

## Quality classification of the muscle *rectus abdominis* of Charolais Heifers

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### Abstract :

Ninety-nine Charolais heifers were used to study the variation of meat quality attributes. The muscle *rectus abdominis* was excised 24 hours post-slaughter and sensory attributes were analysed at 14 days *post mortem*. Meat quality was characterized by physicochemical measurements and sensory evaluation, using the descriptors of initial and general tenderness, juiciness and flavour intensity. The physicochemical measurements included shear force, intramuscular fat content, collagen content, collagen solubility, lactate deshydrogenase, isocitrate deshydrogenase and cytochrome c oxydase activities, mean fibre area, MyHC isoforms, haem iron content, CIELAB colour measurements. A principal component analysis and a hierarchical cluster analysis were used to establish a meat classification. Five meat types (I, II, III, IV and V) were classified by increasing tenderness, from the type I (low tenderness, low juiciness, medium flavour intensity) to the type V (high tenderness, medium juiciness, high flavour intensity). The types IV and V led to both tender and juicy meat and contained the most intramuscular fat. The lowest level of tenderness (type II) was explained by a high shear force and more total and insoluble collagen. Lactate deshydrogenase, isocitrate deshydrogenase and cytochrome c oxydase activities, fibre size, MyHC isoforms, haem iron content and CIELAB colour measurements were no different between the five meat types.

**Key words :** heifer, meat, quality, cattle, Charolais, *m. rectus abdominis*

### 1. Introduction

Most of the bibliography indicated that there is positive correlation between the quality traits of tenderness, juiciness and flavour intensity of the muscle *longissimus thoracis*. For example, between tenderness and juiciness, a correlation varying between +0.54 and +0.58 was found. Between tenderness and flavour intensity, the correlation varied between +0.29 and +0.34. Moreover, juiciness and flavour intensity were positively correlated, with a coefficient varying between +0.20 and +0.70 (Ockerman et al., 1984 ; Gregory et al., 1994 ; Dransfield et al., 2002 ; Rhee et al., 2004 ; Serra et al., 2004). Thus, the correlations of +0.35 to +0.50 explained that different levels of juiciness and flavour intensity may be found within one level of tenderness.

Physicochemical characteristics commonly tested explained twelve to thirty percent of meat quality traits, in spite of the significant correlations that appears between sensory evaluation and physicochemical measurements (Renand et al., 2001 ; Dransfield et al., 2003 ; Hocquette et al., 2004). These results were obtained by considering individually the different descriptors and so, each descriptor was explained one by one by the different physicochemical characteristics. Thus, the global

quality of meat samples, considering together tenderness, juiciness and flavour intensity, was never evaluated.

The aim of this study was first to constitute a typology of meat by multifactorial statistical analysis on tenderness, juiciness and flavour intensity, and then to explain this typology by physicochemical characteristics of muscles.

## **2. Material and methods**

### *2.1. Slaughter and muscle samples*

A total of 99 Charolais heifers were collected in a cooperative of livestock farmers located in Venarey-Les-Laumes (Côte-d'Or, France). All slaughters occurred in the same industrial slaughterhouse in order to standardize slaughtering, chilling and storing procedures.

The animals were slaughtered between 26 and 43 months of age with an average of 33.4 months and their carcass weight is on average 381 kg (330 – 509 kg).

The pH of each carcass was measured at 24 h *post mortem* in the *longissimus thoracis* and the *rectus abdominis* muscles.

The colour of *rectus abdominis* (RA) muscle was measured 24 hours *post mortem* using a portable spectrophotometer (CR300, MINOLTA). Colour coordinates were calculated in the CIELAB system and results were expressed as lightness (L\*), redness (a\*) and yellowness (b\*). Samples of m. RA were removed from the carcasses for sensory evaluation and physicochemical measurements.

### *2.2. Physicochemical characteristics*

the measures were carried out on the RA collected 24 hours after slaughter. Dry matter content was measured by oven-drying at 103°C for 48 hours. The intramuscular lipid content was determined by the Soxhlet standard method (NF V 04-402, 1968). The haem iron content and the collagen content were evaluated respectively by the method of Hornsey (1956) and by measurement of hydroxyproline content (collagen = 7.5 x hydroxyproline) according to the method of Bergman and Loxley (1963). Collagen in the insoluble part was determined according to a procedure given by Bonnet and Kopp (1992). Collagen solubility was expressed as the percentage of heat-soluble collagen (total – insoluble) to total collagen.

The metabolic muscle type was determined by measuring enzyme activities. The anaerobic glycolytic metabolism was assessed by lactate dehydrogenase (LDH) activity (Ansay, 1974). The aerobic oxidative metabolism was assessed by isocitrate dehydrogenase (ICDH) and cytochrome-c oxydase (COX) activities according to the method of Briand et al. (1981) and Piot et al. (1998).

Muscle mean fiber area was determined on 10-µm thick sections cut perpendicular to the muscle fibers with a cryotome at –25°C. Between 100 and 200 fibers in each of two different locations in the muscle were used to determine the mean fiber area by computerized image-analysis. The different types of myosin heavy chains isoforms were determined on the basis of previously determined

migration pattern (Young and Davey, 1981 ; Picard et al., 1995) on sodium Dodecyl Sulfate polyacrylamide gel electrophoresis (SDS-PAGE) performed by the method of Laemmli (1970).

Contents of  $\mu$ -calpains and proteasome 27K sub-unit were determined by western-blot. The protein concentration of the sample was determined according to the method of Bradford (1976) using bovine serum albumin as standard. The separation gel was a 12.5% polyacrylamide gel (C = 2.6%) (Towbin et al., 1979). Migration was performed at 4°C at 150 V. The proteins were also electro transferred from the SDS gel onto a membrane of polyvinyl. The membrane was saturated 1 hour under. Incubation with the anti  $\mu$ -calpain and anti 27K antibodies, diluted in a solution containing TBS 1X and 10% Tween was performed during one hour at 37°C under shaking. The dilution varied depending on antibody used (1 / 8 000 or 1 / 4 000). The second antibody (anti mouse IgG) was diluted 10 000 fold and applied for 1 hour at room temperature. After washing in TBS 1X Tween 10% and using luminal reagent, development was made on a photo film and analysed with Quantitone software (Biorad).

The textural properties of raw meat were studied in the RA muscle, vacuum packaged and aged at 4°C for 14 days *post mortem*. Shear force was measured according to the method of Salé (1971).

### 2.3. Sensory evaluation

Sensory assessment was conducted on samples of *rectus abdominis* (RA) muscle by a test panel. The aim was to classify the 99 heifers by a monadic test.

The RA muscles were vacuum-packaged and chilled for 14 days at +4°C for ageing. After ageing, the RA muscle was trimmed and cut into 1.5 cm thick homogeneous steaks, then vacuum-packaged a second time and frozen at - 20°C until the sensory analysis.

Meat in the form of 15 mm steaks was thawed and then cooked for 1 minute 45 seconds in an Infra grill Duo Sofraca set at a temperature of 300°C. After cooking, the steaks were cut into 20 mm samples that were served at an internal temperature of 55°C. The jury, trained in meat appreciation, consisted of 16 panellists. The panellists evaluated the cooked samples for initial tenderness (IT), overall tenderness (OT), juiciness and flavour intensity. Initial tenderness was defined as tenderness felt at the first bite, whereas general tenderness indicated the amount of chewing needed before swallowing a meat sample. Each attribute was rated on a 10-point non-graduated scale on an ascending scale of quality with a score of 0 equivalent to tough, lacking juiciness and low flavour, and a score of 10 equivalent to tender, extremely juicy and high flavour. Therefore, at each session, a monadic presentation of 5 samples was done. The sessions were carried out in a sensorial analysis room with individual boxes, under artificial non-coloured lighting.

### 2.4. Statistical analyses

Principal Component Analysis (PCA) implemented in the WINSTAT software was used. Afterwards, the first three factors obtained in the PCA were included in a Hierarchical Cluster Analysis (HCA, Winstat). The item coordinates on the first three dimensions were also used in the HCA. Indeed, it is quite difficult to make visual items or variable categories on more than two factorial dimensions (Lebart

et al., 1995). The centroid method was used as the means of aggregation. The programme identified the cluster, which has the minor within-group variance and the greater variance between groups. Five meat types were identified using their description by the three factorial dimensions of the MCA. Among the five types, muscular characteristics differences were evaluated. Variance analysis and mean multiple comparisons with one factor were carried out using the GLM procedure (general linear model) from SAS 9.1 (2002).

### 3. Results and discussion

Initial tenderness, overall tenderness and juiciness scores were distributed between 3.0 and 7.8 /10. The average scores were respectively 5.7/10, 5.3/10 and 5.3/10. Flavour intensity was noted between 4.3 and 6.4 /10 with an average of 5.7/10 (table 1). The variability of the tenderness and juiciness scores was twice higher than those from the flavour intensity ones, as already observed on the muscle *longissimus thoracis* (Renand et al., 1997 ; Renand et al., 2001 ; Rhee et al., 2004).

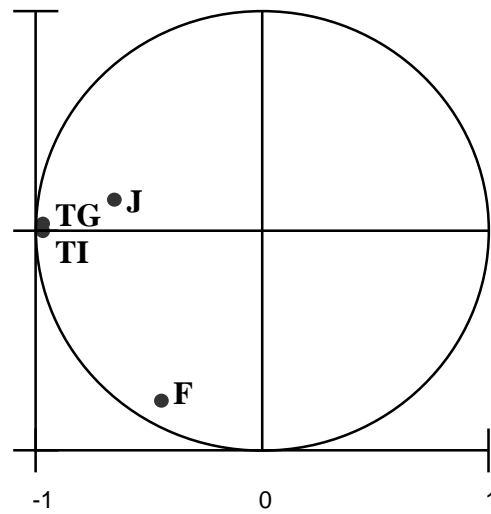
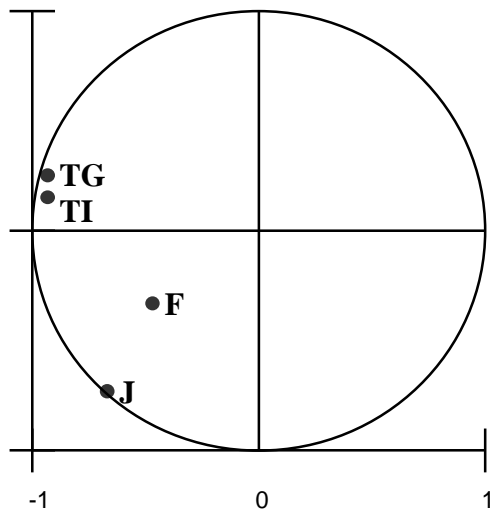
As expected, initial and overall tenderness were highly correlated (+0.97). Juiciness was positively correlated with initial and overall tenderness (+0.52 and +0.46) and with flavour intensity (+0.48). Flavour intensity was also positively correlated with initial and overall tenderness (+0.40 and 0.35). Thus, as meat quality attributes of tenderness, juiciness and flavour intensity were positively correlated, juiciness and flavour intensity scores have probably received a positive influence of the favourable assessment of tenderness scores (Egan et al., 2001). Nevertheless, correlations being between +0.34 and +0.52, it seems possible to have different levels of juiciness and flavour intensity among one level of tenderness.

The principal component analysis (PCA) compared on the first dimension high initial and overall tenderness (negative coordinates) to low initial and overall tenderness (positive coordinates). Juiciness and flavour intensity were well positioned on the second and third dimensions respectively, with increasing scores when going near the negative coordinates (figure 1). Nevertheless, the conclusions about the third dimension had to be moderated, as this dimension explained only 4.8% of total inertia.

Samples of meat were organised in meat types, depending on their sensorial quality. The PCA and the HCA on meat quality traits led to a typology of meat divided into five meat types classified by tenderness, from the type I (low tenderness) to the type V (high tenderness). The five types resulted from a classification made on the first three significant dimensions that explained 99.0% of the total inertia. Tenderness, juiciness and flavour intensity average scores of each meat type were reported in table 1. Meat from type I had both a rather low tenderness and a low juiciness but their flavour intensity was intermediate. Those from type II had a rather low tenderness and a medium juiciness but their flavour intensity was high. Meat from type III had both low tenderness, juiciness flavour intensity scores. Those from type IV were characterised by a medium tenderness, a high juiciness and a medium flavour intensity. To finish with, meat from type V were described as rather tender, with a medium juiciness and a high flavour intensity.

Coord 2 : 18.6 % total inertia

Coord 3 : 4.8 % total inertia



Coord 1 : 75.6 % total inertia

Coord 1 : 75.6 % total inertia

Figure 1 : Representation of meat quality attributes in the two-dimensional space of the principal component analysis defined by factors 1 and 2 and factors 1 and 3.

As expected, the first dimension opposed types I and V (figure 2). On this dimension, types II, III and IV were located successively between types I and V. Types II and III were located on the positive coordinates of the second dimension, as these types were characterised by low juiciness scores. Types I and III characterised by low flavour intensity scores were located on the positive coordinated of the third dimension.

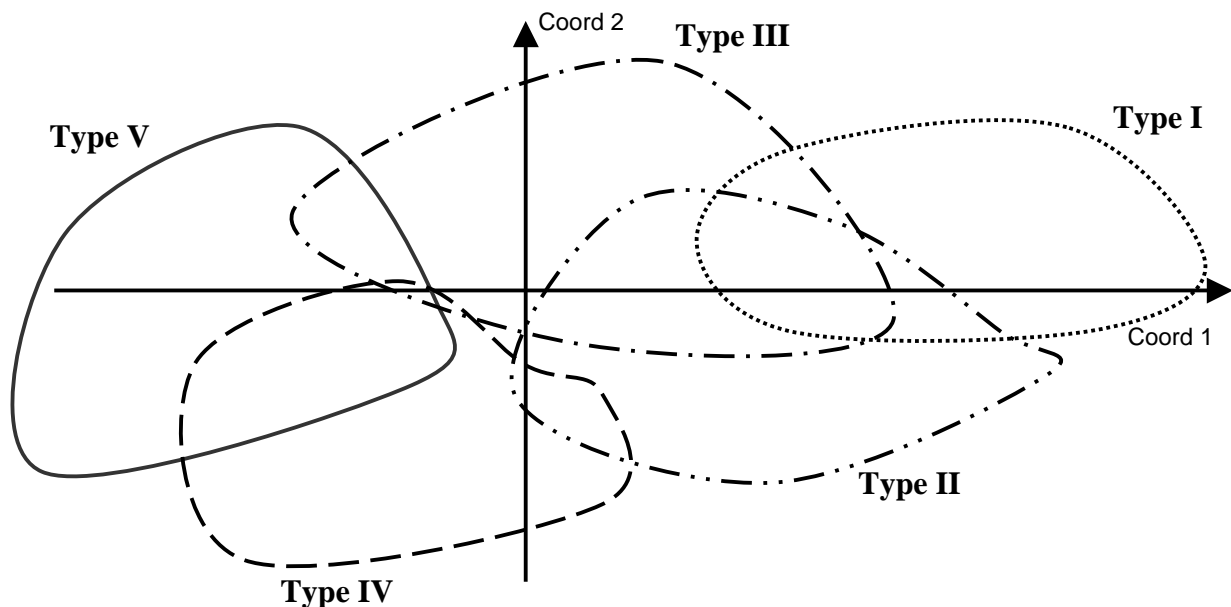


Figure 2 : Representation of meat types in the two-dimensional space of the principal component analysis defined by factors 1 and 2.

Physicochemical characteristics of each meat type were compared in order to establish those that could explain the differences (table 1).

The studied physicochemical characteristics were not sufficient to characterise each meat type, but allowed nevertheless to differentiate some types from the others. Criteria that discriminated meat types were shear force, collagen content, collagen solubility and intramuscular fat content.

Meat from types IV and V, having highest scores for each descriptor than those from types I and III, had highest intramuscular fat content. This observation may be explained by the positive correlation that appears in the *longissimus thoracis* muscle between intramuscular fat content and meat quality traits of tenderness and juiciness (Dikeman et al., 1986 ; Silva et al., 1999 ; Renand et al., 2001 ; Dransfield et al., 2002 ; Geay et al., 2002 ; Jeremiah et al., 2003). Thus, the intramuscular fat content seems to be the factor that discriminate the most the meat types.

Meat from type II had the highest content in total collagen in comparison to the others types (23.9 vs 20.1 to 22.0 mg/g DM). In addition, these meat had the lowest content in soluble collagen (13.8 vs 15.5 to 16.7 % total collagen). Type II led the highest shear forces in comparison to the others types (7.9 vs 6.3 to 6.5 daN). Indeed, shear force is known to be respectively positively and negatively correlated with total and soluble collagen content (Silva et al., 1999 ; Renand et al., 2001 ; Torrecano et al., 2003 ; Rhee et al., 2004). Nevertheless, meat from type II have the same tenderness scores than meat from types I and III.

The highest intramuscular fat content of meat from type II in comparison to the types I and III (17.0% DM vs 15.9% and 15.0% DM) could have a positive impact on tenderness scores. It may have offset a part of the negative impact of shear force and collagen properties (high content and low solubility). This hypothesis is improved by the highest juiciness and flavour intensity scores for meat from type II than those from types I and III.

The five meat types had similar characteristics for mean fiber area, myosin heavy chain I, IIa and IIx percentages, isocitrate dehydrogenase, lactate dehydrogenase and cytochrome c oxydase activities,  $\mu$ -calpain and 27K proteasome sub-unit content, haem iron content and colour measurements (L\*, a\*, b\* and). This result may be linked to the few significant correlations that exist between meat quality traits and physicochemical characteristics, probably because this work was done on an homogeneous population of heifers. Moreover, it seems that the *rectus abdominis* is a muscle with many specificities, especially on muscular fibres, that could have reduced the differences between meat types (Picard et al., 2003).

Table 1.  
Meat quality attributes and physicochemical characteristics of meat types

	Mean	Meat types					Test P =
		I	II	III	IV	V	
N	99	26	12	16	20	25	
<b>Meat quality attributes</b>							
Initial tenderness (score on 10)	5.7	4.97 <sup>a</sup>	5.10 <sup>ab</sup>	5.48 <sup>b</sup>	5.92 <sup>c</sup>	6.55 <sup>d</sup>	<b>0.001</b>
Overall tenderness (score on 10)	5.3	4.54 <sup>a</sup>	4.52 <sup>a</sup>	5.14 <sup>b</sup>	5.54 <sup>c</sup>	6.26 <sup>d</sup>	<b>0.001</b>
Juiciness (score on 10)	5.3	4.51 <sup>a</sup>	5.86 <sup>b</sup>	4.78 <sup>a</sup>	5.94 <sup>c</sup>	5.58 <sup>b</sup>	<b>0.001</b>
Flavour intensity (score on 10)	5.7	5.72 <sup>b</sup>	5.97 <sup>c</sup>	5.16 <sup>a</sup>	5.64 <sup>b</sup>	6.05 <sup>c</sup>	<b>0.001</b>
<b>Physicochemical characteristics</b>							
Shear force - raw meat (daN)	6.7	6.7 <sup>a</sup>	7.9 <sup>b</sup>	6.3 <sup>a</sup>	6.5 <sup>a</sup>	6.5 <sup>a</sup>	<b>0.038</b>

Total collagen (mg/g dry matter)	21.3	21.0 <sup>a</sup>	23.9 <sup>b</sup>	22.0 <sup>a</sup>	20.9 <sup>a</sup>	20.1 <sup>a</sup>	<b>0.011</b>
Soluble collagen (% total collagen)	16.1	16.7 <sup>b</sup>	13.8 <sup>a</sup>	16.6 <sup>b</sup>	16.5 <sup>b</sup>	15.5 <sup>ab</sup>	<b>0.053</b>
Intramuscular fat (% dry matter)	17.8	15.9 <sup>a</sup>	17.0 <sup>ab</sup>	15.0 <sup>a</sup>	20.3 <sup>b</sup>	19.9 <sup>b</sup>	<b>0.007</b>
Mean fibre area (µm <sup>2</sup> )	3380	3600	3383	2805	3314	3568	0.110
Myosin heavy chain I (%)	30.2	29.1	30.3	29.5	30.5	30.8	0.689
Myosin heavy chain IIa (%)	35.2	34.9	36.2	35.7	35.4	36.3	0.955
Myosin heavy chain IIx (%)	34.6	35.9	33.5	34.8	34.1	32.9	0.600
Isocitrate dehydrogenase (µmol/min/g)	1.39	1.41	1.53	1.43	1.31	1.33	0.407
Lactate dehydrogenase (µmol/min/g)	501	498	498	501	506	503	0.994
Cytochrome c oxydase (µmol/min/g)	16.6	16.6	15.7	17.4	15.8	17.2	0.565
µ-calpain (percentage of a control)	100	97	94	99	99	109	0.374
27K proteasome sub-unit (percentage of a control)	145	152	132	156	145	138	0.434
<b>Colour properties</b>							
Haem iron content (µg /g dry matter)	59.2	61.0	61.5	59.4	57.5	58.5	0.500
L*	33.7	34.0	32.3	34.6	33.1	33.7	0.221
a*	20.3	20.1	21.1	20.1	20.1	20.4	0.423
b*	5.8	5.7	5.5	5.8	5.8	5.8	0.991

Within row, means with an identical superscript are not significantly different ( $p>0,05$ )

## 5. Conclusion

Meat types were differentiated in part by physicochemical characteristics and especially by muscles content of intramuscular lipids, total collagen and soluble collagen and shear force. The low differentiation of meat types by physicochemical characteristics may be linked to the low explanation level of each quality descriptors by the physicochemical characteristics. Moreover, according to Hocquette et al. (2004), it seems possible that characteristics that explain tenderness are not the same than those explaining juiciness and/or flavour intensity. Thus, other characteristics as proteases activities, collagen properties (isoforms, proteoglycans) should be interesting in the following works, when studying different types of meat quality.

## Acknowledgment

The authors wish to acknowledge the contribution of the SCICAV Bourgogne Élevage producers, the "Les Éleveurs du Centre-Est" slaughterhouse and the breeders who participated in the present work. The authors wish to thank Mathieu Prun and Jean-Philippe Martin for their valuable help. The authors also wish to thank F. Delamarche and J. Lambert (technicians at the ENESAD laboratory) for muscular analysis.

The work was carried out with the financial support of the Conseil Scientifique de l'ENESAD and the INRA-INAO programme on meat quality.

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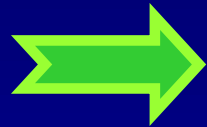
# Quality classification of the muscle *rectus abdominis* of Charolais Heifers

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- Positive correlations inter sensorial descriptors

*Ockerman et al., 1984 ; Dransfield et al., 2002 ; Rhee et al., 2004 ; Serra et al., 2004*

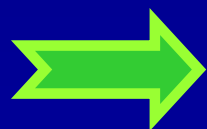
- Correlations from + 0,35 to + 0,50



## Meat types on sensorial properties

- A part of sensorial quality is explained by physicochemical properties of muscles

*Renand et al., 2001 ; Renand et al., 2002 ; Dransfield et al., 2003 ; Maltin et al., 2003 ; Hocquette et al., 2004 ; Hocquette et al., 2005*

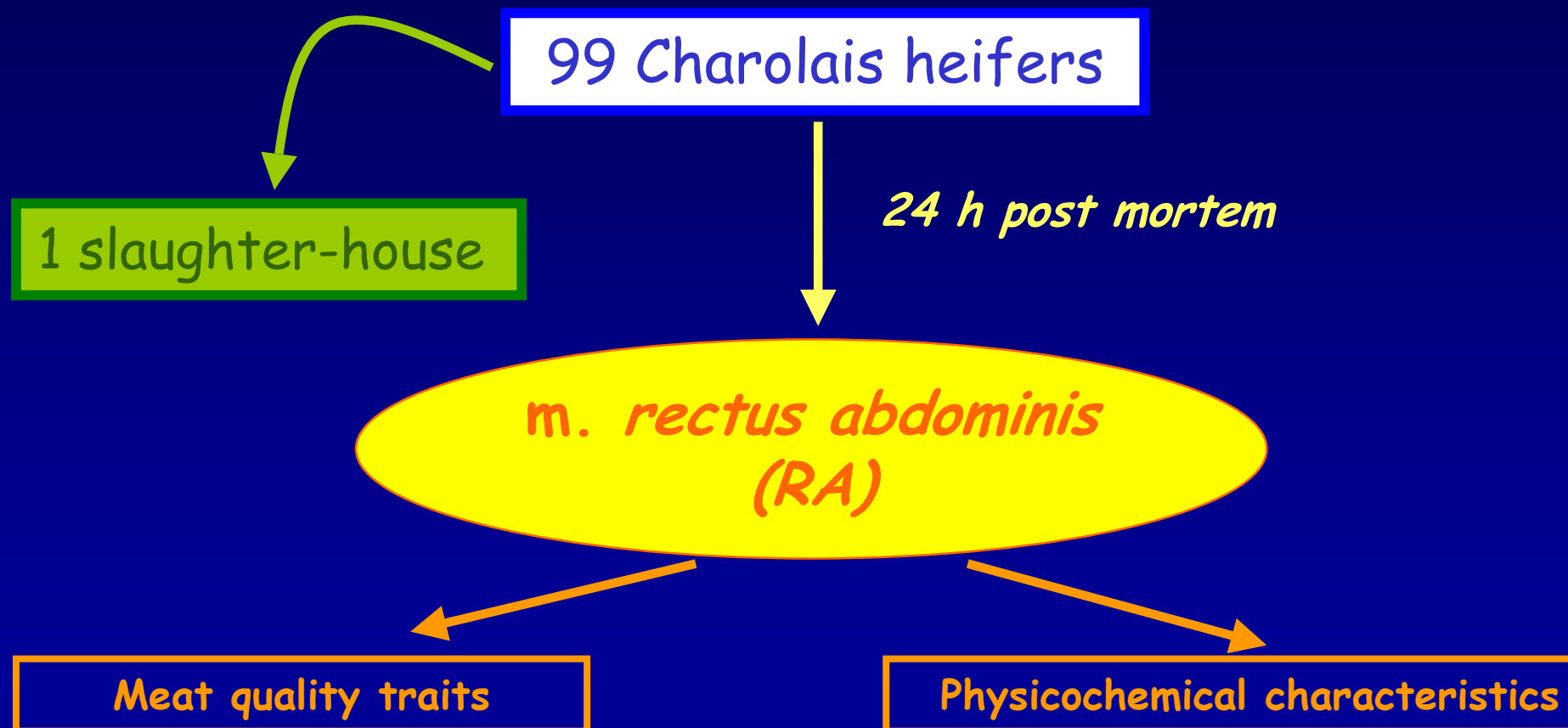


## Relations between physicochemical and sensorial properties

Organise the variability of meat quality traits  
&  
Explain this variability by physicochemical  
characteristics of muscles

# Material and Methods

## - Animals and muscles -



# Material and Methods

## - Meat quality traits -

- ✓ Presentation : **Monadic**
- ✓ Test panel : **16-member trained in the jury**
- ✓ Descriptors
  - ☞ **Initial tenderness (IT)**
  - ☞ **Overall tenderness (OT)**
  - ☞ **Juiciness (J)**
  - ☞ **Flavor Intensity (FI)**
- ✓ Notation : **Non graduated scale (0 to 10)**
- ✓ Samples preparation
  - ☞ **14 days ageing (vacuum-packaged)**
  - ☞ **Cooked on grill**



# Material and Methods

## - Physicochemical Characteristics -

### Ultimate pH

#### FIBRES and ENZYMES

- ✓ Fibre size
- ✓ Myosine isoformes proportions
  - ✓ I ; IIA ; IIX
- ✓ Enzyme activities
  - ✓ LDH ; ICDH ; COX
- ✓  $\mu$ -calpains content
- ✓ 20S proteasome content

#### CONNECTIVE TISSUE

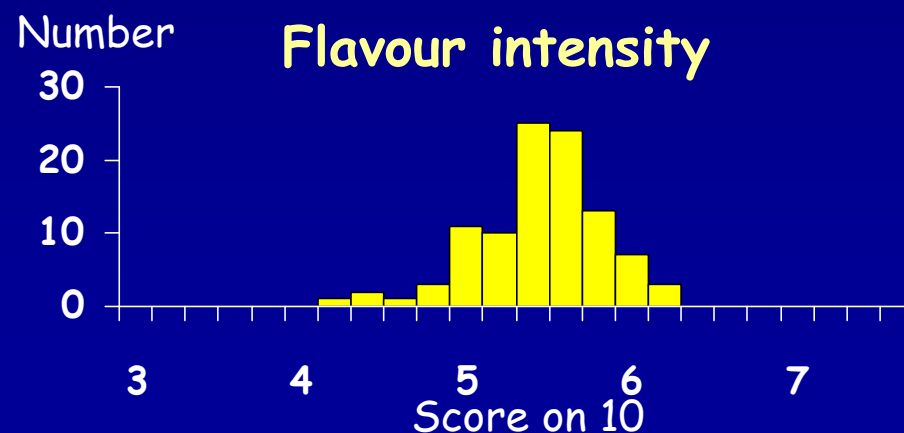
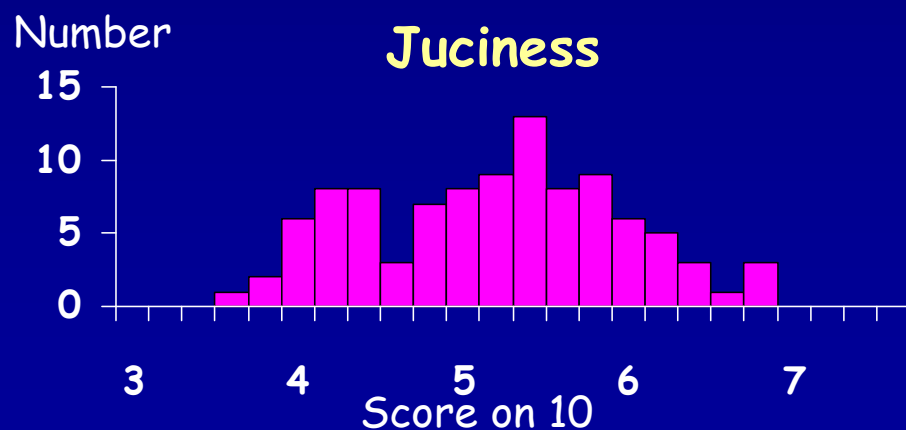
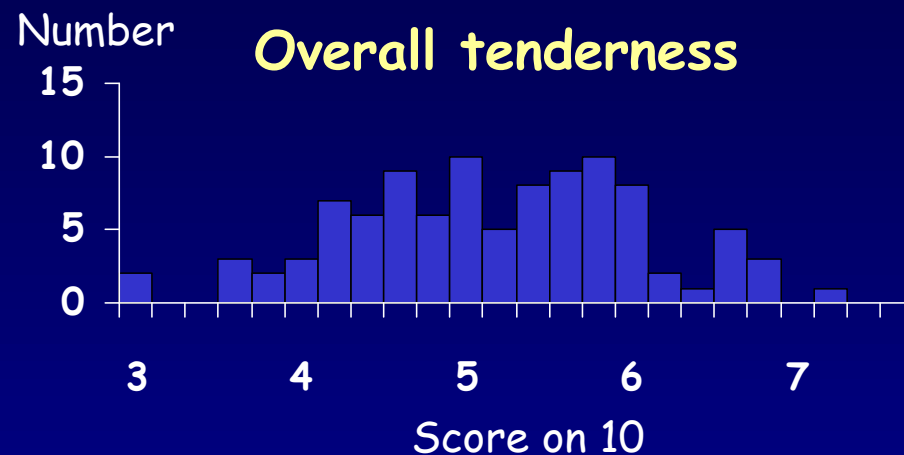
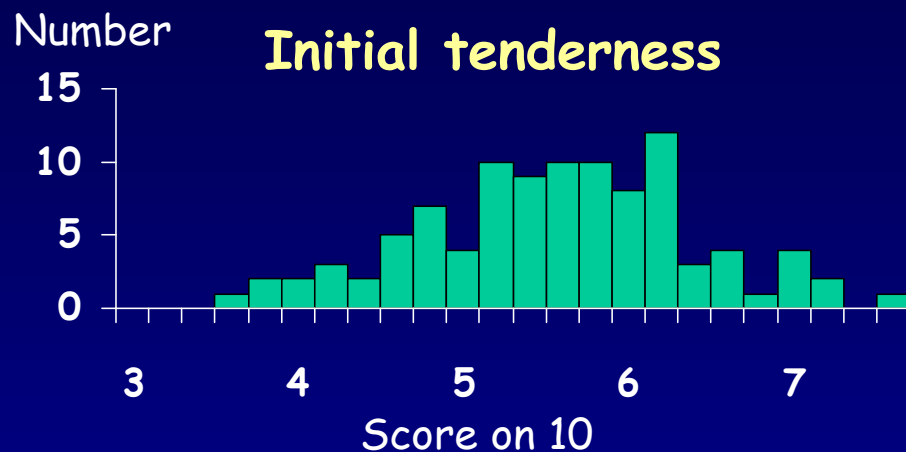
- ✓ Shear force
- ✓ Intramuscular fat content
- ✓ Collagen content
- ✓ Collagen solubility

#### COLOUR

- ✓ CIELAB colour measurements
- ✓ Heam pigment content

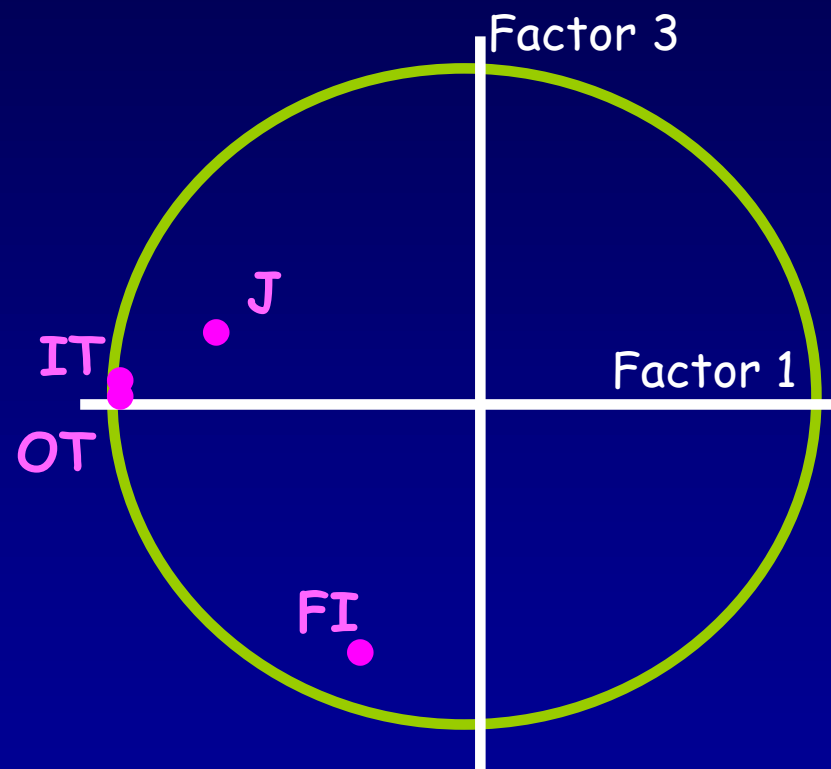
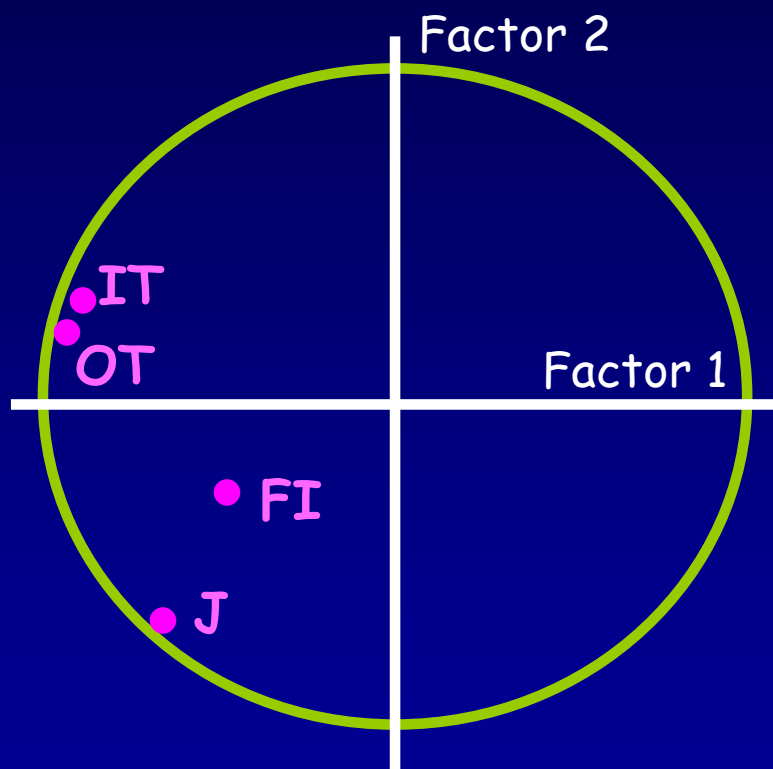
# Results and discussion

# Results : Descriptors scores





# Results : Principal Component Analysis



Representation of meat quality attributes in the two-dimensional space of the principal component analysis defined by factors 1 and 2 and factors 1 and 3

# Results : Correlations coefficients

	Initial tenderness	Overall tenderness	Juiciness
Overall tenderness	+0,97		
Juiciness	+0,52	+0,46	
Flavour intensity	+0,40	+0,35	+0,48

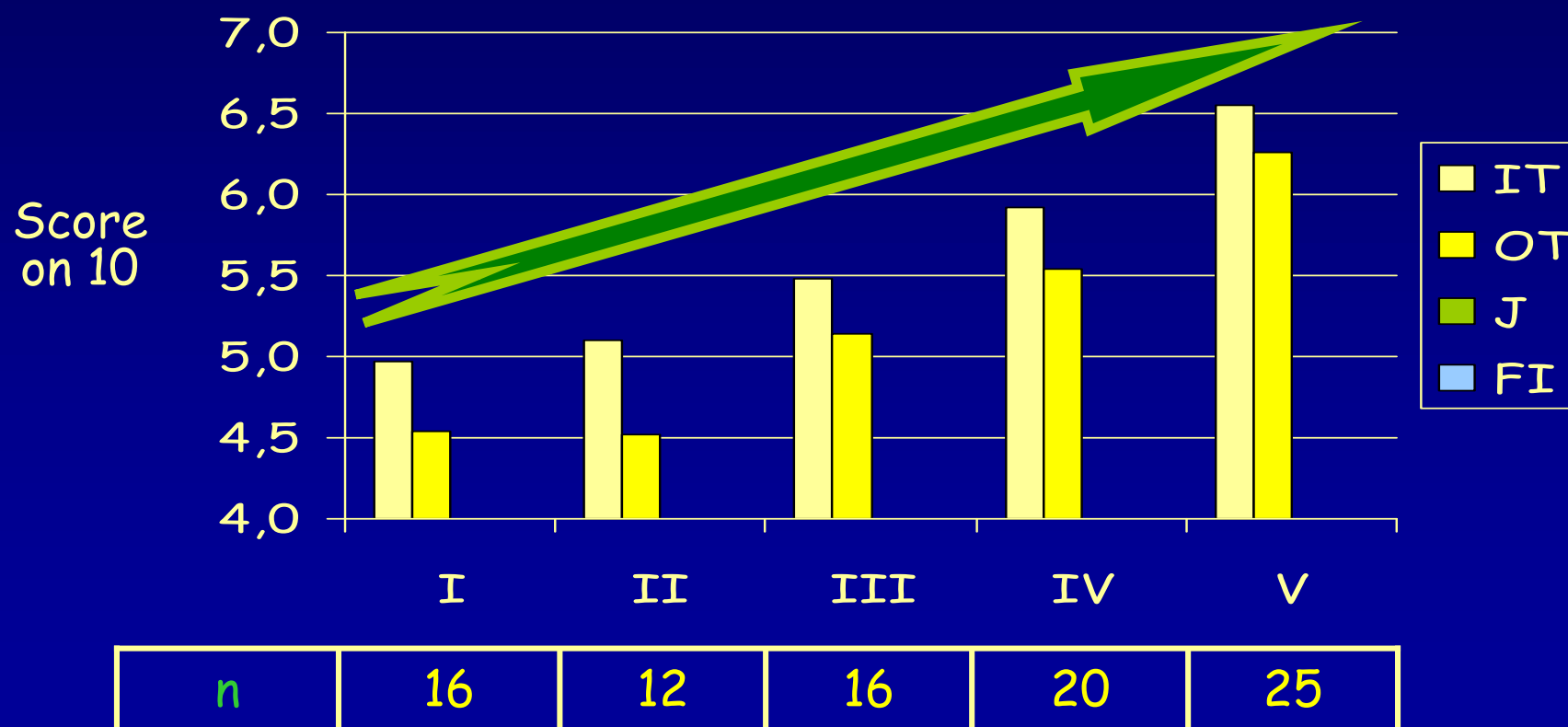
*Ockerman et al., 1984 ; Dransfield et al., 2002 ; Rhee et al., 2004 ; Serra et al., 2004*

# Results : Typology of meat

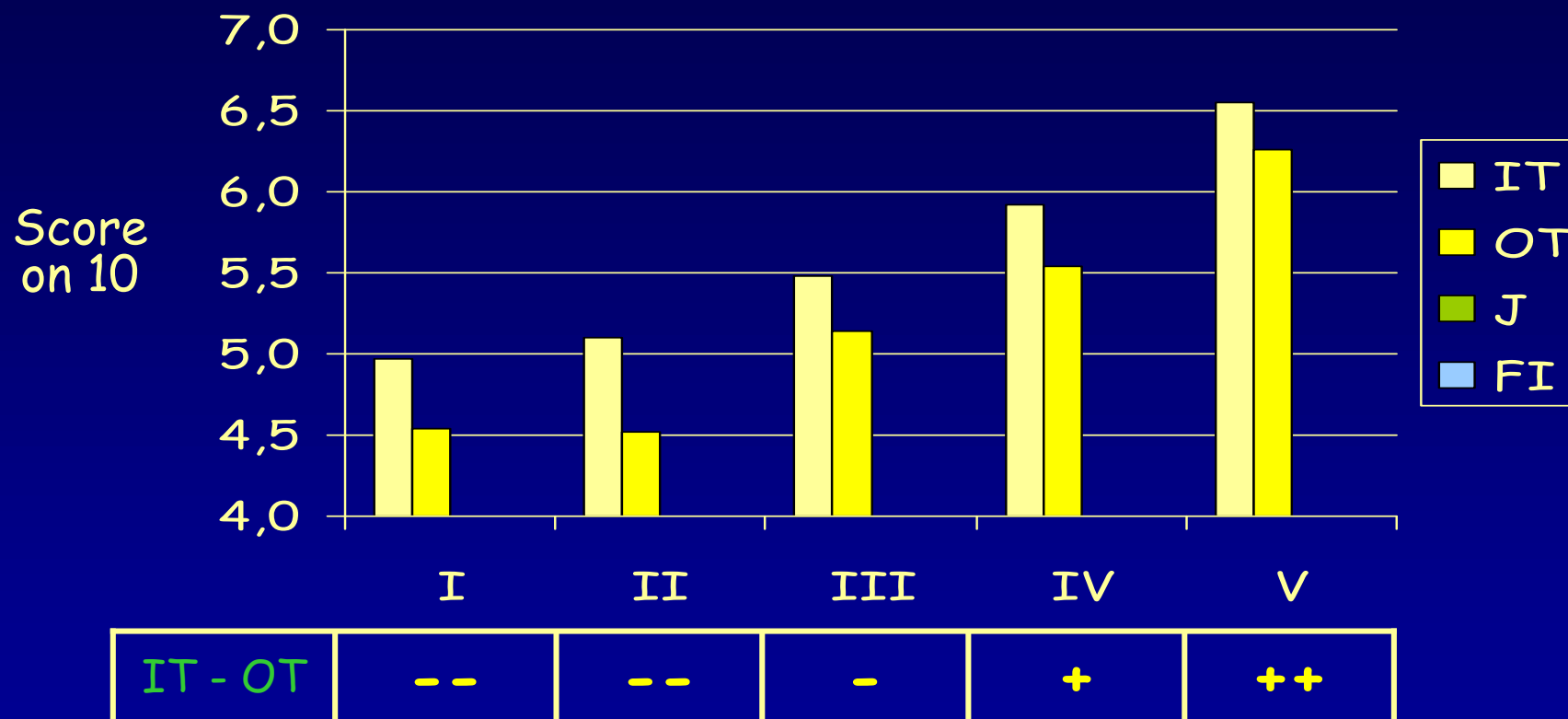
Principal Component Analysis + Hierarchical Cluster analysis



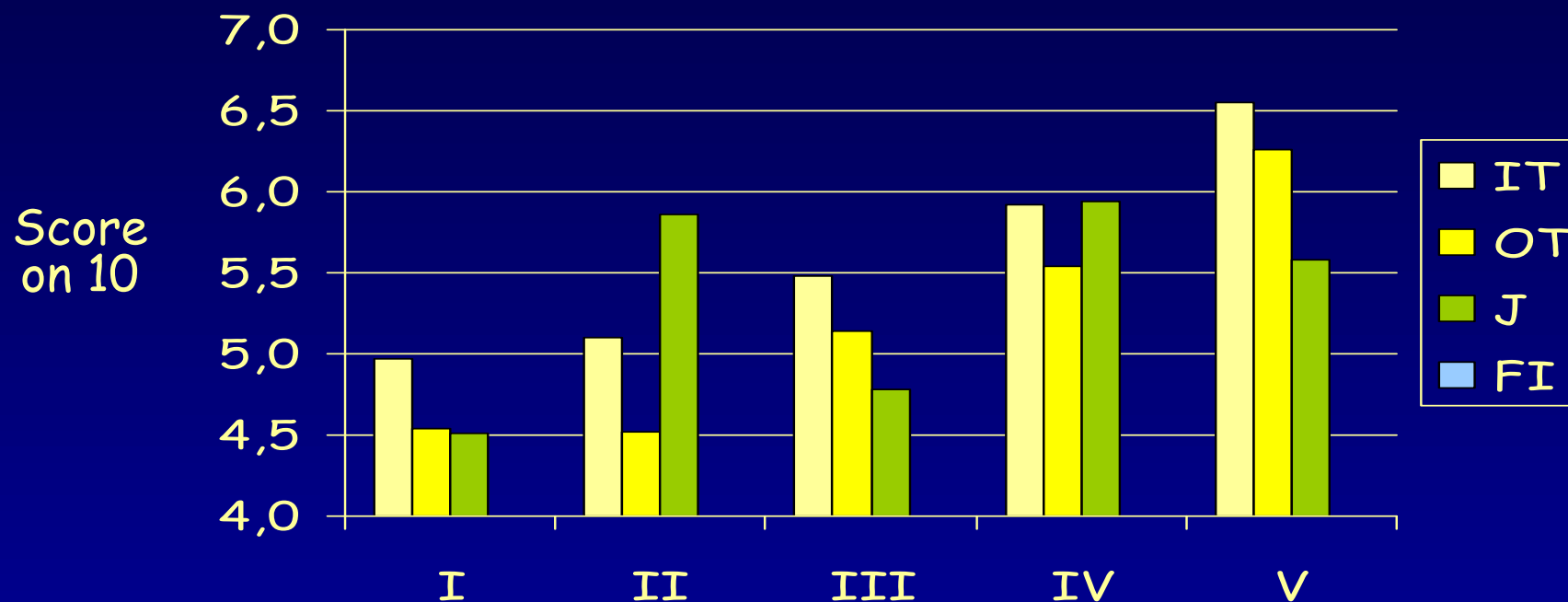
5 types of meat



# Results : Typology of meat

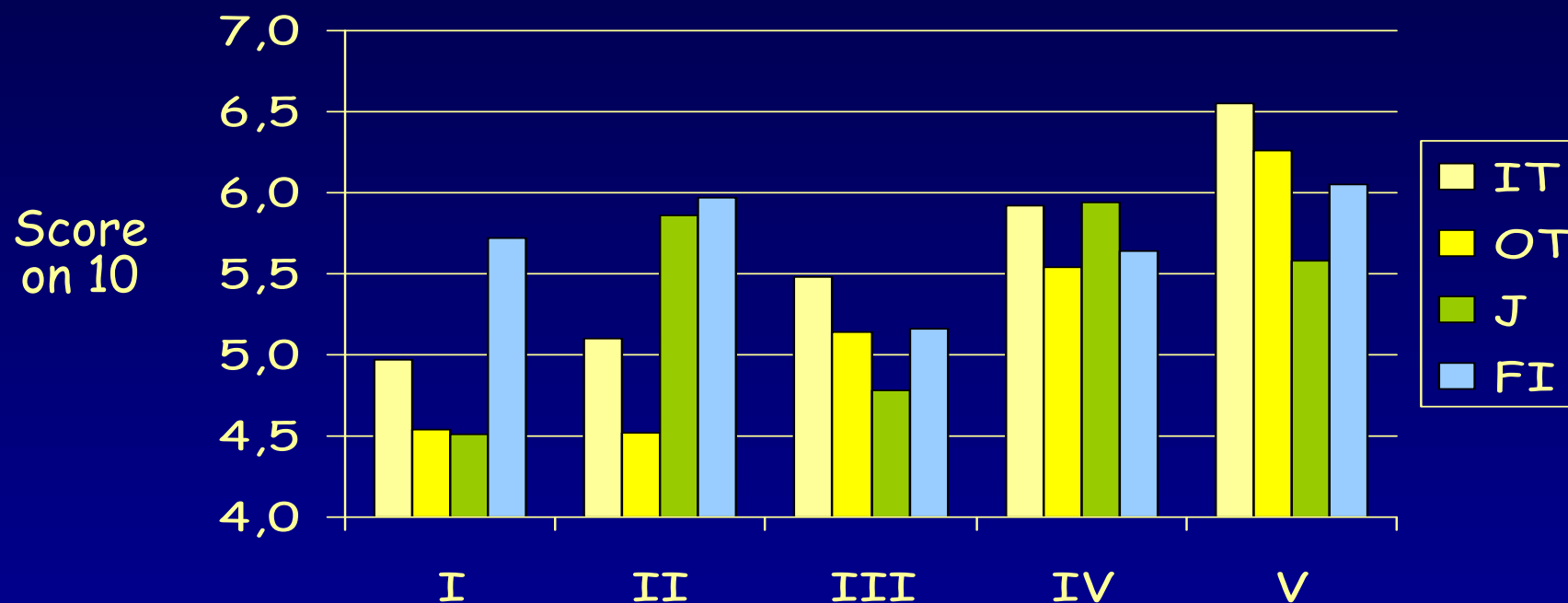


# Results : Typology of meat



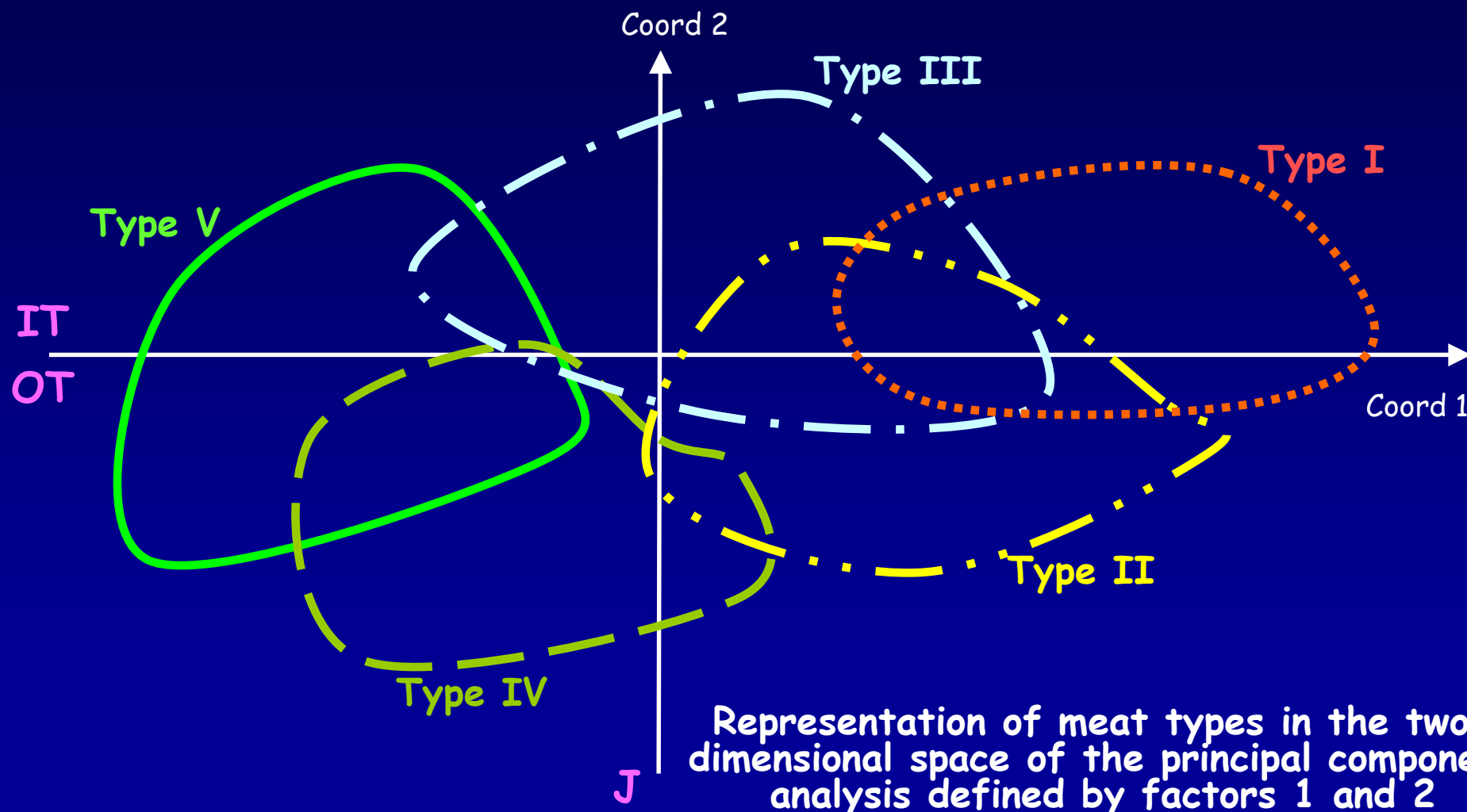
IT - OT	--	--	-	+	++
J	-	=	-	+	=

# Results : Typology of meat



IT - OT	--	--	-	+	++
J	-	=	-	+	=
FI	=	+	-	=	+

# Results : 2-dimensional space of the PCA



# Results : Physicochemical characteristics

Meat types	I	II	III	IV	V
Initial and overall tenderness	--	--	-	+	++
Juiciness	-	=	-	+	=
Flavour intensity	=	+	-	=	+
Intramuscular fat content (% DM)	15,9 a	17,0 ab	15,0 a	20,3 b	19,9 b
Shear Force (daN)	6,7 a	7,9 b	6,3 a	6,5 a	6,5 a
Total collagen content (mg/g DM)	21,0 a	23,9 b	22,0 a	20,9 a	20,1 a
Collagen solubility (% total collagen content)	16,7 b	13,8 a	16,6 b	16,5 b	15,5 ab



# Results : Physicochemical characteristics

Meat types	I	II	III	IV	V
Initial and overall tenderness	--	--	-	+	++
Juiciness	-	=	-	+	=
Flavour intensity	=	+	-	=	+
Haem pigments ( $\mu\text{g}$ /g wet weight)	61.0	61.5	59.4	57.5	58.5
L*	34.0	32.3	34.6	33.1	33.7
a*	20.1	21.1	20.1	20.1	20.4
b*	5.7	5.5	5.8	5.8	5.8

# Results : Physicochemical characteristics

Meat types	I	II	III	IV	V
Initial and overall tenderness	--	--	-	+	++
Juiciness	-	=	-	+	=
Flavour intensity	=	+	-	=	+
Isocitrate dehydrogenase ( $\mu\text{mol}/\text{min}/\text{g}$ )	1.41	1.53	1.43	1.31	1.33
Lactate dehydrogenase( $\mu\text{mol}/\text{min}/\text{g}$ )	498	498	501	506	503
Cytochrome c oxydase ( $\mu\text{mol}/\text{min}/\text{g}$ )	16.6	15.7	17.4	15.8	17.2
$\mu$ -calpain (percentage of a control)	97	94	99	99	109
27K proteasome sub-unit (percentage of a control)	152	132	156	145	138

# Results : Physicochemical characteristics

Meat types	I	II	III	IV	V
Initial and overall tenderness	--	--	-	+	++
Juiciness	-	=	-	+	=
Flavour intensity	=	+	-	=	+
Mean fibre area ( $\mu\text{m}^2$ )	3600	3383	2805	3314	3568
Myosin heavy chain I percentage	29.1	30.3	29.5	30.5	30.8
Myosin heavy chain IIa percentage	34.9	36.2	35.7	35.4	36.3
Myosin heavy chain IIx percentage	35.9	33.5	34.8	34.1	32.9

➡ Fibres of muscle *rectus abdominis* have many specificities  
(Picard et al., 2003)

## Muscular physicochemical characteristics

⇒ allow the differentiation of meat types, thanks the following meat physicochemical characteristics :

shear force,  
collagen amount and solubility,  
intramuscular lipids amount.

⇒ but not the characterization of each meat types

⇒ 12 to 23 % of sensorial properties variability,

⇒ necessity to add others characteristics.

Thank you  
for your attention

