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Correlation of animal diet and fatty acid content in young goat meat by gas chromatography and chemometrics

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Abstract

The meat fatty acids (FA) profiles of caprines submitted to different dietary treatments were determined by gas chromatography. The data were treated by Chemometrics to consider all variables together. The contents of saturated FA (SFA), monounsaturated FA (MUFA), polyunsaturated FA (PUFA), omega-3 (*n*-3) FA, and omega-6 (*n*-6) FA in 32 samples were analyzed. PUFA:SFA and *n*-6:*n*-3 ratios were also considered. The multivariate methods of hierarchical cluster analysis (HCA) and principal component analysis (PCA) were used to analyze the experimental results. HCA can group samples according to their basic composition, and PCA can explain the relationship among the dietary treatments according to the meat fatty acid composition. Treatment 1 presented the highest *n*-6 FA concentration, PUFA:SFA, and *n*-6:*n*-3 ratios, and the lowest MUFA and *n*-3 concentrations. Opposite results were observed for treatment 4. Treatments 2 and 3 were highly similar with differences mainly in SFA and MUFA concentrations.

Keywords: Fatty acids; Caprines; Gas chromatography; Chemometrics

1. Introduction

The interest in chemometric methods has spread to numerous fields of modern analytical chemistry, such as nuclear magnetic resonance (Brescia, Kosir, Caldarola, Kidric, & Sacco, 2003; Duarte, Barros, Almeida, Spraul, & Gil, 2004; Harris & Alam, 2002), infrared (Camacho, Valles-Lluch, Ribes-Greus, & Karlsson, 2003; Parreira, Ferreira, Sales, & Almeida, 2002), Raman (Groot et al., 2001; Ryder, 2002) and mass spectrometries (Moreda-Pineiro, Marcos, Fisher, & Hill, 2001), CG and HPLC chromatographies (Ferreira, Morgano, Queiroz, & Mantovani, 2000; Fraga, Prazen, & Synovec, 2000; Fraga, Bruckner, & Synovec, 2001; Fraga, Prazen, & Synovec, 2001; Héberger & Görgényi, 1999; Hope et al., 2003; Johnson, Prazen, Olund, & Synovec, 2002; Johnson & Synovec, 2002; Lee, Noh, Bae, & Kim, 1998; Prazen, Johnson, Weber, & Synovec, 2001). As most chemical data analysis applications are multivariate by nature, principal component analysis (PCA) (Malinowski, 1991; Sharaf, Illman, & Kowalski, 1986) and the hierarchical cluster analysis (HCA) (Hartigan, 1975; Sharaf et al., 1986) are the most suitable statistic data treatments. Application of these statistic methods to chromatographic data treatments became popular in the last two decades.

Gas chromatography (GC) analysis of fatty acids (FA) was first reported by James and Martin (1952), and since the early fifties, this technique has been

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extensively used for the compositional determination of FAs (Manku, 1983; Sandra & Vanroelenbosch, 1981). Some lipids, e.g., short chain free FAs, can be analyzed by gas chromatography without derivatization. However, most of them must be derivatized before analysis because of their low volatility or poor thermal stability. In this way, standard methodology for the derivatization of FAs is detailed by the International Union of Pure and Applied Chemistry (IUPAC) (Lee et al., 1998). The methodology involves the alkaline saponification of extracted fat to break down their glycerides, and for the subsequent esterification of the liberated FAs in the presence of methanol and boron trifluoride. The formed fatty acid methyl esters (FAMEs) are then extracted with organic solvents and analyzed by GC with flame ionization detector (FID).

Fatty acids (FA) are termed as nonessential or essential. Nonessential FA can be synthesized in the organism and are not required in the animal diet. On the other hand, essential FA (EFA) cannot be synthesized by the organism and must be supplied in the animal diet. Several health problems are associated with EFA deficiencies such as hair loss, eczema-like skin eruptions, susceptibility to infections, arthritis-like conditions, liver or kidney degeneration, growth retardation and vision or learning problems (Andrade, Rubira, Matsushita, & Souza, 1995; Shantha & Napolitano, 1992).

Fatty acids (FA) are classified according to their chemical structure. There are three varieties of FA: saturated FA (SFA), monounsaturated FA (MUFA) and polyunsaturated FA (PUFA). Saturated fatty acids (SFA) are harmful to health, as they tend to elevate LDL-cholesterol level (low density lipoprotein). Additionally, consumers are advised to increase the overall intake of MUFA and PUFA in their diets as these fatty acids help to decrease the amount of LDL-cholesterol. The Department of Health of England (HMSO, 1994) recommends a PUFA:SFA ratio of at least 0.45. On the other hand, omega-3 and omega-6 FA (*n*-3 and *n*-6, respectively) are two important groups of FAs that hold important functions in the organism. The dietary importance of *n*-6 FA and *n*-3 FA has long been recognized. The presence of a high concentration of *n*-3 FA associated with a low concentration of *n*-6 FA can decrease the risk of coronary diseases (Andrade et al., 1995; Shantha & Napolitano, 1992). The Health Department of England (HMSO, 1994) recommends 4.0 as the maximum ratio of *n*-6:*n*-3.

GC data acquired for I samples can be organized in a multivariate data matrix denoted by $X(I,J)$ where the J variables correspond to the different fatty acids. However, this representation is not usually appropriated to visualize the latent structure inherent to the data. To overcome this difficulty, the use of chemometric methods is instrumental.

In this way, the main goal of the present work was to study the relationship of FA content in caprine meat of animals submitted to four different dietary treatments. For this task, GC, PCA, and HCA chemometric methods were exploited. The final sample chemical compositions presented the best animal dietary treatment to produce meat with better FA contents.

2. Materials and methods

Data analyses were performed by using the software PIROUETTE 2.2 (Infometrix, Seattle, WA).

2.1. HCA and PCA principles

The primary goal of Hierarchical Cluster Analysis (HCA) is to display data in such a way that natural clusters and patterns can be shown in a two-dimensional space. The results, qualitative in nature and usually presented in a dendogram form, permit the visualization of clusters and correlations among either samples or variables. In HCA, the Euclidean distances among samples or variables are calculated and transformed into similarity indices ranging from 0 to 1. A small distance corresponds to a large index and means a large similarity (Hartigan, 1975; Sharaf et al., 1986).

On the other hand, PCA is a data compression method based on the correlation among variables. Its aim is to group correlated variables, and replace them by new sets called principal components, PCs. PCs are completely uncorrelated and they are built as a simple linear combination of the original variables. It should be stressed that PCs contain most of the data set variability, but in a much lower-dimensional space. The first principal component, PC_1, is defined as the direction of maximum variance of the whole data set. PC_2 is the direction that describes the maximum variance in the orthogonal subspace to PC_1. The subsequent components are taken orthogonally to the ones previously chosen and describe the maximum of the remaining variance. When redundancy is removed, only the first few principal components are required to describe most of the information contained in the original data set. The data matrix, X $(I \times J)$, where I corresponds to the number of samples and J to the number of measurements taken on each sample, is decomposed into two matrices, T and L, so that $X = TL^{T}$.

The matrix T, known as the "score" matrix, represents the positions of the compounds in the new coordinate system, i.e., in the PC coordinate system. L is the "loading" matrix whose columns describe how the new axes, i.e., the PC's, are built from the old axes. The algorithms for PCA can be found in standard chemometric books and tutorials (Ferreira et al., 2000; Malinowski, 1991; Sharaf et al., 1986).

2.2. Animals and diets

In this experiment, 32 young *Saanen* caprines, 16 males and 16 females, weaned at 60th day of age, aged 75 days and with mean initial live weight of 15.3 kg were divided in four groups. Each group was submitted to four diets with different energetic levels (Table 1). All animals were slaughtered at Iguatemi Experimental Farm of Maringa State University. Meat samples were taken from *Longissimus dorsi* muscle at the 11th rib, vacuum-packed, rapidly frozen, and stored at −18 °C until chemical analysis was carried out.

2.3. Muscle composition

Moisture, ash, and protein contents were determined as described by AOAC (1990). Lipids were extracted from muscle tissues by the method described by Folch, Lees, and Stanley (1957). The meat samples (15.00 ± 0.01 g) were homogenized with 90 ml of chloroform–methanol (2:1, v/v) solution for 2 min. After blending, 30 ml of chloroform and 30 ml of deionized water were added and the mixture was homogenized. Then, an NaCl aqueous solution at 0.98% (m/v) was added to the homogenate to separate the lipid-containing chloroform layer from the methanol–water phase. The lipid extract was transferred to a 250-ml flask and the solvent was evaporated under nitrogen stream. The lipid content was then gravimetrically determined.

2.4. Transesterification and fatty acid composition

Methyl esters were prepared by transmethylation according to ISO 5509 (1978) method, using KOH 2 mol l^{-1} in methanol and *n*-heptane medium. Fatty acid methyl esters (FAME) were analyzed using a Shimadzu 14A (Japan) gas chromatograph equipped with flame ionization detector and Varian (USA) fused silica capillary column (100 m × 0.25 mm and 0.20 μm of biscyanopropyl polysiloxane, CP-Sil 88). Column temperature was programmed from 140 to 225 °C at 10 °C min^{-1}. The injection port and detector were maintained at 220 and 230 °C, respectively. Hydrogen at 1.2 ml min^{-1} was used as a carrier gas and nitrogen at 30 ml min^{-1} was used as the make up gas in split mode 1:100.

Identification of normal fatty acids was carried out comparing sample FAME peak relative retention times with those obtained for Sigma (USA) standards. The peak areas were determined by CG-300 computing integrater program (CG Instruments, Brazil). Data were calculated as normalized area percentages of fatty acids.

3. Results and discussion

The use of HCA and PCA multivariate methods afforded a better understanding of the composition of meat samples of goats submitted to different dietary treatments and of their fatty acids profile. Eight samples were collected from each animal and named as follows: samples 1–8 for treatment 1, samples 9–16 for treatment 2, samples 17–24 for treatment 3 and samples 25–32 for treatment 4. It was observed that on the average the fatty acid concentrations followed the PUFA:SFA percent ratio of at least 0.45 recommended by The Department of Health of England (HMSO, 1994). In all chromatograms obtained, the predominant FAs were oleic acid (C18:1*n*-9), palmitic acid (C16:0), and stearic acid (C18:0), which represented more than 80% of the total fatty acids of the samples.

The original data set (Table 2), $X(32, 7)$ consisted of seven variables measured in 32 samples (eight samples for each of the four treatments), where each entry matrix was an average value of three replications. The data were autoscaled prior to analysis (each X column has zero mean and unity variance). The HCA results gave a dendogram (Fig. 1) that grouped all samples of each treatment with a similarity index of approximately 0.9. This indicated that there was no mixing for different treatments. Treatment 1 samples (1–8) resulted in a unique cluster, which indicated that their chemical composition was different from those of the other treatments.

Table 1
Percentage and chemical composition of different dietary treatments (dry material bases)

	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Ingredients (%)				
Oat hay	62.17	48.04	32.65	17.21
Soybean meal	18.86	18.15	17.38	16.61
Corn ground	15.97	30.81	46.97	63.18
Mineral mixture*	3.00	3.00	3.00	3.00
Composition				
Metabolizable energy (MJ/kg)	9.60	10.40	11.60	12.40
Dry matter (%)	90.69	90.36	90.04	89.47
Crude protein (%)	16.78	16.63	16.39	16.55
Neutral detergent fibre (%)	56.30	50.14	40.17	31.69

* Chemistry composition (per kg of product): 120.00 g Ca. 270.00 g Cl. 65.00 g P. 125.00 mg Co. 650.00 mg F. 60.00 mg I. 3.00 g Mg. 4.4 g Mn. 45.00 mg Se. 175.50 mg Na. 4.68 g Zn (commercial product).

Table 2
Original data set of variables measured in all caprine meat samples

Samples	*n*-3	*n*-6	*n*-6:*n*-3	SFA	MUFA	PUFA	PUFA:SFA
1	0.2641	3.7498	14.1964	50.8125	41.5846	7.6029	0.1496
2	0.2686	3.7584	13.9931	50.9835	41.5317	7.4848	0.1468
3	0.2534	3.7288	14.7119	51.2187	41.1364	7.6448	0.1493
4	0.2646	3.8066	14.3885	50.1781	41.9751	7.8568	0.1538
5	0.2674	3.7707	14.1003	51.4442	41.1296	7.4348	0.1445
6	0.2647	3.7492	14.1617	51.7418	40.8164	7.4419	0.1439
7	0.2615	3.7110	14.1934	49.6910	42.6104	7.6985	0.1549
8	0.2574	3.7667	14.4064	50.8604	41.5824	7.5574	0.1486
9	0.3082	3.6688	11.9032	50.1596	44.7324	5.1079	0.1018
10	0.3028	3.6869	12.1748	51.0211	43.7937	5.1851	0.1016
11	0.2986	3.6462	12.2127	50.1743	44.7181	5.1076	0.1018
12	0.3088	3.6534	11.8326	50.3315	44.5703	5.0982	0.1013
13	0.3152	3.6755	11.6604	48.7372	46.3307	4.9321	0.1012
14	0.2974	3.6462	12.2594	49.1212	45.6885	5.1902	0.1057
15	0.3036	3.6621	12.0602	50.3380	45.4279	4.2341	0.1040
16	0.2935	3.6486	12.4330	49.8098	44.9126	5.2776	0.1060
17	0.3265	3.5218	10.4667	53.7498	41.1561	5.0964	0.0948
18	0.3381	3.4948	10.3354	53.3053	41.5914	5.1033	0.0957
19	0.3414	3.5198	10.3096	53.5173	41.4064	5.0761	0.0948
20	0.3352	3.5109	10.4754	52.8601	41.9989	5.1410	0.0973
21	0.3368	3.5314	10.4853	52.3581	42.5652	5.0767	0.0970
22	0.3367	3.5224	10.4602	53.2827	41.6098	5.1076	0.0959
23	0.3361	3.4972	10.4059	53.7520	41.1832	5.0647	0.0942
24	0.3414	3.5098	10.2802	53.5173	41.4064	5.0761	0.0949
25	0.3506	3.1983	9.1235	42.7332	52.4618	4.8050	0.1124
26	0.3525	3.2163	9.1247	42.8315	52.3111	4.8574	0.1134
27	0.3496	3.2131	9.1909	42.4186	52.7001	4.8813	0.1151
28	0.3468	3.2200	9.2858	42.3136	53.1051	4.5812	0.1083
29	0.3527	3.2172	9.1216	43.1662	51.8315	5.0023	0.1159
30	0.3475	3.2290	9.2916	42.8412	52.1960	4.9628	0.1158
31	0.3517	3.1981	9.0936	42.0623	53.1285	4.8092	0.1143
32	0.3536	3.2090	9.0741	42.4960	52.6710	4.8329	0.1137

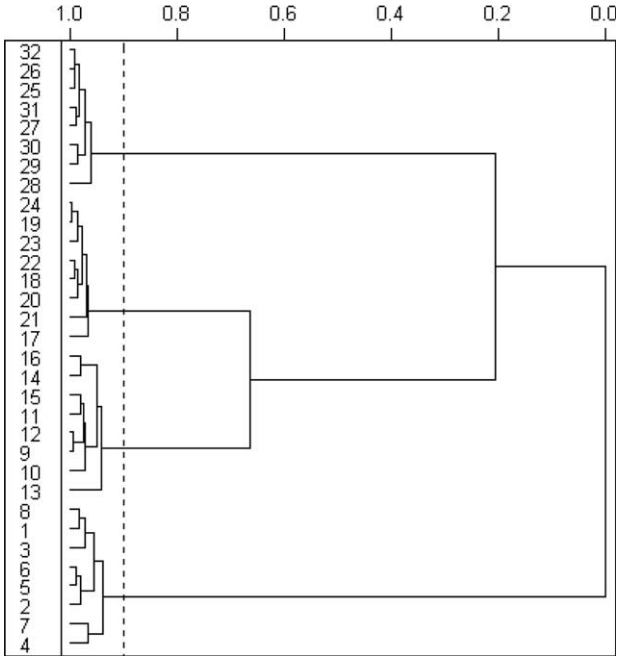

Fig. 1. HCA results for caprine meat of submitted to four different dietary treatments. Samples numbered according to diet treatment: Samples 1–8, 9–16, 17–24, and 25–32 represent treatments 1–4, respectively.

Table 1 shows that all treatments were isoproteic, and that the differences among the treatments were mainly in energy levels. Treatment 1 presented the lowest energy level as well as the lowest nitrogen content.

PCA analysis (Table 3) yielded similar trends and characteristics as those observed in HCA, but with the advantage that the correlation among variables and samples became more evident. PC_1 contains 72.15% of the original information of the data set, and the loadings indicated that there were significant contributions of *n*-6:*n*-3, *n*-6, and PUFA variables with positive loadings, and of *n*-3 and MUFA variables, with negative loadings.

Table 3
Loadings for the first three PCs and their respective variances

Components	PC_1	PC_2	PC_3
n-3	−0.42510	−0.16460	0.36437
n-6	0.41611	−0.18750	−0.46830
n-6:*n*-3	0.43416	0.10224	−0.33290
SFA	0.29152	−0.57420	0.28316
MUFA	−0.35810	0.43388	−0.37900
PUFA	0.39722	0.30407	0.42731
PUFA:SFA	0.29427	0.56286	0.36128
% of total variance	72.1472	23.7765	3.8139
Cumulative % variance	72.1472	95.9238	99.7377

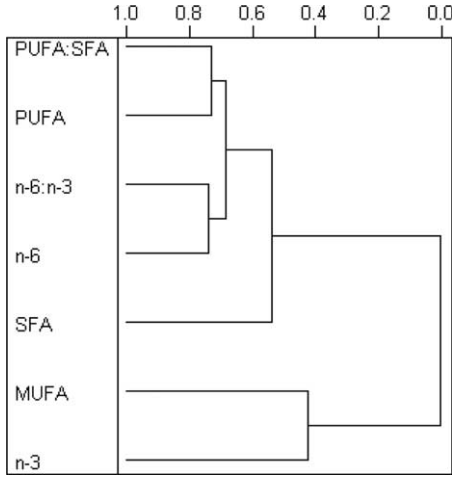

Fig. 2. Dendogram (HCA) for variables. Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-3 fatty acids (*n*-3), and omega-6 fatty acids (*n*-6).

The latter variables were inversely correlated to the three former ones. These correlations were confirmed by HCA analysis (Fig. 2), whose MUFA and *n*-3 variables were in one single cluster. The second PC described 23.78% of the total variance and presented high positive loadings for MUFA and PUFA:SFA variables. Moreover, the SFA variable presented high negative loading in PC_2. It was verified that the first two PCs accounted for almost 96% of the total variance of the data set.

The scores and loading plots for PC_1 vs. PC_2 are presented in Figs. 3 and 4. Definite clusters for each of the four treatments were observed (Fig. 3) and the PC_1 scores discriminated treatment 1 (positive scores) from treatment 4 (negative scores). Treatment 1 (see loadings in Fig. 4) was characterized by a higher ratio of PUFA:SFA, concentration of PUFA, *n*-6 FA, and *n*-6:*n*-3 ratio, and lower MUFA and *n*-3 concentrations, whereas opposite results were observed for treatment 4 (high MUFA and *n*-3 FA concentrations). Furthermore,

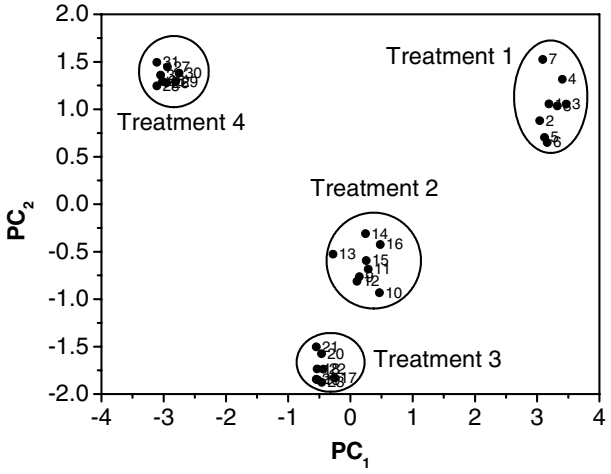

Fig. 3. PC_1 vs. PC_2 score plots.

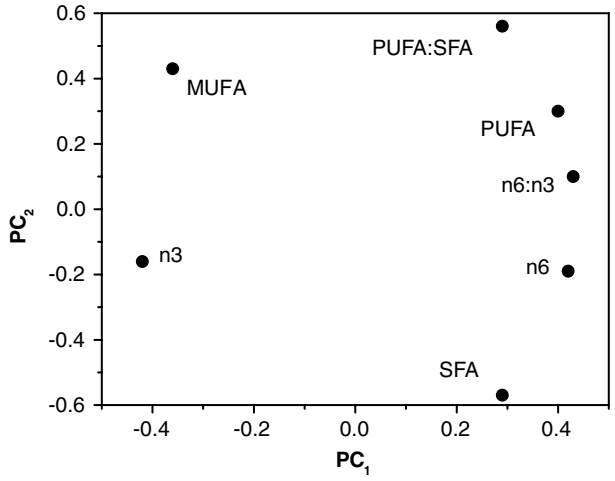

Fig. 4. PC_1 vs. PC_2 loadings plots.

treatment 4 presented lower SFA concentration, as it was highly negatively loaded in PC_2 (Fig. 4). A higher similarity was observed for treatments 2 and 3 when HCA was analyzed. The main differences observed were the higher SFA and the lower MUFA contents when compared with treatment 3.

Treatment 4 presented higher MUFA concentration and the principal fatty acid found was from the oleic acid group. This treatment also presented lower *n*-6:*n*-3 fatty acid ratio and lower SFA concentration. As the reduction of these variables is essential to decrease the incidence of diseases, for example, coronary diseases (Bucher, Hengstler, Schindler, & Meier, 2002), treatment 4, can be considered as the best and should be chosen.

4. Conclusions

This report demonstrated that chemometrics is an important tool to investigate the relationship of FA contents in meat of caprine submitted to different diets. The association of experimental GC data with chemometric tools (HCA and PCA) was essential for a better understanding of the data, especially because in the present work all the variables should be taken into account simultaneously. In summary, analysis of meat FA contents of caprine under the same protein levels, but differing in their metabolizable energy levels showed that the FA composition can differ significantly. As verified, the diet that decreased the *n*-6:*n*-3 FA ratio and the SFA content the most was the one used in treatment 4, which also presented the highest energy level in chemical composition analysis.

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