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Eating quality of beef from biotypes included in the PGI "Ternera Asturiana" showing distinct physicochemical characteristics and tenderization pattern

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ABSTRACT

Different biotypes of the Protected Geographical Indication (PGI) "Ternera Asturiana" were studied to determine if their differences in physicochemical characteristics and tenderization pattern during maturation (3 to 21 days) had an effect on the consumer evaluation of beef palatability. Biotype affected significantly pH, water holding capacity, chemical composition ($P<0.001$) and meat lightness ($P<0.05$). Ageing time affected significantly ($P<0.05$) colour, meat toughness and sensory attributes in a different way within each biotype. Multivariate analysis showed two different meat groups: 1) meat from *mh*-genotypes, characterized by high juice losses, lightness (L^*), protein content and high sensory acceptability at intermediate (7 and 14 days) ageing times; 2) meat from rustic (AM) breed and biotypes free of myostatin mutation (AV (+/+) and AV×AM), showing higher intramuscular fat, myoglobin content, and instrumental toughness and requiring longer storage times (21 days). This should be taken into account for the proper post-mortem management and commercialization of each product to achieve its best sensory quality.

1. Introduction

Human senses act as gatekeepers for food intake. This biological function protects us from eating spoiled or unfit items and encourages eating nutritious or otherwise beneficial items (Breslin & Spector, 2008). As a consequence no food or beverage is worth producing, distributing or marketing without at least an approximate idea that its sensory quality is accepted by consumers (Tuorila & Monteleone, 2009). Within the modern meat chain, consumers occupy a crucial position being the end users but also at the start of the chain as inspiration for a consumer-driven or market-oriented chain organisation (Gellynck, Verbeke, & Viaene, 2004).

In the case of fresh meat, it is known that consumers consider its sensory characteristics, its nutritional value, its convenience and its impact on health as the main motivators for liking and purchasing (Verbeke & Viaene, 1999; Wood et al., 2003). From these, eating satisfaction is one of the most important characteristics by which consumers judge meat quality after purchase (Grunert, Bredahl, & Brunso, 2004), tenderness, flavour and juiciness being the main components of beef palatability (Dransfield et al., 1984; Monsón, Sañudo, & Sierra, 2005; Serra et al., 2008; Muchenje et al., 2009).

Since 1992, the European agricultural quality policy promotes label systems known as PDO (Protected Designation of Origin), PGI (Protected Geographical Indication) and TSG (Traditional Speciality Guaranteed) that protect food names as a way to keep consumers confidence on food safety and quality. The quality label "Ternera Asturiana", which ranks second in the PGI market of fresh meat in Spain, both in production (more than 18,000 animals slaughtered per year) and economical value (over 20 million euros) (MAPA, 2007), includes meat products of two local beef breeds from Northern Spain namely "Asturiana de los Valles" (AV) and "Asturiana de la Montaña" (AM) as well as their cross-breed (AV×AM). AV is a late-maturing breed with a high growth rate, high muscle percentage (Franco, 1997; Piedrafita et al., 2003) and low amount of fat (Aldai et al., 2006; Aldai, Nájera, Martinez, Celaya, & Osoro, 2007) mainly due to the high frequency of double-muscling or muscular hypertrophy (*mh*) caused by a myostatine inactivating mutation (Grobet et al., 1998), while AM is a small-to medium sized rustic and early-maturing breed adapted to less favoured mountain areas, which have lower conformation score, higher total fat and darker meat (Gil et al., 2001; Piedrafita et al., 2003; Aldai et al., 2006).

Several works have described the physicochemical characteristics of meat from different biotypes included in "Ternera Asturiana" (AV (*mh*/*mh*), AV (*mh*/+), AV (+/+), AM (+/+)) at a given ageing time (Gil et al., 2001; Oliván et al., 2004a; Aldai et al., 2006) and found an increase in meat lightness and drip loss and a decrease of intramuscular

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fatness with increasing number of *mh*-alleles. Moreover, *mh*-genotypes (*mh*/*mh* and *mh*/+) present a different fatty acid profile, with higher proportion of unsaturated and polyunsaturated fats (Aldai et al., 2006). These differences may influence the sensory quality of meat at a given post-mortem time, but also its evolution during ageing, as the different pigment and fat contents and profiles may affect oxidation processes and consequently the development of desirable or undesirable odour and flavour notes (Calkins & Hodgen, 2007; Dransfield, 2008).

There is a general belief that post-mortem ageing increases beef palatability, mainly due to its positive effect on tenderness (Dransfield, 1998; Campo, Sañudo, Panea, Albertí, & Santolaria, 1999; Monsón, Sañudo, & Sierra, 2004; Shanks, Wulf, & Maddock, 2002) and having a significant effect on sensory parameters such as odour, flavour and juiciness (Campo et al., 1999; Monsón et al., 2005; Díez et al., 2006; Revilla & Vivar-Quintana, 2006). During ageing, muscles undergo a series of physical and biochemical changes which are responsible for their conversion to meat mainly due to the action of endogenous peptidases (calpains, cathepsins, proteasomes, caspases, matrix metallopeptidases, and serine peptidases) degrading the myofibrillar structure (Sentandreu, Coulis, & Ouali, 2002; Ouali et al., 2006; Kemp, Sensky, Bardsley, Buttery, & Parr, 2010).

The evolution of these processes may vary depending on pre-, peri- and post-mortem factors, mainly related to production (breed, sex, age, feeding) and technological (chilling, ageing, packaging...etc) management of animals and carcasses. Thus, the post-mortem tenderization pattern should be studied for every product as the detection of an optimum ageing time would increase consumer satisfaction and give added value to the final product.

Previous research with "Ternera Asturiana" biotypes have shown different post-mortem tenderization patterns, being faster in *mh*-genotypes (Campo et al., 1999; Caballero et al., 2007), probably due to faster muscle acidification which influences positively earlier activation of the lysosomal acidic peptidases cathepsins (Caballero et al., 2007), while longer ageing times have been suggested for tenderization of meat from normal (+/+) AV and AM animals (Sierra et al., 2006).

The objective of this work was to determine if the differences in physicochemical and textural traits for the wide variety of biological types protected by the PGI "Ternera de Asturias" have an effect on consumer evaluation of beef palatability and its variation during post-mortem ageing. Knowledge of the optimum ageing time for each meat category would be a key tool for guaranteeing the distribution of every product at its best in order to meet consumer expectations and keep their confidence in this quality label.

2. Materials and methods

2.1. Animals and meat samples

Forty meat samples were obtained from the *Longissimus dorsi* (*LD*) muscle of yearling bulls of five different biological types from breeds "Asturiana de los Valles" (AV) and "Asturiana de la Montaña" (AM) and the cross-breed (AV × AM) all protected by the PGI "Ternera Asturiana". Within the AV breed, animals had different double-muscling character, being homozygous (*mh*/*mh*), heterozygous (*mh*/+) and normal (+/+). Eight animals of each type (AV (*mh*/*mh*), AV (*mh*/+), AV (+/+), AM (+/+), (AV × AM)), were used. Calves were managed with their mothers from birth (winter) to weaning (October). Afterwards calves were fed on concentrate (84% barley meal, 10% soya meal, 3% fat, 3% minerals, vitamins and oligo-elements) and barley straw *ad libitum* for about five months and slaughtered between 14 and 18 months of age, at approximately 500 kg live weight.

Slaughtering was performed in a commercial abattoir according to routine procedures. At 24 h post-slaughter, the left half of the carcass was quartered between the 5th and 6th thoracic vertebrae and the ultimate pH (pH 24 h) of the *LD* muscle of the 5th rib was recorded using a penetration electrode. Then, the rib joint was excised from between the 6th and 12th ribs and transported to the laboratory.

The *LD* muscle was then sliced into 2.5 cm thick (250 g) steaks, vacuum packed (if not indicated otherwise) and kept at 4 °C ± 1 °C for different times (3, 7, 14 and 21 days). After ageing, the steaks were frozen and stored at −20 °C for subsequent analysis.

2.2. Physicochemical analysis

At 48 h post-mortem, the *LD* muscle from the 6th rib was placed in a poliexpan tray, over-wrapped with oxygen-permeable film, aged at 4 °C for 7 days in order to simulate average retail conditions and then minced with an electrical chopper (Moulinex 327, Spain) for chemical analysis. A portion of 10 g was used for the determination of myoglobin pigment concentration assessed by absorbance measurements at 512 nm (Hornsey, 1956), and the rest of the sample was analysed for chemical composition (moisture, intramuscular fat and protein) by near infrared spectroscopy (NIRS) with the application of chemometric equations calibrated by reference methods (Oliván, de la Roza, Mocha, & Martínez, 2002).

Meat colour was measured on the *LD* of the 7th rib, extracted at 48 h post-mortem, then covered with plastic film permeable to O_2 and stored at 4 °C. Measurements were made on the muscle surface at 0 h, 24 h, 6 days and 14 days after meat cutting, using a Minolta CM-200 colorimeter (Konica Minolta Sensing Inc., Tokyo, Japan) with C illuminant and 2° standard observer in the CIE L^*, a^*, and b^* space (L^* *lightness* is a measure of the light reflected (100 = white; 0 = black); a^* redness, measures positive red and negative green; and b^* yellowness, measures positive yellow, negative blue) (Commission International De I' Eclairage, 1976).

Meat water holding capacity was determined by two different methods: Drip Loss (DL, % exudate) recorded on 50 g samples from the *LD* of the 9th rib aged 48 h post-mortem (Honikel, 1998); Expressible Juice (EJ, g/kg) measured on 5 g minced samples from the *LD* of the 6th rib aged 7 days post-mortem, according to the filter-paper press method of Grau and Hamm (1956), modified by Sierra (1973).

Thiobarbituric reactive substances (TBARS, mg malonaldehyde/kg muscle) were measured on *LD* samples at different post-mortem ageing times (3, 7, 14 and 21 days) by the method of Botsoglou et al. (1994), using derivative spectrophotometry.

LD samples (steaks) for instrumental texture analysis, randomly obtained at different locations along the muscle and aged for 3, 7, 14 and 21 days post-mortem were thawed at 4 °C for 24 h, cooked at 75 °C for 45 min by immersion in their vacuum packed plastic bags in a water bath and then allowed to cool under tap water to 18 °C. Samples for the Warner–Bratzler (WB) shear test were obtained by cutting eight pieces, 1 cm^2 in cross-section, parallel to the longitudinal orientation of the muscle fibres. These pieces were subjected to a WB shear blade in an Instron 1011 machine (Instron Limited, High Wycombe, Bucks, UK). The maximum load (kg) required for total split was recorded. Results are expressed as the mean WB maximum load value for each steak.

2.3. Sensory analysis

For sensory evaluation, *LD* samples (steaks) were randomly obtained at different locations along the muscle, vacuum packed, aged at 4 °C for 3, 7, 14 and 21 days and frozen at −20 °C. Before analysis, steaks were thawed overnight at 4 °C ± 1 °C, then wrapped in aluminium foil and cooked at 200 °C in a convention oven until an internal temperature of 70 °C, measured by a thermocouple probe inserted at the steak midpoint, was reached. Once cooked, the core portion of each steak was cut into ten portions (2 × 2 × 2 cm) that were immediately wrapped in aluminium foil, marked with a random 3-digit code and kept at 60 °C (Guerrero, 2005).

The sensory analysis was performed in individual booths under red lighting to mask differences in colour (ISO 8589-1988; UNE 87-004-79). The panel included 140 untrained consumers (62 males and

78 females, aged between 18 and 65 years) organized in 14 sessions of 10 consumers each. A comparative multisample test, with six samples each time, was used by means of a balanced incomplete block design, as described by Meilgaard, Civille, and Carr (1991), which allowed study of the effect of biotype and ageing time and its interaction. Each consumer was involved in only one session. For all six samples evaluated in each session, the portion of meat from different steaks given to one consumer was from the same location, avoiding position influence on the marks.

Consumers were asked to evaluate each sample for flavour, juiciness, tenderness and overall acceptability, following the order printed in the questionnaire, which was designed to avoid the effect of order presentation and first-over and carry-over effects (Macfie, Bratchell, Greenhoff, & Vallis, 1989). A 9-point hedonic scale was used, being 1—"Dislike extremely", 2—"Dislike very much", 3—"Dislike moderately", 4—"Dislike slightly", 5—"Neither like nor dislike", 6—"Like slightly", 7—"Like moderately", 8—"Like very much" and 9—"Like extremely". Before tasting each sample, consumers were required to eat some unsalted toasted bread and then rinse their mouths out with water. Additionally, meat consumption habits of consumers were recorded.

2.4. Statistical analysis

The effect of biotype on physicochemical traits measured at a given ageing time was analysed by Analysis of Variance (ANOVA) using the General Linear Model (GLM) procedure of SPSS (2006). For variables measured during ageing (L^*, a^*, b^*, TBARS, and WBSF) the model included the effects of biotype, ageing time and its interaction.

Data from sensory hedonic ratings were analysed by ANOVA using the GLM procedure of SPSS, by considering biological type, ageing time, and its interaction as main effects. Once the interaction between biotype and ageing was proved, the effect of biotype (with consumer as random factor) and the effect of ageing time (with animal and consumer as random factors) were tested over the residuals of the session effect. When significant, differences were analysed by means of Tukey post-hoc test (Games–Howell test when variances were not homogeneous).

In order to establish different clusters of consumers a hierarchical cluster analysis was used based on the overall acceptability with the XLStat program of Microsoft Excel (XLStat, 2009). The effect of cluster on the consumer acceptability scores for every meat product (biotype × ageing time) was analysed by one-way ANOVA.

Relationship between physicochemical and sensory variables was evaluated using Pearson's correlation coefficient. Furthermore, principal component analysis (PCA) was performed, to describe the relationships between variables and biotypes, by XLStat (2009).

3. Results and discussion

3.1. Physicochemical characteristics

Biological type affected significantly most physicochemical traits studied at a given ageing time (Table 1). Meat from animals with muscular hypertrophy (AV (*mh*/*mh*) and AV (*mh*/+)) showed significantly lower ultimate pH ($P<0.001$) and lower water holding capacity, measured as increased drip ($P<0.001$) and expressible juice losses ($P<0.001$). These results agree with previous findings showing a higher glycolytic metabolism and an increased proportion of fast-twitch glycolytic fibres in the muscles of animals with muscular hypertrophy (Gagnière, Picard, Jurie, & Geay, 1997; Oliván et al., 2004a; Bouley et al., 2005).

Breed and *mh*-genotype also affected meat composition, with meat from AV (*mh*/*mh*) bulls having significantly lower IMF ($P<0.001$) and higher moisture ($P<0.001$) and protein contents ($P<0.001$) than any other type. Previous reports have described extremely low IMF contents in meat from (*mh*/*mh*) bulls, not only in the AV breed (Campo et al., 1999; Oliván et al., 2004a; Aldai et al., 2006) but also in other double-muscled breeds such as Belgian Blue White and Piemontese (Destefanis, Barge, & Brugiapaglia, 1996; Clinquart, Hornick, van Eenaeme, & Istasse, 1997).

Higher amounts of IMF and greater water holding capacity (decreases in drip and expressible juice losses) were found in meat from biological types free of myostatin mutation, that is, the rustic AM breed and the normal (+/+) AV genotype, as well as their cross-breed AV × AM. These results support the hypothesis proposed by Oliván, Osoro, Martinez, and Guerrero (2003) and supported by Aldai et al. (2006) that there is a significant positive relationship between IMF content and water holding capacity of meat when comparing different breeds and *mh*-genotypes, with increasing juice loss as the IMF content decreases.

With respect to colour, it will depend on the amount of myoglobin present in the muscle and its oxidative state and light scattering resulting from surface moisture or fibre composition (Swatland 2003). In this work, there was a significant effect of biological type on the myoglobin content of meat (Table 1), with AV (*mh*/*mh*) animals showing the lowest concentration while the highest amount was from bulls from the rustic AM breed and its cross AV × AM. This agrees with earlier studies that described a decrease in haem pigment content in meat of double-muscled animals with respect to normal and heterozygous animals (Bailey, Enser, Dransfield, Restall, & Avery, 1982; De Smet et al., 2000; Aldai et al., 2006). Others have also shown that rustic breeds, such as AM, are characterized by high pigment concentration and darker colour (Insausti et al., 1999; Gil et al., 2001; Aldai et al., 2006; Vieira, Diaz, Martínez, & García-Cachán, 2009). In this study, no significant differences between biological types were found for a^* and b^* (Table 2), while there were significant differences in lightness (L^*), meat from AM bulls being the darkest, and lightness increasing with the number of *mh*-alleles in the AV breed. This could relate in part to differences in muscle structure rendering lower water holding capacity and higher surface moisture in meat from *mh*-animals that may be partially responsible for increased meat surface reflectance. In addition, the light path through the muscle of *mh*-animals may change due to hyperplasia (higher total number of muscle fibres), hypertrophy (increase in myofibre diameter) and changes in fibre composition (higher proportion of fast-twitch glycolytic fibres type IIX) (Bouley et al., 2005), affecting light scattering and absorption.

Table 1
Effect of biotype on physicochemical variables.

	Biological type						
	AV (*mh*/*mh*)	AV (*mh*/+)	AV (+/+)	AM	AV × AM	s.e.m.	Sign.
pH (24 h)	5.57^{a}	5.58^{ab}	5.62^{b}	5.60^{b}	5.59^{b}	0.02	***
Drip loss (%)	2.57^{c}	2.13^{bc}	1.22^{a}	0.91^{a}	1.59^{b}	0.27	***
Expressible juice (%)	26.30^{c}	24.44^{cb}	23.41^{b}	20.55^{a}	23.12^{b}	0.85	***
Moisture (%)	74.32^{b}	73.56^{a}	73.85 ^{a}	73.28^{a}	73.51^{a}	0.26	***
IMF (%)	1.71^{a}	2.70^{b}	2.77^{bc}	3.73^{d}	3.19^{cd}	0.31	***
Protein (%)	22.85^{b}	22.82^{b}	22.51^{a}	22.41 ^{a}	22.46^{a}	0.11	***
Myoglobin (mg/kg)	3.24^{a}	4.26^{bc}	3.73^{b}	4.78^{c}	4.36^{c}	0.25	***

$^{a-d}$Means in the same row followed by different superscripts are significantly different at $P<0.05$; ***$P<0.001$; s.e.m.: standard error of means; IMF: Intramuscular fat.

Table 2
Effect of biotype (in rows) and ageing time (in columns) on colour parameters (L^*, a^*, and b*), lipid oxidation (TBARS) and meat toughness (WBSF). For colour, ageing time refers to hours and days after meat cutting while in the case of TBARS and WBSF it refers to post-mortem time.

		Biological types						
	Ageing	AV(*mh*/*mh*)	AV(*mh*/+)	AV(+/+)	AM(+/+)	AV×AM	s.e.m.	Sign.
L^*	0 h	43.93 C	42.14 BC^{ab}	38.81 AB^{a}	36.62 A^{a}	40.96 BC	0.93	***
	24 h	44.07 B	42.79 AB^{b}	40.63 AB^{b}	38.89 A^{b}	41.66 AB	1.03	*
	6 days	45.62 C	42.72 BC^{b}	41.62 B^{b}	38.40 A^{ab}	42.18 B	0.76	***
	14 days	45.83 B	40.31 A^{a}	40.10 A^{ab}	39.58 A^{b}	39.34 A	0.94	***
	s.e.m.	0.72	0.57	0.44	0.51	0.85		
	Sign.	NS	*	**	**	NS		
a^*	0 h	20.04^{bc}	20.65^{b}	19.03^{b}	19.48^{b}	21.14^{b}	0.58	NS
	24 h	23.26^{c}	23.84^{c}	22.49^{c}	23.84^{c}	23.90^{c}	0.81	NS
	6 days	19.52^{b}	20.92^{b}	22.01^{c}	19.80^{b}	20.82^{b}	0.95	NS
	14 days	10.80^{a}	13.74^{a}	13.11^{a}	13.86^{a}	13.56^{a}	1.02	NS
	s.e.m.	0.95	0.73	0.56	0.90	0.78		
	Sign.	***	***	***	***	***		
b^*	0 h	3.51^{a}	3.20^{a}	1.89^{a}	2.44^{a}	2.87^{a}	0.40	NS
	24 h	9.67^{b}	9.63^{b}	8.31^{b}	10.05^{b}	9.20^{b}	0.67	NS
	6 days	10.48^{b}	9.67^{b}	9.64^{b}	9.80^{b}	9.73^{b}	0.50	NS
	14 days	10.52^{b}	8.81^{b}	7.80^{b}	9.45^{b}	8.72^{b}	0.79	NS
	s.e.m.	0.54	0.47	0.62	0.22	0.66		
	Sign.	***	***	***	***	***		
TBARS (mg MDA/kg)	3 days	0.21^{a}	0.15^{a}	0.15	0.19	0.17	0.60	NS
	7 days	0.19^{a}	$0.19^{a,b}$	0.14	0.19	0.19	0.32	NS
	14 days	0.33^{b}	$0.28^{a,b}$	0.24	0.20	0.17	0.26	NS
	21 days	0.39^{b}	0.37^{b}	0.24	0.24	0.29	0.33	NS
	s.e.m.	0.03	0.05	0.03	0.03	0.04		
	Sign.	***	*	NS	NS	NS		
WBSF (kg)	3 days	5.27	5.14^{b}	6.05^{b}	7.35^{b}	5.88^{b}	0.05	NS
	7 days	4.62	5.12^{b}	5.10^{a}	5.34^{a}	5.10^{ab}	0.05	NS
	14 days	4.25	$4.84^{a,b}$	5.01^{a}	4.88^{a}	4.79^{ab}	0.06	NS
	21 days	4.39	4.17^{a}	4.84^{a}	4.40^{a}	4.52^{a}	0.07	NS
	s.e.m.	0.27	0.19	0.18	0.33	0.31		
	Sign.	NS	**	*	***	*		

For a given ageing time, means in the same row followed by different capital letters are significantly different at $P<0.05$.
For a given biological type and variable, means in the same column followed by different superscripts are significantly different at $P<0.05$.
*$P<0.05$; **$P<0.01$; ***$P<0.001$; NS: not significant.
s.e.m.: standard error of means.

With meat ageing for each genotype (Table 2), the a^* value decreased significantly between 6 and 14 days, while for b^* value the main change was a significant increase between the moment of cutting (0 h) and after 24 h blooming. The decrease of a^* value after 6 days post-mortem is in accordance with previous findings by Demos and Mandingo (1996), who studied the discoloration of ground beef stored in oxygen-permeable packages and showed that by day seven approximately 65% of myoglobin was converted into metmyoglobin.

There was no significant effect of genotype on the TBARS index, which measures the proportion of malonaldehyde (MDA) derived mainly from lipid oxidation in the muscle, although there was a general TBARS increase with storage time (Table 2), being significant for (*mh*/*mh*) ($P<0.001$) and (*mh*/+) ($P<0.05$) genotypes of the AV breed, probably due to their higher PUFA proportion in the intramuscular fat (Aldai et al., 2006) which are more sensitive to oxidation (Cosgrove, Church, & Pryor, 1987). However, in general, the level of lipid oxidation was low as even after 21 days, the MDA content was below the threshold for rancidity (2 mg MDA/kg) proposed by Campo et al., (2006). These low values are probably due to the vacuum packaging minimizing lipid oxidation and therefore avoiding the development of rancid or off-flavours detectable by consumers.

The instrumental toughness, measured as Warner–Bratzler shear force (WBSF) on cooked meat during ageing (Table 2), showed different tenderization patterns depending on biological type, although there was no significant interaction biotype × ageing. The presence of the myostatin mutation in AV (*mh*/*mh*) and AV (*mh*/+) biotypes tended to produce the most tender meat (5.27 and 5.14 kg WBSF, respectively), at the earliest ageing time (3 days post-mortem). The highest toughness values correspond to meat from the rustic AM breed (7.35 kg), although the differences between biological types were not significant at any ageing time, probably due to high intra-group variations (Table 2). These data support the idea that meat from *mh*-genotypes tenderizes in a very short post-mortem period, which is consistent with previous reports by Campo et al. (2000) and Oliván et al. (20004b) who found that meat tenderization was faster in double-muscled bulls than in other genotypes.

When considering ageing effects on WBSF, toughness progressively decreased during maturation, the effect being significant for all biotypes except AV (*mh*/*mh*) (Table 2). Long ageing times reduced the toughness differences between biotypes, which agrees with the findings of Sañudo et al. (2004) on different cattle biotypes (double-muscled, fast-growth, dual-purpose and rustic).

It is important to note that meat from AM animals had the highest tenderization rate (40%), that is, the highest decrease in toughness from 3 to 21 days post-mortem (7.35 kg to 4.40 kg), which indicates a very efficient tenderization process during this period. The lowest tenderization rate was found for meat from *mh*-genotypes (17% and 19% in *mh*/*mh* and *mh*/+, respectively), which were the ones with lower initial toughness, probably due to exhaustion of the enzymes implicated in tenderization at earlier post-mortem times (from 0 h to 3 days post-mortem). Similar results were found by Sañudo et al. (2004) in bulls slaughtered at 300–350 kg, with tenderization rates of 22% for meat of double-muscled genotypes (AV breed) and 42% for rustic types (Avileña, Morucha and Retinta breeds).

It is necessary to stress that meat from *mh*-animals showed no significant effect of ageing on WBSF (Table 2), reaching the lowest (and almost final) toughness at 3 days, although as discussed later this was not perceived as negative by consumers.

3.2. Sensory quality

Biological type affected significantly most of the sensory traits (juiciness, tenderness and overall acceptability) at long ageing times (14

Table 3
Effect of biotype (in rows) and post-mortem ageing time (in columns) on sensory attributes evaluated by consumers.

		Biological types						
	Ageing	AV(*mh*/*mh*)	AV(*mh*/+)	AV(+/+)	AM	AV×AM	s.e.m.	Sign.
Flavour	3 days	5.68	5.70^{a}	5.85	5.84	6.30	0.29–0.32	NS
	7 days	6.37	6.42^{b}	6.00	6.07	5.97	0.31–0.34	NS
	14 days	6.14	6.50^{b}	6.05	6.00	6.45	0.31–0.35	NS
	21 days	6.01	6.19^{ab}	6.09	6.08	6.24	0.29–0.32	NS
	s.e.m.	0.18–0.21	0.20–0.22	0.18–0.20	0.21–0.25	0.25–0.25		
	Sign.	**	*	*	NS	NS		
Juiciness	3 days	4.95^{a}	5.23^{a}	5.80	5.36	5.53^{a}	0.21–0.23	NS
	7 days	5.50^{ab}	5.92^{b}	5.40	5.70	5.53^{ab}	0.24–0.27	NS
	14 days	6.14 AB^{c}	6.60 B^{b}	5.475 A	5.85 AB	6.25 B^{b}	0.29–0.32	***
	21 days	5.55^{bc}	5.83^{ab}	5.86	6.10	5.42^{ab}	0.26–0.29	*
	s.e.m.	0.19–0.22	0.23–0.26	0.22–0.25	0.24–0.28	0.26–0.26		
	Sign.	***	**	**	NS	**	0.25–0.27	
Tenderness	3 days	4.75^{a}	4.87^{a}	5.87	5.18^{a}	5.70	0.25–0.28	NS
	7 days	5.77^{b}	5.90^{b}	5.57	5.65^{ab}	5.50	0.30–0.33	NS
	14 days	6.06 AB^{b}	6.62 B^{b}	5.32 A	5.85 AB^{ab}	6.32 B	0.26–0.29	**
	21 days	5.60^{b}	5.95^{b}	5.82	6.33^{b}	5.65		*
	s.e.m.	0.21–0.24	0.26–0.29	0.24–0.27	0.24–0.28	0.31–0.31		
	Sign.	***	***	NS	***	NS		
Acceptability	3 days	4.95^{a}	5.18^{a}	5.80	5.56	5.88	0.21–0.24	NS
	7 days	5.90^{b}	5.90^{ab}	5.65	5.78	5.58	0.27–0.30	NS
	14 days	5.98 ABC^{b}	6.48 C^{b}	5.48 A	5.73 AB	6.35 BC	0.29–0.32	***
	21 days	5.45 A^{ab}	5.80 AB^{ab}	5.98 AB	6.38 B	5.63 AB	0.27–0.27	**
	s.e.m.	0.21–0.24	0.23–0.25	0.20–0.22	0.24–0.28	0.28–0.28		
	Sign.	**	**	**	NS	NS		

For a given ageing time, means in the same row followed by different capital letters are significantly different at $P<0.05$.
For a given biological type and variable, means in the same column followed by different superscripts are significantly different at $P<0.05$.
*$P<0.05$; **$P<0.01$; ***$P<0.001$; NS: not significant.
s.e.m.: standard error of means.

to 21 days) (Table 3). However, at short ageing times (3 days) significant differences were not found, although there was a tendency of meat from *mh*-genotypes to achieve the lowest valuation of sensory characteristics. This could be due to the low amount of IMF in meat from these genotypes which may jeopardize its sensory quality, especially when tenderization processes are incomplete. However, and probably due to its earlier tenderization, meat from *mh*-genotypes tended to show the highest scores for flavour, tenderness and overall acceptability at 7 days post-mortem. Moreover, AV (*mh*/+) and AV×AM reached the highest scores for juiciness ($P<0.001$), tenderness ($P<0.01$) and acceptability ($P<0.001$) at 14 days of ageing.

At 21 days, biotype was significant for juiciness ($P<0.05$), tenderness ($P<0.05$) and acceptability ($P<0.01$), with meat from the AM breed presenting the highest marks for overall acceptability.

The data show different evolution rates during maturation of the sensory quality traits from these biotypes (Table 3), the effect of ageing was significant for all sensory traits in the case of *mh*-genotypes, for flavour, juiciness and overall acceptability in AV (+/+), for tenderness in AM and for juiciness in AV×AM. For *mh*-genotypes the best sensory scores were reached at 7–14 days, while the other meat types reached significantly higher scores at 14 (AV×AM) and 21 days (AV (+/+) and AM).

Most previous research on beef sensory quality has shown that tenderness tends to increase as ageing time increases (Campo et al., 1999; Monsón et al., 2005; Muchenje et al., 2008; Spanier, Flores, McMillin, & Bidner, 1997), however the present data shows that while true for meat of AV(+/+) and AM biotypes, meat from *mh*-genotypes and AV×AM showed a peculiar increasing pattern for sensory scores (juiciness, tenderness and acceptability, less so for flavour) up to 14 days and then declined. These results, together with the significant interaction biotype×ageing in sensory attributes (flavour, $P<0.01$; juiciness, tenderness and overall acceptability, $P<0.001$) are in clear agreement with the assumption that double-muscling is highly related to a faster metabolism requiring shorter ageing times than other meat types (Campo et al., 1999; Caballero et al., 2007). This faster metabolism in mh-genotypes is also supported by a faster rate of meat oxidation (TBARS) which could improve its sensory quality at shorter ageing times, as some oxidation is needed for a proper meat flavour development, but could influence negatively sensory quality at longer ageing times (21 days) although the oxidation levels reached in this work were below the threshold value for rancidity (Campo et al., 2006).

As for AV×AM meat, the results seem to indicate similar characteristics, in terms of tenderization pattern to that of the AV (*mh*/+) genotype,

Table 4
Frequencies (%) of socio-demographic characteristics and meat consumption habits of the global consumer population and each cluster, identified with respect to overall acceptability.

	Total	Cluster 1	Cluster 2
n	140	73	67
Age			
<25	21	26	15
26–35	38	34	42
36–45	22	19	27
46–55	16	17	15
>55	3	4	1
Gender			
Male	44	43	46
Female	56	57	54
Living place			
Country side	51	52	49
City	49	48	51
Consumption of beef			
<1 per week	18	11	26
2–3 times per week	63	66	59
>4 per week	19	23	15
Preferred meat			
White	32	34	31
Red	21	20	22
Both	47	46	47

Table 5
Effect of cluster on beef overall acceptability for every product (biotype × ageing) studied.

Biotype	Ageing	Cluster 1	Cluster 2	
AV (*mh*/*mh*)	3 days	4.96	4.94	NS
	7 days	6.04	5.75	**
	14 days	6.23	5.70	***
	21 days	5.33	5.58	*
AV (*mh*/+)	3 days	5.27	5.07	NS
	7 days	6.10	5.68	**
	14 days	6.55	6.39	NS
	21 days	5.65	5.97	*
AV (+/+)	3 days	5.93	5.65	NS
	7 days	5.79	5.49	*
	14 days	5.68	5.26	**
	21 days	6.02	5.94	NS
AM	3 days	5.75	5.36	***
	7 days	6.08	5.44	***
	14 days	5.97	5.46	***
	21 days	6.24	6.52	*
AV × AM	3 days	6.03	5.70	*
	7 days	5.75	5.38	***
	14 days	6.59	6.09	***
	21 days	5.56	5.70	NS

*$P<0.05$; **$P<0.01$; ***$P<0.001$; NS: not significant.

although for most physicochemical traits it was intermediate between AV and AM biotypes.

The highest sensory scores for all the attributes tested were for meat from heterozygous (*mh*/+) AV animals matured 14 days (mean scores 6.5 or higher for all sensory traits), followed by meat from the AM breed aged 21 days (mean scores from 5.8 to 6.5) (Table 3).

To identify if this information reveals the existence of consumer segmentation, a hierarchical cluster analysis was conducted. Two groups or clusters were identified with respect to overall acceptability. These clusters did not display any relevant differences due to demographic variables (such as age, gender, living place or beef consumption habits) (Table 4), although some tendencies were observed: a higher percentage of young people (<25 years old) was included in cluster 1 while more consumers between 26 and 45 years were included in cluster 2. Furthermore, there was a higher percentage of consumers in cluster 1 who ate meat more often (over twice per week) than in cluster 2.

When looking at the effect of cluster on the overall acceptability of the different products (biotype × ageing) studied (Table 5), the results show that consumers from cluster 1 (with more young people and higher frequency of beef consumption) tended to prefer beef reaching an appropriate level of tenderness at short-to-medium ageing times (3–14 days), regardless of biotype. However, consumers from cluster 2, who tended to be older with lower beef consumption, gave significantly higher scores for beef aged longer (21 days), rated with higher flavour acceptability, which could be due to the detection of pleasant flavour notes, coming from numerous chemical components (sugars, organic acids, peptides and free amino acids) produced during post-mortem proteolytic, hydrolytic and oxidative ageing processes (Koohmaraie, 1994; Calkins & Hodgen, 2007). This agrees with recent findings showing the importance of flavour, in addition to tenderness, for meat overall ratings (see Calkins & Hodgen, 2007).

3.3. Relationships between physicochemical and sensory parameters

Pearson's correlation coefficients between sensory parameters and physicochemical characteristics are shown in Table 6. There was a significant and positive relationship ($r>0.7$, $P<0.01$) between all sensory traits used, tenderness and juiciness being the best correlated to overall acceptability ($r=0.889$ and $r=0.904$, respectively) at different ageing times. This agrees with Monsón et al. (2005) who showed high correlations between flavour and tenderness ($r=0.78$ and $r=0.52$ respectively) and overall acceptability for beef from different breeds. Consumers may not discriminate in detail between different sensory attributes, but they provide useful information concerning product acceptability or preference for one kind of meat over another (Wheeler, Shackelford, & Koohmaraie, 1997) focusing on how meat can contribute to his/her personal satisfaction.

Table 6
Pearson's correlation coefficients between sensory attributes and physicochemical traits at different ageing times.

	Flavour				Juiciness				Tenderness				Acceptability			
	3 days	7 days	14 days	21 days	3 days	7 days	14 days	21 days	3 days	7 days	14 days	21 days	3 days	7 days	14 days	21 days
pH	−0.400	−0.211	0.026	−0.296	**−0.541**	0.056	0.023	−0.350	**−0.460**	0.148	−0.006	−0.229	−0.402	0.009	0.005	−0.246
Moisture	−0.057	0.041	0.158	**−0.437**	−0.171	−0.007	0.117	−0.411	−0.137	0.153	0.075	−0.364	−0.188	0.185	0.186	**−0.534**
IMF	0.166	−0.170	−0.094	**0.466**	0.205	0.035	−0.116	0.328	0.197	−0.202	−0.032	**0.446**	0.317	−0.174	−0.164	**0.572**
Protein	−0.410	0.018	−0.118	−0.302	−0.408	−0.266	−0.002	−0.122	**−0.436**	0.038	−0.040	**−0.440**	**−0.586**	−0.012	0.021	−0.308
TBARS3	0.105	−0.018	0.419	−0.133	−0.068	−0.176	0.305	0.244	−0.118	−0.090	0.067	0.236	−0.071	−0.064	0.167	−0.014
TBARS7	0.270	0.169	**0.484**	0.233	0.152	−0.058	0.306	**0.466**	0.183	−0.016	0.103	**0.488**	0.156	0.040	0.248	0.277
TBARS14	−0.387	0.236	0.451	−0.335	−0.434	0.289	**0.468**	0.110	−0.380	0.264	0.156	−0.066	−0.371	0.334	0.328	−0.184
TBARS21	0.063	−0.165	0.391	0.216	−0.153	−0.341	0.249	0.263	−0.003	−0.379	0.051	0.287	−0.139	−0.309	0.196	0.139
WBSF3	−0.228	−0.418	0.122	−0.060	−0.348	−0.140	−0.029	−0.003	−0.326	−0.135	−0.140	−0.113	−0.205	−0.043	0.022	0.011
WBSF7	0.022	**−0.540**	−0.059	0.254	−0.312	−0.368	0.076	−0.031	−0.282	**−0.572**	−0.028	−0.013	−0.168	−0.428	−0.040	0.050
WBSF14	−0.197	**−0.593**	−0.336	0.293	−0.211	−0.298	−0.327	0.291	−0.292	−0.241	−0.351	−0.079	−0.104	−0.262	−0.337	0.186
WB21	**−0.472**	−0.374	−0.264	0.019	**−0.527**	−0.217	−0.341	−0.062	**−0.451**	0.042	−0.269	−0.204	−0.416	0.017	−0.232	−0.048
Flav3		0.285	0.629	0.284	**0.670**	0.034	0.463	0.294	**0.493**	−0.424	0.157	0.454	**0.749**	−0.306	0.400	0.138
Flav7			**0.904**	−0.177	−0.023	**0.679**	**0.908**	0.328	−0.102	**0.547**	**0.733**	0.346	0.002	**0.660**	**0.839**	−0.059
Flav14				−0.206	0.383	0.540	**0.816**	0.130	0.217	0.205	**0.587**	0.254	0.315	0.356	**0.848**	−0.120
Flav21					0.290	−0.010	−0.435	**0.652**	0.366	−0.065	−0.374	**0.505**	0.432	−0.195	−0.340	**0.815**
Juic3						0.011	0.173	0.451	**0.839**	−0.137	−0.026	0.385	**0.904**	−0.233	0.227	0.330
Juic7							0.466	0.116	0.012	**0.739**	0.446	0.237	0.128	**0.774**	0.515	0.074
Juic14								0.068	0.005	0.038	**0.805**	0.329	0.101	0.355	**0.903**	−0.178
Juic21									0.233	0.261	0.008	**0.756**	0.412	0.001	0.041	**0.854**
Tend3										−0.043	−0.053	0.451	**0.873**	−0.103	0.126	0.350
Tend7											0.277	0.206	−0.108	**0.817**	0.261	0.221
Tend14												0.458	−0.133	0.568	**0.889**	0.003
Tend21													0.497	0.242	0.327	**0.812**
Accep3														−0.167	0.128	0.406
Accep7															0.520	0.007
Accep14																−0.082

Coefficients in captive bold are significant at least at the 0.05 level.

Of to the relationships between sensory and physicochemical traits, beef flavour at 21 days was positively correlated to IMF ($r=0.466$, $P<0.05$) and beef flavour at 14 days to TBARS ($r=0.484$, $P<0.05$), showing the influence of the quantity and composition of intramuscular fat and its oxidation state on the development of meat flavour notes, as many compounds that contribute to meat smell and flavour are lipid breakdown products (Calkins & Hodgen, 2007). Flavour showed a negative relationship with WBSF at different ageing times ($r=-0.472$ to $r=-0.593$, $P<0.05$) as more pleasant flavour notes were detected by consumers in more tender (lower WBSF) meat.

With respect to meat juiciness, at short ageing times (3 days) it was negatively related to pH ($r=-0.541$, $P<0.05$) probably due to the greater weakening of the muscle structure at low pH, as an acidic environment favours the action of acidic proteases, such as cathepsins, that can minimise water retention between actin and myosin filaments, thus producing higher juice losses during chewing. This is supported by the fact that tenderness at short ageing times was negatively related to pH ($r=-0.460$, $P<0.05$). However, meat juiciness after longer storage periods (14 and 21 days) showed a positive significant relationship with TBARS at 7 ($r=0.466$) and 14 days ($r=0.468$), suggesting that fat oxidation, which depends both on the amount and composition of total fat, may affect the perception of juiciness during chewing.

The results showed positive correlations of ultimate tenderness with IMF ($r=0.446$, $P<0.05$) and TBARS at 7 days ($r=0.488$, $P<0.05$). It has been proposed that IMF indirectly affects meat tenderness perception where it is deposited between fascicules, thereby disrupting the structure of the endomysium (Webb & O'Neill, 2008) and by promoting the secretion of saliva (Warris, 2000) which increases the perception of quality during chewing. The reason for significant correlation of IMF, a constant variable, with ultimate tenderness (also flavour and acceptability) could be due to a confounding effect of the different patterns of sensory scores for the various biotypes, because meat from AV and AM types, that is, those with higher IMF contents, gave the highest sensory notes at the longest ageing time. A similar effect could explain the relationship between TBARS at 7 days and tenderness at 21 days.

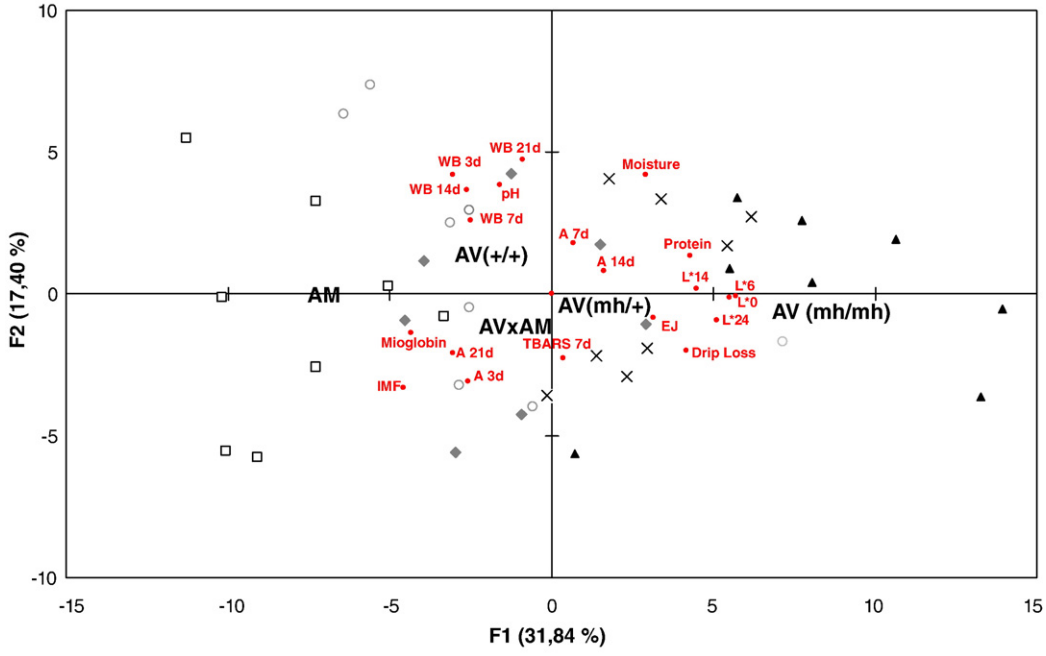
As expected, tenderness at 7 days was negatively related to WBSF at 7 days ($r=-0.572$, $P<0.01$) and tenderness at 3 days negatively related to ultimate WBSF ($r=-0.451$, $P<0.05$). In the literature, correlations between WBSF and sensory assessment of beef tenderness are variable, with r values ranging from −0.32 to −0.94 (Caine, Aalhus, Best, Dugan, & Jeremiah, 2003).

The overall acceptability of meat, was positively related to IMF ($r=0.572$, $P<0.01$) and negatively to moisture and protein, due to their inverse proportions in meat composition. These results show the great influence that IMF has on the eating quality of beef from the biological types studied, due especially to the extremely low amount of IMF in *mh*-genotypes (a minimum fat content of 3% was recommended in meat products by Savell and Cross (1988)), and agree with previous research by Webb and O'Neill (2008), who concluded that reduction of the fat content in meat may adversely affect eating satisfaction.

The biplot obtained by PCA, which was defined by Destefanis et al. (2000) as the best effective procedure to obtain a synthetic judgement of meat quality, describes the relationships between acceptability scores and the main physicochemical traits (pH, EJ, drip, chemical composition, pigment content, colour variables, TBARS and WBSF) together with the different biotypes (Fig. 1). The first component explained 31.84% of the variability and is distinguished in the negative side by meat samples from the rustic type (AM) and other genotypes free of the myostatin mutation (AV (+/+) and AV×AM) which correlated positively to IMF and myoglobin content and to higher instrumental WBSF. In the positive side were placed samples of double-muscled homozygous (*mh*/*mh*) and heterozygous (*mh*/+) animals, which were correlated positively to EJ, drip loss, lightness (L^*) and protein content. This influenced the perception of overall acceptability by consumers, who rated positively meat properly tenderized at intermediate (7 and 14 days) ageing times (*mh*-genotypes) but preferred meat with slower tenderization patterns and higher IMF contents both at short (3 days) and long (21 days) ageing times. That is, when IMF effects on flavour and texture improve meat quality and mask the high toughness due to incipient tenderization, and also after a long storage period, when differences in toughness were diminished.

4. Conclusions

In the studied conditions, meat products protected by the PGI "Ternera Asturiana" may be grouped in two classes: 1) meat from *mh*-



WB: Warner Bratzler toughness; **A:** Overall acceptability; **L*:** Lightness; **IMF**: intramuscular fat content, **EJ:** Expressible juiciness.

Fig. 1. Principal component analysis biplot of variables (physicochemical and overall acceptability) and biotypes (▲ AV(*mh*/*mh*); X AV (*mh*/+); ○ AV (+/+), □ AM; ◆ AV×AM).

genotypes, characterized by high juice losses and lightness, low IMF and faster tenderization processes rendering high sensory acceptability at intermediate (7 and 14 days) ageing times; 2) meat from the rustic AM breed and other genotypes free of myostatin mutation (AV(+/+) and AV×AM), with higher IMF and myoglobin contents but slower tenderization pattern, that require longer storage times (21 days) to achieve the best sensory quality.

Furthermore, among consumers, two different groups were identified, with younger people showing preference for beef at short-to-medium ageing times, while older people preferred beef aged for longer. This may be useful for the development of marketing strategies aimed at specific segments of the population.

It is concluded that appropriate combinations of biotype and ageing should be taken into account for optimal management of each meat product included in the PGI "Ternera Asturiana". This will be a key tool for guaranteeing distribution of every product in its best condition to achieve high sensory quality.

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