

Shelf-life of Halal fresh and minced beef meat packaged under modified atmosphere

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Abstract

The shelf-life of Halal fresh cut and minced beef meat, packaged under modified atmosphere (MAP) was evaluated. The microbial profile of the carcasses intended for cutting and mincing was investigated by detecting spoilage and pathogenic bacteria. Samples of diced meat (DM), marrowbones (MB), steaks (S) and minced meat (MM) were packaged in MAP (66.0% O₂, 25.0% CO₂ and 9.0% N₂) and stored at +2 and +8°C. At 0, 7 and 14 days, gas composition of headspace was measured. Moreover, in all the samples colony count at 30°C, *Enterobacteriaceae*, lactic acid bacteria (LAB) and *Pseudomonas* spp. were determined. The carcasses contamination was in compliance with the criteria fixed by EC Reg. 2073/2005. Gas composition of the headspace changed significantly during the storage, mainly at +8°C, where a significant decrease of O₂ (until 0.1-0.6%) and an increase of CO₂ (until 81.0-89.0%) were recorded. This could be related to the level of LAB and *Pseudomonas* spp. Less significant changes were observed at +2°C. At 7 days of storage colony count, mean values were higher than >10⁷ CFU/g in the samples at +8°C, and also at 14 days at +2°C, presumably due to the high levels of *Pseudomonas* spp., that was dominant at the end of the test. Overall, the microbial mean counts were higher than those detected in similar products packaged under vacuum. In order to extend the shelf-life of the fresh meat and meat preparations, differentiated gas mixtures, and particularly a higher percentage of CO₂, could be employed.

Introduction

Food choice reflects aspects of lifestyle, culture, religion, diet and health concerns (Nakyinsige *et al.*, 2012). Islam dietary laws determine which foods are permitted (*i.e.* Halal) or forbidden (*i.e.* Haram). For Muslims,

the dietary laws are binding and must be observed at all times (Simoons, 1994; Bonne and Verbeke, 2008). In the past, meat for Muslim consumption was sold fresh at easily recognisable joints, overwrapped for short-term chilled storage. Today, the food chain has become longer and people's lifestyle, included those of people who follow religious dietary laws, have greatly changed.

To extend the shelf-life of fresh meat, meat preparations and meat products stored at refrigeration temperature (Nakyinsige *et al.*, 2012), modified atmosphere packaging (MAP) is still one of the most widely used technologies. For fresh red meat a gas mixture with 80% O₂ and 20-30% CO₂ is commonly used (McMillin, 2008). The purpose of the high-oxygen content is to maintain the bright red oxymyoglobin colour of the meat, although aerobic chilled storage supports the growth of Gram-negative, aerobic rod-shaped bacteria, including *Pseudomonas* spp. and other spoilage organisms, that can reduce the shelf-life and generate off-flavours (Esmer *et al.*, 2011).

The aim of this study was to determine the shelf-life of halal fresh and minced beef meat packaged in MAP. The research was carried out in collaboration with a meat processing plant placed in Sardinia, with the view to supply a retail outlet of halal beef, sheep and goat meats. With this aim, we considered the contents of annex II of EC Regulation 2073/2005 (European Commission, 2005) regarding the studies that food business operator shall conduct, in order to guarantee the compliance with the food safety criteria throughout the shelf-life. In particular, we referred to the Technical Guidance Document on shelf-life laboratory durability and challenge studies for *Listeria monocytogenes* in ready-to-eat foods (European Community, 2008).

Materials and Methods

The study was carried out from October 2010 to July 2011. Samples obtained from two batches (L1 and L2) of beef meat, coming from 12 animals slaughtered with the religious Halal method in a slaughterhouse placed in Sardinia, were examined.

Measurements at slaughterhouse

Carcass pH and temperature recording were measured one hour after slaughtering, in *M. longissimus dorsi* between the 4th and the 7th rib, using a portable pH-meter, equipped with a pH-electrode fixing and a temperature probe (Crison GLP21; Crison Instruments, Alella, Spain). As for microbial sampling of carcasses, each carcass was sampled after evisceration and before chilling by sponge, according to ISO

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17604 (Reg. EC 2073/2005) (European Commission, 2005) at shoulder, brisket, rump and loin sites. All the sponge samples coming from carcasses were analysed for colony count at 30°C (ISO 4833:2004) (UNI, 2004b), *Enterobacteriaceae* (ISO 21528-2:2004) (UNI, 2004a), Verocytotoxin-producing *E. coli* (VTEC), (Mazzette *et al.*, 2012), *E. coli* (ISO 16649:2001) (ISO, 2001), *Salmonella* spp. (ISO 6579:2002) (ISO, 2002), and *Listeria monocytogenes* (ISO 11290-1:1996 and 11290-2:1998) (ISO, 1996, 1998).

Measurements at meat processing plant

Carcass pH and temperature were measured 24 h after slaughtering, as previously described.

Shelf-life studies

After storage of the carcasses at +2±2°C for 15 (L1) and 34 days (L2), the following products were obtained: diced meats (DM), marrowbones (MB), steaks (S) and minced meat (MM). After processing, samples were packaged in MAP, using polystyrene two mm thick rigid trays that, after flushing with the selected gas mixture (66% O₂, 25% CO₂ and 9% N₂), were closed by heat-sealing in a high barrier film (oxygen transmission rate of 1.8 cm³/m²/24 h/bar). All the products were divided into two batches and stored in the dark at +2±2°C (ideal retail condition storage) and +8±2°C (simulating a temperature abuse condition). During storage period, the tempera-

ture was monitored by datalogger (Tynitac Plus).

Sampling and analysis were conducted at the following intervals: zero (T0), seven (T7) and 14 (T14) days. Overall, 40 samples were analysed, 10 for each kind of product. Prior to lab analysis, the following organoleptic characteristics of the packaged samples were evaluated: i) package collapse; ii) drip loss; iii) meat and fat colour. The gas composition of headspace in packages was measured with a digital O₂ and CO₂ analyser, Combi checkmate 9900 (PBI Dansensor; Dansensor, Ringsted, Denmark), and expressed as percentage. The remaining gas was calculated as N₂.

Samples were analysed for colony count at 30°C, *Enterobacteriaceae*, *Salmonella* spp., *E. coli*, VTEC, *L.monocytogenes* as previously described; *Lactobacillus* spp. (LAB) by using Man Rogosa Sharpe medium Agar (pH 5.6) (Oxoid, Milan, Italy), at 30°C for five days; *Pseudomonas* spp. on *Pseudomonas* agar base

with ceftrimide, fucidine and cephaloridine (CFC) (Oxoid), at 25°C for 48 h; *Salmonella* spp., *L.monocytogenes* and *E.coli* VTEC, as previously described.

Moreover, an analysis of variance (ANOVA) using the general linear model (GLM) procedure was performed for all considered variables, and when F-values were significant at the P<0.05 level, mean differences were separated by the least significant differences (LSD).

Results

Measurements at slaughterhouse and at processing plant

Carcass pH and temperature showed a regular dynamic. The pH values (minimum and maximum) ranged from 6.1 to 6.8, and from 5.5 to 5.7, respectively one and 24 hours after

slaughtering.

The investigated parameters of the carcasses showed low mean values. The mean counts were 3.51±0.46 and 1.36±1.35 Log CFU/g for colony count at 30°C and *Enterobacteriaceae* respectively, in compliance with the criteria fixed by EC Reg. 2073/2005 (European Commission, 2005). VTEC, *Salmonella* spp. and *L. monocytogenes* were not detected in any of the samples.

Shelf-life studies

During the storage period of the samples at +2°C, temperatures ranging between 0 and +4.7°C were registered, while for those stored at +8°C, the maximum recorded value was +9°C.

Organoleptic characteristics of the samples

Incomplete package collapse was detected at seven days of storage in a MB sample stored at

Table 1. Mean counts (log CFU/g) of total viable count, *Enterobacteriaceae*, lactic acid bacteria and *Pseudomonas* of Halal beef samples stored under modified atmosphere.

		Storage temperature (°C)		Days of storage*		
				0°	7 ^{#,§}	14 ^{#,§}
Colony count at 30°C	MB	+2		4.19±0.87	6.61±1.20 B, x	8.06±0.08 A, x
		+8			7.43±0.18 x	7.91±0.28 x
	DM	+2		4.49±1.39	5.93±2.48 B, x	8.12±0.46 A, x
		+8			7.48±0.37 x	8.06±0.08 x
	S	+2		4.49±0.83	5.81±0.15 x	7.95±0.70 x
		+8			7.66±0.26 x	6.63±2.91 x
MM	+2		4.27±1.07	6.47±1.20 B, x	7.94±1.17 A, x	
	+8			7.57±0.12 x	8.49±0.40 x	
<i>Enterobacteriaceae</i>	MB	+2		0.95±0.49	4.34±1.52 x	4.88 2.81 x
		+8			5.39±1.17 B, x	6.43±1.19 A, x
	DM	+2		0.50±0.71	3.34±2.11 x	3.59±0.41 x
		+8			5.22±0.08 y	5.90±0.46 y
	S	+2		2.91±2.10	4.07±1.36 x	4.67±1.47 x
		+8			4.99±0.30 x	4.67±0.29 x
MM	+2		2.31±2.17	3.70±1.06 x	4.73±2.75 x	
	+8			4.94±0.15 x	3.52±1.85 x	
LAB	MB	+2		-	1.63±1.31 A, x	3.29±1.40 B, x
		+8			4.16±1.40 y	4.22±1.27 x
	DM	+2		0.50±0.71	1.48±1.42 x	2.11±0.72 x
		+8			3.90±0.88 y	4.36±0.59 y
	S	+2		1.50±1.70	0.35±0.49 x	4.55±3.67 x
		+8			3.84±1.22 x	5.52±2.46 x
MM	+2		1.85±0.08	3.24±3.48 B, x	5.24±1.54 A, x	
	+8			5.75±0.58 x	6.29±0.79 x	
<i>Pseudomonas</i> spp.	MB	+2		4.82±1.03	7.03±0.16 B, x	8.05±0.35 A, x
		+8			7.40±0.17 x	7.98±0.38 x
	DM	+2		5.21±1.04	6.26±2.01 B, x	8.23±0.46 A, x
		+8			7.64±0.06 x	8.18±0.29 x
	S	+2		5.03±0.78	5.50±1.02 B, x	7.20±1.68 A, x
		+8			7.62±0.51 y	8.33±0.40 x
MM	+2		4.49±0.94	5.52±1.79 B, x	7.19±1.66 A, x	
	+8			7.10±0.39 x	8.02±0.30 x	

MB, marrowbones; DM, diced meats; S, steaks; MM, minced meats; LAB, lactic acid bacteria. *All values are expressed as mean±standard deviation; °samples at day 0 were stored at +2°C; A,B,C - means within rows with different capital case letters are significantly different (P<0.01); #x, y - means within columns with different capital case letters are significantly different (P<0.01; P<0.05).

+2°C and in a DM stored at +8°C. The same characteristic was recorded at 14 days of storage in 87.5% of the samples stored at +8°C. Drip loss was detected starting from the 7th day, particularly in samples stored at +8°C. Meat color showed a progressive darkening during the storage period, particularly in samples stored at +8°C.

Headspace composition

The gas composition of each package changed significantly during the storage period, related to the temperature of storage and the growth of certain microbial groups (LAB and *Pseudomonas* spp.). Oxygen concentration decreased with a different trend in the various products. At the end of storage at +2°C, O₂ content decreased to values approaching 10% for MB, DM and S samples, and 34% for MM samples. Such decrease was more evident in the samples stored at +8°C with a O₂ concentration of 0.1-0.6 %, except for S samples in which a final value of 11.0% was recorded. Carbon dioxide concentration showed a different trend with the temperature of storage. In the samples stored at +2°C, such trend was irregular for all the kind of products, and the final CO₂ values were comprised between 15.0 and 18.0%. On the other hand, an increase was observed, at the end of storage, in samples stored at +8°C that showed final values ranged from 81 to 89%.

Microbiological analyses

The results of the viable counts of the targeted microbial groups in the different kind of samples packaged under MAP (Log CFU/g; mean±sd) are showed in Table 1.

Marrowbones

Colony count at 30°C, *Pseudomonas* spp. and LAB mean counts of samples stored at +2°C increased gradually (P<0.01) with the storage time. At seven days of storage *Enterobacteriaceae* and *Pseudomonas* spp. showed levels of 10⁴ and 10⁷ CFU/g respectively, irrespective of the storage temperature and *Pseudomonas* spp. was the dominant species at the end of storage. LAB presented a less significant increase respect to *Pseudomonas* spp., showing mean counts of 10³ CFU/g at the 14th day.

Diced meat

Mean levels of the targeted microbial groups were lower than in MB samples, but in the samples stored at +8°C, *Enterobacteriaceae* counts were >10⁵ CFU/g. Colony count at 30°C (P<0.01) and *Pseudomonas* spp. counts increased significantly during storage irrespective of the temperature.

Steaks

Microbial profile was similar to DM samples, but in those stored at +2°C mean values were lower for all the targeted microbial groups, par-

ticularly for *Pseudomonas* spp.

Minced meat

Colony count at 30°C (P<0.01), LAB (P<0.05) and *Pseudomonas* spp. (P<0.05) showed a significant increase with storage time. LAB attained final counts higher than 5 log CFU/g at the end of storage, at both temperatures. *L. monocytogenes*, *E.coli* and VTEC were not detected in any of the samples. *Salmonella* spp. (*S. Muenchen*) was detected in a MB sample at zero day of storage.

Discussion

Results of our study seem to be influenced by the variety of the investigated products. Aerobic Gram-negative bacteria are responsible for spoilage and are the predominant flora in fresh meat and meat preparations with 60% O₂ MAP (McMillin, 2008; Esmer *et al.*, 2011). Some authors report that microbial spoilage of food occurs when colony count at 30°C and/or *Enterobacteriaceae* counts reach 10⁷ CFU/g. In the present work, colony count at 30°C levels reached 10⁷ CFU/g at 7 days at +8°C and at 14 days at +2°C. This can be explained with the high levels of *Pseudomonas* spp., that was the dominant population at both temperatures at the end of the storage time. Moreover, colony count at 30°C and *Pseudomonas* spp. mean counts were higher than those detected in the same kind of products packaged under vacuum. The gas composition of each package changed significantly during storage period. It is known that gaseous environment within a modified atmosphere pack is not static. It may be the result of microbial growth, the permeability of packaging material, and respiration of the product or the gas absorption by the food (McMillin, 2008). It is an expected result for CO₂ concentration to decrease for the absorption by the water and lipid portions of meat until saturation or equilibration is reached. However, in our work, such decrease could be observed only for samples stored at +2°C, presumably due to the microbial growth. During the storage the majority of microorganisms present in meat uses the available oxygen in the headspace, while others such as *Brochothrix thermosphacta* and LAB, produce carbon dioxide (Esmer *et al.*, 2011).

Conclusions

In order to extend the shelf-life it could be considered to use higher CO₂ concentration, with the aim to prevent the growth of Gram-negative bacteria which are generally more sensitive to this gas respect to Gram-positive.

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