mayonnaise-based deli-salads and cooked meat products can be classified as intermediate risk foods, smoked fish as a high risk food.
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1. Introduction

A significant increasing trend has been observed over the past 5 years in listeriosis incidence at the EU level (EFSA, 2007; Goulet et al., 2008). Most of the cases are sporadic and are reported in the age group of 65 years and older. Only a few outbreaks have been reported. Listeriosis acquired from food is mostly due to the consumption of ready-to-eat (RTE) foods which support growth of *Listeria* and develop a high concentration of *Listeria* along the food chain (FDA/FSIS, 2003; Lianou and Sofos, 2007; EFSA, 2008). According to the Community Zoonoses Reports (EFSA, 2006, 2007), the highest frequency of positive samples in RTE foods was found in meat and fish products: 2.7% for meat products and 7.5% for fish products in 2005; 3.5% for bovine meat product, 2.7% for pork meat products and 4.9% for fish products in 2006. Regarding RTE foods, listeriosis cases are associated with processed foods with a long shelf-life that are kept at refrigeration temperatures (Huss et al., 2000). However, often *Listeria monocytogenes* is able to grow to only a limited extent in these

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RTE foods since very few of them contained *L. monocytogenes* bacteria at levels exceeding the legal safety criterion of 100 CFU per gram (EC, 2005). Samples exceeding this limit were more frequently found in RTE fishery products (1.7%) followed by cheeses (0.1–0.6%) as well as in some RTE meat products (0.1%) (EFSA, 2007).

However, the mere fact that some ready-to-eat food categories are more frequently contaminated with *L. monocytogenes* than others does not imply that these food categories are more likely to cause listeriosis. In particular, it is necessary to estimate the point of time when sampling occurred in relation to the shelf-life and whether these food types support growth of *L. monocytogenes* (and to what extent). EC Regulation No. 2073/2005 (EC, 2005) defines a number of conditions for which it is accepted that such RTE foods do not support the growth of *L. monocytogenes*: i) pH $\leq$4.4; ii) $a_w \leq 0.92$; iii) pH$\leq$5.0 and $a_w \leq 0.94$; for which the criterion of 100 CFU/g is used on the market throughout the shelf-life. For a variety of RTE foods with an extended shelf-life under refrigeration, growth inhibition of *L. monocytogenes* is based upon hurdle technology (formulation of the food, microbial ecology and storage conditions). By consequence, this will need to be judged on a case by case basis.

Since the safety of the food is the responsibility of the food business operator (FBO), it is for these particular types of RTE foods up to the FBO to demonstrate the compliance with the limit of 100 CFU/g throughout the shelf-life. This triggers the question on how the FBO i) decides if the product is able or unable to support the growth of *L. monocytogenes*, ii) demonstrates compliance with the 100 CFU/g limit throughout the shelf-life, and iii) sets its own specifications for *L. monocytogenes* for its particular food product at the end of production line that should be low enough to guarantee that the limit of 100 CFU/g is not exceeded at the end of the shelf-life.

Tools available (still according to EC Regulation No. 2073/2005) are i) a record of safety of end product testing indicating the control of *L. monocytogenes* contamination in the production process, ii) literature data with regard to the effect of hurdle technology applied in growth inhibition of *L. monocytogenes*, iii) predictive modeling of *L. monocytogenes* growth potential, and iv) challenge testing.

This manuscript provides an overview of prevalence data and challenge tests related to *L. monocytogenes* for three categories of RTE food i) mayonnaise-based deli-salads, ii) cooked meat products, and iii) smoked fish, based on data records generated by various food business operators in Belgium in the frame of the validation and verification of their HACCP plans. The objective is to illustrate how these food categories can be ranked with regard to the risk they pose for listeriosis exploiting the above mentioned data sets and appropriately selected predictive microbiology models.

2. Materials and methods

2.1. Prevalence data on L. monocytogenes

The prevalence of *L. monocytogenes* in the three food categories i) mayonnaise-based deli-salads, ii) cooked meat products, iii) smoked fish were compiled from the accredited lab section of LFMFP-UGent where microbial analysis is performed at the request of the FBO in the frame of raw material and end product testing for verification of HACCP. Data were collected over the period 2005–2007 from 9 FBO for mayonnaise-based deli-salads (in total 1187 samples) and from 4 FBO for cooked meat products (in total 597 samples). From 4 FBO involved in production of mayonnaise-based deli-salads, samples of cooked meat product (in total 42 samples) and smoked fish (in total 58 samples) intended for use as a raw material in deli-salad preparation were also obtained.

For smoked fish, from one Belgian retailer, samples were obtained for *L. monocytogenes* testing in the frame of verification of safety of smoked fish from their suppliers throughout the shelf-life. For this purpose, 45 duplicate samples of freshly produced smoked fish were obtained and a shelf-life study was performed, i.e. one sample was analysed immediately, while the other sample was kept at 4 °C (with an intermediate abuse of 4 h at room temperature after 1/3 of shelf-life) until the end of the indicated shelf-life of the product prior to analysis. These data of *L. monocytogenes* in smoked fish (90 samples in total, 45 analyses at start of shelf-life, 45 analysis at end of shelf-life) were included both for assessment of prevalence data in smoked fish (together with other smoked fish samples serving as raw materials for deli-salads as mentioned above) and for assessment of potential outgrowth of *L. monocytogenes* in (non-inoculated) smoked fish as can potentially be derived from these 45 shelf-life studies executed.

Presence/absence testing of *L. monocytogenes* in 25 g was performed using AFNOR validated VIDAS LMO method (Bio-12/9-07/02), an enzyme linked fluorescent assay (ELFA) proprietary method (Biomérieux, Marcy-l'Etoile, France). If present in 25 g, the sample was examined for compliance to the limit of 100 CFU/g by presence/absence testing of *L. monocytogenes* in 0.01 g using the same AFNOR validated VIDAS method (Bio-12/9-07/02) for samples analysed in the period January 2005–June 2006. Presence per 0.01 g indicated presence of a level of contamination with *L. monocytogenes* exceeding 100 CFU/g. From the period July 2006–November 2007, due to the publication of EC Regulation No. 2073/2005 (EC, 2005) in November 2005, enumeration of *L. monocytogenes* positive samples (in 25 g) as determined by VIDAS LMO method was performed according to ISO 11290-2 using a reduced detection limit. In the protocol with the reduced detection limit as recommended in EC Regulation No. 2073/2005 (EC, 2005) 1 ml of the primary 10-fold diluted suspension of the food (30 g) in peptone water is plated on three ALOA (Fiers, Belgium) spread plates of 90 mm diameter providing a detection limit of 10 CFU/g instead of the usual detection limit of 100 CFU/g obtained by the standard plating of 0.1 ml of the primary food suspension on an ALOA agar spread plate.

A database was set up containing the test results of all food samples with their corresponding supplier, sample size, storage conditions and the food category they belong to. Based on this table, prevalence data for each food (sub)category and each supplier were determined. The standard deviation of a sample proportion was calculated as:

$$s_{\hat{p}} = \sqrt{\frac{\hat{p}(1-\hat{p})}{n}}$$

with $\hat{p}$ the proportion of positive samples, and n the number of samples tested.

2.2. Challenge testing

Challenge testing aims to assess the growth of *L. monocytogenes* in a food by artificially inoculating the specific foodstuff with a known quantity of this target micro-organism, followed by determining growth of the micro-organism during a defined time period (shelf-life) at given temperatures as recommended by the FBO. Challenge testing was performed in the accredited lab section of LFMFP-UGent on the demand of the FBO in the frame of product development and compliance testing with the limit of 100 CFU/g during shelf-life as demanded by EC Regulation No. 2073/2005 (EC, 2005) according to a protocol developed in the lab of LFMFP-UGent (Uyttendaele et al., 2004). This protocol was also the basis of a challenge testing protocol recommended by the Belgian Federal Agency for the Security of the Food Chain (FASFC) as available from September 2006 (Daube, 2006). In short, three *L. monocytogenes* strains were grown for 24 h at 30 °C in Brain Heart Infusion broth (Oxoid, Basingstoke, Hampshire, England) and appropriately diluted to ca. 5000 CFU/ml. Afterwards, a mixed inoculum of these 3 strains was prepared and the inoculum level was established by surface plating on Tryptone Soy Agar (Oxoid, Basingstoke, Hampshire, UK). An appropriate volume of the mixed inoculum (0.3–1.0 ml) was inoculated on the surface (meat and fish product) or as in depth (deli-

salad) inoculation in ca. 100 g of a representative food sample, as delivered by the FBO (day of production or maximum kept for 24 h at 0–2 °C), in order to obtain an inoculum level of ca. 50–100 CFU/g. For each sampling day an inoculated sample was prepared. Samples were (re) packed under air, vacuum or modified atmosphere conditions as indicated by the FBO's using a gas packaging unit consisting of a gas mixer (WITT KM 100-4 MEM, Witt Gasetechnic, Witten, Germany) and a gas packaging chamber machine (Multivac Sepp Haggenmüller, Model C 300, Wolferschwenden, Germany). The oxygen gas (Freshline) and nitrogen gas (RT-X50S-Food) were delivered by Air products (Brussels, Belgium). Packed samples were kept during the actual shelf-life at 4 or 7 °C or a variable temperature schedule of e.g. 1/3 of shelf-life at 4 °C and 2/3 of shelf-life at 7–8 °C as defined by FBO. Samples were taken at day 0 (before and after inoculation) and at the end of shelf-life. Enumeration of *L. monocytogenes* was performed according to ISO 11290-2 using a reduced detection limit as described in EC Regulation No. 2073/2005 (EC, 2005). If no colonies were recovered by the enumeration technique (detection limit being 10 CFU/g) AFNOR validated VIDAS method (Bio-12/9-07/02) was performed in order to establish presence/absence testing in 25 g. The growth potential is the difference between the $\log_{10}$ counts at the end of shelf-life and the $\log_{10}$ of the initial concentration. If the difference between the counts at “day 0” and “day end of shelf-life” did not exceed 0.5 $\log_{10}$, the changes in the count values may be attributed to the measurement uncertainty of the microbiological enumeration and hence this was not regarded as significant growth of the pathogen (EU CRL *L. monocytogenes*, 2008). Indeed, according to ISO/TS19036 (ISO, 2006), and to its appendix, the median of the reproducibility standard deviation in solid food products is 0.26 $\log_{10}$ (CFU/g), so that the measurement uncertainty U is $2\times0.26\approx0.5$ $\log_{10}$. Samples were mixed in a blender and pH was measured with a stab electrode (Knick pH meter, Berlin, Germany) using 15–20 g of the homogenate. From the same mixed samples the a_w was determined with an a_w-kryometer Typ AWK-20 (NAGY messysteme GmbH, Gaufelden, Germany) using 40–50 g of the homogenate.

Results obtained from challenge tests were compared to model predictions, in order to evaluate their predictive capacity. Predictions calculated from Combase Predictor (part of the Modelling Toolbox of www.combase.cc) (Baranyi and Tamplin, 2004) were contrasted with dedicated predictive models (i.e. published models that have been validated in specific food matrices). In this case, three dedicated models were selected for the corresponding food categories mayonnaise-based deli-salads (*Listeria* growth/no growth model from Vermeulen et al., 2007a), cooked meat products (growth model from Devlieghere et al., 2001), and smoked salmon and halibut (growth rate and growth boundary model of Mejlholm and Dalgaard, 2007). *Listeria* growth rates ($\log_{10}$ CFU/h) and lag phases (h) (if included in the model) were calculated for each combination of environmental factors along the storage time studied. Through these data, predicted growth (expressed as $\log_{10}$ CFU/g increases, i.e., the difference between the final and the initial microbial concentration) were obtained and they were compared to the observed growth (obtained as a result of the challenge test) in order to evaluate the model predictions for each food category.

3. Results

3.1. Prevalence testing

3.1.1. Mayonnaise-based deli-salads

In total 1187 samples of mayonnaise-based deli-salads were analysed for presence of *L. monocytogenes*. In 80 samples (6.7%), the pathogen was detected in 25 g; however, no sample exceeded 100 CFU/g. Vegetable salads, being analysed in a relatively limited number ($n=53$), had the highest prevalence level (13%, SE 4.7%) (Fig. 1a). More important to note is the significant higher prevalence of *L. monocytogenes* in fish salad (10%, SE 1.4%) than in meat salad (4%, SE 0.9%). Regarding the sources of mayonnaise-based deli-salads samples, they were supplied by 9 FBO, i.e., H (constituted 36% of samples), C (13%), D (11%), I (10%), while the remaining 5 FBO (A, B, E, F, G) each supplied less than 10% of samples. The highest prevalence of *L. monocytogenes* was noted for samples obtained from FBO G (15%), providing mainly fish and meat samples, followed by I (11%), also providing mainly fish and meat samples and being the largest provider of vegetable samples, and H (9%), providing mainly surimi and fish samples, while the remaining FBO (A, B, D, F) showed a prevalence of 8% or less each, with no *L. monocytogenes* positive samples detected for FBO C and E (Fig. 1b).

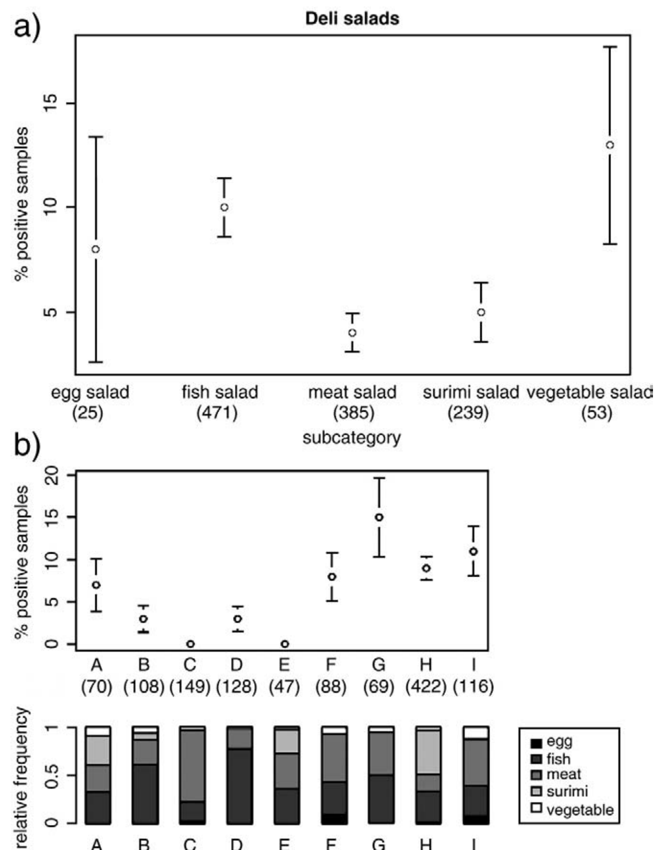

Fig. 1. Proportion of *L. monocytogenes* positive samples (presence in 25 g) of mayonnaise-based deli-salads, with error bars indicating the standard deviation of the proportion a) per type of deli-salad, and b) per food business operator.

3.1.2. Cooked meat products

In total 639 samples of cooked meat products (the majority pre-packed and sliced) were analysed for the presence of *L. monocytogenes*. In 7 samples (1.1%) the pathogen was detected in 25 g; however, no sample exceeded the limit of 100 CFU/g. Although in the present study 352 samples of pâté were included in the analysis, no *L. monocytogenes* was detected in any of the samples (Fig. 2). However, the majority of these pâté samples ($n=346$ over a 2 years period) were provided by one supplier (J) so data obtained were biased towards taking samples from one dominant FBO. Cooked ham samples were obtained from 8 FBO (A, B, F, H, I, K, L, M), luncheon meat from 5 FBO (A, H, K, L, M) and other cooked meat products (including cooked tongue, cooked lard, meat loaf, roast meat, cooked chicken/turkey meat, kassler, etc.) also from 5 FBO (A, I, K, L, M). It should be noted that 4 FBO (B, F, H, I) were in fact producers of mayonnaise-based deli-salads and forwarded the cooked ham and luncheon meat samples ($n=42$) for analysis to the lab as part of their control plan on their suppliers of raw materials. Two of these 42 samples (obtained from F and H) showed presence of *L. monocytogenes*. Among the 4 FBO with activities in production of

cooked meat products for retail sale, A ($n=98$) and L ($n=47$) did not have any positive results for *L. monocytogenes*. K ($n=58$) and M ($n=36$) showed a prevalence of respectively 5% (SE 2.9%) and 6% (SE 3.8%).

3.1.3. Smoked fish

In total 33/58 samples (56.9%) of smoked fish used as a raw material in deli-salad preparation revealed presence of *L. monocytogenes*. Samples were provided by 4 FBO (H, B, F, I). This high prevalence of *L. monocytogenes* in the smoked fish raw materials may explain also the significant higher prevalence of *L. monocytogenes* in the fish salads compared to the meat salads (Fig. 1a) as mentioned above. Indeed, for example FBO H provided 137 of in total 471 samples of fish salads investigated. Of these 137 fish salads, 24 were mayonnaise-based deli-salads with smoked fish, the other samples of fish salads were made with tuna, shrimp, herring, crab, langoustines, etc. 12 out of these 24 smoked fish salads were positive for *L. monocytogenes* whereas for H overall only 16/137 fish salad samples showed detection of the pathogen. FBO's F and G provided each 30 fish salads, among which respectively 9 and 8 smoked fish salads which accounted for 5 out of 6 and 3 out 6 of their respective *L. monocytogenes* positive fish salads. Nevertheless, in none of these salads, nor in the smoked fish as a raw material numbers of *L. monocytogenes* exceeded the limit of 100 CFU/g.

In total 45 smoked fish products were subjected to a shelf-life study for which one sample was analysed for presence (in 25 g) of *L. monocytogenes* at the start of shelf-life and a duplicate sample at the end of shelf-life. Results are shown in Table 1.

At the start of shelf-life (day 0): 13 samples tested positive (*L. monocytogenes* detected in 25 g) of which 3 smoked salmon, 5 smoked halibut and 5 other smoked fish. Of these 13 positive samples (in 25 g), 7 samples were further subjected to presence/absence testing for *L. monocytogenes* per 0.01 g (methodology in use in the period January 2005 until end of June 2006). As such, 2 samples showed presence of the pathogen per 0.01 g indicating a level of contamination with *L. monocytogenes* exceeding the according to EU Regulation 2073/2005 safety limit of 100 CFU/g. The other 6 positive samples (in 25 g) (in the period July 2006 until November 2007) were because of the publication of the EU Regulation 2073/2005 at the end of November 2005 further analysed using enumeration according to ISO 11290-2 with reduced detection limit (10 CFU/g). Out of these 6 samples, only one held 15 CFU/g while the other 5 showed a level of contamination below the detection limit of the enumeration method (<10 CFU/g).

Fig. 2. Proportion of *L. monocytogenes* positive samples (presence in 25 g) of cooked meat products, with error bars indicating the standard deviation of the proportion.

Of these 13 samples in which *L. monocytogenes* was detected at the start of shelf-life (in 25 g), on 6 occasions, the duplicate sample from the same batch stored at 4 °C throughout the shelf-life was found negative for *L. monocytogenes*. These 6 samples included two samples of smoked salmon, one sample of smoked halibut, three samples of other smoked fish (smoked eel, smoked sprat, smoked trout). This inconsistency in finding positive samples (in 25 g) for smoked fish at the start versus the end of shelf-life could be explained by a heterogeneous distribution of *L. monocytogenes* contamination in the batch (a separate package of smoked fish was sampled at the start and the end of shelf-life). It was remarkable that even one of the initially highly contaminated samples (smoked halibut with presence in 0.01 g) was not found positive (in 25 g) at the end of shelf-life in the duplicate package. Of the other 7 samples that were found positive (in 25 g) at the start of the shelf-life study, one sample (a smoked salmon sample) still held *L. monocytogenes* in 25 g but remained to test negative in 0.01 g. Of the 4 smoked halibut samples initially found positive (in 25 g), 2 supported growth from <10 CFU/g to respectively 65 and 80 CFU/g, one sample supported growth from 15 CFU/g to 350 CFU/g exceeding thus the safety limit of 100 CFU/g and one sample remained positive but at levels below 10 CFU/g. The smoked eel sample initially found to be highly contaminated (presence in 0.01 g) was confirmed to be highly contaminated at the end of shelf-life (again tested positive in 0.01 g; however, at the time no enumeration of *L. monocytogenes* was performed). The smoked trout sample initially positive (in 25 g) at the start of shelf-life remained positive (in 25 g) at the end of shelf-life but still did not exceed the safety limit of 100 CFU/g.

Of the 32 smoked fish samples that were initially *L. monocytogenes* free (absence in 25 g), 6 samples showed a positive test result (per 25 g) for the pathogen at the end of shelf-life. These samples included 2 smoked salmon samples, 2 smoked halibut samples, 1 smoked eel sample and 1 sturgeon sample. Except for one of the smoked halibut samples in which 60 *L. monocytogenes* CFU/g were enumerated, all samples carried *L. monocytogenes* at a level below 10 CFU/g at the end of shelf-life.

3.2. Challenge testing

As indicated in Table 2, although the majority of challenge tests performed involved mayonnaise-based deli-salads, only on few occasions (9.9%, $n=182$) the mayonnaise-based deli-salads supported growth of *L. monocytogenes* (i.e. more than 0.5 $\log_{10}$ increase throughout shelf-life). The cooked meat products in most of the occasions (66.3%, $n=92$) supported growth of the pathogen as was also the case for smoked fish (48%, $n=25$).

Detailed data about the challenge tests for a selection of samples of mayonnaise-based deli-salads, cooked meat products and smoked fish can be found in Table 3. From these data it can be noticed that whether a food supports the growth of *L. monocytogenes* or not is mainly determined by the physico-chemical factors (pH, a_w, packaging atmosphere) of the food matrix rather than being defined as such by the food type (cooked meat, salmon, fish salad, etc.). Model predictions obtained from Combase predictor, and dedicated predictive models (Vermeulen et al., 2007a; Devlieghere et al., 2001; Mejlholm and Dalgaard, 2007) are also shown in Table 3. It can be seen that, in general, predictions from Combase Predictor provided an overestimation of *Listeria* growth in most of the food types compared. In contrast, dedicated predictive models predicted log increases closer to the data observed.

3.2.1. Mayonnaise-based deli-salads

Regarding mayonnaise-based deli-salads, it can be noticed that growth is quite influenced by the combination of pH and a_w values. The majority of the deli-salads subjected to challenge testing were formulated as such to have an acid pH (usually pH 5.0–5.5) in

Table 1
Prevalence and enumeration of *L. monocytogenes* in smoked fish samples at start of shelf-life and after storage for a defined period as recommended by the food business operator (the shelf-life) at 4 °C.

Sample description	Presence/absence testing in 25 g at start of shelf-life[a]	Enumeration or estimation of level of contamination[b]	Shelf-life (days) at 4 °C	Presence/absence testing in 25 g at the end of shelf life[a]	Enumeration or estimation of level of contamination[b]
Smoked eel	Presence/25 g	Presence/0.01 g	13	Presence/25 g	Presence/0.01 g
Smoked eel	Absence/25 g	/	20	Absence/25 g	/
Smoked eel	Presence/25 g	Absence/0.01 g	16	Absence/25 g	/
Smoked eel	Absence/25 g	/	20	Presence/25 g	<10/g
Smoked halibut	Presence/25 g	15/g	12	Presence/25 g	350/g
Smoked halibut	Presence/25 g	Presence/0.01 g	13	Absence/25 g	/
Smoked halibut	Absence/25 g	/	9	Presence/25 g	60/g
Smoked halibut	Absence/25 g	/	5	Absence/25 g	/
Smoked halibut	Absence/25 g	/	19	Presence/25 g	<10/g
Smoked halibut	Presence/25 g	<10/g	7	Presence/25 g	80/g
Smoked halibut	Absence/25 g	/	12	Absence/25 g	/
Smoked halibut	Presence/25 g	<10/g	6	Presence/25 g	65/g
Smoked halibut	Absence/25 g	/	12	Absence/25 g	/
Smoked halibut	Presence/25 g	<10/g	11	Presence/25 g	<10/g
Smoked halibut	Absence/25 g	/	5	Absence/25 g	/
Smoked herring	Absence/25 g	/	15	Absence/25 g	/
Smoked herring	Absence/25 g	/	5	Absence/25 g	/
Smoked mackerel	Absence/25 g	/	17	Absence/25 g	/
Smoked mackerel	Absence/25 g	/	12	Absence/25 g	/
Smoked mackerel	Absence/25 g	/	20	Absence/25 g	/
Smoked mackerel	Absence/25 g	/	19	Absence/25 g	/
Smoked salmon	Presence/25 g	<10/g	13	Absence/25 g	/
Smoked salmon	Absence/25 g	/	16	Absence/25 g	/
Smoked salmon	Presence/25 g	Absence/0.01 g	13	Absence/25 g	/
Smoked salmon	Absence/25 g	/	13	Presence/25 g	Absence/0.01 g
Smoked salmon	Presence/25 g	Absence/0.01 g	11	Presence/25 g	Absence/0.01 g
Smoked salmon	Absence/25 g	/	13	Absence/25 g	/
Smoked salmon	Absence/25 g	/	7	Absence/25 g	/
Smoked salmon	Absence/25 g	/	17	Absence/25 g	/
Smoked salmon	Absence/25 g	/	22	Absence/25 g	/
Smoked salmon	Absence/25 g	/	13	Absence/25 g	/
Smoked salmon	Absence/25 g	/	5	Absence/25 g	/
Smoked salmon	Absence/25 g	/	7	Absence/25 g	/
Smoked salmon	Absence/25 g	/	24	Absence/25 g	/
Smoked salmon	Absence/25 g	/	22	Absence/25 g	/
Smoked salmon	Absence/25 g	/	13	Presence/25 g	Absence/0.01 g
Smoked sprat	Presence/25 g	<10/g	11	Absence/25 g	/
Smoked sturgeon	Absence/25 g	/	19	Presence/25 g	Absence/0.01 g
Smoked sturgeon	Absence/25 g	/	11	Absence/25 g	/
Smoked trout	Absence/25 g	/	29	Absence/25 g	/
Smoked trout	Absence/25 g	/	15	Absence/25 g	/
Smoked trout	Absence/25 g	/	11	Absence/25 g	/
Smoked trout	Absence/25 g	/	11	Absence/25 g	/
Smoked trout	Presence/25 g	Absence/0.01 g	13	Absence/25 g	/
Smoked trout	Presence/25 g	Absence/0.01 g	13	Presence/25 g	Absence/0.01 g

[a] Presence/absence testing of *L. monocytogenes* in 25 g tested using the AFNOR validated VIDAS LMO method (Bio-12/9-07/02).
[b] If present in 25 g, the sample was examined for compliance to the limit of 100 CFU/g by presence/absence testing of *L. monocytogenes* in 0.01 g using the AFNOR validated VIDAS method (Bio-12/9-07/02) for samples analysed in the period January 2005–June 2006. Presence per 0.01 g indicated presence of a level of contamination with *L. monocytogenes* exceeding 100 CFU/g. From the period July 2006–November 2007, due to the publication of EC Regulation No. 2073/2005 (EC, 2005) in November 2005, enumeration of *L. monocytogenes* was performed in positive samples (in 25 g) according to ISO 11290-2 using a reduced detection limit (1 ml of the primary 10-fold diluted suspension of the food (30 g) in peptone water is plated on three ALOA (Fiers, Belgium) spread plates of 90 mm diameter providing a detection limit of 10 CFU/g).

combination with a reduced a_w (often a_w 0.96–0.98). Under these conditions no growth of *L. monocytogenes* was observed during prolonged shelf-life (up to 35–42 days) at 4–7 °C (e.g. salad with smoked salmon). Nevertheless, this combination does not fulfill the requirements mentioned in EU regulation 2073/2005 under category 1.3 – note 8 (i) pH$\leq$4.4; ii) $a_w\leq$0.92; iii) pH$\leq$5.0 and $a_w\leq$0.94. The reason is that pH and a_w are not the sole factors inhibiting microbial growth. The presence of organic acids is shown to have a strong negative effect on *Listeria* growth (Buchanan et al., 1994; Giannuzzi and Zaritzky, 1996; Carrasco et al., 2006). Mayonnaise-based deli-salads contain often organic acids, predominantly acetic acid. This acid has a strong inhibitory effect on *L. monocytogenes* growth even at very low levels (0.2% (w/w)) (Vermeulen et al., 2007a). Other organic acids, such as sorbic and benzoic acid, are often added to deli-salads as chemical preservatives, mainly to prevent yeast proliferation. These preservatives are also proven to have a strong inhibitory effect on *L. monocytogenes* growth even at concentrations far below the maximum allowed concentration (Vermeulen et al., 2007b).

From the challenge studies performed, it is clear that if the a_w of a salad increases to 0.98–0.99 (and pH remains at 5.0–5.5) there is potential for limited growth (e.g. fish based salad) which is less the case if an increased a_w 0.98–0.99 is combined with a more acid pH of 4.5–5.0 (e.g. for the vegetarian salad). However, if pH is above 5.5 and a_w is 0.97 or higher, there is clearly a potential for growth (ca. 0.5–1.0 $\log_{10}$ CFU/g in 42 days at 7 °C) (e.g. poultry based salad, meat salad, salad with shrimps). Variability in growth potential (e.g. salad with tuna) might be explained by the fact that the combinations of intrinsic and extrinsic factors applied in the hurdle technology are close to the growth/no growth boundary of *L. monocytogenes* (Vermeulen et al., 2007a).

Regarding model predictions, as shown in Table 3, a growth/no growth model of *L. monocytogenes* from the study of Vermeulen et al.

Table 2
Growth potential of *L. monocytogenes* in three RTE food categories assessed by challenge testing.

Mayonnaise-based deli-salads	Number of challenge tests	Number of samples supporting growth	Cooked meat products	Number of challenge tests	Number of samples supporting growth	Smoked fish	Number of challenge tests	Number of samples supporting growth
Egg	4	0	Turkey/chicken	9	5	Halibut	3	3
Meat	76	9	Cooked ham	28	18	Salmon	16	6
Fish	86	8	Cooked tongue	7	6	Mackerel	6	3
Vegetable	16	1	Luncheon meat	7	5			
			Pâté	27	21			
			Other	14	6			
Total	182	18 (9.9%)	Total	92	61 (66.3%)	Total	25	12 (48%)

The growth potential is the difference between the $\log_{10}$ at the end of shelf-life and the $\log_{10}$ of the initial concentration.
Difference between the counts at "day 0" and "day end of shelf-life" equal to or below 0.5 $\log_{10}$ is not regarded as significant growth of the pathogen.

(2007a), was compared to results obtained from Combase Predictor software. The predicted log increase ($\log_{10}$ CFU/g) was not applicable in the case of the growth/no growth model, since only qualitative information can be obtained from it. Therefore, the probability of growth was calculated for each combination of factors. It can be seen that growth/no growth predictions agreed with observed growth in the challenge tests. However, some discordant predictions were obtained (i.e. salad with tuna, fish-based salad in which growth was observed while no growth was predicted by the model), but observed growth of *Listeria* in these cases was less than 1 $\log_{10}$ CFU/g (respectively 0.33 $\log_{10}$ and 0.60 $\log_{10}$ increase). According to results from Combase Predictor, an important growth potential was predicted in all but one case (for the meat salad a moderate 0.2 $\log_{10}$ increase was predicted). Often, even more than 6.00 $\log_{10}$ increase was predicted, while minor growth potential (0.33–1.07 $\log_{10}$) or no growth was observed during challenge tests. Thus, the growth/no growth model of Vermeulen et al. (2007a) provided better predictions than Combase Predictor regarding the growth of *Listeria* in mayonnaise-based deli-salads.

3.2.2. Cooked meat products

Overall, cooked meat products are more susceptible to support the growth of *L. monocytogenes* mainly because of their intrinsic higher pH (pH 6.0–6.5). Generally, especially two factors influenced growth

Table 3
Physico-chemical characteristics, storage temperature, shelf-life and growth of *Listeria monocytogenes* (expressed as $\log_{10}$ CFU/g increase) for a selection of samples from the three RTE food categories subjected to challenge testing.

Observed results									Predictions			
Food types selected	pH	a_w	Atmosphere packaging[a] (%)	Storage temperature (°C)	Shelf-life (days)	$\log_{10}$ CFU/g increase[b]	Growth rate (h^{-1})	Lagphase (h)	Predicted $\log_{10}$ CFU/g increase	Growth rate (h^{-1})	Lag phase (h)	Predicted $\log_{10}$ CFU/g increase
Mayonnaise-based deli-salads							Combase predictor			Vermeulen et al. (2007a) G/NG (%)[e]		
1 Salad with smoked salmon	5.68	0.968	Air	7	42	NS[i]	0.0137	131.2	>6.00	G (4%)		NA[f]
2 Salad with smoked salmon	5.16	0.978	Air	7	35	NS[i]	0.0112	163.7	>6.00	NG (=0%)		NA
3 Fish-based salad	5.44	0.984	Air	4	42	0.60	0.0090	206.3	>6.00	NG (=0%)		NA
4 Vegetarian salad	4.60	0.998	Air	4	42	−0.60	0.0032	416.7	1.86	NG (=0%)		NA
5 Meat salad	5.83	0.990	66/25/0	4	7	0.65	0.0023	77.1	0.20	G (100%)		NA
6 Poultry-based salad	5.80	0.988	Air	4	42	1.07	0.0124	148.8	>6.00	G (99%)		NA
7 Poultry-based salad	5.66	0.988	Air	4	42	1.04	0.0113	163.0	>6.00	G (75%)		NA
8 Salad with shrimps	5.71	0.976	Air	7	42	0.88	0.0171	107.3	>6.00	G (37%)		NA
9 Salad with tuna	5.56	0.979	Air	7	42	0.69	0.0164	112.1	>6.00	G (2%)		NA
10 Salad with tuna	5.54	0.975	Air	4	31	NS[i]	0.0080	219.9	3.94	NG (=0%)		NA
Cooked meat products							Combase predictor			Devlieghere et al. (2001)[g]		
1 Pâté	6.02	0.969	Air	5/8[c]	23/19[c]	4.99	0.0132	183.4	>6.00	0.028/0.050[c]	11.9/4.7[c]	>6.00
2 Pâté	6.12	0.961	0/50/50	5/8[c]	23/19[c]	NS[i]	0.0095	412.4	5.57	0.018/0.037[c]	25.1/9.7[c]	>6.00
3 Pâté	6.11	0.939	Air	5/8[c]	23/19[c]	0.90	0.0089	431.8	5.05	0.023/0.041[c,g]	16.1/6.4[c,g]	>6.00
4 Pâté	6.28	0.959	Air	2/4[d]	14/21[d]	2.25	0.0078	337.8	3.84	0.018[g]	23.2[g]	1.8
5 Cooked ham	6.23	0.982	Air	4	47	4.00	0.0138	133.3	>6.00	0.028	9.5	>6.00
6 Cooked pork tongue	6.42	0.982	Air	4	44	5.90	0.0146	125.9	>6.00	0.028	9.5	>6.00
7 Cooked ham	6.38	0.978	0/50/50	4	28	0.85	0.0067	233.2	2.89	0.020	18.0	2.0
8 Cooked pork meat	6.65	0.984	Air	4	46	3.10	0.0157	117.3	>6.00	0.030	8.7	>6.0
9 Luncheon meat	5.97	0.977	0/50/50	4	28	NS[i]	0.0057	259.4	2.26	0.020	18.9	2.0
Smoked fish							Combase predictor			Mejlholm and Dalgaard (2007)[h]		
1 Smoked salmon	6.00	0.939	Air	4	28	0.74	0.0025	325.4	0.83		NG	NA
2 Smoked salmon	6.00	0.965	Air	4	28	2.05	0.0081	207.6	3.64		0.0053	1.54
3 Smoked halibut	6.39	0.973	Air	4	23	1.34	0.0120	148.9	4.54		0.0046	1.10

[a] Atmosphere packaging is expressed as % $O_2/CO_2/N_2$, Air indicates packed in air.
[b] $\log_{10}$ CFU/g increase was calculated as the difference between the highest $\log_{10}$ concentration reached and the initial inoculum level.
[c] The product was stored at 5 °C for 23 days and subsequently at 8 °C during 19 days.
[d] The product was stored at 2 °C for 14 days and subsequently at 4 °C during 21 days.
[e] Predictions from the Vermeulen model relate to probability of G (%) or indicate No Growth (NG) (=0%) potential under the defined conditions.
[f] NA = Not applicable.
[g] Predictions from the Devlieghere model in food types no. 3 and 4 (pâté) were made by considering the minimal a_w level (0.9622) inside the range of the model. Likewise, pH was assumed to be constant at 6.2, minimum temperature to be taken up the model is 4 °C.
[h] Predictions from the growth boundary model of Mejlholm and Dalgaard (2007) in cold-smoked salmon (vacuum-packaged) were made assuming 0.70% water phase lactate, 0 ppm of nitrite and 6 ppm of phenol (see Table 4 in that paper), while for cold-smoked halibut 0.125% water phase lactate, 18.15 ppm phenol, and 0 ppm of nitrite are assumed (as indicated in the abstract and in Table 1 of that paper). For the last two predictions, the interaction between the influencing factors turned out to be significant ($0.5<\Psi<1$).
[i] NS indicates No Significant growth and indicates a $\log_{10}$ increase situated between −0.5 and 0.5.

potential of cooked meat products, i.e. its a_w and the presence of CO_2 in the packaging atmosphere. For example, at a_w levels between 0.93–0.94, 0.5–1.0 log_{10} growth in pâté in 42 days was observed; in the same way, if a_w is 0.96–0.97 then ca. 5 log_{10} growth in pâté in 42 days was noticed (Table 3). There was a significant growth of *Listeria* in the investigated cooked meat products at a_w values higher than 0.96 (e.g. cooked ham, cooked pork tongue) unless the cooked meat samples were packed under modified atmosphere (MAP). For example, if packed under MAP, *L. monocytogenes* did not grow on pâté (a_w 0.961) during 42 days (Table 3). Next to the concentration of CO_2 in the atmosphere and the water activity, other factors can influence significantly the potential outgrowth of *L. monocytogenes* on cooked meat products. Especially sodium lactate has a pronounced effect on the growth of the pathogen. More recently, other preservatives, such as diacetate, are also applied as antimicrobial compounds in cooked meat products (Abou-Zeid et al., 2007).

The combined effect of CO_2, a_w, sodium lactate and temperature on the growth (lag phase and specific growth rate) has been described through the development of a predictive model (Devlieghere et al., 2001). Predictions obtained from this growth model were compared to the growth increase estimated by Combase Predictor. Results are shown in Table 3. It can be seen that, in general, predictions from Combase Predictor and Devlieghere et al. (2001) are quite similar and overall provide an important (ca. 1 to sometimes more than 5 log_{10}) overestimation of *Listeria* growth potential. Overestimation in a number of particular cases might be ascribed to the fact that the actual conditions of the food did not fit the growth boundaries of the model. For example, pâté 2 with an a_w of 0.939 is providing more growth inhibition (resulting in established restricted 0.90 log_{10} increase in the challenge test) than the minimal a_w level of 0.9622 which is the lower boundary in the model of Devlieghere et al. (2001), which may account for the overestimation of growth (>6 log_{10}) in this particular case. Growth by predictive models will be overestimated as well if all growth inhibitory conditions present in the food that could be taken up in the predictive model were not available. For example, if it is assumed in pâté 3 that 2% (w/w) sodium lactate is present on the water phase (as is commonly used nowadays in cooked meat processing but for the particular pâté 3 no information was available) calculated lag and growth rates by the Devlieghere et al. (2001) model changed considerably (lag phase at 5 °C increased from 25.1 to 100.58 days and growth rate decreased from 0.018 and 0.037 at respectively 5 and 8 °C to 0.012 and 0.021), resulting in prediction of no growth within the shelf-life. No growth was established in the respective challenge test of pâté 3. Another reason for overestimation of growth by predictive models may have been the presence of competing flora (such as lactic acid bacteria) in the foods during challenge testing accomplishing growth inhibition.

3.2.3. Smoked fish

Similar to cooked meat products, smoked fish supported in many cases the growth of *L. monocytogenes* (Table 3). This is due to a rather neutral pH (6.0–6.5) combined with overall rather high a_W values (0.96–0.98) (except for one with a_w of ca. 0.94). Phenolic compounds found in smoked foods, can diminish the growth of *L. monocytogenes*. However, even a concentration as high as 2 mg/100 g of phenolic compounds may still not be inhibitory for *L. monocytogenes* at 8 °C (Cornu et al., 2006). If smoked fish is produced with pH 5.5–6.0 combined with lower a_W values (0.93–0.94), the product can be categorised as a food which can accomplish a growth limitation of *L. monocytogenes* as shown for one of the smoked salmon samples in Table 3. However, these product characteristics also affect the sensory characteristics and may not be feasible to meet consumer expectations.

In Table 3, predictions obtained by Combase Predictor and the growth boundary model of Mejlholm and Dalgaard (2007) are described. It can be observed that Combase Predictor predictions are quite close to the growth potential observed in the challenge tests. Predictions for the dedicated smoked fish model of Mejlholm and Dalgaard (2007) are rather accurate as the differences between predictions and observed growth in challenge testing are smaller than 0.5 log_{10} for a time window of 28 or 23 days. This model is especially interesting as it makes a realistic difference between the average lactate and phenol contents of the smoked salmon and smoked halibut and because it includes an interaction term enabling to go from a growth rate model to a growth boundary model (following the approach of Le Marc et al., 2005). Unfortunately, the lactate and the phenol contents were not measured for the smoked fish in the present challenge test studies.

4. Discussion

Evaluation of these three RTE food categories with regard to the relative risk they pose for listeriosis should be based upon the prevalence of *L. monocytogenes* in the food category and the ability to support growth of the pathogen to numbers exceeding the safety limit of 100 CFU/g under the foreseen conditions of storage and consumption.

In the first part of the discussion the prevalence of *L. monocytogenes* observed in the present study is discussed in relation to other studies including previous reports from Belgian monitoring studies with regard to *L. monocytogenes* in the selected food categories.

In 80 out of 1187 samples (6.7%) of mayonnaise-based deli-salads the pathogen was detected in 25 g; however, no sample exceeded 100 CFU/g. Several studies have shown the presence of *L. monocytogenes* in 3 to 21% of samples for a variety of mayonnaise-based deli-salads (Harteminkand Georgsson, 1991; McCarthy, 1997; Guerra et al., 2001; Levine et al., 2001; Gombas et al., 2003). In a former study in Belgium (Uyttendaele et al., 1999), we had found a prevalence of 21.3% ($n=874$) in these deli-salads. Overall, the prevalence of *L. monocytogenes* in deli-salads in the present study seems to be lower potentially due to increased awareness by FBO's of the potential risk of the pathogen in these types of RTE food, which is reflected in the introduction of specific legislative criteria for *L. monocytogenes*, and their subsequent attention to the preventive approach. A variation in prevalence depending upon the FBO was noted, indicating that control on ingredients, production environment and processing conditions/practices – resulting in an effective food safety management system based on PRP's and HACCP – varied in efficiency to prevent *L. monocytogenes* contamination in the end product. Also in former studies in Belgium where samples were obtained from retail shops, a higher prevalence of the pathogen was found in fish and shrimp salads (27.3%, $n=362$) (Uyttendaele et al., 1999) and tuna salad (Van Coillie et al., 2004) (28.6%, $n=14$) compared to ham salads (20.7%, $n=159$) (Uyttendaele et al., 1999). In the present study, a prevalence of 5% was noted in surimi-salad which is much lower than the prevalence (25.0%, $n=12$) found by Van Coillie et al. (2004) in imitation crab salad. *L. monocytogenes* positive samples were often associated with smoked fish deli-salads. This might be explained by the high prevalence (56.9%, $n=58$) of the pathogen in the smoked fish used as raw material for these mayonnaise-based deli-salads.

In 7 out of 639 samples (1.1%) of cooked meat products (the majority pre-packed and sliced) the pathogen was detected in 25 g. No sample exceeded the limit of 100 CFU/g. A reduction in prevalence of *L. monocytogenes* in cooked meat products was noted compared to a similar study in Belgium dating from 1999 (4.9% positive samples in 1999, $n=3405$) (Uyttendaele et al., 1999). Van Coillie et al. (2004), also taking samples at retail in Belgium noted a prevalence of 14.3%. Lake et al. (2002) mentioned a contamination of 15.6% ($n=250$) in cooked meat products from New Zealand. A survey on pâté found 5.4% of samples ($n=182$) contaminated (Dominguez et al., 2001). It was shown in the present study that it is feasible by means of Pre-requisite Programs (PRP's) and implementation of HACCP to market *L. monocytogenes*-free (absence in 25 g) pâté (0/346 samples in a 2-years time period). Prevalence of *L. monocytogenes* in cooked meat

products is most of the time related to an (occasional) post-contamination after the cooking process. A post-pasteurization step on the surface of the cooked meat product in the final package (Chilled Food Association, 2006) may eliminate the pathogen. Other alternatives are being proposed to mitigate post-contamination of *Listeria* spp. such as the use of biopreservatives or active packaging (Aymerich et al., 2008).

Although the prevalence of *L. monocytogenes* in smoked fish samples offered at retail was high (28.8%, $n=90$), this prevalence is lower than the 56.9% ($n=58$) prevalence of positive *Listeria* samples found in the smoked fish used as raw material for mayonnaise-based deli-salads. This might indicate that the overall quality of smoked fish used as raw material is different if compared to smoked fish sold as retail product, probably because of the lower added value of mayonnaise-based deli-salads. In a former study in Belgium, Van Coillie et al. (2004) demonstrated a prevalence of 21% ($n=81$) in smoked fish. Several studies revealed a relatively high prevalence of *L. monocytogenes* in smoked fish ranging from 4% to 60%, with most of them indicating 15 to 20% (Johansson et al., 1999; Inoue et al., 2000; Dominguez et al., 2001; Gombas et al., 2003; Besse et al., 2004; Nakamura et al., 2004; Beaufort et al., 2007; Latorre et al., 2007). The wide range may possibly be due to variation of factors such as type of fish and the smoking process (Jørgensen and Huss, 1998). From our data of the smoked fish offered at retail, among the different types of fish, smoked halibut had the highest prevalence (50%, 11/22), followed by the subcategories other smoked fish (25%, 9/36) and smoked salmon (19%, 6/32). This finding coincided with the findings of Van Coillie et al. (2004) in which among fish products they tested, the highest prevalence was also observed in smoked halibut (33%, $n=18$), followed by smoked salmon (19%, $n=42$).

Despite the high prevalence of *L. monocytogenes* in smoked fish, the contamination levels were generally below 100 CFU/g. Nevertheless, in the present study, four samples (of in total 90 retail samples analysed) exceeded the limit of 100 CFU/g (as indicated by enumeration or showed presence in 0.01 g) namely two halibut and two eel samples. Van Coillie et al. (2004) also detected such levels in three smoked fish (two halibuts and one salmon) of in total 81 samples taken at retail in Belgium. In the present study, a contamination level of 350 CFU/g was detected in a halibut sample. There were no quantitative analyses available for the other three samples but only an indication of presence in 0.01 g. Inoue et al. (2000), Nakamura et al. (2004) and Beaufort et al. (2007) reported a generally low level of contamination in smoked salmon. Besse et al. (2004) – using an improved enumeration method by means of membrane filtration – further confirmed that the contamination levels were usually low, i.e., below 100 CFU/g. Gombas et al. (2003) found concentrations exceeding 100 CFU/g in 9 of the 114 positive smoked seafood samples. Dominguez et al. (2001) and Johansson et al. (1999) on the other hand reported more than 100 CFU/g in approximately 50% of their *L. monocytogenes* contaminated smoked fish samples.

The smoking process of fish does not guarantee a 100% inactivation of the *L. monocytogenes* cells present on the raw product (Besse et al., 2004). Moreover, the many steps during the processing of smoked fish entail a reasonable danger for cross contamination. Therefore, contamination at low levels may be inevitable. Vogel et al. (2001) showed that contamination with *L. monocytogenes* was mostly due to direct contact with contaminated processing equipment. Lappi et al. (2004) demonstrated how dedicated plant-specific control strategies for four smoked fish plants enabled to reduce contamination to a (collectively achieved) 2% contamination level. Especially salting and slicing machines have been reported to be difficult to clean and keep *Listeria*-free (Johansson et al., 1999).

However, the mere fact that some ready-to-eat food categories are more frequently contaminated with *L. monocytogenes* than others does not imply that these food categories are more likely to cause listeriosis. Human listeriosis is mainly linked to *L. monocytogenes* levels markedly above the 100 CFU/g limit (WHO, 2004; EFSA, 2008). Listeriosis cases are mostly due to the consumption of ready-to-eat foods which support growth of *Listeria* and develop a high concentration of *Listeria* along the food chain. As such, for risk ranking also the ability to control the growth in the food product during shelf-life should be taken into account. In the second part of this discussion the use of challenge testing and predictive modeling as a tool to acquire information on the ability of the selected food categories to support the growth of *L. monocytogenes* is discussed.

The growth potential of *L. monocytogenes* in a particular food product may be estimated or assessed based on specifications of physico-chemical characteristics of the product, consultation of the available scientific literature, predictive mathematical modeling, challenge testing and periodic shelf-life studies (EC, 2005; EU CRL *L. monocytogenes*, 2008). Predictive mathematical modeling may be helpful in some situations, although the existing predictive models may not necessarily include all the growth limiting factors present in the particular product. Through internet, some predictive microbiology tools with a user-friendly interface can be accessed, such as Combase Predictor (www.combase.cc) or Seafood Spoilage and Safety Predictor (http://www.dfu.min.dk/micro/sssp/Home/Home.aspx). The latter model is an example of product dedicated models. Other product dedicated models described in literature (and included in the present study) are the growth/no growth model of Vermeulen et al. (2007a) predicting the growth probability of *L. monocytogenes* in mayonnaise-based deli-salads, the model of Devlieghere et al. (2001) estimating the growth potential of the pathogen in cooked meat products, and the growth boundary model of Mejhom and Dalgaard (2007) developed especially for lightly preserved seafood. The use of dedicated predictive models for estimating growth potential of *Listeria* in food matrices is recommended. However, it is still recommended to validate the outcome of the predicted growth by challenge testing.

In the present study challenge testing in a limited setup was performed to provide information on the growth potential of the pathogen in the particular food products during their established shelf-life. The challenge tests had as a primary objective to provide an answer about growth/no growth of the pathogen in the particular product. If growth was established, an indication of the growth potential was obtained from the difference between the $\log_{10}$ at the end of shelf-life and the $\log_{10}$ of the initial concentration. In order to be able to estimate with a high degree of certainty the exact growth characteristics (lag phase and generation time) of the pathogen in the particular product, extended experiments would need to be set up and a large number of data points would need to be assembled which was out of the scope of the verification studies commanded by the respective FBO's. However, this data set on challenge testing together with available scientific literature regarding the growth and survival of the pathogen for these three selected food categories along with the information obtained from predictive models, may help to estimate the potential for exposure to high levels of *L. monocytogenes* during the shelf-life.

From the many challenge tests executed (182 test results) for the mayonnaise-based deli-salads, only in 9.9% of cases growth of *L. monocytogenes* occurred. It was shown that if the product formulation is appropriate, growth of *L. monocytogenes* is not supported. Rather than the particular food commodity (whether it involves a meat salad, fish salad or a vegetable salad) within this food category, these are the intrinsic factors that determine the growth potential of *L. monocytogenes*. Previously, it has been demonstrated that in the presence of 0.2% (w/w) acetic acid in the water phase (which is generally added to deli-salads), all conditions at pH $\leq$ 5.4 and $a_w \leq 0.985$ can be considered as safe (Vermeulen et al., 2007a). Alternatively, the addition of 1500 ppm sorbic and benzoic acid in a 1:1 ratio is sufficient to inhibit growth of *L. monocytogenes*, even if the pH is as high as 5.6 and no other organic acids are present (Vermeulen et al., 2007b). These are examples of hurdle technology (differing from the established 'no growth' categories

specified in the EU-legislation – EU Regulation 2073/2005) that do not allow growth. Based on the inability for growth of the pathogen in the majority of the challenge tests performed, the presence of preservatives or organic acids in the mayonnaise-based deli-salads is assumed. This fact is corroborated by the predictions of the growth/no growth model of Vermeulen et al. (2007a), since the effect of acetic acid is included as a significant factor to inhibit *Listeria* growth. Therefore, the limited growth observed in challenge tests can be mainly attributed to the effect of organic acids. On the contrary, predictions from the Combase Predictor did not take into account the presence of organic acids (only pH level), thus providing an overestimation of kinetic growth parameters of *Listeria* in mayonnaise-based deli-salads.

As shown in the present study, cooked meat products, depending upon the exact product composition (pH, a_w), its packaging atmosphere and its shelf-life, may or may not allow the growth of *L. monocytogenes*. Consequently, no generalized conclusions about potential for growth can be made based upon the food belonging to this food category.

One pâté brought to the market by one FBO might differ in its intrinsic and extrinsic characteristics from a second pâté sold by another (or the same) FBO. It is possible to formulate in specific cases cooked meat products which are not supporting growth of *L. monocytogenes* although they do not belong to the 'no growth' categories specified in the EC Regulation No. 2073/2005 (EC, 2005). For example, based on available scientific literature and predictive modeling (Devlieghere et al., 2001), one can conclude that in cooked meat products no growth of *L. monocytogenes* occurs during a shelf-life of 6 weeks at 7 °C when pH ≤ 6.2, $a_w \leq 0.960$, and an atmosphere of minimum 50% of CO_2 is used in combination with minimum 1.5% sodium lactate in the water phase of the product. These combinations (hurdle technology) guarantee no growth of *L. monocytogenes* and thus, even if *L. monocytogenes* is present at moderate levels (1–10 CFU/g), compliance with the 100 CFU/g limit during the products shelf-life is enabled. Other combinations might be available to prohibit the growth of *L. monocytogenes* during shelf-life. Van der Veen et al. (2008) showed, based on testing growth of 138 *L. monocytogenes* strains, none of the strains grew at pH ≤ 5.2 and sodium lactate ≥ 2%, and suggested it as an additional criterion prohibiting growth that could be taken up to the growth limits of *L. monocytogenes* as set out by the European Union. However, it is shown that if for example the cooked ham product formulation does not inhibit *Listeria* growth, pathogen concentration can exceed the limit of 100 CFU/g during storage (Koseki et al., 2007). Further efforts with regard to control of *L. monocytogenes* in cooked meat products are needed as more mildly processed foods with extended shelf-life under refrigeration come to the market. For example, the food industry seeks to reduce salt content (an important growth inhibitory factor) in response to recommendations of food safety agencies that aim at reducing the average salt intake per day in order to prevent hypertension-related diseases (Goulet et al., 2008).

Predictions from the Combase Predictor and Devlieghere et al. (2001) were more or less in the same order of magnitude and most of the time overestimated growth. Sometimes actual conditions of the food did not fit the growth boundaries of the predictive model. Furthermore by lack of data (only growth of *L. monocytogenes* was monitored in the obtained foods and physicochemical measurements were restricted to pH and a_W and measurement of gas composition in the atmosphere) the Devlieghere predictive model could not be fully exploited in its potential i.e. taking into account inhibitory effect accomplished by the presence of sodium lactate or the effect of competitive flora (lactic acid bacteria).

An important number of samples (11/45) of smoked fish as established in the present study carries *L. monocytogenes* at a contamination level between 0.04 and 100 CFU/g at the start of shelf-life. This is a concern as challenge testing showed the ability of significant growth of *L. monocytogenes* during the shelf-life in ca. 50% of the smoked fish samples. Predictions obtained from the Mejlholm and Dalgaard (2007) growth boundary model indicated a slight (smaller than 0.5 logs) underestimation of the growth of *Listeria* in smoked fish in comparison with observed results in challenge tests. However, challenge testing might overestimate the growth potential of *L. monocytogenes* in this particular product. In the naturally contaminated smoked fish samples only on one occasion levels above the safety limit of 100 CFU/g (i.e. 350 CFU/g) at the end of shelf-life was established (whereas it was below this safety limit of 100 CFU/g (i.e. 15/g) in the sample from the same batch at the start of shelf-life). Differences between growth of *Listeria* spp. in naturally contaminated smoked salmon versus challenge tests are well known (Dalgaard and Jørgensen, 1998; Ross et al., 2000; Beaufort et al., 2007). The competition between *L. monocytogenes* and background flora is one of the major reasons proposed to explain this difference (Ross et al., 2000; Beaufort et al., 2007). In addition, next to the competition effect, Beaufort et al. (2007) suggested that in shelf-life studies, the naturally in low numbers occurring stressed cells may have longer lag times than inoculums of cells, often at higher levels, in challenge tests. In the present study, challenge tests were performed using a lower inoculum level of ca. 50–100 CFU/g and inoculation performed using samples of ca. 100 g thus providing ca. $5 \cdot 10^3$–10^4 cells per sample, which is a high enough number of cells to eliminate effects on the outcome of challenge tests by individual cell lag phase distribution (Francois et al., 2006).

5. Conclusions

Mayonnaise-based deli-salads can be categorized as an intermediate risk product taking into account only the prevalence data (6.7% and no samples exceeding 100 CFU/g limit). The prevalence seemed to be variable depending upon the FBO and the type of deli-salad; smoked fish salad showed an increased risk. It can be concluded that if the product formulation of the deli-salads confers to the safe combinations described in literature, deli-salads do not support the growth of *L. monocytogenes*. Although, as shown by the prevalence data, occasional contamination of *L. monocytogenes* is established, the inability for growth if properly formulated underpins the categorization of deli-salads as a low risk food product for listeriosis if taking into account both aspects (prevalence and growth potential). Deli-salads have occasionally been involved in foodborne outbreaks. However, evidence is not always straightforward because if sandwiches are involved, it is not clear what exactly has caused the outbreaks (e.g. vegetable portion, the cooked egg, the sauce, etc.).

Because cooked meat products may or may not have a product formulation that supports the growth of *L. monocytogenes* (and if supportive, significant outgrowth may occur), but show a low prevalence of *L. monocytogenes* (1.1%), they are recommended to be categorized as an intermediate risk food product for listeriosis. Cooked meat products are implicated more frequently in listeriosis cases as shown by epidemiological data, hence possibly suggesting that there are other important factors apart from exposure enhancing the epidemiological association of a food category with listeriosis, such as consumption frequencies (ILSI, 2005). In Belgium for example 56.3% of the respondents consume meat and meat products at least once a day while 35.4% of the respondents eat fish products 2–4 times a week (Debacker et al., 2007).

In the light of the results obtained, it might be concluded that smoked fish (28.8% prevalence and 4/90 samples exceeding 100 CFU/g limit) can be evaluated as a high risk product. Referring to available scientific literature with regard to contamination and control of *L. monocytogenes* in smoked salmon, it seems that high prevalence is intrinsic to the food category of smoked fish. As smoked fish in many cases may also enable growth of the pathogen, for these risk products compliance to criterion of <100 CFU/g at the end of shelf-life should be obtained by restriction of the shelf-life along with explicit labeling to keep the cold chain and communication to the consumer/food handler to create awareness of consequences of rupture of the cold

chain. Zero risk is not feasible but limitation of the risk can be accomplished. Although smoked fish can be concluded to be a high risk food, only a limited number of outbreaks are associated with smoked fish (to our best knowledge, in the period of 1991–2000 overall 27 cases were reported involved in 5 outbreaks).

Prevention of listeriosis demands a combined effort of the whole food chain from processing company to retailer and consumer. In the last 10 years, efforts have been made leading to indeed less large foodborne outbreaks (EFSA opinion, 2008) and a reduced prevalence of *L. monocytogenes* in the food chain as observed in the present study compared to former studies performed in Belgium (Uyttendaele et al., 1999; Van Coillie et al., 2004). Probably this can be (at least partially) attributed to the setting of the new European hygiene legislation and the implementation of food safety management systems in the food producing companies. Still, continuous monitoring is needed to follow-up the trend in the prevalence of the pathogen in RTE foods. Further studies should consider not only presence/absence testing but also the level of contamination, preferably by enumeration methods with decreased detection limit, for example through carefully developed membrane filtration techniques (Besse et al., 2004, 2008), in order to gather quantitative data for risk assessment studies and thus contribute to improve attribution of risk for listeriosis to selected food categories.

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