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# Bresaola made from Cinisara cattle: effect of muscle type and animal category on physicochemical and sensory traits

Giuseppe Maniaci<sup>a</sup>, Marco Alabiso<sup>a</sup>, Nicola Francesca<sup>a</sup>, Cristina Giosuè<sup>b</sup>, Antonino Di Grigoli<sup>a</sup>, Onofrio Corona<sup>a</sup>, Cinzia Cardamone<sup>c</sup>, Graziella Graci<sup>c</sup>, Baldassarre Portolano<sup>a</sup> and Adriana Bonanno<sup>a</sup>

<sup>a</sup>Dipartimento Scienze Agrarie, Alimentari e Forestali, University of Palermo, Palermo, Italy; <sup>b</sup>National Research Council of Italy - Institute of Anthropic Impacts and Sustainability in marine environment (IAS-CNR), Campobello di Mazara, Italy; <sup>c</sup>Istituto Zooprofilattico Sperimentale della Sicilia (IZSSi), Palermo, Italy

### ABSTRACT

The physicochemical characteristics and sensory traits of Cinisara bresaola were investigated, to explore a new commercial opportunity for autochthonous dairy cattle farms. *Semimembranosus, Semitendinosus* and *Biceps brachii* muscles, from adult cows (AC) and grazing (GB) or housed (HB) young bulls of Cinisara breed, were processed to made bresaola. Differences due to animal category and muscle type were observed. Bresaola from AC was richer in fat and volatile organic compounds. The bresaola from *Semitendinosus* showed higher colorimetric parameters, fat and, when from grazing animals, Warner-Bratzler shear force than those made from other muscles. In general, all bresaola were well appreciated. The principal component analysis performed using selected physicochemical and sensory traits was able to discriminate bresaola produced from different animal categories, effect of muscle type was relevant only for AC. These results evidenced the possibility to obtain bresaola from the meat of different animal categories, comparable for sensory properties and appreciable by consumers.

# Bresaola hecha de ganado Cinisara: efecto del tipo de músculo y la categoría del animal en los rasgos fisicoquímicos y sensoriales

#### RESUMEN

El presente estudio se propuso investigar las características fisicoquímicas y los rasgos sensoriales de la bresaola de ganado Cinisara, con el propósito de explorar nuevas oportunidades comerciales para las granjas autóctonas de ganado lechero. Con el fin de elaborar la carne bresaola se procesaron los músculos Semimembranosus, Semitendinosus y Biceps brachii de vacas adultas (AC), de toros jóvenes que pastan (GB) y de toros jóvenes confinados (HB) de la raza Cinisara. El estudio constató la existencia de diferencias atribuibles a la categoría de los animales, así como al tipo de músculo empleado. La bresaola de AC resultó ser más rica en grasa y compuestos orgánicos volátiles. La bresaola de Semitendinosus mostró parámetros colorimétricos más intensos, mayor contenido de grasa y, cuando provenía de animales de pastoreo, [mayor] fuerza de cizallamiento de Warner-Bratzler que las bresaolas elaboradas con otros músculos. En general, todas las bresaolas fueron bastante apreciadas. El análisis de los componentes principales, realizado utilizando rasgos fisicoquímicos y sensoriales seleccionados, fue capaz de discriminar la bresaola producida a partir de diferentes categorías de animales; el efecto del tipo de músculo sólo fue evidente en el caso de las AC. Estos resultados ponen de manifiesto la posibilidad de obtener varias bresaolas a partir de carne de diferentes categorías de animales, las cuales son comparables por sus propiedades sensoriales. Todas ellas son apreciadas por los consumidores.

## 1. Introduction

Bresaola is a typical made in Italy dry meat product, similar to other products such as Turkish pastirma (Kaban, 2009) and Kazakh dry-cured beef (Sha et al., 2017), although the latter is usually boiled or fried before consumption. Bresaola is guaranteed by Protected Geographical Indication (PGI) community trademark "Bresaola della Valtellina," and is much appreciated in the national and international market (Marino et al., 2015) for its nutritional and sensorial characteristics. It shows low fat and calories contents, and high amount of protein, iron, vitamins and minerals (Zhang, 2015), meeting the expectative of current consumers oriented towards protein food low in fat and carbohydrates, but with high-value for sensorial quality (Jimenez-Colmenero et al., 2001). Bresaola is traditionally made by curing cuts from the hind quarter (in particular *Semimembranosus, Semitendinosus* and *Quadriceps femoris* muscles) of horses, donkeys or cattle (Paleari et al., 2003), through different production steps. The meat is deprived by visible fat, then massaged with dry salt and natural flavors, before to be dried and cured under adequate environmental conditions (temperature, relative humidity and air velocity) for 3 weeks (Braghieri et al., 2009). The Bresaola quality depends on the initial properties of the meat and its treatment. In particular, the meat fat content and its fatty acid profile is strongly related to feeding system, diet composition, age and

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PALABRAS CLAVE bresaola; raza Cinisara; compuestos orgánicos volátiles; análisis sensorial

CONTACT Marco Alabiso amarco.alabiso@unipa.it Dipartimento Scienze Agrarie, Alimentari e Forestali, University of Palermo, Viale delle Scienze, 90128 Palermo, Italy

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breed of animals (Di Grigoli et al., 2019; Lengyel et al., 2003; Nuernberg et al., 2005), influencing nutritional and sensorial characteristics of Bresaola (Marino et al., 2015).

Cinisara is a Sicilian autochthonous breed inserted in a Registry recognized by the European Union and established for the protection and conservation of breeds with limited diffusion. The Cinisara is reared in the western part of Sicily according to the traditional livestock system, based on the exploitation of natural resources at pasture. It is considered a dairy breed, and its milk is mainly used to make the Caciocavallo Palermitano cheese (Alabiso et al., 2005; Giosuè et al., 2005; Di Gregorio et al., 2017). Nevertheless, recent studies showed that Cinisara meat presents similar physicochemical traits to those of meat breeds, with a good amount of protein and intramuscular fat (Liotta et al., 2015). However, the market of fresh meat from Cinisara cattle is negatively affected by the competition of the meat from specialized breeds; therefore, processed products, such as salami (Gaglio et al., 2016) and bresaola (Liotta et al., 2015), enhancing the commercial offer with typical local products, would allow to increase the economic profitability of farms.

The aim of this study was to provide a contribution to define the manufacturing process to obtain high-quality Cinisara bresaola, by assessing its physicochemical and sensory properties in relation to the origin of processed meat, in terms of animal category (cow at end of its productive career, grazing young bull or housed young bull) and type of muscle (*Semimembranosus* and *Semitendinosus* from the hind quarter, and *Biceps brachii* from the fore quarter).

#### 2. Material and methods

#### 2.1. Meat and bresaola

The meat used in the experiment was taken from carcasses of Cinisara breed cattle selected after slaughter on the basis of the livestock system of the farm of origin; due to the small consistency of Cinisara breed, only six carcasses could be contemporarily found corresponding to the purpose of the research. The animals were slaughtered at an EU-licensed abattoir, according to the standard handling procedures. In particular, the carcasses belonged to two adult cows (AC) (10 years old) and two grazing young bulls (GB) (18 months old), which were fed pasture-based diets supplemented with hay and concentrate until slaughter; the other two carcasses were obtained from two housed young bulls (HB) (18 months old), fed with hay and concentrate in the final phase.

The carcasses were stored in a cooling room at  $4-8^{\circ}$ C for a 7-day ageing period, and then were dissected (day 0) to remove *Semimenbranosus* (SM), *Semitendinosus* (ST) and *Biceps brachii* (BB) muscles used for the experiment. The muscles of the right half, properly cleaned from fat and external tendons, were processed to produce bresaola, while those of the left half were sampled to assess the physicochemical traits of fresh meat (day 0).

The manufacturing process of bresaola was as follows: on day 0, the meat cuts were submitted to the first dry salting, rubbing by hand their surface with a mixture composed of sodium chloride (1.5%), natural flavorings (0.1%), dextrose (0.35%), potassium nitrate (0.075%), sodium nitrate (0.05%), and sodium ascorbate (0.11%), and then were stored in a cooling room at 4–6°C; on day 6, the second dry salting of cuts was carried out as the previous one; on day 14, the cuts were placed to drain at 4°C; on day 25, the cuts were wrapped with dried and glued natural casing, tied with a rope and transferred to drying cells where they were initially dripped (10 hours at 24°C) and successively dried (24 hours at 22°C and 62% of relative humidity (RH)), and then every day the temperature was reduced ( $-1^{\circ}$ C) and the RH was increased (+2%); on day 32, the bresaola was transferred to the ripening room (10°C and 90% of RH) for 4 weeks, until day 60.

All the bresaola were produced at the "Lipari salami factory" in Alcamo (Sicily, Italy). For the purposes of sensory evaluation, a commercial bresaola was purchased and used as a control.

#### 2.2. Sampling

For each animal category and muscle type, three specimens of 2.5 cm of fresh meat were cut transversely to the direction of the muscle fibers, to determine physical and chemical characteristics. At the end of ripening, the bresaola was sampled using the same procedure; moreover, a portion of each bresaola was conserved for sensory evaluation.

All samples of fresh meat and bresaola were placed in sterile vacuum containers, immediately refrigerated and transported at 8°C to laboratory to be homogenized by stomacher (LAB Blender 400, Seward Medical, London, United Kingdom) for 2 minutes at maximum speed; then samples were frozen at  $-20^{\circ}$ C and freeze-dried for successive analysis (SCANVAC Coolsafe 55–9, Labogene Aps, Lynge Denmark).

## 2.3. Physical and chemical parameters

Each muscle was weighed fresh (day 0) and at the end of the ripening phase (day 60). The weight loss was expressed as a percentage of the initial weight.

pH, water activity ( $a_w$ ), colorimetric parameters, and tenderness were measured at day 0 on fresh meat, and at day 60 on ripened bresaola. The pH was detected with a digital pH meter (Thermo Orion 710 A +, Cambridgeshire, United Kingdom), equipped with a penetration probe. The  $a_w$  was measured with a dew-point hygrometer HygroLab 3 (Rotronic, Huntington, NY, USA); calibration was performed using five saturated solutions of known  $a_w$ .

Colorimetric parameters were measured with a Chroma Meter (CR-300, Minolta, Osaka, Japan) using illuminant C, with calibration based on a white standard such as  $L^* = 100$  (equivalent to BaSO4) and aperture size Ø 8 mm; results were expressed as lightness (L\*, range from 0 (black) to 100 (white)), redness (a\*, range from red (+a) to green (-a)), and yellowness (b\*, range from yellow (+b) to blue (-b)), according to the International Commission on Illumination (CIE, 1975). The values of a\* and b\* were used for the calculation of Chroma  $[C = (a^{*2} + b^{*2})^{o.5}]$  and Hue (H = arctg b\*/a\*).

The Warner-Bratzler shear force (WBS) was measured with an Instron Universal Testing Machine (Instron tester 5564, Trezzano sul Naviglio, Milan, Italy) equipped with Warner-Bratzler device; for each muscle, 5 samples with  $10 \times 10$  mm cross section and 25 mm length were detected.

The chemical analyses were performed in triplicate on freeze-dried samples of fresh meat and bresaola at the end of ripening. Moisture, fat, protein and ash contents in fresh meat and bresaola, and salt content only in bresaola were determined according to the AOAC methods (2012). The nitrogen was converted to protein using the conversion factor of 6.25. Non-protein nitrogen (NPN) was determined at day 0 and day 60 analyzing the supernatant after precipitating the protein with 5% trichloroacetic acid. The proteolysis index (PI) was calculated as the percentage ratio between NPN and total nitrogen (TN).

# 2.4. Volatile organic compounds

Volatile organic compounds (VOC) were determined in triplicate on ripened bresaola by Solid Phase Micro-Extraction technique in Head Space, followed by Gas Chromatography/ Mass Spectrometry (HS-SPMEGC/MS) (Kataoka et al., 2000). Samples of homogenized bresaola (0.50 g) were transferred into 2 mL vials with pierceable silicone rubber septa coated with polytetrafluoroethylene (PTFE) film. A quantity of 50  $\mu$ L of 2-pentanol-4-methyl methanol solution (0.981  $\mu$ g/mL) was used as the internal standard. A Supelco SPME (Bellefonte, PA) holder and fiber was coated with divinylbenzene/carboxen/polydimethylsiloxane. The vials were heated at controlled temperature (40  $\pm$  0.5°C) in order to reach equilibrium and 30 min exposure time. The GC-MS conditions were used as described by Corona (2010). Collected data were processed with the instrument data system.

Bresaola VOC was identified by comparison of the retention times with those of the reference compounds (NIST/EPA/MSDC Mass Spectral Database, T.G. House, Cambridge, UK). Semiquantitative determination was carried out by the method of internal standard. The calibration curve was constructed with readings on five 2-pentanol-4-methylmethanol solutions with concentrations ranging from 1.5 to 8  $\mu$ g/mL (R<sup>2</sup> 0.994).

## 2.5. Sensory evaluation

The evaluation of the sensory profile of the aged bresaola was performed following the ISO guidelines (2005) by twelve judges (6 females and 6 males, 25–35 years old) with experience in the assessment of meat products. The judges were trained in preliminary sessions to gain consensus on the sensory descriptors and the use of scale, with a detailed description of each attribute (Cenci-Goga et al., 2012). A total of 16 descriptors were included in the analysis for the external aspect (color intensity, brightness), flavor (acid, rancid, mold, lactic, bitter, sweet, spicy, salty and intensity), rheology (fattiness, elasticity, fibrosity, chewiness, and tenderness), as well as overall acceptability.

The bresaola samples were randomly evaluated in individual booths under incandescent white light, by assigning to each descriptor a score from 1.00 (the absence of sensation) to 9.00 (extremely intense). Each judge evaluated 18 experimental bresaola and a commercial bresaola. The individual scores for each assessor were then averaged to give a score for the taste panel as a whole. Each evaluation was carried out in different test sessions at the same time of day (Cenci-Goga et al., 2012).

#### 2.6. Statistical and explorative multivariate analysis

Data were statistically processed using the SAS 9.2 software (Institute, 2010). Physicochemical traits and VOC were analysed according to a MIXED model including the fixed effects of animal category (A, with three levels: GB, HB and AC), type of muscle (M, with three levels: SM, ST and BB), their

interaction A\*M, and the animal within category as a random effect. If the interaction was significant (p < .05), Tukey's test was used to compare the means.

In order to compare the sensorial scores of each experimental bresaola to those of commercial bresaola (CB), the data of sensory evaluation were processed using the generalized linear model (GLM) SAS procedure to assess the fixed effect of bresaola type (B, with 10 levels: CB, GB-SM, GB-ST, GB-BB, HB-SM, HB-ST, HB-BB, AC-SM, AC-ST and AC-BB); when the effect was significant (p < .05), means comparisons were performed by Tukey's test.

Pearson's correlation coefficients were calculated between physicochemical and sensory variables.

The principal component analysis (PCA), performed using the PRINCOMP SAS procedure, was based on physicochemical variables, VOC and sensory attributes in order to assess their specific contribution in explaining the differences among bresaola type, due to the different animal category and muscle type. The variables used in the analysis were identified on the basis of a stepwise selection using the STEPDISC SAS procedure, after they were standardized multiplying them by the inverse of standard deviation (1/SD). The number of main components was selected according to Kaiser's criterion and only those with Eigenvalues above 1.00 were retained.

#### 3. Results and discussion

# 3.1. Physical and chemical parameters

The chemical parameters were referred considering the fresh meat (day 0) and bresaola at the end of ripening (day 60), in relation to the animal category and the type of muscle (Table 1).

On overall, animal category, which includes the effects of animal age and farming system, and muscle type were both responsible of differences in the chemical composition of fresh meat and bresaola. However, fresh meat showed more marked differences than those recorded in bresaola, especially due to the higher fat level recorded in all AC muscles, to which corresponded lower moisture percentages than in the younger categories. Among fresh muscles, ST resulted in the fattest for all animal categories.

The moisture content of the bresaola resulted less variable in relation to category and muscle than that of fresh meat; the values recorded were similar to that reported by other Authors for bovine bresaola, and higher than that found in other species (Paleari et al., 2003). However, a higher water level, of around 60%, was reported on bresaola produced from donkey (Marino et al., 2015) and buffalo (Paleari et al., 2003).

Similarly to fresh meat, bresaola from AC showed higher levels of fat compared to those from GB and HB, especially those manufactured with ST and BB muscles, which reached a level equal to 5.70% and 4.37%, respectively. However, also these fat levels were lower than those reported by Liotta et al. (2015) for the Cinisara bresaola and those of the other breeds or species (Paleari et al., 2003; Zhang, 2015).

The PI showed differences among animal categories, being lower in HB for both fresh meat and bresaola; however, the PI of bresaola from HB was lower in SM and BB muscles, whereas that of ST muscle was equal to that of AC. Although PI increased adequately from fresh meat to Table 1. Chemical parameters of fresh meat (day 0) and ripened bresaola (day 60) in relation to animal category and muscle.

Tabla 1. Parámetros químicos de la carne fresca (día 0) y de la bresaola madura (día 60) en relación con la categoría del animal y el músculo.

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Animal categories (A) <sup>a</sup>	I categories (A) <sup>a</sup> GB				HB			AC		S	Significance <sup>f</sup>			
Muscles (M) <sup>b</sup>	SM	ST	BB	SM	ST	BB	SM	ST	BB	SEM <sup>e</sup>	А	М	A*M	
Fresh meat (day 0)														
Moisture, %	75.6 <sup>c</sup>	75.9 <sup>b</sup>	75.5°	76.5 <sup>a</sup>	75.9 <sup>b</sup>	76.5 <sup>a</sup>	74.9 <sup>d</sup>	74.3 <sup>e</sup>	74.5 <sup>e</sup>	0.041	***	***	***	
Fat' %	0.72 <sup>g</sup>	0.82 <sup>f</sup>	0.79 <sup>f</sup>	1.10 <sup>e</sup>	1.17 <sup>d</sup>	1.08 <sup>e</sup>	1.94 <sup>c</sup>	2.31ª	2.19 <sup>b</sup>	0.009	***	***	***	
Protein' %	22.3 <sup>ab</sup>	22.0 <sup>bc</sup>	22.5ª	21.1 <sup>e</sup>	21.5 <sup>d</sup>	21.1 <sup>e</sup>	21.9 <sup>c</sup>	22.1 <sup>bc</sup>	22.0 <sup>bc</sup>	0.048	***	*	***	
PI <sup>d</sup> ,%	12.2 <sup>a</sup>	12.3ª	12.1ª	10.9 <sup>e</sup>	11.7 <sup>b</sup>	11.5 <sup>c</sup>	11.3 <sup>d</sup>	11.9 <sup>b</sup>	11.8 <sup>b</sup>	0.031	***	***	***	
Ash, %	1.10 <sup>e</sup>	1.14 <sup>c</sup>	1.18 <sup>b</sup>	1.14 <sup>c</sup>	1.28 <sup>a</sup>	1.15 <sup>c</sup>	1.09 <sup>e</sup>	1.11 <sup>d</sup>	1.12 <sup>d</sup>	0.001	***	***	***	
Bresaola (day 60)														
Moisture, %	56.3 <sup>ab</sup>	54.5 <sup>ab</sup>	55.5 <sup>ab</sup>	51.8 <sup>b</sup>	54.4 <sup>ab</sup>	54.7 <sup>ab</sup>	52.1 <sup>b</sup>	58.0 <sup>a</sup>	56.9 <sup>ab</sup>	0.871	NS	*	*	
Fat' %	1.41 <sup>d</sup>	1.71 <sup>d</sup>	1.58 <sup>d</sup>	2.10 <sup>cd</sup>	2.31 <sup>cd</sup>	2.20 <sup>cd</sup>	2.72 <sup>c</sup>	5.70 <sup>a</sup>	4.37 <sup>b</sup>	0.165	**	***	***	
Protein' %	36.3 <sup>ab</sup>	37.1 <sup>ab</sup>	35.9 <sup>ab</sup>	39.0 <sup>a</sup>	36.4 <sup>ab</sup>	36.2 <sup>ab</sup>	33.5 <sup>b</sup>	35.2 <sup>ab</sup>	32.2 <sup>b</sup>	0.572	**	*	*	
Pl <sup>d</sup> ,%	15.9 <sup>ab</sup>	15.8 <sup>bc</sup>	16.2 <sup>ab</sup>	13.9 <sup>d</sup>	14.7 <sup>cd</sup>	13.7 <sup>d</sup>	17.1 <sup>a</sup>	14.7 <sup>cd</sup>	15.8 <sup>bc</sup>	0.199	**	**	***	
Ash, %	5.81	6.34	6.64	6.69	6.53	6.75	5.21	6.62	6.13	0.210	NS	*	NS	
NaCl, %	4.54 <sup>bc</sup>	5.51 <sup>ab</sup>	5.82 <sup>a</sup>	5.15 <sup>ab</sup>	5.55 <sup>ab</sup>	5.60 <sup>ab</sup>	4.12 <sup>c</sup>	5.52 <sup>ab</sup>	5.01 <sup>ab</sup>	0.198	NS	**	*	

<sup>a</sup>Animal categories: GB = grazing young bull; HB = housed young bull; AC = adult cow.

<sup>b</sup>Muscles: SM = Semimembranosus; ST = Semitendinosus; BB = Biceps brachii.

<sup>c</sup>The results indicate mean values of the measurements performed on two replicates for animal category.

<sup>d</sup>PI = proteolysis index.

<sup>e</sup>SEM = standard error of the means.

 $f^*P \le 0.05$ ,  $**P \le 0.01$ ,  $***P \le 0.001$ ; NS = not significant; a, b, c, d, e, f, g = P \le 0.05.

<sup>a</sup>Categorías de animales: GB = toro joven que pasta; HB = toro joven confinado; AC = vaca adulta.

<sup>b</sup>Músculos: SM = Semimembranosus; ST = Semitendinosus; BB = Biceps brachii.

<sup>c</sup>Los resultados indican los valores medios de las mediciones realizadas en dos réplicas para la categoría de animales.

<sup>d</sup>PI = índice de proteólisis.

<sup>e</sup>SEM = error estándar de las medias.

 $^{f*}P \le 0.05$ ,  $^{**}P \le 0.01$ ,  $^{***}P \le 0.001$ ; NS = no significativo; a, b, c, d, e, f, g = P \le 0.05.

bresaola, the reached values were always lower than those registered for products with longer maturation, such as ham (Schivazappa et al., 2004), and comparable with that detected on bovine salami (Gaglio et al., 2016).

The ash content of fresh meat was comparable to that found by Paleari et al. (2003), whereas the bresaola showed higher values than that found by the same authors, due to the greater amount of salt used (3% vs 2.5%).

The percentage of NaCl in the bresaola was influenced by the muscle, resulting in a lower average in SM muscles than in BB and ST ones. Probably, the smaller size of BB and the smaller diameter of ST compared to SM, characterized by greater diameter and weight, could have favored the salt absorption. Among the parameters of the centesimal composition, only the salt content did not fall within the limits of the production disciplinary of the bovine "Bresaola della Valtellina P.G.I.", resulting slightly higher than 5% in most cuts.

The physical parameters of fresh meat and bresaola, referred to animal category and type of muscle are reported in Table 2.

Although the initial weight of muscles ranged from 1641 to 3010 g, it was not affected statistically; however, it can be noticed that AC animal category showed the lighter muscles and, among muscles, the BB registered the lower weight.

Fresh meat was significantly affected by animal category for pH, which was lower in AC than in the other animals, and by muscle type for water activity (a<sub>w</sub>), which was higher in SM than in the other muscles; however, these same effects were not detected in bresaola. On the contrary, the lightness (L\*) was affected by muscles type similarly in fresh meat and bresaola, especially due to the lower values that BB muscles showed before and after ripening, especially in grazing animal category.

At the end of ripening, the weight loss did not differ statistically, although it was higher in GB than in AC and HB; in particular, although AC was the fattest, the lower weight of the muscles determined probably a weight loss intermediate between GB and HB. Weight loss ranged from 34.1% to 40.5%, whereas the moisture content decreased from 74.5% to 76% in fresh meat to 51.8–58.0% in final products. These results are consistent with those found in dry meat with longer aging, probably related to the low fat content of bresaola (Schivazappa et al., 2004).

The pH of bresaola showed minimal differences between the muscles (from 5.61 to 5.86), as was found in bresaola from beef and buffalo meat (Paleari et al., 2003), but in contrast with the trend observed in bresaola from horse meat (Cattaneo et al. 1995) and in beef salami (Gaglio et al., 2016). The differences were also not significant among the different animal categories (on average 5.76, 5.77 and 5.71 for GB, HB and AC, respectively). These results confirm the considerable variability often associated with the production plant (Frustoli et al., 2007), which differ in structures, equipment and mainly in the environmental microflora.

The water activity (a<sub>w</sub>) in the bresaola was not influenced by the animal category and the type of muscle; thus, it did not maintain the differences found in fresh meat. A reduction of water activity to values around 0.90, reached after 60 days of maturation, can be attributed to dehydration and diffusion of salt (Schivazappa et al., 2004). The limited curing time and the low fat content, both denoting a high moisture content, could be responsible of final a<sub>w</sub> values higher than those of other cured meats (Schivazappa et al., 2004). This aspect requires a greater hygienic-sanitary control from the raw matrix to the final product, to achieve microbiological safety standards at the end of the ripening process (Paleari et al., 2003).

The color parameters were mainly affected by the muscle. The BB recorded lower values for L\* in both fresh meat, as already mentioned, and bresaola, whereas ST was higher for L\*, b\* and H in bresaola. The values of L\* were higher than

Table 2. Physical parameters of fresh meat (day 0) and ripened bresaola (day 60) in relation to animal category and muscle.

Animal categories (A) <sup>a</sup>		GB			HB			AC			S	ignificar	nce <sup>f</sup>
Muscles (M) <sup>b</sup>	SM	ST	BB	SM	ST	BB	SM	ST	BB	SEM <sup>e</sup>	А	М	A*M
Fresh meat (day 0)													
lnitial weight <sup>c</sup> , g	2972	3010	2243	2883	2955	2146	1977	1641	1460	473.4	NS	NS	NS
pH	5.56	5.61	5.60	5.61	5.69	5.57	5.53	5.49	5.59	0.043	*	NS	NS
a <sub>w</sub>	0.99	0.96	0.96	0.98	0.97	0.97	0.99	0.97	0.98	0.014	NS	**	NS
L <sup>*</sup> , lightness	37.1	38.9	34.7	37.4	40.3	37.1	37.3	39.0	34.7	0.862	NS	**	NS
b*, yellowness	4.48	5.78	5.74	4.73	5.54	4.48	5.74	5.50	4.56	0.687	NS	NS	NS
Chroma	21.4	22.8	22.5	22.7	23.1	21.4	22.5	23.0	22.1	0.716	NS	NS	NS
Hue	9.07	13.0	14.3	11.8	14.7	10.3	14.9	14.6	14.6	1.575	NS	NS	NS
WBS <sup>d</sup> (N/cm <sup>2</sup> )	30.6	38.9	28.0	25.4	34.5	26.7	24.6	45.3	28.6	7.202	NS	NS	NS
Bresaola (day 60)													
Final weight loss <sup>c</sup> , %	39.3	39.1	40.5	37.3	34.1	35.1	38.4	35.8	35.4	2.065	NS	NS	NS
pH	5.83	5.75	5.74	5.81	5.72	5.79	5.66	5.61	5.86	0.074	NS	NS	NS
a <sub>w</sub>	0.92	0.90	0.89	0.90	0.89	0.88	0.92	0.89	0.90	0.011	NS	NS	NS
L <sup>*,</sup> lightness	31.9	34.8	28.6	31.9	32.5	30.3	31.6	34.6	30.3	0.876	NS	**	NS
a*, redness	15.2	16.2	14.2	15.6	16.6	16.0	14.6	16.7	16.3	0.594	NS	NS	NS
b*, yellowness	5.67	7.27	6.82	5.79	6.86	5.88	5.65	7.88	5.59	0.830	NS	*	NS
Chroma	16.2	18.0	14.8	16.1	17.6	17.2	16.0	18.4	16.5	0.814	NS	NS	NS
Hue	20.5	23.9	14.9	21.7	19.4	19.0	20.0	24.9	18.6	2.004	NS	*	NS
WBS <sup>d</sup> (N/cm <sup>2</sup> )	18.1	43.4	14.7	33.4	34.0	19.6	28.2	54.9	16.5	5.858	NS	***	NS

<sup>a</sup>Animal categories: GB = grazing young bull; HB = housed young bull; AC = adult cow.

<sup>b</sup>Muscles: SM = Semimembranosus; ST = Semitendinosus; BB = Biceps brachii.

The results indicate mean values of the measurements performed on two replicates for animal category.

<sup>d</sup>WBS = Warner-Bratzler shear force.

<sup>e</sup>SEM = standard error of the means.

 $f^*P \le 0.05$ ,  $**P \le 0.01$ ,  $***P \le 0.001$ , NS = not significant.

<sup>a</sup>Categorías de animales: GB = toro joven que pasta; HB = toro joven confinado; AC = vaca adulta.

<sup>b</sup>Músculos: SM = Semimembranosus; ST = Semitendinosus; BB = Biceps brachii.

<sup>c</sup>Los resultados indican los valores medios de las mediciones realizadas en dos réplicas para la categoría de animales.

<sup>d</sup>WBS = Fuerza de cizallamiento de Warner-Bratzler.

<sup>e</sup>SEM = error estándar de las medias.

 $f_*P \le 0.05$ ,  $**P \le 0.01$ ,  $***P \le 0.001$ , NS = no significativo.

those reported for beef bresaola (Zhang, 2015) and lower than those found on commercial bresaola (Rød et al., 2012), considering similar ripening time and water content.

The WBS showed no difference between the fresh meat samples, whereas it differed among bresaola from different muscles (P < .001), being higher in bresaola from ST meat particularly for AC and GB categories. These differences, compared to HB, could be due to the intensive physical activity to which the ST muscle is involved during grazing (Dannenberg et al., 2006). Despite the higher age of AC in comparison with GB and HB, no differences between animals emerged, probably due to the greater amount of fat in AC. On the whole, the WBS values were lower than those reported for bresaola obtained from Cinisara cattle (Liotta et al., 2015) and for other beef bresaola (Schivazappa et al., 2004; Zhang, 2015).

#### 3.2. Volatile organic compounds (VOC)

Table 3 shows the bresaola VOC in relation to animal category and muscle. On the whole, the VOC content was higher for AC (2534.51 mg/kg) than for HB (2055.01 mg/kg) and GB (1759.97 mg/kg). The higher VOC content recorded in bresaola from AC can be attributed to their higher content in fat, in which these fat-soluble compounds are concentrated.

Terpenes were the major group, representing 65.5%, 67.3% and 76.3% of the total VOC, respectively, for GB, HB and AC, as found in bresaola processes from buffalo meat (Rapacciuolo et al., 2006) and in other cured meats (Misharina et al., 2001).

The other chemical groups identified were hydrocarbons 16.0%, 15.2% and 6.2%; esters 11.6%, 11.4% and 10.5%; aldehydes 2.9%, 2.7% and 2.6%; carboxylic acids 2.0%, 2.0%

and 1.7%; alcohols 1.9%, 1.0% and 1.6%; and ketones 0.2%, 0.2% and 0.8%, respectively, for GB, HB and AC.

Several factors are involved in the accumulation of volatiles in animal tissues, and among them animal diet plays an important role.

Proteolytic and lipolytic enzymatic reactions generate, directly or indirectly, non-volatile and volatile flavour compounds in processed products (Marušić et al., 2011; McSweeney & Sousa, 2000). Most of the volatile compounds are the result of chemical or enzymatic oxidation of unsaturated fatty acids and further interactions with proteins, peptides and free amino acids. Other volatile compounds result from Stecker degradation of free amino acids and Maillard reactions (Toldrá, 1998).

The presence of terpenes found in the meat of ruminants has been linked to the feeding regimen, since these compounds are almost exclusively synthesized in the plants (Vasta & Priolo, 2006). Nevertheless, the relevant presence of terpenes detected in this experiment can be mainly attributed to the spices used in the preparation of bresaola. Indeed, the influence of the use of natural spices in the bresaola production process on the profile and quantity of VOCs has been proved, although the data reported in the literature are often conflicting, due to the different composition of the spices used in the few studies investigating the aromatic profile of bresaola (Rapacciuolo et al., 2006).

## 3.3. Sensory analysis

Table 4 shows the results of the sensorial analysis in which the different types of ripened bresaola were compared among them and with a commercial product. Table 3. Volatile organic compounds (mg/kg) of ripened bresaola in relation to animal category and muscle.

Tabla 3. C	ompuestos	orgánicos	volátiles	(mq/kq)	de bresaola	madura	en relación	con la	categoría	del animal	y el	múscu	ılo
				·									

Animal categories (A) <sup>a</sup>		GR	) ue presa		HR		egona del a		usculo.		Sic	nce <sup>d</sup>	
	C14	ст		C14	ст.		<u></u>	лс ст		CENC		, initedi	A ¥ 8 4
Muscles (M) <sup>2</sup>	SIM	51	BR	SM	51	BR	SM	51	BR	SEM	A	M	A*M
Alcohols	_			_	_		_	_	_				
Isoamylalcohol	10.89 <sup>c</sup>	11.14 <sup>c</sup>	35.08 <sup>0</sup>	10.47 <sup>c</sup>	10.48 <sup>c</sup>	n.d.	11.66 <sup>c</sup>	11.10 <sup>c</sup>	57.46ª	2.209	*	***	**
2,3-Butanediol	11.54	11.98	11.08	16.12	15.37	11.72	11.41	11.80	7.67	1.955	NS	NS	NS
Aldehydes													
Nonanal	39.05	39.56	16.72	16.69	17.43	69.70	62.97	68.91	18.74	18.892	NS	NS	NS
trans-2-Octenal	6.06 <sup>bc</sup>	6.47 <sup>b</sup>	6.74 <sup>0</sup>	3.22 <sup>ca</sup>	3.05 <sup>cd</sup>	9.45ª	4.41 <sup>bcd</sup>	4.42 <sup>bcd</sup>	2.59 <sup>a</sup>	0.573	*	*	**
Benzaldehyde	11.33	10.83	15.46	12.97	13.71	20.20	15.48	15.83	8.05	3.168	NS	NS	NS
Hydrocarbons													
Undecane	21.43	20.78	14.48	35.67	37.49	17.83	15.03	15.41	20.63	6.684	NS	NS	NS
Phenylethylalcohol	16.77	16.98	21.12	20.73	22.27	22.86	40.97	43.31	38.87	9.942	*	NS	NS
Phenol	12.79 <sup>ab</sup>	12.14 <sup>ab</sup>	9.87 <sup>b</sup>	10.69 <sup>bc</sup>	10.11 <sup>bc</sup>	16.73ª	16.04 <sup>a</sup>	14.78 <sup>ac</sup>	12.95 <sup>ab</sup>	1.325	NS	NS	*
Dodecane	200.40	213.11	243.41	243.55	230.99	238.07	114.83	122.30	n.d.	29.138	NS	NS	NS
Styrene	5.26	5.08	8.25	11.57	10.93	8.71	19.76	18.73	13.15	3.114	*	NS	NS
Terpenes													
a-Terpineol	50.89	50.57	27.73	84.11	86.85	68.20	102.15	102.51	96.90	15.296	*	*	NS
a-Terpinolen	21.05	19.94	46.94	71.86	72.67	112.63	66.62	69.67	9.11	16.580	*	NS	NS
α-Terpinene	28.08	27.77	25.53	56.60	56.12	48.17	43.82	45.59	44.36	2.690	***	NS	NS
Terpinen-4-ol	206.02	198.06	154.54	318.52	323.44	325.11	357.41	331.30	305.01	53.608	NS	NS	NS
α-Terpineol acetate	24.23	23.55	7.59	42.73	46.11	41.84	45.79	43.12	n.d.	7.728	*	NS	NS
α-Phellandrene	17.41	17.31	20.88	28.57	28.41	45.74	32.80	33.06	24.83	10.493	NS	NS	NS
Eugenol	110.67	106.06	42.25	149.73	158.63	116.94	176.41	168.37	140.34	29.868	NS	*	NS
p-Cymene	92.58	98.42	45.86	78.21	74.88	100.80	97.56	94.82	32.95	19.030	NS	NS	NS
2,5Dimethyl styrene	12.26	12.04	7.30	19.94	19.31	10.45	25.23	24.45	10.22	2.610	*	**	NS
Caryophyllen	657.96	616.12	436.83	511.08	487.59	302.09	970.85	933.71	904.59	99.929	NS	NS	NS
α-Caryophyllene	75.93	70.68	28.34	50.60	48.14	10.50	119.72	113.56	94.43	23.339	NS	*	NS
Linalol	38.33	39.57	23.21	47.03	46.07	65.40	53.82	56.17	14.41	11.234	NS	NS	NS
Ketones													
Acetophenon	4.12 <sup>b</sup>	4.03 <sup>b</sup>	4.61 <sup>b</sup>	4.70 <sup>b</sup>	4.46 <sup>b</sup>	5.90 <sup>b</sup>	29.06 <sup>a</sup>	27.33 <sup>a</sup>	6.38 <sup>b</sup>	3.880	**	NS	*
Esters													
Methyloctanoate	30.44	28.61	7.65	37.27	38.85	50.87	58.37	55.40	33.69	13.328	NS	NS	NS
Methylhexanoate	11.44	10.78	n.d.	n.d.	n.d.	11.26	49.20	51.39	22.30	4.716	*	NS	NS
Ethyloctanoate	25.42	26.55	34.51	39.46	39.08	36.75	34.94	36.38	28.13	7.588	NS	NS	NS
Methyldecanoate	83.01	87.33	66.83	81.07	87.13	75.24	102.57	110.08	63.36	12.962	NS	NS	NS
Ethyldecanoate	70.25	73.17	56.66	69.91	69.33	65.57	52.80	56.06	53.16	7.383	NS	NS	NS
Carboxylic acids													
Acetic acid	36.05	36.35	22.91	31.38	30.42	51.39	34.06	35.42	47.33	8.857	NS	NS	NS
Butanoic acid	3.89 <sup>b</sup>	4.04 <sup>b</sup>	2.96 <sup>b</sup>	2.38 <sup>b</sup>	2.31 <sup>b</sup>	6.47 <sup>a</sup>	3.35 <sup>b</sup>	3.56 <sup>b</sup>	4.29 <sup>b</sup>	0.453	NS	*	*

The results indicate mean values of three measurements performed on each of the two replicates for animal category, and are expressed as 4-methyl-2-pentanone (n.d. = non detected).

<sup>a</sup>Animal categories: GB = grazing young bull; HB = housed young bull; AC = adult cow.

<sup>b</sup>Muscles: SM = Semimembranosus; ST = Semitendinosus; BB = Biceps brachii.

<sup>c</sup>SEM = standard error of the means.  $d*P \le 0.05$ ,  $**P \le 0.01$ ,  $***P \le 0.001$ ; NS = not significant; a, b, c, d = P \le 0.05.

Los resultados indican los valores medios de tres mediciones realizadas en cada una de las dos réplicas para la categoría de animales y se expresan como 4-metil-2-pentanona (n.d. = no detectado).

<sup>a</sup>Categorías de animales: GB = toro joven de pastoreo; HB = toro joven confinado; AC = vaca adulta.

<sup>b</sup>Músculos: SM = Semimembranosus; ST = Semitendinosus; BB = Biceps brachii.

<sup>c</sup>SEM = error estándar de la media.

 ${}^{d}*P \le 0.05, \, {}^{**P} \le 0.01, \, {}^{***P} \le 0.001; \, NS = no \, significativo; \, a, \, b, \, c, \, d = P \le 0.05.$ 

No significant difference was observed between products for overall acceptability and the flavor and taste attributes, similar to what has been found by other authors (Braghieri et al., 2009). On the contrary, significant differences were observed among bresaola for descriptors regarding color intensity, elasticity and tenderness (P < .001), chewiness (P < .01), and fattiness (P < .05).

Color intensity resulted highest in BB from GB and AC, both fed at pasture, presumably because this muscle is highly activated during grazing. This result can be associated to the lower lightness detected in the BB muscle from grazing animals (Table 2); indeed, the scores attributed to color intensity resulted negatively correlated to some colorimetric parameters, such as lightness (r = -0.743, P < .001), chroma (r = -0.743, P < .001) and hue (r = -0.643, P < .01).

Elasticity score was higher for ST, especially when obtained from GB, and resulted positively correlated to WBS (r = 0.486, P < .05). Chewiness and tenderness scores were lower in bresaola produced from AC, in line with the higher values of WBS; these results, confirmed by the significant and negative correlations linking WBS to both chewiness (r = -0.478, P < .05) and tenderness (r = -0.487, P < .05), give an indication of the lower appreciation for bresaola from adult animals.

The experimental products, compared to the commercial bresaola, proved to be quite comparable, with the unique differences due to fattiness, that was higher in CB than in all other bresaola types (P < .05) and the elasticity, which was greater inST muscles than CB (P < .001), attributable to the origin of muscle of the commercial bresaola.

Commonly appearance features, especially visible fat and marbling, play an important role in orienting consumer preference before consumption (Fortin et al., 2005). Nevertheless, the lower fatness of experimental bresaola, together with their other attributes, did not influence the overall satisfaction of the evaluators, that was not significantly different than that scored

Table 4. Sensory attribute scores of experimental ripened bresaola compared to a commercial bresaola (CB).

Tabla 4. Puntuaciones de los atributos sensoriales de la bresaola madura experimental comparada con una bresaola comercial (CB) Los resultados indican los valores medios de puntuación de la evaluación de cada una de las dos réplicas para la categoría de animales.

Animal categories <sup>a</sup>			GB			HB			AC			Sd
Bresaola (B) <sup>b</sup>	CB	SM	ST	BB	SM	ST	BB	SM	ST	BB	SEM <sup>c</sup>	В
Color intensity	5.78 <sup>bcd</sup>	5.48 <sup>bcd</sup>	5.95 <sup>abc</sup>	6.61 <sup>a</sup>	6.03 <sup>ab</sup>	5.35 <sup>cd</sup>	5.96 <sup>abc</sup>	6.01 <sup>ab</sup>	5.21 <sup>d</sup>	6.57 <sup>a</sup>	0.242	***
Brightness	2.72	3.42	3.05	3.18	2.51	2.97	3.09	3.09	2.51	2.90	0.314	NS
Acid flavor	0.69	0.61	0.51	0.40	0.68	0.60	0.53	0.77	0.66	0.62	0.130	NS
Rancid flavor	0.14	0.46	0.16	0.22	0.24	0.23	0.24	0.29	0.15	0.23	0.110	NS
Mold flavor	0.14	0.10	0.06	0.10	0.10	0.11	0.08	0.08	0.06	0.09	0.033	NS
Lactic flavor	2.77	2.87	3.11	3.49	3.15	2.76	3.08	3.00	3.17	3.21	0.298	NS
Bitter	0.48	0.51	0.53	0.50	0.51	0.74	0.45	0.67	0.42	0.44	0.141	NS
Sweet	1.79	2.27	1.89	1.92	2.03	2.02	2.13	2.30	2.04	2.10	0.276	NS
Spicy	4.10	4.47	4.47	4.43	4.79	3.62	4.73	4.09	4.42	3.86	0.362	NS
Salty	2.77	2.65	2.75	3.21	3.37	3.05	2.80	3.24	2.69	2.87	0.276	NS
Fattiness	2.77 <sup>a</sup>	1.54 <sup>b</sup>	1.35 <sup>b</sup>	1.72 <sup>b</sup>	2.01 <sup>ab</sup>	1.56 <sup>b</sup>	1.72 <sup>b</sup>	1.77 <sup>b</sup>	1.61 <sup>b</sup>	1.70 <sup>b</sup>	0.268	*
Elasticity	1.02 <sup>d</sup>	1.61 <sup>cd</sup>	3.50 <sup>a</sup>	2.14 <sup>bcd</sup>	1.96 <sup>bcd</sup>	2.76 <sup>ab</sup>	1.22 <sup>d</sup>	1.41 <sup>cd</sup>	2.37 <sup>bc</sup>	1.15 <sup>d</sup>	0.380	***
Fibrosity	2.70	2.76	3.36	3.13	3.06	3.41	2.54	2.64	2.81	2.44	0.336	NS
Chewiness	3.80 <sup>abc</sup>	3.07 <sup>bc</sup>	3.53 <sup>abc</sup>	3.91 <sup>ab</sup>	3.34 <sup>abc</sup>	4.23 <sup>a</sup>	3.22 <sup>bc</sup>	2.77 <sup>cd</sup>	2.88 <sup>cd</sup>	2.09 <sup>d</sup>	0.348	**
Tenderness	3.23 <sup>abc</sup>	2.93 <sup>bc</sup>	3.68 <sup>ab</sup>	3.92 <sup>a</sup>	3.56 <sup>ab</sup>	4.00 <sup>a</sup>	2.47 <sup>c</sup>	2.55 <sup>c</sup>	2.90 <sup>bc</sup>	2.04 <sup>c</sup>	0.336	***
Overall acceptability	4.51	4.60	4.52	4.42	5.10	3.97	4.54	4.56	4.40	4.80	0.395	NS

The results indicate mean scoring values of assessment of each of the two replicates for animal category.

<sup>a</sup>Animal categories: GB = grazing young bull; HB = housed young bull; AC = adult cow.

<sup>b</sup>Bresaola: CB = commercial bresaola; SM = Semimembranosus; ST = Semitendinosus; BB = Biceps brachii.

<sup>c</sup>SEM = standard error of the means.

<sup>d</sup>Significance = \*P  $\leq$  0.05, \*\*P  $\leq$  0.01, \*\*\*P  $\leq$  0.001; NS = not significant; a, b, c, d = P  $\leq$  0.05.

<sup>a</sup>Categorías de animales: GB = toro joven que pasta; HB = toro joven confinado; AC = vaca adulta.

<sup>b</sup>Bresaola: CB = bresaola comercial; SM = Semimembranosus; ST = Semitendinosus; BB = Biceps brachii.

<sup>c</sup>SEM = error estándar de la media.

<sup>d</sup>Significación = \*P  $\leq$  0.05, \*\*P  $\leq$  0.01, \*\*\*P  $\leq$  0.001; NS = no significativo; a, b, c, d = P  $\leq$  0.05.

for the commercial bresaola, as found in other tests where the experimental bresaola was compared with the commercial one (Braghieri et al., 2009).

# 3.4. Multivariate analysis

The plot generated by PCA, implemented to assess the ability of selected physicochemical variables, VOC and sensory attributes to discriminate among the bresaola manufactured with meat from different animal categories and muscles, is shown in Figure 1.

The first two principal components accounted for 75.17% of the total variance. The length of each vector measures the contribution of each variable to the main components. The first principal component, explaining 45.22% of the total variance, was able to discriminate the bresaola from HB to those from GB on the basis of the main contributions of aterpinolen, moisture, undecane and, to a lesser extent, fat, ash and tenderness. Instead, the second principal component was responsible for the separation of bresaola from AC category, especially due to the positive contribution of PI, phenol, acetic acid and protein. The differentiation of bresaola on the basis of the muscle of origin was more evident in the products from AC, among which BB was characterized by positive PI and protein, whereas SM by VOC, such as acetic acid and phenol. On the whole, the contribution to the qualitative traits of bresaola of animal category, which is associated with specific age and feeding system, resulted more relevant than that of muscle type, although the effect of this latter was quite evident among the products obtained from AC.

# 4. Conclusion

The bresaola showed differences in the physicochemical and sensory characteristics due to the animal category and the muscular cut from which the processed meat was obtained. In particular, the use of meat cuts from adult cows fed at pasture positively influenced the chemical composition of the products, which were more rich in fat and VOC than those from young bulls. Moreover, the bresaola produced by Semitendinosus muscles was fatter and characterized by higher color parameters and WBS force. Despite these differences, the sensory acceptance scored for all the manufactured products resulted comparable to that recorded for a commercial bresaola. Although further investigations are required to better characterize the product in relation to the origin area, these first results suggest that Cinisara bresaola could be produced from muscular cuts of different animal categories, loading products characterized by different physicochemical traits but similar sensory properties, well appreciated by consumers.

Accordingly, bresaola production could contribute to the process of valorization of meat of indigenous cattle breeds in the market, taking in consideration also the positive effects of a feeding regimen based on fresh forage of natural pasture until slaughter, in terms of reduction of meat production costs and, especially, improvement of animal welfare and nutritional properties of meat.

#### **Disclosure statement**

No potential conflict of interest was reported by the authors.

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Figure 1. PCA analysis based on the values physicochemical and sensory traits detected on ripened bresaola from different animal categories (GB = grazing young bull; HB = housed young bull; AC = adult cow.) and muscle (SM = Semimembranosus; ST = Semitendinosus; BB = Biceps brachii). The length of each vector is proportional to its contribution to the main components.

Figura 1. Análisis de PCA basado en los valores fisicoquímicos y sensoriales detectados en bresaola madura elaborada a partir de diferentes categorías de animales (GB = toro joven que pasta; HB = toro joven confinado; AC = vaca adulta.) y músculo (SM = Semimembranosus; ST = Semitendinosus; BB = Biceps brachii). La longitud de cada vector es proporcional a su contribución a los principales componentes.

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