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Application of multivariate analysis to the study of mechanically deboned chicken meat (MDCM)

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Abstract

Mechanically deboned chicken meat (MDCM) is a raw material produced with specific mechanical deboning equipment using cheaper chicken parts such as the back, the neck and meat clinging to the bones. Mechanical separation changes the composition of the original raw material, resulting in meat with higher fat and mineral contents; then, determination of proximate and mineral composition of MDCM obtained from different chicken parts, lots and suppliers permitted evaluation of the variability of raw material used in manufacture of chicken meat products. The objective of this work was to study the possible variations on composition of MDCM derived from different suppliers and different chicken parts using principal component analysis. The results obtained were interpreted using multivariate analysis and showed that there is variability among the raw materials, especially in respect of different chicken parts and different manufacturing lots. The proximate and mineral compositions of MDCM products were affected especially by processing conditions.

Introduction

For many different reasons people can prefer eating chicken than beef or pork, since chicken can be consumed in the form of whole carcasses, chicken parts or processed products (USDA, 2014). The mechanical deboning generates parts of low commercial value such as back and neck, but mechanical separation causes a considerable change in the composition of the original raw material. Many authors have observed lower protein content and higher values of total lipids and cholesterol (three to five-fold higher levels) in mechanically deboned poultry as compared to the manually deboned poultry due to incorporation of lipids from the bone marrow and the layer of subcutaneous fat (Froning, 1976; Botka-Petrak *et al*., 2011; Pereira *et al*., 2011; Biohaz, 2013; Song *et al*., 2014; Irshad and Sharma, 2015). Beyond the higher fat content mechanical separation incorporates skin which has a substantial amount of protein (e.g. collagen) and is eliminated together with the bones during the process of mechanical separation, thereby reducing the amount of connective tissue in the end product (CFIA, 2014).

During mechanical separation bone particles are incorporated into the mechanically deboned meat (MDM) thereby increasing its calcium content which has been used as a measure of the amount of bone present in MDM (Froning, 1981; Biohaz, 2013). MDM meat has also increased levels of fluoride and iron (Komrska *et al*., 2011), since iron content is twice as high as of the manually deboned meat due to bone marrow incorporation and the increased content of the heme pigment affects the color of MDM making it redder and darker (Field, 1981). Then, the composition of MDM can present several variations concerning composition which are primarily due to the type raw materials, presence of skin or lack thereof and the meat/bone ratio incorporation from mechanical deboning, as well as type of the mechanical separator equipment used (Nagy *et al*., 2007).

Food is multivariate in nature and the product quality is a resultant of sensory and instrumental attributes (Los *et al.,* 2014). The combination of experimental design with multivariate methods such as principal component analysis (PCA) can be powerful tools for improving the process of product development (Song *et al*., 2014). Ultimately, the purpose of most multivariate analysis is to develop a model that accurately characterizes some properties that are difficult to measure directly, thereby leading to more comprehensive product and raw material testing (Hair *et al*., 1995). On the other hand, PCA is based on the correlation among variables, since it maps samples through scores and variables by

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the loadings in a new space defined by the principal components. The PCs are simple linear combination of original variables operating by scoring plots allow sample identification, checking if they are similar or dissimilar, typical or outlier. The first principal component, PC1, is defined in the direction of maximum variance in the data set, and the subsequent components are orthogonal (uncorrelated) to another and maximize the remaining variance. Once the redundancy is removed only the first few principal components are required to describe most of the information contained in the original data. Interpretation of the loadings vectors (PCs) obtained through PCA become often easier when they are rotated to better match with the original variable's directions which is called Varimax Rotation (Sharaf *et al*., 1986) that maximizes the variance in each PC and the last effect of this rotation is to decrease the effect of those variables with intermediate loadings and increase the effect of those with large (positive and negative) loadings in each factor (Ferreira *et al*., 2000).

The most widely used multivariate statistical tool in sensory analysis, PCA is applied to acceptability studies in which the input data consists of a sample (rows) by consume (columns) matrix, and the result is known as internal preference mapping (Greehoff and MacFie, 1994). When applied to descriptive analysis, the input data is a sample (rows) by descriptor (columns) matrix, usually constructed from the mean values over assessors. PCA reduces the number p original variables (columns) into a fewer number of k unobservable variables (PCs) that are linear combinations of the original ones. The main objective of PCA is the explanation of as much of the variability of the original data as possible with as few of these principal components as possible (although it is possible to obtain as many of them as them as there are original variables (Borgognone *et al.*, 2001; Song *et al.*, 2014).

Analyses of the main components (Moita and Moita, 1998) and of the hierarchical grouping (Bruns and Faigle, 1985) are complementary techniques: the first one, through objective criteria, reduces the analyzed variables to a new set of parameters, allowing the construction of two-dimensional graphs that contain more statistical data; the second technique interconnects the samples through their similarities yielding a dendrogram in which similar samples according to the parameters chosen are sorted amongst themselves (Moita and Moita, 1998; Härdle and Simar, 2015). The use of these techniques is becoming increasingly frequent in the field of food science, especially in meat foods (Karlsson, 1992; Fongaro *et al.*, 2015).

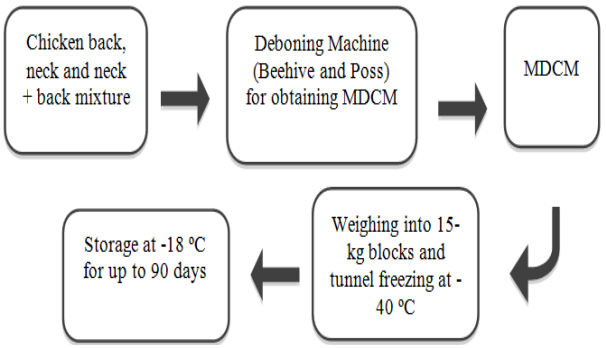

Figure 1. Process for obtaining MDCM

The main objective of this work was the application of multivariate analysis to the study of mechanically deboned chicken meat (MDCM) in order to investigate possible variability of proximate and mineral composition regarding MDCM from different chicken parts or distinct manufacturing lots.

Material and Methods

Material

This study used mechanically deboned meat obtained from different chicken parts, three different suppliers, coded as 1, 2 and 3, and different manufacturing time periods (lots) as shown below. The samples processed by supplier 1 were especially supplied for the execution of this research; whereas the samples obtained from suppliers 2 and 3 came out of production lots used by meat product industries. The MDCM from supplier 1 had the following chicken parts: neck with skin (Nws), back with skin (Bws), a mixture of neck and back with skin (Mws), neck with no skin (Nns), back with no skin (Bns), and a mixture of neck and back with no skin (Mns). Four different lots (1, 2, 3 and 4) were obtained from supplier 1. Considering supplier 2, two distinct parts, e.g., a mixture of back and neck with skin (Mws coded with the letter S) was used. Seven different lots (1, 2, 3, 4, 5, 6 and 7) were obtained from that supplier. In a single lot, the MDCM from supplier 3 contained a mixture of back and neck with skin (coded with the letters GM).

The MDCM was processed as shown on Figure 1, immediately after slaughter (-18ºC in up to 48 h) using specialized equipment owned by the slaughterhouse in three different plants. The samples for this experiment were removed in 0.5 kg portions, packaged in polyethylene bags, frozen through ultra-rapid processes and stored at -18oC for subsequent analysis. At the time of the analyses samples were defrosted in a refrigerator over a 24-hours period of time, homogenized and subsequently analyzed.

Table 1. Proximate composition (g/100g) and pH of the MDCM

Supplier	Sample	Moisture	Ash	Fat	Protein	pH
1	Nws1	67.92	0.68	20.73	11.41	6.69
1	Nws2	67.08	1.05	19.42	13.32	6.60
1	Nws3	65.70	1.04	19.59	12.99	6.49
1	Nws4	70.59	0.74	18.61	11.03	6.51
	X ± sd	67.82±2.06	0.88±0.20	19.59±0.87	12.19±1.14	6.57±0.09
1	Bws1	64.39	0.72	23.49	12.22	6.54
1	Bws2	64.91	1.31	21.30	12.83	6.59
1	Bws3	63.90	0.88	24.14	11.58	6.40
1	Bws4	69.67	0.81	17.14	13.14	6.39
	X ± sd	65.72±2.67	0.93±0.26	21.52±3.16	12.44±0.69	6.48±0.08
1	Mws1	65.25	0.72	21.75	11.70	6.61
1	Mws2	66.53	1.13	19.39	13.27	6.66
1	Mws3	63.87	0.83	22.97	12.70	6.49
1	Mws4	71.05	0.82	15.52	13.34	6.62
	X ± sd	66.68±3.11	0.88±0.18	19.91±3.28	12.75±0.76	6.60±0.07
1	Nns1	66.85	0.83	20.90	12.01	6.57
1	Nns2	67.65	0.98	18.62	13.22	6.79
1	Nns3	66.58	0.90	21.44	11.88	6.20
1	Nns4	70.37	0.71	15.91	12.56	6.41
	X ± sd	67.86±1.73	0.86±0.11	19.22±2.52	12.42±0,61	6.49±0.25
1	Bns1	65.55	0.71	22.82	11.53	6.44
1	Bns2	65.02	0.82	21.72	12.61	6.62
1	Bns3	63.02	0.82	24.80	11.77	6.40
1	Bns4	73.28	0.61	15.32	11.45	6.45
	X ± sd	66.72±4.51	0.74±0.10	21.17±4.10	11.84±0.53	6.48±0.10
1	Mns1	66.61	0.84	20.54	12.72	6.61
1	Mns2	68.77	0.86	17.50	12.99	6.68
1	Mns3	63.34	0.80	23.60	11.42	6.30
1	Mns4	69.65	0.65	18.83	11.40	6.51
	X ± sd	67.09±2.81	0.79±0.10	20.12±2.63	12.13±0.84	6.53±0.17
2	S1	63.44	0.77	24.42	11.50	6.38
2	S2	64.67	0.79	23.52	11.49	6.51
2	S3	65.36	0.94	22.48	11.23	6.40
2	S4	64.60	0.82	22.97	11.12	6.50
2	S5	64.40	0.94	23.26	11.05	6.30
2	S6	63.50	0.85	25.57	10.22	6.40
2	S7	66.96	1.05	20.02	12.04	6.30
	X ± sd	64.70±1.20	0.88±0.10	23.18±1.73	11.24±0.56	6.40±0.08
3	GM	64.05	0.79	23.01	11.87	6.60
Total	X ± sd	66.39±2.62	0.85±0.15	20.98±2.78	12.05±0.82	6.50±0.13
	CI (95%)	0.94	0.05	1.00	0.30	0.05

X: medium, sd: standard deviation, CI: confidence interval;
Nws: neck with skin, Bws: back with skin, Mws: mixture of neck and back with skin,
Nns: neck with no skin, Bns: back with no skin, Mns: mixture of neck and back with no skin;
S: seven different lots from supplier 2 of Mws;
GM: single lot from supplier 3 of Mws

Chemical analysis

The determination of moisture, fat, protein, and pH content was carried out in accordance with the Analytic Standards of Adolfo Lutz Institute (Instituto Adolfo Lutz, 2005). Mineral content (calcium, iron, phosphorus, magnesium and zinc) was determined using an Inductively Coupled Argon Plasma Atomic Emission Spectrometer (ICP), Optima 3000 DV model, Perkin Elmer brand, as recommended by the Association of Official Analytical Chemists (AOAC, 2012). Instrumental conditions and operating parameters were: radiofrequency power (1350 W), nebulization flow (0.72 L/min), auxiliary flow (0.50 L/min), pump flow (1.50 mL/min), plasma (15.00 L/min) and specific wavelength values (in nm) for each element (Ca [422], Fe [260], P [215], Mg [280], and Zn [213]).

Statistical analysis

Data from proximate composition and minerals were analyzed by one-way analysis of variance and means were compared by the Duncan's multiple range test. Principal Component (PC) loadings and rotated factor matrix were calculated by SPSS (Statistical Package for the Social Sciences) for windows (release 10.0) and double cross-validation was performed in order to define the number of significant components in principal component analysis (PCA).

Results and Discussion

Proximate composition

Proximate composition of mechanically deboned meat from different chicken parts (neck, back, mixture of neck and back, with and without skin), from supplier 1 and from a mixture of neck and back with skin from suppliers 2 and 3, are presented in Table 1. Moisture, ash, protein, and lipid (g/100g) content of MDCM in the current study were different to data obtained by Cortez-Vega *et al.* (2013) (66.39 x 89.30; 0.85 x 0.50; 12.05 x 10.40; 20.98 x 1.50). Souza et al. (2009) found higher lipid and protein levels but lower ash and moisture values in MDCM compared to those found in the present study. The

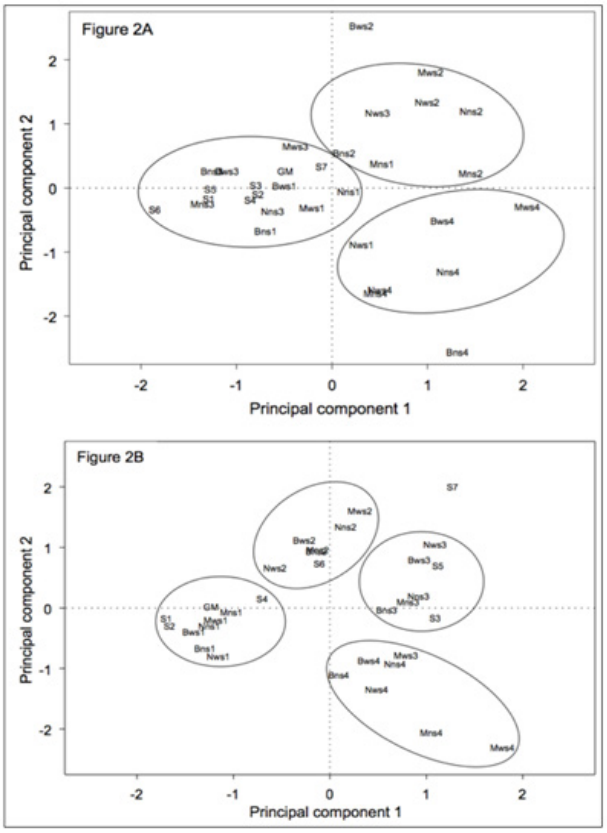

Figure 2. (A) Principal component 1 vs. principal component 2 based on proximate composition and pH; (B) Principal component 1 vs. principal component 2 based in mineral composition for the raw materials

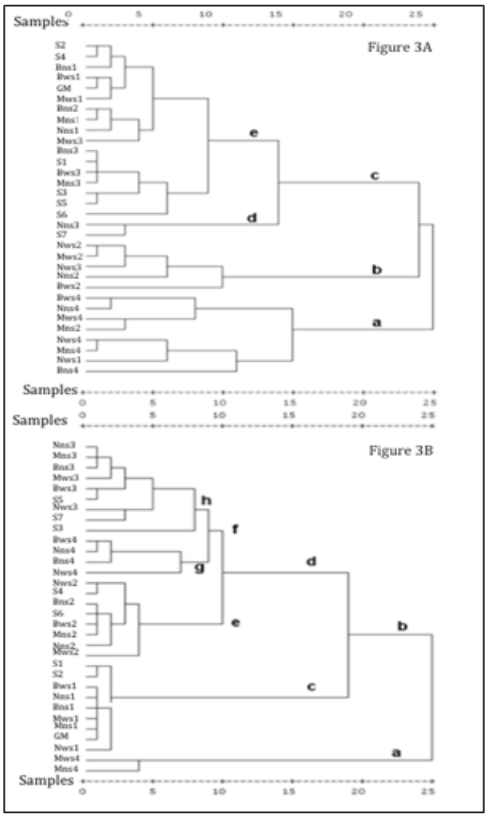

Figure 3. (A) Dendrogram obtained through the variables of proximate composition and pH; (B) Dendrogram obtained through the variables of mineral composition for the raw materials

MDCM produced by Souza (2009) had 37% of fat, which surpasses the 30% permitted by Brazilian norms (BRASIL, 2000). Compared to the MDCM obtained by Ionescu *et al.* (2003), the meat product from the current study had similar values of moisture and ash, but increased content of fat (21% x 14.1%) and decreased values of protein (16.6% x 12.1%).

Proximate composition of the raw materials from the different chicken parts from supplier 1 for all parameters (moisture, ash, fat, protein and pH) presented little variation. Nonetheless, one can evaluate the variability of the raw materials better using multivariate analysis. For it we used a data matrix consisting of five variables (moisture, ash, fat, protein and pH) and 32 samples. Therefore, five new variables were generated, denominated principal components (PC). Each principal component was constructed based on a combination of the five original variables where the weight of each of these variables in the construction of the two most important principal components were describe below: Ash PC1 (0.07619), PC2 (0.86581); Fat PC1 (-0.93891), PC2 (0.24825); pH PC1 (0.57731), PC2 (0.31712); Protein PC1 (0.73029), PC2 (0.55274); and Moisture PC1 (0.82387), PC2 (-0.50933).

Analyzing the weight of principal component 1 it should be noted the samples that were poor in fat (-0.94) but rich in moisture (+0.82) and protein (+0.73) are on the graph, along the positive axis of this component. Component 2 showed differences between samples regarding their ash content (0.87) but not pH or protein. The percentage of variance accrued on the two first principal components is 78.2%. This means that the results for principal component 1 vs. principal component 2 showed the 32 samples of a statistically privileged window (78.2% of the statistical information).

Figure 2A presents the samples distributed on the graph according to principal components 1 and 2. Samples provided by supplier 1 are distributed across the entire graph reflecting higher heterogeneity of these samples. Proximate composition did not provide evidence of similarity between same source samples (neck, back or a mixture of neck and back), but rather between same lot samples, as can be seen in connection with lot 2, which is found in the upper right-hand quadrant. The same phenomenon can be seen with regard to the 4th lot in the lower right-hand quadrant. Lots 1 and 3 are more similar to each other and to the other samples from other suppliers. Based on the location of lots 2 and 4 on the positive axis of principal component 1, we can say that these lots

Table 2. Mineral composition (mg/100g) of MDCM

Supplier	Sample	Ca	Fe	P	Mg	Zn
1	Nws1	39.2	2.5	129.4	11.2	1.6
1	Nws2	108.5	2.2	170.8	14.2	1.4
1	Nws3	171.1	3.4	229.6	21.9	1.8
1	Nws4	49.7	3.1	177.3	18.3	2.8
	X ± sd	92.1±60.8	2.8±0.6	176.8±41.1	16.4±4.7	1.9±0.6
1	Bws1	38.1	1.9	128.6	11.9	1.3
1	Bws2	146.7	2.4	181.9	14.2	1.5
1	Bws3	159.7	2.9	216.2	19.5	2.3
1	Bws4	46.7	3.2	193.9	21.9	1.9
	X ± sd	97.8±64.3	2.6±0.6	180.1±37.2	16.9±4.6	1.7±0.4
1	Mws1	48.2	2.0	142.6	12.8	1.3
1	Mws2	155.4	2.3	226.4	17.4	1.5
1	Mws3	94.3	3.9	191.1	20.3	2.3
1	Mws4	68.6	6.0	210.3	22.6	2.9
	X ± sd	91.6±46.5	3.6±1.8	192.6±36.3	18.3±4.2	2.0±0.7
1	Nns1	49.3	2.0	134.6	12.0	1.4
1	Nns2	165.3	2.4	199.9	15.3	1.8
1	Nns3	137.2	3.4	202.8	22.0	2.1
1	Nns4	64.4	3.9	199.0	22.2	1.9
	X ± sd	104.0±56.0	2.9±0.9	184.1±33.0	17.9±5.1	1.8±0.8
1	Bns1	32.4	2.2	129.0	12.2	1.4
1	Bns2	138.1	2.4	181.0	16.7	1.5
1	Bns3	120.6	3.5	193.5	19.5	2.0
1	Bns4	37.9	3.5	178.8	20.1	1.7
	X ± sd	82.2±54.9	2.9±0.7	170.6±28.5	17.1±3.6	1.7±0.3
1	Mns1	61.7	2.1	148.9	12.8	1.4
1	Mns2	131.1	2.3	191.8	15.5	1.6
1	Mns3	126.5	3.3	200.8	21.5	2.1
1	Mns4	50.3	5.1	186.2	22.3	2.4
	X ± sd	92.4±42.3	3.2±1.4	181.9±22.8	18.0±4.6	1.9±0.5
2	S1	46.8	2.0	121.6	10.0	1.0
2	S2	49.1	2.3	119.3	9.7	1.0
2	S3	152.6	5.3	207.6	20.0	1.7
2	S4	87.5	2.4	155.1	14.5	1.4
2	S5	172.8	3.2	214.0	19.5	2.5
2	S6	128.2	2.5	179.3	17.4	1.5
2	S7	220.0	2.6	239.4	21.5	2.1
	X ± sd	122.4±65.0	2.9±1.1	176.6±46.7	16.1±4.8	1.6±0.6
3	GM	51.6	1,9	146.0	13.4	1,1
Total	X ± sd	98.4±53.0	2.9±1.0	179.0±34.0	17.0±4.2	1.8±0.5
	CI (95%)	19.1	0.4	12.3	1.5	0.2

X: medium, sd: standard deviation, CI: confidence interval;
Nws: neck with skin, Bws: back with skin, Mws: mixture of neck and back with skin,
Nns: neck with no skin, Bns: back with no skin, Mns: mixture of neck and back with no skin;
S: seven different lots from supplier 2 of Mws;
GM: single lot from supplier 3 of Mws

differ from the set of the other samples due to a lower fat content and/or a greater moisture and/or protein content.

The seven samples from supplier 2 are more homogeneous and are closer to each other in the left quadrant of principal component 1. The sample coded as S7, from the same supplier, stands out from the others in that it has higher moisture, ash and protein content but a lower fat content. Sample S6 also stands out from the others due to a higher fat content and a lower protein content. The unique sample from supplier 3 had a central distribution on graph of the principal components, indicating that its composition is close the average composition of the set of 32 samples.

We can see the similarity between the analyzed samples through the Figure 3A dendrogram with scale of similarity of the dendrograms generated by the SPSS program ranges from zero (greater similarity) to 25 (lower similarity).The dendrogram shows three different groupings: group a, which basically consists on the samples from lot number 4; group b, comprising the samples from lot 2; and group c, which consists of all the other samples. In sub-group d we see sample S7, which is different from the other samples from this supplier, which appear in sub-group e.

In summary, the current MDCM had higher content of fat and lower content of protein, which can limit its use in meat products. Excessive lipid content in meat products contribute to undesirable dietary intake of fat that is associated with increasing levels of blood cholesterol and triglycerides as well as enhanced risk of overweight, obesity, and cardiovascular diseases (Kopčevoká *et al*., 2015; Marangoni *et al*., 2015; Odunayia *et al.*, 2015).

Mineral composition

Mineral composition of the raw materials of the different chicken parts from the three different suppliers is showed at the Table 2. Present data indicates that there is a great deal of raw material variation where mineral content is concerned. This is more noticeable in connection with the calcium content, which ranges from 32.4 to 220.0 mg/100g. As we mentioned previously, calcium is the mineral

that reflects the composition of MDCM the most. In Brazilian norms, calcium levels in MDCM should not surpass 1.500 mg/100g (on a dry basis), as well as protein levels should be at least 12% of the product (BRASIL, 2000). Previous studies reported both lower and higher calcium values in MDCM meat produced in Brazil compared to the present data. Gonçalves *et al.* (2009) found 10 to 460 mg/100g of calcium in MDCM produced in Goias State, Brazil, and Kolsarici *et al.* (2010) found calcium values in MDCM produced in Turkey ranging from 52.1 to 179.52 mg/100mg. As occurred regarding calcium (52.07 mg/100g to 179.2 mg/100g), iron (1.61 mg/100g to 3.37 mg/100g) and phosphorus (206.79 mg/100g to 295.94 mg/100g) values obtained by Kolsarici *et al.* (2010) were similar to those found in the current study. Similarly to proximate composition, we do not perceive skin influence on mineral content for the same chicken parts.

The behavior of the results can be better evaluated by means of multivariate analysis, and to do this, we employed a data matrix consisting of the five (5) variables (calcium, iron, phosphorus, magnesium and zinc) and 32 samples. The weight of each of these variables in the construction of the two most important principal components are described below: Calcium PC1 (0.58038), PC2 (0.78900); Iron PC1 (0.76550), PC2 (-0.53018); Phosphorus PC1 (0.92374), PC2 (0.34032); Magnesium PC1 (0.94742), PC2 (-0.11361); and Zinc PC1 (0.84910), PC2 (-0.30478).

In the linear combination that generated principal component 1, all of the variables had a significant weight, especially magnesium, phosphorus and zinc. As all component 1 variables have a large weight, principal component 2 differentiates the samples. The positive axis of component 2 will differentiate the samples that are calcium-rich (+0.79) and/or the ones that are iron-poor (-0.53). The accrued variance percentage of the first two components is 90.4%.

Figure 2B shows a clear separation between the four lots of samples from supplier 1, as they occupy different graph quadrants, e.g., supplier 2 samples form groups close to lots 1 or 3, with sample S6 standing apart, that is close to lot 2, and sample S7 also standing apart, that is richer in calcium and phosphorus than the entire set. This sample, however, has a more homogenous behavior, reflecting more controlled processing conditions than supplier 1 samples. Only supplier 3 sample is close to the samples from supplier 2 and lot 1 from supplier 1. Regarding supplier 1 raw materials, they reflect no similarities between the samples coming from the same chicken part; however, the similarity is found in samples from the same lot, prepared under the same processing conditions.

We can observe the similarity between the samples through the Figure 3B dendrogram, since two samples that make up group a stand out from the other samples in the set due to their higher iron content. The other groupings reflect the same behavior identified in the analysis of principal components. Groups c, e, h and g contain lots 1, 2, 3 and 4, respectively. Thus, one sees that the final composition of the product is quite heavily affected by processing conditions, as a function of two different sets of variables (centesimal composition and mineral content), which point to similar conclusions regarding the analyzed samples.

Although many authors suggested that differences in raw materials are key factors in MDCM composition (Botka-Petrak *et al.*, 2011; Ng and Huda, 2011; Komrska *et al*., 2011; Nitipong *et al*., 2014; Song *et al*., 2014) the present study showed that processing conditions rather than different chicken parts were the most important determinants of composition differences in MDCM.

Conclusions

Greater variation among raw materials of mechanically deboned chicken meat was found primarily from different processing lots, rather than from different chicken parts. Therefore, the product's final composition was affected by processing conditions as revealed by both the proximate and mineral composition differences. This behavior became even more evident in the samples from supplier 1. In the present study, the major variation in the analyzed parameters was especially concerning calcium, minimal and maximal calcium values in MDCM samples (on a dry basis) varied from 2 to 15-fold higher than the permitted values of Brazilian legislation, which could provide an excessive incorporation of that raw material in preparation of sausages and other meat products. Although many authors discuss the influence of source or type of raw material in the final composition of the product, one can see that processing conditions were the greatest source of influence.

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