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An improved version of the risk profiling for traditional smoked, fermented meat product

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An improved version of the risk profiling for traditional smoked, fermented meat product

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Summary

A risk profiling for smoked fermented sausages was developed for the following pathogenic bacteria: *Salmonella* spp., pathogenic *Escherichia coli*, and *Listeria monocytogenes*. The risk profile was developed with the use of Risk Ranger, a spreadsheet software format that embodies established principles of food safety risk assessment, i.e., the combination of probability of exposure to a food-borne hazard, the magnitude of hazard in a food when present, and the probability and severity of outcomes that might arise from the level and frequency of exposure. Risk Ranger scores of *Salmonella* spp., *L. monocytogenes* and *E. coli* EHEC were low for low risk population while moderate risk scores were obtained for high risk population. This may explain the fact that smoked fermented sausages are considered as generally safe products. Only in case of use of cross-contaminated ingredients and application of inadequate fermentation process, *Salmonella* spp., *L. monocytogenes* and *E. coli* EHEC may survive posing a health risk for consumers (moderate risk ratings). Previously (in D3.3.2) a risk profile was implemented based on the information collected so far in TRUEFOOD project (physico-chemical, microbiological data and consumer survey) for traditional Hungarian meat products. An expert estimate was developed with the risk profile techniques, which structured the available data and highlighted which product characteristics and process parameters had the greatest impact on the level of risks based on the flow chart of the HACCP plan, which determined the structure of MRA. The estimation produced in D3.3.2 was developed further by the use of probabilistic approach to get a more precise estimation of Exposure Assessment and Risk Characterization.

An improved version of the risk profiling for traditional smoked, fermented meat product

1 Risk profiling for traditional smoked fermented meat products

Background and rationale

Meat and meat products support the growth of several spoilage and pathogenic bacteria. Intrinsic factors such as pH and water activity do not prevent the growth of these microorganisms because of their initial values. The presence of pathogens on raw meat is the result of their contamination from the live animal, equipment, employees and environment. *Salmonella* spp., *Listeria monocytogenes* and pathogenic *Escherichia coli* have been implicated in food-borne outbreaks associated with the consumption of meat. *Salmonella* constitutes the most common agent involved in human outbreaks. In 2005, *Salmonella* was accounted for 64% (3406 out of 5355 outbreaks) of the reported food-borne outbreaks. Pathogenic *E. coli* (60 outbreaks) and *L. monocytogenes* (5 outbreaks) were other important causes of the recorded outbreaks in European Union (EU). However, the importance of a human illness is not dependant only on the incidence in the population because other factors such as severity of illness and mortality rate are also important. For example, while verotoxin producing *E. coli* (VTEC) and *L. monocytogenes* cause a relatively low number of cases (3314 and 1439 cases, respectively) compared to salmonellosis (176395 cases), nevertheless these infections are considered important because of their severity and their higher mortality rate. Listeriosis accounted for the highest number of reported fatalities (107) in 2004 (EFSA 2007).

Smoked fermented sausages are shelf stable meat products with extended shelf life. They are considered as safe products because the process used in their production are inhibitory to many pathogenic microorganisms. Thus, traditional fermented sausages are characterized by both improved quality and reduced risk of causing illness. However, when fermentation process is not adequate then there is a potential health risk because some pathogens may survive during fermentation (Adams and Mitchell 2002).

Risk analysis is a valuable tool in the management of microbial food safety issues and can provide a systematic approach for the regulatory authorities and the food industry to control the risk posed by a pathogen in a particular food commodity. Risk analysis consists of three elements: risk assessment, risk management and risk communication. Risk assessment is the scientific part of the process in which the hazards are identified and the risk posed by that particular hazard (i.e. pathogen) is calculated. The principle of risk assessment includes four stages, namely hazard

identification, exposure assessment, hazard characterization and risk characterization. In the case of fermented sausages each of the stages is presented below.

Hazard Identification

Smoked fermented sausages are made from raw materials such as meat which as previously mentioned can be contaminated from many sources and therefore several pathogens may be present. This may lead in a large list of pathogens potentially associated with fermented sausages. However, the number of pathogens present in fermented sausages may be limited after examination of various epidemiological data where fermented sausages have been implicated in food-borne outbreaks. The most common pathogens present in fermented sausages and therefore pose a greater health risk are *Salmonella*, *E. coli* (VTEC) and *Staphylococcus aureus*. As shown in a study, *L. monocytogenes* may also survive fermentation if the process is not adequate (Drosinos *et al.* 2006). Therefore, from the above pathogens only these considered in the project (*Salmonella*, pathogenic *E. coli* and *L. monocytogenes*) will be examined.

Salmonella spp.

Salmonella spp. is accounted for the most reported food-borne outbreaks. Eggs-egg products and meat are the main sources of outbreaks. *S. enteritidis* and *S. typhimurium* are the most frequently reported serovars. *S. enteritidis* is mainly associated with eggs and broiler meat, whereas *S. typhimurium* is most often associated with pig, poultry and bovine meat. Positive to *Salmonella* spp., up to 3-5%, ready to eat (RTE) meat products have occasionally been found which constitute a risk for human health (EFSA 2007). Temperature growth range is ranged between 7 and 50°C but it survives for long periods under refrigeration (4°C). *Salmonella* spp. is a heat-sensitive microorganism (60°C for 1-2 min) but it can survive in dry environments (growth range 0.94 to 0.99) for long time. pH growth range is between 3.8 and 9.5. Slow acidification (e.g. fermented sausages) may increase tolerance of the microorganism (acid adapted) to low pHs. Growth under aerobic conditions is faster in comparison with anaerobic conditions. Nitrites-nitrates at the usual concentrations allowed by the legislation do not inhibit growth (NZFSA 2007).

Pathogenic Escherichia coli

Pathogenic *E. coli* include the following sub-categories: entero-pathogenic *E. coli* (EPEC), entero-invasive *E. coli* (EIEC), entero-toxigenic *E. coli* (ETEC), entero-aggregative *E. coli* (EAEC) and entero-haemorrhagic *E. coli* (EHEC). The latter belongs to verotoxigenic *E. coli* (VTEC) producing vero-toxins or shiga-toxins (STEC). VTEC *E. coli* are of great concern because include the *E. coli*

O157:H7 which is the most common serotype isolated from the reported cases (EFSA 2007). *E. coli* O157:H7 has been frequently isolated from beef which is believed to be the main vehicle. However, other meats (e.g. pork) have also been implicated in outbreaks with *E. coli* O157:H7 or *E. coli* non-O157 STEC (EFSA 2007). Heat treatment and/or fermentation process are usually sufficient for producing a safe finished product. However, if these processes are not adequate then *E. coli* O157:H7 or *E. coli* non-O157 STEC may survive during manufacturing if the microorganisms are initially present in the raw material (Adams and Mitchell 2002). *E. coli* O157:H7 or *E. coli* non-O157 STEC grow within the temperature range of 7 and 46°C, and survive at chilled or frozen temperatures. They can also survive at low pH values (such as 3.6; growth range 4.4 to 9.0) and water activity (minimum for growth 0.95) (NZFSA 2007).

Listeria monocytogenes

L. monocytogenes is widely distributed in the environment and it can be found in many food commodities (NACMCF 1991). Post-processing contamination from the plant environment (equipment, personnel, floors, etc) is the most frequently reason for its presence on meat surface (Gudbjornsdottir *et al.* 2004). Various foods have been associated with *L. monocytogenes* outbreaks. Sliced raw meat products required cooking prior their consumption or sliced processed meat products are at greater risk for cross-contamination with *L. monocytogenes* than non sliced raw meat products (EFSA 2007). The pathogen is usually killed during pasteurization ($D_{70}^{\circ C} = 10$ to 15 sec) but it is capable to grow in foods stored at refrigeration temperatures (temperature range for growth -1.5 to 45°C). High salt concentrations (up to 10-11.5%), acid conditions (as low as 4.4) and water activity (0.92) do not allow *L. monocytogenes* growth but it may survive even under these stressful environmental conditions (ICMSF 1996). Fermentation process usually destroys *L. monocytogenes*. However, under inadequate fermentation conditions (e.g. inadequate pH reduction owned to low content of sugars) *L. monocytogenes* is capable of surviving (Drosinos *et al.* 2006).

Hazard Characterization

Hazard characterisation is deal with the effect of a hazard that will have on humans. It gives a description of the frequency, nature, severity and duration of illness caused by the hazard. However, there is considerable variability in virulence between pathogenic strains of the same organism. Human infection can differ between high (young healthy adults, the very young, the very old, the sick, pregnant women and the immuno-compromised) and low risk subpopulations (healthy people). The food vehicle can also play a significant role by protecting the pathogen from the effects of the stomach's acidity. In some outbreaks associated with fermented foods such as salami,

the level of pathogen present in the implicated food has been very low and this may be due to the protective effects of fat (Adams and Mitchell 2002).

Salmonella spp.

Salmonellosis is characterized by diarrhea, fever, vomiting and nausea. Long-term effects (e.g. reactive arthritis) can also be observed after gastrointestinal symptoms. Salmonellosis is usually self-limiting and mortality rate is very low (0.8%). Young (≤ 14 years), elderly ($\geq 60-65$ years) and immuno-compromised people are usually at higher risk than general population. Although high doses (10^5-10^6 cfu/g) are required to cause disease to the average consumer, low doses (5-50 cells) have been implicated in human outbreaks (high risk population) (NZFSA 2007).

Pathogenic Escherichia coli

The microorganisms cause illness due to toxin production after their invasion through the gut. Three severe forms of the disease are observed: haemorrhagic colitis (HC) (bloody diarrhea, abdominal pain, and vomiting), haemolytic uraemic syndrome (HUS) (kidney is the primary target of the toxins produced causing renal insufficiency) which is more often in children, and thrombocytopenic purpura, a version of HUS mainly observed in elderly (HUS symptoms, skin coloration, nervous system disorders and fever). Mortality rate is relatively low ($<1-5\%$). All group ages can be infected with children (≤ 4 years) and elderly people ($\geq 60-65$ years) to be more susceptible. Dose required to cause illness is relatively high (6×10^5 cfu/g), but for sensitive people the dose is below 100 cfu/g (NZFSA 2007).

Listeria monocytogenes

Two types of illness are associated with *L. monocytogenes*; invasive (fever, diarrhea, vomiting, headache, abortion and meningitis) and non-invasive (fever, diarrhea, headache, muscle pain and sometimes vomiting). Invasive type is more severe than non-invasive having a high mortality rate (ca. 30%). High risk population to invasive type includes elderly ($\geq 60-65$ years), immuno-compromised people, infants and pregnant women. Low levels of *L. monocytogenes* can cause listeriosis. In general, *L. monocytogenes* population of 10^2-10^3 cfu/g can cause listeriosis to high risk people. On the contrary, non-invasive type (febrile gastroenteritis) can occur in anyone, but a high number ($>10^5-10^6$ cfu/g) of *L. monocytogenes* is needed. (ILSI 2005; NZFSA 2007).

Exposure Assessment

Exposure assessment assesses the likely intake of a particular pathogen. To achieve this, the estimation of the level of pathogen or toxin in the food at the time of consumption and details of its consumption pattern are required (Adams and Mitchell 2002).

Pathogens prevalence and concentration

Prevalence and concentration (at the processing level) of pathogens taken into account during the preparation of the smoked fermented sausages risk profile were: *Salmonella* spp., 1.6%; pathogenic *E. coli*, 1.2%; and *L. monocytogenes*, 12.3% (EFSA 2007; NZFSA 2007). Initial load was taken into consideration per pathogen, in order to evaluate the increase needed to cause infection. Initial microbial load was considered after processing (*Salmonella* spp., 1-10 cfu/g; pathogenic *E. coli*, 0.04-0.4 cfu/g; *L. monocytogenes*, 0.04-0.1 cfu/g).

Food consumption patterns

Food consumption patterns were determined based on the 2005 FAOSTAT (2007) statistical data for the European population. Apart from EU population (466,085,066 people), meat consumption (1.85 kg per day per capita), production, imports and exports were also taken into consideration. It was also assumed that 25% of meat is converted to meat products (NZFSA 2007) in order to estimate the amount of available meat products for consumption. It was found that half (50%) of the population consumes meat products on a monthly basis. High risk population (infants, very young, elderly and immuno-compromised) was assumed to represent the 25% of the general population (Buchanan *et al.* 1997).

Risk factors, sources and places of infection

According to EFSA (2007) 2004 and 2005 reports, catering services, restaurants and institutions such as schools and aged care facilities were significant places of exposure to pathogens. Major reasons were bad food handling and/or preparation of meal by infected personnel. Meat and meat products were responsible for the 17-25% of the food-borne outbreaks in Europe and US during the period 1993-1998. Bovine, pork and poultry were accounted for the 7-10%, 5-7% and 3-5%, respectively (Olsen *et al.* 2000; Schmidt and Tirado 2001). For the smoked fermented sausages, it was assumed that 27%, from the 11% of smoked fermented sausages manufactured with contaminated ingredients, receive an inadequate fermentation or cooking process (Schmidt 1996).

Risk Characterization

The last step in the risk profile is the evaluation of the information derived in the previous stages in order to produce an estimate of the probability of illness and its severity in a given population. This can be quantitative, qualitative or semi-quantitative in the case of absence of quantitative data. In this work semi-quantitative estimation of the risk was performed by the mean of Risk Ranger software (Ross and Sumner 2002). It is an Excel-based software program which can be used in ranking the risk of various product/pathogen combinations answering a series of questions in relation to food safety and factors contributing to food safety risk such as susceptibility of the population of interest, severity of the hazard under concern, probability of exposure to the hazard under concern and probability of food containing an infectious dose (Table 1). The answers to the questions were relied on the data presented in the previous sections. In addition, the initial page of the Risk Ranger software with the relevant questions to be answered during the risk profiling process is presented in Figure 1.

Semi-quantitative estimation of risk in smoked fermented sausages

Total population considered was 466,085,066 people. The risk was estimated separately for high (25%) and low (75%) risk population because the hazards influence differently the population. Answers to questions 1 (hazard severity) and 2 (how susceptible is the population of interest) were based on the data of the Hazard Identification and Hazard Characterization for each pathogen. Questions 3 (frequency of consumption) and 4 (proportion of population consuming the product) were answered based on the food consumption patterns data. The size of consuming population in the question 5 was calculated according to total population considered, food consumption patterns and data from risk factors related to smoked fermented sausages. For example, the size of population consuming smoked fermented sausages was determined as follows: total population, 466,085,066 people; consumption frequency, 50% on monthly basis (233,042,533 people); products manufactured from contaminated ingredients, 11% (25,634,679 people); inadequate fermentation process, 27% (6,921,364 people). Thus, the population considered in the question 5 for the smoked fermented sausages were 6,921,364 people. Questions 6 (probability of contamination of raw product per serving) and 10 (increase needed in the post-processing contamination level to cause infection or intoxication) were answered according to the prevalence-concentration and Hazard Characterization data, respectively. The effect of processing (question 7) was answered as “process usually eliminates the hazard (99% in case of inadequate process)” and for the potential for recontamination after processing (question 8) the “No” option was selected because contamination after processing is unlikely for fermented sausages. The answer in the question 9 (how effective is

the post-processing control system) was “not relevant” because fermented sausages are stored at ambient temperatures. Finally, question 11 was answered based on food preparation before eating. Usually no preparation is made before consumption. However, growth of pathogen during storage is unlikely whereas further inactivation may occur under the conditions prevail in fermented sausages. Therefore, the option “preparation slightly reduces hazard” was selected. Risk was characterized as low (Risk Ranger ratings lower than 32), medium (Risk Ranger ratings from 32 to 48) or high (Risk Ranger ratings higher than 48) according to Sumner and Ross (2002).

Risk Ranger risk estimations

The results of risk estimation made by the Risk Ranger tool per pathogen for the smoked fermented sausages are shown in Table 1. Risk categorization was performed according to Sumner and Ross (2002) as described previously. Risk Ranger scores of *Salmonella* spp., *L. monocytogenes* and *E. coli* EHEC were low (lower than 32) for low risk population while moderate risk scores (32 to 48) were obtained for high risk population. This may explain the fact that smoked fermented sausages are considered as generally safe products. Only in case of use of cross-contaminated ingredients and application of inadequate fermentation process, *Salmonella* spp., *L. monocytogenes* and *E. coli* EHEC may survive posing a health risk for consumers (moderate risk ratings).

Table 1. Risk estimation for high and low risk population due to consumption of contaminated smoked fermented sausages.

Risk Ranger	<i>Salmonella</i> spp.		<i>Pathogenic Escherichia coli</i>		<i>Listeria monocytogenes</i>	
	High	Low	High	Low	High	Low
Question 1	Mild	Mild	Moderate	Moderate	Moderate	Mild
Question 2	Slight	General	Slight	General	Slight	General
Question 3	Monthly	Monthly	Monthly	Monthly	Monthly	Monthly
Question 4	Some (25%)	Most (75%)	Some (25%)	Most (75%)	Some (25%)	Most (75%)
Question 5	Other: 6921364	Other: 6921364	Other: 6921364	Other: 6921364	Other: 6921364	Other: 6921364
Question 6	6921364	6921364	6921364	6921364	Other: 12.3%	Other: 12.3%
Question 7	Other: 1.6%	Other: 1.6%	Other: 1.2%	Other: 1.2%	Usually	Usually
Question 8 ^c	Usually	Usually	Usually	Usually	eliminates	eliminates
Question 9	eliminates	eliminates	eliminates	eliminates	No	No
Question 10	No	No	No	No	Not relevant	Not relevant
Question 11 ^d	Not relevant	Not relevant	Not relevant	Not relevant	Other: 2.5×10 ⁴	Other: 2.5×10 ⁷
Probability of illness ^a	Other: 5×10 ¹	Other: 10 ⁶	Other: 2.5×10 ³	Other: 1.5×10 ⁷	Slightly reduces	Slightly reduces
Total predicted illnesses ^b	2.63×10 ⁻⁷	2.63×10 ⁻¹²	33	33	4.04×10 ⁻⁹	8.09×10 ⁻¹³
Risk ranking	33.2	0.00498	3.95×10 ⁻⁹	1.32×10 ⁻¹³	0.511	0.00153
	38	16	0.498	0.000249	33	13
			33	15		

^a Per day per consumer of interest

^b Per annum in population of interest

^c Contamination after processing is unlikely for fermented sausages

^d Because growth during storage is unlikely whereas further inactivation may occur under the conditions prevail in fermented sausages

Figure 1. Initial page of the risk profiling software 'Risk Ranger' with the 11 questions used for the estimation of risk.

A. SUSCEPTIBILITY AND SEVERITY

1 Hazard Severity

SEVERE hazard - causes death to most victims
 MODERATE hazard - requires medical intervention in most cases
MILD hazard - sometimes requires medical attention
 MINOR hazard - patient rarely seeks medical attention

2 How susceptible is the population of interest ?

GENERAL - all members of the population
SLIGHT - e.g., infants, aged
 VERY - e.g., neonates, very young, diabetes, cancer, alcoholic etc
 EXTREME - e.g., AIDS, transplant recipients, etc.

C. PROBABILITY OF FOOD CONTAINING AN INFECTIOUS DOSE

6 Probability of Contamination of Raw Product per Serving

Rare (1 in a 1000)
 Infrequent (1 per cent)
Sometimes (10 per cent)
 Common (50 per cent)
 All (100 per cent)
 OTHER

If "OTHER" enter a percentage value between 0 (none) and 100 0.0001%

7 Effect of Processing

The process RELIABLY ELIMINATES hazards
 The process USUALLY (99% of cases) ELIMINATES hazards
 The process SLIGHTLY (50% of cases) REDUCES hazards
 The process has NO EFFECT on the hazards
The process INCREASES (10 x) the hazards
 The process GREATLY INCREASES (1000 x) the hazards
 OTHER

indicates the extent of risk increase 1.00E-03

8 Is there potential for recontamination after processing ?

NO
YES - minor (1% frequency)
 YES - major (50% frequency)
 OTHER

If "OTHER" enter a percentage value between 0 (none) and 100 9.00%

9 How effective is the post-processing control system?

WELL CONTROLLED - reliable, effective, systems in place (no increase in patho)
 CONTROLLED - mostly reliable systems in place (3-fold increase)
 NOT CONTROLLED - no systems, untrained staff (10-fold increase)
 GROSS ABUSE OCCURS - (e.g. 1000-fold increase)
NOT RELEVANT - level of risk agent does not change

B. PROBABILITY OF EXPOSURE TO FOOD

3 Frequency of Consumption

daily
weekly
 monthly
 a few times per year
 OTHER

If "OTHER" enter "number of days between a 100g" 10

4 Proportion of Population Consuming the Product

all (100%)
 most (75%)
some (25%)
 very few (5%)

5 Size of Consuming Population

Australia
 ACT
 New South Wales
 Northern Territory
 Queensland
 South Australia
 Tasmania
 Victoria
 Western Australia
 OTHER

Population considered: 19,500,000

If "OTHER" please specify: 6,500,000

10 What increase in the post-processing contamination level would cause infection or intoxication to the average consumer ?

none
 slight (10 fold increase)
moderate (100-fold increase)
 significant (10,000-fold increase)
 OTHER

If "other", what is the increase (multiplicative) needed to reach an infectious dose ? 1.E+02

11 Effect of preparation before eating

Meal Preparation RELIABLY ELIMINATES hazards
 Meal Preparation USUALLY ELIMINATES (99%) hazards
 Meal Preparation SLIGHTLY REDUCES (50%) hazards
 Meal Preparation has NO EFFECT on the hazards
OTHER

If "other", enter a value that indicates the extent of risk increase 1.00E-03

RISK ESTIMATES

probability of illness per day per consumer of interest	$(P_{inf} \times P_{exp})$ 1.42E-07
total predicted illnesses/annum in population of interest	2.54E+02
RISK RANKING (0 to 100)	40

2. An improved version of risk profile for traditional Hungarian meat products

The estimation provided in D3.3.2 for traditional Hungarian meat products was improved by the probabilistic approach through the application of the *Sym'Previus* software (Leporq *et al.* 2005) that is also under continuous development in the context of WP3 to get more precise estimations. At this stage the 'Hazard identification' and the 'Hazard characterization' steps are not detailed as these have been reported previously in D3.3.2. Therefore 'Exposure assessment' and 'Risk Characterization' were further developed for traditional meat products with the *Sym'Previus* platform that was used for simulations in order to determine the behaviour of a given microorganism in the food matrix.

Exposure assessment:

At first challenge tests were carried out, where the results of Risk Profiling technique indicated that additional analyses would be needed. *Sym'Previus* was chosen for the Exposure Assessment step because bacterial growth in a given food matrix can be determined by just only one challenge test reducing thus the required number of expensive microbiological experiments. Based on the results of this fitting, further growth simulations can be carried out to have a realistic estimation of the possible level of pathogens at the end of shelf life.

Challenge tests

Challenge tests of traditional meat products were carried out based on an agreed procedure that was thoroughly discussed in WP3. We were surprised that many of the traditional Hungarian meat products (flamed sausages, dried and fermented sausages) did not support the growth of pathogens under consideration (*S. aureus*, *L. monocytogenes*) so no simulation on these products was feasible. A high water activity (*ca.* 0.95) traditionally fermented sausage was specifically manufactured for the purpose to record *Listeria monocytogenes* growth, but probably due to the competitive microflora sharp reduction in the numbers of the pathogen occurred. The detailed procedure of the testing and the results of challenge tests for validating the existing predictive models will be discussed in D3.3.6 "Report on the assessment of quantitative predictive models and validation under dynamic temperature profiles" (due to M36).

Pig cheese

There was only one product where growth of *L. monocytogenes* was experienced and this was vacuum-packed pig-cheese ('Disznósajt' in Hungarian), which is actually not a cheese but a meat

product. Small pieces of meat are mixed (especially from the head of the pig) with bacon pieces and stuffed inside the pig's stomach, then cooked in spicy water. There are many spices used, but mainly red paprika. Finally it is smoked like sausages and ham.

Challenge test procedure of pig cheese

This vacuum-packed, sliced product has 21 days of shelf-life as determined by the manufacturer. Prior to inoculation and during the challenge test, the physico-chemical properties of the product were measured, but hardly any change was evident:

- Salt content: 1.98 - 2.07%
- pH: 6.3 – 6.5
- a_w : 0.968 – 0.969

The products were inoculated with *L. monocytogenes* (ca. 10^3 cfu/g) isolated previously from meat products, and stored under controlled (5°C) and dynamic (fluctuating) temperature conditions. The dynamic conditions were simulated in a ventilated refrigerator according to a temperature program that was based on a real temperature fluctuation in the Hungarian cold chain (results presented in D3.1.5). Enumeration of *L. monocytogenes* and total viable counts was carried out in duplicate samples of pig cheese at 8 sampling times for an overall period of 28 days.

The results of the challenge tests are presented on Figure 2. It can be concluded from the graph that a rapid growth starts at day 7 and hardly any difference is evident between storage under dynamic and static (5°C) conditions.

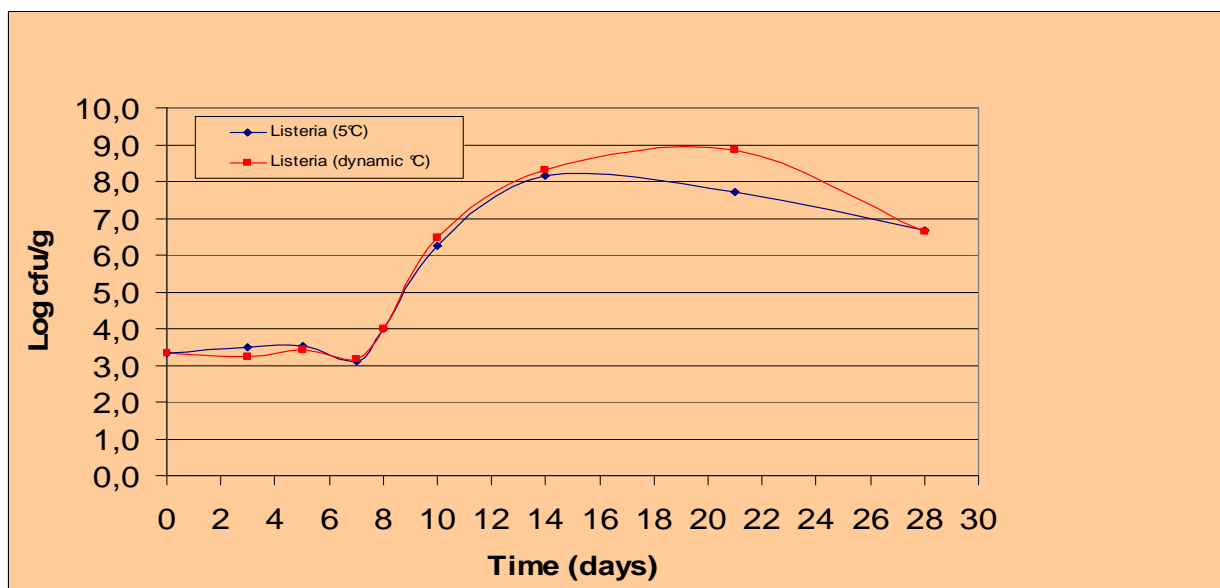


Figure 2. Results of *L. monocytogenes* challenge test in pig cheese stored at 5°C and under dynamic temperature conditions.

Growth curve fitting and simulation

The data points of the challenge test at 5°C were fitted with *Sym'Previus* and the kinetic values were determined. The results are presented in Figure 3.

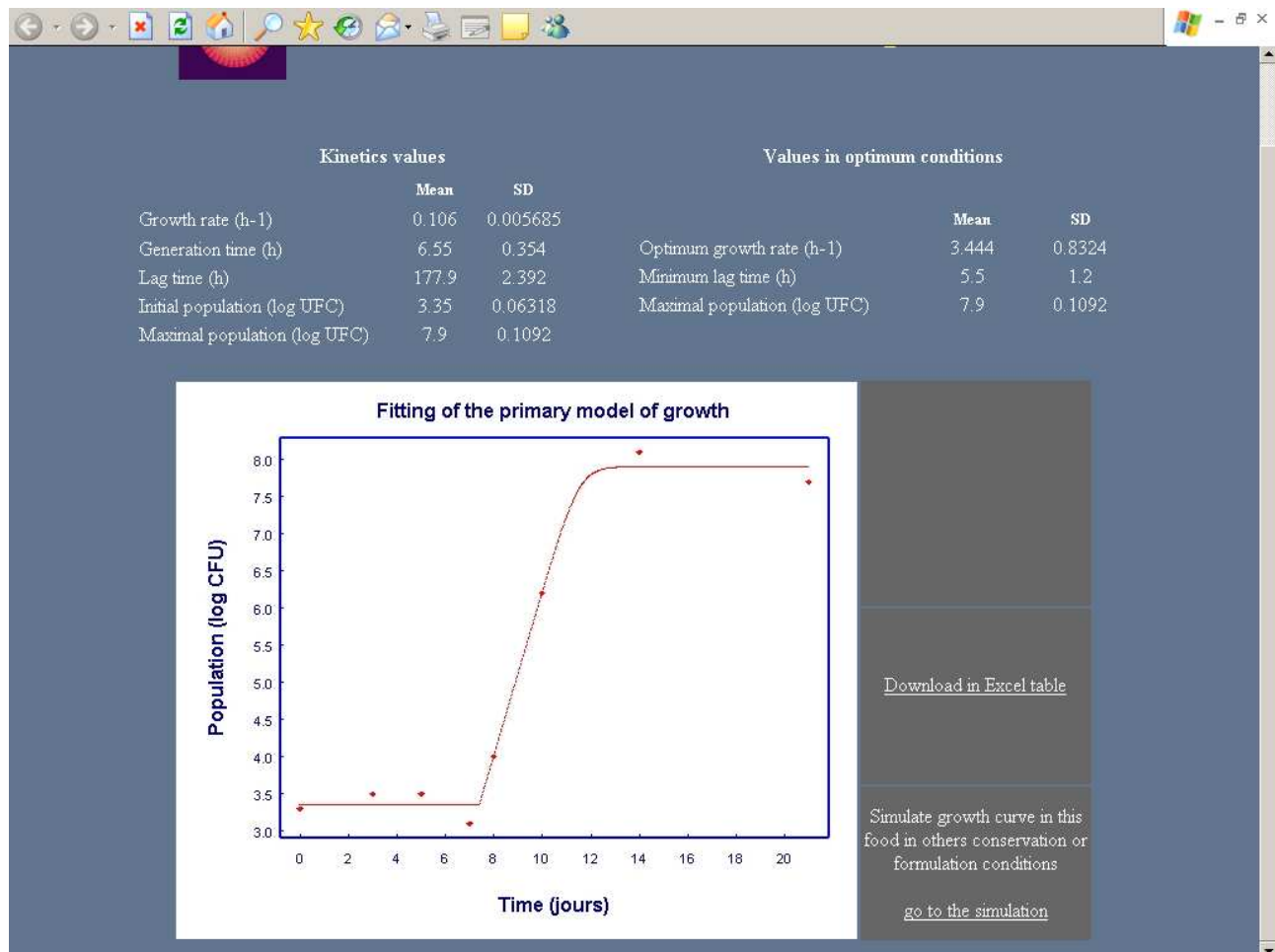


Figure 3. Results of fitting with *Sym'Previus* on the data points of pig cheese challenge test.

For the simulation, the probabilistic module of *Sym'Previus* was used where the kinetic values determined with the primary fitting were used to simulate accurately the growth of *L. monocytogenes* in pig cheese. A great advantage of *Sym'Previus* platform is that simulation can be carried out based on fragmented data. So a simulation can be carried out having only the results of one challenge test and the additional information of routine laboratory tests (e.g. presence/absence of a given microorganism). For the so called 'Autocontrol data' (presence/absence) option of the platform, the 2006 Monitoring Data on Traditional Meat Products of the authority database (Central Agricultural Office Food and Feed Safety Directorate) was used. According to this database 8 *L. monocytogenes* (1.5%) positive samples were found out of 539 surveyed cooked and smoked products like pig cheese.

Based on the food safety criteria of 2073/2005/EC regulation, the maximum contamination of *L. monocytogenes* at the end of shelf life cannot exceed 2 log cfu/g, and this value was taken as the

end point of the simulation. The weight of the final product was taken as 150 ± 5 g and dynamic temperature profile was based on the real temperature fluctuation in the Hungarian cold chain (T_{average} : 5.8°C , T_{min} : 4.5°C , T_{max} : 7°C).

The result of the probabilistic simulation with 90% confidence interval and the criterion limit set to 2 log cfu/g is presented in Figure 4.

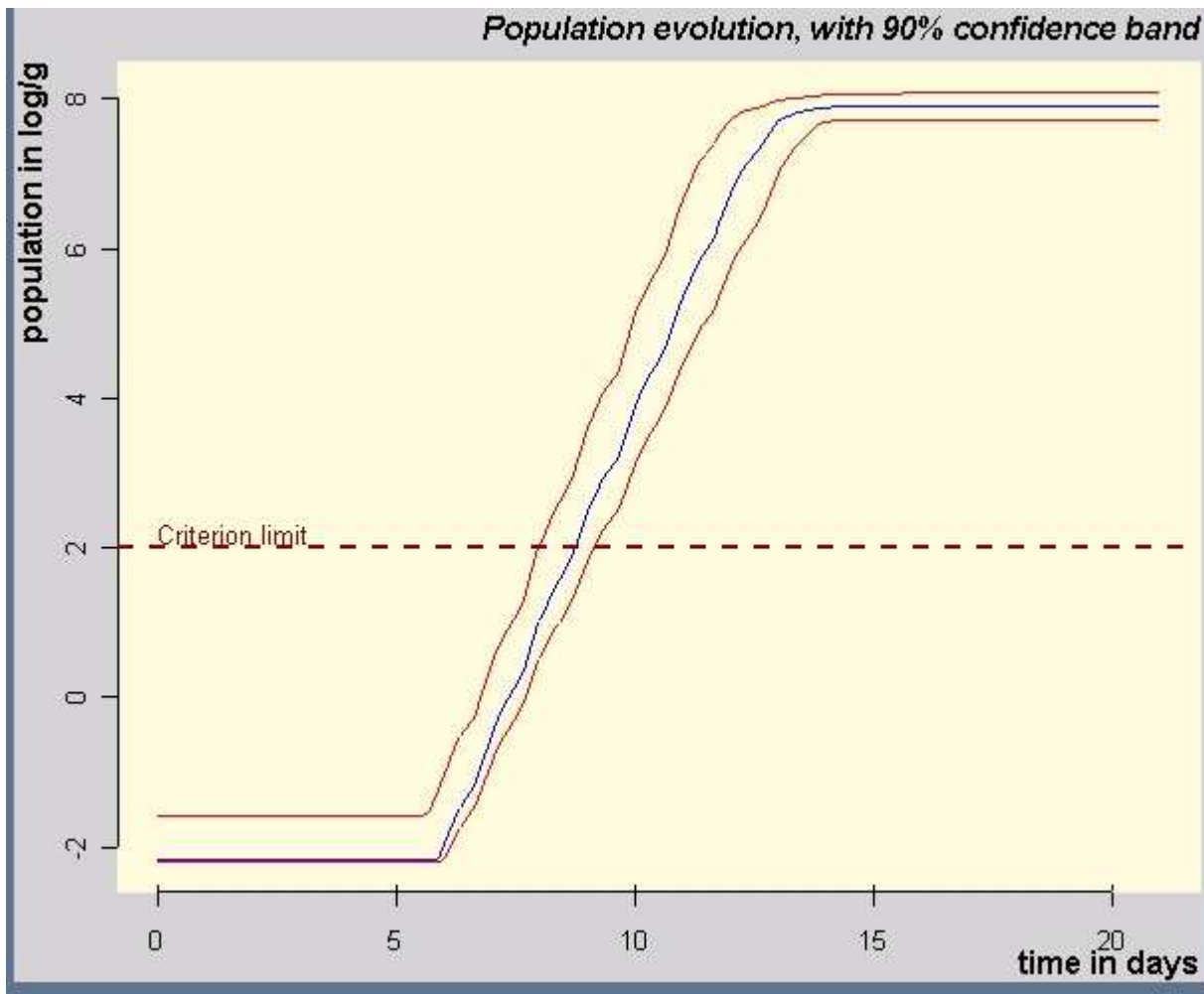


Figure 4. Result of *L. monocytogenes* growth simulation in pig cheese.

It is obvious based on the available 1) prevalence data of *L. monocytogenes* in cooked, smoked meat products in Hungary, 2) time-temperature distribution of the Hungarian cold chain, 3) the food safety criteria of *L. monocytogenes* set in 2073/2005/EC regulation, and 4) fitting results of a pig cheese challenge test with *Sym'Previus* that the manufacturer's shelf-life estimation (21 days) is not realistic and should be changed according to the result of the simulation.

Risk characterization

Based on Figure 5, which shows the evolution of the contamination distribution in terms of time, at 9.3 days and under dynamic (periodically fluctuating) temperature profile, 98.6% of the products on the market are over the safety limit set by the EU, which obviously poses a high risk for consumers.

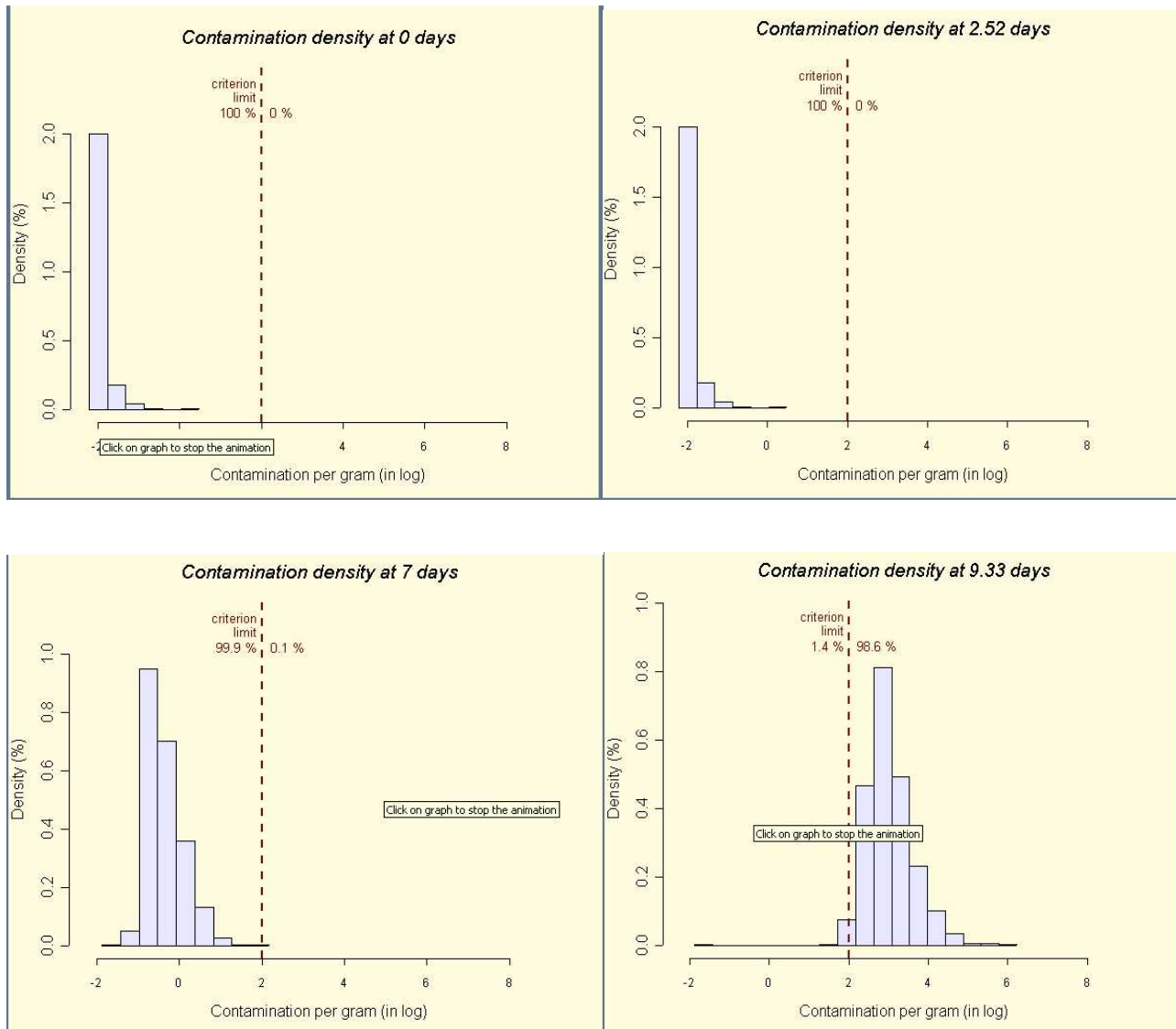


Figure 5. Evolution of *L. monocytogenes* contamination distribution over time in pig cheese under dynamic temperature condition

It is difficult to estimate the actual risk posed by the consumption of this product as: (1) traditional pig cheese is also prepared during pig killings according to several 'in-house' recipes, (2) no consumption data are available on a national level, and (3) the number of human listeriosis from the consumption of pig cheese is not registered. Furthermore it should be noted that the simulation

results of one challenge test based on the predictive model of *Sym'Previus* does not take into account the following factors: (1) human susceptibility, (2) strain variability, (3) competitive microflora and other ingredients, and (4) the variability in manufacturing processes. Despite the many uncertainties in the estimation, it is alarming how favorable conditions are for *L. monocytogenes* to grow in the pig cheese supply chain.

The microbiological safety of this product is based on the right sequence of hurdles. Hurdle technology is the application of many controlling factors to product in order to inhibit microbiological growth. Each of the hurdles is non-lethal on their own, but collectively they exert inhibitory power to prevent microbiological growth. Hurdle technology usually means inhibition of growth but not complete destruction of organisms.

The cooking step may inactivate any *Listeria* cells that are present (6D reduction), and any other vegetative form of microorganisms including the natural competitive microflora of the sausage, which represents a hurdle against growth of *Listeria* in the case of a potential cross contamination.

The next step in the production is to slice the product. It is well established that slicing and other handling operations associated with cooked and uncooked meats, can cross-contaminate with *Listeria* and other microorganisms at any operation and in any factories. Generally the risks are evaluated higher if the product has been heat-treated before slicing (i.e. the natural competitive microflora has been inactivated) and if the product will not be heat-treated before consumption. Most frequently cross contamination of the sliced meat products is caused by the slicing machine and the attached steps. Potential cross contamination cannot be fully avoided at slicing of any cooked/deli meat product at any operation, only the likelihood can be minimized to an acceptable level by maintaining high standards of hygiene – meeting requirements in high risk operations.

3 Concluding Remarks

The results of risk estimation made by the Risk Ranger tool per pathogen for the smoked fermented sausages showed that scores of *Salmonella* spp., *L. monocytogenes* and *E. coli* EHEC were low for low risk population, whereas moderate risk scores were obtained for high risk population. Only in case of use of cross-contaminated ingredients and application of inadequate fermentation process, *Salmonella* spp., *L. monocytogenes* and *E. coli* EHEC may survive posing a health risk for consumers. In addition, the results of the improved (probabilistic) version of risk profile for traditional Hungarian meat products suggested that most of the traditional meat products that were surveyed are safe due to the processing technology applied and the intrinsic inhibitory factors. The

method of the probabilistic assessment of traditional Hungarian meat products can be limitedly used, as the available modelling tools can't take into account many growth factors e.g.: competitive microflora. For the probabilistic assessment many challenge tests were carried out and surprisingly only one of the tested products (sliced, vacuum-packed pig cheese) supported the growth of the relevant pathogens (*Listeria monocytogenes* for this product). Even a high water activity ($a_w \sim 0.95$) traditionally fermented sausage was specifically manufactured for the purpose to record *Listeria monocytogenes* growth, but probably due to the competitive microflora sharp reduction in the numbers of the pathogen occurred. The probabilistic assessment for sliced, vacuum-packed pig cheese, which can be cross contaminated during slicing, showed that *L. monocytogenes* can grow above the safety limit set by the EU within half of the shelf-life time declared by the manufacturer.

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