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Research Note

Effect of High-Barrier Packaging Films with Different Oxygen Transmission Rates on the Growth of *Lactobacillus* sp. on Meat Fillets

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ABSTRACT

The goal of this study was to determine the combined effect of (i) the oxygen transmission rate (OTR) of packaging film, often called oxygen film permeability or film permeability and (ii) temperature on the growth rate of the main prevailing organism, *Lactobacillus* sp., in 100% CO_2-packed sterile meat fillets. Multifactorial experiments were designed to study the effect of OTR and temperature (0, 5, 8, and 10°C) on the growth rate of *Lactobacillus* sp. inoculated on sterile meat fillets under 100% CO_2 and aerobic conditions. The packaging conditions (air or 100% CO_2) and the film OTR significantly affected the growth rate of *Lactobacillus* sp. only at temperatures higher than 0°C. Low-permeable films with different OTRs did not affect the final population of the bacterium, but the growth rate was significantly changed. The correlation of an ephemeral microbial association with a low spoilage potential (e.g., lactic acid bacteria) or their growth retardation cannot always be assumed unless other determinants (e.g., OTR) of equal importance are taken into account. The present study provides information that can be of benefit to industry and the consumer.

It is well known that any combination of carbon dioxide and oxygen in modified atmosphere packaging (MAP) is able to inhibit or restrict the growth of pseudomonads (aerobic spoilage flora) and promote the growth of gram-positive bacteria *(10, 12).* This is fortuitous in the sense that lactic acid bacteria (LAB) possess a relatively slow and less ''offensive'' spoilage potential compared to the typical spoilage profile of the aerobic pseudomonads *(10, 11).* The efficacy of MAP is thought to depend on the contribution of the three main gases, CO_2, N_2, and O_2, in the gas mixture. However, several factors, such as the properties of packaging materials (e.g., the oxygen transmission rate [OTR] of the packaging film, often called oxygen film permeability or film permeability *(2, 4, 13, 14)*) have been underestimated. The lack of consistency with respect to the final microbial association *(12)* in published results that demonstrate the extension of the storage life of fresh meat stored under identical MAP conditions might be attributed to the effect of the different OTR values of the packaging films, even if they are characterized as high-barrier films *(7–9, 12).* Consideration of these characteristics not only addresses consumer concerns, ecological concerns, and compliance with the European legislation *(13)* but also addresses the maintenance of the balance of the flushed gaseous atmosphere in the headspace of the pack *(2).*

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Because temperatures are not always kept constant throughout the food chain *(6),* there is a need to determine or even model the combined effect of packaging films and temperature values on the growth rate of ephemeral spoilage association of meat. The goal of this study was to determine (i) the combined effect of temperatures and the OTR of packaging films (high and low) on the growth of the prevailing bacterium in 100% CO_2-packed meat, *Lactobacillus* sp., inoculated on sterile meat fillets packed under air or 100% CO_2 and (ii) the effect of different OTR values of high-barrier films at 8°C with respect to changes in the growth rate of *Lactobacillus* sp. under the packaging conditions of task (i).

MATERIALS AND METHODS

Bacterial strains. For use in our experiments, we recovered the prevailing LAB from fresh meat packaged with its naturally acquired microflora in 100% CO_2 and stored at 0°C. At the end of the storage period, deMan Rogosa Sharpe (MRS) agar plates were examined for typical colony types and morphological characteristics. Colonies were selected from petri dishes that had an average of 30 to 50 colonies, and they were transferred into MRS broth and inoculated at 30°C until visible growth. Purity was checked by streaking the strains on MRS agar, and the strains were kept in MRS broth plus 20% (wt/vol) glycerol at −20°C for further studies. Each strain under examination was subcultured twice overnight in MRS broth at 30°C and initially characterized by the following criteria *(14)*: Gram stain reaction; cell morphology and

motility (phase-contrast microscopy); and oxidase and catalase reaction. Gram-positive, nonmotile, fermentative, and oxidase- and catalase-negative strains were classified as LAB and further examined by a limited number of tests *(14)*: gas production from glucose; arginine hydrolysis; production of dextran from sucrose; growth at 3, 15, 30, and 45°C in MRS broth; growth in 8 and 10% NaCl; growth on acetate agar; growth on MRS at pH 3.9; and carbohydrate fermentation by means of the 50CH API tests (bioMérieux, Marcy l'Etoile, France).

Preparation of beef fillets. A piece of deboned beef (ca. 10 kg) was obtained from a local processing plant and transported under refrigeration (3°C) to our laboratory within 1 h. The surface of the meat was sprayed with 100% alcohol and ignited with a gas burner to reduce the initial microbial load of the meat piece. The burnt surface tissue was removed aseptically, and the sterile tissue below was excised and cut into 40- to 50-g fillets.

Preparation of inocula and inoculation. The strain used in this study was an atypical homofermentative streptobacterium, *Lactobacillus* sp. *(14)*. This isolate was subcultured twice in 100 ml of MRS broth (catalog no. 1.10661, Merck, Darmstadt, Germany) and incubated at 30°C for 18 h. Cells were harvested and washed by centrifugation with sterile quarter-strength Ringer solution (Lab M, Bury, UK). Each meat fillet was immersed in an appropriate volume of a bacterial suspension in Ringer solution to inoculate the meat at a level of 5 log CFU g^{-1}. The inoculated fillets were drained on a sterile grid.

Microbiological analysis. Samples (25 g) from meat were weighed aseptically, added to sterile quarter-strength Ringer solution (225 ml), and homogenized in a stomacher (Lab Blender 400, Seward Medical, London, UK) for 60 s at room temperature. For the enumeration of *Lactobacillus* sp., duplicate 1-ml samples of the appropriate decimal dilutions in quarter-strength Ringer solution were poured on MRS agar (catalog no. 1.10660, Merck), overlaid with the same medium, and incubated at 25°C for 96 h under anaerobic conditions. For the total viable count, duplicate 0.1-ml samples of the same dilutions were also spread on plate count agar (catalog no. 1.05463, Merck) and incubated at 25°C for 72 h. All plates were examined for the phenotypic characteristics that are associated with the inoculated microorganism.

Experimental design. Two main experiments were performed. For the first experiment, a three-way analysis of variance was designed. The growth responses of *Lactobacillus* sp. were evaluated for two packaging conditions (air and 100% CO_2), two values of the OTR of the packaging film (2,600 and 28 cm^3 m^{-2} 24 h^{-1} at 23°C and 75% relative humidity [RH]), and four temperatures (0, 5, 8, and 10°C). For the second experiment, the effect of film packaging with different OTRs (85, 35, 28, 17, and 2.5 cm^3 m^{-2} 24 h^{-1} at 23°C and 75% RH) was studied on the growth of *Lactobacillus* sp. inoculated on sterile meat fillets and packed in 100% CO_2 at 8°C. In both sets of experiments, an equal number of inoculated samples was packaged individually under the previously stated conditions by means of a HencoVac Machine (Howden Food Equipment B.V., Noord-Scharwoude, The Netherlands). For aerobic storage, the samples were placed in sterile petri dishes, and uninoculated samples were used as controls to ensure the sterility of meat fillets.

The entire experimental procedure was performed twice. The growth data (10 to 12 sampling points from each treatment) from plate counts were transformed to log values. The Baranyi model *(1)* was fitted to the logarithm of the viable cell concentration. For curve fitting, the proprietary program DMFit was used, which was kindly provided by Dr. J. Baranyi. The significance ($P < 0.05$) of the main treatment effects was determined by the SYSTAT (Systat, Evanston, Ill.) program.

Effect of temperature on specific growth rate. The specific growth rates were fitted by linear regression to an Arrhenius model:

$$\mu = A \exp(-\mathrm{Ea}/R \times T) \quad (1)$$

where μ is the specific growth rate, T is the absolute temperature, Ea is the activation energy (kilojoules per mole), A is the regression coefficient, and R is the gas content.

RESULTS

A total of 88 strains that were isolated at the initiation and at the end of storage of 100% CO_2-packed fresh meat with its background microflora at 0°C were gram-positive, catalase- and oxidase-negative non–spore-forming bacteria. The lactic acid isolates were categorized into two groups, heterofermentative group A (data not shown) and homofermentative group B, on the basis of the production of gas from glucose. Because most (63.6%) of the isolated strains from packed meat occurred in group B, this group was further subcategorized according to its cell morphology and typical phenotypic properties. Table 1 shows the distribution of isolates of group B and the population of LAB at the beginning and end of the storage period for packed meat, as well as the phenotypic properties of subgroup B_1, which consisted of 50 rod-shaped cells, and subgroup B_2, which consisted of six cocci. The lactic isolates of subgroup B_1 were characterized as atypical streptobacteria, *Lactobacillus* sp., because of their inability to ferment lactose and mannitol, the sugars that typical streptobacteria ferment. One strain of *Lactobacillus* sp. was further selected, and its growth was monitored on meat fillets under aerobic conditions and 100% CO_2 with a high-permeable film (2,600 cm^3 m^{-2} 24 h^{-1} at 23°C and 75% RH) and a low-permeable film (28 cm^3 m^{-2} 24 h^{-1} at 23°C and 75% RH) at 0, 5, 8, and 10°C.

The counts of the test bacterium on the corresponding selective agar agreed well (within 1 log CFU g^{-1}) with those on the plate count agar at each sampling time, whereas no colonies were evident on the uninoculated (sterile) meat samples throughout the storage period. The kinetic parameters of the *Lactobacillus* sp. for each combination of temperature and packaging film OTR are shown in Table 2.

The OTR of the packaging film played a rather significant role in the kinetics of this bacterium (Table 3). For example, the decrease in OTR from 2,600 to 28 cm^3 m^{-2} 24 h^{-1} at 23°C and 75% RH resulted in an extension of the lag period and a decrease in the growth rate of *Lactobacillus* sp. This was evident at higher temperatures of storage (≥5°C) but not at 0°C. The temperature also affected the kinetic parameters of the bacterium. Indeed, as the temperature increased, the growth rate of *Lactobacillus* sp. increased, and the lag period was reduced (Table 2). None of the storage conditions had any effect on the final population of the bacterium. The temperature dependence of growth was further modeled by use of the Arrhenius equation (Fig. 1). *Lactobacillus* sp. showed the highest Ea values under both aerobic storage and 100% CO_2 packaging conditions

TABLE 1. *Morphological, physiological, and biochemical characteristics of the homofermentative lactic isolates*[a]

	Group B	
	B_1	B_2
Total no. of strains	50	6
Distribution of strains	B: 18; E: 32	B: 2; E: 4
Cell morphology	Lactobacilli	Cocci
Population (CFU/g)	3.04	6.78
Production		
CO_2 from glucose	−	−
NH_3 from arginine	−	−
Dextran from sucrose	−	−
Growth at (°C):		
3	(+)	(+)
15	+	+
30	+	+
45	(−)	−
Growth at:		
8% NaCl	−	d
10% NaCl	d	d
Acetate agar, pH 5.6	+	+
MRS, pH 3.9	(+)	−
API 50 CHL system		
L-Arabinose	+	+
Ribose	+	+
Galactose	+	+
D-Glucose	+	+
D-Fructose	+	(−)
D-Mannose	+	+
Ramnose	−	−
α-Methyl-D-glucoside	d	−
N-acetylglucosamine	+	+
Amygdaline	+	(±)
Arbutine	+	+
Esculine	+	+
Salicine	+	+
Cellobiose	+	+
Maltose	+	−
Melibiose	+	+
Saccharose	+	+
Trehalose	d	+
D-Raffinose	−	−
β-Gentobiose	+	(±)
D-Turanose	d	(±)
Gluconate	(±)	(±)
2-Ceto-gluconate	d	−

[a] B, beginning of storage; E, end of storage; +, 90 to 100% of the strains were positive; (+), 75 to 89% of the strains were positive; d, 26 to 74% of the strains were positive; (−), 11 to 25% of the strains were positive; −, 0 to 10% of the strains were positive; ±, no clear indication.

with the high-permeable film and the lowest Ea values under 100% CO_2 packaging conditions with the low-permeable film (Table 3). Low storage temperature (0°C) and packaging in air and in 100% CO_2 with either the high- or the low-permeable film had no significant effect on the growth of *Lactobacillus* sp. (Fig. 1). On the other hand, at 8 and 10°C, the growth rate of this bacterium was more significantly reduced in the samples packed in low-permeable film than in those packed in high-permeable film.

The effect of various films that are all classed as being high barriers against oxygen but having different OTR values (85, 35, 28, 17, and 2.5 $cm^3\ m^{-2}\ 24\ h^{-1}$ at 23°C and 75% RH) on the growth of *Lactobacillus* sp. on sterile meat fillets packed under 100% CO_2 at 8°C was further investigated (Table 3). With the same packaging conditions (100% CO_2) and storage temperature (8°C), the OTR of the packaging film affected the growth rate of *Lactobacillus* sp. but not its final population (Table 2). The greatest growth rates of this bacterium were obtained when packaging films with OTRs of 85 and 35 $cm^3\ m^{-2}\ 24\ h^{-1}$ at 23°C and 75% RH were used. On the other hand, the packaging films with OTRs of 28, 17, and 2.5 $cm^3\ m^{-2}\ 24\ h^{-1}$ at 23°C and 75% RH did not significantly affect ($P > 0.05$) the growth of the bacterium (Table 3).

DISCUSSION

Spoilage of modified atmosphere packed meat, especially under 100% CO_2 conditions, is typically associated with the growth and dominance of LAB *(10, 11).* Several authors have reported that atypical streptobacteria make up one of the major groups of LAB that cause spoilage in modified atmosphere packed and vacuum-packed meat and meat products *(12, 14).* The succession dynamics of a particular LAB population in packed meat are complex, because, on the one hand, one ephemeral LAB strain may replace another without an observable decline in total LAB numbers *(5, 10).* On the other hand, the growth rate of these ephemeral LAB and their subsequent metabolic activity, which will inevitably lead to the spoilage of packed meat, are strongly affected by prevailing extrinsic, intrinsic, and implicit factors *(10, 11, 14).* Among these factors, the selection of a packaging film with the appropriate specifications is important in extending the shelf life of a modified atmosphere packed meat product, because it ensures that either (i) the growth of a bacterial population with a low spoilage potential is facilitated or (ii) a retardation in the growth rate of the aerobic spoilage microorganisms is facilitated. The present study showed that in a meat model system that simulates the dominance of LAB, the combined effect of the OTR of the packaging film and temperature has a significant impact on the growth rate of *Lactobacillus* sp.

The primary functions that a packaging film material must meet are to (i) present the product to the consumer in an attractive manner, (ii) optimize the shelf life of the product, and (iii) maintain both the sensory quality and the microbiological safety of the product through its barrier properties *(10).* The last function can be achieved by ensuring that the initial composition of the atmosphere within the package is maintained, i.e., with low-permeable packaging film. Indeed, the relatively lower growth rate of *Lactobacillus* sp., which was observed at 8 and 10°C in samples with low-permeable film compared to samples packed with high-permeable film, showed that the former could assist in

TABLE 2. *Effect of storage temperatures and packaging on the maximum growth rate (days^{-1}), final population (log CFU/g), and lag period (days) on the growth of* Lactobacillus *sp. inoculated on sterile meat fillets*

Packaging condition	Temp (°C)	Growth rate (days^{-1})	Lag period (days)	Final population (log CFU/g)
Air	0	0.155 ± 0.01[a]	0	7.90 ± 0.16
	5	0.374 ± 0.02	0	8.00 ± 0.01
	8	0.874 ± 0.15	0	7.62 ± 0.08
	10	1.072 ± 0.27	0	7.80 ± 0.11
2,600 cm^3 m^{-2} 24 h^{-1}	0	0.150 ± 0.01	4.90 ± 1.07	7.55 ± 0.14
	5	0.427 ± 0.07	3.07 ± 0.59	7.00 ± 0.08
	8	0.760 ± 0.16	0	7.30 ± 0.09
	10	1.082 ± 0.22	0	7.33 ± 0.07
28 cm^3 m^{-2} 24 h^{-1}	0	0.145 ± 0.02	9.67 ± 1.10	7.07 ± 0.12
	5	0.303 ± 0.08	4.82 ± 0.52	7.18 ± 0.09
	8	0.418 ± 0.13	3.05 ± 0.83	6.81 ± 0.17
	10	0.513 ± 0.08	0	7.30 ± 0.09

[a] Standard deviation.

maintaining the initial atmosphere of the packaged meat through its barrier properties (Table 3 and Fig. 1).

In our study, all samples were inoculated identically (e.g., same strain and inoculum size) and stored under the same packaging conditions, so that changes in the kinetics of *Lactobacillus* sp. would be dependent only on variations in the packaging film. Indeed, only at >5°C did the decrease of the OTR of film packaging from 2,600 to 28 cm^3 m^{-2} 24 h^{-1} at 23°C and 75% RH result in a decrease in the growth rate of *Lactobacillus* sp. However, under all the storage temperatures, the duration of the lag phase was dependent on the packaging conditions and the film OTR, whereas the final population of the bacterium remained approximately at the same level (Table 3). The highest Ea values for *Lactobacillus* sp. were observed under aerobic storage and 100% CO_2 packaging conditions with the high-permeable film, and the lowest Ea values were observed under 100% CO_2 packaging conditions with the low-permeable film (Table 2). Differences in Ea values have also been reported by Giannuzzi et al. *(3)* for psychrotrophic microorganisms grown in vacuum-packed beef, including LAB, when the O_2 permeability of the packaging material varied between high and low values. The relatively ''discontinuous'' position of the regression lines in the Arrhenius plots of *Lactobacillus* sp., which are expressed when Ea is held statistically invariant, leads to the conclusion that the temperature dependence of its growth rate is significantly affected by the film OTR (Fig. 1 and Table 3). Indeed, it has been reported that the OTR increases with rises in temperature *(15).*

It is generally accepted that the selection of an impermeable film will reinforce the advantageous effects of MAP and, as a consequence, guarantee the extension of shelf life and maintain the quality of meat. However, a large volume of packaging materials are commercially available that fulfill the above criteria. The use of high-barrier films that range from 85 to 2.5 (28 cm^3 m^{-2} 24 h^{-1} at 23°C and 75% RH) showed that the growth rate of *Lactobacillus* sp. was significantly reduced in samples that were packaged in 100% CO_2 when films with a permeability of less than 35 cm^3 m^{-2} 24 h^{-1} at 23°C and 75% RH were used. This

TABLE 3. *The effect of film[a] permeability (fm) on the growth kinetics of* Lactobacillus *sp. inoculated on sterile meat fillets and stored in 100% CO_2 at 8°C*

Packaging	fm	Final population (log CFU/g)	Lag period (days)	Growth rate (days^{-1})
AIR		7.62 ± 0.08[b]	0	0.87 ± 0.15
	85	7.07 ± 0.19	3.92 ± 0.94	0.61 ± 0.30
	35	6.95 ± 0.12	4.12 ± 0.59	0.49 ± 0.19
MAP	28	6.88 ± 0.17	3.05 ± 0.83	0.41 ± 0.11
	17	7.05 ± 0.25	1.47 ± 1.11	0.42 ± 0.08
	2.5	7.06 ± 0.09	1.31 ± 0.49	0.43 ± 0.07

[a] OTR cm^3 m^{-2} 24 h^{-1} at 23°C and 75% RH.
[b] Standard deviation.

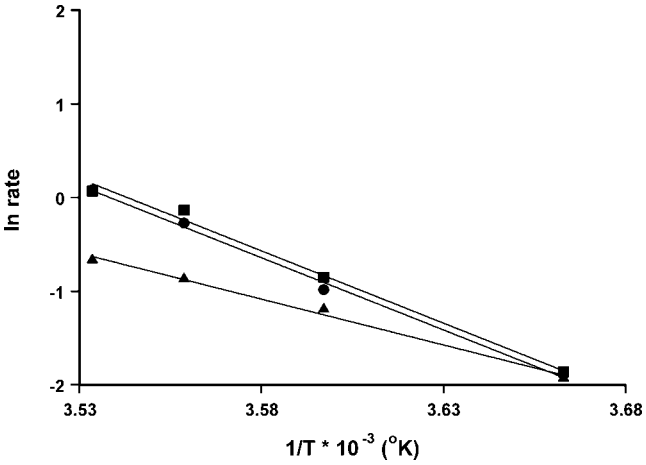

FIGURE 1. *The effect of temperature (Arrhenius model) on the maximum growth rate of* Lactobacillus *sp. inoculated on sterile meat fillets and stored in air (■) and in 100% CO_2 when packaging films with OTRs of 2,600 (●) and 28 (▲) cm^3 m^{-2} 24 h^{-1} at 23°C and 75% RH are used.*

factor subsequently affected the potential spoilage of a meat ecosystem that simulates dominance of LAB (Table 3).

Given the results of our studies, the usefulness of arbitrarily requiring high-barrier packaging films to control storage organisms may be of concern. Instead, it may be more useful to outline a specific range of OTRs and consider how this specification could be influenced during the storage conditions, because the advantageous outcome of MAP and vacuum-packaging conditions cannot always be ensured under the existence or inappropriate selection of several determinants, such as film permeability. This is more evident at temperature-abused conditions, even if a low-permeable film with a relatively high OTR is used.

Further microbiological and sensory analysis should be performed in naturally contaminated meat.

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