Original study

Generalized procrustes analysis (GPA) as a tool to discriminate among sheep breeds

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Abstract

Forty male lambs of five Southern Spanish breeds were used to study the effects of the breed in their sensorial characteristics. The used breeds were: Segureña, Spanish Merino, Grazalema Merino, Churra Lebrijana and Montesina breeds. Milk lambs were slaughtered at 12 kg of live weight. A descriptive sensory evaluation was developed using the *longissimus lumborum* from each animal by a panel of 12 experts and a Generalized Procrustes Analysis (GPA) was used to discriminate among them. Generalized Procrustes Analysis clearly differentiated Churra Lebrijana of out the rest breeds. Churra Lebrijana was defined as more tender, juicier and with less lamb odour than the rest of the Southern Spanish lamb breeds. Thus, GPA is able to discriminate among breeds.

Introduction

Labelled meats are perceived by the consumers as healthy food, particularly if it is possible to associate them to the breed and the rearing land (Dias *et al.* 2008). Spanish consumers show great interest in origin-linked products, since they associate these products with high-quality, healthy foods (MAGRAMA 2014). In this way, since the implementation of the labelled meat under »Protected Geographical Indicators«, producers were encouraged to continue producing according to the traditional methods, because products are better accepted by the consumers. In the last years, demand for meat obtained from organic, natural and biological livestock production systems has increased (Estévez *et al.* 2003). Native breeds are important for the development of their rearing areas and the producers developed strategies to use local resources, and make their production sustainable. As an alternative to promote those native breeds' production, one or more quality labels could be developed to reinforce their position in the market.

However, in the field of raw meat there are still few breeds protected under these labels, producing a loss of information for the consumer who cannot distinguish among different products because raw meat is sold as a generic product. Thus, there is an urgent need to refocus the lamb market on the customer's requirements.

Breed evaluation provide information that is essential for the development and use of the native resources. Meat products will have an added value if they belong to selected breeds and are produced with a particular traditional method (Sacco *et al.* 2005). However, breeders' associations and meat industry must be provided with fast and economic tools to be able to discriminate and control meat from their animals.

Breeds characterization should include sensory traits to provide relevant information for highly competitive markets because attributes of lamb meat affect whether consumers choose lamb instead of other species (Rhee & Yiprin 1996).

Sensory data, obtained using assessments by taste panels, are arguably the most relevant traits to identify meat that consumers will prefer and will choose to purchase again. This method is ideally recognized as the »gold standard« for evaluating meat eating quality (Lambe *et al.* 2009).

Generalized Procrustes Analysis (GPA) is a powerful multivariate technique that takes into account free-choice profiling. This statistical tool is used extensively in sensory evaluation (Gower 1975, Rodrigues & Teixeira 2009, Moreno-Indias *et al.* 2012, Rodrigues & Teixeira 2013). With this tool, problems typically encountered with sensory analysis (such as variation among panellists) can be corrected (Stone & Sidel 1985). To generate consensus among panellists, GPA transforms the individual data matrices to allow for optimal comparability.

Therefore, the main objective of this study was to assess a sensorial analysis by GPA to discriminate between the five most widespread sheep breeds in Southern Spain.

Material and methods

Animal management

The studied breeds were the five most widespread sheep breeds in southern Spain and they are the base of lamb meat production in this area. The choice of which breed is used depends on the tradition and available resources. According to the breeds official Catalogue of Spain

(annex 1, RD 2129/2008) our breeds of interest are classified into »local breed of promotion« within we find: Spanish Merino (ME) which has a mix aptitude: wool, meat and milk with a mean milk yield per lactation of 275 kg and an average daily gain of 300 g; and Segureña (SE) with a mix aptitude: milk and wool with a mean milk yield per lactation of 45 kg and an average daily gain of 216.1 g; and the »native breed endangered« within we find: Grazalema Merino (GM) which is a dairy sheep breed, with a mean milk yield per lactation of 120.22 kg and an average daily gain of 262 g; Churra Lebrijana (CL) which is a meat sheep breed (350 mL/day); and Montesina (MO), a meat sheep breed (300 mL/day) (Information provided by the National Breeders' associations, FEAGAS). However, all breeds are used for meat production to increase the income, although they are not always meat breeds.

Forty male lambs from the five Spanish sheep breeds abovementioned were selected for the study (eight per breed). Each animal was reared at its local place in the same period of the year. Kids suckled their mothers' milk only and remained indoors while the mothers grazed for 8 h a day. Concentrate, hay, forage or other supplements were not given to lambs. Ewes' lactation diet was composed of the local pastures when available and the same concentrate (15.5 % protein, 2.3 % fat, 6.8 % cellulose and 6.7 % ash) when pasture was not enough.

When the animals reached the target live weight (approximately 12 kg live weight), they were transported in accordance with welfare specifications and slaughtered in a slaughterhouse according to the EU Council Directive 86/609/EEC that establishes guidelines regarding the protection of animals used for experimental and other scientific purposes. All the lambs were slaughtered in the same EU accredited slaughterhouse when the required live weight was reached. Carcasses were chilled at 4 °C for 24 h. *Longissimus lumborum* samples were collected 24 h post-slaughter from the left side of carcasses, vacuum packed and aged at 2 °C for 72 h. After that, samples were stored at −18 °C until analysis.

Sensory analysis

On the day of analysis, samples were thawed inside their vacuum bags (4 h) in tap water until the internal temperature reached 15-17 °C. Following this, the samples were taken out of the vacuum packaging, trimmed of external fat, wrapped in aluminium foil and cooked in a double plate grill preheated at 200 °C until the internal temperature reached 70 °C. Each sample was then cut into 2×2 cm^2 samples. The sensory test was performed over seven sessions in a standardized tasting room equipped with individual booths and, to mask any differences in meat colour, under red lighting. 20 plates were served with three different breed samples, in a comparative multi-sample test using a completely balanced design; the samples were served randomly to a trained 12-member sensory panel (ISO 8586-1:1993). To avoid the possible effects of the order of presentation, samples were presented to panellists in different orders. Sensory profile and specific training was developed in an additional session using samples from animals on each treatment.

The panellists used a 10 point scale to evaluate the samples for tenderness (1-extremely tough, 10-extremely tender), initial and sustained juiciness (1-extremely dry, 10-extremely juicy), chewiness (1-non-chewy, 10-extremely chewy), length in mouth (1-no persistence or very weak, 10-extremely persistence or very strong), salivation (1-low secretion, 10-extremely high secretion), and lamb flavour intensity (1-no aroma, 10-very intense) (AMSA 1995).

Statistical analysis

Using GPA analysis, the data matrices of five breeds by seven sensory attributes for the 12 assessors (configurations) were matched to find a consensus using the Microsoft Office Excel add-in software, XLSTAT (Trial Version 2009, Addinsoft, Paris, France).

Results

In the sensory profile, meat samples were described by panellists using seven attributes (tenderness, initial juiciness, maintained juiciness, chewiness, length in mouth, salivation and lamb flavour intensity). Although a training period was carried out in order to minimize differences among assessors, no training can eliminate variation among panellists (Stone & Sidel 1985). Thus, a GPA was used to ensure the consensus.

The efficacy of the training may be confirmed in Table 1. The low level of variance on the residual analysis from the panellists and the homogeneity of the scaling factors explained that panellists followed the same methodology for the evaluation. On the other hand, the residuals by object (Table 2) showed that the higher consensus was reached around CL followed by MO.

The first two principal components from GPA explained 86.47 % of the variability in the model. The first component explained 76.91 % and the second component 9.56 %. Each of the remaining components was not taken into account due to the low explanation of total variance. Generalized Procrustes Analysis was used to find a real consensus among panellists. Table 3 shows the means and standard errors of sensory attributes for all meat samples, as well as the correlations between sensory attributes and the principal components (F1 and F2). At this point, it can be observed that F1 explained most of the attributes, whereas F2 was less representative. Vectors associated with long- lasting juiciness and tenderness were directed towards positive F1 values; whereas salivation, chewiness, maintained juiciness, duration in mouth and lamb flavour intensity were directed towards negative F1. F2 explained some of the variance of initial juiciness (r=0.408).

Table 1
The Residual variance, scaling factor, and the variation percentage that is explained by the first two principal components of each panellist are shown

Panellist	Residual	Scaling factor	F1	F2
1	2.486	0.991	50.307	26.568
2	0.727	0.784	75.763	4.759
3	2.261	0.853	48.705	39.936
4	0.969	1.191	75.585	11.235
5	0.784	1.023	81.394	6.003
6	0.741	1.248	84.533	9.750
7	1.489	1.068	83.667	7.292
8	0.643	0.953	74.178	13.053
9	0.520	1.321	82.588	5.513
10	1.069	1.195	60.013	5.496
11	1.272	0.994	82.439	7.965
12	0.979	0.823	76.076	2.832

F1: first principal component of the GPA,   F2: second principal component of the GPA

Table 2
Residual variance of each breed

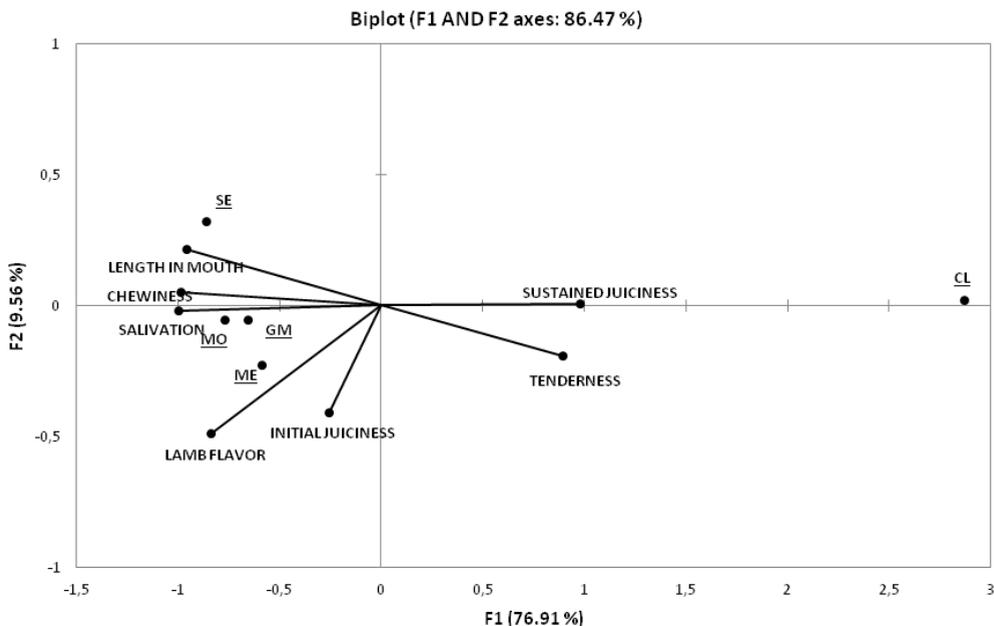| Breed	Breed residual
Churra Lebrijana	1.699
Spanish Merino	3.403
Grazalema Merino	3.248
Montesina	2.473
Segureña	3.117

Figure 1
Consensus configuration: representation of correlations between sensory traits, breeds, and the top two components of the consensus variation. F1: first principal component of GPA, F2: second principal component of the GPA

Observing the consensus configuration (Figure 1), the reached main result is that CL was clearly separated from the other breeds. The measured parameters seemed to be quite homogeneous. Churra Lebrijana is associated with positive values in tenderness and long-lasting juiciness, while the other breeds were quite homogeneous around F1 parameters, without finding any discriminant parameter which could define them, but basically characterized by a worse valorated lamb flavour record, length in mouth and chewiness. About F2, the most different breeds resulted SE and ME, getting SE the highest score for initial juiciness. Thus, all breeds, with the exception of CL, may be considered as similar breeds in relation to sensory characteristics.

Table 3

The correlation between sensory traits and factors produced in the GPA and sensory scores (means±SEM) of each breed

| Sensory traits | F1 | F2 | Breeds | | | | |
			CL	ME	GM	MO	SE
Tenderness	0.895	−0.192	6.195±0.135	5.372±0.158	5.109±0.148	5.267±0.138	5.208±0.148
Initial juiciness	−0.256	−0.408	5.594±0.141	5.101±0.162	5.289±0.142	5.191±0.141	5.016±0.140
Maintained juiciness	0.980	0.008	5.109±0.148	4.562±0.155	4.742±0.144	4.450±0.151	4.500±0.134
Chewiness	−0.983	0.049	5.492±0.151	5.558±0.150	5.828±0.136	5.756±0.143	5.736±0.136
Lengh in mouth	−0.955	0.216	4.669±0.135	5.504±0.139	5.742±0.133	5.802±0.137	5.832±0.135
Salivation	−0.997	−0.021	4.969±0.120	5.411±0.115	5.375±0.127	5.392±0.119	5.480±0.119
Lamb flavour	−0.837	−0.490	4.704±0.175	5.373±0.178	5.127±0.177	5.240±0.158	5.050±0.176

F1: first principal component of the GPA, F2: second principal component of the GPA

Discussion

Traditionally, discrimination among breeds has been carried out by physicochemical parameters. Martínez-Cerezo *et al.* (2005a) found that the breed had a significant effect on the physicochemical characteristics of lamb meat and should be considered in programs to improve meat quality. However, the use of only these parameters to characterize the meat quality is too simplified. Meat quality is something very complex and cannot be evaluated by only one characteristic. That is why it is necessary to study different quality levels. In this way, Juárez *et al.* (2008) went one step further by studying the same breeds of this manuscript. They found out that the five breeds could be differentiated just by visible spectroscopy, but combining this information with physicochemical parameters improved the accuracy.

Nowadays, with the normalization of the fatty acid profiles, the discrimination of the different breeds seems to be concluded. Intramuscular, intermuscular and subcutaneous fats significantly affect flavour, juiciness and texture of meat (Wood, 1984). Thus, Juárez *et al.* (2010) reported that the use of the fatty acid profile of one fat depot was not able to assign 100 % of the carcasses to their origin, but using the information from two depots led to a reliability of 100 %. However, the use of the fatty acid profile to discriminate different breeds, although conclusive, could not be affordable for producers.

Fatty acid profile is related to sensorial properties (Moreno-Indias *et al.*, 2012), so that sensorial analysis could be an excellent discriminator. The breeds' differentiation has been carried out by sensorial analysis in other occasions by some authors (Martínez-Cerezo *et al.* 2005b), although the results have not been always conclusive (review in Crouse *et al.* 1981). Although the main attributes to explain the overall acceptability of meat are the same among all breeds: tenderness, flavour and juiciness, it has been reported that genotype did not affect the sensory traits (Safari *et al.* 2001). However, in the present study, it has been demonstrated that breeds may be separated.

Southern Spanish breeds studied are characterized by their rusticity (Sañudo *et al.* 1998). They are reared under an extensive system with natural resources. The breeds catalogued under the »local breed of promotion« distinction have reached their own PGI label: ME as »Cordero de Extremadura« (Boletín Oficial del Estado 2009), and SE is commercialized as »Cordero Segureño« (Boletín Oficial del Estado 2011). These two breeds are studied deeply in order to promote them (SE: Domenech *et al.* 1989, Domenech *et al.* 1990, Peña *et al.* 2005; ME: Martínez-Cerezo *et al.* 2005a,b). But for the »native breed endangered«, efforts have not been the same. Grazalema Merino is starting to be studied (Juárez *et al.* 2005), like CL (Romero *et al.* 2007), and MO is starting to be taking into account. In this manuscript, we are going to focus on CL, because it has been the breed which could be differentiated from the other breeds.

Churra Lebrijana is a breed which produces meat at low costs (Romero *et al.* 2007), because it is well-known for its robustness and adaptability to harsh environments (such as the high salinity of the *marismas* where CL is reared). Possibly, CL is the most extreme of all breeds. The meat of CL has been characterized here as more tender and juicier than that of the other breeds. Juárez *et al.* (2008) reported about high values of moisture in this breed (CL). The better perception of juiciness reached in CL was potentially due to a higher amount of water, which results in meat that is perceived juicier (Bañón *et al.* 2006). On the other hand, Ripoll *et al.* (2012) found that breed does not influence meat flavours, but affects juiciness in goat

kids. Moreover, Juárez *et al.* (2008) who studied the same breeds as in the current study, also reported about low percentages of protein and fat for this breed, data related with the least score in lamb odour. These parameters are in accordance with the harsh environment where CL is reared, corroborated by the fact that CL is the breed that needs more time to reach the slaughter weight (Juárez *et al.* 2008). However, in the study by Juárez *et al.* (2008) it is observed that contrary to this data, the Warner-Bratzler Shear Force of CL was not the smallest, although in another study from these authors CL obtained better scores in suckling lambs (Juárez *et al.*, 2009).

Generalized Procrustes Analysis has shown the capacity to differentiate among the five sheep breeds tested, although the great similarity between some of them makes it necessary to use another extra method to increase the accuracy. We found great differences between CL breed and the rest of the breeds tested. Churra Lebrijana belongs to the Churra genetic branch, while the other breeds belong to the Merino genetic branch (MAGRAMA 2014). These breeds are more similar among themselves with similar growth patterns and tissue distribution in a particular weight and diet. We found out that the main parameters that characterized CL are its tenderness and the long-lasting juiciness. On the other hand, the rest of the analysed breeds in this study exhibita high homogeneity among themselves.

In conclusion, breed effect is clearly demonstrated by GPA. Churra Lebrijana is a breed with different characteristics compared to the rest of sheep breeds studied. However, when breeds are genetically close, as in the case of the other breeds studied (Grazalema Merino, Spanish Merino, Montesina and Segureña), and reared under the same conditions, GPA would need another external discriminator. Churra Lebrijana focuses its difference in tenderness and juiciness, two parameters highly appreciated by the consumers. Thus, CL might be studied deeper to assure its existence, production and of course its survival.

Acknowledgements

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