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Applicability of a microbial Time Temperature Indicator (TTI) for monitoring spoilage of modified atmosphere packed minced meat

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ABSTRACT

The applicability of a microbial Time Temperature Indicator (TTI) prototype, based on the growth and metabolic activity of a *Lactobacillus sakei* strain developed in a previous study, in monitoring quality of modified atmosphere packed (MAP) minced beef was evaluated at conditions simulating the chill chain. At all storage temperatures examined (0, 5, 10, 15 °C), the results showed that lactic acid bacteria (LAB) were the dominant bacteria and can be used as a good spoilage index of MAP minced beef. The end of product's shelf life as revealed by the sensory evaluation coincided with a LAB population level of 7 $\log_{10}$ CFU/g. For all temperatures tested, the growth of *L. sakei* in the TTI resembled closely the growth of LAB in the meat product, with similar temperature dependence of the μ_{max} and thus similar activation energy values calculated as 111.90 and 106.90 kJ/mol, for the two systems, respectively. In addition, the end point of TTI colour change coincided with the time of sensory rejection point of the beef product during its storage under isothermal chilled temperature conditions. The estimated activation energy, E_α, values obtained for parameters related to the response of ΔE (total colour change of the TTI) describing the kinetics of colour change of the TTI during isothermal storage (i.e. the maximum specific rate of ΔE evolution curve, $\mu_{\Delta E}$, and also the reciprocal of t_i, time at which half of the maximum ΔE is reached), were 112.77 and 127.28 kJ/mol, respectively. Finally, the application of the microbial TTI in monitoring the quality deterioration of MAP minced beef due to spoilage was further evaluated under dynamic conditions of storage, using two separate low temperature periodic changing scenarios, resembling the actual conditions occurring in the distribution chill chain. The results showed that the end point of TTI, after storage at those fluctuating temperature conditions, was noted very close to the end of product's sensorial shelf life. This finding points to the applicability of the developed microbial TTI as a valuable tool for monitoring the quality status during distribution and storage of chilled meat products, which are spoiled by lactic acid bacteria or other bacteria exhibiting similar kinetic responses and spoilage potential.

1. Introduction

Nowadays, the food industry is promoting the development of new technologies trying to meet consumers' expectations for food products of improved safety and sensory quality. Consumers also desire foods of increased functional and nutritional properties combined with extended shelf life, as well as products containing fewer calories and additives which additionally offer convenience (Nychas et al., 2008; Taoukis, 2001; Taoukis and Labuza, 2003). In this context, the development and application of structured quality and safety assurance systems based on prevention through monitoring, recording and controlling of critical parameters during the entire life cycle of the products, seems to be a prerequisite (Koutsoumanis et al., 2005; Taoukis, 2001). Unlike other parameters affecting food quality (e.g. pH, water activity, redox potential, gas composition), temperature of chilled products may vary extensively during transportation, retail and domestic storage, and often deviates from the recommended storage conditions, affecting greatly the shelf life of foods. Thus, a cost effective way to monitor the temperature conditions of individual food products throughout distribution is required to indicate their real safety and quality. Time Temperature Indicators (TTI) could potentially fulfill the above requirement (Taoukis and Labuza, 2003).

A TTI based system provides data and information on the time-temperature history down to the product unit level, allowing for the determination of product quality at any point of the chill chain, and is shown by an easily measurable time and temperature dependent change (Taoukis et al., 1999). In order to ensure that the adopted TTI matches the quality loss of the food, systematic kinetic modeling of the effect of temperature on the shelf life of the chilled product as well as a complete kinetic study of the TTI response is required (Taoukis, 2001; Taoukis and Labuza, 2003). In particular, the activation energy (E_α), indicating the temperature sensitivity of the TTI response,

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should be similar to the E_α of the food deterioration process, and the endpoint of the TTI should be close to the end of product's shelf life. This approach, based on the thorough kinetic study on both the shelf life of food product and the TTI, has been successfully applied in the optimization of the chill chain management of meat and fish products in many studies using different TTI types (Giannakourou et al., 2005; Giannakourou et al., 2001; Koutsoumanis et al., 2005; Taoukis, 2001; Tsironi et al., 2008).

Several TTI types differing in their working principle have been developed and these have been reviewed by Taoukis (2001) and Taoukis and Labuza (2003). The commercially available patented TTI prototypes are systems that provide temperature dependent changes based on molecular diffusion (3 M Monitor Mark® and Freshness Check indicators by the 3 M Company, USA), polymerization reactions (Lifelines Fresh-Check®, and Freshness Monitor® by Lifelines Inc., USA), enzymatic activity (CheckPoint TTI by the VITSAB Company, Sweden) and microbial growth (TRACEO and eO by CRYOLOG S.A., France) (Kerry et al., 2006; Taoukis, 2001; Taoukis and Labuza, 2003).

Microbial TTIs prevail among other TTI types since their response is directly related to food spoilage process. In these systems, the occurred bacterial growth and metabolism is translated in a TTI response which reflects the microbial growth responsible for the quality loss due to spoilage of the traced food product (Vaikousi et al., 2008). Such a microbial growth - TTI prototype, based on the growth and metabolic activity of a *Lactobacillus sakei* strain for monitoring the microbial quality of chilled foods, has been recently developed by Vaikousi et al. (2008). In this particular TTI system, an irreversible colour change of a chemical indicator from red to yellow progressively occurs due to the pH decline, as a result of microbial growth and metabolism of a selected growth medium. In addition, the complete kinetic study conducted for the effect of temperature on the TTI response (colour change described above), showed that the proposed TTI could be used as an effective tool for monitoring shelf life during distribution and storage of food products that spoil by the growth and metabolic activity of lactic acid bacteria or other bacteria of similar kinetic behavior (Vaikousi et al., 2008). Indeed, lactic acid bacteria (LAB) have been reported as the main cause of spoilage in meat products packed under vacuum or modified atmospheres (Borch et al., 1996; Devlieghere et al., 1998; Devlieghere et al., 2000; Guerrero and Chabela, 1999; Koutsoumanis et al., 2008; Nychas and Drosinos, 1999; Nychas et al., 2008; Mataragas et al., 2006; Samelis et al., 2000). Modified atmosphere packaging (MAP), widely used by the meat industry, is well known as an effective tool for extending the shelf life of meat products. The presence of CO_2 in the headspace of modified atmosphere meat packages inhibit microbial growth and the dominant microflora is shifted to bacterial groups (lactic acid bacteria and or *Brochothrix thermosphacta*) with reduced spoilage potential, which increase the shelf life of food products (Koutsoumanis et al., 2008). The resulting spoilage by these bacteria is mainly characterized by a sour, acid, or cheesy odour due to the transformation of the metabolized glucose into organic acids (Borch et al., 1996; Nychas and Drosinos, 1999).

As a follow up of the previous study on the development of the microbial TTI conducted by Vaikousi et al. (2008), the present work aimed at investigating the applicability of the TTI to monitor microbial quality of MAP minced beef stored under isothermal or dynamic conditions simulating the chilled distribution chain. For this reason, the kinetics of MAP beef spoilage and the microbial TTI were examined in parallel.

2. Materials and methods

2.1. Preparation of MAP minced beef samples

Fresh minced beef of normal pH (5.8–6.0) purchased at a local butcher shop was used in this study. Meat was divided into portions of 100 g and 50 g for microbiological and sensory analysis, respectively, placed on plastic trays, flushed with gas atmosphere composed of 60% CO_2 — 20% O_2 — 20% N_2, and enclosed into low-permeability polyethylene plastic film. The samples were stored under controlled isothermal conditions (0, 5, 10 and 15 °C) or programmed fluctuating temperature protocols in high-precision (±0.2 °C) low-temperature incubators (model MIR 153; Sanyo Electric Co., Ora-Gun, Gunma, Japan). For the dynamic storage experiments, two non-isothermal low-temperature storage profiles were used in order to simulate a continuously changing storage environment and potential abuse periods in the chilled chain. The first storage protocol included a periodically alternating 24 h cycle of 18 h at 5 °C and 6 h at 15 °C, and the second profile involved storage for 24 h at 0 °C, followed by an abrupt change of temperature for 6 h at 15 °C and finally for a 12 h period at 10 °C. The temperature of samples was monitored throughout the entire storage period by using electronic temperature recording devices (Cox Tracer; Cox Technologies, Belmont, NC).

Duplicate packages of the MAP meat product, from each storage temperature, were sampled at appropriate time intervals to allow for efficient kinetic analysis of microbial growth, pH measurements and sensory evaluation of colour and odour for the study of microbial spoilage of meat stored under either isothermal or dynamic storage conditions. All experiments were conducted twice.

2.2. Microbiological analysis

Samples (25 g) of minced meat were aseptically weighed, added to 1/4 strength Ringer's solution (225 ml), and homogenized in a stomacher (Lab Blender 400, Seward Medical, London, United Kingdom) for 60 s at room temperature. Decimal serial dilutions in quarter strength Ringer's solution were prepared and duplicate 1 ml or 0.1 ml samples of appropriate dilutions were poured or spread on the surface of the appropriate media in petri dishes for enumeration of (i) total aerobic viable count (TVC) on Plate Count Agar (PCA; Merck, Darmstadt, Germany), incubated at 25 °C for 72 h, (ii) *Brochothrix thermosphacta* on STAA medium supplemented with streptomycin sulfate, thallous acetate and cycloheximide (actidione; this medium was made from basic ingredients in the laboratory, and incubated at 25 °C for 72 h), (iii) Lactic acid bacteria (LAB) on Man Rogosa Sharpe agar (MRS) (Merck, Darmstadt, Germany) overlaid with the same medium and incubated at 25 °C for 96 h, (iv) *Pseudomonas* spp. on cetrimide-fucidin-cephaloridine (CFC) agar (Oxoid, CM559 supplemented with selective supplement SR 103E, Basingstoke, UK) incubated at 25 °C for 48 h, and (v) *Enterobacteriaceae* on Violet Red Bile Dextrose/Glucose (VRBG) agar (Merck, Darmstadt, Germany) overlaid with the same medium and incubated at 37 °C for 24 h. All plates were examined visually for typical colony types and morphological characteristics that were associated with each growth medium. In addition, the selectivity of each medium was checked routinely by Gram staining and microscopic examination of smears prepared from randomly selected colonies obtained from all media.

2.3. Measurement of pH and gas atmosphere in beef samples

The pH values were recorded by a pH meter (Russel, Moder RL150), with the glass electrode being immersed in the homogenate of minced meat after the end of microbiological analysis.

The gas headspace analysis of the MAP minced beef packages was made with a Gaspace 2 gas analyzer (Gaspace 2, Madderlake scientific Ltd, UK).

2.4. Sensory analysis

Sensory evaluation of minced beef samples was performed during storage at isothermal or dynamic conditions, by a five member sensory panel composed of staff from the laboratory. The same trained persons were used in each evaluation session, and all were blinded to the age and temperature history of the product being tested. The

sensory evaluation was carried out under artificial light and the temperature of packed product approximated the ambient temperature. The meat product was cooked in aluminium foil at 180 °C for 20 min. Special attention was given to the colour of raw and the taste and odour of cooked meat. Taste and odour were judged and recorded in appropriate forms with descriptive terms, reflecting the organoleptic evolution of quality deterioration (Taoukis et al., 1999). A simple three point scoring system was adapted (Dalgaard et al., 1993; Taoukis et al., 1999). Each attribute was scored on a continuous 0 to 3 hedonic scale with 0 being the highest quality score, 1 given to the acceptable product, 2 being the limit of product acceptance or rejection point, and 3 the unacceptable meat sample.

2.5. Measurement of the TTI response

The microbial TTI consisted of the following components: a mixture of nutrient broth enriched with 2% (w/v) glucose and 0.5% (w/v) yeast extract, and the aseptically incorporated into the sterilized medium chlorophenol red solution (0.2% w/v in 0.00944 M NaOH) at 1. 5% v/v, inoculated with the appropriate concentration of the *L. sakei* strain. The TTI system was prepared as described by Vaikousi et al. (2008) and stored isothermally under 0, 5, 8, 10, 12, and 15 °C and at dynamic conditions together with the MAP beef samples, in order to examine the applicability of the developed TTI to describe the spoilage process of meat. The *L. sakei* strain was inoculated at the level of 10^4 log CFU/ml in the growth medium, a level similar to the initial population of lactic acid bacteria enumerated in the beef product. The microbial TTI used is based on the irreversible colour change of the chemical indicator in the growth medium, caused by the pH decrease due to the microbial growth and metabolic activity of the *L. sakei* strain inoculated in the selected medium (Vaikousi et al., 2008). Thus, the growth of lactic acid bacteria, the pH and colour changes were recorded at appropriate time intervals to monitor the kinetic responses of the microbial TTI. The LAB growth of the TTI samples was tested on MRS agar as described above, while the pH was determined at the same time intervals with the microbiological analysis sampling.

The distinct irreversible colour change of the TTI from the initial red to final yellow (end point of TTI) was used as the measurable response of change. The kinetics of colour evolution of the TTI system (microorganism, growth medium and chemical indicator) was assessed using a hand-held colorimeter (chroma meter CR-410, Konica Minolta Sensing Inc., Japan) as described by Vaikousi et al. (2008), to determine the CIE colour space co-ordinates, i.e. the visible colours to the human eye, as specified by the International Commission on Illumination (*Commission Internationale d'Eclairage, CIE*), L^*, a^*, b^*. In particular, L^* represents the lightness of the colour (with 0 yielding black and +100 indicating diffusive white), α^* is the colour's position between green (−60) and red (+60) and b^*, its position between blue (−60) and yellow (+60).

The colour change of the TTI system was expressed as the index of total colour change, ΔE (in arbitrary units):

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (\alpha^* - \alpha_0^*)^2 + (b^* - b_0^*)^2} \quad (1)$$

with L_0^*, α_0^* and b_0^* being the CIE colour values at time $t=0$, and L^*, a^* and b^* the colour coordinates at time t. The change in chromaticity response ΔE with time was described by the four parameter logistic equation using the Table Curve 2D software (SPSS Inc.) as follows:

$$\Delta E = \Delta E_{min} + \frac{\Delta E_{max} - \Delta E_{min}}{1 + exp[-\mu_{\Delta E}(t - t_i)]} \quad (2)$$

where ΔE_{min} is the initial ΔE value at time $t=0$, ΔE_{max} is the maximum ΔE value as estimated by the logistic model, $\mu_{\Delta E}$ is the maximum rate for ΔE evolution, t is time (hours) and t_i is the time (hours) when half of the maximum ΔE is attained.

2.6. Data analysis

The growth data of all spoilage bacteria tested in the meat product and those of LAB in the microbial TTI were modelled as a function of time using the model of Baranyi and Roberts (1994). For curve fitting the in-house program DMFit of IFR (Institute of Food Research, Reading, UK), kindly provided by Dr. J. Baranyi, was applied. Curve fitting allowed for the estimation of the kinetic parameters, i.e. the initial cell concentration N_{min} (CFU/g), the maximum specific growth rate μ_{max} (h^{-1}), the duration of the Lag phase (h), and the maximum bacteria population N_{max} (CFU/g) were estimated.

The temperature dependence of a) the maximum specific growth rate μ_{max} of lactic acid bacteria grown in MAP beef, b) the μ_{max} of LAB growth in the TTI, c) the maximum rate for ΔE evolution, $\mu_{\Delta E}$, describing the colour change of the TTI, and d) the reciprocal of the parameter t_i ($1/t_i$), involved in the same ΔE response function (logistic equation), were modeled using the modified Arrhenius equation:

$$lnY = lnY_{ref} - \frac{E_\alpha}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right) \quad (3)$$

where Y is one of the responses given above, T is the absolute temperature (Kelvin), Y_{ref} is the response value at the reference temperature (h^{-1}), E_α is the activation energy (kJ/mol K), R is the universal gas constant, and T_{ref} a reference temperature (273 K).

3. Results and discussion

3.1. Spoilage of MAP minced beef

The experimental data for the growth of the different measured bacteria of the natural beef microflora packed under modified atmosphere and stored at 5 and 15 °C are shown in Fig. 1. Similar microbial profiles were observed at 0 and 10 °C (data not shown). It is evident that LAB constituted the predominant part of the total natural microflora of MAP minced beef. Starting from an initial level of 10^4 CFU/g in raw fresh meat, LAB reached high populations of 10^7–10^8 CFU/g at the end of the storage period. The dominance of LAB in the CO_2 rich atmosphere has been repeatedly reported in the literature for meat and meat products (Borch et al., 1996; Devlieghere et al., 1998; Devlieghere et al., 2000; Guerrero and Chabela, 1999; Luño et al., 2000; Nychas and Drosinos, 1999; Nychas et al., 2008; Patsias et al., 2006; Patsias et al., 2008; Samelis et al., 2000; Soldatou et al., 2009; Sørheim et al., 1997). The modified atmosphere applied in this study, partly inhibited the growth of pseudomonads which reached moderate numbers ($10^{4.5}$–$10^{5.6}$ CFU/g at 0 and 5 °C, respectively), in agreement with previous reports for MAP meat (Drosinos and Board, 1995; Gill, 1996; Patsias et al., 2006; Patsias et al., 2008; Soldatou et al., 2009). In contrast, under aerobic storage of chilled meat, the *Pseudomonas* spp. are generally considered as the main spoilage organisms (Nychas and Drosinos, 1999; Nychas et al., 2008; Koutsoumanis et al., 2006; Koutsoumanis et al., 2008) due to their fast growing, reaching population levels of 10^7–10^8 CFU/g. Opposed to pseudomonads, the growth of *B. thermosphacta* was slightly affected by the applied modified atmosphere reaching populations between $10^{5.4}$ and $10^{6.8}$ CFU/g during isothermal storage in the range of 0 to 15 °C, confirming that along with LAB it is the favoured flora of facultative anaerobic groups that grow in MAP products (Drosinos and Board, 1995; Guerrero and Chabela, 1999; Hansen and Bautista, 1999; Nychas and Drosinos, 1999; Nychas et al., 2008; Soldatou et al., 2009). Finally, the *Enterobacteriaceae* group of bacteria remained at low population levels of 3.2–3.7 $\log_{10}$ CFU/g during storage of meat

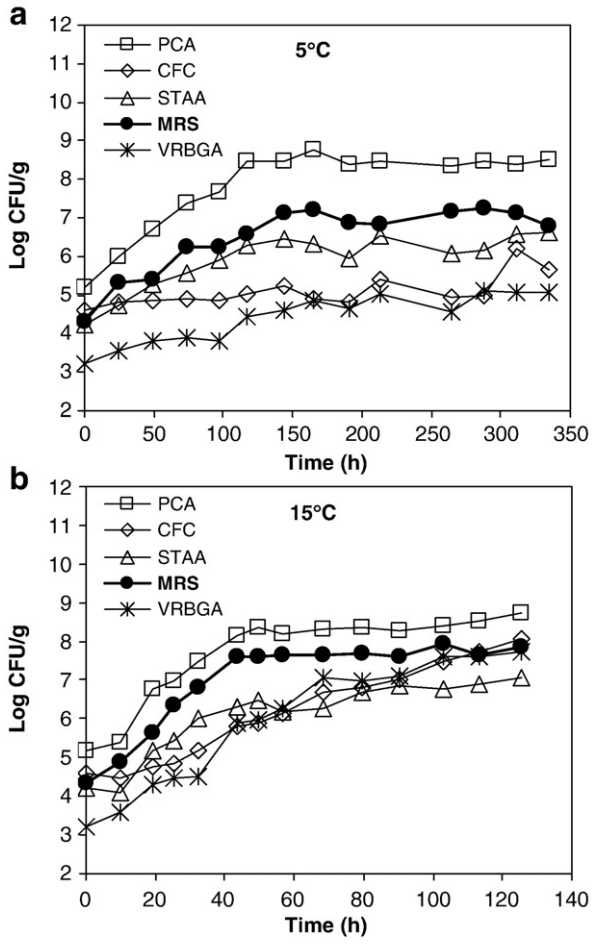

Fig. 1. Bacterial counts of specific groups of bacteria in Modified Atmosphere Packaged (MAP) minced beef samples stored at 5 (a) or 15 (b) °C. PCA, total aerobic bacteria; CFC, pseudomonads; STAA, *Brochothrix thermosphacta*; MRS, lactic acid bacteria; VRBG, *Enterobacteriaceae*.

samples at 0 °C (data not shown). This group of bacteria exhibited increased counts (up to 10^5–10^7 CFU/g) and accelerating growth rates, when the meat samples were stored in the range of 10 to 15 °C. Despite this, the estimated maximum specific growth rates of *Enterobacteriaceae* were the lowest among all tested bacteria at all storage temperatures except at 15 °C.

The storage temperature affected significantly microbial growth rate. The estimated by the primary model values of μ_{max} were fitted to the Arrhenius equation (Eq. (3)). The Arrhenius model allowed for the estimation of the temperature dependence of the maximum specific growth rates, in terms of activation energy, E_α. The calculated E_α values were 77.84, 85.69, 92.78 and 106.90 kJ/mol for pseudomonads, *Br. thermosphacta*, *Enterobacteriaceae*, and lactic acid bacteria, respectively, grown in MAP beef. The storage temperature had no significant effect on the maximum concentration N_{max} of any of the bacterial groups that were quantified. The estimations of this specific parameter for lactic acid bacteria remained constant under all storage conditions with an average value of 7.36 ± 0.33 $\log_{10}$ CFU/g (mean ± standard deviation).

Organoleptic evaluation of MAP minced beef took place in parallel with the microbial analysis. The results of sensory score given at each sampling time for meat products at all storage temperatures are shown in Fig. 2. The average calculated score was linearly related to time and the product's shelf life was estimated as the time at which the linear regression line, describing the sensory response, reached the value of 2 (rejection point). As expected, the shelf life of beef product decreased with increasing the storage temperature from 413.7 ± 15.8 h (mean ± standard deviation) at 0 °C to 41.4 ± 0.6 h at 15 °C. The primary growth model equation was used to calculate the levels of bacteria populations at the time of organoleptic rejection of the product by setting time equal to shelf life. The estimated values of the microbial association spoilage bacteria at the end of shelf life (N_s) revealed that LAB were the dominating microorganisms, reaching an average level of 7.08 ± 0.34 $\log_{10}$ CFU/g (mean ± standard deviation) at all storage temperatures. Similar population levels at the end of shelf life have been also reported for other vacuum packed and MAP meat products spoiled by LAB (Borch et al., 1996; Devlieghere et al., 1998; Devlieghere et al., 2000; Fernández-López et al., 2008; Korkeala et al., 1987; Koutsoumanis et al., 2005; Mataragas et al., 2006; Nychas et al., 2008; Samelis et al., 2000). The levels for the rest of bacteria at rejection point were lower at all tested temperatures and calculated as 6.21 ± 0.45, 5.23 ± 0.36, 4.54 ± 0.96 $\log_{10}$ CFU/g for *B. thermosphacta*, pseudomonads and *Enterobacteriaceae*, respectively, all lower than the level of LAB.

The sensory analysis showed that the growth and metabolic activity of LAB in the meat product contributed a lot to the final sensory features of the rejected product. Indeed, meat samples were characterised as having a sour taste and pungent odour and in some cases a cheesy odour and flavour, at the end of storage time. These sensory characteristics were in agreement with the pH evolution of the meat samples which decreased from the initial 5.8–6.0 to a final value of 5.25–5.40 at all tested temperatures. The above results in combination with the dominance of LAB at the time of rejection show that LAB can be defined as the specific spoilage organism (SSO; the fraction of total microflora which dominates over a range of storage conditions and is considered responsible for the spoilage of a certain food product) for the minced beef packed under modified atmosphere. Other studies have also shown the spoilage potential of LAB in MAP meat products (Gill, 1996). *Lactobacillus* spp. has been identified as the dominant microorganisms in MAP products due to their faster growth than the competitors because they are not significantly affected by pH and antimicrobial products such as lactic acid, H_2O_2 and antibiotics. They utilize glucose for growth and produce organic acids, mainly lactic acid, but also acetic acid and formic acid, depending on the homofermentative or heterofermentative metabolic feature of the genus species and growth conditions (Borch et al., 1996; Nychas and Drosinos, 1999).

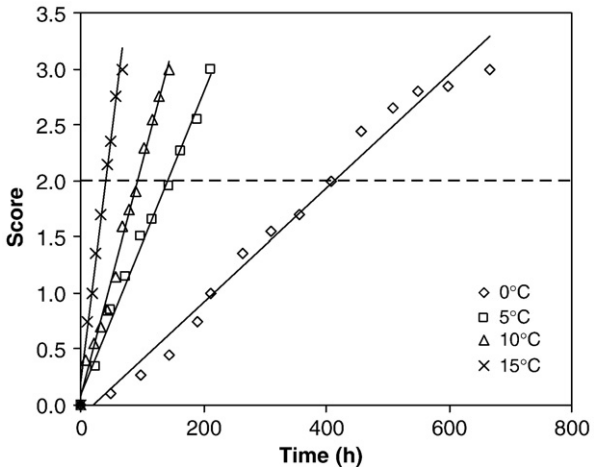

Fig. 2. Sensory evaluation score of MAP minced beef during storage at 0, 5, 10, 15 °C (data points) and linear regression lines describing the evolution of sensory evaluation scores as a function of time at each storage temperature; each data point represents the mean value given by five panelists, while the horizontal line depicts the score limit for organoleptic rejection of the product.

3.2. Comparison of MAP beef spoilage and TTI response

A comparison between the growth of LAB in beef and that occurring in the TTI, along with the changes observed in other TTI characteristics (evolution of the chromaticity response, ΔE, and declining pH profile) when both systems were stored isothermally at 15 °C, is shown in Fig. 3. Being able to adjust the initial level of the inoculated microorganism in TTI as close as possible to the initial count of LAB obtained for raw beef, i.e. 10^4 CFU/ml, a good agreement was obtained between the growth curves of the two systems. LAB growth in beef at 15 °C coincided with *L. sakei* growth in the TTI, exhibiting similar specific growth rate values of 0.21 and 0.27 h^{-1}, respectively. The growth of *L. sakei*, in the TTI, progressively resulted in lactic acid production with a subsequent decline of the pH in the substrate medium, visually observed as an irreversible colour change of the incorporated chemical indicator from the initial red to orange and finally yellow, as shown by the respective ΔE evolution curve in Fig. 3. According to Vaikousi et al. (2008) the pH decrease was attributed solely to lactic acid production as a result of the growth of the homofermentative *L. sakei* strain, since no additional organic acids, like acetic acid, was detected in the fermentation medium by the HPLC analysis conducted in that study. The respective colour change of the TTI occurred when the pH of the TTI medium was close to 5.3. At the end point of TTI, i.e. the time at which the distinct colour change occurred (or time for ΔE to reach the value of 20, as has been defined by Vaikousi et al., 2008), the LAB population in both the TTI and the beef product reached the level of 10^7–10^8 CFU/ml or /g. As it has been already mentioned, at this bacterial population LAB spoil the MAP beef, defining the end of product's shelf life. Similar observations were made comparing LAB growth and changes in TTI response at all tested temperatures. Indeed, the temperature dependence of the maximum specific growth rates, μ_{max}, of LAB grown in beef or in TTI, as described by the Arrhenius equation, resulted in similar activation energy values of 106.90 and 111.90 kJ/mol for the two systems, respectively (Table 1). Comparable E_α values have been reported for the growth of lactic acid bacteria in various food products (Koutsoumanis et al., 2000; Koutsoumanis et al., 2006). This finding further supports the reliability of the microbial TTI in describing the spoilage process of MAP minced beef stored under isothermal chilling conditions between 0 and 15 °C.

The initial contamination level of the SSO has been reported to affect the shelf life of a food product at a certain temperature along with other significant factors such as pH, water activity, and the presence of antimicrobials (Koutsoumanis et al., 2000; Koutsoumanis and Nychas, 2000; Koutsoumanis et al., 2002; Koutsoumanis et al., 2006). In the case of MAP minced beef the initial microflora can vary depending on the microbial quality of raw meat and the handling and processing conditions followed by the meat industry. However, a relatively accurate estimation of the average initial microbial level of a product batch is easy in a meat industry where handling and processing conditions are well controlled and information on the raw material quality is available. The average initial microbial level can be used for the appropriate adjustment of the initial concentration of the inoculated microorganism in the TTI medium. For example, increased initial contamination level of the SSO in a food product, results in a shorter shelf life since the time required for the SSO to reach spoilage levels decreases. This can be followed by the microbial TTI. By changing the initial concentration of the inoculated *L. sakei*, an adjustment of the endpoint of the TTI is possible, in order to coincide with the time for organoleptic rejection. It is important also to note that any change to the initial microbial level in the food does not affect the E_α value of the spoilage process. Therefore, the microbial TTI can be used as a valuable tool in monitoring the shelf life of food products with different initial microbiological quality (Vaikousi et al., 2008).

The effect of temperature on the colour evolution of the TTI, as expressed by the chromaticity value ΔE, is shown in Fig. 4. The experimental data of the TTI response, fitted to the logistic equation, were in good agreement with the predicted by the model changes in ΔE response (lines in Fig. 4), at all tested temperatures. The fitting procedure of colour evolution data to the logistic model allowed also for the estimation of the model parameters (i.e., ΔE_{min}, ΔE_{max},

Table 1
Parameters and statistics of the Arrhenius model (Eq. (3)) for the effect of temperature on the maximum specific growth rate (μ_{max}) of lactic acid bacteria grown in ground beef, *L. sakei* grown in TTI and on the parameters related to the chromaticity response, ΔE, describing the colour development in the TTI.

Parameter	$\mu_{ref,}\ 1/t_i\ ^{c}_{ref}$ (h^{-1})	E_α^{d} (kJ/mol)	CL[e] (±)	R^{2f}
$\mu_{max\ LAB\ in\ beef}$	0.019	106.90	11.50	0.989
$\mu_{max\ L.\ sakei\ in\ TTI}$	0.023	111.90	9.47	0.986
$\mu^{a}_{\Delta E}$	0.009	112.77	11.68	0.979
$1/t_i\ ^{b}_{\Delta E}$	0.002	127.28	14.89	0.973

[a] $\mu_{\Delta E}$, rate of ΔE chromaticity response.
[b] $1/t_{i\ \Delta E}$, reciprocal of time at which half of the maximum ΔE is attained.
[c] The subscript $_{ref}$ indicates values of the relative parameters, μ and $1/t_i$, at reference temperature (0 °C).
[d] E_α, activation energy.
[e] CL, 95% confidence limits for the estimated value of activation energy.
[f] Coefficient of determination.

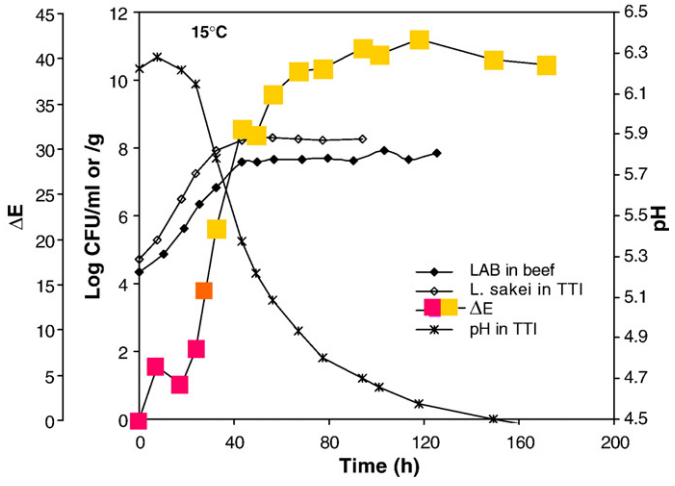

Fig. 3. Representative growth curves of lactic acid bacteria (LAB) in MAP minced beef and *L. sakei* in TTI during isothermal storage of both systems at 15 °C, as well as pH and colour changes of the TTI system stored at the same conditions; data points in ΔE evolution curve are substituted with colour squares representing the respective colour of the TTI system at that time.

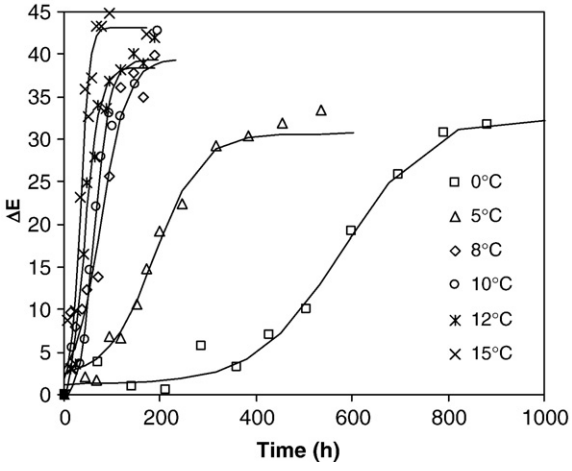

Fig. 4. Evolution of the chromaticity response, ΔE, upon storage of the TTI system isothermally at 0, 5, 8, 10, 12, and 15 °C; points depict experimental data and solid lines are the logistic model fittings (Eq. (2)).

$\mu_{\Delta E}$, $t_{i\,\Delta E}$). The colour change of the microbial TTI occurred at pH values of 5.2–5.3, at the point where the chemical indicator change is usually observed under all storage conditions. Increasing of the storage temperature resulted in shorter times of the microbial TTI colour change, and thus in decreased endpoint (time at which the ΔE response reached the value of 20). The parameter of the TTI endpoints was used in the previous study of Vaikousi et al. (2008) to describe the temperature dependence of the developed microbial TTI response, while here the parameters of colour development kinetics, i.e. $\mu_{\Delta E}$ and $1/t_{i\,\Delta E,}$ were adopted. The Arrhenius formalism applied on these two parameters is depicted in Fig. 5. The estimated values of E_α were 112.77 and 127.28 kJ/mol (Table 1) for the rate of colour development ($\mu_{\Delta E}$) and $1/t_{i\,\Delta E}$, respectively, being in the range of activation energy values of other non-microbial TTIs reported in the literature (Shimoni et al., 2001; Taoukis and Labuza, 1989; Taoukis et al., 1999; Yan et al., 2008).

The parallel positions of the Arrhenius plots obtained for LAB growth rates in beef and in TTI, in conjunction with the plots of TTI response parameters (Fig. 5), imply similar temperature dependence between spoilage occurring in the beef product and the TTI responses. The almost identical E_α values observed for the TTI responses and the quality loss of the beef product (Table 1) make the microbial TTI a good candidate in monitoring the shelf life of MAP minced beef. This view is further supported by the fact that the endpoint of TTI is closely related to the end of the product's shelf life as has been previously shown (Fig. 3).

3.3. Validation of the applicability of the microbial TTI under dynamic conditions

The applicability of the microbial TTI in describing the spoilage of MAP minced beef was also validated under non-isothermal conditions, simulating temperature fluctuations which may occur in the chill chain. Samples of MAP beef and TTI were stored together under dynamic temperature profiles. The growth of LAB in beef and the changes observed in the TTI are shown in Fig. 6. At both fluctuating temperature scenarios, lactic acid bacteria, starting with an initial (in beef) contamination level of $10^{4.5}$ CFU/g, grew and finally spoiled the

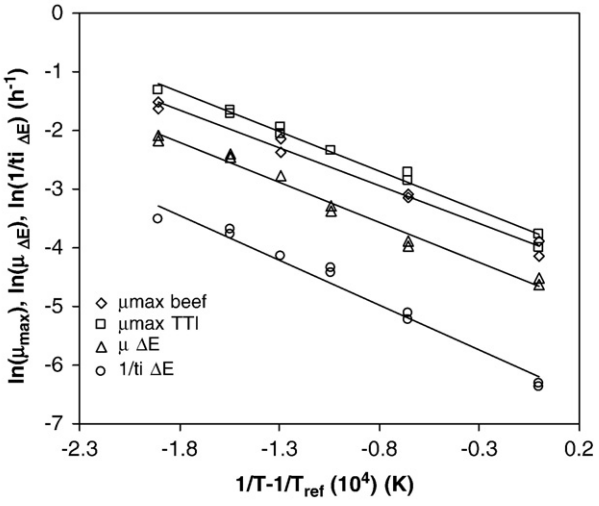

Fig. 5. Arrhenius plots describing the temperature dependence of the maximum specific growth rate (μ_{max}) of lactic acid bacteria observed in beef, and *L. sakei* in TTI, as well as the temperature dependence of the maximum growth rate of ΔE evolution curve and the reciprocal of the t_i parameter ($1/t_i$) involved in the logistic equation of ΔE response function; lines represent the regression plots obtained after fitting the Arrhenius model (Eq. (3)) to the respective growth rate data, as calculated at different storage temperatures.

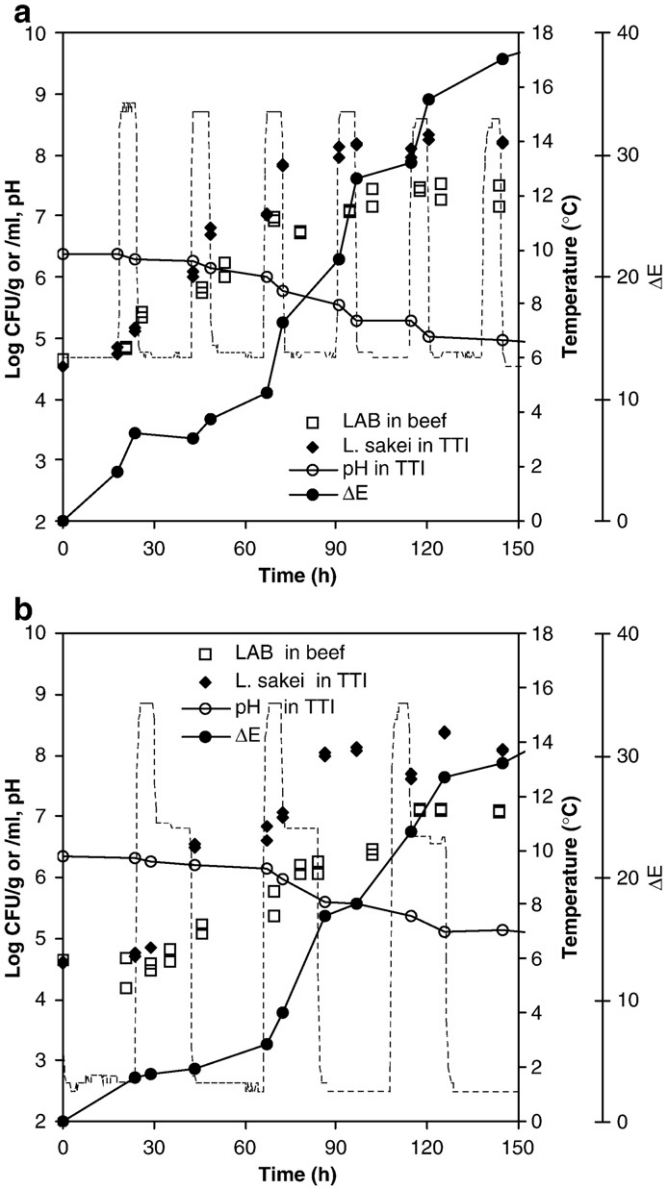

Fig. 6. Comparison of lactic acid bacteria (LAB) grown in MAP beef and *L. sakei* in TTI, in conjunction with the observed changes in pH and ΔE response of the microbial TTI stored under the same periodically changing temperature conditions; the first storage protocol included a periodically alternating 24 h cycle of 18 h at 5 °C and 6 h at 15 °C (a), and the second involved storage for 24 h at 0 °C, 6 h at 15 °C and finally 12 h at 10 °C (b).

product which was characterized as having a sour and acid taste, similar to the sensory characteristics previously observed for meat samples stored under isothermal conditions. The end of shelf life, as estimated by the average sensory score given by the panel, was noted after storage for 101 h under the first variable temperature regime and 114 h for the second fluctuating temperature profile (data not shown), which coincided with population levels of LAB reaching 7 $\log_{10}$ CFU/g in the meat product in both cases. The *L. sakei* in the TTI system followed closely the growth of SSO in MAP beef for the largest part of the storage period; however, higher final population levels were attained for *L. sakei* grown in TTI compared to the beef product, probably due to the lack of antagonism for the same nutrients in the former system. The observed deviation in growth curves of LAB in meat and *L. sakei* in TTI was more pronounced under the second dynamic storage protocol (Fig. 6b). Despite that, the colour change of the TTI ($\Delta E = 20$) occurred when LAB in beef were close to the spoilage level (i.e. 6.5 $\log_{10}$ CFU/g). Therefore, the microbial TTI seems

to be applicable in the MAP meat product and follows reasonable well the spoilage occurring at fluctuating temperature regimes, representing real chilling conditions in the distribution chain.

4. Conclusion

Overall, the microbial TTI responses reflecting the bacterial growth and metabolism occurring in the TTI system itself can be directly related to the microbial spoilage of MAP minced beef. Lactic acid bacteria were proved to be the dominant microflora which spoiled the product when grown to the level of 7 log CFU/ml. The microbial TTI prototype managed to describe adequately the end of product's shelf life as assessed by sensory analysis, under isothermal or dynamic storage conditions, via a progressive and irreversible colour change of the TTI response from red to yellow. The temperature dependence of LAB growth in meat and the TTI responses (*L. sakei* growth in the TTI system and its colour change) was similar, exhibiting almost identical activation energy values of the above mentioned parameters. Thus, the microbial TTI can be used as an effective tool for monitoring microbial quality of fresh ground meat stored under MAP conditions. The microbial TTI may serve as active shelf-life labeling devices in conjunction with the "used-by-date" labeling, when attached to individual product units, or may be used to optimize distribution control and management of the stock rotation system, reducing food waste (Giannakourou et al., 2001; Giannakourou et al., 2005; Koutsoumanis et al., 2005; Tsironi et al., 2008).

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