Analyse multivariée de la variabilité de la qualité de viande de porc selon la race et le système d'élevage

Motivation

Les avancées technologiques permettent aujourd'hui de générer des données issues de plateformes différentes. Ainsi l'analyse de données interdisciplinaires visant à mieux comprendre et maîtriser les différentes fonctions biologiques à tous les niveaux d'intégration auxquels elles se manifestent au niveau du même système biologique est maintenant possible. Cette approche analytique globale, appelée biologie intégrative, a pour but d'étudier un ou des organismes en intégrant des données de sources multiples, et ainsi de mieux appréhender les différents processus cellulaires très complexes inhérents au système.

Les objectifs d'une telle analyse intégrative sont les suivants (Steinfath *et al.,* 2008) :

1. comprendre les interactions entre variables de même type ;
2. relier ces variables de même type au phénotype (ensemble de caractéristiques observables caractérisant un être vivant) ;
3. comprendre les relations entre des variables de types différents.

Ces objectifs rendent l'analyse statistique peu aisée compte tenu du nombre limité d'échantillons et du grand nombre de variables mesurées.

Les méthodes appliquées pour résoudre ces problèmes d'intégration de multiples données biologiques sont très souvent des méthodes classiques de projection multivariées, puisqu'elles permettent de projeter les données dans des espaces de dimension plus petite. Le biologiste peut alors plus facilement interpréter les résultats grâce à des représentations graphiques résumant l'information.

Méthodes d'analyse multivariées

Il existe de nombreuses méthodes dites d'analyse multivariées : analyse en composantes principales (ACP), analyse factorielle des correspondances (AFC), analyse des correspondances multiples, analyse factorielle multiples (AFM)…Le lecteur pourra se référer à Escofier et Pagès (1988 et 1998) pour une description de ces approches.

Dans le cadre de ce travail, nous nous sommes principalement focalisés sur deux approches.

La méthode d'analyse à un tableau : ACP

L'Analyse en Composantes Principales (Jolliffe, 2002) est la méthode de projection multivariée la plus connue. En général elle ne s'applique que sur un seul tableau X de taille n x p où le vecteur x^j représente les mesures des variables j de type X pour chaque échantillon.

L'ACP est utilisée comme un outil préliminaire pour visualiser de façon rapide si les échantillons biologiques peuvent être séparés au niveau de l'expression des variables suivant les conditions biologiques mesurées lors de l'expérience (Steinfath *et al.,* 2008).

Rappelons que le but de l'ACP est de trouver des combinaisons linéaires des variables initiales appelées composantes principales, qui maximisent la variance du jeu de données. Les composantes principales sont orthogonales entre elles et représentent de nouvelles variables artificielles non corrélées. Ainsi, nous recherchons les vecteurs unitaires $v_1 \ldots v_H$ tels que :

$$\arg\max_{v'_h v_h=1} \mathrm{var}(Xv_h)$$

où les v_h, h = 1…H, H < p, sont les vecteurs appelés facteurs principaux ou « loadings » et les composantes principales associées sont les Xv_h. La plus grande partie de la variance est, par construction, expliquée par les premières composantes principales H. Notons une propriété très utile des facteurs principaux qui est la correspondance directe entre leur coordonnées et l'importance des variables dans le modèle, dans le cas de variables homogènes ou réduites.

Une limite de l'ACP lorsque l'on dispose de données de grande dimension est l'interprétabilité et le manque de lisibilité des résultats lorsque le nombre de variables devient trop grand.

Dans le cadre de données à très grandes dimensions, nous nous sommes inspirés d'une variante dite sparse PCA qui permet de réduire le nombre de variables en fixant des simple seuillages, et qui consiste, de manière empirique, à annuler les coefficients des variables dont les valeurs absolues sont inférieures à un seuil donné (Cadima et Joliffe, 1995). Dans le cadre de notre travail, l'approche sparse PCA choisie était celle proposée par Zou *et al.* (2006) : sparse PCA Elastic Net.

L'analyse factorielle multiple : AFM

Dans le cadre de l'AFM les données sont constituées d'un ensemble d'individus, {*i* ; *i*=1, *I*}, décrits par plusieurs groupes de variables. Ces données peuvent être regroupées sous forme d'un tableau unique structuré en sous-tableaux (Figure 4.1). On note :

X : le tableau complet ;

K : l'ensemble des variables ;

J : l'ensemble des sous-tableaux ;

Kj : l'ensemble des variables du groupe *j* ;

Xj : le tableau associé au groupe *j*.

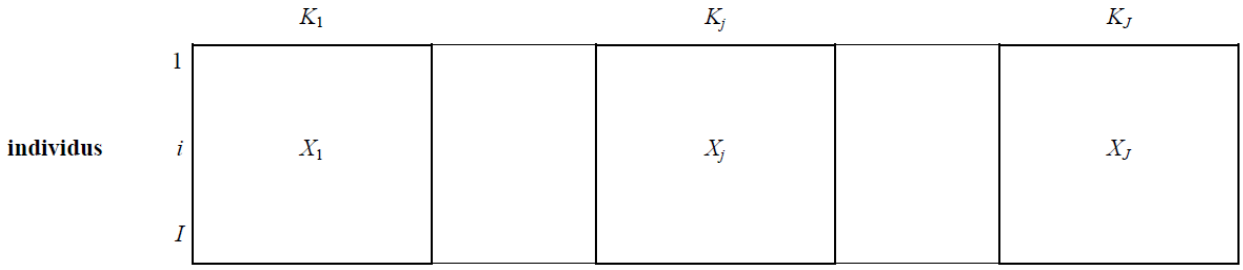

Figure 4.1 - Structure des données dans le cadre d'une AFM

Au tableau *X* correspond le nuage des individus, N_I, situé dans l'espace R^K. A chaque groupe de variables, correspond un nuage d'individus, dit partiel et noté N_i^j, situé dans un espace de dimension K_j.

Si l'on plonge le nuage N_I^j dans l'espace R^K les coordonnées de chacun des individus de ce nuage se trouvent au sein du tableau, noté $\tilde{X}_j$, de dimension (I, K), dans lequel X_j est complété par des 0.

L'AFM propose une représentation superposée des nuages partiels. Le cœur de cette analyse est constitué par une ACP effectuée sur le tableau complet *X*, dont les variables sont pondérées. La pondération utilisée consiste à diviser le poids initial de chaque variable du groupe *j* par λ_i^j (en notant λ_i^j l'inertie projetée sur le premier axe de l'analyse séparée du groupe *j*). On obtient ainsi une représentation du nuage N_I , comme dans toute ACP. A cette représentation, on superpose les nuages N_I^j en introduisant les tableaux $\tilde{X}_j$ en

supplémentaires dans l'ACP du tableau complet *X*. Cette représentation a au moins deux propriétés intéressantes :
- elle s'inscrit dans une méthode générale qui fournit de nombreux points de vue sur l'analyse simultanée de plusieurs tableaux en particulier de nombreuses aides à l'interprétation ;
- il existe pour cette représentation des relations de transition dites partielles.

Objectifs de l'étude

Afin de générer la variabilité nécessaire pour étudier les relations entre un très grand nombre de variables habituellement utilisées pour l'évaluation de la qualité de viande de porc, un dispositif expérimental incluant 50 animaux provenant de deux races contrastées a été utilisé : une race classique (Large White, LW), et une race locale (basque, B) du sud-ouest de la France qui est génétiquement éloignés des autres races européennes (Laval *et al.,* 2000). Les animaux ont été élevés dans trois systèmes d'élevage différents : le système conventionnel, alternatif et extensif. Ces systèmes sont connus par leur impact sur les paramètre de la qualité de viande (Guéblez *et al.,* 2002; Alfonso *et al.,* 2005; Lebret *et al.,* 2006).

Notre objectif était d'identifier la structure des corrélations entre un très grand nombre de variables de mesure de la qualité de viande (~ 255 variables) classées dans 9 sous groupes distincts : transcriptomiques, protéomiques, chimiques, composition d'acides gras, protéolyse, fibres et de réactivité à l'abattage.

Les analyses multivariées, notamment l'ACP et l'AFM, semblent les plus appropriées pour explorer les relations existant entre ces variables tout en considérant l'ensemble des données simultanément. Ainsi, et après avoir analysé les corrélations entre les groupes de variables de manière globale par le biais de l'ACP, nous nous sommes concentrés sur cette structure des corrélations qui discriminent les classes de race x système d'élevage, indépendamment de la variance résiduelle en considérant les moyennes des classes. Enfin, nous avons cherché à identifier quels sont les groupes de variables qui expliquent la variabilité intra-classes en appliquant une AFM sur les résidus.

Résultats et discussion

Nos résultats ont montré que les variables discriminant les cinq classes de race x système d'élevage étaient les mêmes que celles qui discriminaient les individus entre eux, en l'occurrence les variables transcriptomiques, chimiques, technologiques et celles de mesure des acides gras. Le groupe composé des variables relatives à la réactivité à l'abattage discriminaient principalement les systèmes d'élevage, en particulier la classe des porcs basques élevés dans le système extensif.

Les résultats obtenus dans le cadre de cette étude confirment l'intérêt de ce genre d'analyses pour l'exploitation et l'exploration de grands volumes de données.

Aujourd'hui, une grande quantité d'information est recueillie et généralement stockées dans des bases, et qui peuvent être organisées selon une hiérarchie. Avec l'AFM, les structures dans lesquelles s'inscrivent ces données peuvent être fructueusement étudiés. Ainsi, l'AFM peut être considérée comme un outil de «data-mining». Sa principale caractéristique consiste en sa capacité à fournir:

- ✓ Une représentation graphique résumant l'ensemble des données ;
- ✓ Des représentations partielles qui reflètent les variables impliquées dans les différentes classes des individus ;
- ✓ Un aperçu graphique montrant comment ces différentes classes sont reliées les unes aux autres.

Les analyses multivariées peuvent être considérées comme des analyses préliminaires mettant en évidence les principales relations entre les groupes de variables, et qui peuvent être complétées par des analyses quantitatives de ces relations par d'autres méthodes statistiques, telles que l'analyse de variance (ANOVA) ou l'analyse de variance multivariée (MANOVA).

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A multivariate analysis comparing pig meat quality traits according to breed and rearing system

A multivariate analysis comparing pig meat quality traits according to breed and rearing system

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Abstract

Meat quality traits covering a large range of biological features were recorded in 50 castrated Large White and Basque pigs raised in three rearing systems: conventional, alternative and extensive (the last only containing Basque animals). Five classes were then defined by breed x rearing system. Traits were classified into nine groups: transcriptomic and proteomic data, sensorial, technological, chemical, fatty acids, muscle fibre, lipid and protein oxidation, and slaughter reactivity traits. Multivariate analysis, and in particular Multiple Factor Analysis (MFA), was performed on all traits. An extension of MFA in which data are organized into a hierarchical structure is discussed. This method of analysis balances the role of the groups of variables at each level of the hierarchy and provides outputs that can be interpreted from an overall perspective (overall hierarchical structure) and from perspectives relating to both the various levels of the hierarchy (i.e. between-class analysis) and individual variability (i.e. within-class analysis).

Our results showed that the variables which discriminated the five classes were the same as those discriminating individuals. Indeed a first group composed of transcriptomic, chemical and technological variables discriminated breeds. Slaughter reactivity variables discriminated rearing systems, especially the Basque pigs reared in the extensive system.

Key words: pork, meat quality, multiple factor analysis, sparse principal component analysis, rearing system, breeds.

1. Introduction

The quality of pig meat depends on several interactive effects including genetic background (Sellier and Monin, 1994), rearing conditions (Lebret et al., 2006), pre-slaughter handling (Álvarez et al., 2009) and carcass- or meat-processing. However, the biological characteristics associated with sensory quality have not yet been clearly identified. To improve that quality we need a better understanding of which biological phenomena govern tissue characteristics and the way those phenomena impact on quality. The attributes that define the quality of meat have become very diverse, and today many definitions of meat quality can be found in literature: proposed definitions have focused on palatability, on technological aspects, and on safety (Mullen, 2002). Hoffman (1990) described meat quality as the sum of all quality factors in meat, including sensory, nutritive, hygienic and toxicological and technological properties (Aaslyng, 2002).

To introduce the variability needed to investigate relationships between the large number of variables usually used for pork meat quality assessment, an experimental design using two contrasting breeds was used. The breeds were: a conventional breed (Large White, LW); and a local breed (Basque, B) from the south west of France which is genetically distant from the other European breeds (Laval et al., 2000) and gives high eating quality in fresh pork and other pork products (Promeyrat et al, 2011). The animals were reared in three different rearing systems which are known to affect meat quality (Guéblez et al., 2002; Alfonso et al., 2005; Lebret et al., 2006, 2008).

Our aim was to identify the 'correlation structure' within a very large range of variables of meat quality by grouping those variables and considering all data sets in a global Multiple Factor Analysis (MFA). We subsequently focused on this correlation structure between classes of breed x rearing systems independently of the residual variance by working on the mean averages of each class. Finally, we studied the groups of variables explaining the intra-class variability by working on residuals.

Today MFA is a well established method (for a brief introduction, see Escofier and Pagès (1988, 1998)). It has been applied to data of various kinds — in particular, sensory profiling data (Pagès and Husson, 2001; Le Dien and Pagès, 2003), omics data (Tayrac et al., 2008), and, in pork husbandry, sow farrowing data (Canario et al., 2009). Its main features are twofold: first, the balancing of sets of variables; and second, outputs specific to the partition

of the variables in different sets — mainly, the superimposed representations of individuals and categories, and the group's representation.

2. Material and methods

2.1. Animals and rearing systems

A total of 50 pigs were used for the experiment: 20 LW and 30 B finishing castrated boars. Of these, 10 pigs of each breed were reared in a conventional system (C: slatted floor, 1.0 m^2/pig) and a further 10 of each breed were reared in an alternative system (A: bedding with free access to an outdoor area, 2.4 m^2/pig) at the French National Institute for Agricultural Research (INRA) experimental farm (Saint-Gilles, France). Moreover, 10 Basque pigs were reared in the extensive (E: free range) production system of B pigs (south west of France). Thus, we distinguish five classes of breed x rearing system:

	Conventional system	Alternative system	Extensive system
Basque	BC	BA	BE
Large White	LWC	LWA	

In order to standardize the period to animal slaughter, and taking into account differences in growth rates between the breeds and systems estimated by Guéblez et al. (2002) and Alfonso et al. (2005), the BE animals were 3 months older at slaughter than the BAs and BCs, and the LWA and LWC animals were two months younger at slaughter than the BAs and BCs.
All animals were slaughtered at an average live weight of 150 kg, according to standard procedures followed in the INRA experimental slaughterhouse; they were fasted for 36 h prior to slaughter (a Basque industry practice) and stunned with low voltage electricity.

2.2. Groups of variables

2.2.1.Slaughter reactivity

Blood temperature was recorded after slaughter (Thermometer JTEK, Cole Parmer Instrument Company, Chicago, IL). The blood was collected in EDTA tubes, centrifuged immediately, and stored at −20°C until plasma ACTH could be determined using a 2-site ^{125}I immunoradiometric assay (Nichols Diagnostic Institute, San Juan Capistrano, CA). The quantification limit of the assay was 6 pg/mL of plasma, and the intra- and inter-assay CVs were 3.0 and 7.8%, respectively, at 35 pg/mL. Blood was also collected in heparinized tubes, immediately centrifuged, and stored at −20°C until plasma cortisol could be determined using a competitive ^{125}I RIA kit (Immunotech, 13276 Marseille, France). The quantification limit of the assay was 8 ng/mL of plasma, and the intra- and inter-assay CVs were 4.2 and 10.0%, respectively, at 71 ng/mL.

Plasma concentrations of glucose and lactate (bioMerieux kits, Marcy l'Etoile, France) and FFA (Wako Chemicals GmbH, Neuss, Germany), as well as creatine kinase activity (bioMerieux kit), were all determined on the blood samples collected in heparinized tubes using a multichannel spectrophotometric analyzer (Cobas Mira, Hoffmann-LaRoche, Basel, Switzerland).

2.2.2.Lipid and protein oxidation

Lipid and protein changes were measured in *Longissimus lumborum* pig muscles 24 h after animal slaughter, after 3 days of subsequent refrigerated storage at 4°C, and after 30 min of cooking at 100°C. All biochemical measurements were performed in duplicate.

Lipid oxidation was evaluated by measuring the ThioBarbituric Acid Reactive Substances (TBARS) according to the method described by Mercier et al. (1998). The results were expressed as mg of malondialdehyde (MDA) per kg of meat (TBA units).

Basic amino acids oxidation was evaluated by measuring protein carbonyl groups according to the method first described by Oliver et al. (1987) and then modified for measurements in meat samples by Mercier et al. (1998). Carbonyl groups were detected by reactivity with 2,4 dinitrophenylhydrazine (DNPH) to form protein hydrazones. The results were expressed as nanomoles of DNPH fixed per milligram of protein.

Thiol oxidation was measured with a modification of Ellman's method using 2,2'-dithiobis (5-nitropyridine) DTNP (Morzel et al., 2006). The results were expressed as nanomoles of free thiol per milligram of protein.

Aromatic amino acids (tryptophan, and tyrosine) were determined using the method described by Gatellier et al. (2008) using second-derivative UV spectroscopy on meat extracts prepared in guanidine. The values were expressed as percentage of amino acid in meat (g/100g of meat).

Protein surface hydrophobicity was determined on myofibrillar proteins with a hydrophobic probe (bromophenol blue, BPB) according to the method proposed by Chelh et al. (2006). The results were expressed as micrograms of BPB bound to proteins.

Protein aggregation was determined on myofibrillar proteins using the method suggested by Long et al. (2008). Fluorescence light scattering was measured for the same excitation and emission wavelengths (λex and λem = 300 nm) on a PERKIN ELMER, Luminescence Spectrometer LS 50 B. The values were expressed in arbitrary units.

2.2.3.Sensory analysis

The day after slaughter a piece of the right loin of each carcass lying between the 2^{nd} / 3^{rd} and 9^{th} /10^{th} vertebrae was trimmed of external fat, kept at 4°C for 3 subsequent days, put under vacuum, and frozen at −20°C until sensory analyses could be performed at INRA-EASM (Le Magneraud, France). So the frozen loins were cut into chops, individually vacuum packaged, and stored frozen. After thawing (for 48 h at 4°C) a slice was taken for visual assessment of the intensity and homogeneity of red coloration and the marbling of the raw meat on a scale from 0 (absent) to 10 (high). The remaining chops were grilled with a double-contact grill at 250°C for 10 min. Samples comprising a third of the muscle part of the deboned chop with the remaining external fat (3–5 mm depth) were assessed by a 12-member, trained taste panel for odour (normal and abnormal odours of lean and fat), marbling, tenderness, juiciness, fibreness, and typical and abnormal flavours on a scale from 0 (absent) to 10 (high). The samples were served in daylight. Panellists were served water and bread to rinse their palates between samples. Individual panellist scores were averaged, and the mean scores from each sample were used in the subsequent statistical analysis.

2.2.4. Technological/Chemical

The internal temperature of *longissimus muscle* (LM) was recorded (temperature probe Pt1000, Knick, Berlin, Germany) 30 min after slaughter, and samples of this muscle were taken, frozen immediately in liquid nitrogen, and stored at −80°C until determinations of pH 30 min post-mortem (pH1) and glycolytic potential (GP), as described by Lebret et al. (2006), could be made. The pH1 was determined after the homogenization of 2 g of muscle in 18 mL

of 5 mM Na iodoacetate, pH 7.0 (Ingold Xerolyte electrode, Knick pH-meter, Berlin, Germany).
The following day, transverse sections of LM were taken for direct determination of ultimate pH (pHu) using the same apparatus as described above. Colour was also evaluated on these muscle samples through the value determination of coordinates CIE L* (lightness), a* (redness), b* (yellowness) and c* (chroma) and h° (hue angle); the average of 3 different determinations per sample, using a chromameter Minolta CR 300 (Osaka, Japan) with a D65 illuminant and a 1-cm diameter aperture, was calculated.
Muscle slices were then trimmed of external fat, minced, and freeze-dried; following this their intramuscular lipid content (Lebret et al., 2006) was determined. A second part of the meat sample was lyophilized and minced to determine its total collagen content and the thermosolubility of the collagen as described by Lebret et al. (2001).
On the same day, 3 slices (1.5 cm depth) of LM muscle were taken at the level of the last rib, trimmed of external fat and perimysium, weighed, and kept at 4°C in plastic bags until drip loss at 2 and 4 days post-mortem (Honikel, 1998) could be determined.

The day after slaughter a piece of the left loin of each carcass lying between $2^{nd}/3^{rd}$ and $6^{th}/7^{th}$ (LW pigs) or $7^{th}/8^{th}$ (B pigs) vertebrae was removed, trimmed and kept for ageing at 4°C for 3 days. On the fourth day this section was vacuum packaged, frozen and kept at –20°C for objective texture assessment (INRA-QuaPA). Measurements of cooking loss and shear force were determined in the way recommended by Honikel (1998).
Muscle lactate content was determined using the method previously described in connection with plasma lactate. Muscle glycogen content was determined from glucose determination after hydrolysis by amyloglucosidase, as described by Talmant et al. (1989). For muscle GP determinations, samples from 1 replicate were analyzed in a single assay. Lactate, free glucose, and glucose-6-phosphate, together with glucose from glycogen hydrolysis, were expressed as micromoles per gram of wet tissue; GP was expressed as micromoles of equivalent lactate per gram of wet tissue.

The activities of lactate dehydrogenase (LDH), citrate synthase (CS), and β-hydroxy-acyl-CoA dehydrogenase (HAD) were determined on LM samples taken at 30 min post-mortem as described previously (Lebret et al., 1999). These enzymes were chosen so as to assess the glycolytic pathway, Krebs cycle activity, and fatty acid β-oxidation potential, respectively.

Activities were measured at 30°C and expressed as micromoles of substrate degraded per minute per gram of fresh tissue.

2.2.5. Transcriptomic

LM samples were taken 30 min after exsanguination, frozen immediately in liquid nitrogen and stored at −80°C until the RNA could be isolated. Total RNA was extracted from LM samples (Chomczynski and Sacci (1987)) and purified using RNeasy MinElute Kit (Qiagen, Hilden, Germany). The RNA concentration was evaluated by ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE), and RNA quality was assessed using an Agilent Bioanalyser 2100 (Agilent Technologies, Santa-Clara, CA).

The RNA samples and the reference (pool of an equal amount of the 50 LM RNA) were labelled according to Agilent manual with Cy3 and Cy5 dye, respectively. The samples were hybridized to the Agilent custom 15K microarray designed for muscle tissue and washed according to Agilent procedure.

Hybridized microarrays were scanned at 5 µm/pixel resolution on a DNA Microarray scanner (Agilent Technologies, Santa-Clara, CA). Image analyses were performed with Agilent Feature Extraction Software (v9.5). The intensities of selected spots were transformed into log(Cy3/Cy5), and data were normalized, by both spot and chip, by the weighted linear regression (LOWESS) method, using the microarray software package GeneSpring GX 7.3.

After processing, 11429 probe sets were retained for further analysis.

2.2.6.Fatty acid composition

The fatty acid composition of lipid fractions was determined by gas-liquid chromatography of methyl esters (Morrison and Smith, 1964). Fatty acid composition was expressed as % total fatty acids. The following parameters were calculated from fatty acid composition: i) the sum of saturated, monounsaturated and polyunsaturated fatty acids; ii) the ratio of polyunsaturated fatty acids to saturated fatty acids; iii) the unsaturation coefficient defined as the average number of double bounds of unsaturated fatty acids; iv) the chain length coefficient calculated using the formula $\Sigma p_i c_i/100$ where p_i and c_i are, respectively, the percentage and the number of carbon atoms of each fatty acid i; v) the ratio of n-6 fatty acids to n-3 fatty acids.

2.2.7.Proteomics

The two-dimensional gel electrophoresis method was used to individualize and quantify separately the relative amount of muscle proteins. Analysis was performed on the

Longissimus lumbrum samples taken 30 min post-mortem. The soluble protein extraction and the two-dimensional gel electrophoresis were performed in the way described by Promeyrat et al. (2011).

A 2-D gel from each animal (n = 50) was analyzed. Gel images were acquired using a GS-800 imaging densitometer (BioRad). Digitalized images of stained gels were aligned and protein spots were detected and quantified following the method described by Promeyrat et al. (2011). Spot volumes, relative to total spot density, were used as quantitative data.

The protein identification was made by spectrometry in the way described by Promeyrat et al. (2011).

2.2.8.Fibre measurements

Histological measurements were performed on 10-µm-thick serial transverse sections cut on a cryostat (2800 frigocut N, Reichert-Jung, Heidelberg, Germany) at −20°C. Histoenzymology was undertaken on both biopsies and 30-min post-mortem samples of LM. The identification of types I, IIA, and IIB fibre, together with a further fibre type, was processed for succino-dehydrogenase in order to identify red oxidative (R) and white glycolytic (W) fibres, as described previously (Lefaucheur et al., 2004). Both I and IIA fibres are oxidative (type R), whereas IIB fibres can be either moderately oxidative (type R) or not oxidative (type W). Myofibres were classified as types I, IIA, IIBR, and IIBW as a result of both stains (Larzul et al., 1997). For an accurate estimation of the proportions of type I, IIA, IIBR and IIBW fibres several entire bundles of myofibres from 30-min post-mortem samples were selected in 3 random fields to obtain a total of at least 1,000 fibres that were counted using a projection microscope (Visopan, Reichert, Heidelberg, Germany).

A total of 500 fibres from 3 random fields were used to evaluate the cross-sectional area (CSA) of the different fibre types classified by histoenzymology, or immunocytochemistry, or both, on biopsies and 30 min post-mortem samples of LM.

2.3. Statistical analysis

The analysis of a high-dimensional data, partitioned in different groups of variable, and in different classes of individual, leads to relevant questions: (a) What variables are the most important in describing the variability? (b) How is the variability structured according to classes of individuals? (c) How is the variability structured according to the groups of

variables? We propose to answer these questions from within the general framework of factorial multivariable methods on the basis of the following procedures:

2.3.1. Selecting the most influential variables, and facilitating the interpretation of results: sparse principal components analysis (SPCA)

We feel it is desirable to reduce both the dimensionality and size of explicitly used variables by selecting a subset of influential variables contributing most to the overall variability of the animals. We adopt the *elastic net* approach proposed by Zou and Hastie (2003), which is a variable selection technique simultaneously producing accurate and sparse models. Sparse principal component analysis (SPCA) uses the *elastic net* to produce modified principal components with sparse loadings. The SPCA function of the *elasticnet* package (R-software) makes it possible to estimate the sparse principal components and the number of variables to keep per axis by using an alternating minimization algorithm to minimize the SPCA criterion. In the case of transcriptomic and proteomic variable groups, we limited our selection to the first five principal components with a constraint of 12 variables per axis. Thus, we retained a total of 60 data sets for both groups. We obtained a data table in which a single set of individuals (50 animals) is described by nine groups of variables: transcriptomic (60 variables), proteomic (60 variables), slaughter reactivity (7 variables), sensorial (12 variables), technological (18 variables), chemical (19 variables), fatty acid composition (29 variables), protein and lipid oxidation (21 variables) and muscle fibre (type and sizes, 29 variables).

2.3.2. Studying the different groups of variables: MFA

Multiple factor analysis (MFA) (Escofier and Pagès, 1988-1998; Pagès, 2002, Tayrac et al., 2008)) can be used to analyze several groups of variables which describe the same samples. At the core of MFA is a principal component analysis (PCA) applied to the whole set of variables in which each group of variables is weighted; this makes it possible to analyze different points of view by taking them equally into account. We consider the merged data set: $K = [K_1, K_2, \ldots, K_9]$, where each Kj (j=1, …, 9) corresponds to the data table of one of the groups of variables.

First, separate analyses are performed by PCA on each group *j* of variables. Secondly, a global analysis is carried out: each variable belonging to a group *j* is weighted by $1/\lambda_1^j$, where λ_1^j denotes the largest eigenvalue of the matrix of variance-covariance associated with each data table K*j*. The first dimension's variance relative to each data table is then equal to one. The rationale of the scaling is that, through it, information that is common to the data tables emerges. Besides, no data table can generate the first dimension of the global analysis by itself. In this way MFA provides a balanced representation of each individual according to the joint data table *K*, but also a partial representation of each individual according to each group *j* of variables. The corresponding graphical displays (Individual Factor Map and Variables Representation) are read as they are in PCA. The partial individual $i^{[j]}$ is on the side of the variables of the group *j* for which it takes high values, and on the opposite side of the variables of the group *j* for which it takes low values. Partial representations of one and the same individual are all the more close that they do express the same information. And, the balanced representation of an individual *i* is located in the exact barycentre of its partial points corresponding to the groups of variables.
MFA also provides a representation of each matrix of variables (Groups Representation) which allows specific and common structures to be visualized. Consequently, it is possible to get an overall picture of the common structure emerging from the entire dataset.

This representation of the groups is presented as a graphical display of the groups of variables as points in a scatter plot. It has to be read as follows: the closer the coordinate of a given group is all the more close to 1 than the more correlated variables of this group are highly correlated with the dimension issued from the MFA (either positively or negatively).Hence, two groups are all the more close than the structures they induce on the observations are close.

2.3.3. Studying the different classes of individuals: Between-class and within-class analyses

When classes of samples are involved, between-class analysis can be seen simply as the MFA of the table of class means. Its aim is to highlight differences between classes, and row scores maximize the between-class variance. Within-class analysis is the reverse of between-class analysis: it is the MFA of the residuals between initial data and class means. It removes the effect of the grouping variable and analyses the remaining variability.

In the present study we applied these methods to analyze the effect of the five classes of breed (LW and B) x rearing system (C, A and E). Initially, a within-class MFA — i.e. an MFA on data expressed as a deviation from class means — was carried out to estimate within-class variability and to identify the correlation structure between variable groups when the effect of breed x rearing system had been excluded. Next, a between-class MFA was performed to investigate the differences according to the correlation structure between classes, to estimate between-class variability, and to classify the main variable groups involved in these differences by decreasing order of magnitude. The influence of each class could then be investigated by comparing the results of the between-MFA with those from the global MFA performed on the pooled data set.

All of the multivariate analyses were performed with the ade4 package of the R software (R Development Core Team, 2008; Chessel et al., 2004; Dray et al., 2007). In all the analyses the number of components retained was determined by a scree test (Cattell, 1966).

3. Results and discussion

3.1. Total Multiple Factor Analysis

Between-class and a within-class PCAs of breed x rearing system were performed on the whole data set. Most of the variability (73%) was found to have within-class origin. However, the between-class variability was not negligible (27%).

Projection of the first two components of the between-class PCAs on the first two axes of the standard PCA of the whole data set showed that there was a strong pairwise correlation between the first two components (0,98 and 0,95, respectively). The correlation between the first axes of the within-class PCA and those of the standard PCA was very low (0.42 and 0.13, respectively, between the principal axes 1 and 2). These results show that the variables which discriminated the five classes were the same as those discriminating individuals. The variables explaining the residual variability were different, however.

To have displayed all 253 variables on the basis of the same plot would have been impractical. It was more effective to use the MFA to overcome the problem of structuring of variables into separate groups.

Figure 1 shows the projected inertia; it also shows the links between the nine groups of variables and the reference plane defined by the first two principal components of the MFA compromise. Notice that the first component of the MFA compromise (52% of the total inertia) is mainly composed of the groups of transcriptomic, chemical, technological and fatty acids variables. The slaughter reactivity variables differ from the other groups of variables and are mostly responsible for the second component of the compromise. We can distinguish an intermediate group composed of sensorial, fibre and oxidation variables which are moderately correlated with the first axis. The correlation coefficients between the nine groups of variables and the first two components of the MFA compromise are cited in Table 1. Interestingly, in our analysis, proteomic variables were located near the origin of this reference plane (Figure 1), suggesting that they did not discriminate the animals. A recent study, made by Promeyrat et al. (2011) on the same animals, disclosed a relation between the muscle proteome and the protein oxidation during ageing and cooking. These results are inconsistent with our conclusions, which suggest that, from an overall point of view, proteomic variables are not correlated with the oxidation variables which are correlated with the first axis of the compromise. However, the Promeyrat study correlated only one oxidation parameter (carbonyls) with proteome; in the present study oxidation is described by 21 variables.

The superimposed representation of the partial projection of individuals represented as partial clouds of individuals belonging to the same class of breed x rearing system is depicted in Figure 2. This representation allows for the precise comparison of clouds N_I^J (I=1,...,5; J=1,…,9). Figure 2 suggests that the first axis of the compromise discriminated breeds. It opposed LW and Basque animals. The second axis discriminated rearing systems. In other words, transcriptomic, chemical, technological and fatty acids variables discriminate mainly LW and B animals, when the slaughter reactivity variables opposed the rearing systems, especially, the extensive system where only the B animals were reared.

These findings are in line with those of Lebret et al. (2011), who found that the plasma concentrations of ACTH, cortisol and creatine kinase hormones, reflecting levels of stress in the animals they investigated, were significantly different in BE animals as compared with the other classes (BA, BC, LWA and LWC), despite maximum standardization of the slaughter conditions on two sites (they used an experimental site for animals reared in alternative and conventional systems and an industrial site for the animals reared in the extensive system.)

The authors explain this important distinction of the BE (Figure 2 and Figure 3) by referring to the pre-slaughter conditions. In the case of industrial slaughter, aggression and fights during pre-slaughter could explain the variation in hormonal concentrations, and especially the creatine kinase hormone concentration in the BE animals. The variation would reflect a high physical activity for this group.

Proteomic variables are located near the origin, which corroborates our previous suggestions regarding their limited importance in discriminating between breed and rearing system.

It is worth noting that, within the same breed, there was no notable distinction between the conventional and alternative rearing systems. Indeed projections of the barycentres of both systems within the same breed were very close (Figure 2). These results were similar to those of Lebret et al. (2011), who concluded that there was no significant difference between conventional and alternative systems, and that both systems were significantly different from the extensive system.

3.2. Between-class structure

This approach was applied to the mean averages of the five classes of breed x rearing system for all the variables to highlight the groups of variables discriminating the classes after residual variability had been excluded. Figure 3 superimposes the partial projections of the five classes on the factorial plan of between-MFA. There are numerous similarities in the results of the total MFA (Figure 2) and the between-class MFA (Figure 3): notice, for example, the high levels of discrimination between breeds on the first component of both of their compromises, and the second axis, which is mainly composed of slaughter reactivity variables that discriminate rearing systems. Figure 4 illustrates the projected inertia; it also shows the links between the nine groups of variables and the reference plane defined by the first two principal components of the between-class MFA compromise.

In the total MFA some groups of variables, such as proteomic and sensorial groups of variables, did not distinguish the classes of breed x rearing system; by contrast, in the between-class MFA all groups of variables except slaughter reactivity were highly linked with the first axis, i.e. all of the groups of variables except one contributed equally to the discrimination of breeds.

Between-class variability explains most of the total variance in the group of sensorial variables, and especially the breed differences, as a result of the high correlation of this group with the first factorial axes of the total and between-class MFA analyses discriminating most

strongly between breeds. Performance differences between LW and B animals regarding sensorial attributes have been highlighted by several studies (Guéblez et al., 2002; Alfonso et al., 2005; Lebret et al., 2011).

The correlation between transcriptomic data sets and drip loss as a technological attribute was investigated by Wyszynska et al. (2009), using data from the same experiment as our study. They found a significant correlation between the expression of CAV3 (Caveolin-3, a gene encoding the sodium channel regulator activity protein) and PGM1 (a gene encoding phosphoglucomutase 1, an enzyme which catalyzes the isomerization of glucose 1-phosphate to glucose 6-phosphate). The authors speculate that these gene expressions could affect drip loss by indirectly influencing muscle lactate production. The Wyszynska study also showed that drip loss is weakly correlated with pHu. These findings highlight the potential connection between transcriptomic, chemical and technological groups of variables.

The most important contrast between total MFA and between-MFA analyses concerns the proteomic variables. These variables are highly correlated with the first two components of the compromise of the between-MFA (with correlation coefficients of 0.86 and 0.98, respectively). The results of the partial PCA of the proteomic variables imply that the between-variance represents only 10% of the total of this group of variables. Given these findings, we suggest that the discriminatory power of proteomic data was hidden by the residual variability. Moreover, the high correlation between slaughter reactivity and proteomic groups of variables could be explained by the fact that both groups contain variables measured immediately after slaughter: after all, this timing would ensure that both groups of variables reflect the level of stress suffered by animals during this step. The link between slaughter reactivity and proteomic variables could be also explained by the findings of Boles et al. (1992). These findings suggest that pH drop, which is more pronounced when animals are stressed, is associated with high muscle temperature denatured proteins which become insoluble.

3.3. Within-class structure

In this section we address the following question: Which groups of variables scatter the animals whatever class of breed x rearing system is considered? Figure 5 illustrates the projected inertia; it also shows the links between the nine groups of variables and the

reference plane defined by the first two principal components of the within-class MFA compromise.

Our results suggest that individual variability is explained chiefly by chemical and fatty acids variables which are highly correlated with the first principal component of the compromise of the within-class MFA. The slaughter reactivity variables and proteomic variables are responsible for the second axis. According to Terlouw (2005), differences in stress responses exist not only between animals of different breeds or rearing systems: pigs of a similar genetic type (but different genetic make-up) and the same rearing unit (but different social and other experiences) display different stress reactivities. Generally, individuals are showing a certain consistency in their reaction to stress.

Technological measurements are also correlated with the second axis, i.e. they are highly correlated with slaughter reactivity. This result is consistent with the findings of earlier studies, which found a correlation between the reactivity of animals to slaughter, expressed by plasma hormone concentrations, such as lactate and cortisol, on the one hand, and technological attributes, represented by pH decline, colour and drip loss, on the other (Hemsworth et al., 2002; Terlouw, 2005; Terlouw and Rybarczyc, 2008).

The correlation between slaughter reactivity, proteome, oxidation and technological groups of variables on the second axis (Figure 5) accords with the findings of Foury et al. (2005), whose account of the relationship between levels of stress hormone and carcass composition and muscle quality allows that relationship to be explained by the action of these hormones on energy and protein metabolism.

The lack of individual variations related to transcriptomic group of variables prevented us from establishing a relationship between the molecular genetic markers and other groups of variables. However, it has been established that genetic factors influence individual variations in stress behavioural responses (Mormède et al., 2002). This discrepancy suggests that there is a need for further investigations in which the number of animals is increased in order to establish a link between individual variations and genetic factors.

Unlike the transcriptomic data, the proteomic data contained individual variations that will make further analyses, in which proteomic markers are identified in order to predict the biological mechanisms involved in meat quality, possible. This is in keeping with the

conclusions of Pomeyrat et al. (2011), who report that there is a significant relationship between post-mortem modifications of the muscle proteome and protein oxidation during ageing and cooking. On the basis of these results, we suggest that the proteomic approach should be extended to other targets, and that this will lead to better control of pork quality.

Conclusion

Nowadays, a large amount of information is gathered and generally stored in databases which can be organized into a hierarchy. With MFA, the structures into which these data fit can be fruitfully investigated. MFA can be seen as a tool for 'data mining'. The main value of MFA consists in its ability to provide:

- ✓ An overall graphical display representing the whole data set
- ✓ Partial representations that reflect the variables involved in the various nodes
- ✓ A graphical display showing how the various nodes are related to each other.

The method of analysis illustrated here, in which large numbers of data sets were structured into groups, allowed existing correlations between these groups to be assessed by taking into account all the information simultaneously. The multivariate analyses are perhaps best regarded as preliminary investigations highlighting the main relationships between groups of variables. With complementary statistical methods, such as ANOVA (Analysis of Variance) and MANOVA (Multivariate Analysis of Variance), it should be possible to advance beyond these initial investigations and quantify the relationships.

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Table 1. The correlation coefficients between the nine groups of variables and the first two components of the total-MFA compromise.

Groups of variables	Factorial axis 1	Factorial axis 2
Transcriptomic	0.82	0.29
Proteomic	0.21	0.15
slaughter reactivity	0.05	0.61
Sensorial	0.55	0.17
Fatty acids	0.78	0.27
Fiber	0.49	0.25
Proteolysis	0.46	0.37
Technological	0.78	0.36
Chemical	0.88	0.29

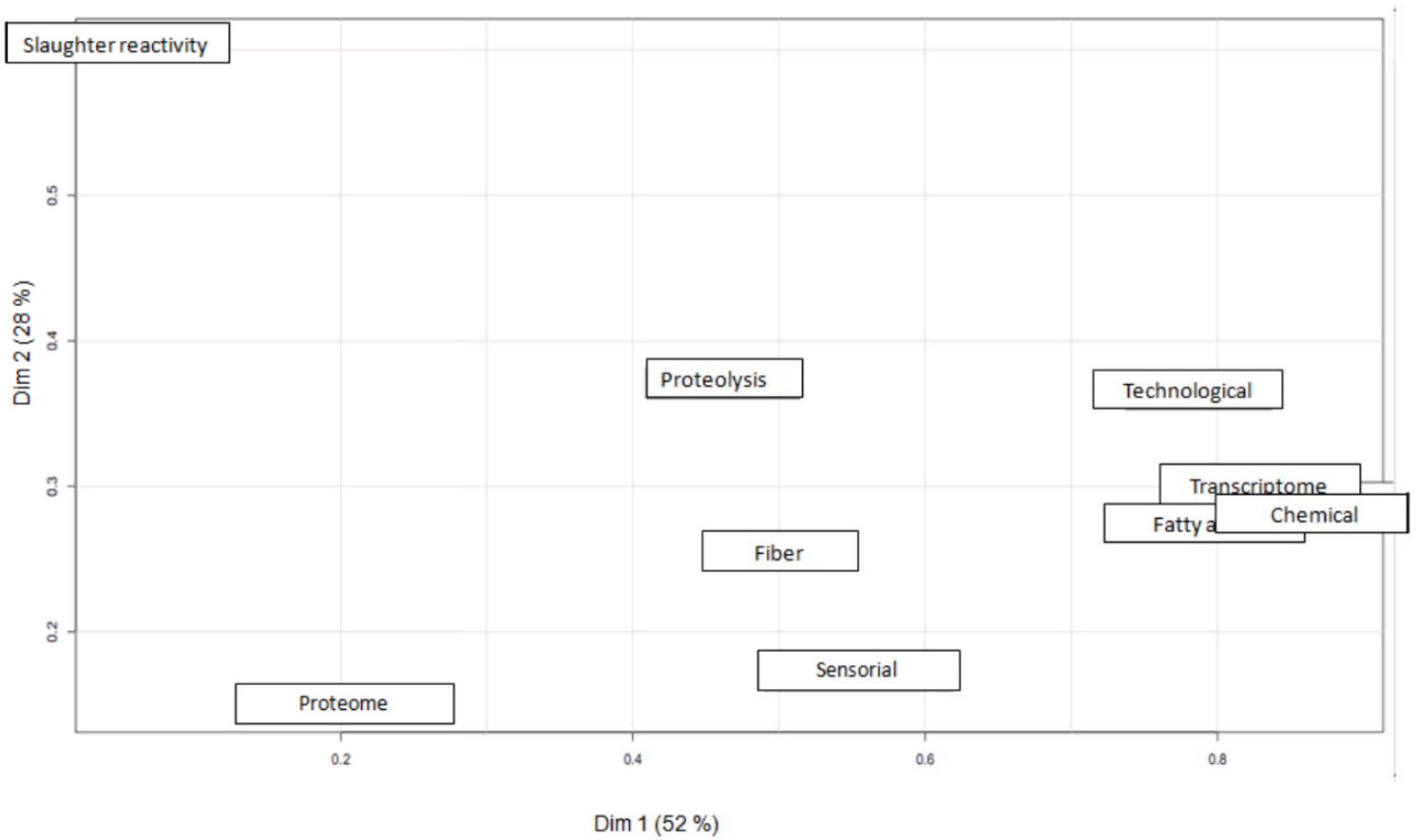

Figure 1 . Plot of the links between the projected inertia of the 9 groups of variables and the reference plan composed of the first two principal components of the compromise of the total-MFA .

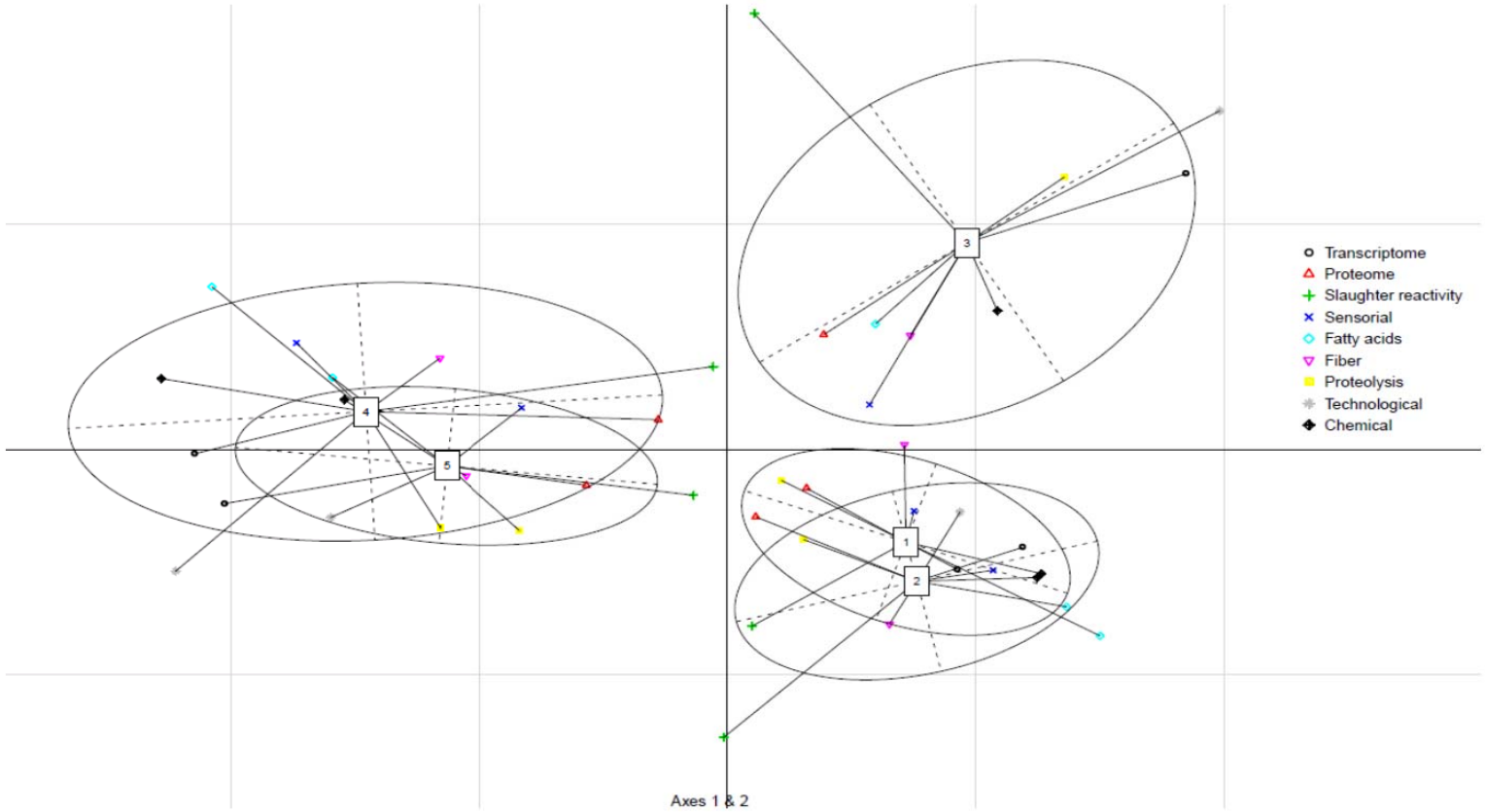

Figure 2. Total-MFA scatterplot (Axis 1 and 2). Consensus and partial representations of the five clouds of breed x rearing system classes according to the nine variable groups (B conventional (1), B alternative (2), B extensive (3), LW indoor (4), LW alternative (5)).

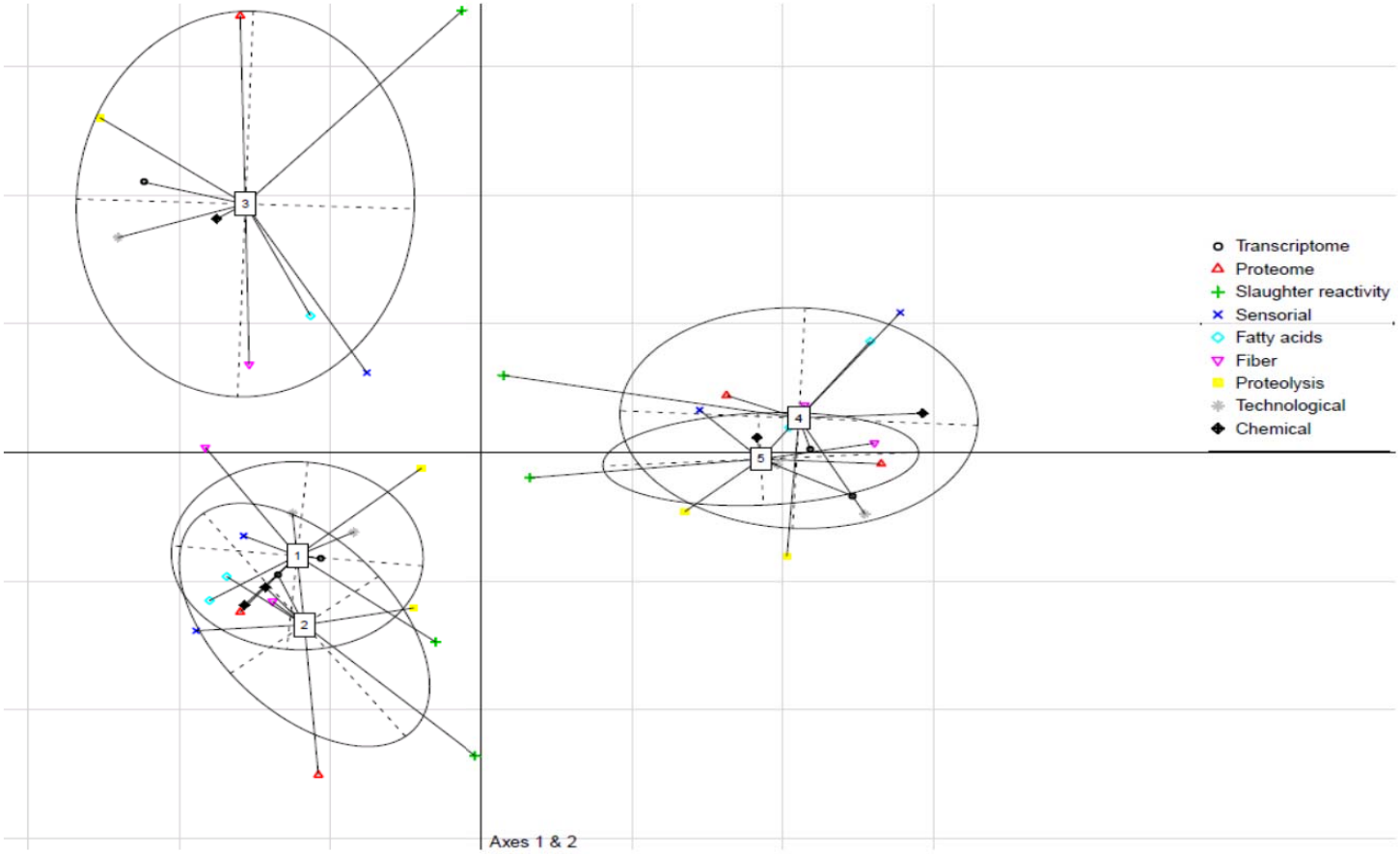

Figure 3. Between-MFA scatterplot (Axis 1 and 2). Consensus and partial representations of the five clouds of breed x rearing system classes according to the nine variable groups (B conventional (1), B alternative (2), B extensive (3), LW indoor (4), LW alternative (5)).

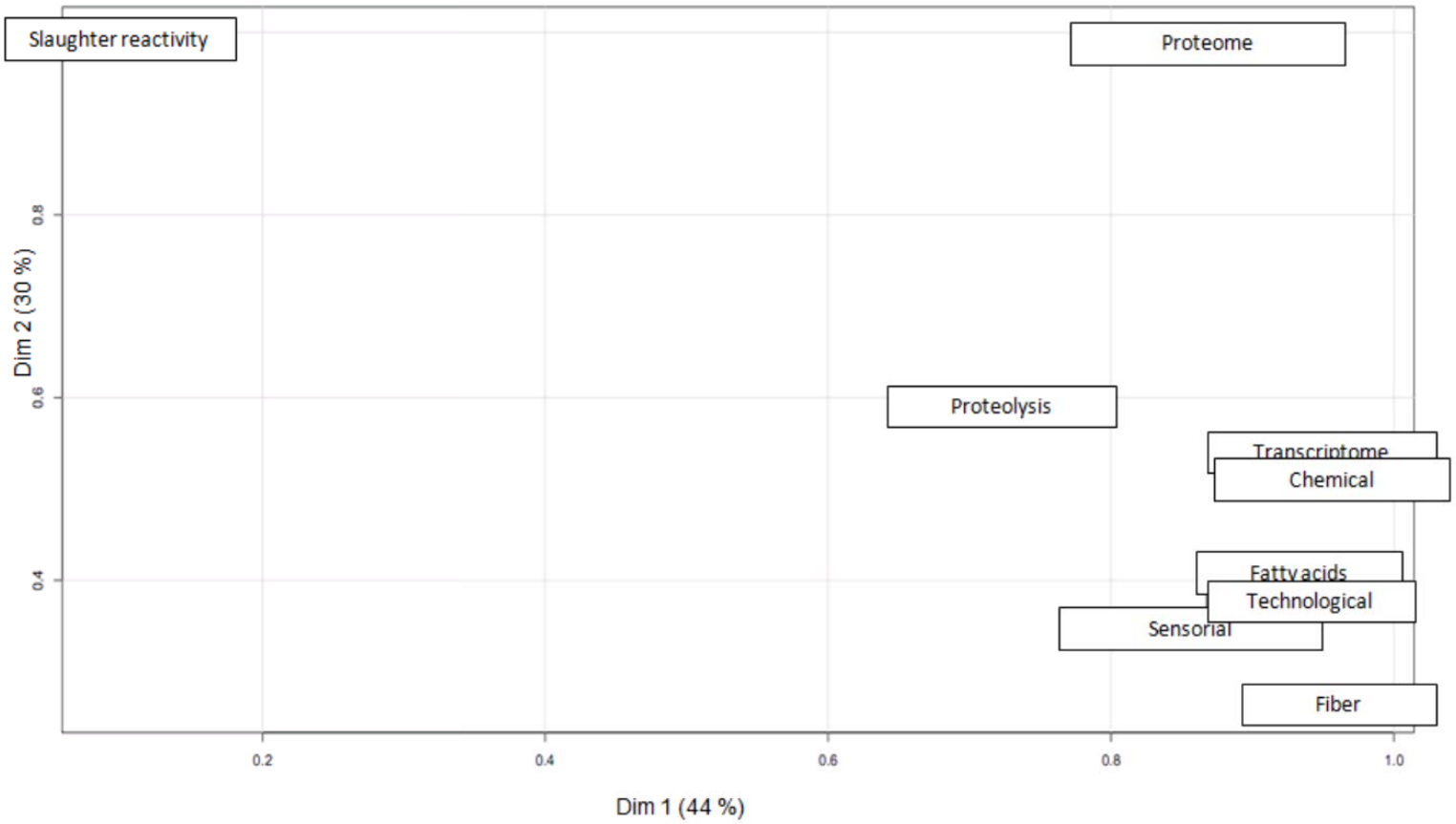

Figure 4 . Plot of the links between the projected inertia of the 9 groups of variables and the reference plan composed of the first two principal components of the compromise of the between-MFA .

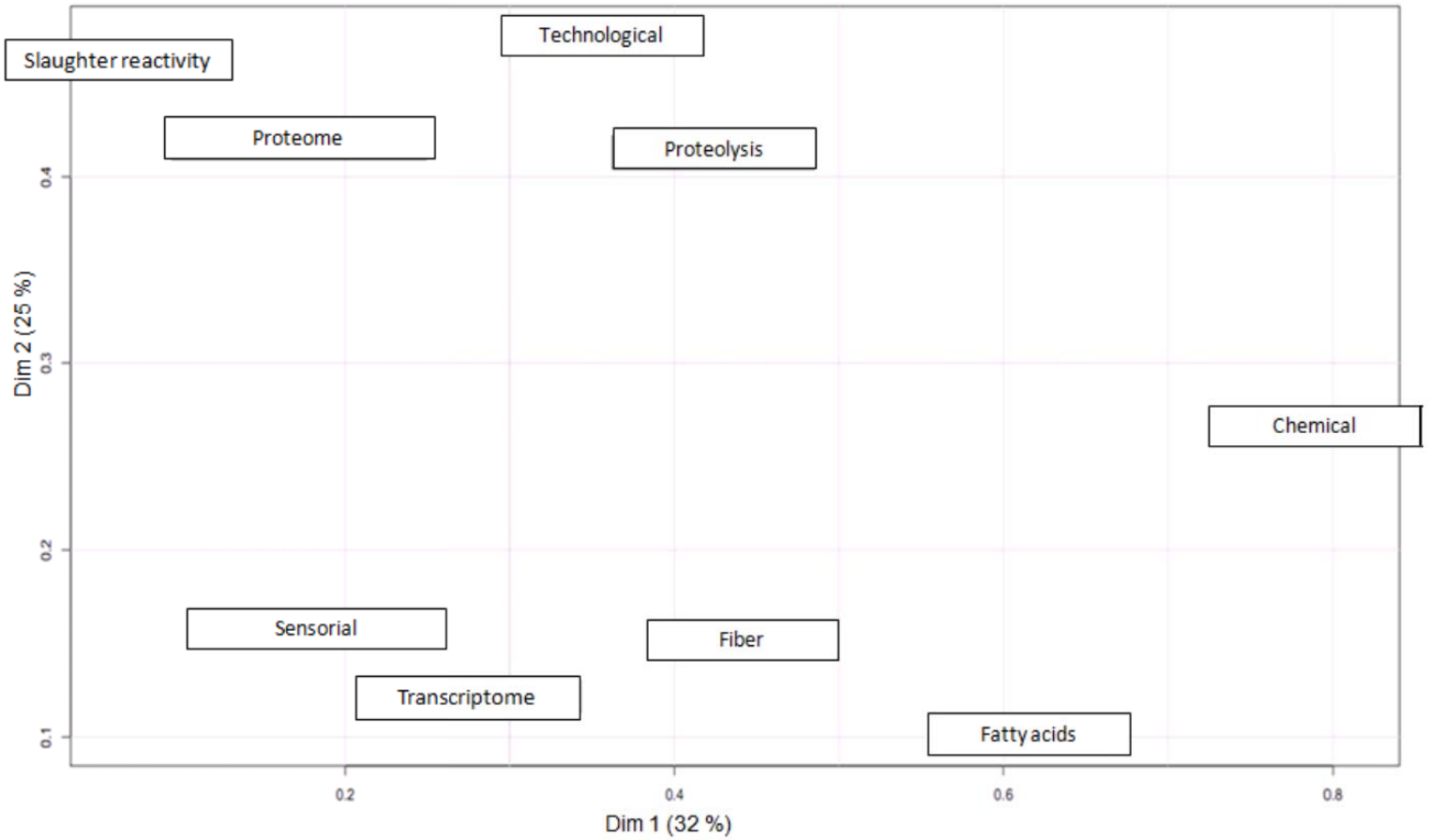

Figure 5 . Plot of the links between the projected inertia of the 9 groups of variables and the reference plan composed of the first two principal components of the compromise of the within-MFA .