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Research Article

Cull Beef Cow from Southern Portugal: High Amount of n-3 PUFA

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Abstract

The goal of this research work was to characterize carcass, meat quality traits, proximate composition and lipid profile of cull beef cows reared in extensive systems across various seasons. Mature beef cows (n=65) with mean age of 14.12 years and culled from commercial farms were slaughtered in the Spring, Summer, Autumn and Winter, after a finishing period of about 4 weeks. The meat quality traits were analyzed at 7 d *postmortem* in *Longissimus thoracis, Longissimus lumborum, Semimembranosus, Psoas major* and *Semitendinosus* muscles. The highest carcass yield was obtained in Winter (P <0.05), but the Intramuscular Fat (IMF) means were similar in the different seasons. The meat samples showed high average Warner-Bratzler shear force-WBSF (between 5.41-6.86kg), which may be considered as tough (according to the scale used), except for the *Longissimus thoracis* which showed intermediate average (5.13kg). However, *Psoas major* obtained the highest overall acceptance scores. Samples of meat from animals kept in extensive system, natural conditions of Southern Portugal, present high amounts of n-3 PUFA. In Autumn and Summer, portions of 100 g/day of meats with 4.03% and 4.25% of IMF with 1.12 and 2.20% of n-3 PUFA in total fatty acids, can provide between 72.96 and 143.31mg of n-3 PUFA or 31.99 and 61.21mg/d of EPA plus DHA, which corresponds to 12.47% and 24.48% of the requirements recommended by EFSA authorities (250mg/d of EPA plus DHA).

Introduction

Mature or cull cows are generally animals that have ended their productive life, are poorly suited for breeding or are old. The total amount of beef from cull beef cows is variable and, under normal conditions, the proportion of meat from this animal category is between 20 and 25 % in Portugal. However higher values have been described in Brazil in 2003 (44.4 %) and France in 2019 (50.0 %) [1,2]. Compared to other cattle categories, cull cows present a lower performance in feed efficiency, weight gain and carcass yield and the finishing time of these animals is generally very short (around 30 days) [3], for it to be profitable. On the other hand, carcasses with low fat cover are more susceptible to losses during cooling (dripping or evaporation) and/or to cold shortening, possibly resulting in meat with high shear force and low tenderness [4,5].

The meat from the carcasses of cull cows is used mainly in the manufacture of processed products, due to low tenderness and dark color [2]. In this case, low tenderness may result from several factors, including the high number of age-related cross-links between collagen structures, but also due to the possible occurrence of cold shortening (carcasses subjected to intense *postmortem* cooling regimes in the absence of adequate electrical stimulation, with low subcutaneous fat) [4]. However, some authors describe similar quality traits between cull cows and heifers [6] if high-energy finishing diets are offered for relatively long periods [7]. With this procedure, some carcass quality prerequisites are met, including the standardization of the fattening rate of the animals, which impact subcutaneous and intramuscular fat proportions and meat quality, by delaying carcass cooling rate (thus reducing the degree of muscle shortening) and improving flavour and colour. In sensorial analysis, high-energy fishing results in higher values of tenderness/juiciness [8], as well as higher carcass yield, commercial parts yield and, consequently, improved carcass commercial value. Mature cows kept in extensive systems are exposed to different environmental challenges, and seasonal effects have been shown to exert a significant impact on the productive aspects of cattle and lamb, due to exposure to stress from heat and cold, to the nutritional variability of pastures [9,10] and to the large variability of productive management aspects involving cull cows, as described by Couvreur et al. [2]. However, little is known about the quality of beef produced by cull cows, particularly regarding the impact of seasonal effects [11]. The present study aimed to characterize meat quality and carcass traits of cull beef cows reared in extensive systems across various seasons, as a possible way to add value to their products.

Material and Methods

Animals and treatments

This work was carried out with 65 cows from the commercial herd of six farms (place of origin-PO) in southern Portugal, District de Portalegre, in a region of the agroforestry system called Montado (named Dehesa in Spain). In this region, animals are usually raised and maintained in extensive systems (grazing), so that feed supply depends on the availability of natural pastures, which is, in turn, influenced by the environmental conditions of a typical Mediterranean climate, characterized especially by a hot and dry Summer. Under these conditions, the average daily maximum/minimum temperatures are 12-18 / 5-7 °C in the Winter, 20-28 / 8-12 °C in the Spring, 29-35 / 15-16 °C in the Summer and 15-22 / 7-12 °C in the Autumn. The average monthly rainfall for the same seasons is 55-80, 25-60, 2-20 and 65-85 mm, respectively. In this productive

process, the commercial herd is formed by animals of meat breeds crossed with the Alentejana breed, an autochthonous group adapted to seasonal variations, such as: availability of pasture/ acorn fruit, temperature, humidity, etc. In general, pastures in this region produce somewhere between 3 and 9 tons of DM/ha/year, with higher production rates in the Spring (65-85 % of the total DM) and Autumn (15-35 % of the total DM) [12]. Generally, the most representative forage species are: *Trifolium* spp., *Medicago* spp; *Ornithopus* spp; *Vulpia* spp., *Bromus* spp., *Aegilops* spp., *Hordeum* spp. And *Avena* spp. Grazing was carried out in oak (*Quercus ilex*) or cork oak (*Quercus suber*) understory areas, with about 15 to 30 trees per hectare and acorn production between November and February (Winter). Traditionally, during periods of reduced fodder availability, animals are supplemented with commercial feed, where most ingredients are regionally produced.

In this context, with an average age of 14.12 ± 2.81 years and separated from the commercial herd in a proportion between 18-22 % throughout the year, cull cows were kept in grazing pens with a stocking density of less than 560 kg live weight/ha and for periods of about 4 weeks. Thus, the sixty-five cows were finished and slaughtered during Spring (n=22), Summer (n=14), Autumn (n=15) and Winter (n=14). In this finishing period, the animals were supplemented with concentrate feed with 14.5 % crude protein, 2.5 % ether extract, 12 % crude fiber and 5 % ash, using corn, wheat bran, barley, sunflower meal, soybean cake, rice bran and molasses. The concentrate was offered twice a day (morning and afternoon) ad libitum allowing approximately 7-8 % daily refusals. After the finishing period and before transport to the slaughterhouse, individual Live Weight (LW) was recorded.

Slaughter and carcass information

The cows were slaughtered in groups of 3 to 5 cows per week in a commercial slaughterhouse, following EU regulations on the protection of animals during transport and related operations (Regulation EU 2017/625 of 15/03), and at the time of killing (EC Council Regulation 1099/2009 of224/09). After arrival to the slaughter plant, animals were submitted to a period of 8-10 h of rest and solid feed fasting, after which they were mechanically stunned and slaughtered. During bleeding, the carcasses were subjected to electrical stimulation with low-voltage current (85 V, 14 Hz), applied for approximately 30 seconds. In this process, the clamp-shaped positive electrode was attached to the nasal septum and grounding was applied to the left leg of the suspended animal. After evisceration and longitudinal sectioning of the carcass, the two half carcasses were then weighed, to record hot carcass weight, and cooled to a temperature of 2.1 °C. After 48 h *postmortem*, the two half carcasses were weighed again, to determine Cold Carcass Weight (CCW). They were then boned, with commercial cuts obtained in accordance with Portuguese guidelines [13,14], and grouped in the categories of extra, first-class and second-class meat [15,16]. The muscles corresponding to the different categories of meat cuts were as follows: *Psoas major* (PM) and *Longissimus lumborum* (LL) for the extra-class meat cuts; *Longissimus thoracis* (LT), *Gluteus medius* (GM), *Biceps femoris* (BF) and *Semitendinosus* (ST) for first-class meat cuts; and all other muscles for second-class meat cuts. Carcass yield (CY) was calculated for each animal as CY=(CCW/LW)x100. Meat yield (MY) was determined relative to cold carcass weight as MY=(weight of total cuts/CCW)x100, and the percentages of extra-class, first-class and second-class meat cuts were computed from the weight of the pieces in each category relative to MY.

After boning, marbling was determined in the LT surface between the 10th and 11th ribs, using a 12-point scale (1= absence, 2=traces, ..., 12=extremely abundant fat) [17]. Marbling was determined by two judges and the mean score was used in statistical analyses. For the laboratory analyses, primal cuts [13,14] were collected when boning the carcass, and then vacuum-packed individually, identified, stored at 2 °C, and transported to the laboratory. To perform the chemical composition and lipid profile determinations, the samples were minced, individually vacuum-packed, identified and frozen at temperatures between -30 to -35 °C and stored at -20 °C until further analyses. For the determination of physical characteristics and for sensory analysis, samples weighing about 600 g were cut from each muscle (transversal direction), individually vacuum packed, identified, frozen at -30 to -35 °C and stored at -20 °C until further analyses. Therefore, when samples were later on thawed for different analytical procedures, they corresponded to an evaluation of meat at 7 d postmortem.

Laboratory analysis

Proximate composition: Meat samples from the LT, PM, SM, and LL muscles were subjected to proximate composition analysis (moisture, protein, fat, and ash) at 7 days *postmortem*. The analyses were performed in duplicate, using AOAC [18] methods. Briefly, protein was quantified using the micro-Kjeldahl method (method 954.01) with a block digester and nitrogen distiller; fat content (or IMF) was determined by the Soxhlet method (method 920.39), using a Soxhlet extractor (VELP Scientifica SER 148/6 Solvent Extractor, Italy); moisture content was determined in an oven, at a temperature of 105 °C, until a constant sample weight was obtained (method 950.46); and ash determination was performed by carbonization and incineration of the samples in a muffle furnace, at a temperature of 550 °C (method 920.153).

Minerals: For the determination of macro and trace minerals, test portions (1.5g) of fresh meat samples were incinerated in a muffle furnace at 550 °C [19], and the ashes dissolved in hydrochloric acid. Any silicon compounds presented were removed by precipitation and filtration. The precipitate was dissolved in hydrochloric acid and diluted to the desired volume. The Calcium (Ca), Sodium (Na), Potassium (K), Magnesium (Mg), and Iron (Fe) contents were determined by flame (air-acetylene) Atomic Absorption Spectrometry (Shimadzu AA 680®), following the specifications for each mineral [20]. The absorption of each element was measured by comparison to the absorption of the same element in calibration solutions. Phosphorus (P) was determined by UV/VIS Spectrometer (Unicam UV2®) after the colour reaction of the ammonia nitrovanado-molybdate reagent [21]. Fe was expressed as µ/g, Ca was expressed as mg/100g and the other minerals were expressed as g/100g of product.

Lipid profile: Fat for lipid profile determination was extracted from LT, PM and LL muscles according to Folch et al. [22] and esterified according to Raes et al. [23]. The Fatty Acid Methyl Esters (FAME) were submitted to gas-liquid chromatography on an Agilent HP 6890 chromatographer (Agilent technologies Inc., Palo Alto, CA) equipped with a 100 m capillary column (CP-Sil 88, 100m x 0.25mm x 0.20µl, Varian Inc., Walnut Creek, CA) and flame ionization detector. The chromatographic conditions applied were described by Bessa et al. [24]. FAME were identified by comparing the retention time of the sample peaks to the retention times of known standard peaks. Individual quantification of fatty acids was based on the total peak area identified in each chromatogram.

Physical characteristics: Meat pH was measured at 7 d *postmortem* by making a scalpel incision in the LT, ST, PM, SM and LL muscle, and inserting a glass electrode, model FC200 (Hanna Instruments, Leighton Buzzard, UK), attached to a portable pH meter, approximately 1.0cm into the muscle. Three pH measurements were taken from each sampled point, and the mean of these measurements was used for statistical analysis. Objective colour measurement was performed at 7 d *postmortem* on the surface of the exposed area of the samples, using the CIE L* a* b* system, with the aid of the Minolta Chroma Meter CR-400 (Minolta Camera Co., Ltd., Osaka, Japan), illuminant D_{65}, 10º standard observation, reading area of 1cm Ø. Colour coordinates were obtained after 60min of blooming at 4 °C, by averaging three readings performed in the median region of each sample, at regular distance intervals in the mid-space of 45mm, which corresponds to the average diameter of each piece. The lightness (L*), redness (a*) and yellowness (b*) of the surface were recorded, according to the CIE colour scores.

Instrumental analysis of meat quality was performed after the thawing of the pieces at 4 °C, for 24 hours. To determine the Warner-Bratzler Shear Force (WBSF), samples were subjected to cooking, according to the procedures recommended by AMSA [25]. Briefly, samples (200 ± 25g) were boiled in water at 80.0 ± 0.2 °C until they reached an internal temperature of 75.0 °C, measured with an Omega RDXL4SD type T fine-gage thermocouple (Omega Engineering, Inc., Manchester, UK). Cooked samples were stored at 4 °C for temperature stabilization, and after 24h they were cut parallel to the direction of muscle fibers (1 × 1 × 3cm). These subsamples were sheared with a TA.XT2 texture analyzer (Stable Micro Systems, Surrey, England), equipped with a Warner-Bratzler shearing device at a crosshead speed of 300mm/min, and results were expressed in kilograms. The average value of 15 to 25 measurements per sample was used for statistical analysis.

Sensory analysis: Due to logistic constraints, the meat samples assessed by the sensory panel originated from animals slaughtered in Winter and four muscles were represented (LT, PM, SM, LL). Samples of meat aged for 7 d were used for sensory analysis. Sensory evaluation was carried out by a trained panel of 10 members, both male (n = 4) and female (n = 6), ranging in age from 30 to 60 years. The panelists were chosen from the staff of the research station, and they were all experienced in the profile assessment of different meats and trained according to ISO 8586 [26]. There were 30 trial sessions, 1 per day. In each session, six to eight different samples were served to all judges in random order, including at least one sample from the four cuts under evaluation. Samples of the LT, PM, SM and LL muscles were thawed at 4.0 ± 1.0 °C for about 20 hours. After removal of all visible external fat, the samples were cut into a cobblestone shape (200 ± 25g) and placed in aluminium trays, with an Omega RDXL4SD type T fine-gage thermocouple (Omega Engineering, Inc., Manchester, UK)

inserted into the center of each sample. The samples were roasted in a preheated, electric, natural convection oven at 163 °C, to an endpoint core temperature of 71 °C [26]. After 10min of stabilization at 40 °C, the external surface of the roast was discarded, and the remainder sliced (thickness 1.0cm). The slices were sectioned in cubes (1 × 1 × 1cm), and two cubes were placed in a preheated glass Petri dish, covered and maintained at 40 °C until evaluation (no longer than 30min). Judges were given samples in groups of two, along with an evaluation card sheet, where different meat properties were scored in a scale of 1 to 8, according to AMSA [25]. Meat attributes evaluated were juiciness (1 = extremely dry; 8 = extremely juicy), tenderness (1 = extremely tough; 8 = extremely tender), flavour intensity (1 = extremely mild; 8 = extremely intense), flavour acceptability and overall acceptability (1 = extremely inacceptable; 8 = extremely acceptable).

Statistical analysis

Carcass traits were assumed to be affected by place of origin, season and the linear effect of carcass weight and were analysed with the GLM procedure of SAS 9.4 (SAS Institute Inc., Cary, NC) in analyses of variance. Data on the physicochemical properties of meat were assumed to have been affected by place of origin and carcass weight, and to have originated from a factorial treatment arrangement, combining muscles and seasons. In all cases, the four seasons were considered, while the number of muscles varied according to the characteristic analysed: five muscles (LT, ST, PM, SM, and LL) were considered for physical traits and colour; four muscles (LT, PM, SM, and LL) were considered for chemical composition and minerals; and three muscles (LT, PM, and LL) were used for determination of fatty acid profile. For these groups of traits, a linear model including the main effects of muscle, season and their interaction, in addition to the effect of place of origin and carcass weight (linear covariate) was used. The meat samples assessed by the sensory panel originated from animals slaughtered in Winter, and four muscles were represented (LT, PM, SM and LL), with 10 panelists assessing meat palatability. Therefore, the sensory variables were analyzed with the MIXED Procedure of SAS, using a mixed model which included the fixed effect of muscle and the random effect of panellist, in addition to the effect of place of origin and carcass weight (linear covariate).

Results

Analysis of variance (ANOVA)

In the present study the coefficients of determination (R^2) found were relatively high (Table 1), ranging from 0.40-0.48 for proximate composition, 0.43-0.65 for minerals, 0.42-0.43 for pH and Warner-Bratzler Shear Force, 0.50-0.74 for colour coordinates, and 0.49-0.74 for fatty acids. Coefficients of determination with slightly lower values (0.30-0.46) were found for sensory attributes and some carcass characteristics (meat yield and marbling). Higher R^2 was found for carcass yield (0.61), however for yield of the various meat cut categories (extra, first-class and second-class meat) the R^2 was low (<0.15). Regarding the covariate effects, most meat traits were not affected by carcass weight (Table 1). On the other hand, the place of origin had a significant effect (P<0.05) on all response variables of proximate composition and lipid profile (except for C18:0 fatty acid). The effect of place of origin on mineral composition and sensory attributes was not significant in most cases.

Table 1: P-values for the effect of muscle (M), slaughter season (S), interaction MxS, place of origin (PO) and carcass weight (CW), and coefficient of determination (R^2) in analyses of variance of meat quality traits in cull beef cows reared in extensive systems.

Group of Traits	Trait	P-Value					R^2
		M	S	M x S	PO	CW	
Yield	Carcass	-	<0.001	-	0.01	-	0.614
	Meat	-	0.082	-	0.014	0.971	0.309
	Extra-class meat	-	0.073	-	0.892	0.883	0.147
	First-class meat	-	0.609	-	0.749	0.906	0.085
	Second-class meat	-	0.187	-	0.711	0.87	0.141
	Marbling	-	0.002	-	<0.001	0.641	0.449
Proximate composition	Moisture	<0.001	0.015	0.138	0.016	0.144	0.401
	Protein	<0.001	0.008	0.003	<0.001	0.658	0.484
	Fat	<0.001	0.372	0.315	<0.001	0.358	0.408
	Ashes	<0.001	0.001	0.082	<0.001	0.121	0.442
Minerals	Ca	0.104	0.001	0.601	0.008	0.771	0.494
	P	<0.001	0.02	<0.001	0.838	0.972	0.648
	K	<0.001	0.212	0.076	0.391	0.561	0.435
	Na	<0.001	0.13	0.285	0.818	0.289	0.611
	Mg	<0.001	0.153	0.022	0.417	0.344	0.573
	Fe	<0.001	<0.001	0.411	0.002	<0.001	0.622
Physical traits	pH	<0.001	0.019	0.039	<0.001	<0.001	0.432
	WBSF	<0.001	<0.001	0.04	0.086	0.406	0.419
Colour coordinates	L*	<0.001	0.756	0.271	0.01	0.001	0.504
	a*	0.016	<0.001	0.017	0.293	0.184	0.553
	b*	0.178	<0.001	<0.001	0.136	0.117	0.74

Fatty acids	C16:0	<0.001	0.077	0.45	0.002	0.072	0.703
	C18:0	<0.001	<0.001	0.975	0.186	0.416	0.631
	C18:1t11	0.199	<0.001	0.964	<0.001	0.619	0.738
	C18:1c9	0.001	0.011	0.772	<0.001	0.009	0.585
	C18:2c9t11	0.02	<0.001	0.919	<0.001	0.65	0.645
	SFA	0.033	0.038	0.86	0.002	0.067	0.487
	MUFA	<0.001	0.07	0.433	0.009	0.173	0.513
	PUFA	<0.001	0.087	0.584	0.011	0.178	0.599
	n-6	<0.001	0.449	0.621	<0.001	0.223	0.626
	n-3	0.016	<0.001	0.6	<0.001	0.234	0.634
	PUFA:SFA	0.001	0.1	0.764	0.007	0.138	0.569
	PUFA:MUFA	<0.001	0.083	0.328	0.025	0.361	0.613
	MUFA:SFA	0.007	0.057	0.647	0.003	0.125	0.494
	n-6:n-3	0.046	0.107	0.986	<0.001	0.198	0.676
Sensorial attributes	Juiciness	<0.001	-	-	0.045	0.114	0.371
	Tenderness	<0.001	-	-	0.608	0.811	0.354
	Flavour	<0.022	-	-	0.229	0.282	0.465
	Overall acceptance	<0.001	-	-	0.079	0.182	0.296

Muscles and seasons affected (P<0.05) a total of 28 (90.3 %) and 20 (57.1 %), respectively, of the 31 and 35 response variables studied. On the other hand, the effect of the interaction between muscle and season was negligible (P>0.05) in most of the traits and was only significant (P<0.05) for protein, a* and b* colour coordinates, pH, WBSF and for two of the six minerals analysed. Given the absence of a consistent indication of a significant muscle x season interaction, it was ignored when least square means were obtained for main effects in all variables, to allow a more uniform analysis and presentation of results.

Carcass and meat yields

Season affected the results of carcass yield (P<0.05), meat yield (P = 0.082) and extra-class meat (P = 0.073, Table 1). Highest mean carcass yield (about 8.0 % higher) and extra-class meat yield (about 0.46 % higher) were obtained in animals slaughtered in the Winter (Table 2), but differences were minor in the other seasons. For meat yield, the highest mean values were observed in animals slaughtered in Autumn and Spring, while for first and second-class meat no effect of slaughter season was detected. Although marbling was higher (P<0.05) in carcasses from animals slaughtered in the Spring, the averages were very low, with values between 1.62 and 2.64 (in a scale of 1-12).

Table 2: Least square means ± SEM for carcass and meat yield and for marbling score (*longissimus thoracis* surface) in carcasses from cull beef cows slaughtered in different seasons.

Carcass trait	Season			
	Winter (n=14)	Autumn (n=15)	Spring (n=22)	Summer (n=14)
Carcass yield (%)	$55.3^{a} \pm 1.00$	$46.0^{b} \pm 0.92$	$46.1^{b} \pm 0.04$	$47.8^{b} \pm 1.18$
Meat yield (%)	$67.8^{ab} \pm 1.08$	$69.0^{a} \pm 0.90$	$67.3^{ab} \pm 0.82$	$65.6^{b} \pm 1.18$
Extra-class cuts (%)	$7.36^{a} \pm 0.21$	$6.89^{ab} \pm 0.17$	$6.76^{b} \pm 0.15$	$6.90^{ab} \pm 0.22$
First-class cuts (%)	37.83 ± 0.55	37.33 ± 0.46	37.12 ± 0.41	37.10 ± 0.60
Second-class cuts (%)	54.81 ± 0.58	55.78 ± 0.44	56.18 ± 0.44	55.99 ± 0.63
Marbling score1	$1.62^{b} \pm 0.20$	$1.77^{b} \pm 0.16$	$2.64^{a} \pm 0.15$	$1.64^{b} \pm 0.22$

[a,b,c]Means without a common letter differ (P<0.05)

[1]Score of 1 – 12, where higher scores correspond to more abundant fat.

Chemical composition

The moisture, protein, fat and ash results differed (P<0.05) among the various muscles studied. Moisture content was lower in LT and LL muscles, by about 1 % and 1.7 %, when compared with PM and SM, respectively (Table 3-A). For fat content, the LT, PM and LL muscles had an average amount of the IMF in the range of 4.2 to 4.7 %, while in the SM muscle this value was 2.4 %. Protein content showed the opposite pattern, with a higher mean (P<0.05) in SM relative to the other muscles, while the amount of ash was higher in PM (P<0.05). Regarding the seasons, moisture measurements show significant effect (P<0.05), with differences of less than 1%, and fat content was similar between the seasons in study (P<0.05). Meat from animals slaughtered in the Autumn showed higher content of protein (P<0.05), while ashes were higher in Winter-slaughtered animals (P<0.05), both in comparison with the other seasons.

Overall, the mean concentrations were 24.15µg/g for Fe, 5.04mg/100 g for Ca, 0.254g/100 g for K, 0.19g/100 g for P, 0.065g/100 g for Na and 0.023g/100 g for Mg. All minerals were affected (P<0.05) by type of muscle, except for Ca. In general, higher mean values for minerals (P, Na, Mg, and especially Fe) were found in the PM and SM muscles. Regarding

seasons, high levels of Ca and low levels of Fe were found (P<0.05) in Spring compared to the other seasons (Table 3-B), but the content of K, Na and Mg was not influenced by season.

Table 3-A: Least square means ± SEM by muscle and season for chemical composition, physical traits (Warner-Bratzler Shear Force-WBSF) and colour coordinates (CIE L* a* b* system) in different muscles (Table 3A) from cull beef cows slaughtered in different seasons (Table 3B)[1].

	Muscles[2]				
	LT	**ST**	**PM**	**SM**	**LL**
Prox. Composition (%)	**(n=65)**		**(n=65)**	**(n=65)**	**(n=65)**
Moisture	$71.83^{c} \pm 0.16$	-	$72.81^{b} \pm 0.16$	$73.48^{a} \pm 0.17$	$71.78^{c} \pm 0.16$
Protein	$22.74^{b} \pm 0.12$	-	$21.47^{c} \pm 0.12$	$23.18^{a} \pm 0.13$	$22.54^{b} \pm 0.12$
Fat	$4.15^{b} \pm 0.19$	-	$4.69^{a} \pm 0.19$	$2.40^{c} \pm 0.21$	$4.56^{ab} \pm 0.19$
Ashes	$1.04^{c} \pm 0.01$	-	$1.10^{a} \pm 0.01$	$1.06^{b} \pm 0.01$	$1.04^{c} \pm 0.01$
Minerals	**(n=65)**		**(n=65)**	**(n=65)**	**(n=65)**
Ca mg/100 g	5.05 ± 0.212	-	5.17 ± 0.210	4.49 ± 0.210	4.92 ± 0.210
P g/100 g	$0.19^{b} \pm 0.004$	-	$0.19^{b} \pm 0.004$	$0.21^{a} \pm 0.004$	$0.18^{c} \pm 0.004$
K g/100 g	$0.26^{ab} \pm 0.008$	-	$0.23^{a} \pm 0.021$	$0.23^{c} \pm 0.008$	$0.26^{b} \pm 0.008$
Na g/100 g	$0.053^{c} \pm 0.002$	-	$0.076^{a} \pm 0.004$	$0.062^{b} \pm 0.008$	$0.061^{b} \pm 0.002$
Mg g/100 g	$0.022^{b} \pm 0.001$	-	$0.024^{a} \pm 0.008$	$0.024^{a} \pm 0.001$	$0.022^{b} \pm 0.001$
Fe µ/g	$23.85^{c} \pm 0.83$	-	$29.23^{a} \pm 0.83$	$28.27^{ab} \pm 0.83$	$26.77^{b} \pm 0.83$
Physical traits	**(n=63)**	**(n=40)**	**(n=40)**	**(n=41)**	**(n=52)**
pH	$5.67^{d} \pm 0.01$	$5.79^{a} \pm 0.01$	$5.72^{bc} \pm 0.01$	$5.73^{b} \pm 0.01$	$5.69^{cd} \pm 0.01$
WBSF (kg)	$5.13^{c} \pm 0.19$	$6.86^{a} \pm 0.21$	-	$5.88^{b} \pm 0.21$	$5.96^{b} \pm 0.20$
Colour Coordinates	**(n=52)**	**(n=14)**	**(n=14)**	**(n=14)**	**(n=20)**
L*	$34.97^{b} \pm 0.38$	$38.46^{a} \pm 0.87$	$38.03^{a} \pm 0.87$	$38.03^{a} \pm 0.87$	$38.56^{a} \pm 0.63$
a*	$19.08^{a} \pm 0.30$	$18.30^{ab} \pm 0.69$	$17.62^{bc} \pm 0.69$	$17.62^{bc} \pm 0.69$	$17.35^{bc} \pm 0.51$
b*	$8.15^{a} \pm 0.18$	$8.46^{a} \pm 0.41$	$8.68^{a} \pm 0.41$	$7.96^{a} \pm 0.41$	$8.46^{a} \pm 0.30$

[1]For a given main effect, values shown are means pooled across the various levels of the other main effect.
[2]LT = *Longissimus thoracis*, ST = *Semitendinosus*, PM = *Psoas major*, SM = *Semimembranosus*, LL = *Longissimus lumborum*.
[a,b,c]Means for muscles without a common letter differ (P<0.05).

Table 3-B: Least square means ± SEM by muscle and season for chemical composition, physical traits (Warner-Bratzler Shear Force-WBSF) and colour coordinates (CIE L* a* b* system) in different muscles (Table 3A) from cull beef cows slaughtered in different seasons (Table 3B)[1].

	Season			
	W	**A**	**SP**	**SU**
Prox. Composition (%)	**(n=56)**	**(n=60)**	**(n=88)**	**(n=56)**
Moisture	$72.18^{B} \pm 0.21$	$72.81^{A} \pm 0.18$	$72.93^{A} \pm 0.18$	$71.98^{B} \pm 0.24$
Protein	$22.81a^{A} \pm 0.15$	$22.16^{B} \pm 0.13$	$22.37^{AB} \pm 0.12$	$22.60^{AB} \pm 0.17$
Fat	3.78 ± 0.25	4.03 ± 0.21	3.72 ± 0.20	4.25 ± 0.27
Ashes	$1.08^{A} \pm 0.01$	$1.04^{B} \pm 0.01$	$1.05^{B} \pm 0.01$	$1.05^{B} \pm 0.01$
Minerals	**(n=56)**	**(n=60)**	**(n=88)**	**(n=56)**
Ca mg/100 g	$5.34^{A} \pm 0.48$	$5.21^{A} \pm 0.26$	$5.75^{A} \pm 0.14$	$3.71^{B} \pm 0.23$
P g/100 g	$0.21^{B} \pm 0.009$	$0.19^{A} \pm 0.005$	$0.19^{A} \pm 0.004$	$0.18^{A} \pm 0.009$
K g/100 g	0.268 ± 0.006	0.253 ± 0.006	0.241 ± 0.005	0.256 ± 0.006
Na g/100 g	0.052 ± 0.002	0.063 ± 0.003	0.067 ± 0.003	0.071 ± 0.002
Mg g/100 g	0.023 ± 0.001	0.023 ± 0.001	0.023 ± 0.001	0.022 ± 0.001
Fe µ/g	$29.0^{C} \pm 1.89$	$29.2^{A} \pm 1.02$	$20.9^{B} \pm 0.82$	$29.1^{A} \pm 1.83$
Physical Traits	**(n=70)**	**(n=52)**	**(n=81)**	**(n=34)**
pH	$5.70^{B} \pm 0.01$	$5.74^{A} \pm 0.01$	$5.74^{A} \pm 0.01$	$5.69^{B} \pm 0.01$

WBSF (kg)	5.99^{AB} ± 0.26	6.30^{A} ± 0.23	5.41^{BC} ± 0.23	6.13^{AB} ± 0.24
Colour Coordinates	**(n=67)**	**(n=18)**	**(n=14)**	**(n=15)**
L*	37.14 ± 0.45	36.13 ± 0.77	36.71 ± 1.02	36.87 ± 0.90
a*	21.31^{A} ± 0.36	18.47^{B} ± 0.62	16.80^{B} ± 0.82	17.15^{BC} ± 0.66
b*	10.40^{A} ± 0.21	8.18^{B} ± 0.36	7.14^{C} ± 0.48	7.64^{B} ± 0.40

[1]For a given main effect, values shown are means pooled across the various levels of the other main effect.
[2]W = Winter, A = Autumn, SP = Spring, SU = Summer.
^{A,B,C}Means for seasons without a common letter differ (P<0.05).

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Physical traits

The means for WBSF and pH in muscles from cull beef cow were affected (P<0.05) by season and type of muscle (Table 3-B and Table 3-A, respectively). Higher pH values (P<0.05) were found in Autumn and Spring (5.74), when compared with Winter and Summer (5.69 to 5.70). For the various muscles studied, ST presented a pH value (5.79) higher than the other muscles. The WBSF values of meat from beef cows were affected (P<0.05) by slaughter season and muscle type. Considerable differences in mean WBSF were observed be-tween muscles, with the highest WBSF observed in ST (6.86 kg) and the lowest in LT (5.13 kg). When the effect of slaughter season was considered, the lowest WBSF values were found in Spring (5.41 kg), while the highest value was observed in Autumn (6.30 kg). The variability between samples for WBSF was large. An example is the individual variation found in the *Longissimus lumborum* results (Figure 1). In this case, WBSF ranged from 3.17 to 11.20 kg, i.e. from extremely tender to extremely tough.

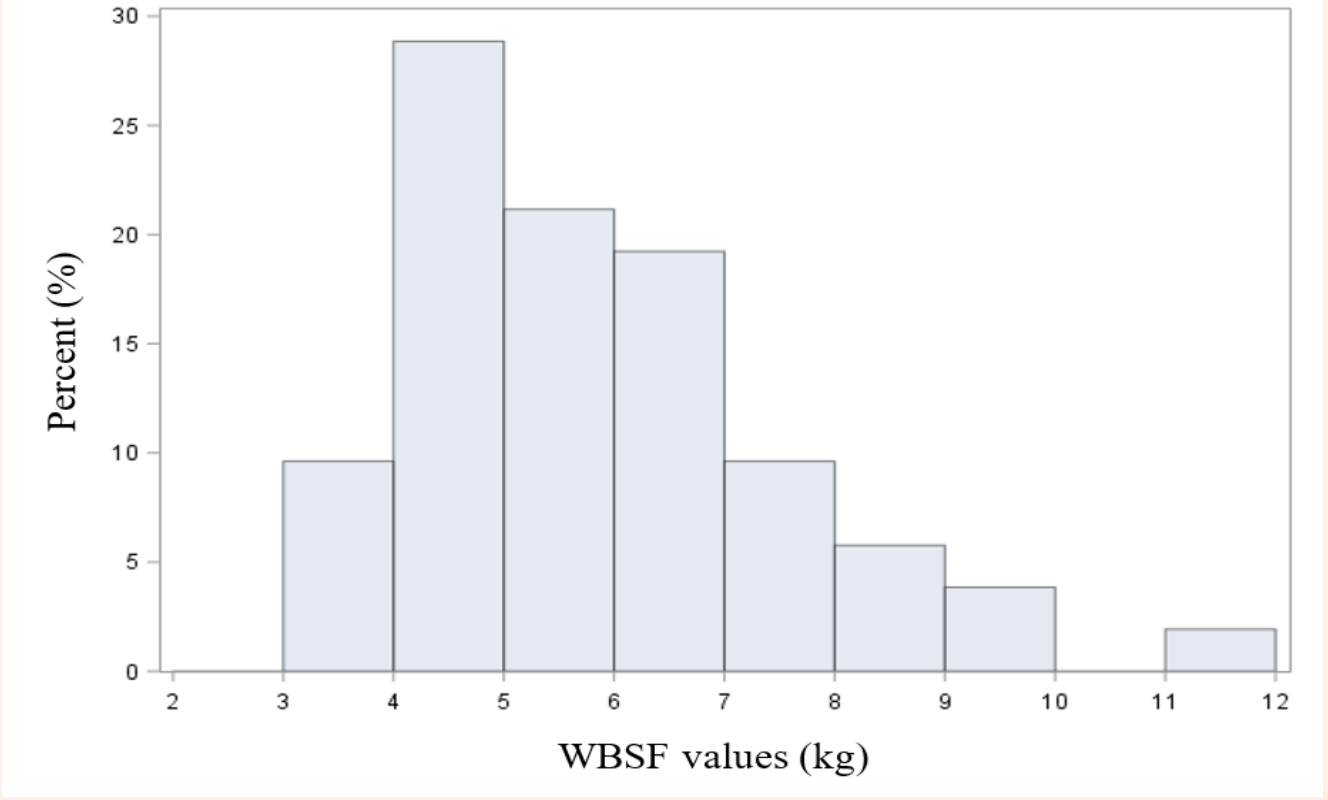

Figure 1: Frequency distribution (%) for Warner-Bratzler Shear Force (WBSF – kg) values after 7 days of ageing in individual samples of the *Longissimus lumborum* muscle from cull beef cows (n=52) slaughtered in different seasons.

Regarding meat colour, the values determined for coordinates a* and b* were affected by slaughter season (P<0.05), while coordinates L* and a* were affected by the type of muscle (P<0.05). The samples of animals slaughtered in Winter showed the highest values for a* (21.31) and b* (10.40), while animals slaughtered in Spring and Summer showed the lowest values for a* and b* (16.80 and 17.15 for a*, and 7.14 and 7.64 for b*, respectively). For the various muscles, LT presented the lowest value for L* (34.97) and the highest for a* (19.08), while the PM, SM and LL muscles showed high L* values (between 38.0 and 38.5) and low a* values (between 17.35-17.62).

Fatty acids methyl esters (FAME)

The predominant Saturated Fatty Acids (SFA) in all muscles were C16:0 (ranging from 24.76 to 27.44 % of total FAME) and C18:0 (13.57 to 17.93 %), while the predominant monounsaturated fatty acid (MUFA) was C18:1c9 (35.41 to 37.96 %) (Table 4). Among the Polyunsaturated Fatty Acids (PUFA), the group of n-6 PUFA contributed with percentages ranging from 4.26 to 6.03 % in the different muscles. The seasons significantly (P<0.05) affected trans fatty acids (C18:1t11 and C18:2c9t11) as well as n-3, C18:0 and total SFA. However, the mean results for n-6 did not differ (P>0.05) between seasons. Higher amounts of n-3, C18:1t11 and C18:2c9t11were found in samples from animals slaughtered during

the Summer. The overall means percentages of C20:5n-3 (EPA) plus C22:5n-3 (DHA) were between 0.25 and 0.43 % of total fatty acids (data not shown). On the other hand, higher C18:0 and SFA were observed (P<0.05) in animals slaughtered in the Spring.

Table 4: Least square means ± SEM by muscle and season for individual and major groups of fatty acids in muscles from cull beef cows slaughtered in different seasons[1].

	Muscle[2]			Season[3]			
Fatty Acids	LT (n=21)	PM (n=25)	LL (n=23)	W (n=13)	A (n=16)	SP (n=28)	SU (n=12)
C16:0	27.20^{a} ± 0.44	24.76^{b} ± 0.39	27.12^{a} ± 0.40	25.53 ± 0.51	27.44 ± 0.99	26.33 ± 0.42	26.15 ± 0.95
C18:0	15.89^{a} ± 0.46	16.62^{a} ± 0.41	13.57^{b} ± 0.42	14.04^{B} ± 1.03	15.03^{B} ± 0.55	17.93^{A} ± 0.44	13.57^{B} ± 0.98
C18:1t11	1.34 ± 0.12	1.51 ± 0.11	1.25 ± 0.11	0.94^{C} ± 0.15	0.65^{C} ± 0.15	1.45^{B} ± 0.12	2.41^{A} ± 0.26
C18:1c9	35.41^{b} ± 0.64	34.76^{b} ± 0.57	37.73^{a} ± 0.59	37.96^{A} ± 1.45	37.57^{A} ± 0.77	33.73^{B} ± 0.52	34.61^{AB} ± 0.82
C18:2c9t11	0.37^{b} ± 0.03	0.41^{b} ± 0.03	0.47^{a} ± 0.03	0.36^{B} ± 0.07	0.27^{B} ± 0.04	0.36^{B} ± 0.03	0.66^{A} ± 0.07
SFA	48.88^{a} ± 0.81	47.28^{ab} ± 0.72	46.15^{b} ± 0.75	44.81^{B} ± 1.83	48.19^{AB} ± 0.97	50.42^{A} ± 0.78	46.23^{B} ± 1.75
MUFA	42.27^{b} ± 0.72	43.95^{b} ± 0.63	47.16^{a} ± 0.63	47.41 ± 1.61	45.45 ± 0.85	42.65 ± 0.69	44.98 ± 1.53
PUFA	6.92^{b} ± 0.42	8.77^{a} ± 0.33	6.69^{b} ± 0.39	7.78 ± 0.95	6.36 ± 0.41	6.93 ± 0.41	8.78 ± 0.90
n-6	4.60^{b} ± 0.35	6.03^{a} ± 0.31	4.26^{b} ± 0.32	5.32 ± 0.79	4.51 ± 0.42	4.49 ± 0.33	5.53 ± 0.76
n-3	1.55^{b} ± 0.10	1.83^{a} ± 0.09	1.49^{b} ± 0.09	1.57^{A} ± 0.22	1.12^{B} ± 0.12	1.60^{A} ± 0.09	2.20^{C}± 0.21
PUFA/SFA	0.144^{b} ± 0.01	0.187^{a} ± 0.009	0.147^{b} ± 0.009	0.17^{6} ± 0.023	0.137 ± 0.010	0.139 ± 0.010	0.185 ± 0.022
PUFA/MUFA	0.156^{b} ± 0.010	0.200^{a} ± 0.009	0.114^{b} ± 0.008	0.162 ± 0.023	0.136 ± 0.012	0.166 ± 0.010	0.201 ± 0.022
MUFA/SFA	0.914^{a} ± 0.028	0.935^{b} ± 0.025	1.022^{a} ± 0.025	1.049 ± 0.062	0.950 ± 0.027	0.901 ± 0.021	0.881 ± 0.031
n-6/n-3	3.070a^{b}± 0.193	3.414^{a} ± 0.171	2.924^{b} ± 0.177	3.44 ± 0.43	3.66 ± 0.28	2.91 ± 0.19	2.54 ± 0.41

[1]For a given main effect, values shown are means pooled across the various levels of the other main effect.
[2]LT = M. Longissimus thoracis, PM = M. Psoas major, LL = M. Longissimus lumborum
[3]W = Winter, A = Autumn, SP = Spring, SU = Summer.
^{a,b,c}Means for muscles without a common letter differ (P<0.05).
^{A,B,C}Means for seasons without a common letter differ (P<0.05).

All individual FA, major groups, and ratios of fatty acids varied significantly (P<0.05) in the IMF of the muscles studied (LT, PM, and LL), except for C18:1t11. Higher amounts of C18:0, C18:1t11, n-3, n-6, and total PUFA were found in the PM muscle, and the same happened with the PUFA/SFA, PUFA/MUFA, and n-6/n-3 ratios. On the other hand, the LL had a lower mean SFA and higher MUFA, when compared with the LT and PM (P<0.05).

Sensory analysis

The mean results for sensory attributes, evaluated in Winter-slaughtered animals, showed differences (P<0.05) between the muscles studied (Table 5). The PM muscle received the highest scores for juiciness, tenderness and overall acceptance attributes, followed by the SM muscle. On the other hand, the LL muscle received the lowest scores in all criteria assessed.

Table 5: Least square means ± SEM for sensory attributes assessed by a taste panel in different muscles from cull beef cows reared in extensive system and slaughtered in the Winter.

	Muscle[1]			
Sensory Attributes[2]	LT (n = 50)	PM (n = 64)	SM (n = 56)	LL (n = 37)
Juiciness	4.35^{b} ± 0.17	5.91^{a} ± 0.17	5.55^{a} ± 0.18	4.36^{c} ± 0.21
Tenderness	4.95^{bc} ± 0.18	6.35^{a} ± 0.18	5.16^{b} ± 0.19	4.45^{c} ± 0.21
Flavour	4.29^{c} ± 0.17	4.82^{ab} ± 0.16	5.00^{a} ± 0.18	4.45^{bc} ± 0.20
Overall acceptance	5.27^{bc} ± 0.15	6.23^{a} ± 0.14	5.53^{b} ± 0.15	5.07^{c} ± 0.18

^{a,b,c}Means without a common letter differ (P <0.05).
[1]LT = M. *Longissimus thoracis*, PM = M. *Psoas major*, SM = M. *Semimembranosus*, LL = M. *Longissimus lumborum*.
[2]Samples scored in a scale of 1 to 8, higher scores being more desirable.

Discussion

The meat quality in cull cows can be affected by many factors. An extensive review was conducted by Couvreur et al. [2]. In the group of factors preceding slaughter, the most important are breed, age at slaughter, environmental conditions, production system and diet. In animals raised and finished in extensive systems, the grass availability is closely related to edaphoclimatic conditions and seasonality, which determine the adapted forage species and the variations in production cycles throughout the seasons [27-29]. In the present work, the factors considered (place of origin, carcass weight, season and type of muscle) accounted for 40 to 74 % of the variability in physicochemical traits and lipid profiles, and for 30 to 46 % of the variability in sensory attributes of meat. The variability between places of origin was important for meat chemical composition and lipid profile, but less relevant for some of the physical properties and colour coordinates of meat. The percentages of different meat cuts (extra, first and second-class cuts) had a lower coefficient of determination, indicating that it was mostly independent of the effects considered. This high residual variability of meat cuts in our analyses may reflect a lack of accuracy of measurements in this data set [30], which in this case may be associated with variability inherent in the experimental animals [31] or to an insufficient standardization of procedures in a commercial slaughter, associated with mature cow carcasses.

When compared with animals slaughtered in other seasons, those slaughtered in the Winter presented carcass yield higher by about 8 %. Generally, the mean carcass yield in animals slaughtered in seasons other than Winter ranged from 46 to 48 %, in line with the values described for Charolais and Nellore cull cows (46.1 % and 49.6 %) [31]. In cattle, the increase in carcass yield is usually associated with greater fat deposition in the carcass [32]. In commercial slaughtering in the United States, a higher frequency of adult carcasses with yield higher than 58.3 % was found in the period between November and March, compared to the rest of the year, when there was a higher incidence of low yield carcasses [33]. In our study, a higher percentage of extra-class meat was also found in carcasses slaughtered during the Winter. This higher carcass yield and extra-class meat may result from higher supplementation levels [1], in association with acorn availability. In general, moisture and ash content

of beef do not show a lot of variation, with mean values of approximately 75 % for moisture and 1 to 2 % for minerals. However, more variability is expected in protein and fat content of meat. In our work, the proximal composition results obtained were close to the values reported in the literature [34]. The fat amount in the LT, PM and LL muscles was almost twice as much as the fat observed in the SM muscle. However, the SM muscle showed higher moisture and protein. The fat amount in the LT, PM and LL muscles was almost twice as much as the fat observed in the SM muscle. However, the SM muscle showed higher moisture and protein. This is expected [35] because there is a negative correlation between IMF, on one side, and moisture and protein content on the other side [36,37]. The differences between muscles for moisture, protein, fat and ash can be attributed to physiological differences associated with muscle functions and anatomical structure. The IMF may vary due to nutritional [34,38,39], environmental [40] and physiological factors, such as the muscles function in the body and the types of muscle fiber [35]. The IMF, also assessed by marbling, is positively correlated with the succulence, tenderness, and overall acceptability of beef [41].

Regarding mineral composition, cattle meat is a source of many minerals, such as Fe, Zn, Se and P [38]. The mineral profile and content of the different elements in animal tissues can be affected by several factors, such as animal management, muscle, breed and diet [42], but the major source of variability in mineral composition of meat is the diversity of soil and climatic conditions, physiological state of the cow and forage composition. Overall, meat exhibits natural variations in the amounts of minerals [43] and a large variation in the total Fe contents is reported in the literature [44]. The pH of meat is associated with colour characteristics, water retention capacity and tenderness, and it is dependent on the amount of glycogen stored in the muscle during the slaughter period [45,46]. Thus, in the postmortem period, meat pH evolves from values near 7.0 to values between 6.4-6.8 after 5 h, and between 5.5-5.8 after 24 hours. In general, the final pH is indicative of the conditions of energy intake and animal welfare in the pre-slaughter period [34] and affects the shelf life of the final product. In our study, all meat samples presented pH values below 5.9, which is a limit value often adopted for quality control of fresh meat [47]. Additionally, the variations in pH values between muscles or slaughtering seasons were narrow and possibly associated with the intrinsic differences of each muscle (muscle fiber types and biochemical metabolism). When compared to other beef categories, cull cows may have a higher incidence of dark meat (by about 1 to 3 %), which is due to low glycogen reserves (preslaughter) associated with high pH values at 24 h postmortem [46]. Alterations in colour parameters imply low market acceptance of the meat [48]. Generally, consumers assume that a bright red colour is indicative of fresh meat (adequate quality). Meat colour can be determined instrumentally by the CIE L* a* and b* system, where a* quantifies redness and L* quantifies luminosity. Together the a* and L* values provide the colour impressions of the meat surface [49]. In our study, the results for L* and a* coordinates in various muscles (34.97 - 38.56 and 17.35 - 19.08, respectively) are between normal values described in the literature for cattle meat [48]. Concerning season, a greater redness was found in samples obtained from animals slaughtered in Winter, compared to samples obtained from animals slaughtered in other seasons. However, this does not seem to be related to low glycogen reserves, because the pH found in samples obtained in Winter (5.70) showed intermediate values compared to the other seasons. The amounts of myoglobin and mitochondrial metabolism can change depending on physiological aspects [50], and the red color of the meat increases as the animals age [51]. Myoglobin and mitochondrial activity in muscle tissue show a mutual regulation mechanism, in which the number of mitochondria can be changed in a cell to meet energy demands [52]. In our study, high b* values were found in animals slaughtered in Winter. In general, the b* values are related to the yellow pigments of meat (carotenoids) and high yellowness are found in meats from pasture-fed cattle [53]. Under these conditions, the visible fat of meat can present a yellow coloration, which can be associated, by the consumer, to meats from mature animals [54].

Regarding fatty acids, the means found for C16:0, C18:0, C18:1c9, total SFA, MUFA, PUFA, C18:1t11 and C18:2c9t11 are consistent with the results described for beef [29,55-59]. Generally, ruminant meats contain high amounts of SFA, which represent about 50 % of total FA, and are associated with several health disorders [60]. Compared with other species, these high amounts of SFA result from the extensive biohydrogenation that occurs with FA C18:1c9, C18:2n-6 and C18:3n-3 [61], but differences in feeding management will have an influence on beef FA composition [56,58,59]. In ruminant fat, rumenic (18:2c9t11) and vaccenic fatty acids (18:1t11) usually appear in amounts representing 60-80 % of total trans fatty acids [61]. These trans fatty acids are related to beneficial health effects, such as cancer prevention, decreased atherosclerosis and improved immune response [55]. On the other hand, consumption of trans FA derived from industrial oil hydrogenation is associated with increased risks of cardiovascular disease [62]. Regarding the n-6 and n-3 PUFA fatty acids of cattle meat, which are of dietary origin, about 60-75% of n-3 PUFA come from pastures and about 53-65% of n-6 PUFA come from grains [63]. In our study, the place of origin of the animals was initially disregarded due to the proximity between the six farms (less than 50 km of distance) and similarity in aspects of breeding and finishing system. However, when the covariate place of origin was included in the experimental model, the coefficient of determination (R^2) showed an important increase, so that the factors included in the model began to explain more accurately the variation factors of the results. Except for C18:0, all fatty acids were influenced by the place of origin.

In this context, for the overall data throughout the year (between seasons), there was no difference in the results of C16:0, MUFA and n-6 PUFA. These results suggest that in this period, the energy available in the metabolism of cull cows and used for fat biosynthesis (C16:0) was similar between seasons. Similar amounts of MUFA suggest that there was not a significant variation in body reserve fat deposition (IMF), and possibly in the processes involved in fat deposition (elongation and desaturation). In turn, similar n-6 PUFA values, suggest that there was no major variation in concentrate intake in terms of quantity and quality (diet ingredients), as expected. In contrast, significant effects of season on fatty acids were found in Spring and Summer. Higher amounts of C18:0 and total SFA were found in Spring and high amounts of C18:1t11, 18:2c9t11 and n-3 PUFA were found in Summer. In young cattle finished in extensive systems and comparing the effect of Spring and Autumn seasons, the results found in the literature are contradictory. High amounts of C16:0, C18:0, trans FA, n-6 and n-3 PUFA were found in Spring in Limousin × Charolais crossbred animals [64]. However, similar values of C16:0, C18:0, trans FA, n-6 and n-3 PUFA were described in Barrosã-PDO breed slaughtered in Spring and Autumn [65].

In general, meats from ruminants finished in extensive system show high amounts of 18:2c9t11, n-3 PUFA and low levels of SFA [27]. However, our data differ from this information and only meats from carcasses obtained in Summer show this lipid profile. Possibly during the Summer period, the animals had access to a greater amount of the natural source for n-3 PUFA, which by biohydrogenation resulted in high amounts of rumenic acid, vaccenic acid and n-3 PUFA in the meat [55]. This is consistent with Boufaied et al. [63], who reported that pastures (legumes or grasses) have a higher percentage of n-3 PUFA in Summer than in Spring, and that forage conservation processes do not significantly reduce n-3 PUFA ratios. In any case, meat from ruminants, particularly lamb and beef, can be a source of n-3 PUFA, with favorable n-6:n-3 ratio, especially when animals are grazing [28]. Regarding n-3 PUFA and the prevention of cardiovascular diseases, the EFSA authorities [66] recommend a daily dose of 250 mg of C20:5n-3 (EPA) plus C22:5n-3 (DHA). In this sense and in our results, meat obtained in Autumn and Summer, with 4.03 % and 4.25 % of IMF and 1.12 % and 2.2 % of n-3 PUFA in total fatty acids (lowest and highest averages), respectively, means that portions of 100 grams/day can provides between 72.96 and 143.31 mg/day of n-3 PUFA or 31.99 and 61.21 mg/d of EPA plus DHA (data not shown), which corresponds to 12.47% and 24.48% of the daily requirements recommended of EPA plus DHA.

Several factors influence the tenderness of the meat. In practical terms, the four most important general factors are postmortem proteolysis, IMF or marbling, connective tissue, and muscle contractile state. These factors explain the great differences found in meat characteristics between species, breeds, age of the animals, influence of technological aspects and types of muscles in the same carcass [48,49,51]. In Charolais steers slaughtered at 14-16 months of age and meat stored at 2 °C until 14 d post mortem, muscles with highest tenderness were *Psoas major* and *Longissumus dorsi* and muscles with lowest tenderness were *Supraspinatus*, *Semitendinosus* and *Biceps femoris* [16]. Our WBSF results for the *Longissimus thoracis* and *Semitendinosus* muscles confirm the literature reports [15,16]. The large difference observed between muscle groups in a carcass possibly results from variations in connective tissue composition, associated with muscle function and the physiological aging process [48]. The *Psoas major* muscle received the best scores for tenderness, juiciness, and overall acceptance in the taste panel. This was expected, since the PM is usually the more tender muscle in the carcass. On the other hand, juiciness is related to the ability of the muscle to release during mastication both its constitutive water (initial juiciness) and the infiltrated fat content (sustained juiciness), and the higher juiciness found in PM meat could be associated with its higher IMF content. Indeed, when meat is tasted, melted lipids generate a broth, which, when combined with water, stimulates the flow of saliva, thus improving the juiciness of meat [67]. The sensory score differences in tenderness between muscles are in line with the WBSF results and could be explained by the difference in cross-links between collagen fibers, which may affect collagen solubility [68] and thus meat tenderness.

Meat tenderness is one of the most important features defining consumer satisfaction when consuming beef. In our study, the WBSF was highest in the

Semitendinosus muscle, intermediate in *Semimembranosus* and Longissimus lumborum muscle and lowest in *Longissimus thoracis* muscle, with differences of about 1 kg between the WBSF of each of the three muscle groups. It was suggested by Destefanis et al. [48] that meat tenderness can be classified in 5 categories according to consumer perception of tenderness and the corresponding WBSF. These categories are as follows: 1) very resistant meat (WBSF>6.38 kg); 2) resistant meat (6.37>WBSF>5.38 kg); 3) meat of intermediate resistance (5.37>WBSF>4.37); 4) tender meat (4.36>WBSF>3.36 kg); 5) very tender meat (WBSF<3.36 kg). Our results indicate that, at 7 d *postmortem*, meat cuts from cull cows raised extensively are mostly in the tougher categories, as only the *Longissimus thoracis* could be considered of "intermediate resistance", while the other cuts were less tender, and the Semimembranosus muscle was in the harder category. It is expected that meat from mature cows will present lower tenderness, which can be ascribed to collagen restructuring and to an increase in the number of thermo-resistant interconnections in this molecule, that leads to noticeable hardness in cow meat. Still, beef tenderness can be improved by ageing for a period of at least 3 weeks, as the decline in WBSF is linear from 5 to 21 days of ageing and drops thereafter [69]. Therefore, even though meat from old cows is unquestionably tougher, some of their meat cuts can be sold as primal cuts rather than as mincemeat, if they are properly aged [70]. Further studies are warranted to clarify these points, particularly for extensively managed cull cows.

Conclusion

Under the conditions of our study, the centesimal composition of cull cows finished throughout the year is quite homogeneous and the IMF varies between 3.72% and 4.25%. Muscles also show homogeneous IMF values, values between 4.15% and 4.56%, except for the *Semimembranosus* (2.4 %). Samples of meat from animals kept in extensive system in the South of Portugal (Mediterranean climate conditions) present high amounts of n-3 PUFA. In Autumn and Summer, meat portions of 100 grams / day with 4.03% and 4.25% of IMF and 1.12% and 2.2% of n-3 PUFA, respectively, can provide between 72.96 and 143.31 mg/day of n-3 PUFA or 31.99 and 61.21 mg/d of EPA plus DHA, which corresponds to 12.47% and 24.48% of the daily requirements recommended of EPA plus DHA. Regarding WBSF, at 7 days *postmortem*, samples from cull beef cows were tough (5.41-6.86 kg), except for the *Longissimus thoracis* (5.13 kg), considered intermediate tough, emphasizing a possible benefit of adequately ageing the cuts. *Psoas major* muscle presented the best scores in sensory assessment for juiciness, tenderness and overall acceptance. However, *Psoas major* was the darkest muscle, with lower luminosity (L*) and higher redness (a*).

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Author Contributions

Conceptualization, M.C.B., J.A.M. and L.T.G.; data collection and curation, M.C.B., J.A.M., A.P.P., J.S.S. and O.M.; statistical analyses, E.M.C.S. and L.T.G.; investigation, M.C.B., J.S.S and L.T.G.; writing-original draft preparation, E.M.C.S. and M.C.B.; writing-review and editing, M.C.B., S.S.J., A.T.B., S.H. and LTG; funding acquisition, M.C.B. and L.T.G. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

Ethical review and approval were waived for this study because it was conducted under commercial conditions that did not involve additional manipulations to those required by the European Union regulations for the transport and slaughter of cattle, which are mandatory.

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