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Effect of storage temperature and gas permeability of packaging film on the growth of lactic acid bacteria and *Brochothrix thermosphacta* in cooked meat emulsions

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Abstract

The effect of gas permeability of packaging film on the growth of lactic acid bacteria and *Brochothrix thermosphacta* in cooked meat emulsions stored at 0, 8 and 15 °C was investigated. The estimated parameters from Gompertz equation for the assayed temperature–oxygen permeability combinations showed LAB development to be significantly greater than those of *B. thermosphacta*. The influence of the two sources of variation (oxygen permeability of packaging film and temperature) on the growth parameters of LAB and *B. thermosphacta* was analysed showing a significant effect ($P<0.001$) of the temperature on both bacterial population while the film permeability had only a significant influence ($P<0.001$) on *B. thermosphacta* growth. Under the conditions of this study the packaging film influenced the maximum counts and growth rates of both organisms. Since the inhibition of *B. thermosphacta* occurred when the meat product was vacuum-packaged in films possessing high oxygen permeability and the effect of pH was found not to be associated with the growth inhibition, accumulation of hydrogen peroxide produced by LAB may possibly be one of the main factors responsible for *B. thermosphacta* inhibition. Shelf-life of vacuum-packaged cooked meat emulsions in high oxygen transmission rate films will be guarantied and a temperature abuse will not result in an increase of spoilage by LAB.

Keywords: Cooked sausages; Lactic acid bacteria; *B. thermosphacta*; Predictive microbiology; Temperature effect; Gas permeability effect

1. Introduction

The quality and shelf life of cooked meat foods are determined by the growth of micro-organisms. To control microbial survival and outgrowth of micro-organisms in foods, food preservation procedures are used. Vacuum-packaging has been shown to be very effective in extending the shelf-life of perishable foods such as meat products (Church and Parsons, 1995). Under these conditions the oxygen supply will be restricted, the gas phase being determined by the rate of gas permeation through the film and the rate of oxygen consumption in the package, these changes having a selective effect on the microbial population (Farber, 1991; Labadie, 1999). Storage of meat products in gas-impermeable packs restricts the growth of *Pseudomonas* so that lactic acid bacteria (LAB), *Brochothrix thermosphacta* and *Enterobacteriaceae* becomes the major component of the spoilage microflora (Taylor, 1996; Korkeala and Björkroth, 1997; Hansen and Bautista, 2000; Nychas and Drosinos, 2000).

LAB were identified as the major spoilage population of vacuum-packaged emulsion-type sausages and other processed meats stored at refrigeration temperatures (Korkeala and Björkroth, 1997; Samelis et al., 2000). *Brochothrix thermosphacta* is also found to be a numerical significant component of the microflora of

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meat and meat products stored under these conditions (Nielsen, 1983; Kotzekidou and Bloukas, 1995, Samelis et al., 2000), this bacterium changing from a major contaminant to a minor part of the final population after storage (Collins-Thompson and Rodriguez Lopez, 1980). The growth and metabolism of *B. thermosphacta* on meat and meat products depends on factors such as pH, temperature, gas environment and substrate availability (Dainty and Hibbard, 1983; Blickstad and Molin, 1983; Grau, 1980; Pin et al., 2002). LAB and *B. thermosphacta* significantly influence the quality of meat and meat products, both being associated with spoilage in this products. Under anaerobic conditions LAB may cause souring, slimy, swelling of the pack and/or greening, while *B. thermosphacta* produce mainly lactic acid, ethanol and only small amounts of short-chain fatty acids causing off-odours (Hitchener et al., 1979; Blickstad and Molin, 1983; Pin et al., 2002). Temperature is a major factor on food deteriorative reactions, especially for microbial spoilage since specific growth rate and lag phase are highly temperature dependent (Wijtzes et al., 1995; Devlieghere et al., 1998; Cayré et al., 2003). However, the bacterial development in the package is not only influenced by temperature, oxygen availability and water activity also determine the quantities and the type of micro-organisms growing on meats (Labadie, 1999).

Predictive modeling has been extensively used mainly to predict bacterial growth as a function of environmental factors such as temperature, pH and a_w (McMeekin et al., 1987; Zwietering et al., 1994; Rosso et al., 1995) and more recently to predict fungus growth (Panagou et al., 2003). Most of the research in this area has focused on modeling the effect of intrinsic and extrinsic parameters on the growth/inactivation of food pathogens (Pond et al., 2001; Castillejo-Rodríguez et al., 2002). Predictive modeling can also be applied to predict the shelf-life of foods, this being based on a prediction of the growth of the responsible spoilage bacteria in specified product. Sigmoidal curve such as Gompertz function has often been used to model microbial numbers on foods as a function of time. Combined with different statistical approaches, the Gompertz equation can be used to describe single and multiple effects of different growth conditions (Skinner and Larkin, 1994; Linton et al., 1996; Cayré et al., 2003).

In this study, the growth of LAB and *B. thermosphacta* on cooked meat emulsion vacuum-packaged under different oxygen permeability films and stored at different temperatures were monitored. Four growth parameters were estimated by fit the experimental data to primary growth model and the effect of oxygen permeability of packaging film and temperature on each were analysed.

2. Materials and methods

2.1. Sausage samples and storage

Cooked sausages were prepared in a local meat processing plant by traditional techniques. The composition of the food product as supplied by the manufacturer was 46% beef; 35% pork; 15% pork fat; 2% NaCl, powder milk 0.8%; nitrite and nitrate 0.02%; binding and flavoring additives 1%. Two separate batches were prepared by thoroughly mixing the ingredients. The mix was then emulsified, filled into natural casings and cooked to a core temperature of 75 °C this procedure resulting in sausages that were 13 cm long and 2 cm in diameter with an average weight of 35 g. Immediately after cooking samples were taken and transported to the laboratory under refrigeration conditions and vacuum-packaged using three packaging films: (a) Maraflex, oxygen transmission rate of $19\,cm^3\,m^{-2}\,24\,h^{-1}\,atm^{-1}$ at 25 °C and 75% Relative Humidity (RH) and (b) Triplon, oxygen transmission rate of 70 and $150\,cm^3\,m^{-2}\,24\,h^{-1}\,atm^{-1}$ at 25 °C and 75% RH. The films were supplied by ENVARIL S.A.I.C.I.F., Argentine. The packages were sealed to a final vacuum of 0.95 mm of Hg using a RAPI-VAC S-750 vacuum machine (SERVIVAC S.R.L Argentine). Same samples were immediately analysed (day 0) while the remaining were stored at 0 °C for 75 days, and 8 and 15 °C for 52 days. A new package was opened at each sampling time. The data presented are the result of two experiments.

2.2. Microbiological analysis and pH determination

Microbial evaluation of cooked sausages was performed by aseptically transferred 10 g of sample to a Stomacher bag containing 90 ml of sterile 0.1% (w/v) peptone-water and homogenized in a Stomacher (Lab-Blender 400, Seward, London, UK) at room temperature for two minutes. Serial decimal dilutions of the samples were prepared and duplicate 0.1 ml aliquots of appropriate dilutions were spread on agar plates. Total LAB were determined on MRS agar (Merck, Darmstadt, Germany) after incubated at 30 °C for 72 h while *B. thermosphacta* was enumerated on selective Streptomycin Thallous Acetate Agar base (STAA) whit full selective supplement (Oxoid, Basingstoke, England) and incubated at 25 °C for 48 h. Two separate determinations were performed and results were expressed as log colony forming units per gram ($cfu\,g^{-1}$). For pH determination 10 g of sample were blended with 90 ml distilled water for 30 s. Readings of pH values were taken using a digital pH meter (Orion Model 525A, Boston, Massachussets, USA).

2.3. Modeling of microbial growth

For each temperature–oxygen permeability combination the experimental data were fitted to the modified-Gompertz equation (Gibson and Roberts, 1989) whose expression is

$$\log N = A + C \exp(-\exp(-B(t - M))), \quad (1)$$

where $\log N$ is the decimal logarithm of microbial counts at time t (cfu g^{-1}), A the asymptotic log count as time decreases indefinitely (cfu g^{-1}), C is the log count increment as time increases indefinitely (cfu g^{-1}), B is the relative maximum growth rate at time M (day^{-1}) and M is the time required to reach the maximum rate growth (day).

2.4. Statistical analysis

All models were obtained by fitting the data to Eq. (1) with the Marquardt algorithm. Confidence intervals were calculated by using the Student t value. Residual analysis and lack of fit test were used to assess the statistical acceptability of the models. Data collected for A, C, B and M parameters were analysed by a two-factor factorial design (3^2), the factors being temperature and oxygen permeability of packaging films. Appropriate transformations were performed for establishing the variance and/or to normalize the experimental data. When main effects were significant Tukey test was used to determine the relative significance of the means. In order to compare both micro-organism growth patterns, a paired-sample comparison was used to determine significant differences using the estimated parameters from Gompertz equation. All the tests were performed by using STATGRAPHICS® *Plus* version 4.0 (Statistical Graphics Corp.) software.

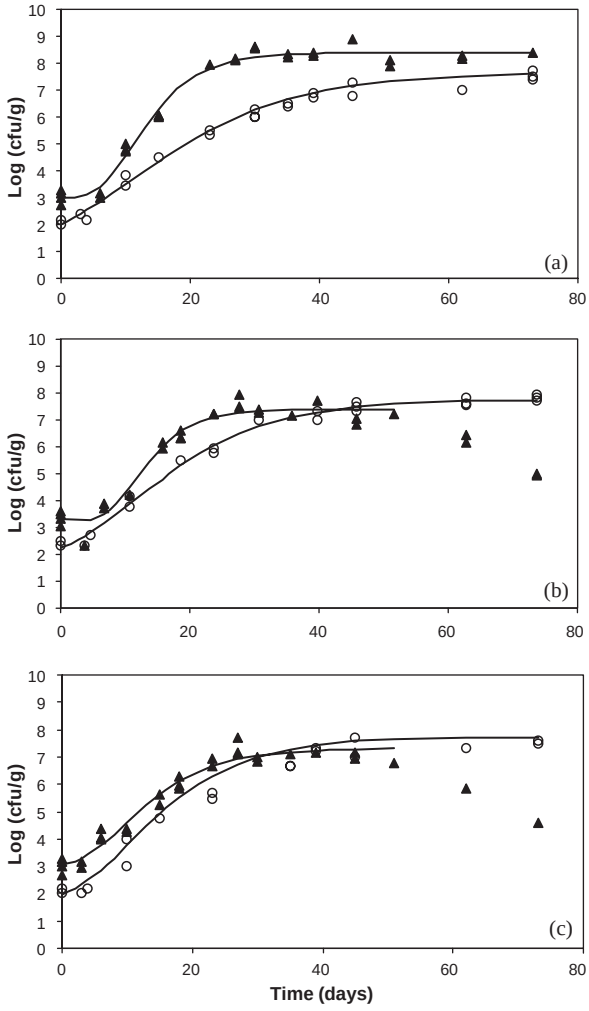

Fig. 1. Growth of LAB (○) and *Brochothrix thermosphacta* (▲) at 0 °C on cooked meat emulsions vacuum-packed in different oxygen permeability films; (a) 19, (b) 70 and (c) 150 $cm^3 m^{-2} 24 h^{-1} atm^{-1}$ to oxygen at 25 °C and 75% RH.

3. Results

Figs. 1–3 show the growth of LAB and *B. thermosphacta* in cooked meat emulsions packaged in different oxygen permeability films and stored at 0, 8 and 15 °C, respectively. Independently of temperature and oxygen permeability, the LAB counts increased from 2.2 ± 0.4 to 8.0 ± 0.5 cfu g^{-1} remaining stable up to the end of the storage time. Maximal growth rate both for LAB population and *B. thermosphacta*, were observed to be highly dependent of temperature, being maximal at 15 °C. On the other hand and regardless temperature and storage time, *B. thermosphacta* showed a different development picture during the storage period. At the three storage temperatures, *B. thermosphacta* grew and reached a maximal population of 7.1 ± 0.2 log cfu g^{-1} after which a dramatic decline in cell counts was observed when the product was vacuum-packaged under high oxygen transmission rate films (70 and 150 $cm^3 m^{-2} 24 h^{-1} atm^{-1}$), the population reaching zero between 35–50 days (8 °C) (Fig. 2b,c) and 20–25 days (15 °C) (Fig. 3b,c). Results from microbial growth curves shown that LAB would remain as the dominant flora in cooked meat emulsions stored under vacuum in high oxygen permeability films (70 and 150 $cm^{-3} m^{-2} 24 h^{-1} atm^{-1}$) while the population in the same product packaged in low gaseous permeability film (19 $cm^3 m^{-2} 24 h^{-1} atm^{-1}$) involved both, LAB and *B. thermosphacta*.

Table 1 shows the changes in pH of cooked meat emulsion vacuum-packaged in different permeability films during storage at 0, 8 and 15 °C. The initial pH values of the meat product were between 6.37 and 6.43. At the end of the storage time, pH values showed to be lower when higher temperatures and film permeability were applied. At 15 °C, pH reached values of 5.99, 4.69

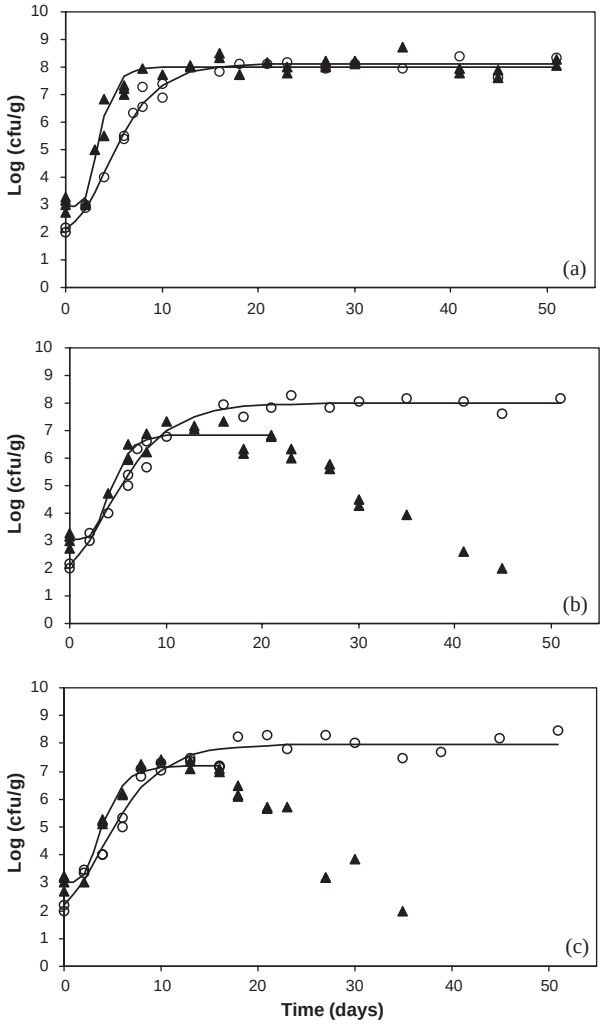

Fig. 2. Growth of LAB (○) and *Brochothrix thermosphacta* (▲) at 8 °C on cooked meat emulsions vacuum-packed in different oxygen permeability films; (a) 19, (b) 70 and (c) 150 $cm^3 m^{-2} 24 h^{-1} atm^{-1}$ to oxygen at 25 °C and 75% RH.

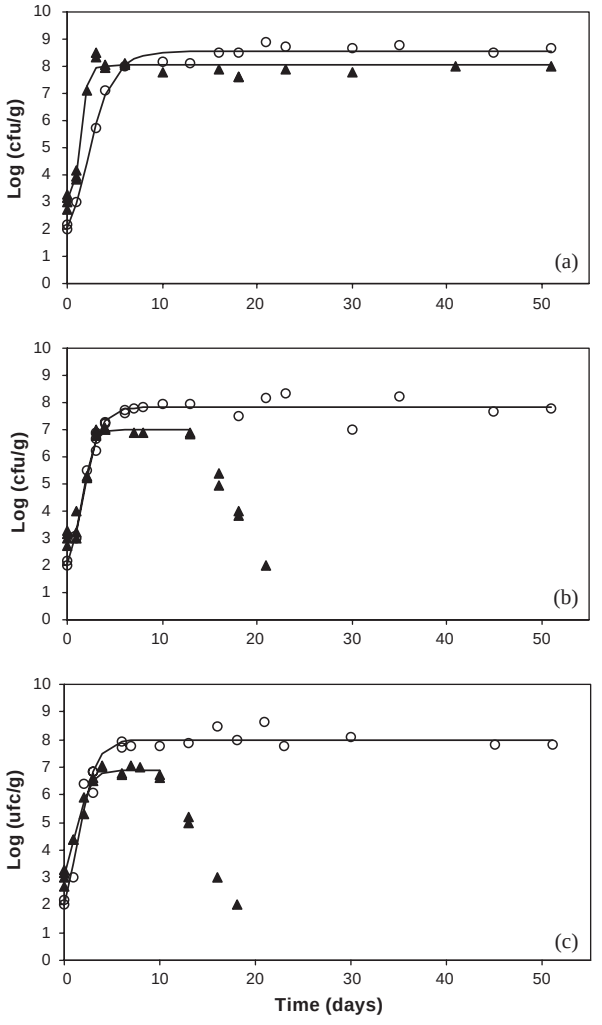

Fig. 3. Growth of LAB (○) and *Brochothrix thermosphacta* (▲) at 15 °C on cooked meat emulsions vacuum-packed in different oxygen permeability films; (a) 19, (b) 70 and (c) 150 $cm^3 m^{-2} 24 h^{-1} atm^{-1}$ to oxygen at 25 °C and 75% RH.

and 4.59 for 19, 70 and 150 $cm^3 m^{-2} 24 h^{-1} atm^{-1}$ oxygen transmission rates, respectively. The same pattern was also observed at 8 °C. These data are correlated with the growth picture observed for LAB at 8 and 15 °C whose population reached high final cell counts which could account for the acid production. Analysis of pH after 75 days of storage at 0 °C, and after 52 days at 8 and 15 °C showed that values were between 5.45 and 5.42 and below 5.00, respectively. pH values at 8 and 15 °C reached in the high permeability packs (70 and 150 $cm^3 m^{-2} 24 h^{-1} atm^{-1}$) at the time in which *B. thermosphacta* population started to decrease or be inhibited showed that they were higher than the corresponding values for low permeability film (19 $cm^3 m^{-2} 24 h^{-1} atm^{-1}$) where inhibition did not occur. These results indicated that the observed *B. thermosphacta* inhibition could not only be explained in terms of acidity.

The fittings of the modified-Gompertz equation to microbial counts are showed in Figs. 1–3 (continues line). In all cases, good determination coefficients were obtained, thus indicating Gompertz equation can explain a high percentage of the total variation of log counts during the storage time. The different parameters of the growth curves based on the Gompertz equation for different temperature–oxygen permeability combinations were analysed by a two-factor factorial design. In this work the estimated values of *B* and *M* for LAB and *B. thermosphacta* were transformed to ln(*B*) and ln(*M+1*) in order to homogenize the variance. Analysis of variance for the effect of temperature and oxygen permeability of packaging film on estimated parameters or their transformations are summarized in Tables 2 and 3 for *B. thermosphacta* and LAB, respectively. It can be seen that oxygen permeability of packaging film and its double interaction (A × A) significantly affected *C*

Table 1
Changes in pH of cooked meat emulsions vacuum-packaged in different oxygen permeability films during storage at different temperatures

Storage temperature	Oxygen permeability[a]	Storage time (days)	*Initial pH*	pH[b]	Final pH
	19		6.38		5.45
0 °C	70	75	6.38		5.43
	150		6.40		5.42
	19		6.39	5.25[c]/5.31[d]	4.93
8 °C	70	52	6.37	5.92	4.73
	150		6.43	5.34	4.62
	19		6.38	5.46[c]/5.50[d]	4.99
15 °C	70	52	6.39	5.66	4.69
	150		6.42	5.89	4.59

[a]Expressed as $cm^3\,m^{-2}\,24\,h^{-1}\,atm^{-1}$.
[b]Comparative pH of samples packaged in $19\,cm^3\,m^{-2}\,24\,h^{-1}\,atm^{-1}$permeability film at the time at which inhibition of *B. thermosphacta* packaged in [(c)] 70 and [(d)] $150\,cm^3\,m^{-2}\,24\,h^{-1}\,atm^{-1}$ permeability film was observed.

Table 2
F-values for the effect of oxygen permeability of packaging film and temperature on estimated parameters obtained from Gompertz equation for *Brochothrix thermosphacta* growing in cooked meat emulsions

Source of variation	Growth parameters			
	A	*C*	Ln (*B*)	Ln (*M*+1)
A[a]	0.07^{NS}	22.84^{***}	2.29^{NS}	0.72^{NS}
B[b]	0.08^{NS}	3.13^{NS}	150.49^{***}	601.45^{***}
A × A	0.10^{NS}	20.38^{***}	0.23^{NS}	0.40
A × B	0.18^{NS}	0.03^{NS}	6.96^{*}	0.03
B × B	0.01^{NS}	0.47^{NS}	1.25^{NS}	9.23^{***}

A: initial bacterial numbers; *C*: log count increment; *B:* maximum growth rate; *M*: time to reach maximum growth rate.
[*]Significant at $P<0.05$; [**]significant at $P<0.01$; [***] significant at $P<0.001$; [NS] not significant.
[a]Oxygen permeability of packaging film.
[b]Temperature (°C).

Table 3
F-values for the effect of oxygen permeability of packaging film and temperature on estimated parameters obtained from Gompertz equation for LAB growing in cooked meat emulsions

Source of variation	Growth parameters			
	A	*C*	Ln (*B*)	Ln (*M*+1)
A[a]	0.01^{NS}	0.27^{NS}	0.05^{NS}	0.35^{NS}
B[b]	0.28^{NS}	0.11^{NS}	170.90^{***}	99.05^{***}
A × A	0.04^{NS}	0.31^{NS}	0.18^{NS}	0.02^{NS}
A × B	0.04^{NS}	0.02^{NS}	0.52^{NS}	1.02^{NS}
B × B	0.10^{NS}	0.01^{NS}	0.01^{NS}	0.19^{NS}

A: initial bacterial numbers; *C*: log count increment; *B:* maximum growth rate; *M*: time to reach maximum growth rate.
[*]Significant at $P<0.05$; [**]significant at $P<0.01$; [***]significant at $P<0.001$; [NS] not significant.
[a]Oxygen permeability of packaging film.
[b]Temperature (°C)

parameter (total increment of micro-organisms) for *B. thermosphacta* (Table 2) while neither oxygen permeability nor temperature had effect on *C* parameter for LAB population (Table 3). The *C* values estimated for LAB were significantly ($P<0.01$) higher than those estimated for *B. thermosphacta*, even in samples packaged in films with low oxygen permeability (Table 4), these results being in accordance with the fact that LAB

Table 4
Effect of temperature and oxygen permeability on growth parameters of *B. thermosphacta* and LAB growing in cooked meat emulsions

Factor	Growth parameter							
	Brochothrix thermosphacta				LAB			
	A	*C*	Ln(*B*)	Ln (*M*+1)	*A*	*C*	Ln(*B*)	Ln (*M*+1)
Temperature*								
0 °C	2.95[a]	4.63[a]	−1.73[a]	2.45[a]	1.61[a]	5.82[a]	−2.34[a]	2.44[a]
8 °C	2.99[a]	4.35[a]	−0.27[b]	1.48[b]	1.86[a]	6.16[a]	−1.23[b]	1.57[b]
15 °C	3.00[a]	4.30[a]	0.50[c]	0.82[c]	1.86[a]	6.26[a]	−0.20[c]	0.93[c]
Film permeability**								
19	2.98[a]	5.14[a]	−0.31[a]	1.56[a]	1.82[a]	6.38[a]	−1.33[a]	1.70[a]
70	3.02[a]	3.95[b]	−0.44[a]	1.66[a]	1.73[a]	6.02[a]	−1.25[a]	1.63[a]
150	2.94[a]	4.19[b]	−0.74[a]	1.52[a]	1.78[a]	5.84[a]	−1.19[a]	1.61[a]

A: initial bacterial numbers; *C*: log count increment; *B:* maximum growth rate; *M*: time to reach maximum growth rate.
*Each number represents the average value of each parameter for all samples stored at the same temperature.
**Each number represents the average value of each parameter for all samples packed in films with the same oxygen permeability.
[a–c]Means within the same column with different superscript letters are different ($P<0.05$—Tukey Test).

growth was significantly greater than those of *B. thermosphacta* at all assayed oxygen permeability–temperature combinations. Ln (*B*) (maximal growth rate) calculated for both micro-organisms was observed to be significantly affected by temperature (Tables 2 and 3), the higher the temperature the higher the ln (*B*) value (Table 4). Even when no effect of oxygen permeability of packaging film on ln (*B*) was observed, the interaction temperature–oxygen permeability was significant for *B. thermosphacta* (Table 2) indicating that the effect of temperature for *B. thermosphacta* was dependent on the level of oxygen permeability of packaging film. Temperature and its double interaction (B × B) were the unique factor significantly affecting ln (*M*+1) (time for maximum growth rate) for *B. thermosphacta* and LAB (Tables 2 and 3), the higher the temperature the lower the time required to reach the maximum rate growth. No significant difference ($P<0.05$) were found between ln (*M+1*) values for LAB and *B. thermosphacta* at the different temperature levels.

4. Discussion

The effect of gas permeability of packaging film on the growth of LAB and *B. thermosphacta* in cooked meat emulsions stored at 0, 8 and 15 °C is reported. LAB population grows well at the three assayed temperatures and this growth is in agreement with those reported in the literature for vacuum-packaged beef (Giannuzzi et al., 1998; Sakala et al., 2002) and different cooked meat products (Nielsen, 1983; Kotzekidou and Bloukas, 1995; Samelis et al., 2000; Cayré et al., 2003). On the other hand, the development of *B. thermosphacta* in the same conditions showed two different patterns depending on the packaging permeability of the film. At low gaseous transmission rates, the growth of *B. thermosphacta* paralleled LAB growth but when film permeability changed to high oxygen transmission rates a dramatic decline of the former was produced, which was strongly dependent on the storage temperature. The higher the oxygen transmission rate of the film the shorter the time *B. thermosphacta* population fall to zero, this time depending of the storage temperature. Our findings indicate that film permeability more than storage temperature account for *B. thermosphacta* population decline. Storage in high oxygen permeability films allowed LAB to remain as the dominant flora in cooked meat emulsions at the three assayed temperatures (0, 8 and 15 °C). In contrast, when low gaseous permeability films were used no decline of *B. thermosphacta* was observed, both populations being present at the assayed temperatures. Many associated growth studies involving the contaminant *B. thermosphacta* and the lactic acid microflora in meat stored under vacuum using different gaseous permeability films were reported. Most of them found that lactobacilli markedly restricted the growth of *B. thermosphacta* when fresh meat or meat products were vacuum-packaged in films with low oxygen transmission rate. In contrast our findings showed that no inhibition of *B. thermosphacta* was observed in the least permeable film. Collins-Thompson and Rodriguez Lopez (1980) found a decline in *B. thermosphacta* levels at the time at which LAB reached levels of $10^6\,g^{-1}$ in bologna vacuum-packed in vinyldene chloride–vinyl chloride copolymer bags (low gaseous permeability) during 2 weeks. A decline in *B. thermosphacta* population after two weeks was also observed in beef stored at 2 °C and vacuum-packaged in a low permeability film ($2–3\,ml\,m^{-2}\,24\,h^{-1}\,atm^{-1}$ at 20 °C), different species of LAB remaining the dominant population up to six weeks of storage (Sakala et al., 2002).

Many factors such as pH changes, temperature, the presence of inhibitory substances, product composition,

packaging material/environment inside the package has been suggested to contribute to the decline of *B. thermosphacta* during storage at low temperature. Campbell et al. (1979) reported that this micro-organism is unable to grow on beef under anaerobic conditions at pH values below 5.8 and this inability depends on the inhibition by undissociated lactic acid (Grau 1980). On the other hand, Blickstad and Molin (1983) found that *B. thermosphacta* failed to grow anaerobically in broth at pH 5.30 and 8 °C and that at such low pH the growth may be inhibited by the pH per se. In the present study, pH changes during storage for all temperature–oxygen permeability combinations were from 6.37–6.42 to 4.59–5.45 indicating that the growth inhibition of *B. thermosphacta* cannot be explained only in terms of acidity. However the amount, as well as the nature of organic acid produced may be important since the undissociated form of acids highly depends of the external pH. Lower changes in external pH cause higher changes in undissociated form of organic acids. The inability of *B. thermosphacta* to compete against lactic acid bacteria in chill-stored beef under anaerobic conditions has been noted by several researchers (Collins-Thompson and Rodriguez Lopez, 1980; Nissen et al., 1996; Sakala et al., 2002). In this study, the inhibition of *B. thermosphacta* occurs after LAB counts overcome 10^7 cfu g^{-1} in the cocked meat emulsions. However, not only LAB numbers but also the species involved will determine the inhibition. The product type strongly select the growth and the composition of the spoilage lactic acid microflora in cold-stored vacuum or modified atmosphere packaged meat and meat products (Samelis et al., 2000; Susiluoto et al., 2003; Björkroth et al., 1998). *Lactobacillus* spp., *Leuconostoc* spp. and *Carnobacterium* spp. have mainly been associated with the spoilage of this products (Korkeala et al., 1988; Dainty and Mackey, 1992). In addition to the production of acid by LAB, the ability to produce inhibitory metabolites other than lactic acid during its growth is reported elsewhere. Among this substances, hydrogen peroxide can be produced by LAB in the presence of oxygen (Kandler, 1983) and with some exceptions LAB lack peroxidase (Kono and Fridovich, 1983) allowing hydrogen peroxide or other formed oxygen metabolites to accumulate acting as powerful inhibitors. Since the inhibition of *B. thermosphacta* in cooked meat emulsions occurred when the product was vacuum-packaged in film possessing high oxygen permeability, it can be hypothesized that hydrogen peroxide can be produced by LAB inhibiting *B. thermosphacta* since oxygen was reported to affect the metabolism of this contaminant (Blickstad and Molin, 1983). On the other hand, LAB associated with meat products are important natural bacteriocin producers (Stiles, 1996) and they are antagonistic against a number of gram positive pathogens or contaminants. However, as the oxygen concentration in the package is not a determinant factor for bacteriocin production, this ability of LAB could not fully account for *B. thermosphacta* inhibition. From the results, accumulation of hydrogen peroxide produced by LAB may possibly be one of the main factors responsible for *B. thermosphacta* inhibition.

The estimated parameters from Gompertz equation for the growth of LAB and *B. thermosphacta* for the temperature–oxygen permeability combinations (Table 2) are in agreement whit the experimental values for both bacterial populations. When the influence of the two sources of variation (oxygen permeability of packaging film and temperature) on the growth parameters of LAB and *B. thermosphacta* were analysed, a significant effect ($P<0.001$) of the temperature on both bacterial population was obtained while the film permeability only showed a significant ($P<0.001$) influence on *B. thermosphacta* growth. The dependence with temperature of LAB growth rate in vacuum-packaged cooked meat products was also stated by Nielsen (1983) and Cayré et al. (2003). From the results storage temperature would be the main factor controlling the rate of growth of both populations in meat emulsions since other factors such as nutrient status and available water are non-limiting (Gill and Newton, 1977). Although for *B. thermosphacta* the two source of variation, the individual effects and interactions, were observed to be significant, the decline of its growth after reached the maximal population level was due to an indirect phenomenon. As was discussed above, the fast growth rate of lactic acid flora present in the meat emulsions at the assayed temperatures and under the three different oxygen permeability films was the responsible for *B. thermosphacta* inhibition, the oxygen transmission rate of the film would be the selective factor that allow the formation of hydrogen peroxide.

More quantitative studies should be done to assess acid production as well as other inhibitory metabolites by strains able to develop on vacuum-packed cooked meat emulsions. However, based on the obtained results, the shelf-life of vacuum-packaged meat emulsions stored at 0, 8 and 15 °C using high oxygen transmission rates films will be guarantied and since the final number of LAB did not show significant changes with temperature after the storage time, a temperature abuse during storage will not result in an increase of spoilage by LAB.

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