AN ESSENTIAL PART OF THE FRISBEE SOFTWARE TOOL: IDENTIFICATION AND VALIDATION OF MODELS QUANTIFYING THE IMPACT OF THE COLD CHAIN ON RTE PORK PRODUCTS

V. STAHL[a], G. ALVAREZ[d] , E. DERENS[d], H. M. HOANG[d], A. LINTZ[a], B. HEZARD[a], M. EL JABRI[b], J.F. LEPAGE[b], F.T. NDOYE[d], D. THUAULT[b], S.G. GWANPUA[c], P. VERBOVEN[c], A. GEERAERD[c]

[a] Aérial, rue Laurent Fries 67412 Illkirch, France (v.stahl@aerial-crt.com)

[b]ADRIA Développement Z.A Creac'h Gwen 29196 Quimper, France

[c]BIOSYST-MeBioS, K.U.Leuven, Leuven, Belgium (annemie.geeraerd@biw.kuleuven.be)

[d]Irstea. Refrigeration processes engineering research unit, 1 rue Pierre-Gilles de Gennes 92761 Antony, France.

ABSTRACT

Food business operators producing Ready-To-Eat (RTE) foodstuffs must be able to demonstrate that the products will comply to regulatory specifications in terms of food safety. At the same time, various food quality aspects are also important to ensure the economic position of the food producers. For refrigerated products, it is obvious that the actual time-temperature profiles a food product undergoes in the cold chain, is of paramount importance for guaranteeing food safety and quality. This work is part of the EU-FP-7 project FRISBEE. More specifically, the objective of this work is to develop, identify and validate kinetic models for RTE pork meat products like pasteurized ham, pâté and raw ham. Hereto, dedicated storage experiments are designed and conducted. Attributes were identified: quality indicators including texture, drip-loss, water-loss, colour and microbiological indicators including *Listeria monocytogenes* and lactic acid bacteria. In parallel, heat transfer models were also identified and validated allowing to link the temperature in a certain step of the cold chain with the actual temperature as experienced by the food products. The developed models are an essential part of the FRISBEE software tool: a user-friendly software application that allows to mimic the effect of realistic time-temperature profiles in the cold chain on the final product safety and quality when reaching the consumer, and that at the same time calculates the energy requirements and environmental impact of the cooling technologies being part of the simulated cold chain.

1. INTRODUCTION

Many models have been developed to explain the temperature evolution and resulting food product quality and safety along cold chains, but they have not been yet combined into a user-friendly software from upstream to downstream of the food chain. Moreover, although refrigeration is very important in extending the shelf life of perishable products, it has a setback of being a major user of energy and a contributor to global warming. This FRISBEE part of work focuses on a framework that is currently being developed to evaluate energy consumption, environmental impact and associated food quality and safety attributes in the European cold chain. As a first step, reference products were to be chosen for different product categories classified as chilled, frozen and super-chilled/super-frozen. Within the category "chilled products", apple was selected as reference fruit type, ready-to-eat (RTE) pork meals as reference pork meat product and salmon fillets as reference fish product. The quality and safety indicators for the different products were selected. Quality and safety aspects are a real concern for RTE foods, partly because they will not receive a heat-treatment (ensuring the destruction of major bacteria) before consumption. Food business operators are responsible of the compliance with the safety criteria throughout the shelf-life (Anonymous, 2005).

More specifically, the objective of this work was to develop, identify and validate kinetic models for RTE pork meat products like pasteurized ham, pâté and raw ham. Kinetic data at several temperatures over a fairly large temperature range were required in order to test the development and applicability of the models and in order to study the effect of temperature. Dedicated storage experiments are designed and conducted. Relevant attributes were identified: quality indicators including texture, drip-

loss, water-loss, colour and microbiological indicators including *Listeria monocytogenes* and lactic acid bacteria.

2. MATERIALS AND METHODS

2.1. Bacteria strains, *Listeria monocytogenes* and lactic acid bacteria

Listeria monocytogenes (SOR 100; n° 352 Aérial (F)) is a reference strain of the national French program in predictive microbiology Sym'Previus (Couvert et al., 2010), isolated from industrial meat food environment. *Lactobacillus sakeii* 1322 and *Leuconostoc mesenteroïdes* 74 are two strains from the Aerial collection (stored at -80°C), isolated from chilled meat products.

2.2 Ready-to-eat pork meals products

The following food products packed under modified atmosphere (50% CO_2 – 50% N_2), obtained from manufacturers, were studied: i) sliced cooked ham, ii) sliced cooked pâté, iii) sliced raw, salted and smoked ham.

2.3 Optical density data

The aim is to estimate cardinal temperature values. Several approaches have been proposed to predict microbial growth in foods. Among those, Cardinal Parameter Models used parameters having a biological significance. They are considered intrinsic to the strain and independent of the growth medium in which growth takes place (Rosso et al, 1995). The effect of T is described by T_{min} (minimal value to growth), T_{opt} (optimal value to growth), and T_{max} (maximal value to growth). The methodology is described for the species *Listeria monocytogenes* (Pinon et al, 2004). The methodology has been applied on the two lactic acid bacteria strains with an adaptation to the incubation medium of the strain: Elliker medium was used instead of BHI (Brain Heart Infusion broth). Turbidity growth curves of the strains were generated with an automatic Bioscreen C reader (Labosystemes Honeycomb 2 France SA).

2.4 Microbiological challenge-tests

(1) Experimental *Listeria monocytogenes (Lm)* challenge testing were performed at 5°C; 8°C; 12°C; 15°C, under modified atmosphere (50% CO_2 and 50% N_2) in one batch for each RTE product per origin producer. Challenge tests used a reference strain of *L. m* SOR 100. One physiological state was used in this study: cells at the end of the exponential growth phase. Two subcultures of *L. m* were grown in Brain Heart Infusion (BHI) at 37°C for respectively 16 h and for 8 h. A third subculture was carried out at 8°C for 6 days, in Brain Heart Infusion broth to obtain, for the inoculum 10^9 CFU/ml in the late exponential growth phase. Samples of 10 g of product were inoculated with 0.5ml of the diluted subculture to obtain an initial concentration of approximately 10^2 CFU/g at the surface of the food product. Control samples were constituted without inoculation with *L. m*. Natural lactic acid flora [(1ml was plated into MRS (according to NF V 04-503)], aerobic microorganisms (1ml was plated into PCA, 30°C three days, pH [Hanna instruments HI 213 model (5g); according to NF V04-108),], and a_w (GBX-FA-St 1 model; NF ISO 21807: 2004) were quantified at three days during challenge testing to characterize the variability of the physico-chemical characteristics and natural flora of the batch of the products.Twelve growth curves of *Lm* in mixed-culture (potential presence of natural flora) were obtained at four levels of temperature (5°C ; 8°C ; 12°C ; 15°C) under modified atmosphere (50% CO_2 and 50% N_2). 10g samples were homogenized with 90ml of tryptone salt solution using a stomacher blender. Ten-fold serial dilutions were carried out and 0,1ml of the appropriate dilution was plated onto Compass *L. monocytogenes* (37°C; 48h)] for the quantification of *L. monocytogenes*. The enumerations of *L. monocytogenes* were performed according to the ISO 11290-2 standard on three samples at 10 or 11 different times during the lag, the exponential and the stationary phases of the growth curve.

(2) Lactic acid bacteria challenge-testing were performed with one batch of pasteurized ham, one batch of pasteurized pâté from one producer. Three growth curves were obtained at 8°C under modified atmosphere (50% CO_2 and 50% N_2) in "pure-culture" for each strain inoculated separately (i.e, challenge-testing in the ionized product (Aérial, France/BGS, Germany)). Three subcultures of *Lb. sakeii* 1322 and *Lc. mesenteroïdes* 74 strains were grown at respectively 30°C; 30°C and 8°C in

Elliker media. Samples of 10g of product were inoculated with 0,5ml of the diluted subculture to obtain an initial concentration of approximately 10^2 CFU/g in the foodstuff. *Lb. sakeii* 1322 and *Lc. mesenteroïdes* 74 were quantified at regular time intervals to obtain data points representing all of the parts of the growth curve (lag, exponential and stationary phases). The 10g samples were homogenized with 90ml of tryptone salt solution using a Stomacher blender. Ten-fold serial dilutions were carried out, then 0,1ml of the appropriate dilution was plated onto MRS (according NF V 04-503) for the enumeration of the both strains inoculated separately. pH and aw were measured. Control samples were constituted without inoculation with lactic acid bacteria.

2.5 Physico-chemical quality attributes measurements

Colour, firmness, water retention capacity and water content have been followed during time depending on static temperature conditions (3, 8, 12 and 15°C). Quantification of the colour change was based on measurement of CIE Lab values (CIE, 1978) with a CM-2002 Minolta Spectrophotometer® (Minolta Co., Japan). A standard white plate (Calibration plate CM-2002) was used to standardize the instrument under "C" illuminant condition according to the CIE. Three pieces of each product (pâté, cooked ham, raw ham), were measured at frequent time intervals during their storage at the different temperatures conditions. The water content has been determined on weighed samples of each product (around 10g), placed during 24h in an oven at 103°C and weighed again. The water activity (aw) has been measured with an aw-meter (Decagon Aqualab 4TE, AquaLab International, U.S.) on thin samples of each product, avoiding the fat to obtain better repeatability in the measurements. The accumulated drip loss measurement may be an easy way to observe a global effect of alteration in the meat product matrix, allowing a leaking of fat or water to the outside. In order to estimate the exudates of fat and water of each product along storage time depending of temperature conditions, the change of weight before and after wiping has been measured. The surface of around 200g of product has been wiped with absorbing paper. At each temperature and step time, and for each product, three measurements were realized on three different slices. Firmness was measured using a compression test on the pâté, a penetration test on the two hams. Each measurement was realized on products at ambient temperature (20°C). For the compression test on a cylindrical sample of pâté (2 cm in height, 2.5 cm in diameter), a cylinder of 25 mm diameter was selected to compress the product. Constant penetration speed was applied on the pâté until rupture. The cylinder probe approaches the sample at a speed of 0.2 mm/sec and mashes the sample of pâté. A force-distance curve is obtained and texture parameter (firmness) is determined as the maximum strength until rupture. For penetration tests on raw salted ham type bacon and cooked ham, a conical probe was selected to penetrate the product. Constant penetration speed was applied on the hams until perforation. The probe approaches the sample at a speed of 0.2 mm/sec and penetrates through the slice of ham. A force-distance curve is obtained and texture parameter (firmness) is determined as the maximum strength until rupture.

2.6 Bacterial model parameters estimation and model validation

2.6.1 Cardinal values of temperature

The cardinal values of *L. monocytogenes* were those acquired in Sym'Previus. It corresponds to a pathogen strain which has the ability to grow at low temperature. For lactic acid strains, the cardinal values were estimated from growth kinetics obtained by Bioscreen (2.3). A secondary model which describes the influence of temperature on the growth rate (Pinon et al., 2004) was employed to estimate the cardinal values of temperature (T) (1):

$$\gamma(T) = \begin{cases} \dfrac{(T - T_{\max}).(T - T_{\min})^2}{(T_{opt} - T_{\min})\left[(T_{opt} - T_{\min}).(T - T_{opt}) - (T_{opt} - T_{\max}).(T_{opt} + T_{\min} - 2.T)\right]} & \text{if} \quad T_{\min} < T < T_{\max} \\ 0 & \text{otherwise} \end{cases}$$

2.6.2 Bacteria growth kinetics and modeling

The growth kinetics were acquired at different static levels of temperature. The population size as function of time was described by the primary model of Rosso (Rosso et al., 1996). This model provides a good accuracy using only four descriptive parameters: lag time (lag), growth rate (μ_{max}), initial population size ($\mathbf{N_0}$) and the maximum population size **(**N_{max}**)** (2)

$$\ln(N) = \begin{cases} \ln(N_0) & ,t \le lag \\ \ln(N_{\max}) - \ln\left(1 + \left(\frac{N_{\max}}{N_0} - 1\right)e^{-\mu_{\max}(t-lag)}\right) & ,t > lag \end{cases}$$

The differential form of this equation is given (3) $\begin{cases} \frac{dN}{dt} = 0 & ,t \le lag \\ \frac{dN}{dt} = \mu_{\max}.N\left(1 - \frac{N}{N_{\max}}\right) & ,t > lag \end{cases}$

2.6.3 Estimation of growth parameters and validation

The maximum growth rate (μmax) and the lag time (*lag*) were estimated for each growth curve obtained at 8°C by fitting the logistic with delay growth model (Rosso et al., 1996; Pinon et al., 2004). The optimal growth rate μ_{opt} and the minimal lag time, lag_{min} are the maximum specific growth rate and lag time values when T, pH and aw are set to their optimal values. They depend on both strain and food matrix (Pinon et al., 2004). They are calculated according to the following equations**:**

$\mu_{max} = \mu_{opt}.\gamma(T,pH,aw)$ (4) and $lag = lag_{min} / \gamma(T,pH,aw)$ (5)

where γ(.) are gamma factors that help to quantity effect of environmental factors.

These values were then used to validate the estimation of growth parameters μ_{max} and *lag phase* at three temperature levels (5°C, 12°C and 15°C). The growth rate μ_{max} is estimated with Rosso model (2). It was then compared with the estimation using gamma concept of Zwietering (Zwietering et al., 1996) allowing the decomposition of μ_{max} (6):

$$\mu_{\max}(T) = \mu_{opt}.\gamma(T)\gamma(pH)\gamma(aw)\gamma(\text{int})$$

For a given temperature T(°C), the lag phase was estimated according the kinetic growth model of Rosso (1) and it was then compared to the lag time estimated according to the following equation (7):

$$Lag(T) = \frac{\mu_{opt}.Lag_{\min}}{\mu_{\max}(T)}$$

Root Mean Squared Error (RMSE) statistical indicator was then used to compare all these estimations.

2.6.4 Estimation of the bacterial growth under dynamic temperature

At each given time-temperature couple, the growth rate (μ_{max}) was estimated following the equation showing the gamma concept (eq. 6). The lag phase under dynamic conditions was evaluated following the model which calculates the lag time under fluctuating temperature. It takes into account the influence of the physiological state in predictive models based on the following equation (8):

$$\lambda\mu = \mu_i \cdot (lag_i - t_i),\ t_i \le lag_i$$

Where μ_i (h^{-1}) is the maximum specific growth rate of the inoculum at the temperature T_i (°C), lag_i(h) is lag time of the inoculum at the temperature Ti, and ti(h) is the duration of the pre-incubation at the temperature Ti. (Augustin et al., 2000). Considering μi*lagi = μopt*lagmin **(9),** the remaining lag_{min} could be evaluated by the following equation:

$$Lag^{r}_{\min} = Lag_{\min} - \frac{t_i\,\mu_i}{\mu_{opt}} \textbf{ (10)}$$

The model predictions of *L. monocytogenes* were compared with Sym'Previus (Couvert et al, 2010) tool dealing with *L. monocytogenes* in the considered food products.

3. RESULTS & DISCUSSION

3.1 Bacterial kinetic models of chilled ready-to-eat pork meals

3.1.1 Cardinal temperature values

Cardinal temperature values of the strain of *L. monocytogenes* and for both lactic acid bacteria strains, *Leuconostoc (Lc.) mesenteroïdes* 74 and *Lactobactillus (Lb.) sakeii* 1322 were acquired in liquid microbiological media (table 1).

Table 1. Cardinal temperature values (T°C) of *L. monocytogenes*, *Leuconostoc mesenteroïdes* 74 and *Lactobactillus sakeii* 1322 and estimated in liquid microbiological media

Cardinal T(°C) values	*L.monocytogenes*	*Lc. mesenteroïdes* 74	*Lb. Sakeii 1322*
T_{min}	-2,50	-0,53	0,25
T_{opt}	38,22	28,04	33,02
T_{max}	42,84	36,05	39,12

3.1.2 Estimation of bacterial growth parameters in cooked ham and pâté, raw salted, smoked ham

The process of cooking has been demonstrated to be sufficiently destructive to the Gram-positive and Gram-negative foodborne pathogens and several major background flora like lactic acid bacteria. Industrially-produced cooked ham and pâté products are exposed to the environment post-lethality and handled prior to final packaging (Mejlholm et al., 2010). Raw bacon has been shown to *contain L. monocytogenes* (Angelidis et al., 2006). The evolution of *L. monocytogenes* in "mixed-culture (i.e., in the non-ionized foodstuff inoculated), of *Lb.sakeii* 1322 and of *Lc. mesenteroïdes* 74 (i.e., in the ionized foodstuff inoculated) at different levels of temperature during storage period were studied for the three RTE pork meals. A significant increase in the concentration of *Lm* was observed during the study period at each temperature studied for pasteurized ham and pâté. Initial pH values and initial a_w values classify the products in the food category allowing growth of *Lm*. Physico-chemical parameters (pH, a_w) did not significantly evolve along the studied periods. Whatever the temperature, other microflora (natural lactic acid bacteria, aerobic mesophilic bacteria) were naturally present on pasteurized ham and pâté. They did not significantly affect growth potential of *Lm a*s maximum level reached were above 8 $\log_{10}$ (cfu/g).

An increase in the concentration of the *Lb. Sakeii* 1322 and *Lc. mesenteroïdes* 74 studied independently is also observed at 8°C in "pure-culture (without endogen flora), during the study period. The maximum levels reached were above 8 $\log_{10}$ (cfu/g). pH and aw values permit growth.

All the curves at each temperature, for each strain and each foodstuff, were fit separately with the primary model and permitted to assess values of μ_{opt} and lag_{min}. Growth parameters estimated were summarized in table 2 and table 3.

Table 2. Challenge tests performed to study the behavior of *L. monocytogenes* and growth parameters obtained at different temperatures in RTE pork meals, under modified atmosphere, 50 % CO_2; 50% N_2

Ham [(a)]	T°C	N_0[(b)]	N_{max}[(b)]	Lag(h)	$\mu_{max}(h^{-1})$	$\mu_{opt}(h^{-1})$	$Lag_{min}(h)$	γ(T)
pH 6.31 ; SD 0.04	5	2.34	8.56	100	0.0233			
a_w 0.98 ; SD 0.005	8	2.51	9.01	73.92	0.0361	0.7525	3.55	0.0828
	12	2.58	9.11	22.9	0.0746			
	15	2.35	8.67	12.1	0.103			

Pâté [(a)]	T°C	N_0[(b)]	N_{max}[(b)]	Lag(h)	$\mu_{max}(h^{-1})$	$\mu_{opt}(h^{-1})$	$Lag_{min}(h)$	γ(T)
pH 6.45 ; SD 0.01	5	2.75	8.77	10.56	0.0230			
a_w 0.986 ; SD 0.003	8	2.75	8.39	18.62	0.0472	0.7537	1.17	0.0828
	12	2.75	9.18	8.74	0.0974			
	15	2.75	9.05	7.87	0.1260			

(a) Characteristics; SD: Standard Deviation (b) in log10(cfu/g)

It should be noticed that both the mean and the coefficient of variation of μ_{opt} are consistent with those obtained by Augustin et al. (2011) for cooked ham and cooked pâté.

Table 3. Challenge tests performed to study the behavior of *Lb. sakeii (Lb. s.)* 1322 and *Lc. mesenteroïdes (Lc. m.)* 74 and growth parameters (in pure-culture) obtained at 8°C in RTE ham (a) and pâté (b), under modified atmosphere (50 % CO_2; 50% N_2)

*Lb. s.*1322	$N0^{(a)}$	$N_{max}^{(a)}$	Lag(h)	$\mu_{max}(h^{-1})$	$\mu_{opt}(h^{-1})$	$Lag_{min}(h)$	γ(T)
Ham	1.8	8.91	7.368	0.108	2.84	0.28	0.0798
Pâté	1.69	8.67	37.68	0.0942	1.97	1.80	0,0798

Lc. m. 74	$N0^{(a)}$	$N_{max}^{(a)}$	Lag(h)	$\mu_{max}(h^{-1})$	$\mu_{opt}(h^{-1})$	$Lag_{min}(h)$	γ(T)
Ham	2.38	9.07	1.1304	0.0896	1.4	0.07	0.1486
Pâté	2.27	9.18	5.34	0.0969	1.17	0.44	0,1486

No growth of *L. monocytogenes* in "mixed-culture" raw, salted, smoked ham from producer 1 at 3 levels of temperature (5°C; 8°C; 15°C) is observed during the study period at each temperature (Table 4). Aw levels of raw ham from producer 1 (0.904 mean value) do not permit growth. No growth of *Lm* is also observed during the study period at each temperature for the origin 2. pH and aw levels permit potential growth. Initial natural lactic acid bacteria population was enumerated at high levels (9 log cfu/g). These environmental conditions are correlated with the process of origin 2. The hypothesis is that several factors in interaction inhibit *Listeria monocytogenes*. Other inhibitors but also high level of endogen lactic acid bacteria could potentially be the cause of the inhibition of the pathogen bacteria (Cornu et al., 2011).

Table 4. Challenge tests performed to study the behavior of *L. monocytogenes* and growth parameters obtained at different temperatures in raw, salted, smoked ham, packed under modified atmosphere 50 % CO_2; 50% N_2

Raw, salted, smoked ham (characteristics mean; SD)	T(°C)	$N0^{(a)}$	$N_{max}^{(a)}$	Lag(h)	$\mu_{max}(h^{-1})$
Origin producer 1-process 1	5	2.22	2.22	∞	0
pH 6.19/ SD 0.16	8	2.15	2.15	∞	0
aw 0.904/ SD 0.014	15	2.25	2.25	∞	0

Raw, salted, smoked ham (characteristics mean; SD)	T(°C)	$N0^{(a)}$	$N_{max}^{(a)}$	Lag(h)	$\mu_{max}(h^{-1})$
Origin producer 2 – process 2	5	2.56	2.56	∞	0
pH 5.72/ SD 0.02	8	2.54	2.54	∞	0
aw 0.969/ 0.001	12	2.61	2.61	∞	0
	15	2.6	2.6	∞	0

3.1.3 Validation

Optimal growth rate and lag phase (μopt and lag_{min}) determined at 8°C were used to validate the estimation of growth parameters μ_{max} and lag phase at the three temperature levels, 5°C, 12°C and 15°C. The growth rate μ_{max} was estimated with Rosso model (1). It was then compared with the estimation using gamma concept of Zwietering (Zwietering et al., 1992) allowing the decomposition of μmax (Eq. 6). For a given temperature T(°C), the lag phase was estimated according the growth kinetic model of Rosso (Eq. 2) and it was then compared to the lag time estimated according to the following equation :

$$Lag(T) = \frac{\mu_{opt}.Lag_{\min}}{\mu_{\max}(T)}$$

Root Mean Squared Error (RMSE) statistical indicator was then used to compare all these estimations. Table 5 show the results of both estimated and predicted parameters obtained in the case of pasteurized pâté. There is no significant difference observed between estimated growth rates (μmax)

and predicted (μmax) at different temperatures. The RMSE value of μmax is relatively small in the two cases. The statistical indicator, RMSE used permit to validate the approach. With regard to the lag time results, significant differences are observed between estimated and predicted lag times. In most cases the model overestimates this parameter. For lag time, the prediction is larger than the estimated one and therefore unsafe. The simulation of the lag is complex in predictive microbiology as it depends on many factors. Taking into account the estimations of μ_{opt} and lag_{min} temperature allowed to estimate the growth parameters in other temperature conditions in the case of *Listeria monocytogenes*. Good results of growth rate were obtained also in the case of pasteurized ham.

Table 5. Pasteurized pâté – RMSE indicator results

T°C	Estimated μ_{max}	Predicted μ_{max}	Estimated lag	Predicted lag
5	0.0230	0.0240	10.56	38.22
8	0.0472	0.0470	18.62	18.62
12	0.0974	0.0891	8.74	9.02
15	0.1260	0.1292	7.87	6.98
	RMSE(μ_{max})	0.0045	RMSE(lag)	13.92

3.1.4 Development and validation of models or equations

Models, their parameters and the initial conditions used to describe bacterial behavior per RTE pork meals are summarized in table 6.

Table 6. Models, their parameters and the initial conditions used to describe *L. monocytogenes*, *Lb. sakeii* 1322 and *Lc. mesenteroïdes* 74 per pasteurized ham and pâté and raw ham

Predicted parameter	Equation or model	Used parameters
Bacterial population	Eq. (3)	Fixed: pH, a_w (initial mean, SD)
Maximum growth rate	Eqs. (4) and (1)	Calculated : N_o(log CFU/g), N_{max}(log CFU/g), μ_{max} (h^{-1}), lag(h)
Lag time	Eqs. (8) and (10)	T_{min}, T_{opt}, T_{max}
		μ_{opt} (h^{-1}) Lag_{min}(h) Gamma (T)

These equations are valid for static and dynamic temperatures between 0°C and 15°C.

3.1.5 Growth simulation of *Listeria monocytogenes* under dynamic temperature

The algorithm implemented in Matlab developed under Frisbee project (the model used allows to describe the influence of temperature on the lag time for the regrowth of *Lm* (Augustin et al, 2000)), to predict the bacteria behavior under dynamic temperature. A temperature profile 1/3 at 4°C and 2/3 at 8°C over shelf life period of 21 days is chosen for pâté and ham. The algorithm used in Matlab is different from that of Sym'Previus and the simulation results obtained with both matlab code and Sym'Previus software are convergent. This approach could be applied for *Lactobacillus sakeii 1322* and *Leuconostoc mesenteroïdes 74.*

3.2 Physico-chemical quality attributes evolution for chilled ready to eat pork meals and equations

Color, firmness, water retention capacity (drip loss) and water activity were measured for the three RTE foodstuffs. Storage experiments were conducted at static temperatures, 3°C, 8°C, 12°C and 15°C. The objective was to identify and develop kinetic models or equations of the selected quality indices to quantify the impact of temperature. The results are summarized table 7. RTE pork meals colour was constant during the storage period at each T. For all RTE products studied, the water content remains the same along their shelf life. The water activity relates to the quantity of water not strongly bound to the matrix, and which is able to react with the environment and in biological or physico-chemical reactions. For all products studied, the water activity remains the same during the experiments. The values of drip loss change during the storage. The greater loss appears for the pasteurized ham (a loss lower than 3g in the most unfavorable conditions of storage (15°C)). The loss is not relevant in regard

of the 200g of studied product. For the raw salted ham type bacon and the pâté, the losses obtained are even lower, <0,3g and <0,4g respectively. These variations, in %, in regard of the standard errors are not relevant to discriminate different behaviour between each product. The loss in g measured are really small compared to the initial mass, and thus should not be considered significant. The firmness values were studied. The burst strength (=maximum force to rupture) was found to be constant when plotted with time for every storage temperature studied. Same observations have been made in the publications (Jeremiah et al.,1997; García-Esteban et al., 2004).

Table 7: Equations, parameters describing physico-chemical properties of raw, salted, smoked ham like bacon(a), pasteurized ham (b) and pasteurized pâté (c), under modified atmosphere.
These equations are valid for static and dynamic temperatures between 0°C and 15°C. The threshold levels indicate the level where the change in the quality indicator is considered significant.

(a)

Predicted parameter	Equation or model	Used parameters
Drip loss	Y = constant	Mean Y = 0,27 % ; Std = 0.31% Threshold: 5% of the initial mass
Water content	Xw=constant	Mean X w = 52,0%; Std = 2,4% Threshold: variation of 5 g of water per gram of product
Water activity	aw=constant	Mean aw= 0,902 ± 0,009. Threshold : variation in (+/-)2 *SD
Color	L*, a*, b* =constant	Mean L* = 46,0(+/-)2,4 Mean a* =13,1(+/-)0,9 Mean b* = 13,3(+/-)1,1 Threshold : depending formulation; variation (+/-) SD
Firmness	Fmax (t) = constant	Mean Fmax=2,47 +/- 0,63N Threshold : depending formulation; variation (+/-) SD

(b)

Predicted parameter	Equation or model	Used parameters
Drip loss	Y = constant	Mean Y = 0,11 % ; Std = 0.03 % Threshold: 5% of the initial mass
Water content	Xw=constant	Mean X w = 73.4 %; Std = 0.6 % Threshold: variation of 5 g of water per gram of product
Water activity	aw=constant	Mean aw= 0,982 ± 0,002. Threshold : variation in (+/-)2 *SD
Color	L*, a*, b* =constant	Mean L* = 53.7 (+/-) 2,8 Mean a* = 7.5 (+/-) 0,6 Mean b* = 6.6 (+/-) 0.4 Threshold : depending formulation; variation (+/-) SD
Firmness	Fmax (t) = constant	Mean Fmax= 0.23 +/- 0,03N Threshold : depending formulation; variation (+/-) SD

(c)

Predicted parameter	Equation or model	Used parameters
Drip loss	Y = constant	Mean Y = 0,13 % ; Std = 0.03 % Threshold: 5% of the initial mass
Water content	Xw=constant	Mean X w = 55.1 %; Std = 1.4 % Threshold: variation of 5 g of water per gram of product
Water activity	aw=constant	Mean aw= 0,973 ± 0,003. Threshold : variation in (+/-)2 *SD
Color	L*, a*, b* =constant	Mean L* = 44.1 (+/-) 0.6 Mean a* = 7.3 (+/-) 0,6 Mean b* = 9.7 (+/-) 0.6 Threshold : depending formulation; variation (+/-) SD
Firmness	Fmax (t) = constant	Mean Fmax= 7.34 +/- 0,72N Threshold : depending formulation; variation (+/-) SD

4. CONCLUSION

This work focuses on the development and validation of kinetic models and equations for selected RTE pork meat products, pasteurized pâté, pasteurized ham and raw salted ham, packed under modified atmosphere. The impact of four static temperature storing conditions on quality and safety attributes of the products has been followed during storage (four to six weeks). Quality indicators like firmness, drip-loss, colour, water activity were constant during the storage period at each temperature. Kinetic data of *Listeria monocytogenes SOR 100*, *Lactobacillus sakeii 1322 and Leuconostoc mesenteroïdes 74* at four static temperatures were acquired in order to test the development and the applicability of the models. Parameters of the models were also estimated. These results are a part of the future FRISBEE tool: a user-friendly software application that allows to mimic the effect of realistic time-temperature profiles in the cold chain on the final product safety and quality when reaching the consumer, and that at the same time calculates the energy requirements and environmental impact of the cooling technologies being part of the simulated cold chain (Gwanpua et al., 2013).

Acknowledgements

The research was supported by the FRISBEE project - Grant Agreement N° 245288 and is a part of the Work Package 3 (WP3 Leader, KU Leuven (Belgium) -Department of Biosystems (BIOSYST) - Division MeBioS). FRISBEE coordinator is Irstea (France).

REFERENCES

Angelidis, A.S.,Koutsoumanis,K.,2006. Prevalence and concentration of *Listeria monocytogenes* in sliced ready- to-eat meat products in the Hellenic retail market. J.Food Prot.69, 938-942.

Anonymous, 2005. Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Official Journal of the European Union, L 338, 22/12/20051–26.

Augustin, J.-C., Rosso, L., Carlier, V., 2000. A model describing the effect of temperature history on *lag* time for *Listeria monocytogenes*. International Journal of Food Microbiology 57, 169-181.

Augustin, J.-C., Bergis, H., Midelet-Bourdin, G., Cornu, M., Couvert, O., Denis, C., Huchet, V., Lemonnier, S., Pinon, A., Vialette, M., Zuliani, V., Stahl, V., 2011. Design of challenge testing experiments to assess the variability of *Listeria monocytogenes* growth in foods. Food Microbiology 28, 746-754.

Couvert, O., Pinon, A., Bergis, H., Bourdichon, F., Carlin, F., Cornu, M., Denis, C., Gnanou-Besse, N., Guillier, L., Jamet, E., Mettler, E., Stahl, V., Thuault, D., Zuliani, V., Augustin, J.-C., 2010. Validation of a stochastic modelling approach for *Listeria monocytogenes* growth in refrigerated foods. International Journal of Food Microbiology 144, 236-242.

Cornu M., Billoir E., Bergis H., Beaufort A., Zuliani V. 2011. Modeling microbial competition in food: Application to the behavior of *Listeria monocytogenes* and lactic acid flora in pork meat products. Food Microbiology 28, 639-647.

García-Esteban M., Ansorena D., IAstiasarán ciar , 2004. Comparison of modified atmosphere packaging and vacuum packaging for long period storage of dry-cured ham: effects on colour, texture and microbiological quality Original Research Article. Meat Science, Volume 67, Issue 1, May 2004, Pages 57-63.

Gwanpua S.G, Verboven P., Verlinden B., Van der Sluis S., Wessink E., Evans J., Brown T., Leducq D., Alvarez G., Taoukis P., Stahl V., Thuault D., Claussen I., Indergård E., Nicolaï B. , Geeraerd A. ,2013. A quality, energy and environmental assessment tool for the European cold chain. IIR conference.

Jeremiah L.E., Gibson L.L. ,1997. The influence of controlled atmosphere storage on the flavor and texture profiles of display-ready pork cuts Original Research Article. Food Research International, Volume 30, Issue 2, March 1997, Pages 117-129.

Mejlholm, O., Gunvig, A., Borggaard, C., Blom-Hanssen, J., Mellefont, L., Ross, T., Leroi, F., Else, T., Visser, D., Dalgaard, P., 2010. Predicting growth rates and growth boundary of *Listeria monocytogenes* - An international validation study with focus on processed and ready-to-eat meat and seafood. Int. J. Food Microbiol. 141, 137-150.

Pinon, A., Zwietering, M., Perrier, L., Membré, J.M., Leporq, B., Mettler, E., Thuault, D., Coroller, L., Stahl, V., Vialette, M., 2004. Development and Validation of Experimental Protocols for Use of Cardinal Models for Prediction of Microorganism Growth in Food Products. Applied and Environmental Microbiology 70, 1081-1087.

Rosso L, Lobry JR, Bajard S, Flandrois JP. 1995. Convenient model to describe the combined effects of temperature and pH on microbial growth. Appl. Environ. Microbiol. 61:610-616.

Rosso, L., Bajard, S., Flandrois, J.P., Lahellec, C., Fournaud, J., Veit, P., 1996. Differential growth of *Listeria monocytogenes* at 4 and 8°C: Consequences for the shelf life of chilled products. Journal of Food Protection 59, 944-949.

Zwietering, M.H., De Wit, J.C., Notermans, S., 1996. Application-of predictive microbiology to estimate the number of *Bacillus cereus* in pasteurised milk at the point of consumption. International Journal of Food Microbiology 30, 55-70.