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Effect of muscle type and animal category on fatty acid composition of bresaola made from meat of Cinisara cattle: preliminary investigation

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ABSTRACT

The bresaola could represent an alternative commercial opportunity for autochthonous dairy cattle farms. Therefore, a study was carried out to investigate the fatty acid (FA) composition of bresaola made using *Semimembranosus* (SMm), *Semitendinosus* (STm), and *Biceps brachii* (BBm) muscles from grazing young bulls (GB), housed young bulls (HB) or grazing adult cows (AC) of Cinisara breed. Animal category, fat content, feeding system, and type of muscle influenced the FA composition of bresaola. Fatter products, made from AC and HB meat, or with STm and BBm muscles showed lower polyunsaturated/saturated FA ratio. The bresaola from young bulls fed pasture-based diet showed an increased polyunsaturated FA content, a reduction of n-6/n-3 ratio, and improved health indexes. However, the rumenic acid, isomer of conjugated linoleic acid, did not show differences related to the use of pasture. Bresaola made from lean meat cuts of grazing animals seems to show the best health-related FA profile.

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raza Cinisara; carne; bresaola; grasa; ácidos grasos

Efecto del tipo de músculo y la categoría del animal en la composición de los ácidos grasos de la bresaola hecha de carne de ganado Cinisara: investigación preliminar

RESUMEN

La bresaola podría representar una oportunidad comercial alternativa para las granjas autóctonas de ganado lechero. Por este motivo, el presente estudio se propuso investigar la composición de ácidos grasos (FA) de la bresaola elaborada con músculos *Semimembranosus* (SMm), *Semitendinosus* (STm) y *Biceps brachii* (BBm) de toros jóvenes de pastoreo (GB), toros jóvenes alojados (HB) y vacas adultas de pastoreo (AC) de la raza Cinisara. La categoría del animal, el contenido de grasa, el sistema de alimentación y el tipo de músculo influyeron en la composición de los FA de la bresaola. Se constató que los productos más grasos, elaborados con carne AC y HB, o con músculos STm y BBm, contienen menor proporción de FA poliinsaturados/saturados. La bresaola de toros jóvenes alimentados con una dieta basada en pastos resultó tener mayor contenido de FA poliinsaturados, menor proporción de n-6/n-3 y mejores índices de salud. Sin embargo, el ácido ruménico, isómero del ácido linoleico conjugado, no mostró diferencias relacionadas con el uso de pastos. La bresaola hecha de cortes de carne magra de animales de pastoreo parece exhibir el mejor perfil de FA en relación con la salud.

1. Introduction

Cinisara cow is a Sicilian breed traditionally reared in the western part of Sicily, adopting a feeding system based on the prevalent exploitation of natural resources. It is considered a dairy breed, and its milk is mainly used to produce the Caciocavallo Palermitano cheese (Alabiso et al., 2005; Bonanno et al., 2013; Giosuè et al., 2005; Di Gregorio et al., 2017). Its fresh meat production is strongly penalized by competition with those of specialized breeds; otherwise, processed products, such as salami (Gaglio et al., 2016) and bresaola (Liotta et al., 2015), could increase the farms' profitability, diversifying the offer in the market.

Bresaola is a typical Italian product based on dried meat, and it is obtained by processing the *Semimembranosus*, *Semitendinosus*, *Quadriceps femoris* and *Brachial biceps* muscles of different animal species, in particular equine and cattle (Alabiso et al., 2019; Braghieri et al., 2009; Marino

et al., 2015; Paleari et al., 2003). It is similar to other products such as Turkish pastirma (Kaban, 2009) and Kazakh seasoned beef (Sha et al., 2017).

Different factors can influence the meat quality attributes, and especially the fatty acid (FA) profile has a relevant importance in nutrition and human health. Indeed, several benefits have been shown by consuming products with high degree of unsaturation. In particular, a high concentration of n-3 FA determines a reduction in the incidence of cancer, cardiovascular disease, hypertension, and arthritis and also an improvement in visual acuity (Nestel et al., 2002; Ragni et al., 2014). The FA composition influences also the meat flavor, and the grass-fed beef has naturally high levels of long-chain FA, producing a 'grass fed' taste in which other components of grass are also involved (Wood et al., 2004).

The variations of FA composition in fresh meat depend on the animal's feeding system, age, and gender (Lengyela

et al., 2003; Malau-Aduli et al., 1998; Nuernberg et al., 2005; De Smet et al., 2004; Steen & Porter, 2003). In particular, grazing improves the organoleptic and health quality of both fresh and processed meat products, influencing positively the FA profile, through the increase of beneficial FA, such as n-3 FA and conjugated linoleic acids (CLA) (Nuernberg et al., 2005). Otherwise, the products obtained by animals fed with concentrate-based diets present generally high levels of n-6 FA, mainly linoleic acid (LA, 18:2 n-6) (Wood et al., 2008).

The increase in fat content proportionally reduces the incidence of phospholipids, high in linoleic acid (LA), of the cell membrane. Therefore, PUFA decrease compared to saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) (Wood et al., 2008). Thus, the amount of phospholipids and, as a consequence, PUFA may be proportionally greater in low-fat fresh or processed meat.

The bresaola is made by processing whole muscles, and during ripening the microbial contribution is minimal and lipolysis is almost exclusively attributable to endogenous enzymes (Toldrá, 1998). This affects the FA composition, reducing the PUFA and MUFA and increasing the SFA, as observed in both salami and bresaola produced from Kazakh beef (Sha et al., 2017) and raw ham (Motilva et al., 1993).

The present study investigated the FA profile of bresaola made processing *Semimembranosus*, *Semitendinosus* and *Biceps brachii* muscles from grazing or housed young bulls and grazing adult cows of Cinisara breed. This survey was oriented to provide additional qualitative information to those reported by Alabiso et al. (2019) and Maniaci et al. (2020) on the physicochemical and sensory properties of bresaola, with the aim to better characterize this product.

2. Materials and methods

2.1. Meat and bresaola

The livestock system (use or non-use of pasture) and the age (adult/young) were the criteria used to select the carcasses of Cinisara breed. Due to the low consistency of Cinisara breed, only six carcasses corresponding to the purpose of the research could be contemporarily included in the study; they were obtained from the following animals:

- (1) two grazing young bulls (GB) (18 months old), which were fed pasture-based diets (from 6 months) supplemented with hay and concentrate in the final phase (16–18 months);
- (2) two housed young bulls (HB) (18 months old), which were fed pasture-based diets (from 6 months) and only with hay and concentrate in the final phase (16–18 months);
- (3) two adult cows (AC) (10 years old), which were fed pasture-based diets (from 6 months) supplemented with hay and concentrate from the beginning of the reproductive career (24 months).

The animals were slaughtered at an EU-licensed abattoir, according to the standard handling procedures, in respecting EU regulations (EC Regulation No 1099/2009) on the protection of animals at the time of slaughter. The carcasses were stored in a cooling room at 4–8°C for a 7-day aging period, and then were dissected (day 0) to remove *Semimembranosus* (SMm), *Semitendinosus* (STm), and *Biceps brachii* (BBm) 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. The bresaola was processed as follows: on day 0, the meat cuts were submitted to the first dry salting, rubbing by hand their surface with a mixture 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 singular cuts were wrapped with handkerchiefs of glued natural casing, tied with a rope and hung in 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°C) and the RH was increased (+2%); on day 32, the bresaola were 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).

2.2. Sampling

For each animal category and muscle type, three specimens of 2.5 cm of bresaola, at the end of ripening (day 60), were cut transversely to the direction of the muscle fibers. All samples were placed in sterile vacuum containers, immediately refrigerated, and transported at 8°C to laboratory to be homogenize by stomacher (LAB Blender 400, Seward Medical, London, United Kingdom) for 2 minutes at maximum speed; then samples were frozen at –20°C and freeze-dried for successive analysis (SCANVAC CoolSafe 55–9, Labogene Aps, Lyngby Denmark).

Moisture and total fat levels were determined in triplicate on freeze-dried samples of bresaola according to the AOAC methods (AOAC International, 2012).

2.3. Fatty acids composition

Fatty acids (FA) were extracted according to the method developed by O'Fallon et al. (2007). C23:0 (Sigma-Aldrich) was used as internal standard (0.5 mg/g freeze-dried sample) for the FA quantification. One microliter of each sample was injected by autosampler into an HP 6890 gas chromatography system equipped with a flame-ionization detector (Agilent Technologies Inc., Santa Clara, CA). Fatty acid methyl esters from all samples were separated using a 100-m length, 0.25-mm i.d., 0.25-μm capillary column (CP-Sil 88; Chrompack, Middelburg, the Netherlands). The injector temperature was kept at 255°C and the detector temperature was kept at 250°C, with an H₂ flow of 40 mL/min, air flow of 400 mL/min, and a constant He flow of 45 mL/min. The initial oven temperature was held at 70°C for 1 min, increased at 5°C/min to 100°C, held for 2 min, increased at 10°C/min to 175°C, held for 40 min, and then finally increased at 5°C/min to a final temperature of 225°C and held for 45 min. Helium, with a head pressure of 158.6 kPa and a flow rate of 0.7 mL/min (linear velocity of 14 cm/s), was used as the carrier gas (2).

Fatty acid methyl ester hexane mix solution (Nu-Chek Prep Inc., Elysian, MN) was used to identify each FA. The

identification of CLA isomers was performed using a commercial mixture of *cis*- and *trans*-9,11- and 10,12-ocadecadienoic acid methyl esters (Sigma-Aldrich, Milano, Italy). Each individual FA was expressed as g/100 g total detected FA.

The thrombogenic index (TI) was calculated according to Ulbricht and Southgate (1991) as follows: $TI = (C14:0 + C16:0 + C18:0) / ((0.5 \times \Sigma MUFA) + (0.5 \times \Sigma PUFA \text{ n-6}) + (3 \times \Sigma PUFA \text{ n-3}) + ((n-3)/(n-6)))$. The health-promoting index (HPI) was calculated as suggested by Chen et al. (2004): $\text{total unsaturated FA} / (C12:0 + (4 \times C14:0) + C16:0)$.

2.4. Statistical analysis

Data were statistically processed using the SAS 9.2 software (SAS Institute, 2010). Fatty acid composition were analyzed 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: SMm, STm and BBm), 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.

The principal components analysis (PCA), performed using the PRINCOMP SAS procedure, was based on FA composition in order to assess the specific contribution of individual FA 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 Eigen values above 1.00 were retained.

3. Results and discussion

3.1. Fatty acid composition

The total FA content (%DM) (Table 1) was on average higher in AC (8.90%) than in HB (4.37%) and GB (3.13%) ($P < .001$), according to the fat level (Table 1). Among muscles, the total FA, as well as the fat content, were lower in bresaola made with SMm than with STm, in line with what was found in the buffalo bresaola (Diaferia et al., 2006), and this difference was more evident between SMm and STm bresaola from AC. The SFA (Table 1) were on average higher in AC and HB bresaola than in GB ones ($P < .001$), especially when made with SMm. In particular, the myristic (C14:0) and palmitic (C16:0) acids were on average higher in AC than in HB and GB ($P < .001$), while the stearic acid (C18:0) was lower in AC respect to GB ($P < .001$) (Table 2). In muscles, myristic acid was lower in SMm, although in a lesser extent for bresaola from GB; moreover, palmitic acid was higher in STm than BBm and SMm ($P < .05$), while stearic acid was higher in BBm than in SMm and STm ($P < .001$) (Table 2).

Among SFA, the myristic acid (C14:0) would be the most undesirable, presenting an important hypercholesterolemic effect for human health unlike palmitic acid (French et al., 2003) and stearic acid, which is transformed in oleic acid (OA, C18:1 n-9) in the body tissues, both beneficial for their hypocholesterolemic action (Sinclair, 1993). On the basis of these results, the bresaola made by GB would show a better SFA profile than those made from HB and AC.

The MUFA (Table 1) were on average higher in AC than in GB bresaola, with intermediate values in HB products ($P < .001$). The most representative FA for all animal categories was OA (Table 3), which followed the same decreasing trend of MUFA from AC to HB and GB ($P < .001$); OA was also higher in STm than in BBm and SMm ($P < .001$).

Specifically, the MUFA content in muscle neutral lipids and total fat of cattle were found to be affected by age, with

Table 1. Effects of animal category and muscle on the level of bresaola fat and its fatty acids profile (g/100 g FA) and health indexes.

Tabla 1. Efectos de la categoría de los animales y el músculo en el nivel de grasa de la bresaola y su perfil de ácidos grasos (g/100 g FA) e índices de salud.

Animal categories (A) ^a	GB			HB			AC			SEM ^h	Significance ⁱ		
Muscles (M) ^b	SMm	STm	BBm	SMm	STm	BBm	SMm	STm	BBm		A	M	A*M
Moisture, %	56.3 ^{ab}	54.5 ^{ab}	55.5 ^{ab}	51.8 ^b	54.4 ^{ab}	54.7 ^{ab}	52.1 ^b	58.0 ^a	56.9 ^{ab}	0.871	NS	*	*
Fat, %	1.41 ^d	1.71 ^d	1.58 ^d	2.10 ^{cd}	2.31 ^{cd}	2.20 ^{cd}	2.72 ^c	5.70 ^a	4.37 ^b	0.165	**	***	***
Total FA, % DM	2.83 ^c	3.24 ^c	3.32 ^{ce}	3.89 ^{ce}	4.54 ^{cd}	4.67 ^{de}	6.10 ^d	11.10 ^a	9.49 ^b	0.566	***	***	***
SFA^c	39.32 ^c	40.42 ^c	45.46 ^{ab}	44.00 ^{bd}	46.37 ^{ab}	45.35 ^{ab}	46.14 ^{ab}	41.85 ^{cd}	47.61 ^a	0.836	***	***	**
MUFA^d	23.84	27.44	23.72	30.28	34.95	31.72	38.12	49.41	39.99	1.188	***	***	NS
PUFA^e	36.93	31.90	30.58	25.29	17.98	21.88	14.84	6.65	10.88	1.399	***	***	NS
MUFA^e/SFA^c	0.61	0.66	0.52	0.68	0.75	0.70	0.83	1.18	0.84	0.036	***	***	NS
PUFA^e/SFA^c	0.94 ^c	0.79 ^c	0.67 ^c	0.56 ^{ce}	0.36 ^{df}	0.46 ^{de}	0.32 ^f	0.16 ^a	0.23 ^b	0.105	***	***	***
n-6	30.21	26.40	25.89	21.76	15.57	19.28	12.00	5.56	8.99	1.161	***	***	NS
n-3	6.51 ^a	5.45 ^b	4.68 ^c	3.57 ^d	2.54 ^{efg}	2.90 ^{def}	3.12 ^{de}	1.70 ^g	2.48 ^f	0.214	***	***	*
n-6/n-3	4.64 ^c	4.86 ^c	5.53 ^c	6.11 ^b	6.12 ^b	6.66 ^a	3.84 ^d	3.27 ^e	3.62 ^d	0.087	***	***	***
TI^f	0.69	0.78	0.89	0.95	1.15	1.11	1.11	1.12	1.29	0.055	***	**	NS
HPI^g	3.16	2.91	2.98	2.31	1.81	1.99	1.83	1.71	1.65	0.146	***	**	NS

The results indicated mean values of three measurements performed on each of the two replicates for animal category.

^aAnimal categories: GB = grazing young bull; HB = housed young bull; AC = adult cow.

^bMuscles: SMm = *Semimembranosus*; STm = *Semitendinosus*; BBm = *Biceps brachii*.

^cSFA = saturated fatty acids; ^dMUFA = monounsaturated fatty acids; ^ePUFA = polyunsaturated fatty acids; ^fTI = thrombogenic index; ^gHPI = health-promoting index.

^hSEM = standard error of the means. ⁱ* = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$; NS = not significant; a, b, c, d, e, f, g = $P \leq 0.05$.

Los resultados indican los valores medios de tres mediciones realizadas en cada una de las dos réplicas para la categoría de animales.

^aCategorías de animales: GB = toro joven de pastoreo; HB = toro joven alojado; AC = vaca adulta.

^bMúsculos: SMm = *Semimembranoso*; STm = *Semitendinoso*; BBm = *Biceps braquial*.

^cSFA = ácidos grasos saturados; ^dMUFA = ácidos grasos monoinsaturados; ^ePUFA = ácidos grasos poliinsaturados; ^fTI = índice trombogénico; ^gHPI = índice de promoción de la salud.

^hSEM = error estándar de la media. ⁱ* = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$; NS = no significativo; a, b, c, d, e, f, g = $P \leq 0.05$.

Table 2. Effects of animal category and muscle on saturated fatty acids (g/100 g FA) of bresaola fat.**Tabla 2.** Efectos de la categoría de animal y del músculo en los ácidos grasos saturados (g/100 g FA) de la grasa de bresaola.

Animal categories (A) ^a	GB			HB			AC			SEM ^d	Significance ^e		
	SMm	STm	BBm	SMm	STm	BBm	SMm	STm	BBm		A	M	A*M
Others SFA ^c	6.85	3.51	8.09	6.59	6.00	4.70	4.84	1.88	2.71	1.532	**	NS	NS
C14	0.64 ^b	0.99 ^b	0.90 ^b	1.58 ^b	2.14 ^a	2.05 ^{ac}	1.79 ^{bc}	2.46 ^a	2.05 ^{ac}	0.123	***	***	***
C15	0.82	0.99	1.04	0.74	0.75	0.91	0.87	0.99	1.02	0.056	*	**	NS
C16	14.30	16.61	14.68	18.22	21.00	19.28	22.41	23.23	23.22	0.639	***	*	NS
C17	1.48 ^{bd}	1.61 ^a	1.52 ^b	1.33 ^c	1.29 ^c	1.41 ^{cd}	1.51 ^{bd}	1.49 ^{bd}	1.71 ^a	0.044	***	*	*
C18	14.99 ^{bd}	16.57 ^b	19.13 ^a	15.25 ^{bd}	14.94 ^{bd}	16.78 ^b	14.40 ^d	11.48 ^c	16.62 ^b	0.579	***	***	*
C20	0.16	0.12	0.13	0.09	0.08	0.13	0.11	0.11	0.09	0.098	ns	ns	ns
C22	0.13	0.11	0.08	0.21	0.14	0.17	0.11	0.09	0.10	0.011	***	*	NS

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

^aAnimal categories: GB = grazing young bull; HB = housed young bull; AC = adult cow.

^bMuscles: SMm = *Semimembranosus*; STm = *Semitendinosus*; BBm = *Biceps brachii*.

^cSFA = saturated fatty acids.

^dSEM = standard error of the means. ^e* = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$; NS = not significant; a, b, c, d = $P \leq 0.05$.

Los resultados indican los valores medios de tres mediciones realizadas en cada una de las dos réplicas para la categoría de animales.

^aCategoría de animales: GB = toro joven de pastoreo; HB = toro joven alojado; AC = vaca adulta.

^bMúsculos: SMm = *Semimembranoso*; STm = *Semitendinoso*; BBm = *Bíceps braquial*.

^cSFA = ácidos grasos saturados.

^dSEM = error estándar de la media. ^e* = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$; NS = no significativo; a, b, c, d = $P \leq 0.05$.

Table 3. Effects of animal category and muscle on unsaturated fatty acids (g/100 g FA) of bresaola fat.**Tabla 3.** Efectos de la categoría de animal y del músculo en los ácidos grasos insaturados (g/100 g FA) de la grasa de bresaola.

Animal categories (A) ^a	GB			HB			AC			SEM ^c	Significance ^d		
	SMm	STm	BBm	SMm	STm	BBm	SMm	STm	BBm		A	M	A*M
C14:1	0.19	0.31	0.20	0.34	0.51	0.38	0.38	0.94	0.40	0.029	***	***	NS
C15:1	0.61	0.45	0.40	0.39	0.23	0.28	0.24	0.09	0.14	0.030	***	***	NS
C16:1	1.28 ^e	1.93 ^d	1.38 ^e	2.28 ^{cd}	2.90 ^{bc}	2.32 ^c	3.01 ^b	5.58 ^a	3.18 ^b	0.163	***	***	***
C17:1	1.29 ^a	1.07 ^b	0.97 ^{bc}	1.24 ^a	0.99 ^{bc}	1.05 ^{bc}	0.90 ^c	1.04 ^{bc}	0.88 ^c	0.051	**	**	*
C18:1 c9, OA	15.98	19.18	16.20	21.94	26.00	23.16	29.52	36.62	30.63	0.919	***	***	NS
Others C18:1	4.08	4.16	4.38	4.01	4.19	4.43	3.94	4.97	4.62	0.191	NS	*	NS
C18:2	0.00	0.16 ^c	0.29 ^{bc}	0.25 ^{bc}	0.37 ^{bc}	0.55 ^b	0.49 ^b	1.07 ^a	0.78 ^a	0.066	***	***	*
C18:2 n-6, LA	20.84	18.60	18.87	14.26	10.19	12.88	7.12	3.38	5.50	0.761	***	***	NS
C18:2 c9t11 RA, CLA	0.30	0.27	0.24	0.23	0.21	0.22	0.23	0.55	0.30	0.027	NS	*	NS
C18:2 CLA isomers	0.12	0.17	0.10	0.12	0.13	0.13	0.09	0.11	0.08	0.16	NS	NS	NS
C18:3 n-3 ALA	3.08 ^a	2.58 ^b	2.48 ^b	1.20 ^{cd}	0.94 ^e	1.09 ^{de}	1.31 ^c	0.97 ^e	1.21 ^{cd}	0.057	***	***	**
C18:3 n-6	0.12 ^c	0.16 ^{ab}	0.17 ^a	0.12 ^c	0.11 ^c	0.13 ^c	0.12 ^c	0.14 ^{bc}	0.13 ^c	0.007	***	***	*
C20:1 c9	0.41 ^a	0.32 ^b	0.18 ^c	0.09 ^d	0.06 ^e	0.07 ^{de}	0.07 ^{de}	0.04 ^e	0.06 ^e	0.009	***	***	***
C20:2 n-6	0.26	0.22	0.20	0.19	0.13	0.15	0.11	0.06	0.08	0.012	***	***	NS
C20:3 n-6	1.29	1.00	0.88	1.02	0.68	0.80	0.91	0.36	0.61	0.072	***	***	NS
C20:4 n-6, AA	6.65	5.58	4.95	5.46	3.87	4.67	3.17	1.18	2.16	0.344	***	***	NS
C20:5 n-3, EPA	1.08 ^a	0.93 ^a	0.72 ^b	0.75 ^b	0.52 ^{ce}	0.60 ^{bc}	0.62 ^{bc}	0.23 ^d	0.39 ^{de}	0.055	***	***	*
C22:2 n-6	0.26	0.24	0.24	0.17	0.12	0.14	0.07	0.04	0.06	0.007	***	***	NS
C22:4 n-6	0.44	0.36	0.30	0.44	0.29	0.34	0.23	0.09	0.16	0.027	***	***	NS
C22:5 n-3, DPA	2.35	1.94	1.49	1.61	1.08	1.22	1.12	0.46	0.76	0.117	***	***	NS

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

^aAnimal categories: GB = grazing young bull; HB = housed young bull; AC = adult cow.

^bMuscles: SMm = *Semimembranosus*; STm = *Semitendinosus*; BBm = *Biceps brachii*.

^cSEM = standard error of the means. ^d* = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$; NS = not significant; a, b, c, d, e = $P \leq 0.05$.

OA = oleic acid; LA = linoleic acid; ALA = α -linolenic acid; RA = rumenic acid; CLA = conjugated linoleic acid; AA = arachidonic acid; EPA = eicosapentaenoic acid; DPA = docosapentaenoic acid.

Los resultados indican los valores medios de tres mediciones realizadas en cada una de las dos réplicas para la categoría de animales.

^aCategoría de animales: GB = toro joven de pastoreo; HB = toro joven alojado; AC = vaca adulta.

^bMúsculos: SMm = *Semimembranoso*; STm = *Semitendinoso*; BBm = *Bíceps braquial*.

^cSEM = error estándar de la media. ^d* = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$; NS = no significativo; a, b, c, d, e = $P \leq 0.05$.

OA = ácido oleico; LA = ácido linoleico; ALA = ácido linolénico; RA = ácido ruménico; CLA = ácido linoleico conjugado; AA = ácido araquidónico; EPA = ácido eicosapentaenoico; DPA = ácido docosapentaenoico.

a consequent increase of the MUFA/SFA ratio with age (Wood et al., 2008). Therefore, the higher OA content recorded in AC, which contributed to an increase in MUFA and in the MUFA/SFA ratio, would be related to the greater age of AC than HB and GB animals (Wood et al., 2008).

The levels of PUFA (Table 1) were on average higher in GB than in HB and AC ($P < .001$), and comparable with the range found on *Semimembranosus* muscle of Belgian Blue, Limousin and Irish beef (33.8, 24.8 and 9.93 g/100 g FA, respectively)

(Raes et al., 2003). For all animal categories, LA was the most representative among PUFA, and was on average higher in GB compared to HB and AC, and in SMm than STm and BBm ($P < .001$) (Table 3). In accordance with Wood et al. (2008), the LA content seems to be negatively related to the trend of bresaola fat, which decreased from AC to HB and GB bresaola. Besides LA, also the arachidonic acid (AA, C20:4 n-6), which derived from LA through the action of $\Delta 5$ and $\Delta 6$ desaturase enzymes and elongase, was on

average higher in GB compared to HB and AC ($P < .001$), similarly to its precursor. Moreover, α -linolenic (ALA, C18:3 n-3), eicosapentaenoic (EPA, C20:5 n-3) and docosapentaenoic (DPA, C22:5 n-3) acids were found in important concentrations, and on average were higher in GB than HB and AC ($P < .001$). These results can be referred to the feeding system, since grazing is recognized to have a positive effect in increasing the PUFA contents of beef, and in general in products obtained by ruminants (French et al., 2000). Indeed, GB animals grazed for longer time than HB subjects. Although AC used grazing until slaughter, the bresaola of this animal category showed lower concentrations of ALA and PUFA than GB. The same differences were found between HB and GB which differed in feeding pattern. These results could be linked to the greater age of AC as found in fallow deers of different ages, in which the older ones recorded higher concentrations of fat in the muscles and lower PUFA levels than the younger ones, in the same feeding conditions (Volpelli et al., 2003). Ruminants preferentially deposit PUFA in the phospholipids of cell membranes (De Smet et al., 2004), and phospholipids amount may be higher in bresaola with low fat. Therefore, in leaner animals, as GB, the higher incidence of phospholipids determines higher LA and PUFA contents, as also found in cattle of different breeds, in which LA reaches values above 20% (Raes et al., 2003). Conversely, the increase of intramuscular fat, consisting mainly of MUFA and SFA, reduces the proportion of PUFA (Sharma et al., 1987).

Based on these results, the bresaola produced with meat from grazing animals, due to its higher PUFA content, should be highly exposed to lipid oxidation; however, in this case, it can be assumed that natural antioxidants transmitted from pasture, such as vitamins from group A, C and especially E or phytochemicals such as carotenoids and flavonoids, could protect the PUFA against lipid oxidation (Descalzo et al., 2000; Gatellier et al., 2004).

The content of CLA isomers, in particular rumenic acid (RA, C18:2 c9t11), which is known for its beneficial anticancer and anti-atherogenic effects on human health (Parodi & Wiley-Blackwell, 2009), are generally higher in ruminant products from animals fed fresh forage on pasture compared to those from confined animals fed dry diets (Hur et al., 2017). The RA content in bresaola was found in the range of 0.2 and 1 g/100 g of total FA, as reported for lamb and beef (Raes et al., 2004), and in this study was on average higher (not significantly) in products made from animals (GB and AC) fed with pasture. However, the use of pasture, and therefore of a diet high in PUFA, did not lead to an increase in CLA, as also observed in other investigations (Raes et al., 2004; Steen & Porter, 2003).

In general, a ratio of PUFA/SFA above about 0.45 and a ratio of n-6/n-3 below 4.0 are required in a diet combating various "lifestyle diseases," such as coronary heart disease and cancers (Simopoulos, 2002). On the basis of the FAO/WHO report, it is possible to deduce an optimal n6/n3 ratio of 4.5–5 (FAO/WHO, 2010). The lower fattening status of GB compared to HB and the use of pasture would have determined a decrease in the n-6/n-3 ratio of muscle fat, as consequence of the increased concentration of n-3 FA, principally ALA (Table 1). This improvement of the acidic profile of the fat for human health was also observed in products from sheep and cattle grazing

with different duration (Bonanno et al., 2016; Noci et al., 2005; Nuernberg et al., 2005; Raes et al., 2004). However, it can be noticed that, despite the lower PUFA content, AC showed a favorable lower n6/n3 ratio (Table 1), especially due to the lower LA level.

The higher percentage of PUFA in GB resulted in a higher PUFA/SFA ratio compared to both HB and AC. This ratio in GB and HB was on average higher than 0.45 (Table 1), which is the minimum reference value for food safety (DOH, 1994; Simopoulos, 2002). A favorable percentage content of PUFA and a beneficial PUFA/SFA ratio was also found in young indigenous Podolica breed bulls (Cifuni et al., 2004). Only in AC, the ratio was lower than 0.45, due to lower PUFA level, but it was analogous to the value found by other authors on grazing animals, equal to 0.20 ± 0.013 (Realini et al., 2004).

The thrombogenic index (TI) and health-promoting index (HPI) are indicators of the health value of dietary fat. A product with high HPI or low TI value is assumed to be more beneficial to human health (Chen et al., 2004; Ulbricht & Southgate, 1991). Both health indices were more favorable in GB than in HB and AC (Table 1); indeed, the GB bresaola showed lower TI and higher HPI, mainly due to the lower content of C14:0 and C16:0 and the greater PUFA level. The value of these indices for HB and AC bresaola were similar to those of bresaola from buffalos reared in confinement (Calabrò et al., 2014).

Muscle type is important in terms of phospholipids content and composition of the meat. Red and dark fiber oxidative muscles contain more phospholipids than white fiber glycolytic muscles (De Smet et al., 2004). On the other hand, MUFA are dominant in the phospholipids' structure of white muscles and PUFA are dominant in the phospholipids' structure of dark muscles (Habeanu et al., 2014). PUFA in bresaola were higher in the SMm, being a dark muscle and less fat than STm and BBm. Consequently, PUFA/SFA ratio, as well as TI and HPI had the same trend (De Smet et al., 2004).

3.2. Multivariate analysis

The plot generated by PCA is shown in Figure 1.

The first two principal components accounted for 88.40% of the total variance. The length of each vector measures the contribution of each variable to the main components. The first principal component, explaining 76.40% of the total variance, was able to discriminate the bresaola from AC to those from GB and HB on the basis of the main contributions of MUFA, OA, PUFA, n-6 FA, and LA. Instead, the second principal component, explaining 12.00% of the total variance, was responsible for the separation of bresaola on the basis of muscle origin, especially due to the contribution of C18:0 and AA. The generated plot is in line with the comparisons among bresaola for their FA profile; indeed, the main contributions in PCA were those resulted significantly different for the effects of animal category and muscle. On the whole, the animal category (associated to specific age and feeding system) contributed more to the qualitative traits of bresaola than the muscle type. This result was more evident for AC, although the effect of muscles was quite observable among the products obtained from GB, and to a lesser extent for AC and HB.

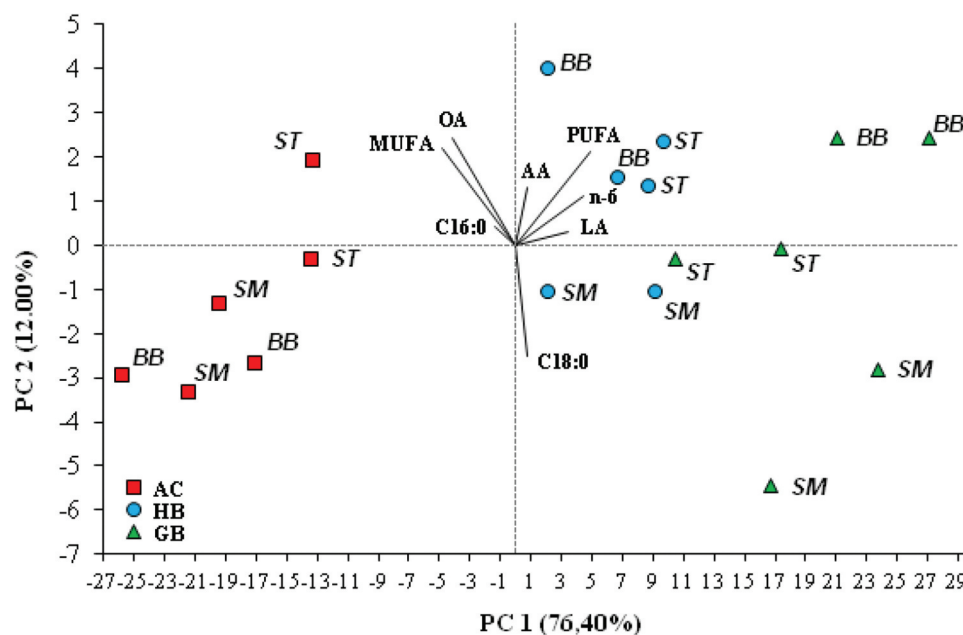


Figure 1. Score plot and loading plot from principal component analysis (PCA) of ripened bresaola. Abbreviations: Animal categories: GB = grazing young bull; HB = housed young bull; AC = adult cow. Muscles: SM = *Semimembranosus*; ST = *Semitendinosus*; BB = *Biceps brachii*; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; OA = oleic acid; LA = linoleic acid; AA = arachidonic acid.

Figura 1. Gráfico de puntuación y gráfico de carga derivados del análisis de componentes principales (PCA) de la bresaola madura. Abreviaturas: Categorías de animales: GB = toro joven de pastoreo; HB = toro joven alojado; AC = vaca adulta. Músculos: SM = *Semimembranoso*; ST = *Semitendinoso*; BB = *Biceps braquial*; MUFA = ácidos grasos monoinsaturados; PUFA = ácidos grasos poliinsaturados; OA = ácido oleico; LA = ácido linoleico; AA = ácido araquidónico.

4. Conclusions

The bresaola showed differences in the acidic composition due to animal category and muscle cut. The bresaola made with muscles from adult cows showed lower PUFA contents than those from young bulls, due to the higher fat and independently of the feeding regime. Considering the young bulls categories, a favorable effect of pasture up to slaughter emerged on the FA profile of bresaola, which showed improvements in terms of PUFA contents, PUFA/SFA ratio, and also for the fractions n-6 and n-3 and their ratio. Therefore, these results suggest that Cinisara bresaola made from lean meat cuts of grazing cattle seems to show the best health-related fatty acids profile, with a major content of RA, ALA, and PUFA, transferring the beneficial effects of fresh grass on the functional quality of the products. The present study represents a first step to evaluate the influence of livestock system, animal age and type of muscle on fatty acid composition of bresaola obtained with meat from Cinisara breed, thus further investigations should be conducted using a superior number of animals.

Disclosure statement

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Author contributions

Conceptualization, Marco Alabiso and Giuseppe Maniaci; Data curation, Marco Alabiso, Giuseppe Maniaci and Antonino Di Grigoli; Funding acquisition, Nicola Francesca and Baldassare Portolano; Investigation, Giuseppe Maniaci, Cristina Giosuè and Adriana Bonanno; Resources, Giuseppe Maniaci; Supervision, Adriana Bonanno; Visualization, Cristina Giosuè, Raimondo Gaglio, Nicola Francesca, Antonino Di Grigoli and Baldassare Portolano; Writing – original draft, Marco Alabiso and

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