Meat Science 83 (2009) 398–404

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Effect of frozen storage conditions (temperature and length of storage) on microbiological and sensory quality of rustic crossbred beef at different states of ageing

C. Vieira [a,*], M.T. Diaz [b], B. Martínez [a], M.D. García-Cachán [a]

[a] Estación Tecnológica de la Carne, ITACyL, Consejería de Agricultura y Ganadería de Castilla y León, Apdo, 58-37770 Guijuelo–Salamanca, Spain
[b] Instituto Nacional de Investigación Agraria y Tecnología Alimentaria (INIA), Ctra, Coruña, km, 7.5 Madrid, Spain

ARTICLE INFO

Article history:
Received 16 July 2008
Received in revised form 5 March 2009
Accepted 5 June 2009

Keywords:
Frozen storage
Freezing temperature
Ageing
Beef quality

ABSTRACT

The effect of frozen storage conditions on meat from 36 Morucha × Charolais crossbred yearlings was studied. Slices of M. *Longissimus thoracis* were randomly assigned to groups arising from the combination of experimental factors. These factors were: ageing extent (3 and 10 days), length of frozen storage (0, 30, 75 and 90 days) and temperature (−20 and −80 °C). Regarding microbiological counts, although values were acceptable in all cases, longer storage time and longer previous ageing extent provided higher phychrotrophic bacteria counts. As frozen storage period increased, colorimetric parameters L^*, a^* and C^* decreased, but H^* increased. Regarding Warner–Braztler shear force and tenderness values, an interaction ($p < 0.05$) between frozen storage and post-mortem ageing resulted from larger differences between frozen storage periods at shorter ageing periods than those at longer ageing periods. Frozen storage for 90 days resulted in a reduction in water holding capacity, without differences in juiciness. No effect of freezing temperature was observed in any of the parameters studied.

1. Introduction

The limit of storage or shelf life of meat has been prevented from being reached by microbiological and physicochemical spoilage under conditions such as refrigeration and freezing. In this sense, in recent years, storing beef under freezing conditions has been increased in order to address market problems, and significant beef stocks have been generated. These stocks are often stored frozen for various times before being thawed to be consumed. Several investigations have reported that frozen storage could affect microbiological quality and physicochemical characteristics such as oxidative stability and sensory properties (Damen & Steenbekkers, 2007; Farouk & Weliczko, 2003; Hinton et al., 1998). In order to provide consumers with a high quality product from frozen/thawed beef, it is necessary to ascertain the effects of factors involved in the freezing process in relation to the quality of meat after thawing. Several authors have pointed out that one of the factors that affect beef quality after thawing is the frozen storage time (Farouk, Weliczko, & Merts, 2003; Shanks, Wulf, & Maddock, 2002). According to several studies (Ngapo, Babare, Reynolds, & Mawson, 1999; Shanks et al., 2002; Wheeler, Miller, Savell, & Cross, 1990), the quality of frozen meat deteriorates progressively over storage. Notwithstanding this, frozen storage has been claimed to improve tenderness, especially in unaged beef (Shanks et al., 2002; Wheeler et al., 1990). Also, freezing rate and storage temperature have been found to be important in the behaviour of ice crystals that could be detrimental to meat quality (Hildrum, Solvang, Nilsen, Froyetein, & Berge, 1999; Mousavi, Miri, Cox, & Fryer, 2007). However, Farouk et al. (2003) and Bertram, Andersen, and Andersen (2007) did not find an effect of frozen storage temperature on most sensory properties in frozen beef. Although recent works (Farouk & Weliczko, 2003; Lagersted, Enfält, Jihansson, & Lundström, 2008; Ngapo et al., 2002; Zhang, Farouk, Young, Weliczko, & Podmore, 2005) have tried to clarify the effects of the freezing process on beef muscle, findings from several studies, as mentioned, show an apparent lack of agreement on some of the points.

On the other hand, it is important to note that the effects of frozen protocol on meat quality after thawing are dependent on the characteristics of meat itself. So, the stability of beef in frozen storage depends on its chemical composition because of the differences in colour stability, sarcoplasmic protein solubility and fat oxidation (Farouk & Weliczko, 2003; Zhang et al., 2005). In this sense, meat from rustic breed crosses such as Morucha × Charolais, could show differential behaviour under frozen storage, and not many studies have evaluated the effect of freezing conditions on this type of meat. Meat from rustic breeds such as Morucha and its crosses is characterized by a high amount of fatness, high water holding capacity and high heme pigment concentration

* Corresponding author. Tel.: +34 923 580 688; fax: +34 923 580 353.
E-mail address: viealce@itacyl.es (C. Vieira).

0309-1740/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.meatsci.2009.06.013

(Albertí et al., 2005; Vieira, García-Cachán, Recio, Dominguez, & Sañudo, 2006), which could have an important influence on the effect of freezing on its quality.

As indicated above, the effect of freezing on meat sensorial properties remains unresolved; especially that from rustic breeds. Thus, the purpose of this study was to study individual and combined effects of different factors in relation to frozen storage, on the characteristics of meat from the Morucha × Charolais breed.

2. Material and methods

2.1. Experimental design and sampling

The experiment was carried out at the Meat Technological Station of the Agronomic Technological Institute of Castilla & Leon (ITACyL) in Guijuelo, Salamanca (Spain). Thirty-six Morucha × Charolais crossbred bulls were slaughtered on the same day at an EU authorized slaughterhouse. Taking into account local market preferences, slaughtering was fixed within a range of age of 13–14 months. Cold dressing percentage was expressed as chilled carcass weight – after chilling at 4 °C for 24 h – percentage of slaughter body weight. Conformation and fatness scores were graded visually following the European Normative (Council Regulation (EC) 1183/2006 and 103/2006). Conformation was assessed according to a scale ranking EUROP using a 15-point scale, from 15 (the best conformation) to 1 (the worst conformation). Fatness score was measured on a 5-point scale from 5 (very high fat) to 1 (very low fat). Carcass grading was determined by trained slaughterhouse staff. pH was measured at 24 h post-mortem in the M. *Longissimus thoracis* at the 6th rib level using a 'penetration' pH-electrode. At 48 h after slaughtering, *L. thoracis* muscle between the 6th and 11th ribs was removed from the right side of each carcass. In order to characterize the samples used, before sampling, a steak of about 100 g was removed from each 6th to 11th rib piece used, and dry matter, ash, ether extract and crude protein were determined according to official procedures (AOAC., 1990). A fatty acid profile was obtained from intramuscular fat by gas chromatography (Perkin-Elmer Auto syst-X.L). The lipid extraction from *L. thoracis* muscle was carried out according to the Bligh and Dyer (1959) technique, with the methyl esters being obtained according to Morrison and Smith (1964). A summary of carcass characteristics and muscle chemical composition is given in Table 1.

The experimental design comprised three factors: ageing extent (3 and 10 days), storage conditions (fresh meat and meat stored for 30, 75 and 90 days under frozen conditions) and temperature during frozen storage (−20 and −80 °C). Once the steak used to evaluate meat composition was separated, *L. thoracis* sections were divided into three pieces, and each one was vacuum packaged in a total of 108 meat pieces. The sample pool of 108 meat pieces of *L. thoracis* from 36 bulls was divided randomly into two, in order to perform the ageing process (half of the samples were aged for 3 days and the remaining samples for 10 days). After a corresponding ageing period, two control groups of non-frozen meat, six samples of each ageing period, were collected to study the characteristics of non-frozen meat. The remaining samples were assigned to four groups according to the factors established in the experimental design: ageing extent (3 and 10 days) and temperature during frozen storage (−20 and −80 °C). The effect of storage extent (30, 75 and 90 days) under frozen conditions was studied in each of the four groups of samples mentioned. After the corresponding storage time, frozen samples were removed from their respective storage packages and thawed for 48 h in a 4 °C cooler before being analyzed. In regard to samples which had not been kept under frozen conditions, analyses were performed just after removing from vacuum ageing packs.

Table 1
Carcass characteristics and *Longissimus thoracis* muscle chemical composition (means and standard deviations).

Parameters	Mean	Standard deviation
Carcass characteristics		
Slaughter weight (kg)	595.7	35.33
Carcass weight (kg)	342.0	22.95
Cold dressing percentage (%)	57.4	1.57
[a]Conformation score	3.8	0.15
[b]Fatness score	3.0	0.00
pH 24 h	5.6	0.04
Longissimus chemical composition		
Moisture (%)	74.60	1.29
Ether extract (%)	3.60	1.24
Crude protein (%)	21.83	0.35
[c]SFA (g/100 g fatty acids identified)	44.72	2.34
[c]MUFA (g/100 g fatty acids identified)	42.50	1.57
[c]PUFA (g/100 g fatty acids identified)	12.77	2.15

[a] Conformation score: 1 = the worst conformation, 15 = the best conformation.
[b] Fatness score: 1 = the lowest fatness grade, 5 = the highest fatness grade.
[c] SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

2.2. Microbiological sampling and analysis

In order to investigate the microbiological quality, all meat samples were treated as follows: the surface of each piece was sampled by swabbing an area of 20 cm^2, with a cellulose acetate sponge that had been moistened with 0.1% peptone sterile water (Scharlau, Spain). Each sponge used for swabbing meat was mixed for 1 min with an additional 10 ml of 0.1% (w/v) sterile peptone water. Serial, 10-fold dilutions of each homogenised fluid were prepared with 1 ml each of the undiluted, 10-fold, 100-fold, and 1000-fold diluted fluids in 9 ml volumes of 0.1% peptone water. Then, the dilutions were plated onto growth media in duplicate. Culturing and incubation conditions for different microorganism groups were as follows. To enumerate aerobic psychrotrophic bacteria, aliquots of 1 ml of each dilution were placed on a plate of Plate Count Agar, PCA (Scharlau, Spain), which was incubated at 7 °C for 10 days. To count enteric bacteria, 1 ml of each dilution was placed on a plate of Violet Red Bile Glucose Agar, VRBGA, medium (Scharlau, Spain) and plates were incubated microaerobically at 37 °C for 2 days. Aliquots of 0.1 ml of each dilution were spread on plates of Man Rogosa Sharpe-MRS- agar (Scharlau, Spain) and incubated microaerobically at 30 °C for 2 days to enumerate lactic acid bacteria. Counts were expressed as the $\log_{10}$ cfu/cm^2.

2.3. Meat colour

Muscle colour was estimated using the M. *L. thoracis* at the 6th rib, after the newly cut surface was exposed to an artificial fluorescent light for 90 min at 10 °C. Colorimetric parameters (L^*, a^* and b^*) were measured on four spots on each sample using a Minolta (Scharlau, Spain) (Minolta camera, Tokyo Minolta CM2002 spectrophotometer) in the CIE$L^*a^*b^*$ space under D65, 10° and SCI conditions. Hue angle (H^*) and Chroma (C^*) for meat were calculated as described by Liu, Scheeller, Arp, Schaefer, and Williams (1996).

2.4. Lipid oxidation (TBARs)

Lipid oxidation was determined by thiobarbituric acid reactive substances (TBARs) value, using the method of Maraschiello, Sarraga, and García Regueiro (1999). Duplicate samples were used and the results were expressed in mg malonaldehyde (MDA)/kg muscle.