Molecular biomarkers, Near infra-red spectroscopy and Computed tomography as new methodologies applied in TREASURE project to predict the quality of pork and pork products from local pig breeds

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Context : Pork quality

- **Pork** : most widely eaten meat, great diversity of products

- **Quality of pork**
  - Carcass: weight & lean/fat ratio → commercial value
  - Meat & products   - sensory (appearance, taste, tenderness)
    - technological (processing ability)
    - nutritional
    - hygienic
    - extrinsic properties : production system

Various expectations

Pig breed/genotype, rearing conditions: feeding level & diet composition, housing…, slaughtering and **meat processing** conditions…
- **Multifactorial determinism**
- **Late (post-mortem), invasive and costly determination**

⇨ **Need for prediction methods / tools**
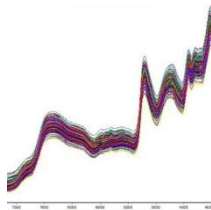
How to predict pork quality?

Approaches

- Assess effects of breed or production system

- Identify underlying biological mechanisms or physical specificities

- Research on reliable predictive indicators of quality traits

➡ Methodologies : biology, physics, data analysis and modeling
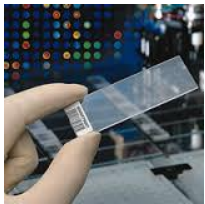
'Omics' Spectroscopy Computed tomography

Impacts

Decision-making and management tools

- Slaughterhouses: better allocate carcasses or cuts to the appropriate process or markets

- Pig production/slaughter : improve practices to optimize the intrinsic tissue traits favorable for quality

- Breeding : select breeds/lines for high quality pork

Recent developments

1. Molecular biomarkers

✓ Genes expressed in muscle tissue : transcripts

2. Spectra methodologies

✓ Near Infra-red Spectroscopy (NIRS)

**3. Computed tomography**

✓ X-ray and 3D imaging

Use in TREASURE project to predict carcass and meat quality (MQ) from local pig breeds
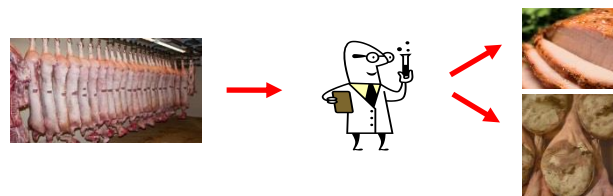
Molecular biomarkers - 1

➢ Evaluate / predict <u>early after slaughter</u> the subsequent sensory and technological quality of pork

➢ Biological level : underlying mechanisms determining meat Q

1. Identification and validation of **biomarkers of MQ traits**: functional genomics (high-throughput & global approach)

DNA → **mRNA** → Proteins → Metabolites → Phenotypes
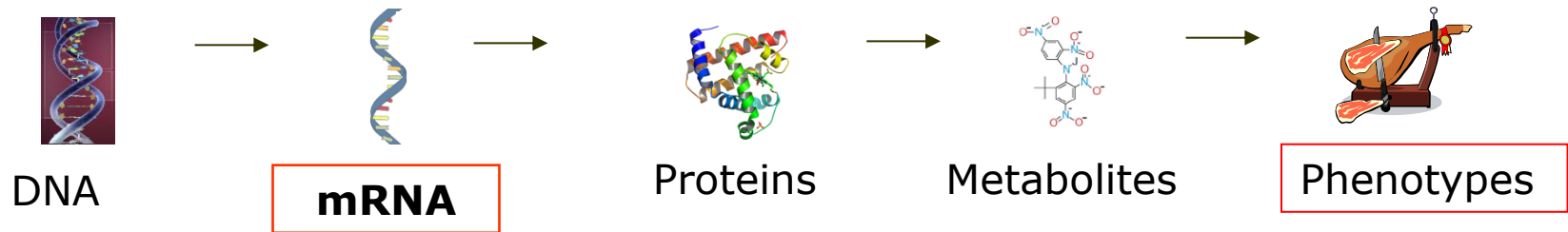
(Te Pas et al 2011, 2017; Picard et al 2015)

2. Identify **predictors of pork quality classes** (levels)

(Lebret et al 2015)

Molecular biomarkers - 2

1. Identification of biomarkers by transcriptomic analysis

Longissimus muscle samples
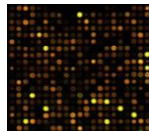high & gradual variability of sensory and technological quality (n=50)
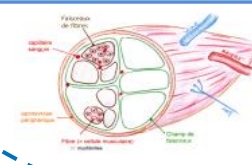
30 min p.m.

1 to 4 d p.m.

RNA: Transcriptomics
Microarray 15K: expression ~9000 genes

MQ: pH, color, intramuscular fat, drip loss, shear force, tenderness, juiciness

Transcripts - traits individual associations
140 (a*) up to 3000 (tenderness) correlations (P- *BH* **<0.10; R²≤ 0.65)**

(Damon et al 2013)

Molecular biomarkers - 3

40 genes, q-RT PCR

Confirmation

(same animals)

113 correlations, R² ≤ 52%

Internal validation

(n=50 complementary data)

60 correlations validated, R² ≤ 46%

External validation

(n=100 commercial pigs)

19 correlations validated, R² ≤ 24%

MQ trait	n genes correlated	R² max. %
pHu	10	46
Drip loss	11	26
Lightness	12	30
Redness (a*)	4	15
Hue angle (h°)	10	38
IM Fat	6	22
Shear force	1	8
Tenderness	4	14

- Identification and validation of **biomarkers of pork quality**
- Improve their **predictive value** → control tools

(Damon et al 2013, Lebret et al 2013)

TREASURE

2. Predictors of sensory & technological pork quality grades

↳ **Previous data set (n=100 pigs) wide & gradual variation in MQ**
Expression level (PCR) of 40 genes
Muscle tissue composition
Sensory & technological quality

↳ **Determination of MQ classes**

- Scientific expertise, literature, expertise of pork chain actors
  → important MQ traits, threshold values of acceptance/rejection

- Multidimensional statistical analyses
  → Traits discriminating MQ levels

Choice of **4 discriminant indicators** and **threshold values**

pH 30 min
pH 24h
drip loss
Intramuscular fat

➡ Decision tree ➡ **3 quality classes** (sensory & techno)
- Low **L**
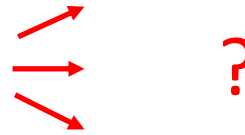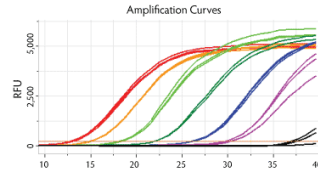- Acceptable **A**
- Extra **E**

(Lebret et al 2014)

Molecular biomarkers - 5

Combinaison of genes expression → discriminate quality classes

✍ **Statistics : multinomial generalized linear model**

- Stepwise selection
- Constraint : probability cut-point to predict class L=0.3 (↘ risk over-ranking)
- Cross validation 'leave one out'

Best predictive model : 12 genes

Cross validation: 76% accurate classification
 (88% for L and 82% for E samples)

		Predicted		
		Low	Acceptable	Extra
Observed	Low	30	2	2
	Acceptable	9	13	3
	Extra	4	2	28

Predictors (at slaughter) of the subsequent level of pork quality : L / A / E
External validation - - - → control tools

(Lebret et al 2015)

Near Infra-red spectroscopy - 1

➢ **Physical analysis methods** based on principle of energy **absorption** of organic molecules (bonds) at **specific wavelenghts** (NIRS : 700-2500 nm)

➢ Applications in agricultural and **food products : composition and quality**

➢ **Fast and non destructive** method with **little or no preparation of sample**

➢ Laboratory or **portable devices → industrial conditions**

➢ Indirect method : **calibration step**

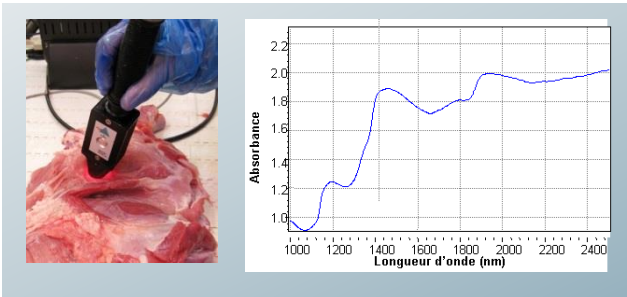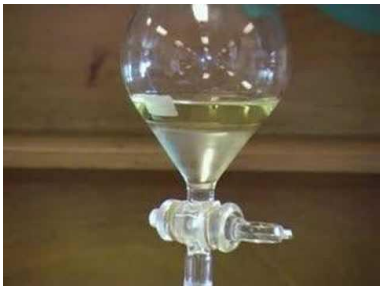$Y = \beta_0 + \beta_1 X + \varepsilon$
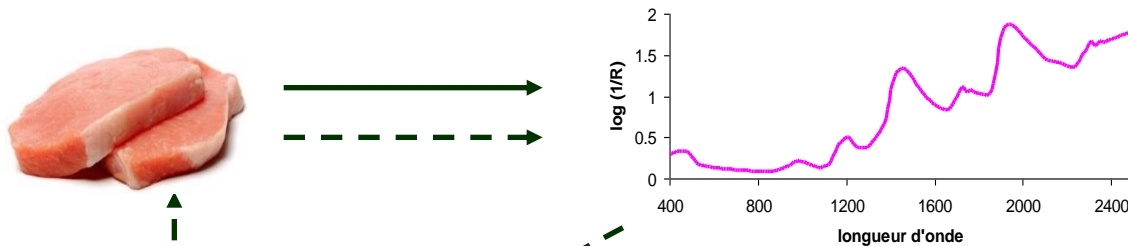
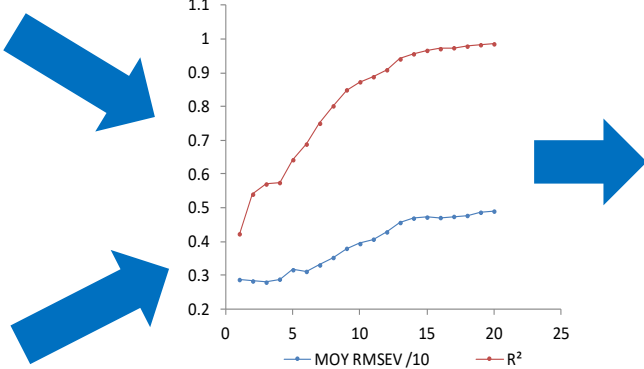R^2, error

Near Infra-red spectroscopy - 2

- ➤ **Calibration** : relationships between trait to be predicted / NIR spectra
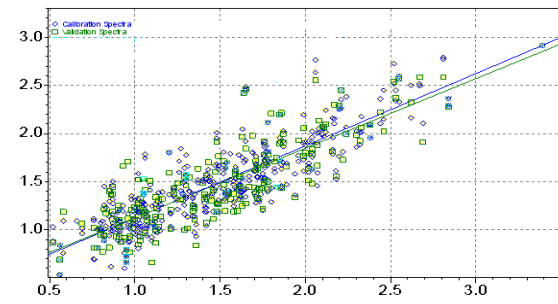- ➤ **Validation** : predicted / observed value

NIRS measurement

Reference method

Calibration
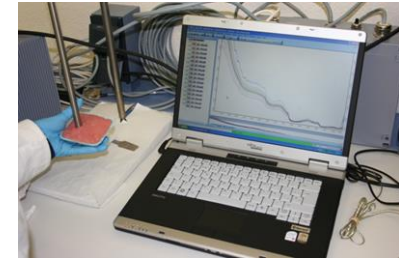Multivariate analyses
(PCA, PLS)

Cross and external
validation

Near Infra-red spectroscopy - 3

➢ **Chemical composition of raw meat : high accuracy**
- Fat, protein, water
- Fatty acid composition of fat or muscle tissue
(intact, minced or homogeneized samples)

➢ **Physical characteristics of raw meat : lower accuracy**
- Physical traits only partly related to chemical composition
- Processing yield of cooked ham : development of prediction method

➢ **Processed products**
- Chemical composition : fat, water, fatty acids, salt…
- Physico-chemical traits related to eating quality : rheology, proteolysis index

(Prevolnik et al 2005, 2010; Font-i-Furnols et al 2015)

Computed tomography : CT- 1

TREASURE

- ➢ **Non invasive technique –** Meat sector : **in vivo or post-mortem**
- ➢ **Combines X-ray and 3D imaging**

  - • **X-Rays:** differential attenuation depending on **density (nature) of tissues**

  - • **CT:** X-ray source and detector rotate around the object

    → matrix of attenuation values (Hounsfield units)

    → **3D image = tomogram : lean / fat / bone tissue**

- • **Evaluate body / carcass composition** ; growth curves

- • **Lipid** content in **raw meat**

- • **Salt** content and diffusion, **water** content and activity in **processed products**

- ➢ **CT : breeding purposes; control** in slaughter/processing plants

(Font-i-Furnols et al 2009, 2015; Fulladosa et al 2010)

Computed tomography - 2

> **Proportion and distribution of lean and fat tissues according to pig genotype**
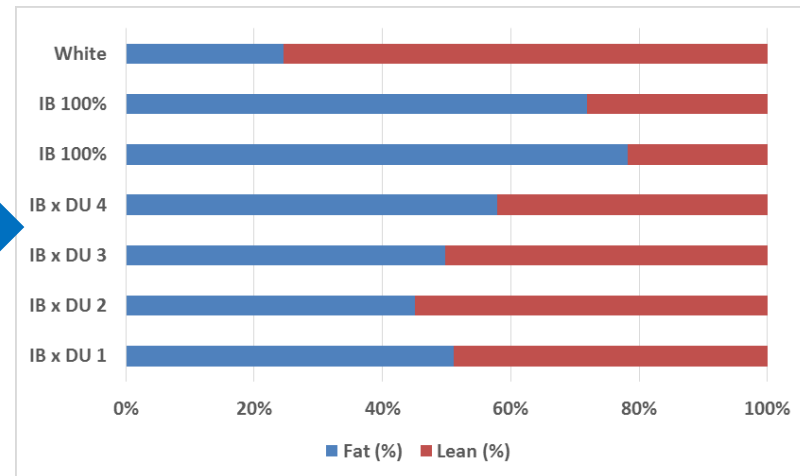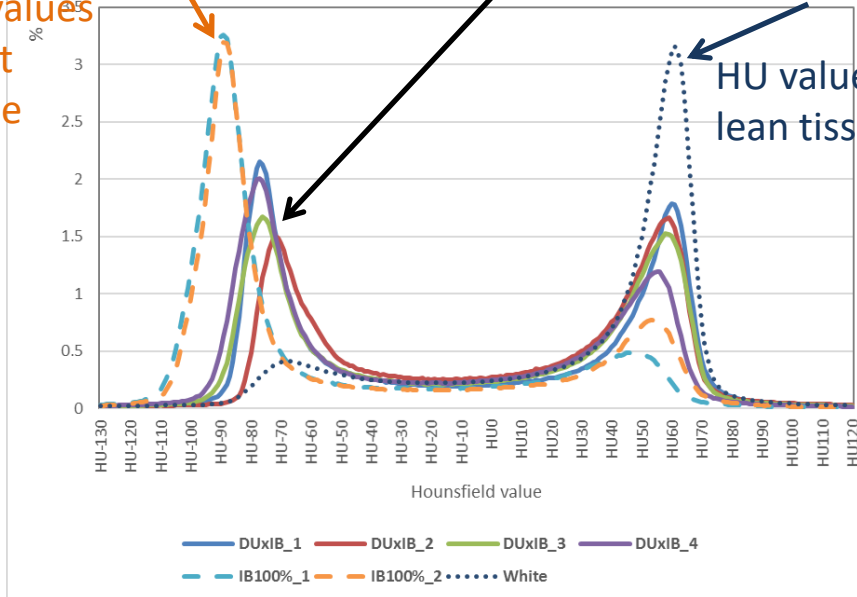
100% Iberian / Duroc X Iberian / Commercial 'white'

(Font-i-Furnols et al 2017)
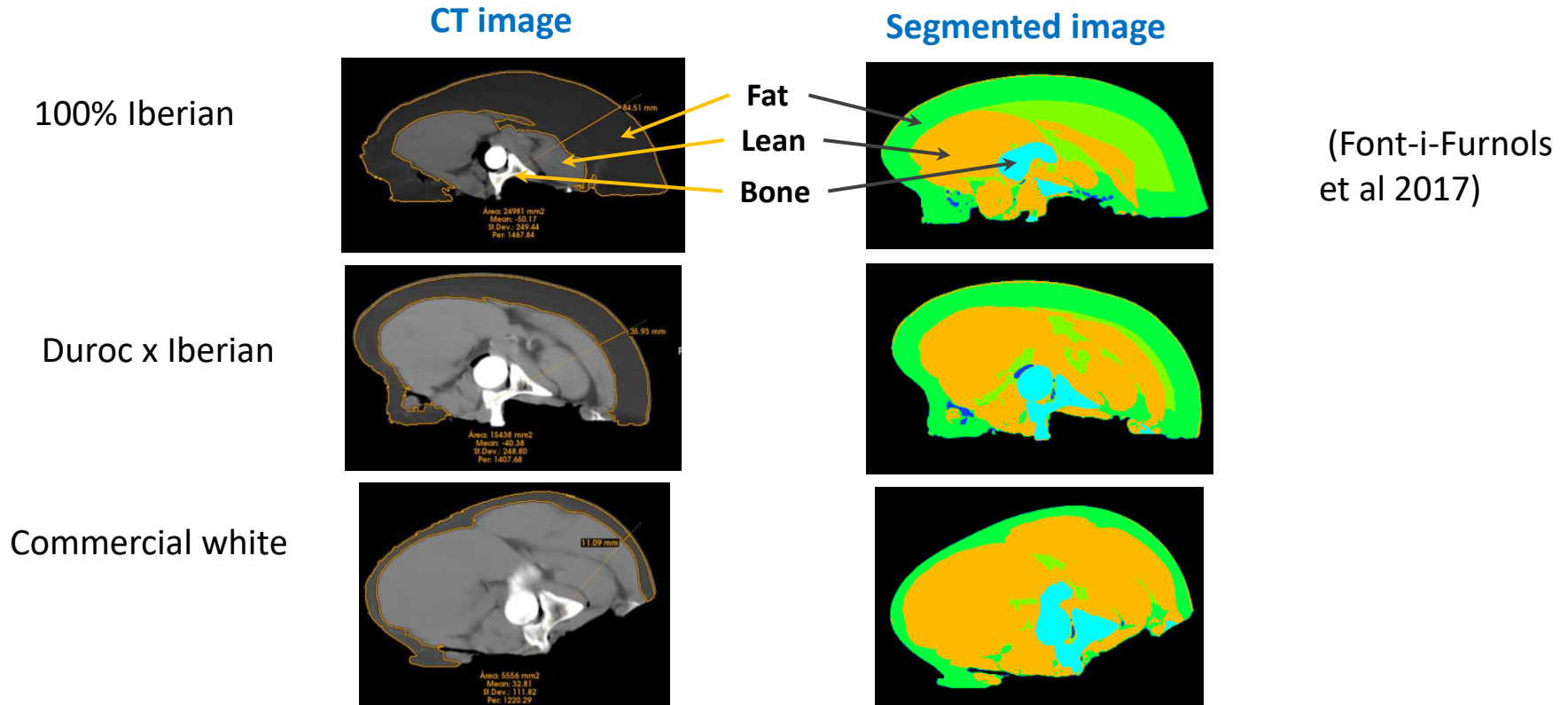

HU values of fat tissue

HU values of lean tissue

Percentages of total fat and lean

Computed tomography - 3

> **Distribution of lean and fat tissues in ham according to pig genotype**

CT image **Segmented image**

100% Iberian — Fat, Lean, Bone

Duroc x Iberian

Commercial white

(Font-i-Furnols et al 2017)

> **CT: non invasive & non-destructive determination of carcass/cuts composition**
> **Measures of tissue area / thickness at anatomical locations of interest**

Applications in TREASURE

✓ **Molecular biomarkers**

– External validation: predictors - individual MQ traits (pH, drip, colour, IMF..)

- MQ classes (Low, Acceptable, Extra)

Samples (n=175) from 4 pure local breeds exhibiting variability in MQ

✓ **NIRS**

– Prediction of lipid content and fatty acid composition of muscle & fat tissue

Samples (n=634) from 17 local breeds, various feeding regimen or production systems

– Prediction of chemical composition and quality traits of processed products

Samples from various products (salami, pancetta, hams) from 4 local breeds

✓ **CT**

– Proportion and distribution of lean & fat tissues in carcass or primary cuts

Samples from pure local or crossbreeds pigs – pilot study

New methodologies / tools to evaluate / predict quality of pork and pork products from local breeds

Thank you for your attention