Effect of modified atmosphere packaging on the growth/survival of *Yersinia enterocolitica* and natural flora on fresh poultry sausage

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Modified atmosphere packaging (MAP) is increasingly used to extend shelf-life of fresh produce. In the present work, we determine the survival and growth of *Yersinia enterocolitica* and aerobic mesophiles on fresh poultry sausage under MAP. Samples were divided in two lots, half inoculated with 10^5 cfu g^{-1} of *Y. enterocolitica* ATCC 9610, and half uninoculated control samples. The inoculated and control samples were packaged in different CO_2 concentrations and storage at 4±1°C, during 19 days. Parameters of microbial growth (lag and log phase) were determined by Baranyi and Roberts's equation. Aerobic mesophiles counts presented similar log and lag phase in all samples, except for the ones packaged with 20% CO_2. In the inoculated samples *Y. enterocolitica* showed negative log phase in 100% CO_2, 80% CO_2, and 40% CO_2, 2.1 days in 20% CO_2, and approximately 0.3 day in air and 100% N_2. The lag phase varied from 0.9 to 12.0 days (air and 100% CO_2, respectively). Results show that MAP slowed the growth of *Y. enterocolitica* due to the bacteriostatic effect of CO_2 on the development of this pathogen in the samples, but MAP itself was not able to eliminate this pathogen.

Keywords carbon dioxide; MAP; pathogen

1. Introduction

Poultry is considered one of the most important animal protein sources for the world population [1, 2]. In Brazil, aviculture has significantly grown for the past ten years. Brazil is already the biggest poultry meat exporters in the world. In the year 2007, Brazil produced 9,700 thousand tons and exported 3,203 thousand tons of poultry meat, excluding industrialized products. In 2000, the Brazilian annual poultry consumption was 5,110 thousand tons, this number rising to 7,120 thousand tons in 2007 [3]. In addition to this, the poultry industry has started to develop new products, such as sausages and other industrialized products [4, 5].

Modified atmosphere packaging (MAP) technology provides a method of offering to consumers fresh products with a longer shelf-life [6]. This technology can be used by the food industry as an efficient tool to launch new products, providing convenience and practicability to them [7, 8]. At the present time, the food sector requires technologies that can replace preservation methods which can alter food chemically and physically by less severe methods, such as MAP technology [9, 10].

However, an extended shelf-life can lead to an increase of the microbiological risk. Any attempt of extending the shelf-life of foods should consider the potential health hazard posed by the growth of the cold-tolerant pathogens [10, 11, 12, 13]. *Y. enterocolitica* is a psychrotrophic pathogen, able to grow and reach high concentrations in a short period of time, when in refrigeration temperature [14, 15]. Yersiniosis is mostly a food borne disease. When acquired by contaminated food this pathogen can cause gastroenteritis with diarrhea and/or vomit, even though, fever and abdominal pain [16, 17, 18]. This microorganism may also cause infection in other sites such as wounds, joints and urinary tract [19].

The objective of this study was to evaluate the growth/survival of aerobic mesophiles and *Y. enterocolitica*, when contaminating fresh poultry sausage, in MAP with different concentrations of CO_2 (100% CO_2, 100% N_2, 20/80 CO_2/N_2, 40/60 CO_2/N_2, 80/20 CO_2/N_2). For this purpose, the conditions and velocity of the growth of this pathogen in MAP environment and in aerobiosis, at 4±1°C, were studied.

2. Materials and methods

2.1 Sausage preparation

Fresh poultry sausages were elaborated at the Laboratory of Meat Technology, Faculty of Veterinary, Universidade Federal Fluminense, according to the following formulation: 85.5% poultry breast, 10% pork belly, 1.5% salt, 0.25% powdered garlic, 0.20% white pepper, and 3% sterilized distilled water. A total of 5 Kg of fresh poultry sausage were elaborated. Chicken breast fillets and pork belly were grinded together in a grinder equipped with a disk of 1 mm of diameter. Grinded meat and pork belly were mixed manually with the condiments and distilled water, until the 5 Kg

sausage mixture was totally homogenized. Then, the sausage mass was divided into two lots of 2.5 Kg each. A suspension of 10^5 cfu g^{-1} of *Y. enterocolitica*, ATCC 9610, was inoculated into one of the batches by manual mixing.

Both control and inoculated batches were stuffed in artificial collagen casings, ´Coria` FSC 21 x 40, using a stuffing machine ´Picelli`, with a 10 mm diameter stuffer. The control batch was stuffed before the inoculated one. A total of 180 small (10.0 x 1.5 cm) sausages was manufactured, 90 control sausages and 90 inoculated sausages.

2.2 *Yersinia enterocolitica* strain

The pathogen strain of *Yersinia enterocolitica* ATCC 9610 [20] was obtained from the Health Quality Control National Institute, at the Oswaldo Cruz Foundation.

2.3 Modified atmosphere packaging

Six lots of fifteen control sausages and fifteen sausages inoculated with *Y. enterocolitica* were packaged with either 1 L of 100% air, 100% N_2, 100% CO_2, or 20/80, 40/60, or 80/20 CO_2/N_2 in an ´FAMABRAS` (Model TEC MAQ sealed in vacuum AP 450) packaging machine, which, finally, heat-sealed the bags. ´White Martins LTDA` supplied the gases. Each sausage was packaged alone in one bag of 20 x 22 cm ´Cryovac` BB4L, with diffusion coefficients, according to the supplier, of 150 $cm^3/24h.m^2.bar$ to CO_2, 35 $cm^3/24h.m^2.bar$ to O_2 and 1.4 $cm^3/24h.m^2.bar$ to N_2 at 22ºC. All packaged sausages were kept under refrigeration, at 4±1ºC.

2.4 Microbial analyses

Microbial analyses were performed on duplicate samples, i.e. two slices from different bags. After opening the bags, slices (10g) were immersed in 90 ml sterile physiological saline solution (0.85% NaCl) and homogenized in a stomacher (Colwoth Stomacher 400 Lab Blender, Seward, U.K.) for 15s. After, decimal dilutions were prepared in saline solution.

Aerobic mesophiles counts were determined by the pour plate technique [21], in Plate Count Agar PCA (Merck), incubated at 35ºC for 48h. *Yersinia enterocolitica* counts were performed by the spread plate technique [21], in *Yersinia* Selective Agar Base (Oxoid) with selective supplement SR109 (cefsulodin 1.5%, igarsan 0.4% and novobiocin 0.25%), at 32ºC for 24h.

Aerobic mesophiles and *Y. enterocolitica* counts were determined after 0, 1, 3, 5, 7, 9, 12, 14, 16, and 19 days of storage. Both control and inoculated samples were analyzed for periodic aerobic mesophiles counts. Only inoculated samples were analyzed for *Y. enterocolitica* counts.

Bacterial growth parameters (lag phase and log phase) were assessed using the Baranyi and Roberts [22] equations and the self-life of meat was defined as days to reach 10^7 cfu g^{-1}.

3. Results

Figure 1 shows the growth curves of aerobic mesophiles in the control and inoculated samples packaged in the six atmospheres assayed in the present work.

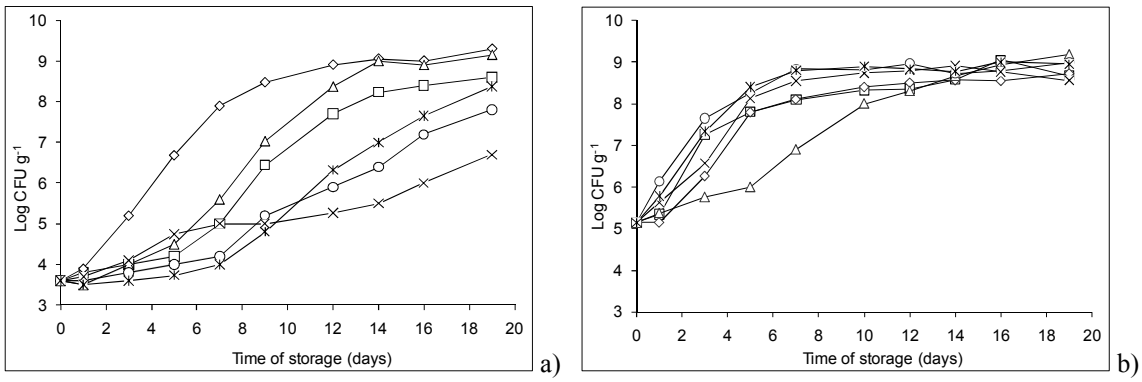

Fig. 1 Aerobic mesophiles growth curves in the control (a) and inoculated (b) samples packaged in: (—□—) 100% air, (—◇—) 100% N_2, (—△—) 20/80 CO_2/N_2, (—○—) 40/60 CO_2/N_2, (—×—) 80/20 CO_2/N_2, and (—✳—) 100% CO_2, stored at 4±1ºC.

In the control samples, the initial count was $4.4x10^3$ cfu g^{-1}. Among the six atmospheres tested during the 19 days of experiment, MAP with 20/80 CO_2/N_2 and with 100% N_2 showed the highest counts of aerobic mesophiles on day 19, reaching $7.5x10^9$ cfu g^{-1} and $9.6x10^9$ cfu g^{-1}, respectively. On the other hand, MAP with 40/60 CO_2/N_2 and 80/20 CO_2/N_2 showed the lowest levels of aerobic mesophiles, reaching $6.2x10^7$ cfu g^{-1} and $5.2x10^6$ cfu g^{-1}, on day 19,

respectively. Thus, both atmospheres (80/20 CO_2/N_2 and 40/60 CO_2/N_2) showed the best behavior for aerobic mesophiles, presenting, together with lower counts, a higher log time (Table 1).

According to Figure 1a, aerobic mesophiles already started to grow on day one in samples packaged in 100% N_2. In samples packed in 100% air and in 20/80 CO_2/N_2 aerobic mesophiles increased their growth rate after day 5. The same happened in samples packaged in 40/60 CO_2/N_2 and in 100% CO_2, on day 7. Samples packaged in 80/20 CO_2/N_2 showed as light growth of aerobic mesophiles after day 1, stabilizing at day 5, and growing again on day 9.

Figure 1b shows the growth curves of aerobic mesophiles in the inoculated samples. Due to the presence of *Y. enterocolitica*, the initial count of mesophiles was much higher than in control samples. For the same reason, in comparison with the control samples, counts reached higher values in less time in the inoculated samples, approximately 10^9 cfu g^{-1} at the end of the experience, except for MAP with 80/20 CO_2/N_2 and 40/60 CO_2/N_2 which achieved a maximum of 10^8 cfu g^{-1}. The atmosphere with the 100% N_2 recorded the highest counts, with $6.6x10^9$ cfu g^{-1} at day 19.

The six curves followed the same pattern, showing expressive growth already on day 1. Counts reached values of approximately 10^8 cfu g^{-1} on day 5 in MAP with 40/60 CO_2/N_2, 80/20 CO_2/N_2, and 100% CO_2, and on day 7 in 100% air and 100% N_2. MAP with 20/80 CO_2/N_2 presented discrete growth until day 7 (10^6 cfu g^{-1}) and more expressive growth at day 10 (10^8 cfu g^{-1}).

Table 1 shows the growth parameters (lag-phase, log-phase and number of cells in the stationary phase) of aerobic mesophiles in the control and inoculated samples. In control samples, the log phase was longer in MAP with 40/60 CO_2/N_2 and 80/20 CO_2/N_2 which also presented lower doubling time and achieved lower counts in the stationary phase.

Table 1 Growth parameters of aerobic mesophiles in the control and inoculated samples packed in 100% air and in different MAP, stored at 4±1ºC.

Atmosphere	Parameter	Control	Inoculated
100% Air	Lag	4.3	0.3
	Log	13.7	11.0
	NC	8.7	8.5
100% N_2	Lag	0.4	1.5
	Log	11.1	9.3
	NC	9.0	8.6
20/80 CO_2/N_2	Lag	4.3	0.5
	Log	6.6	25.5
	NC	9.0	9.2
40/60 CO_2/N_2	Lag	4.6	0.0
	Log	22.9	10.1
	NC	8.2	8.8
80/20 CO_2/N_2	Lag	0.0	0.3
	Log	44.7	11.5
	NC	7.4	8.8
100% CO_2	Lag	6.2	0.0
	Log	16.0	10.3
	NC	8.5	8.9

Lag – Lag-phase (days).
Log – Log-phase (hours).
NC – Number of cells in the stationary phase (log cfu g^{-1}).

In inoculated samples, MAP with 20/80 CO_2/N_2 showed the longest log-phase. But also the highest values in the stationary phase, opposite to what happened in the control samples. In the other five MAP tested, mesophiles presented similar log and lag phases (Table 1).

Figure 2 shows the growth curves of *Y. enterocolitica* in the inoculated samples in the six atmospheres tested. The initial count was $8.2x10^4$ cfu g^{-1}. Inoculated samples packaged in 100% air and in MAP with 100% N_2 presented the highest counts, about 10^7 cfu g^{-1}. The lowest counts of *Y. enterocolitica* were recorded in the MAP with 100% CO_2, decreasing its number from the initial values to $5.3x10^4$ cfu g^{-1}, at day 19.

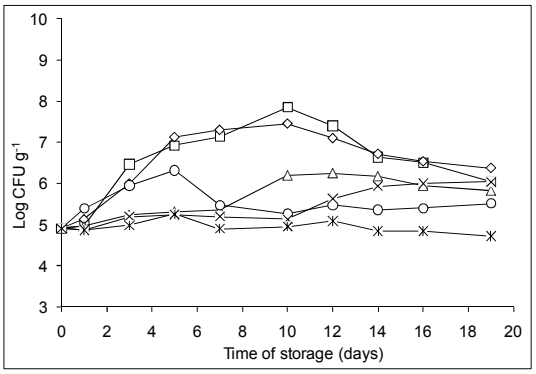

Fig. 2 *Y. enterocolitica* growth curves in the inoculated samples packaged in: (□) 100% air, (◇) 100% N_2, (△) 20/80 CO_2/N_2, (○) 40/60 CO_2/N_2, (×) 80/20 CO_2/N_2, and (✳) 100% CO_2, stored at 4±1ºC.

MAP with 100% CO_2 presented a very discrete growth during the whole experience, even registering the lowest counts at day 19. Growth was delayed in MAP with 80/20 CO_2/N_2 until day 12, reaching 10^6 cfu g^{-1} at day 14. In MAP with 40/60 CO_2/N_2 the maximum growth was observed on day 5 (10^6 cfu g^{-1}) and then a slight decrease was noticed remaining with counts around 10^5 cfu g^{-1} until the end of experiment. In MAP with 20/80 CO_2/N_2 a gradual growth of the pathogen was observed until day 12, reaching its highest value, and then decreasing its number until day 19. The most significant growth of *Y. enterocolitica* was observed in inoculated samples packaged in 100% air and in 100% N_2. In both environments a gradual growth of *Y. enterocolitica* was verified, achieving the maximum values at day 10.

Table 2 shows the growth parameters of *Y. enterocolitica*. This microorganism presented the longest log-phase in MAP with 80/20 CO_2/N_2. Negative log-phases were detected in MAP with 40/60 CO_2/N_2 and 100% CO_2. The pathogen was not capable of growing in both environments. Its population decreased to lower values than the initial counts. *Y. enterocolitica* presented a shorter log-phase and higher counts in the stationary phase in 100% air and 100% N_2.

Table 2 Growth parameters of *Yersinia enterocolitica* in the inoculated samples packaged in 100% air and in different MAP, stored at 4±1ºC.

Atmospheres	Parameter	*Y. enterocolitica*
100% Air	Lag	0.9
	Log	9.5
	NC	6.9
100% N_2	Lag	2.5
	Log	3.4
	NC	6.8
20/80 CO_2/N_2	Lag	2.3
	Log	50.7
	NC	6.1
40/60 CO_2/N_2	Lag	5.0
	Log	-497.6
	NC	4.9
80/20 CO_2/N_2	Lag	1.2
	Log	108.5
	NC	6.0
100% CO_2	Lag	12.0
	Log	-117.5
	NC	4.7

Lag – Lag-phase (days).
Log – Log-phase (hours).
NC – Number of cells on stationary phase (log cfu g^{-1}).

4. Discussion

The growth pattern of aerobic mesophiles observed in the control samples packaged in 100% air and in 100% N_2 (Figure 1a), agrees with the one described by Kakouri and Nychas [23], who also found higher counts in shorter time in packages with 100% N_2 than in samples packaged in 100% air. On the contrary, Mano et al. [24] observed in turkey meat, stored at 7ºC, higher counts in samples packaged with 100% air. However, these differences could be attributed to the storage temperature and the product studied.

Aerobic mesophiles took 16 days to reach 10^7 cfu g^{-1} in packages with 100% CO_2 in Figure 1a. However, Kakouri and Nychas [23], Hudson et al. [25], and Bell et al. [26] found that aerobic mesophiles needed approximately 10 days to

achieve these counts in the same atmosphere in different products, i.e. poultry meat, smoked cod, and red meat, respectively. It stands to reason that the pH of poultry sausage is lower than that of poultry meat itself. The sausage tested in this experiment presented pH 5.9, on day 0, which could have contributed to delay the bacterial growth. Furthermore, poultry sausage contains salt and condiments that can also contribute for retardation and/or the inhibition of microbial growth. The initial counts found by the above mentioned authors were higher than we observed in the present work (Figure 1a – $4.4x10^3$ cfu g^{-1} mesophiles) but the storage temperatures were also different. Both factors certainly influenced the disparity of results found by the different authors.

Zeitoun et al. [27] detected $2.5x10^7$ cfu cm^{-2} of mesophiles around day 6, in poultry meat samples packaged in MAP with 90/10 CO_2/O_2. According to Figure 1a, aerobic mesophiles grew slower under 80/20 CO_2/N_2. The different behavior observed can be explained by the use of O_2 combined with CO_2 instead of N_2. It stands to reason that O_2 favors the growth of aerobic bacteria. Moreover, N_2 does not have the same effect [28].

The inoculated samples showed high initial counts of mesophiles, which could have interfered on the bacteriostatic effect of CO_2. The same was observed by Sarantópoulos et al. [29]. In addition to this, the growth curves of aerobic mesophiles in the inoculated samples packaged in modified atmospheres reached higher values in shorter time in comparison with the control samples (Figures 1a and 1b), and presented pretty short lag and log phases (Table 1).

Y. enterocolitica did not grow in the samples packaged in MAP with 100% CO_2, and even reduced its population. The same was observed by Hudson et al. [25], Bell et al. [26], Doherty et al. [30], Bodnaruk and Draughon [31] and Tassou et al. [32]. These authors assayed different products and storage temperatures, which can confirm the bacteriostatic effect of 100% CO_2 on the pathogen *Y. enterocolitica*.

In the inoculated samples packaged in 100% air and in 100% N_2, *Y. enterocolitica* increased its population up to three logarithmic cycles. Shenoy and Murano [33] also detected the growth of this organism in chopped pork meat packaged in air and stored at 4°C. Doherty et al. [30] detected growth of *Y. enterocolitica* in lamb meat packaged in 100% air, and stored at 0°C and 5°C. Wei et al. [34] also verified the growth of the pathogen in poultry meat packaged in 100% air. Thus *Y. enterocolitica* is capable of growing in 100% air and in MAP with 100% N_2, when stored at 4±1°C.

Although *Y. enterocolitica* can grow in MAP with 20/80 CO_2/N_2, its growth can be strongly influenced by the storage temperature. In the present study *Y. enterocolitica* reached counts up to 10^6 cfu g^{-1} in this atmosphere. Manu-Tawiah et al. [35] also detected growth in chopped pork meat, using the same MAP and temperature, with counts up to 10^7 cfu cm^{-2}. However, Van Den Elzen et al. [36] observed a very discrete growth of this pathogen in pork meat, in MAP with 25/65/10 $CO_2/O_2/N_2$, stored in 3°C, with counts only up to one logarithmic cycle above the initial count. The association of CO_2 and O_2 tends to inhibit the growth of this pathogen, which can explain the differences between the results described above.

In 40/60 CO_2/N_2, *Y. enterocolitica* counts slightly increased until day 5, decreasing afterwards. In addition, it can be observed in Table 2 that the pathogen was not capable of growing. During the stationary phase it decreased in comparison with the initial counts. However, Doherty et al. [30] and Shenoy and Murano [33] detected *Y. enterocolitica* growth in MAP with 50/50 CO_2/N_2. The different gas concentrations, MAP with 50/50 CO_2/N_2 and 40/60 CO_2/N_2, and the different product (pork meat) used in the experiments might explain the disparity of results.

Hudson et al. [25] stated that to inhibit the growth of psychrotrophic pathogens it is necessary an atmosphere with more than 75% CO_2 and total absence of O_2. In the presence of O_2, higher concentrations of CO_2 are necessary. The effect of MAP with O_2 on the growth of *Y. enterocolitica* was not studied in the present work. The results exposed on Figure and Table 2 show that the pathogen was able to grow in MAP with 80/20 CO_2/N_2, which does not agree with Hudson et al. [25]. Nevertheless, it was a discrete growth, presenting a pretty long log-phase (108.5 hours) compared to the growth in samples packaged in 100% air (9.5 hours). These observations confirm the bacteriostatic effect of CO_2.

The influence of competitive microbial population was evidenced by the growth of aerobic mesophiles, which presented much higher counts when compared to *Y. enterocolitica*, during the 19 days of experiment in all the inoculated samples and MAP tested (Tables 1 and 2).

The infective dose of *Y. enterocolitica* in food is still unknown [37]. However, Bhaduri and Turner-Jones [38] stated that the virulent characteristics of this microorganism remain the same, even in anaerobiosis and in mixtures of CO_2 and other gases, being able to cause foodborne disease. The study of *Y. enterocolitica* in MAP food becomes significant to public health, provided that this pathogen can be present in food and is able to grow in those conditions, even in low levels.

5. Conclusions

According to this research, it can be concluded that MAP with 80/20 CO_2/N_2 is the best choice for fresh poultry sausage. This atmosphere controlled the growth of aerobic mesophiles. In those conditions, mesophiles showed a low growth rate and moderate counts. This MAP was also capable of inhibiting *Yersinia enterocolitica*, although other factors can influence its development. Therefore, it can be concluded that MAP itself is not able to eliminate this pathogen in fresh poultry sausage. However, it can collaborate, sometimes precisely, by controlling its development, avoiding its growth and even reducing its population.

Packaging in 100% air and in 100% N_2 should not be used for fresh poultry sausage. Moreover, it enhances the rapid growth of not only the natural microbiota, but also of *Y. enterocolitica*, being able to reach high counts and thus, representing a serious risk for public health.

Further research on the pathogen *Y. enterocolitica* and its behavior in different MAP is still necessary for the development of packaging of different animal origin products. It is likely that the effectiveness of the technique shall be optimized so that ideal concentrations of gases in MAP can be established for each type of product.

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References

[1] Rust RE. Productos embutidos. In: Price JF, Schweigert BS, eds. *Ciencia de la carne y de los productos cárnicos*. Zaragoza: Acribia Press; 1994:415-439.

[2] Lopes MM, Silva LP, Conte-Junior CA, Teodoro AJ, Mano SB, Freitas MQ, Franco RM, Pardi HS. Aspectos bacteriológicos e físico-químicos da lingüiça frescal de frango. *Revista Portuguesa de Ciências Veterinárias*. 2007;102:331-338.

[3] Associação Brasileira dos Exportadores de Frango. The ABEF page. Available at: http://www.abef.com.br/estatisticas. 08. Accessed May 26, 2010.

[4] Madava R, Hoogenkamp H. The role of processed products in the poultry meat industry. In: Richardson IR, Mead GG, eds. *Poultry Meat Science*. Wallingford: CABI Publishing; 1999:396-410.

[5] Silva LP, Lopes MM, Mano SB, Máralém ET, Conte-Junior CA, Teodoro AJ, Guedes WS. Influência da adição de polifosfato em lingüiça de frango. *Revista Brasileira de Ciência Veterinária*. 2008;15:50-55.

[6] Mano SB, García de Fernando GD, López-Gálvez D, Selgas MD, García ML, Cambero MI, Ordoñez JA. Growth/survival of natural flora and *Listeria monocytogenes* on refrigerated uncooked pork and turkey packaged under modified atmosphere. *J. Food Safety*. 1995;15:305-319.

[7] García de Fernando GD, Nychas GJE, Peck MW, Ordóñez JA. Growth/survival of psychrotrophic pathogens on meat packaged under modified atmospheres. *International Journal of Food Microbiology*. 1995;28:221-231.

[8] Conte-Junior CA, Fernández M, Mano SB. Use of carbon dioxide to control the microbial spoilage of bullfrog (*Rana catesbeiana*) meat. In: Mendez-Vilas A, ed. *Modern Multidisciplinary Applied Microbiology: Exploiting Microbes and their Interactions*. Weinheim: Wiley-VCH; 2006:356-361.

[9] Sarantópoulos CIGL, Alves RMV, Contreras CJC, Galvão MTEL, Gomes TC. Use of a modified atmosphere masterpack for extending the shelf life of chicken cuts. *Packaging Technology and Science*. 1998;11:217-229.

[10] Mano SB, Ordónez JA, Garcia de Fernando GD. Growth/survival of natural flora and *Aeromonas hydrophila* on refrigerated uncooked pork and turkey packaged in modified atmospheres. *Food Microbiology*. 2000;17:657-669.

[11] Church N. Developments in modified-atmosphere packaging and related technologies. *Trends in Food Science & Technology*. 1994;5:345-352.

[12] Phillips CA. Review: modified atmosphere packaging and its effects on the microbiological quality and safety of produce. *International Journal of Food Science and Technology*. 1996;31:463-479.

[13] Woods LFJ, Church P N. Strategies for extending the shelf-life of poultry meat and products. In: Richardson IR, Mead GG, eds. *Poultry Meat Science*. Wallingford: CABI Publishing; 1999:297-310.

[14] Nortjé GL, Vorster SM, Greebe RP, Steyn PL. Occurrence of *Bacillus cereus* and *Yersinia enterocolitica* in South Africa retail meats. *Food Microbiology*. 1999;16:213-217.

[15] Tompkin RB, Mcnamara AM, Acuff GR. Meat and poultry products. In: Downes FP, Ito K, eds. *Compendium of methods for the microbiological examination of foods*. Washington: American Public Health Association; 2001:421-428.

[16] Bourgeois C. Conservación en atmosferas modificadas. In: Bourgeois MC, Mescle FJ, Zucca J, eds. *Microbiologia alimentaria aspectos microbiológicos de la seguridad y calidad alimentaria*. Zaragoza: Acribia; 1994:409-421.

[17] Dromigny E. *Campylobacter, Listeria monocytogenes, Yersinia enterocolitica*. In: Bourgeois MC, Mescle FJ, Zucca J, eds. *Microbiologia alimentaria aspectos microbiológicos de la seguridad y calidad alimentaria*. Zaragoza: Acribia Press. 1994:113-129.

[18] Center for Desease Control and Prevention. The CDC page. *Yersinia enterocolitica*. Available at: http://www.cdc.gov/ncidod/dbmd/diseaseinfo/yersinia_g.htm. Accessed May 27, 2010.

[19] United States Food & Drug Administration. The FDA page. *Yersinia enterocolitica*. In: *Foodborne pathogenic microorganisms and natural toxins handbook*. Available at: http://www.fda.gov/Food/FoodSafety/FoodborneIllness/FoodborneIllnessFoodbornePathogensNaturalToxins/BadBugBook/ucm070040.htm. Accessed May 27, 2010.

[20] Krieg NR, Holt JG. *Bergey's manual of systematic bacteriology*. 2nd ed. Baltimore, Md: Williams and Wilkins; 1984.

[21] Vanderzant C, Splittstoesser DF. Compendium of methods for the microbiological examination of foods. 3nd ed. Washington, Md: American Public Health Association; 1992.

[22] Baranyi J, Roberts TA. A dynamic to predicting bacterial growth in food. *International Journal of Food Microbiology*. 1994;23:277-294.

[23] Kakouri A, Nychas GJE. Storage of poultry meat under modified atmospheres or vacuum packs: possible role of microbial metabolites as indicator of spoilage. *Journal of Applied Bacteriology*. 1994;76:163-172.

[24] Mano SB, Ordóñez JA, Fernando, GDG. Aumento de la vida útil y microbiología de la carne de pavo envasada en atmósferas modificadas. *Revista Brasileira de Ciência Veterinária*. 1999;6:55-65.

[25] Hudson AJ, Mott SJ, Penney N. Growth of *Listeria monocytogenes*, *Aeromonas hydrophila*, and *Yersinia enterocolitica* on vacuum and saturated carbon dioxide controlled atmosphere-packaged sliced roast beef. *Journal of Food Protection*. 1994;57:204-208.
[26] Bell GR, Penney N, Moorhead SM. Growth of the psychrotrophic pathogens *Aeromonas hydrophila*, *Listeria monocytogenes*, and *Yersinia enterocolitica* on smoked blue cod (*Parapercis colias*) packed under vacuum or carbon dioxide. *International Journal of Food Science and Technology*. 1995;30:515-521.
[27] Zeitoun AAM, Debevere JM, Mossel DAA. Significance of Enterobacteriaceae as index organisms for hygiene on fresh poultry, poultry treated with lactic acid and poultry stored in modified atmosphere. *Food Microbiology*. 1994;11:169-176.
[28] Guynot ME, Marín S, Sanchis V, Ramos AJ. Modified atmosphere packaging of mold spoilage of bakery products with different pH and water activity levels. *Journal of Food Protection*. 2003;66:1864-1872.
[29] Sarantópoulos CIGL, Alves RMV, Oliveira LM, Gomes TC. *Embalagens com atmosfera modificada*. 2nd ed. Campinas, Md: CETEA/ITAL Press; 1998.
[30] Doherty A, Sheridan JJ, Allen P, Mcdowell DA, Blair YS, Harrington D. Growth of *Yersinia enterocolitica* O:3 on modified atmosphere packaged lamb. *Food Microbiology*. 1995;12:251-257.
[31] Bodnaruk PW, Draughon, FA. Effect of packaging atmosphere and pH on the virulence and growth of *Yersinia enterocolitica* on pork stored at 4^{o}C. *Food Microbiology*. 1998;15:129-136.
[32] Tassou CC, Lambropoulou K, Nychas GJE. Effect of prestorage treatments and storage conditions on the survival of *Salmonella enteriditis* PT4 and *Listeria monocytogenes* on fresh marine and freshwater aquaculture fish. *Journal of Food Protection*. 2004;67:193-198.
[33] Shenoy K, Murano EA. Effect of storage conditions on growth of heat-stressed *Yersinia enterocolitica* in ground pork. *Journal of Food Protection*. 1996;59:365-369.
[34] Wei QK, Fang TJ, Chen WC. Development and validation of growth model for *Yersinia enterocolitica* in cooked chicken meats packaged under various atmosphere packaging and stored at different temperatures. *Journal of Food Protection*. 2001;74:987-993.
[35] Manu-Tawiah W, Myers DJ, Olson DG, Molins RA. Survival and growth of *Listeria monocytogenes* and *Yersinia enterocolitica* in pork chops packaged under modified gas atmospheres. *Journal of Food Science*. 1993;58:475-479.
[36] Van den Elzen AMG, Houben JH, Snijders JMA. Effect of modified atmosphere packaging on the survival of pathogens on artificially contaminated pork. Proceedings of the *40th International Congress of Meat Science Technology*. The Hague, Holland; 1994.
[37] Long C, Jones TF, Vugia DJ, Scheftel J, Strockbine N, Ryan P, Shiferaw B, Tauxe RV, Gould LH. *Yersinia pseudotuberculosis* and *Y. enterocolitica* infections, FoodNet, 1996–2007. *Emerging Infectious Diseases*. 2010;16:566-567.
[38] Bhaduri S, Turner-Jones C. The effect of anaerobic atmospheres on the stability of the virulence-related characteristics in *Yersinia enterocolitica*. *Food Microbiology*, 1993;10:239-242.