MEAT QUALITY FROM FIGHTING BULLS IN SPAIN

Calidad de la carne del toro de Lidia en España

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ABSTRACT

Spaniard beef derived from "running bulls" are consumed during the festival days as has become traditional at every annual San Fermin fair. However, eating quality, safety and chemical composition of beef derived from these animals are generally unknown. The present work characterized beef from San Fermin´s fighting bulls (SFRB) by conducting microbiological test analyses of selected physical-chemical traits including fatty acid composition of intramuscular lipids during ageing. Beef from fighting bulls was characterized by a dark red colour, due to its high pH values (pH$_{48hours}$= 6.2). However, this beef did not show any dry firm dark (DFD) characteristic, as usually occurs with meat from other beef breeds with final pH higher than 6.0. The high myoglobine content (10.6 mg·g-1) and the low intramuscular fat percentage (1.5%) provide this beef with adequate nutritional properties for the diet/health consumer. The fatty acids (FA) profile stands out because of its high nutritional interest, due to the fact that polyunsaturated FA were higher than 20% of total FA in the muscle. The obtained results allow the differentiation of this kind of beef and it will contribute to setting up a quality mark or label because consumer evaluates positively those meat products that come from animals reared in extensive livestock systems.

Key words: Beef, quality, fighting bulls, ageing.

RESUMEN

La carne del toro de lidia es un tipo de carne que se consume durante las fiestas de San Fermin. Sin embargo, es un producto del que apenas se conoce su calidad, seguridad alimentaria y composición química. En este trabajo se ha caracterizado la carne del toro de Lidia mediante la determinación de los principales parámetros relacionados con la calidad físico-química e higiénica de la misma, así como de la composición en ácidos grasos de la grasa intramuscular durante la maduración. La carne del toro de lidia se caracteriza por presentar color rojo oscuro debido a sus elevados pH (pH$_{48horas}$= 6,2), aunque no mostró las características de carne oscura, firme y seca (DFD) como sucede en las otras carnes de vacuno con un pH superior a 6,0. El alto contenido en mioglobina (10,96 mg/g-1) y bajo en lípidos intramusculares (con un valor medio de 1,5%) confieren a esta carne, unas propiedades nutritivas adecuadas para el consumo humano. La composición en ácidos grasos (AG) presenta un adecuado interés nutritivo, ya que el contenido en AG poliinsaturados supera el 20% de los AG totales del músculo. Los resultados obtenidos permiten diferenciar este tipo de carne y por tanto, contribuyen al establecimiento de una marca de calidad con un gran atractivo para el consumidor ya que provienen de animales criados en libertad en un ecosistema natural de dehesa.

Palabras clave: Calidad, carne, toro, Lidia, maduración.

INTRODUCTION

Beef from Spaniard "fighting" bulls (*Bos taurus*) specially those known as San Fermin "running" bulls (SFRB) has a limited supply and demand because it is only consumed locally, as a result of the culinary traditions of the popular festivals. Consumers do not have an appropriate perception of this beef, because the production system and its sensory quality are quite unknown, and there are few published studies on quality from fighting bulls (FB).

Livestock production systems of FB are subjected to the European Union Regulations. This breed contributes to the development and stabilization of genuine Spanish meadow ecosystem models [3].

The carcass production of FB in Spain is around 6,000 tm year-1 and they come from approximately 30,000 animals year-1 from around 1,200 primary producers of FB [38]. The production of beef from FB involves a complex system in which

many productive and technological aspects can influence its sensory quality. Cattle are fed on natural pastures that are not abundant enough to feed animals correctly. So, producers have to supplement with concentrates during some seasons of the year. This is especially strategic during the months previous to the fighting so that bulls can reach the required body conditions and live weight [37].

From the time bulls are transported to the bullring until they are sacrificed they face difficult, stressful conditions.

These conditions can affect the organoleptic characteristics of beef. Butchering SFRB is carried out under the European Regulation for Fresh Meat [39] since the Bovine Spongiform Encephalopathy (BSE) in 2000.

After carcass fabrication meat ageing takes place, changing progressively the original biochemical and sensory characteristics and improving its eating quality [6, 26, 36]. The regulation concerning the production and marketing of cattle animals 4-5 years old from the FB breeds [39] indicates that beef has to be refrigerated or frozen and it has to be stored at a temperature lower than 7°C. However, this regulation does not specify the period of storage or the effect that ageing time can have on meat sensory characteristics. That is why this legislation indicates the need for carrying out research on the specific characteristics of these beef.

The development of marketing, trading and selling strategies of beef from FB could be a solution in order to improve the benefits of livestock productions of FB. The objective of the present work was to characterize meat quality from FB during ageing. This in turn will allow the identification of the potential differentiating traits of beef from SFRB.

MATERIALS AND METHODS

The beef was obtained from 32 FB sacrificed at the San Fermín festivals (Pamplona, Spain) during two consecutive years (2006-2007). The first year, 16 bulls were studied (Assay 1) and the second, 16 bulls (Assay 2). Bulls belonged to eight different livestock producers (4 producers each year) and four bulls per producer were studied. Beef was aged 7 days (Assay 1) and 14 days (Assay 2). Ageing time was longer in Assay 2 because results from Assay 1 showed no WBSF differences in beef aged 7 days.

Bulls were slaughtered and the end of the lidia by the "torero" at the bullring. The carcass pH was measured just after slaughter (45 min) and 48 h post mortem between the 5th and the 6th rib by an Orion Research Potentiometer (290A, Barcelona, España) for solid samples [24]. After slaughter, carcasses were fabricated at the bullring facilities and then they were carried to the slaughterhouse La Protectora (Pamplona, Spain) where they were kept at 4 h at ambient temperature (18°C) and subsequently chilled (Koxka, Premium, Liebherr, España) 24 h at 2°C and 98% relative humidity. After chilling, the carcasses

were weighed (cold carcass weight) reaching 325.29 ± 31.54 kg in Assay 1 and 345.20 ± 31.07 kg in Assay 2. Carcasses stayed at the slaughter house until 48h post-mortem at 7°C. All the meat quality traits were evaluated at the *longissimus dorsi* muscle thoracic region (6th-13th rib), that was removed from the left carcass side 48 h *postmortem*. Then, the muscle was fabricated in 2.5 cm thick steaks that were vacuum packaged (99%) in polyamida/polyethylene (PA/PE) pouches (120 m and 1 mL m^{-2} 24 h^{-1} O$_2$ permeability, 3 mL m^{-2} 24h^{-1} CO$_2$ permeability and 0.5 mL m^{-2} 24 h^{-1} N$_2$ permeability measured at 5°C and 75% relative humidity (Vaessen Schoemaker Ind., Barcelona, Spain) in an Egarvac Basic Machine (Terrassa, Spain). Samples were aged at 2 ± 1°C until 7 and 14 d *postmortem* in Assays 1 and 2, respectively. After each ageing period, samples were frozen (model 7080 225-01, Liebherr, España) at -18°C until subsequent analysis. Proximate and collagen composition of the *longissimus dorsi* muscle was analysed as follows: intramuscular fat [22], moisture [21], protein [23], ashes [25] and soluble and total collagen [1, 13].

Microbiological analysis

Microbiological analysis were carried out in meat aged 48 h and 7 and 14 d (Assay 1 and Assay 2, respectively). Two cores of 10 ± 0.1 g of fresh unfrozen meat were removed using sterile scalpels and forceps to aseptically separate the bag from the beef and blended with a 90 mL of 1% tryptone solution (w/v) for 60 s in a Stomacher (Lab Blender 400; Seward Medical, London, UK). Additional dilutions were made in 1% tryptone (w/v). Then, 1 mL of the undiluted homogenate of each dilution was spread on duplicate plates. Microbiota number was determined from plates bearing 30-300 colonies. Counts were obtained as follows: Aerobic plate counts on Plate Count Agar (Difco®), incubated (UFP 800 TS, Memmert, España) at 32°C for 48 h; *Enterobacteriaceae* on Violet Red Bile Glucose Agar (Difco®) overlaid with the same medium and incubated (Masalles 25, Madrid, España) at 37°C for 24 h.

Physical beef quality traits

Colour, texture and water-holding capacity analysis were evaluated in the *longissimus dorsi* muscle thoracic region. Beef colour (L*, a*, b*, C* y H*) [5] was measured with a Minolta CM2002 spectrophotometer (Tokio, Japan) with a D65 illuminant and 10° standard observer [5] after blooming the steaks for 1 h after 2 and 7 d *postmortem* (Assay 1) and 2 and 14 d *postmortem* (Assay 2). The heme pigment content (myoglobin concentration, Mb) was determined by the method described by Hornsey [17].

For toughness analysis, a model TA-XT2i texture analyser (England) was used. Five to 10 replications were made per animal. Beef toughness was measured as the maximum shear force and it was assessed using a Warner-Bratzler (WBSF) shearing device until samples were cut completely through at 150 mm s^{-1}. Samples having a cross section of 1

cm^2 and 2 cm long were cut with the muscle fibres parallel to the long axis of the sample.

Water-holding capacity (WHC) was measured 48 h *post-mortem* using the method of filter paper press described by Honikel et al. [15]. Cooking (Nahita 5 L, Auxilab SL, España) losses were obtained calculating the difference in sample weight before and after cooking the beef at 70°C internal temperature and they were expressed as percentage of the initial weight. Final weight after cooking was calculated after refrigerating the beef sample for 24 h (2°C) under vacuum. Then, the pouches were opened and samples were weighted after slurring.

Fatty acid composition

Intramuscular lipids were extracted by the procedure of Bligh and Dyer [2]. The lipid extract was mixed with the internal standard (C21:0, methyl ester) and methylated with boron trifluoride-methanol following the procedure described by Morrison and Smith [33]. Analyses of 28 fatty acids as methyl esters were carried out by flame ionization gas chromatography (HP5890 series II, Hewlett-Packard, Madrid, Spain). A capillary column of 60m x 0.25 mm I.D., 0.25 µm) (HP 19091N-136, crosslinked polyethylene glycol, Hewlett-Packard, Madrid, Spain) was used to separate the fatty acids metylated esters (FAMEs) under the following conditions: temperature, programmed from 150° to 210°C at 10°C min-1, from 210° to 240°C at 4°C min-1, held at 240°C for 25 min; detector temperature, 240°C and injector temperature 255°C; carrier gas helium at 1 mL min-1 and splitless injection mode. The Gas chromatography (GC) system was calibrated with a standard FAME (Sigma-Aldrich). Identification of fatty acids was accomplished by comparing the relative retention time of the FAME peaks from samples with those from the standards. Relative retention times were checked every 30 samples. Fatty acids were expressed as area percentage of the total detected area percentage (mg 100-1 g of total fatty acids).

Statistical analysis

Results were analyzed statistically using SPSS V. 13.5 statistical program, [43]. The statistical model was $y_{ik} = \mu + T_i + e_{ik}$, where: y_{ik}= pH 0, 12, 24 h *postmortem;* intramuscular fat, moisture, protein, ashes, soluble and total collagen, myoglobin concentration, and *longissimus dorsi* colour (CIE L*a*b*C*H*), water-holding capacity, hardness and cooking loss and fatty acids; μ= least square mean; T_i = fixed effect due to ageing (i=1 2 d, i=2 7 d, (Assay 1); i=1 2 d, i=2 14 d, (Assay 2); e_{ik}= random residual effect.

RESULTS AND DISCUSSION

The physico-chemical parameters of the *longissimus dorsi* muscle of FB are shown in TABLE I. There was a high variability in the muscle total lipids content, soluble and total

TABLE I
PHYSIC-CHEMICAL CHARACTERISTICS OF THE *LONGISSIMUS DORSI* MUSCLE OF FIGHTING BULLS

	Mean value	Standard deviation	Max. value	Min. value
Moisture (%)	74.97	1.58	76.81	73.12
Protein (%)	21.07	0.25	22.63	19.69
Total lipid (%)	1.50	0.86	4.09	0.54
Ashes (%)	1.44	0.35	1.24	0.41
Total collagen (mg g^{-1})	3.90	0.18	5.11	3.05
Soluble collagen (%)[1]	4.64	0.28	6.30	3.30
Myoglobin (mg g^{-1})	10.96	1.64	12.21	8.31

[1]Soluble collagen was expressed as percentage of total collagen (%).

collagen between the studied animals. They are similar to those reported for beef [28]. These animals are entire bulls of 4-5 years and they are reared in extensive livestock systems and with high muscle percentage for the exercise for the fighting. The low lipid content when compared with other beef breeds at the same age at slaughter (1.5 ± 0.86% *vs* 5-12%) [8] might be due to the fact that FB breeds has very low marbling (intramuscular fat) and to the livestock production system. These beef characteristics could satisfy the Spanish consumer demand that prefers lean carcasses and low marbled beef [46]. Nevertheless, the total lipid content showed high variability in the studied FB (variation coefficient = 57.3%), which is higher than the variabilty observed in other beef breeds (40%) [19].

The total collagen content of beef from the studied FB (3.9 mg g^{-1}) is similar to the amount reported for one year old bulls and fed concentrate and barley straw, both ad *at libitum* (3.8 mg g^{-1}) [29]. However, the soluble collagen percentage (4.64% of total collagen) is lower than the latter (16.4%) [30].The soluble collagen content suggests that beef from these bulls is tougher [29], because collagen does not gelatinise upon cooking and beef does not reach the adequate tenderness level. Spanish consumers know that it needs longer time of cooking for tendering beef structure [11].

The total aerobial counts and *Enterobacteriaceae* counts (log ufc g^{-1}) in beef from FB after 7 (Assay 1) and 14 d (Assay 2) of ageing are shown in TABLE II. It is observed that microbial development was significantly greater in the Assay 2 than Assay 1. These microbiological counts indicated an acceptable hygienic quality of beef from FB. Microbiological counts were lower than the recommended 10^7 CFU g^{-1} by ICMSF [20], due to the correct hygienic and health practices at the slaughter facility where meat was fabricated. The increase in microbiological counts is supposed to take place between days 7 and 14 because there were no differences between days 2 and 7 (Assay 1) . Significant differences were observed between 2 and 14 d (Assay 2) (P≤0.01 and P≤0.05, respectively, for aerobial and *Enterobacteriaceae* counts).

TABLE II
**TOTAL AEROBIAL COUNTS AND *ENTEROBACTERIACEAE* COUNTS (LOG UFC G^{-1})
IN MEAT FROM FIGHTING BULLS DURING AGEING
(7 AND 14 DAYS).**

	Assay 1			Assay 2		
Postmortem	2 d	7 d		2 d	14 d	
Aerobial counts	4.91 ± 0.5	5.0 ± 0.5	ns	4.0 ± 0.3	7.0 ± 0.4	**
Enterobacteriaceae	3.64 ± 0.4	3.9 ± 0.5	ns	1.9 ± 0.2	5.1 ± 0.9	*

Significance level reported in analysis of variance: * P≤0.05; **P≤0.01; ***P≤0.001; ns P≥0.05.

Meat pH from FB after slaughter (45 min and 48 h) is shown in TABLE III. The pH measured at 45 min may be considered as normal or a slightly lower than in beef cattle but later it remained in values close to 6.5. The pH is an important factor determining meat quality because it is related to the biochemical processes involved in the conversion of muscle into beef after slaughter and it has a direct influence on the organoleptic characteristics of the final product [7]. The intense physical exercise that animals do during the fight involves the depletion of the glycogen reserves. This fact may prevent sufficient lactic acid formation after slaughter in order to acidify meat and get an adequate pH value, giving DFD (dark, firm and dry) meats of high pH [44]. This result confirms that there was a glycogen reserve mobilization during the bullfighting. The pH values 48 h after slaughter were higher than 6.0 in all the bulls. The depletion of glycogen prior to slaughter due to the stress caused by exercise and the *fighting*, does not allow pH in the animal muscle to decrease during *post-mortem*. Besides, Micol et al. [32] reported that muscles of the FB breeds present a very oxidative metabolism with a very high content of slow fibres (20-30 *vs* 17% in Charolais), and a small content of glicolitic fibres (15 *vs* 40% in Charolais). The low glicolitic potential of animals of FB agrees with its high pH. Besides, FB are uncastrated animals about four years old and they have a diet consisting on grass, involving an increase in oxidative character and slow fibres content [4,14]. Although FB reached a high final pH, their meat did not show DFD characteristics, as it usually occurs with beef from other beef breeds with final pH higher than 6.0. The ability of these animals to overcome the stress of the FB (general syndrome) has been essential to maintain the quality of beef from FB.

The colour coordinates for the bulls are shown in TABLE IV; there was different development during the ageing in colour coordinates in both assays, and, in general, beef from FB was very red due to a* values of about 20. Beef colour depends on myoglobin concentration and its chemical state, as well as on the pH of beef and the amount of intramuscular fat [40]. The total amount of heam pigments of the *Longissimus dorsi* muscle (10.96 mg g^{-1} of myoglobin, TABLE I) was high when compared with other beef presenting the same fat/protein ratio, for example in yearling animals of Pirenaica breed (4.17 mg g^{-1} myoglobin) [29]. This characteristic is very interesting because

the intake of beef from FB duplicates the iron intake coming from beef of young animals from other Spanish breeds [34].

The coordinates L*, a* and C*, after 2 d under refrigeration (TABLE IV), correspond to a beef with a dark intense red colour when compared to beef from Pirenaica yearling bulls, that showed L*, a* and C* values of 37.94, 15.68 and 18.28, [29]. Micol et al. [32] justified the dark beef colour from FB due to their high pH and low oxidation level and high antioxidative potential in FB muscles. In Assay 1, the values obtained at day 7 were significantly lower than at 2 d, probably because muscles with a high haemoglobin pigment percentage are more oxidative and thus their colour is less stable [42]. Besides, Hernandez et al. [12] stated that the evolution of metmyoglobin, oxymioglobin and myoglobin in beef samples, despite the stressing conditions due to the fighting and to the high pH values, was similar to the values observed in animals slaughtered under usual conditions at the slaughter house. So, the second day of ageing, beef showed high oxidation because of the high relative metmyoglobin percentage on the beef surface. This percentage increased after 7 d of ageing (9.60 and 34.32, P≤ 0.001) [12]. In the same study [12], this evolution during ageing was similar in beef from Pirenaica breed yearling bulls with an increase from 2.53 to 29.43% (P≤0.01) at days 2 and 7 [29]. In Assay 2, the decrease in colour coordinates only took place in L* (P≤0.05). This phenomenon could be due to the higher variability among the studied animals in this assay, which did not allow the detection of significant differences between days 2 and 14 after slaughter.

The evolution of firmness and juiciness (WHC, cooking losses) in beef during ageing (7 and 14 days) is shown in TABLE V. It was observed that Warner Bratzler shear force measured in beef after 14 d of ageing was significantly smaller than shear force measured in beef after 2 d of ageing. However, in Assay 1 beef didn't show changes in hardness between 2 and 7 d of ageing (P>0.05). Similar results were found in WHC, where significant differences were observed between WHC values at 2 and 14 d (Assay 2). Beef showed similar behaviour in cooking loss in both assays, decreasing during ageing (P<0.001). Toughness can be defined as the easiness of beef to be cut and chewed. Hardness is mainly determined by the muscle proteins, the nature and content in collagen and the amount of intramuscular fat [45]. A significant decrease in toughness was observed, measured as Warner Bratzler shear force, after 14 d of ageing (Assay 2, TABLE V), because mus-

TABLE III
pH OF MEAT FROM FIGHTING BULLS AFTER SLAUGHTER (45 MINUTES AND 48 HOURS).

	Assay 1	Assay 2
45min	6.46 ± 0.22^{a}	6.17 ± 0.14^{a}
48 h	6.28 ± 0.21^{c}	6.47 ± 0.28^{c}

Values with different superscript letter show significant differences into the assay (P≤0.05).

cle might likely need more ageing time. Thus, this beef that comes from 4 years old animals and with high muscling needs longer ageing periods than beef from yearling bulls slaughtered with 500 kg live weight, that need 7 d of ageing [29, 46, 47].

Water holding capacity (WHC) and the amount of fat are related to beef juiciness [15]. The significant decrease in cooking losses observed after ageing (TABLE V) and the lower WHC, that was only significant after 14 d of ageing, might be due to the changes that take place in the myofribillar proteins between days 7 and 14 [28] that involve a split up and the break down of sarcomers, giving a more tender beef [16].

Fatty acid composition of intramuscular fat of FB (2 and 7 d of ageing, Assay 1; 2 and 14 d of ageing, Assay 2) is shown in TABLE VI. Twenty six fatty acids were determined. TABLE VI only shows the most abundant fatty acids in the total composition of intramuscular fat (C16:0, C18:0 y C18:1), and the fatty acids that showed the highest differences between the two assays (C18:2n6).

There were very high polyunsaturated fatty acids (PUFA) of around 18 and 25% in Assay 1 and Assay 2, respectively; 38 and 44% saturated fatty acids (SFA) and 42 and 31% monounsaturated fatty acids (MUFA). Besides, ageing affected fatty acid content, specially, unsaturated fatty acids such as linoleic acid (C18:2-6n) and linolenic acid (C18:3-3n). Differences between the PUFAs, MUFAs and SFAs content from both assays could be explained by the different lipid beef content of the animals, so that minor total lipid in beef of Assay 1 (1.05 ± 0.51) vs Assay 2 (2.15 ± 1.21; P≤0.05), could justify the lower content of SFA and higher PUFA in the FB of that Assay 1, because there is a high genetic variability in the FB breeds. It belongs to a big group of animals with a point of union which is the brave behaviour without taking into account the carcass conformation and dressing percentage.

Total fatty acids of SFA, MUFA and PUFA are similar to those found in bulls from local breeds in Spain slaughtered at a slightly lower weight (450-470 kg) (43% of SFA, 41% of MUFA, 16% of PUFA; [19]) and a similar total muscle lipid content (1-1.5). It can be pointed out the different fatty acid composition of the Assays 1 and 2, being results from Assay 1 more similar to Spanish rustic beef breeds. Fattening illustrates the increasing importance of neutral lipids in total lipids as fattening proceeds, the fairly constant level of phospholipids, and the declining importance of dietary fat as a source of muscle fatty acids as fat deposition accelerates [49]. The content and evolution of MUFA is closely related to the percentage of the major monounsaturated fatty acid 18:1n-9. This fatty acid is the final

TABLE IV
EVOLUTION OF COLOUR IN MEAT FROM FIGHTING BULLS DURING AGEING (7 AND 14 DAYS)

	Assay 1			Assay 2		
	2d	7d		2d	14d	
L*	28.94 ± 0.46	28.14 ± 0.66	**	31.73 ± 2.47	29.95 ± 3.38	*
a*	22.31 ± 0.55	17.47 ± 0.76	***	19.20 ± 2.10	20.76 ± 3.03	ns
b*	6.10 ± 0.54	3.78 ± 0.48	***	8.12 ± 2.20	6.91 ± 2.97	ns
C*	23.46 ± 0.69	17.55 ± 0.77	**	21.01 ± 0.58	22.06 ± 0.94	ns
H*	15.23 ± 1.18	12.67 ± 1.90	***	22.55 ± 1.51	22.40 ± 4.25	ns

Significance level reported in analysis of variance: * P≤0.05; **P≤0.01; ***P≤0.001, ns P≥0.05.

TABLE V
EVOLUTION OF HARDNESS AND JUICINESS (WHC, COOKING LOSSES) IN MEAT FROM FIGHTING BULLS DURING AGEING (7 AND 14 DAYS)

	Assay 1			Assay 2		
	2 d	7 d		2d	14d	
WBSF (kg)	6.15 ± 0.97	6.27 ± 0.71	ns	9.47 ± 1.19	7.64 ± 1.44	**
WHC [1]	22.02 ± 2.77	20.01 ± 2.90	ns	20.72 ± 3.32	15.82 ± 2.85	***
Cooking loss (%)	16.63 ± 2.14	15.66 ± 3.90	*	18.49 ± 3.74	17.57 ± 2.99	**

(1) Measured as the percentage of the exudated juice
Significance level reported in analysis of variance: *P≤0.05; **P≤0.01; ***P≤0.001, ns P≥0.05.
(2) WHC: Water holding capacity.

TABLE VI
**FATTY ACID CONTENT (% OF TOTAL FATTY ACID) IN INTRAMUSCULAR FAT OF *LONGISSIMUS DORSI*
MUSCLE OF FIGHTING BULLS DURING AGEING (7 AND 14 DAYS)**

	Assay 1			Assay 2		
	2d	7d		2d	14d	
C16:0	22.81 ± 4.01	22.15 ± 2.74	ns	21.28 ± 5.30	19.29 ± 2.35	ns
C18:0	9.00 ± 3.48	13.59 ± 3.06	**	18.38 ± 4.98	15.46 ± 1.67	*
Total SFA[a]	37.87 ± 6.54	38.78 ± 5.41	ns	46.26 ± 8.66	42.06 ± 3.39	ns
C18:1n9	39.37 ± 6.70	39.43 ± 5.25	ns	24.68 ± 7.74	27.22 ± 3.89	ns
Total MUFA[b]	42.12 ± 7.30	42.80 ± 5.68	ns	30.29 ± 7.44	31.76 ± 3.89	+
C18:2n6	15.45 ± 7.26	10.79 ± 4.36	+	14.95 ± 3.71	13.47 ± 3.34	ns
C18:3n3	0.22 ± 0.13	0.52 ± 0.08	***	0.66 ± 0.48	0.53 ± 0.20	ns
C20:4n6	3.65 ± 0.60	2.57 ± 1.85	ns	3.65 ± 0.90	2.56 ± 1.84	ns
C22:2n6	0.15 ± 0.05	1.78 ± 1.24	***	0.36 ± 0.18	0.09 ± 0.04	***
Total PUFA[c]	20.05 ± 10.0	16.58 ± 7.64	+	25.44 ± 6.24	24.17 ± 6.70	ns
PUFA/SFA	0.65 ± 0.42	0.45 ± 0.27	ns	0.57 ± 0.21	0.63 ± 0.21	ns

a,b,c: Means for the sum of the saturated fatty acids (SFA), sum of the monounsaturated fatty acids (MUFA), sum of the polyunsaturated fatty acids (PUFA).

Significance level reported in analysis of variance: + $P \leq 0.10$; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$, ns $P \geq 0.05$

PUFA/SFA: sum of the polyunsaturated fatty acids (PUFA)/sum of the saturated fatty acids (SFA) ratio.

product of the Δ 9desaturase-elongase enzyme system which transforms the saturated fatty acids 14:0, 16:0 and 18:0 produced from ruminal hydrolysis and saturations, into Δ 9 monounsaturated acid 18:1n-9 [31]. The content of n-3 PUFA was very low because the high proportion of barley and soybean in the diet, rich in 18:2n-6 which is the precursor of the n-6 fatty acids. So, this could explain the high amount of n-6 PUFA compared to the results of Insausti et al. [19], in different rustic breeds in Spain.

PUFA content in muscle total lipid of the studied FB (18 and 25% in the Assays 1 and 2) was greater than those reported by Eichhorn et al. [8] in different phenotipic animals, ranging PUFA from 5.1 (straightbred Hereford) to 12.5% (chi-aninax Hereford) in total lipid extracts of *Longissimus* muscle. Koizumi et al. [27] observed breed differences in intramuscular polyunsaturated fatty acid (PUFA) content of beef: muscles from Yellow and Hereford cattle contained three and four times more n-3 PUFA respectively than those from Japanese Black (Wagyu) cattle. Such a peculiar fatty acid composition may be explained by the high lipid content in the cells in comparison to reserve total lipids which are predominant in the FB muscles. The high PUFA content in the FB determine a PUFA/SFA ratio greater than 0.45 which is the recommended value. This fact, with its low intramuscular fat content, gives thi beef very good nutritional properties [48].

Regarding the fatty acid composition, it is observed that oleic acid (C18:1) was the most abundant (39.4 and 26.2%, in assays 1 and 2), followed by palmitic acid (C16:0, 21.0%) and stearic acid (C18:0, 11.5 and 19.5%). Values obtained in this study were not different (P>0.05) from bulls of other rustic Spanish breeds that were slaughtered with a mean live weight between 450 and 470 kg and 12-14 months old [19]. With regard to PUFA, C22:2 and C20:4, values found in the intramuscular fat of FB are similar to other foreign breeds [8], while PUFA linoleic (C18:2), with a value around 12%, was much higher than other breeds that show values between 4 to 5% [9]. This could be explained because age increases the fat monounsaturation grade, and FB are sacrified at 4-5 years old [18]. Differences observed between Assays 1 and 2 in the changes of fatty acid profile during ageing could be explained by a longer ageing [10, 41] and also to the superior iron content that could increase the oxidative character of the FB muscles as suggested Picard et al. [35] in FB breed.

CONCLUSIONS AND IMPLICATIONS

The livestock production system (breed, weight and age at slaughter) and the bullfighting confer this beef from FB a specific palatability different from other beef breeds. Beef from FB is characterised by a dark colour due to its high pH and

myoglobin content, a high heam iron content and low intramuscular fat with an equilibrated ratio PUFA/SFA from a nutritional point of view. The characteristics of color, visual aspect and fat content have been well accepted frequently by the popular culture but these characteristics have not been studied objectively by analytical methods. Thus, the increase in the value of beef from FB might be possible giving an added value to this kind of livestock by the establishment of a Qualilty Label for this beef from FB breed animals.

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