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EFFECT OF ULTIMATE PH AND PACKAGING ON MICROFLORA OF BEEF OF MARONESA BREED STORED AT 4ºC

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Introduction

Meat is a good support for bacterial growth because of its composition: 75% water and many different metabolites such as amino-acids, peptides, nucleotides and sugars (Labadie, 1999). Spoilage of meat is the result of the microbial activity of several microorganisms whose survival and growth is dependent on the characteristics of the product and the way it is processed and stored, affecting the qualitative and quantitative composition of the spoilage microflora (Huis in´t Veld, 1996). The predominant bacteria associated with spoilage of refrigerated beef are Brochothrix thermosphacta, Carnobacterium spp., Enterobacteriaceae, Lactobacillus spp., Leuconostoc spp., Pseudomonas spp. and Shewanella putrefaciens (Borch et al., 1996).

Among the parameters that affect the growth of microorganisms in meat, it is assumed that the ultimate pH and the gaseous composition of the packaging have an important role (Newton and Gill, 1978; Walker and Betts, 2000). The preservation of foods in modified atmosphere packaging (MAP) generally consists in the enclosure of the food product in gas-barrier materials, in which the gaseous environment as been changed (Gould, 1996) This procedure inhibits spoilage agents minimizing the loss of products and maintaining a higher quality in perishable food during its normal shelf-life or extending it. Nevertheless, MAP should be associated with strict temperature control to achieve maximum microbial inhibition (Tewari et al. 1999).

MAP generally uses three different gases: oxygen, carbon dioxide and nitrogen each with a specific function. Packages containing up to 80% oxygen and 20% carbon dioxide (CO2) (high oxygen MA) will preserve fresh meat colour and prevent anaerobic spoilage but will have only a slightly increase in the shelf-life (Borch et al., 1996). Atmospheres with high concentrations of CO2 are used to benefit from the bacteriostatic effect of this gas; nevertheless it is usually associated with rapid discoloration of meat surface causing sensorial rejection. Vacuum packaging prevents the growth of a few groups of spoilage microorganisms, due mainly to the low availability of oxygen (Church and Parsons, 1995).

Objectives

The aim of the present work was to determine the influence of different meat packaging: (air, vaccum and modified atmospheres with different gaseous compositions)

on spoilage microflora usually associated with spoilage of refrigerated beef (Total viable counts, Lactic Acid Bacteria, Enterobacteriaceae, Pseudomonas spp. and Brochothrix thermosphacta) and the influence of ultimate pH (Normal and High) of beef of maronesa breed on same microflora.

Methodology

M. longissimus thoracis et lumborum of bovine maronesa breed with high ultimate pH values (≥6.2, n=6) and with normal pH values (<5.8, n=6) were cut from carcasses 24h post mortem and kept in cold storage. Muscles were cut into pieces weighing approximately 70g and packed in 5 different types of packaging namely: air (A); vacuum packaging (V); 70%O2 + 20%CO2 + N2 (MAP70/20); 50%O2 + 40%CO2 + 10%N2 (MAP50/40) and 30%O2 + 60%CO2 + 10%N2 (MAP30/60).

Air: Meat cuts were tray-packaged in air overwrapped with polyetilen film.

Vacuum: Beef cuts were individually vacuum packaged in COMBITHERM bags (WIPAK Walsrode, HAFRI) which have an oxygen transmission rate of 63cm^3 m^{-2} d^{-1} atm^{-1} at 23ºC, 0% r.h. and water vapor transmission (WVT) of 1g m^{-2} d^{-1} at 23ºC, 85% r.h, using a SAMMIC V-420 SGA.

Modified atmosphere packaging: Meat cuts were individually placed in COMBITHERM XX bags (WIPAK Walsrode, HAFRI) 0,115 mm thick and OTR of 1 cm^3 m^{-2} d^{-1} atm^{-1} at 23ºC, 0% r.h. and WVT of 1 g m^{-2} d^{-1} at 23ºC, 85% r.h.. The atmosphere in the MA packages was first removed and then flushed with the appropriate gas mixture (Praxair, Portugal) using a SAMMIC V-420 SGA. The final gas to meat ratio was approximately 3:1.

Following packaging, meat samples were stored at 4±1ºC and examined at intervals of 3, 7, 10, 14 and 21 days post mortem (the last not made for packaging A) for microbiological analysis. One extra sampling period (28 days) were made for vacuum and MAP30/60.

Microbiological analysis: Meat cuts were sampled aseptically at each interval. Samples were homogenized with tryptone salt (tryptone 0.3% and NaCl 0.85%) in a Stomacher for 90s. Serial decimal dilutions were prepared in the same solution for microbiological determinations.

Total aerobic counts were determined on Plate Count Agar (OXOID CM325) (30ºC 3 days), Lactic acid bacteria (LAB) on double layer on MRS agar (OXOID CM361) (30ºC 3 days).

Enterobacteriaceae, Brochothrix thermosphacta and Pseudomonas were determined basically according to ISO 5552(1997), ISO 13722 (1996) and NF V 04-504 (1998) on double layer VRBG (OXOID CM485) (37ºC 24h), Brochothrix thermosphacta on STAA agar (CM881, SR151; OXOID) (25ºC 2 days) and Pseudomonads counts on CFC agar (CM559, SR103; OXOID) (25ºC 2 days), respectively.

Statistical analysis

The data were subjected to analysis of variance with GLM procedure in order to test the effect of pH, packaging and interactions for each day of storage using the Systat programme 10.2 (Systat Software Inc., 2002). Tukey test was used to locate differences between means at 5% level of probability.

Results & Discussion

Results of microbial analysis are presented in tables 1 and 2, according the packaging regime and the pH group respectively.

According to results presented in table 1, it is possible to observe that LAB are generally unaffected by the packaging, as indicated by the similar ($P \geq 0.05$) counting along all the period studied.

For the TVC, B. thermosphacta, Enterobacteriaceae and Pseudomonas, the same pattern was generally observed. Thus, the air packaging presents always higher counts than any other packages. The effect of the composition of MAP observed was not as intense as it was expected, namely regarding the similar counts observed in high CO2 packaging for different spoilage groups. The type of packaging has influenced the growth of BT, with significantly differences ($P<0.001$) in day 7 and day 14, and significantly differences ($P<0.05$) in 10 day post mortem. The higher counts values were obtained in air (6.60 log ufc cm-2) at day 14 post mortem and in MAP70/20 (6.27 log ufc cm-2) and in MAP50/40 (6.07 log ufc cm-2) both at day 21 post mortem. These results are in accordance with the resistance of this bacterium to high concentrations of CO2 (Newton and Rigg, 1979). The effect of higher CO2 concentration was noted also in the counting of Pseudomonas at 7, 10 and 21, days, resulting in lower counting. Considering the aerobic character of Pseudomonas it was expected a stronger effect of packaging.

Among all microorganisms, Enterobacteriaceae presented the lowest level of growth in all storage times and type of packaging, being the higher value 3.81 log cfu cm-2 in vacuum at day 28.

Generally, it was observed higher counts – significantly different - of all the groups of microorganisms screened in meat presenting a higher ultimate pH (table 2), except for LAB. That difference is usually pointed by several authors based on the shorter lag phase of spoilage bacteria in those meats. Additionally, the implications that overgrowth have in the sensorial spoilage perception is due also to the smallest amount of available glucose that the meat with high pH presents, inducing bacteria to a rapid shift in metabolism to the use of amino acids. The exception observed in LAB is related to its known tolerance to low pH (Gill, 1983; Walker and Betts, 2000). As observed for the effect of packaging, it was also in the group of BT and Pseudomonas that this effect was more evident, particularly after 7 days pm.

Conclusions

According to the results of the present work it is possible to conclude that the packaging strategy used have implications on the spoilage microflora. The main differences were observed between air, vacuum and the other MAPs. Differences between MAPs were found for the counts of Pseudomonas and Brochothrix thermosphacta. The effect of increasing carbon dioxide concentration on the packaging was not evident on the growth of LAB, Enterobacteriaceae and TVC.

The effect of the ultimate pH of the meat was evident for almost all spoilage groups studied, except for LAB. Higher pH was associated to higher counts.

The packaging more suitable for each type of meat, considering the ultimate pH, should be established, from the microbiological data, as those obtained with this work, but also from the evaluation of the sensory consequences of its growth.

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Tables and Figures

Table 1. Microflora (log cfu/g, means (standard deviation) isolated from beef of different type of packaging.

Microflora	Packaging	Storage time (days)						
		1	3	7	10	14	21	28
Lactic Acid Bacteria	Air	1.05 (1.16)	0.95 (0.90)	2.71 (1.86)	4.05 (2.35)	4.75 (1.64)	nd[2]	nd
	Vacuum	«	0.79 (1.06)	1.58 (1.83)	2.98 (1.71)	4.16 (2.05)	4.77 (2.04)	5.37 (2.30)
	$MAP_{70/20}$	«	1.11 (1.10)	1.62 (1.41)	2.95 (2.10)	4.37 (1.89)	4.87 (1.98)	nd
	$MAP_{50/40}$	«	1.14 (1.21)	1.58 (1.18)	2.64 (2.05)	3.42 (2.15)	5.07 (1.27)	nd
	$MAP_{30/60}$	«	1.29 (0.98)	1.59 (1.14)	2.99 (1.68)	3.62 (2.18)	5.21 (1.72)	5.33 (2.24)
	sig[1]		ns	ns	ns	ns	ns	ns
Brochothrix thermosphacta	Air	0.35 (1.21)	1.13 (1.69)	4.29 a[3] (2.12)	5.31 a (2.56)	6.60 a (1.14)	nd	nd
	Vacuum	«	0.19 (0.66)	2.18 b (1.94)	3.40 ab (2.30)	5.52 ab (1.22)	5.99 81.43)	5.78 (1.79)
	$MAP_{70/20}$	«	0.55 (1.00)	2.07 b (1.97)	3.57 ab (2.10)	5.07 ab (1.97)	6.27 (2.49)	nd
	$MAP_{50/40}$	«	0.47 (1.12)	1.18 b (1.82)	2.69 b (2.31)	4.25 bc (2.03)	6.07 (1.62)	nd
	$MAP_{30/60}$	«	0.22 (0.78)	0.69 b (1.38)	2.08 b (2.41)	2.97 c (2.27)	5.31 (2.42)	5.29 (2.26)
	sig		ns	***	*	***	ns	ns
Enterobactereaceae	Air	0.12 (0.43)	0.48 (0.91)	1.50 a (1.47)	2.52 a (2.03)	3.78 a (2.17)	nd	nd
	Vacuum	«	0.46 (0.94)	0.45 b (1.04)	1.28 ab (1.99)	2.99 ab (2.39)	3.45 a (2.67)	3.81 a (2.95)
	$MAP_{70/20}$	«	0.00 (0.00)	0.08 b (0.29)	0.67 b (1.00)	1.09 bc (1.77)	2.07 ab (2.09)	nd
	$MAP_{50/40}$	«	0.16 (0.55)	0.11 b(0.38)	0.15 b (0.51)	0.56 b (1.34)	1.33 b (1.62)	nd
	$MAP_{30/60}$	«	0.45 (0.68)	0.55 ab (0.74)	0.62 b (1.68)	1.03 bc.(1.31)	1.31 b (1.28)	0.75 b (1.37)
	sig		ns	**	**	***	*	**
Total viable counts	Air	2.52 (0.60)	2.95 (0.80)	6.54 a (1.09)	8.21 a (0.99)	8.79 a (0.63)	nd	nd
	Vacuum	«	2.48 (0.70)	3.41 b (1.29)	4.64 b (1.25)	6.14 b (1.23)	6.69 (1.33)	7.22 (1.11)
	$MAP_{70/20}$	«	2.50 (0.61)	3.21 b (0.96)	4.25 b (1.39)	5.81 bc (1.47)	7.38 (1.24)	nd
	$MAP_{50/40}$	«	2.77 (0.48)	3.11 b (1.10)	3.91 b (1.45)	4.88 bc (1.48)	6.28 (1.72)	nd
	$MAP_{30/60}$	«	2.56 (0.49)	2.86 b (0.83)	3.67 b (1.43)	4.76 c (1.75)	6.32 (2.01)	6.21 (1.62)
	sig		ns	***	***	***	ns	ns
Pseudomonas spp.	Air	1.33 (1.48)	2.28 a (1.56)	6.85 a (1.01)	7.93 a (0.96)	8.70 a (0.75)	nd	nd
	Vacuum	«	1.08 ab (1.24)	2.99 b (1.64)	4.40 b (1.26)	5.08 b (1.11)	5.45 ab (0.83)	5.69 a (0.88)
	$MAP_{70/20}$	«	1.09 ab (1.04)	2.29 b (1.14)	3.22 bc(1.37)	4.41 b (1.63)	6.41 a (1.33)	nd
	$MAP_{50/40}$	«	0.92 b (1.13)	2.04 b (1.42)	2.30 c (1.40)	2.73 c (1.56)	4.26 bc (1.64)	nd
	$MAP_{30/60}$	«	1.10 ab (1.18)	1.57 b (1.14)	1.79 c (1.53)	3.09 c (1.60)	3.09 c (1.60)	3.17 b (1.64)
	sig		*	***	***	***	***	***

[1]Significance: ns - not significantly different ($P \geq 0.05$); * - significantly different ($P < 0.05$); ** - very significantly different ($P < 0.01$); *** - high significantly different ($P < 0.001$).

[2]nd – not determined.

[3]In the same row means without common letters are significantly different ($P < 0.05$).

Table 2. Microflora (log cfu/g, means (standard deviation) isolated from beef of the two pH groups (Normal pH<5.8 and High ultimate pH ≥6,2)

Microflora	pH group	Storage time (days)						
		1	3	7	10	14	21	28
Lactic Acid Bacteria	Normal	0.94 (0.99)	1.03 (0.99)	1.53 (1.37)	2.60 (1.83)	4.36 (1.08)	4.70 (0.96)	5.79 (1.00)
	High	1.15 (1.24)	1.08 (10.9)	2.10 (1.65)	3.64 (2.03)	3.77 (2.58)	5.26 (2.44)	4.92 (2.98)
	sig[1]	ns	ns	ns	ns	ns	ns	ns
Brochothrix thermosphacta	Normal	0.00 (0.00)	0.26 (0.79)	1.31 (1.97)	2.68 (2.52)	4.08 a (2.20)	5.08 (2.18)	4.96 (2.00)
	High	0.70 (1.59)	0.77 (1.34)	2.86 (2.14)	4.14 (2.32)	5.68 b (1.71)	6.74 (1.45)	6.11 (1.92)
	sig	*	ns	**	*	***	**	ns
Enterobacteriaceae	Normal	0.00 (0.00)	0.19 (0.51)	0.34 (0.70)	0.74 (1.26)	1.13 (1.67)	1.33 (1.73)	1.54 (2.21)
	High	0.25 (0.56)	0.43 (0.86)	0.73 (1.23)	1.36 (1.83)	2.65 (2.41)	2.75 (2.45)	3.01 (3.10)
	sig	*	ns	ns	ns	**	*	ns
Pseudomonas spp.	Normal	0.48 (0.67)	0.67 (0.98)	2.80 (2.11)	3.50 (2.56)	3.93 (2.75)	4.38 (1.68)	4.14 (1.77)
	High	1.91 (1.66)	1.91 (1.30)	3.50 (2.44)	4.36 (2.51)	5.32 (2.44)	5.23 (1.94)	4.72 (1.90)
	sig	***	***	*	*	***	*	ns
Total viable counts	Normal	2.44 (0.38)	2.57 (0.51)	3.38 (1.49)	4.29 (2.11)	5.37 a (1.94)	6.04 (1.48)	6.48 (1.27)
	High	2.60 (0.72)	2.74 (0.74)	4.27 (1.85)	5.58 (1.93)	6.79 b (1.77)	7.29 (1.53)	6.95 (1.64)
	sig	ns	ns	**	***	***	**	ns

[1]Significance: ns - not significantly different ($P \geq 0.05$); * - significantly different ($P < 0.05$); ** - very significantly different ($P < 0.01$); *** - high significantly different ($P < 0.001$).