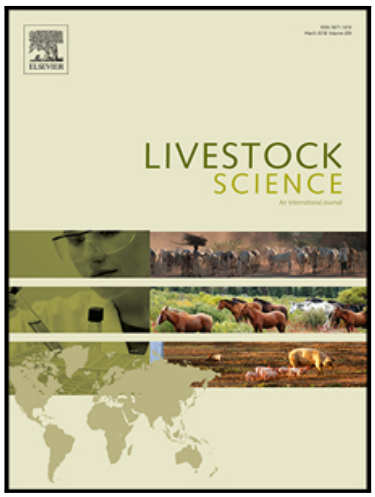

What are the drivers of beef sensory quality using metadata of intramuscular connective tissue, fatty acids and muscle fiber characteristics?

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Highlights

- Cross-links are robustly associated with beef tenderness across experiments
- Cross-links are specifically associated with juiciness and flavour in *Rectus abdominis* muscle
- SFAs and MUFAs are associated with tenderness in *Longissimus thoracis* muscle only
- Lipids and fatty acids (except PUFAs) are associated with juiciness and flavour
- Muscle fibers, except IIA, are weakly associated with beef sensory qualities

What are the drivers of beef sensory quality using metadata of intramuscular connective tissue, fatty acids and muscle fiber characteristics?

Anne Listrat[1*a], Mohammed Gagaoua [1,4**a], Donato Andueza[1], Dominique Gruffat[1], Jérome Normand[2], Guillaume Mairesse[3], Brigitte Picard[1], Jean-François Hocquette[1].

[1] INRAE, Université Clermont Auvergne, VetAgro Sup, Herbivores (UMRH) - UMR1213, F-63122 Saint-Genès-Champanelle, France.

[2] Institut de l'Elevage, Service Qualité des Viandes, 23 Rue Jean Baldassini, F-69007 Lyon, France

[3] Valorex, La Messayais, F-35210 Combourtillé, France

[4]Food Quality and Sensory Science Department, Teagasc, Ashtown Food Research Centre, Ashtown, Dublin, Ireland

[a] Both authors contributed equally to this work

Corresponding authors at

*Dr. Anne Listrat: anne.listrat@inrae.fr

** Dr. Mohammed Gagaoua: gmber2001@yahoo.fr

Abstract

The aim of this integrative study was to investigate the relationships between biochemical traits (total, insoluble and soluble collagens (TCol, ICol, SCol), cross-links (CLs), proteoglycans (TPGs), proportion of fiber types, total lipids (TLips), main fatty acids (FAs) families, the n-6/n-3polyunsaturated FA (n-6/n-3PUFA) ratio and the sensory attributes scores (tenderness, juiciness, flavor) of two muscles from beef: *Rectus abdominis* (RA) and *Longissimus thoracis* (LT). For robust analysis, a database was prepared using samples from three studies from animals raised under different production systems. The analyses were performed either on each study separately or on pooled data per muscle after removing as many studyal effects as possible. The CLs (across the muscles and studies) and, to a lower extent, type IIA muscle fibers (mainly for RA muscles), saturated FAs (SFAs), monounsaturated FAs (MUFAs) (for the LT muscles) were the components most frequently associated with tenderness. The CLs, type IIA muscle fibers (mainly for the RA muscles), TLips, SFAs, MUFAs, conjugated linoleic acids (CLAs) and n-6/n-3PUFA ratio (mainly for the LT muscles) were the components most associated with juiciness. The TLips and CLAs (across the muscles and studies), SFAs, MUFAs (mainly for the LT muscles), CLs (mainly for the RA muscles) and TPGs (mainly for the LT muscles) were the components most associated with flavor. The CLs, CLAs, TLips, SFAs, MUFAs, n-6/n-3PUFA ratio, type IIA and I muscle fibers were the components most frequently associated with the 3 sensory scores taken together. The SCol, TPGs and type IIX+B muscle fibers were little associated with the sensory scores taken together. The TCol, ICol and PUFAs were components least associated with sensory scores. The data of this trial, highlighted for the first time that the CLs were negatively involved in the determination of the three sensory traits mainly in the RA muscles. The muscle fibers in this integrative study had a weak impact on the variations in the beef sensory traits. The type IIA and IIX+B muscle fibers were respectively negatively and

positively associated with the tenderness, negatively associate with the juiciness and flavor. The type I muscle fibers were overall positively associated with the juiciness and flavor and negatively or positively with the tenderness and were muscle and study-dependent. Overall, the TLips and FAs were positively associated with the sensory scores and the n-6/n-3PUFA ratio were negatively associated.

Keywords: Meat quality, Muscle properties, Data integration, Multivariate analyses, Cross-links, Cattle.

1. Introduction

Consumers have a growing demand for meat of high consistent eating quality (hygienic, nutritional, organoleptic). The organoleptic qualities (tenderness, juiciness, flavor) are influenced by the main muscle components of the muscles (Gagaoua et al., 2018; Listrat et al., 2016). The most studied muscular features with a relationship with organoleptic traits, particularly tenderness, are the types of muscle fibers (I, IIA, IIX+B) and intramuscular connective tissues (IMCT), in particular total and insoluble (or soluble) collagen (TCol, ICol, SCol) contents (Gagaoua et al., 2018; Listrat et al., 2016). To a lesser extent, the cross-links (CLs), including the pyridinoline, the main CL of muscle, and proteoglycans (PGs) have been investigated (Dubost et al., 2013b; Mezgebo et al., 2019). The role of these muscle components on the juiciness and flavor is, to our knowledge, much less known than on tenderness. Intramuscular fat (IMF) content, i.e. its content in total lipids (TLips) is well known to have an impact on muscle tenderness, juiciness and beef flavor (Frank et al., 2016; Hocquette et al., 2010). To the best of our knowledge, few authors have considered combined impact of the IMCT components, of muscle fiber type proportions, and TLip content on the

tenderness, juiciness and flavor. However, precise knowledge of the relationships between the muscle components and organoleptic qualities is a prerequisite to understand and control the biological basis of meat quality.

Knowledge on the beef fatty acids (FAs) composition is of growing importance for consumers. Indeed, the FAs influence a range of diseases, including cardiovascular, metabolic such as type 2 diabetes and inflammatory disorders (Calder, 2015). It is worthwhile to note that the results about the relationships between the FAs and sensory attributes of beef meat are contradictory (Cho et al., 2005; Garmyn et al., 2011; Hunt et al., 2016; Hwang and Joo, 2017, 2016).

In this context, the aim of this integrative study was to identify the most generic relationships between the main components of IMCT (TCol, ICol, SCol, CLs, PGs), the proportion of fiber types, the TLips, the main families of FAs and the major beef sensory attributes. To do so, three independent studies were utilised to build a database composed of samples from two muscles (*Rectus abdominis* and *Longissimus thoracis*) from animals raised under different studyal conditions.

2. Material and methods

2.1. Beef production and muscle sampling

The studyal procedures and animal holding facilities respected the French animal protection legislation, including licensing of experimenters. They were controlled and approved by the French Veterinary Services (slaughterhouse and experimental facilities).

2.1.1. Experimental designs and samples

Study 1 (St1): All the experimental procedures performed in this study were approved by the Animal Ethics Comittee of INRA-CIRAD-IFREMER (APAFIS#1765-2015091516305 V3). The study was performed on 32 young Charolais bulls housed in straw bedded pens. The animals were assigned a 6 months basal diet (60% hay and 40% concentrate). They were slaughtered at, on average, 18 months old with a final live weight of 736±38.46 kg.

Sudy 2 (St2): This study was part of the European « ProSafeBeef » Integrated Project (FOODCT-2006-36241). The study was performed on 40 young purebred bulls, Aberdeen Angus (n = 12), Limousin (n = 14) and Blond d'Aquitaine (n = 14), housed in straw bedded pens. Animals were assigned to a 100 days finishing period (75% concentrate and 25% straw). They were slaughtered at, on average, 17 months old with a final live weight of 670±47.32 kg. This experimental protocol was previously described by Dubost et al. (2013a).

The animals of the studies 1 and 2 were raised and slaughtered at the studyal facilities and slaughterhouse of the INRA Research center (license numbers #63 345 01 and #63 345.17, respectively).

Study 3 (St3): This study was performed on 52 animals. They came from 16 different, non-experimental farms. The animals were of two breeds (dairy Holstein and beef Charolais and Rouge des Prés) and of four genders: young bulls (n=15), heifers (n=9), steers (n=6) and cows (n=22). The young bulls were slaughtered at about 19 (for beef animals) and 21 months (for dairy animals) of age, steers at about 35 months, heifers at about 32 months and cows between 6 and 8 years. Diets mainly consisted of grass or concentrate and forage (grass or corn silage, straw). The animals were slaughtered in the same abattoir (Le Lion d'Angers, France, license number #49 176 001), following the same conditions for slaughter and carcasses management. This experimental protocol was previously published by Listrat et al. (Listrat et al., 2020a).

2.1.2. Muscle sampling

For the three studies (n=124 animals), samples were taken between 24 and 48h post-mortem. For the St1 (n=32) and 2 (n=40), samples of *Longissimus thoracis* (LT) muscles were collected between the 5th rib and 9th rib. For the St1 (n=32) and 3 (n=52), samples of *Rectus abdominis* (RA) muscles were removed from the middle part of the muscle. Samples for one analysis were always taken at the same anatomical position from animal to animal and study to study. Carcasses were chilled in a cold room (+2°C). For the sensorial evaluations of the three studies, the meat samples were aged in vacuum-packs at +4 °C for 7 (St1 and 3) or 14 days (St2) according to the study then stored at −20 °C until analyses.

2.2. Biochemical characteristics of Intramuscular Connective Tissue

For proteoglycan (PG), muscle samples (60–80 g) were taken 15 min after exsanguination, of the livestock, close to the piece used for sensory analyses, cut up into small pieces, frozen and powdered in liquid nitrogen, then stored at −80 °C until analysed. For collagen, CLs, lipid and FA measurements, muscle samples (about 150 g) were taken at 24 h post-mortem. They were cut into small pieces, frozen, ground in liquid nitrogen with a mixer grinder (Retch MM 301, Hann Germany) to produce a fine homogeneous powder and then stored at −80 °C until analysed. For collagen and CL measurements, samples, after grinding in liquid nitrogen, were freeze-dried for 96 h in a freeze-drier (Cryotec, France), pulverized in a horizontal blade mill and finally stored at+4 °C in stopper plastic flasks until analysed.

2.2.1. Total, insoluble (soluble) collagen and cross-link measurements

For the TCol and CL contents, about 250 mg of muscle powder were weighed in duplicate, acid hydrolysed with 20 mL of 6 N HCl, overnight, at 110°C in a screw-capped glass tube. The acid hydrolysate was diluted 5 times in 6 N HCl. For ICol, muscle powder (250 mg) was weighed in duplicate and rehydrated for one hour with solubilization buffer (0.23 M NaCl, 25 mM Tris–HCl, pH 7.4) and heated in a water bath at 75°C for one hour. The soluble fraction was separated from the insoluble fraction by filtration (pleated filters in cotton cellulose, VWR 512–0206) and discarded. Insoluble fraction was hydrolysed according to the same method as for TCol content. Hydroxyproline content was measured in TCol and ICol hydrolysates according to the procedure previously described by Dubost et al. (Annabelle Dubost et al., 2013a). The data were expressed in mg of hydroxyproline per g of dry matter (mg OH-pro g-1 DM). OH-Prol was not measured in the soluble fraction, because of the low concentrations that made the determination imprecise, but was determined as follows: Soluble Collagen (SCol) = (TCol-ICol)/TCol)* 100. The CLs were determined by the enzyme-linked immunoassay Metra Pyd EIA kit (Quidel Corporation, USA) according to the manufacturer procedure adapted by Dubost et al. (2013a) for muscular tissues. The results were expressed in nM of pyridinoline per g of DM (nM pyr g^{-1} DM).

2.2.2. Total proteoglycan content

Total PGs (TPGs) content was measured according to procedure adapted by Dubost et al. (2013a). Each sample was measured twice and data were expressed in µg of glycoaminoglycans (GAGs) per g of DM (µg GAGs g-1 DM).

2.3. Myosin heavy chains isoforms quantification by electrophoresis

Myosin heavy chain (MyHC) isoforms were separated with sodium dodecyl sulfate glycerol gel electrophoresis according to Picard et al. (2011)'s method. After migration, the gels were fixed in 30% (v/v) ethanol and 5% acetic acid (v/v) and then stained with colloidal Coomassie Blue R250 for 24 h. After destaining, the gels were scanned and the proportions of the different MyHC bands were quantified by densitometry with ImageQuant Software5500 (Amersham Biosciences/GE Healthcare). The quantification of the bands revealed the existence of MyHC-IIB isoform was found in only 5 animals. Therefore, MyHC-IIB percentage were added to those of MyHC-IIX as described by Gagaoua et al. (2015) creating a new variable, muscle fiber type IIX+B.

2.4. Intramuscular fat content and fatty acid composition

Total lipid contents (TLip) contents were estimated by NIRS using the model reported by Andueza et al. (2019) for lyophilised samples. A total of 88 samples were analysed by the reference method (Folch et al., 1957) to validate the NIRS predictions. The model used was characterised by the following statistics: R2V(validation)=0.93; standard error of prediction (SEP)= 1.01g/100g fresh samples.

Fatty acid extraction and transmethylation into fatty acid methyl esters (FAME) were subsequently performed according to Scislowski et al. (2005). Fatty acid methyl ester analysis was performed with GLC using a Peri 2100-chromatography system (Perichrom Society, Saulx-les-Chartreux, France) fitted with a CP-Sil 88 glass capillary column (Varian, Palo Alto, CA; length = 100 m; diam. = 0.25 mm). The carrier gas was H2, and the oven and flame ionization detector temperatures described by Scislowski et al. (2004) were used. Total FA were quantified using C19:0 as an internal standard. The identification of each individual FAME and the calculation of the response coefficients for each individual FAME were

performed using the quantitative mix C4-C24 Fame (Supelco, Bellafonte, PA). Data were expressed in mg per 100g of fresh matter.

2.5. Sensory analysis

After thawing at 2 to 5 ° C in vacuum packs for at least 24 h before cooking, muscles of St1 and 2 were cut into pieces of 1.5 cm in cross-section then grilled up to an internal temperature of 55°C. Muscles of St3 were cut into pieces of 3 cm in cross-section and cooked in an oven to 250°C. They were removed, as for studies 1 and 2, at an internal temperature of 55 °C. The cooking method used (cooked in an oven for St2 as opposed to grilling for St1 and 3) was without consequence on the results as shown by Lawrence et al. (2001) and tested in our laboratories (result not shown). The samples of each study were presented in sequential monadic sessions involving 12 panellists. At each sensory session, the 12 panellists evaluated 6 samples of the same muscle, randomly selected. The expert panellists were trained in accordance with the ISO standards ISO/TC as described by Gagaoua et al. (2016). The panellists rated global tenderness, juiciness and flavor of the meat on a continuous scale scored from 0 to 10, where 0 refers to extremely tough, dry, not tasty and 10 to extremely tender, juicy, tasty. During sessions, panelists were randomly seated in individual booths, in a sensory analysis room, equipped with individual booths under artificial red light to reduce the influence of the appearance of the samples. The panelists were provided water and unsalted crackers to clean their palate. Each tasting booth was equipped with computer terminal linked to a fileserver running a sensory software (Fizz, version 2.20h; Biosystemes, Couternon, France) that facilitated the direct entry of assessor ratings.

2.6. Statistical analysis

The data from the three studys were analyzed together using XLSTAT 2017.19.6 (AddinSoft, Paris, France). Normal distribution and homogeneity of the dataset was tested by Shapiro–Wilk test ($P > 0.05$). Data were standardized in the same way for all studies to remove the effects of muscle (St. 1), of breed (St. 2) as suggested by Gagaoua et al. (2015) and of breed and gender (St. 3) as in Listrat et al. (Listrat et al., 2020a), which are the major parameters affecting eating quality of beef. The standardization of the data was based on Z-scores, which represent the number of standard deviations for each observation relative to the mean of the corresponding data group amongst the studied factors of each study. Therefore, after this transformation, the data had a mean of 0 and standard deviation of 1. Subsequently, Partial Least Squares (PLS) regression based on Z-scores for the whole variables of the database were used to explain tenderness, juiciness, and flavor traits for each muscle using IMCT, IMF and muscle fiber characteristics. The PLS method aimed to predict data by relating two data matrices X and Y to each other, where in our case, the X consists in the explanatory variables (X-matrix, 14 variables) and Y consists in sensory beef quality traits (Y-matrix, 3 variables). The relationships for each sensory quality (or PLS models) were built first per muscle of each study and second per muscle across studys (St1 + St2 = LT muscle or St1 + St3 = RA muscle). The filter method with the variable importance in the projection (VIP) set at the level of VIP > 0.8 was used for variable selection as described by Gagaoua et al. (2019). For the selection of the variables, the jack-knife method was included in the PLS regression as a selective parameter. Finally, for all the entered variables in the PLS, the standardized regression coefficients (β) were further given.

3. Results

The variability of raw data for the LT muscles from St 1 (n=32) and 2 (n=40) was illustrated in Table 1. Briefly, it was noted that the the LT muscles from the first two studies had

equivalent amounts of TCol, an equivalent n-6/n-3PUFA ratio and tenderness. The LT_St1 had less ICol, TPGs, was less glycolytic, more oxydo-glycolytic (less IIX+B and more IIA muscle fibers), less juicy and more tasty than the LT_St2. On the contrary, the LT_St1 had more SCol, more CLs, TLips, SFAs, MUFAs, PUFAs and CLAs. Overall, coefficients of variations for IMCT components, TLips and FAs were higher for the LT_St2 than for the LT_St1. The variability of raw data for the RA muscles from St 1 (n=32) and 3 (n=52) was illustrated in Table 2. The RA muscles from the St1 and 3 had equivalent amounts of ICol, CLs, TPGs, PUFAs, and of type IIA and IIX+B muscle fibers. On the contrary, the RA_St1 had, on average, more TCol, SCol, a higher n-6/n-3PUFA ratio and less of the other components than the RA_St3. The RA_St3 was, on average, more tender, juicier, tastier. Overall, the coefficients of variation of the RA_St3 were higher for TCol, CLs, PUFAs, the proportions of muscle fiber types and the tenderness.

3.1. Relationship between muscle components and tenderness

The relationships between the muscle components and tenderness are illustrated in Tables 3 and 4. The PLS models explained between 32% (RA_St3) and 66% (LT_St1) of the tenderness variability. The following results were summarized in Table 5. The CLs were the main drivers of tenderness (they were significantly and negatively retained 4 times in PLS models of tenderness, once for each LT muscle and once for each RA muscle. This result is indicated in the “Fr” column inside “tenderness” column). The CLs were followed by type IIA muscle fibers (significantly retained 3 times) then by SFAs, MUFAs, n-6/n-3PUFA ratio, CLAs, type I and IIX+B muscle fiber types (significantly retained twice). The parameters that had the least impact were the TCol, ICol, SCol, TPGs, TLips, PUFAs (retained once). The TCol, ICol, SCol and TPGs were also negatively associated with tenderness but were muscle and study-dependent (TCol and SCol for LT_St1, ICol and TPGs for RA_St3). The association between the muscle fiber types and the tenderness was also muscle and study-

dependent. The type IIA muscle fibers were negatively associated with the tenderness of the LT_St1 and of the two RA muscles, while the type IIX+B were positively associated with the tenderness of LT and RA_St1. The type I muscle fibers were negatively associated with the tenderness of LT_St1 and positively with tenderness of the RA-St3.

The TLips or FAs were frequently associated with the tenderness of LT compared to RA, i.e, the SFAs and MUFAs were positively associated with tenderness of the two LT muscles, TLips and PUFAs with the tenderness of the LT_St2, CLAs with tenderness of the LT_St2 and RA_St1 and n-6/n-3PUFA ratio positively with tenderness of the LT_exp2 and RA_St1. The TLips, SFAs, MUFAs and PUFAs were not associated with the tenderness of RA muscles.

3.2. Relationship between muscle components and juiciness

The relationships between the muscle components and the juiciness were illustrated in Tables 6 and 7. The PLS models explained between 36% (LT_St2) and 57% (RA_St1) of the juiciness variability. The following results were summarized in Table 5. The main drivers of the juiciness were the CLs, type IIA muscle fibers, TLips, SFAs, MUFAs, n-6/n-3PUFA ratio, CLAs (they were significantly retained 3 times in PLS models of juiciness for both LT and RA muscles (column Fr inside column juiciness) followed by SCol and type I muscle fibers (significantly retained twice). The measurements that had least impact were the ICol, TPGs, IIX+B muscle fibers, PUFAs. The TCol was not retained. The CLs were negatively associated with the juiciness of the RA muscles from St1 and 3 and of the LT_St2. The ICol was positively associated with juiciness of the LT-St1, SCol negatively with juiciness of the LT_St1 and RA_St3 and TPGs negatively associated with juiciness of the LT_St2. The type I muscle fibers were positively associated with juiciness of the RA muscles from St1 and 3, but

not of LT muscles from St1 and 2. The type IIA fibers were negatively associated with the juiciness of the RA muscles from St1 and 3 and of LT_St2. The type IIX+B fibers were not associated with juiciness of the LT muscles but negatively associated with the juiciness of the RA_St3. The TLips were positively associated with the juiciness of the LT muscles from St1 and 2 and of RA_St3. The SFAs, MUFAs and CLAs were also positively associated with the juiciness of the LT muscles from St1 and 2 and of RA_St1. The PUFAs were only positively associated with the juiciness of the RA_St1 and n-6/n-3PUFA ratio was negatively associated with the juiciness of the LT muscles from St1 and 2 and of RA_St3.

3.3. Relationship between muscle components and flavor

The relationship between the muscle components and the flavor were illustrated in Tables 8 and 9. The PLS models explained between 36% (for LT_St2) and 63% (for LT_St1) of the flavor variability. The following results were summarized in Table 5. The main drivers of flavor, Tlips, CLAs (they were signicantly retained 4 times in PLS models of flavor for both LT and RA muscles (column Fr inside column flavor) followed by CLs, TPGs, SFAs, MUFAs (significantly retained 3 times), SCol, type I muscle fibers and n-6/n-3PUFA ratio (significantly retained twice). The measurements the least associated were the ICol, IIA, IIX+B muscle fibers and PUFAs, all retained once. The total collagen (TCol) was not retained. The cross-links were negatively associated with the flavor of the two RA muscles and of LT_St2 and the TPGs positively associated with the flavor of the LT muscles from St1 and 2 and negatively with the flavor of the RA St_3. The ICol were negatively associated with the flavor of the LT_St1. The SCol were negatively associated with the flavor of the LT_St1 and of the RA_St3. The type I muscle fibers were positively associated with the flavor of the LT_St2 and RA_St3 and the type IIA and IIX+B, negatively with the flavor of the RA_St3. The TLips and CLAs were positively associated with the flavor whatever the muscles and the studies. The SFAs and MUFAs were associated with the flavor of the two LT muscles but

only of RA_St1. The PUFAs were associated with the flavor of LT_St2 and the n-6/n-3 PUFA ratio negatively with the flavor of the LT and RA_St1.

4. Discussion

Many authors have identified variability in eating quality, especially the tenderness, as one of the primary causes influencing consumers' desire to not re-purchase meat (Maltin et al., 2007). Consequently, only some authors have taken into account the juiciness and flavor in the studies on beef sensory qualities. However, recently, O'Quinn et al. (2018) and Liu et al. (2020) indicated that flavor and to a lower extent juiciness also need to be taken into account in evaluation of overall palatability. The sensory scores (tenderness, juiciness, flavor) are influenced by the main components of muscle tissues *i.e.* the IMCT, IMF and muscle fibers (Gagaoua et al., 2018; Listrat et al., 2016). The development of a beef quality guarantee system may rely on muscle profiling research (Chriki et al., 2013; Seggern et al., 2005). It is the reason why, in this study, we highlighted the role of different components of the IMCT (mainly CLs, but also collagen, TPGs), the proportions of muscle fiber types and IMF (TLips and main fatty acids families) on the beef sensory scores (tenderness, juiciness and flavor). To demonstrate, among others, the important role of CLs, we used three very different data sets from various groups of livestock. The first one was composed of Charolais young bulls, the second one was composed of Aberdeen Angus, Limousin and Blond d'Aquitaine young bulls. The main differences between these two groups was the duration of ageing of their meat (7 days for the first group *vs* 14 days for the second). Meat from LT muscle of animals of the first and second group had an equivalent tenderness. Meat from LT muscle of animals of the first therefore should have been harder than that of animals of the second group due to the difference of ageing. The LT muscles of animals of the first group contained more soluble collagen, less insoluble collagen (probably due to the high average daily weight gain of

animal: >2kg/day (result not shown)) (Archile-Contreras et al., 2010; Fishell et al., 1985) than the animals of the second group. The LT muscles of the livestock of the first group contained also more total lipids and fatty acids. As shown by Fishell et al. (1985) and Nishimura (2010) these differences in amounts of soluble, insoluble collagen and of lipids probably resulted in increased tenderness of meat from LT muscle of animals of the first group which compensated their lower tenderness potential compared to the second group. The third group was composed of an heterogeneous set of animals (young bulls, heifers, cows and stears of three breeds) from several non experimental farms that had been mainly raised in pasture or consigned and fed with different forage. Feeding (for study 3, pasture, grass or corn silage, concentrate *vs*, for study 1, hay and concentrate), age and sexe differences, associated to their high average DWG, could explained the fact that the RA muscles of study 1 contained more collagen and were less oxydative (Monin, 1991). To get rid of main differences between studies, all data were normalized per study but also per breed, sex and cut.

4.1. Impact of IMCT on sensory quality traits with a particular focus on a thermo-stable cross-links, pyridinoline

Collagen is the main protein of IMCT. The collagen fibers are stabilized by inter- and intra-molecular CLs. In adult muscle, there are different types of CLs, including the pyridinoline which is the main thermo-stable CL (Kuypers and Kurth, 1995). The results in the literature on the role of CLs on the tenderness are contradictory (Lepetit, 2007). We previously used data of St2 and 3 to study the role of muscle components, including CLs, on tenderness (Dubost et al., 2013b; Listrat et al., 2020b). The present results confirm the key role of CLs on tenderness of LT and RA muscles. For the first time, the data of this trial provide the evidence that CLs have an impact on the juiciness and flavor, more marked in the RA muscles than LT muscles, perhaps because RA muscles had more CLs than the LT muscles. Dubost et al.

(2013b) had already attempted, by using samples of St2, to show if there was a relationship between the CLs, the juiciness and flavor, but failed to find an association. This difference of result is because Dubost et al. (2013b) had worked on raw data and not on normalized data. To the best of our knowledge, there are no data in the litterature to explain the role of CLs on juiciness and flavor. However, the CLs could affect the juiciness and flavor via relationships that they have with the PGs and lipids. The proteoglycans interact with water molecules (Iozzo and Schaefer, 2015) to create a water compartment around collagen matrix and participate to create (Reese et al., 2013), in association with the CLs (Depalle et al., 2015), a specific force necessary for the adipocytes to differentiate (Cristancho and Lazar, 2011). The proportions of CLs and PGs together with the lipids in muscles could influence the sensory scores.

Bovine muscles contain between 1-10% of collagen (in % of dry matter). Elastic modulus of raw collagen fibers is comprised between 0.5–1 GPa, which gives them a high stiffness (Lepetit, 2008). This has contributed to several authors over several decades to investigate the possible negative impact of TCol on meat tenderness. This study allowed to highlight the consistencies and divergences among studies as described by Lepetit (2007) and to identify the main robust variables to explain beef sensory quality traits. For example, the results of the present paper show that the TCol, ICol and SCol have a negative impact on meat tenderness as in Jeremiah et al. (2003) and Chriki et al. (2012)'s studies, but that their role was muscle- and experimental design-dependent. These results are a confirmation of Holman et al. (2020) statements that muscle collagen content is not a suitable predictor of the tenderness. These authors explained this result by the fact that, as reported by several authors (Jeremiah and Martin, 1981; Starkey et al., 2016) and in this paper, the role of collagen (total, insoluble, soluble) on tenderness is very dependent on the cattle population and on the muscle. In addition, Holman et al. (2020) indicated that cooking temperature could negate the

contribution of collagen to tenderness. In the present study, the negative effect of TCol, ICol and SCol can indeed be explained by the cooking temperature used (55°C). As a matter of fact, complete denaturation of collagen and its gelatinization occurs between 60 and 70°C (Tornberg, 2005). Below 55°C, the beneficial effect of gelatin on tenderness (Chang et al., 2011) does not occur.

In IMCT, collagen makes up a network of fibers embedded in a matrix of PGs. The present study showed that TPGs have a negative effect on tenderness and juiciness, which are muscle and livestock dependent. TPGs had also, according to muscle and study, a positive or negative effect on flavor. This result could be linked to the property of PGs to retain water and to their possible relationships with the TLips and CLs (Cristancho and Lazar, 2011; Depalle et al., 2015; Reese et al., 2013), components that also affect sensory scores. We hypothesise that cooking temperature could act on PGs properties and modify their capacity to hold water by decreasing the amount of water retained in meat and thus juiciness perception.

4.2. Role of muscle fiber types on sensory traits

The results of the present study confirmed the complex relationships between tenderness and muscle fibers observed in various studies (Chriki et al., 2012; Listrat et al., 2020b; Listrat et al., 2016). For the RA muscle, among the muscle fibers, a robust negative relationship (for the two considered studies) was observed between IIA fibers and tenderness. This result was in accordance with several authors who have shown negative associations between IIA fibers and tenderness in different muscles (Chriki et al., 2012; Jurie et al., 2007). The results for the relationship between tenderness and type IIX+B, in this study, were opposite to those of Picard et al. (2014) who showed for the *Longissimus thoracis* muscle (fast oxido-glycolytic muscle) that higher degrees of fast glycolytic properties are associated with lower tenderness,

this relationship being more or less related to the breed. The contradiction between Picard et al. (2014) results and those of this study are due by the fact that the results of this study were analysed irrespective of breed while those of Picard et al. (2014) were analyzed across breeds.

Overall, type IIA and IIX+B muscle fibers were negatively associated with juiciness mainly in RA muscles, whereas type I fibers were, rather, positively associated with juiciness and flavor. This relationship between juiciness and the slow oxidative fibers (type I) have already been described by Waritthitham et al. (2010). The relationship between type I muscle fibers and flavor can be probably explained by the high phospholipid content of type I fibers, since phospholipids are a major determinant of the flavor of cooked meat (Gandemer, 2002). Another explanation could be that high levels of type I muscle fibers would induce high free amino acid contents in muscles that would contribute to intense flavor possibly because of a greater oxidative metabolism (Mashima et al., 2019).

4.3. Role of total lipids and fatty acid composition on sensory traits

The results of our study confirmed that TLips content played a positive role on meat tenderness (muscle and study dependent), juiciness (muscle and study dependent but more marked for LT muscle) and flavor (across muscles and studys). TLips (their adipocytes) would affect indirectly tenderness (Hocquette et al., 2010). Adipocytes, which develop in the perimysium (between muscle fiber bundles), would cause the remodelling of ECM and reduce the mechanical strength of IMCT, contributing to the tenderization of beef (Nishimura, 2010; Roy et al., 2018). We hypothesise that the LT muscles have a thin and slightly branched endomysium and perimysium compared to other muscles (Dubost et al., 2013a), their endomysium and perimysium would be more fragile and then probably easier to break when the amounts of TLips increase, modifying the feeling of tenderness. On the contrary, the

TLips might directly affect juiciness and flavor. When the amount of TLips increases, the water-holding capacity of meat, with which lipids are positively correlated, would also increase (Joo et al., 2002), which could lubricate the muscle fibers during cooking and thus increase the apparent sensation of juiciness. It could also stimulate salivary flow during mastication (Smith and Carpenter, 1974). The mechanism by which the TLips contribute to flavor is well known. Cooked meat characteristic aroma are derived from volatile components thermally induced during Maillard reactions, lipid oxidation and vitamin degradation (Van Ba et al., 2012). The results of this integrative study highlighted a positive role of SFAs and MUFAs on the tenderness, juiciness and flavor of the LT muscles and on the juiciness and flavor of RA muscles in some some experiments and, overall, a negative of n-6/n-3PUFA ratio on sensory qualities. Other authors have studied the relationships between the different FA families and sensory scores and have obtained contradictory results, equivalent or opposite to those of this study. Over five studies (Cho et al., 2005; Garmyn et al., 2011; Hunt et al., 2016; Hwang and Joo, 2017, 2016), three authors showed, as in the present study, a positive relationship between MUFAs (Garmyn et al., 2011; Hunt et al., 2016; Hwang and Joo, 2017), SFAs (Cho et al., 2005; Hunt et al., 2016; Hwang and Joo, 2016) and sensory parameters while four authors showed an opposite relationship between PUFAs and sensory parameters (Cho et al., 2005; Garmyn et al., 2011; Hwang and Joo, 2017, 2016). This was probably due to differences in the muscles, animal types, temperature and cooking modes used or still consumer habits and preferences. The FAs are involved in nutritional quality, some are not beneficial for its improvement, such as the SFAs, others, such as the MUFAs, CLAs or a low n-6/n-3PUFA are beneficial. For SFAs, nutritional recommendations around the world suggest that their intake has to be kept low, while, on the contrary, an increase of unsaturated fatty acids and CLAs and a decrease of n-6/n-3 PUFA ratio in diets should be beneficial for health (Vahmani et al., 2015). Then, it should be possible to improve both

sensory and nutritional quality of meat by changing the composition in FAs by utilising specific breeding protocols.

5. Conclusion

The original statistical approach applied of this integrative study highlighted a preponderant role of CLs in determining tenderness whatever muscle and livestock and in juiciness and flavor mainly for RA muscle. On the contrary, TCol had not a preponderant role in tenderness score since it was associated with tenderness for only one of the LT muscles in this study. TCol had no role in juiciness and flavor. In contrast, ICol and SCol that were associated with tenderness, juiciness and flavor depending on the muscle and study. The three types of muscle fibers were associated with tenderness, juiciness and flavor, but less frequently than the CLs or TLips, SFAs, MUFAs, CLAs. Type IIA muscle fibers were the type of fibers that were the most associated with the three sensory scores. SFAs, MUFAs CLAs and to a lesser extent n-6/n-3PUFA ratio had a preponderant relationship with tenderness, juiciness and flavor mainly in LT muscles.

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Declaration of competing interest

There are no conflicts of interest to declare

Author Contributions: A. Listrat conceived and designed this study, analysed the data, prepared the tables and figures, wrote the paper, and approved the final draft. M. Gagaoua participated in the analyses of the data, reviewed the drafts of the paper and approved the final draft. D. Andueza performed the SPIR measurements on meat samples, participated in the data analyses and reviewed the drafts of the paper, D. Gruffat conceived study 1 and with B. Picard supervised measurements on meat samples and reviewed drafts of the paper, J. Normand and G. Mairesse co-conceived study 3 and J.F. Hocquette was responsible of the study 2 and reviewed the final draft of the paper.

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Declaration of competing interest

We certify that the submission is original work and is not under review at any other publication and there are no conflicts of interest to declare.

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Table 1: Least squares means, coefficient of variation (CV) and range (min–max) of the bovine *Longissimus thoracis* muscle characteristics of studies 1 (St1) (Charolais young bulls) and 2 (St2) (Aberdeen Angus, Limousin and Blond d'Aquitaine young bulls). The analyses have been realized on n=72 animals (n=32 and n=40 for St1 and 2).

Variables	Mean LT_St1	CV	Range	Mean LT_St2	CV	Range
IMCT						
TCol	3.87 [a]	0.25	2.66-7.12	4.15 [a]	0.23	2.94-7.15
ICol	1.55 [b]	0.15	1.19-2.05	3.03 [a]	0.27	2.01-5.34
SCol	58.68 [a]	0.12	43.69-71.29	28.59 [b]	0.42	8.46-65.97
CLs	23.54 [a]	0.11	18.92-29.67	18.97 [b]	0.18	13.93-26.88
TPGs	185.12 [b]	0.31	110.33-308.06	629.34 [a]	0.25	340.34-903.24
Muscle fibers						
I	24.90 [a]	0.22	11.00-36.22	23.57 [a]	0.19	12.26-37.20
IIA	62.02 [a]	0.13	43.72-78.28	35.05 [b]	0.45	16.47-63.87
IIX+B	13.51 [b]	0.64	1.48-36.16	41.38 [a]	0.42	2.52-63.84
Tlips and FAs						
TLips	3.00 [a]	0.43	0.51-6.28	2.01 [b]	0.68	0.78-6.06
SFAs	1285.73 [a]	0.49	541.41-3158.20	585.67 [b]	0.91	106.53-2121.07
MUFAs	1034.68 [a]	0.48	435.39-2459-36	550.57 [b]	0.96	96.20-1975.96
PUFAs	293.66 [a]	0.18	161.50-420.83	195.47 [b]	0.24	147.05-358.24
n-6/n-3PUFAs	4.93 [a]	0.12	3.59-5.97	4.73 [a]	0.32	2.46-8.57
CLAs	12.73 [b]	0.50	5.20-33.73	5.55 [a]	0.94	0.61-19.23
Sensory parameters						
Tenderness	4.69[a]	0.10	3.72-5.85	4.68[a]	0.15	3.17-6.11
Juiciness	3.29[b]	0.09	2.27-3.86	4.68 [a]	0.08	3.47-5.30
Flavor	4.54 [a]	0.07	3.83-5.16	3.89 [b]	0.12	2.88-4.92

Abbreviations: IMCT: Intra Muscular Connective tissue; TCol: Total Collagen (mg OH-proline/g dry matter); ICol: Insoluble Collagen (mg OH-proline/g dry matter); SCol: Soluble Collagen (%); CLs: Cross-Links (nM pyridinoline/g dry matter); TPGs: Total Proteoglycans (µg chondroitin 4-sulfate/g dry matter); FAs: Fatty Acids; TLips: Total Lipids (mg/100g fresh matter), SFAs: Saturated Fatty Acids; MUFAs: Monounsaturated Fatty Acids; PUFAs: Polyunsaturated Fatty Acids; n-6/n-3PUFAs: n-6/n-3Polyunsaturated Fatty Acids; CLAs: Conjugated Linoleic Acids.

All fatty acids were expressed in mg/100g fresh matter.

Intensities of tenderness, juiciness and flavor of the meat were scored on a continuous scale from 0 to 10, where 0 refers to extremely tough, dry, not tasty and 10 to extremely tender, juicy, tasty.

Least squares means in the same row for muscle of each experiment not followed by a common letter differ significantly, $P<0.05$.

Table 2: Least squares means, coefficient of variation (CV) and range (min–max) of the bovine *Rectus abdominis* muscle characteristics of studies 1 (St1) (Charolais young bulls) and 3 (St3) (Holstein, Charolais and Rouge des Prés young bulls, heifers, steers and cows). The analyses have been realized on n=84 animals (n=32 and n=52 for St1 and 3).

Variables	Mean RA_St1	CV	Range	Mean RA_St3	CV	Range
IMCT						
TCol	6.19 [a]	0.18	3.89-8.12	4.57 [b]	0.24	2.68-7.27
ICol	2.52 [a]	0.24	1.30-4.06	2.55 [a]	0.26	1.63-4.51
SCol	59.43 [a]	0.11	44.47-69.89	35.87 [b]	0.25	15.03-52.55
CLs	31.52 [a]	0.16	23.92-46.53	30.13 [a]	0.26	19.70-57.62
TPGs	154.47 [a]	0.34	49.62-290.17	178.95 [a]	0.26	83.69-266-36
Muscle fibers						
I	30.37[b]	0.23	16.50-46.92	36.08[a]	0.27	13.40-63.09
IIA	45.68 [a]	0.16	31.62-64.96	41.97 [a]	0.27	18.02-74.52
IIX+B	24.74 [a]	0.42	0.68-44.40	21.94 [a]	0.50	0.00-46.63
TLips and FAs						
TLips	2.25 [b]	0.61	0.11-5.95	5.28 [a]	0.37	3.38-13.37
SFAs	1326.35 [b]	0.45	483.46-2756.72	2064.20 [a]	0.41	1173.41-5550.02
MUFAs	1173.02 [b]	0.46	372.64-2205.34	2043.30 [a]	0.46	1002.69-5834.08
PUFAs	388.20 [a]	0.23	244.68-648.29	380.42 [a]	0.30	221.25-719.47
n-6/n-3PUFAs	4.95 [a]	0.13	3.69-6.65	3.87 [b]	0.32	1.74-6.88
CLAs	15.91 [b]	0.44	5.69-31.92	22.49 [a]	0.36	11.56-49.58
Sensory parameters						
Tenderness	4.85 [b]	0.11	4.01-6.14	5.49 [a]	0.21	3.47-8.42
Juiciness	3.83 [b]	0.11	2.90-4.77	5.72 [a]	0.07	4.93-6.43
Flavor	4.92 [b]	0.06	4.11-5.53	5.91 [a]	0.07	4.80-6.87

Abbreviations: IMCT: Intra Muscular Connective tissue; TCol: Total Collagen (mg OH-proline/g dry matter); ICol: Insoluble Collagen (mg OH-proline/g dry matter); SCol: Soluble Collagen (%); CLs: Cross-Links (nM pyridinoline/g dry matter); TPGs: Total Proteoglycans (µg chondroitin 4-sulfate/g dry matter); FAs: Fatty Acids; TLips: Total Lipids (mg/100g fresh matter), SFAs: Saturated Fatty Acids; MUFAs: Monounsaturated Fatty Acids; PUFAs: Polyunsaturated Fatty Acids; n-6/n-3PUFAs: n-6/n-3Polyunsaturated Fatty Acids; CLAs: Conjugated Linoleic Acids.

All fatty acids were expressed in mg/100g fresh matter.

Intensities of tenderness, juiciness and flavor of the meat were scored on a continuous scale from 0 to 10, where 0 refers to extremely tough, dry, not tasty and 10 to extremely tender, juicy, tasty.

Least squares means in the same row for muscle of each experiment not followed by a common letter differ significantly, $P<0.05$.

Table 3: Ranking of the retained variables in tenderness PLS models, according to their variable importance in projection (VIP) for the *Longissimus thoracis* (LT) muscle of studies 1 (St1) (Charolais young bulls) and 2 (St2) (Aberdeen Angus, Limousin and Blond d'Aquitaine young bulls). Only the VIP > 0.8 (in bold) were considered as significant. The analyses were carried out using Z-scores. Coefficient (β) indicates if the variables are involved positively or negatively in the models. The analyses have been realized on n=72 animals (n=32 and n=40 for St1 and 2).

	Tenderness								
	LT_St1 R^2=0.66			LT_ St 2 R^2=0.49			LT_ St 1/ St 2 R^2=0.53		
	Rank	VIP	β	Rank	VIP	β	Rank	VIP	β
Variable									
IMCT									
TCol	2	**1.38**	-0.15	8	0.60	-0.04	5	**1.11**	-0.09
ICol	9	0.72	-0.08	12	0.10	0.01	13	0.33	-0.03
SCol	4	**1.22**	-0.13	9	0.45	-0.03	7	**0.93**	0.08
CLs	6	**1.10**	-0.12	5	**1.32**	-0.09	3	**1.39**	-0.12
TPGs	13	0.24	0.02	10	0.34	0.02	14	0.32	0.03
Muscle fibers									
I	8	**0.88**	-0.09	14	0.01	0.00	12	0.48	-0.04
IIA	3	**1.30**	-0.14	11	0.15	0.01	11	0.62	-0.05
IIX+B	1	**1.72**	0.18	13	0.04	0.01	8	**0.91**	0.07
TLips and FAs									
TLips	12	0.24	0.02	6	**1.30**	0.09	9	**0.90**	0.08
SFAs	7	**1.10**	0.11	3	**1.42**	0.10	2	**1.44**	0.12
MUFAs	5	**1.18**	0.13	4	**1.41**	0.10	1	**1.49**	0.13
PUFAs	11	0.53	0.06	2	**1.52**	0.10	4	**1.19**	0.10
n-6 /n-3PUFAs	14	0.07	-0.01	1	**1.57**	0.11	10	**0.89**	0.07
CLAs	10	0.66	0.07	7	**1.05**	0.07	6	**0.98**	0.08

Abbreviations: IMCT: Intra Muscular Connective tissue; TCol: Total Collagen (mg OH-proline/g dry matter); ICol: Insoluble Collagen (mg OH-proline/g dry matter); SCol: Soluble Collagen (%); CLs: Cross-Links (nM pyridinoline/g dry matter); TPGs: Total Proteoglycans (µg chondroitin 4-sulfate/g dry matter); FAs: Fatty Acids; TLips: Total Lipids (mg/100g fresh matter), SFAs: Saturated Fatty Acids; MUFAs: Monounsaturated Fatty Acids; PUFAs: Polyunsaturated Fatty Acids; n-6/n-3PUFAs: n-6/n-3Polyunsaturated Fatty Acids; CLAs: Conjugated Linoleic Acids.

All fatty acids were expressed in mg/100g fresh matter.

Intensities of tenderness of the meat were scored on a continuous scale from 0 to 10, where 0 refers to extremely tough and 10 to extremely tender.

Table 4: Ranking of the retained variables in tenderness PLS models, according to their variable importance in projection (VIP) for the *Rectus abdominis* (RA) muscle of studies 1 (St1) (Charolais young bulls) and 3 (St3) (Holstein, Charolais and Rouge des Prés young bulls, heifers, steers and cows). Only the VIP > 0.8 (in bold) were considered as significant. The analyses were carried out using Z-scores. Coefficient (β) indicate if the variables are involved positively or negatively in the models. The analyses have been realized on n=84 animals (n=32 and n=52 animals for St1 and 3).

	Tenderness								
	RA_St1 (n=32)			RA_ St 3 (n=52)			RA_ St 1/ St 3 (n=84)		
	R^2=0.51			R^2=0.32			R^2=0.38		
	Rank	VIP	β	Rank	VIP	β	Rank	VIP	β
Variable									
IMCT									
TCol	12	0.19	-0.02	8	0.61	-0.03	11	0.46	-0.05
ICol	10	0.34	-0.03	2	**1.63**	-0.09	5	**1.13**	-0.08
SCol	14	0.01	-0.01	14	0.18	0.01	13	0.11	0.01
CLs	1	**2.17**	-0.20	4	**1.58**	-0.09	1	**2.09**	-0.12
TPGs	11	0.27	-0.02	5	**0.92**	-0.05	7	0.68	-0.03
Muscle fibers									
I	8	0.61	-0.06	1	**1.70**	0.09	8	0.66	0.02
IIA	4	**1.44**	-0.14	3	**1.61**	-0.08	2	**1.72**	-0.10
IIX+B	5	**0.86**	0.08	13	0.24	0.01	10	0.60	0.07
TLips and FAs									
TLips	13	0.01	0.01	12	0.35	0.02	12	0.21	0.06
SFAs	9	0.43	0.04	7	0.73	0.04	9	0.65	0.06
MUFAs	6	0.68	0.06	9	0.57	0.03	6	0.70	0.07
PUFAs	7	0.64	0.06	11	0.46	-0.02	14	0.07	-0.01
n-6 /n-3PUFAs	3	**1.45**	-0.15	10	0.52	-0.02	4	**1.19**	-0.09
CLAs	2	**1.65**	0.13	6	0.75	0.04	3	**1.22**	0.06

Abbreviations: IMCT: Intra Muscular Connective tissue; TCol: Total Collagen (mg OH-proline/g dry matter); ICol: Insoluble Collagen (mg OH-proline/g dry matter); SCol: Soluble Collagen (%); CLs: Cross-Links (nM pyridinoline/g dry matter); TPGs: Total Proteoglycans (µg chondroitin 4-sulfate/g dry matter); FAs: Fatty Acids; TLips: Total Lipids (mg/100g fresh matter), SFAs: Saturated Fatty Acids; MUFAs: Monounsaturated Fatty Acids; PUFAs: Polyunsaturated Fatty Acids; n-6/n-3PUFAs: n-6/n-3Polyunsaturated Fatty Acids; CLAs: Conjugated Linoleic Acids

All fatty acids were expressed in mg/100g fresh matter.

Intensities of tenderness of the meat were scored on a continuous scale from 0 to 10, where 0 refers to extremely tough and 10 to extremely tender.

Table 5: Summary of the muscle components positively (in green) or negatively (in red) retained in Partiel Least Square (PLS) models of tenderness, juiciness and flavor from normalized data (Z-score). Number 1 or 2 (in bold) in red or green cases indicates that the muscle components are retained in 1 or 2 predicting models either for *Longissimus thoracis* (LT) of studies (St) 1 or 2 (in brackets, in red or green cases) or for *Rectus abdominis* (RA) of studies 1 or 3 (in brackets in red or green cases). For each sensory parameter, "Frequency (Fr)" column indicates the number of time where the muscle components were retained in models both for LT and RA muscles. Total Fr indicates total amount of times where muscle components were retained in prediction models, per muscle component, for the three sensory parameters.

	Tenderness			Juiciness			Flavor			Total Fr
	LT St _1 /_2	RA St _1 /_ 3	Fr	LT St _1 /_2	RA St _1 /_ 3	Fr	LT St _1 /_2	RA St _1 /_ 3	Fr	
IMCT										
TCol	**1** (1)		1			0			0	1
ICol		**1** (3)	1	**1** (1)		1	**1** (1)		1	3
SCol	**1** (1)		1	**1** (1)	**1** (3)	2	**1** (1)	**1** (3)	2	5
CLs	**2** (1, 2)	**2** (1, 3)	4	**1** (2)	**2** (1, 3)	3	**1** (2)	**2** (1, 3)	3	10
TPGs		**1** (3)	1	**1** (2)		1	**2** (1, 2)	**1** (3)	3	5
Muscle fibers										
I	**1** (1)	**1** (3)	2		**2** (1, 3)	2	**1** (2)	**1** (3)	2	6
IIA	**1** (1)	**2** (1, 3)	3	**1** (2)	**2** (1, 3)	3		**1** (3)	1	7
IIX+B	**1** (1)	**1** (1)	2		**1** (3)	1		**1** (3)	1	4
TLips and FAs										
TLips	**1** (2)		1	**2** (1, 2)	**1** (3)	3	**2** (1, 2)	**2** (1, 3)	4	8
SFAs	**2** (1, 2)		2	**2** (1, 2)	**1** (1)	3	**2** (1, 2)	**1** (1)	3	8
MUFAs	**2** (1, 2)		2	**2** (1, 2)	**1** (1)	3	**2** (1, 2)	**1** (1)	3	8
PUFAs	**1** (2)		1		**1** (1)	1	**1** (2)		1	3
n-6 /n-3PUFAs	**1** (2)	**1** (1)	2	**2** (1, 2)	**1** (3)	3	**1** (1)	**1** (1)	2	7
CLAs	**1** (2)	**1** (1)	2	**2** (1, 2)	**1** (1)	3	**2** (1, 2)	**2** (1, 3)	4	9

Abbreviation: IMCT: Intra Muscular Connective tissue; TCol: Total Collagen (mg OH-proline/g dry matter); ICol: Insoluble Collagen (mg OH-proline/g dry matter); SCol: Soluble Collagen (%); CLs: Cross-Links (nM pyridinoline/g dry matter); TPGs: Total Proteoglycans (µg chondroitin 4-sulfate/g dry matter); FAs: Fatty Acids; TLips: Total Lipids (mg/100g fresh matter); SFAs: Saturated Fatty Acids; MUFAs: Monounsaturated

Fatty Acids; PUFAs: Polyunsaturated Fatty Acids; n-6/n-3PUFAs: n-6/n-3Polyunsaturated Fatty Acids; CLAs: Conjugated Linoleic Acids.

All fatty acids were expressed in mg/100g fresh matter.

Intensities of tenderness, juiciness and flavor of the meat were scored on a continuous scale from 0 to 10, where 0 refers to extremely tough, dry, not tasty and 10 to extremely tender, juicy, tasty.

Table 6: Ranking of the retained variables in juiciness PLS models, according to their variable importance in projection (VIP) for the *Longissimus thoracis* (LT) muscles of studies 1 (St1) (Charolais young bulls) and 2 (St 2) (Aberdeen Angus, Limousin and Blond d'Aquitaine young bulls). Only the VIP > 0.8 (in bold) were considered as significant. Analyses were carried out using Z-scores. Coefficient (β) indicate if the variables are involved positively or negatively in the models. The analyses have been realized on n=72 animals (n=32 and n=40 for St1 and 2).

	Juiciness								
	LT_St1 (n=32) R^2=0.47			LT_St2 (n=40) R^2=0.36			LT_St1/St2 (n=72) R^2=0.35		
	Rank	VIP	β	Rank	VIP	β	Rank	VIP	β
Variable									
IMCT									
TCol	10	0.70	-0.04	12	0.57	-0.06	7	0.76	-0.04
ICol	6	**0.92**	0.06	9	0.78	0.04	14	0.02	-0.01
SCol	1	**1.68**	-0.10	13	0.21	-0.10	5	**1.07**	-0.05
CLs	14	0.08	-0.01	6	**1.02**	-0.01	8	0.72	-0.03
TPGs	8	0.75	0.05	5	**1.05**	-0.02	12	0.27	-0.01
Muscle fibers									
I	13	0.09	0.01	11	0.59	0.01	9	0.44	0.02
IIA	12	0.44	0.02	8	**0.87**	-0.01	10	0.33	-0.01
IIX+B	9	0.70	-0.04	14	0.11	0.01	11	0.32	-0.01
TLips and FAs									
TLips	7	**0.84**	0.05	3	**1.29**	0.03	3	**1.31**	0.06
SFAs	3	**1.49**	0.06	2	**1.64**	0.09	2	**1.91**	0.09
MUFAs	2	**1.52**	0.09	1	**1.66**	0.10	1	**1.94**	0.09
PUFAs	11	0.58	0.03	10	0.71	0.05	6	**0.80**	0.04
n-6 /n-3PUFAs	4	**1.42**	-0.09	4	**1.06**	-0.12	13	0.09	-0.01
CLAs	5	**0.98**	0.06	7	**0.98**	0.09	4	**1.20**	0.06

Abbreviations: IMCT: Intra Muscular Connective tissue; TCol: Total Collagen (mg OH-proline/g dry matter); ICol: Insoluble Collagen (mg OH-proline/g dry matter); SCol: Soluble Collagen (%); CLs: Cross-Links (nM pyridinoline/g dry matter); TPGs: Total Proteoglycans (µg chondroitin 4-sulfate/g dry matter); FAs: Fatty Acids; TLips: Total Lipids (mg/100g fresh matter), SFAs: Saturated Fatty Acids; MUFAs: Monounsaturated Fatty

Acids; PUFAs: Polyunsaturated Fatty Acids; n-6/n-3PUFAs: n-6/n-3Polyunsaturated Fatty Acids; CLAs: Conjugated Linoleic Acids.

All fatty acids were Stressed in mg/100g fresh matter.

Intensities of juiciness of the meat were scored on a continuous scale from 0 to 10, where 0 refers to extremely dry, and 10 to extremely juicy.

Table 7: Ranking of the retained variables in juciness PLS models, according to their variable importance in projection (VIP) for the *Rectus abdominis* (RA) muscles of studies 1 (St_1) (Charolais young bulls) and 3 (St_3) (Holstein, Charolais and Rouge des Prés young bulls, heifers, steers and cows). Only the VIP > 0.8 (in bold) were considered as significant. Analyses were carried out using Z-scores. Coefficient (β) indicate if the variables are involved positively or negatively in the models. The analyses have been realized on n=84 animals (n=32 and n=52 animals for St_1 and_2).

	Juiciness								
	RA_St1 (n=32)			RA_St3 (n=52)			RA_St1/St3 (n=84)		
	R^2=0.57			R^2=0.38			R^2=0.32		
	Rank	VIP	β	Rank	VIP	β	Rank	VIP	β
Variable									
IMCT									
TCol	12	0.25	-0.03	13	0.26	0.02	14	0.02	-0.03
ICol	11	0.37	-0.03	11	0.30	0.02	13	0.03	-0.03
SCol	13	0.10	0.01	3	**0.94**	-0.08	9	0.63	-0.01
CLs	1	**1.90**	-0.17	6	**0.91**	-0.08	3	**1.62**	-0.11
TPGs	9	0.73	-0.06	14	0.02	0.02	11	0.39	-0.04
Muscle fibers									
I	6	**0.99**	0.07	2	**2.00**	0.17	1	**1.78**	0.06
IIA	7	**0.90**	-0.11	3	**0.96**	-0.08	12	0.08	-0.01
IIX+B	8	0.75	-0.05	1	**2.15**	-0.18	2	**1.74**	-0.05
TLips and FAs									
TLips	14	0.05	-0.01	7	**0.82**	0.07	10	0.48	0.02
SFAs	5	**1.07**	0.08	9	0.59	0.05	6	**0.96**	0.07
MUFAs	4	**1.10**	0.08	8	0.63	0.05	3	**1.01**	0.06
PUFAs	3	**1.47**	0.11	12	0.29	-0.03	8	0.64	0.05
n-6 /n-3PUFAs	10	0.62	-0.05	4	**0.96**	-0.08	7	**0.93**	-0.05
CLAs	2	**1.53**	0.12	10	0.33	0.03	4	**1.06**	0.07

Abbreviations: IMCT: Intra Muscular Connective tissue; TCol: Total Collagen (mg OH-proline/g dry matter); ICol: Insoluble Collagen (mg OH-proline/g dry matter); SCol: Soluble Collagen (%); CLs: Cross-Links (nM pyridinoline/g dry matter); TPGs: Total Proteoglycans (µg chondroitin 4-sulfate/g dry matter); FAs: Fatty Acids;

TLips: Total Lipids (mg/100g fresh matter), SFAs: Saturated Fatty Acids; MUFAs: Monounsaturated Fatty Acids; PUFAs: Polyunsaturated Fatty Acids; n-6/n-3PUFAs: n-6/n-3Polyunsaturated Fatty Acids; CLAs: Conjugated Linoleic Acids.

All fatty acids were expressed in mg/100g fresh matter.

Intensities of juiciness of the meat were scored on a continuous scale from 0 to 10, where 0 refers to extremely dry, and 10 to extremely juicy.

Table 8: Ranking of the retained variables in flavor PLS models, according to their variable importance in projection (VIP) for the *Longissimus thoracis* (LT) muscles of studies 1 (St1) (Charolais young bulls) and 2 (St 2) (Aberdeen Angus, Limousin and Blond d'Aquitaine young bulls). Only the VIP > 0.8 (in bold) were considered as significant. The analyses were carried out using Z-scores. Coefficient (β) indicate if the variables are involved positively or negatively in the models. The analyses have been realized on n=72 animals (n=32 and n=40 for St1 and 2).

	Flavor								
	LT_St1 (n=32)			LT_ St 2 (n=40)			LT_ St 1/ St 2 (n=72)		
	R^2=0.63			R^2=0.36			R^2=0.32		
	Rank	VIP	β	Rank	VIP	β	Rank	VIP	β
Variables									
IMCT									
TCol	14	0.26	-0.03	14	0.01	0.00	12	0.33	-0.01
ICol	8	**0.87**	-0.16	13	0.10	-0.01	10	0.48	0.03
SCol	5	**1.18**	-0.07	12	0.13	0.01	3	**1.39**	-0.04
CLs	12	0.37	-0.05	3	**1.25**	-0.06	2	**1.55**	-0.05
TPGs	6	**1.00**	0.06	8	**0.80**	0.04	5	**1.13**	0.06
Muscle fibers									
I	13	0.32	0.03	7	**0.81**	0.04	1	**1.60**	0.03
IIA	11	0.38	0.03	10	0.30	0.02	9	**0.89**	0.02
IIX+B	10	0.56	-0.04	9	0.71	-0.04	6	**1.03**	-0.04
TLips and FAs									
TLips	1	**1.84**	0.13	6	**1.13**	0.06	4	**1.24**	0.10
SFAs	3	**1.36**	0.08	5	**1.13**	0.06	8	**0.93**	0.08
MUFAs	2	**1.38**	0.09	4	**1.24**	0.06	7	**0.98**	0.09
PUFAs	9	0.72	0.05	2	**1.68**	0.09	14	0.03	0.08
n-6 /n-3PUFAs	4	**1.19**	-0.28	11	0.14	0.01	11	0.40	-0.04
CLAs	7	**0.97**	0.07	1	**1.89**	0.10	13	0.18	0.09

Abbreviations: IMCT: Intra Muscular Connective tissue; TCol: Total Collagen (mg OH-proline/g dry matter); ICol: Insoluble Collagen (mg OH-proline/g dry matter); SCol: Soluble Collagen (%); CLs: Cross-Links (nM

pyridinoline/g dry matter); TPGs: Total Proteoglycans (µg chondroitin 4-sulfate/g dry matter); FAs: Fatty Acids; TLips: Total Lipids (mg/100g fresh matter), SFAs: Saturated Fatty Acids; MUFAs: Monounsaturated Fatty Acids; PUFAs: Polyunsaturated Fatty Acids; n-6/n-3PUFAs: n-6/n-3Polyunsaturated Fatty Acids; CLAs: Conjugated Linoleic Acids.

All fatty acids were expressed in mg/100g fresh matter.

Intensities of flavor of the meat were scored on a continuous scale from 0 to 10, where 0 refers to not tasty and 10 to extremely tasty.

Table 9: Ranking of the retained variables in flavor PLS models, according to their variable importance in projection (VIP) for the *Rectus abdominis* (RA) muscles of St1 (Charolais young bulls) and 3 (St3) (Holstein, Charolais and Rouge des Prés, young bulls, heifers, steers and cows). Only the VIP > 0.8 (in bold) were considered as significant. The analyses were carried out using Z-scores. Coefficient (β) indicate if the variables are involved positively or negatively in the models. The analyses have been realized on n=84 animals (n=32 and n=52 for St1 and 3).

	Flavor								
	RA_ St1 (n=32)			RA_ St3 (n=52)			RA_ St 1/ St3 (n=84)		
	R^2=0.41			R^2=0.38			R^2=0.44		
	Rank	VIP	β	Rank	VIP	β	Rank	VIP	β
Variables									
IMCT									
TCol	10	0.50	-0.04	13	0.11	-0.02	7	**0.92**	-0.05
ICol	14	0.03	-0.01	9	0.61	-0.04	8	**0.91**	-0.05
SCol	7	0.75	-0.04	3	**1.35**	-0.09	11	0.48	-0.02
CLs	1	**1.74**	-0.12	5	**0.91**	-0.07	1	**1.87**	-0.10
TPGs	8	0.69	-0.04	4	**1.04**	-0.07	6	**1.01**	-0.05
Muscle fibers									
I	11	0.25	-0.01	1	**2.73**	0.17	5	**1.06**	0.06
IIA	9	0.57	-0.05	8	**0.81**	-0.06	10	0.60	-0.03
IIX+B	13	0.07	0.01	2	**1.40**	-0.10	12	0.35	-0.02
TLips and FAs									
TLips	5	**1.19**	0.07	6	**0.87**	0.06	3	**1.29**	0.07
SFAs	4	**1.30**	0.08	12	0.38	0.03	4	**1.25**	0.07
MUFAs	6	**1.13**	0.07	10	0.55	0.04	2	**1.35**	0.07
PUFAs	12	0.18	0.01	14	0.07	-0.01	14	0.17	-0.01
n-6 /n-3PUFAs	3	**1.42**	-0.08	11	0.40	0.03	9	**0.85**	-0.04
CLAs	2	**1.67**	0.09	7	0.**84**	-0.06	13	0.34	0.02

Abbreviations: IMCT: Intra Muscular Connective tissue; TCol: Total Collagen (mg OH-proline/g dry matter); ICol: Insoluble Collagen (mg OH-proline/g dry matter); SCol: Soluble Collagen (%); CLs: Cross-Links (nM pyridinoline/g dry matter); TPGs: Total Proteoglycans (µg chondroitin 4-sulfate/g dry matter); FAs: Fatty Acids; TLips: Total Lipids (mg/100g fresh matter), SFAs: Saturated Fatty Acids; MUFAs: Monounsaturated Fatty Acids; PUFAs: Polyunsaturated Fatty Acids; n-6/n-3PUFAs: n-6/n-3Polyunsaturated Fatty Acids; CLAs: Conjugated Linoleic Acids ;

All fatty acids were expressed in mg/100g fresh matter.

Intensities of flavor of the meat were scored on a continuous scale from 0 to 10, where 0 refers to not tasty and 10 to extremely tasty.