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Characterization of commercial cooked hams according to physicochemical, sensory, and textural parameters using chemometrics

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Abstract

Cooked ham is considered a high-value product due to the quality of its raw material. Although its consumption is still low in Brazil, it is increasing due to the rising purchasing power of sectors of the population. This study aimed to assess the microbiological, physicochemical, rheological, and sensory quality of cooked hams (n=11) marketed in Brazil. All samples showed microbiological results within the standards established by Brazilian legislation. Eight of the eleven samples studied met all the legal requirements; two samples violated the standards due to the addition of starch; one sample had lower protein content than the minimum required, and another one had sodium content higher than that stated on the label. The use of Hierarchical Cluster Analysis allowed the agglomeration of the samples into three groups with distinct quality traits and with significant differences in moisture content, chromaticity, syneresis, and heating and freezing loss. Principal Component Analysis showed that the samples which correlated to higher sensory acceptance regarding flavor and overall acceptability were those with higher moisture, protein, fat, and luminosity values. This study confirmed the efficacy of multivariate statistical techniques in assessing the quality of commercial cooked hams and in indicating the physicochemical parameters associated with the perception of product quality.

Keywords: microbiology, quality, HCA, PCA.

1 Introduction

According to Brazilian legislation (Brasil, 2000), cooked ham is an industrialized meat product obtained exclusively from deboned, spiced, cooked pork leg. In Brazil, only eight companies produce twelve trademarked cooked hams (Associação Brasileira de Proteína Animal, 2013). Data specifically related to the consumption of cooked ham in Brazil is not available, but the whole class known as ham, which includes pork and chicken cooked ham and similar products, represents approximately 1.31 kg/year of total domestic consumption (Associação Brasileira de Proteína Animal, 2012), which is lower than that of other countries. In Italy, which is one of the largest consumers of cooked ham in Europe, along with France and Spain, the per capita consumption reaches 4.9 kg/year (Casiraghi et al., 2007).

There is a very complex relationship between the constituents of meat products, such as moisture, protein, and fat, which provide the desired sensory attributes, especially in terms of texture (tenderness, cohesiveness, chewiness) and color. Accordingly, many studies have been conducted to establish the relationship between the physicochemical characteristics of meat products and the quality attributes desired by consumers (Cheng et al., 2005). These studies usually adopt correlation analysis combined with one-way analysis of variances, which are uni/bivariate by nature. However, multivariate statistical techniques, such as Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) have also been used to assess the quality of food products, including meats

(Zielinski et al., 2014a; Marušić et al., 2014; Laureati et al., 2014). Ferreira et al. (2000) used PCA and HCA to demonstrate the correlation between the different types of processed turkey products and their fatty acid and mineral composition. The classification of cooked hams according to the origin of the raw material and the percentage of injection was investigated using PCA combined with linear discriminant analysis (Casiraghi et al., 2007; Moretti et al., 2009).

Given that the consumption of cooked ham by Brazilians has increased, monitoring the quality parameters of commercial brands marketed in the country is essential. Thus, the objectives of this study were: i) to evaluate the microbiological, physicochemical, and rheological quality of cooked ham sold on a retail basis in the Brazilian market and ii) to group the samples based on these quality traits using unsupervised statistical methods.

2 Materials and methods

2.1 Physicochemical analysis

To assess the quality of the cooked hams, eleven samples were collected between November 2012 and June 2013, in the region of Ponta Grossa, PR. Brazil. These samples represented more than 90% of the trademarked hams sold in Brazil (Associação Brasileira de Proteína Animal, 2013). The whole ham samples (3.4 kg), were collected and taken immediately to the laboratory, where they were stored at 7 °C until analysis.

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For the physicochemical characterization, the samples were analyzed in triplicate according to IAL (Instituto Adolfo Lutz, 2008) and AOAC (Association of Official Analytical Chemists, 1990) for the following parameters: pH by the potentiometric method; moisture content, gravimetrically at 105 °C; ash content by incineration at 550 °C for 6 hours in a muffle furnace (model 2310 - Jung, Blumenau, SC, Brazil); protein content by the Kjeldahl method; lipids by the Soxhlet method; and sodium content by nitric-perchloric digestion, followed by reading on a flame photometer (model 7000 - TECNOW, São Paulo, Brazil) (Association of Official Analytical Chemists, 1990). Water activity was determined at 25 °C using an Aqualab meter 3TE (Decagon Device Inc., Pullman, WA, USA). The qualitative presence of starch was determined using the Lugol test (Brasil, 2006). All chemicals used were of analytical grade (Merck, Darmstadt, Germany).

2.2 Instrumental color

Instrumental color analysis was performed on a Mini EZ Scan spectrophotometer, (model 4500L HunterLab, Reston, VA, USA) using D65 illuminant and 10° observation angle. The samples were cut into 2.5 cm thick slices, and seven measurements were performed on each slice. The parameters were measured according to the CIE $L^*a^*b^*$ system: L^* (lightness); a^* (redness); b^* (yellowness); chromaticity (C), and hue (h) values were calculated.

2.3 Rheometry

Instrumental texture analysis was performed using a TA.XT plus Universal texturometer (Stable Micro Systems, Godalming, UK) at 20 °C using five replicates. Texture profile analysis (TPA) was conducted according to Tabilo et al. (1999); the samples were cut into 2 cm cubes and subjected to a dual compression of 20% using a P75 cylindrical probe at the constant speed of 1.0 mm.s⁻¹, with a five second interval between the first and second compression. Hardness (N), adhesiveness (N.mm), cohesiveness, elasticity (mm), and chewiness (N.mm) were evaluated.

Samples of sizes $1.5 \times 1.5 \times 4.0$ cm were subjected to shear bond strength testing and were cut using a Warner-Bratzler Blade at 2.0 mm.s⁻¹; Samples of sizes $1.5 \times 1.5 \times 2.0$ cm were compressed at 66.67% of their height using a P36/R probe at a speed of 1.5 mm.s⁻¹ (Pedroso & Demiate, 2008).

2.4 Analysis of technological properties

The water holding capacity (WHC) of the samples was evaluated according to the method proposed by Prestes et al. (2012).

The evaluation of freezing losses was performed according to the method of Lee et al. (2002) and adapted by Prestes et al. (2013). Cylindrical samples of 3 cm diameter \times 1cm height were weighed, wrapped in aluminum foil, and frozen at -18 °C for 72 hours. The samples were then thawed at 25 °C for 4 hours, wrapped in a 12.5 cm diameter paper filter, and compressed for 5 minutes with a 2 kg weight. After pressing, the samples were

removed from the paper filter and weighed again. The mass freezing loss was calculated as the difference in weight (%).

The method proposed by Hachmeister & Herald (1998) was used to evaluate the heat loss. Samples were cut in pieces $(2.0 \times 2.0 \times 6.0 \text{ cm})$, weighed, and submerged for 6 min in 400 mL of boiling water in a 500 mL beaker covered with a watch glass. Subsequently, the samples were drained on paper towels and cooled to 7 °C for 6 minutes. The percentage of heating loss was calculated as the difference in weight (%).

Syneresis was evaluated by the method used by Prestes et al. (2012).

2.5 Microbiological analysis

The samples were subjected to the following microbiological analyses according to Brazilian legislation (Brasil, 2002): thermotolerant coliforms at 45 °C; *Salmonella* sp; *Staphylococcus* coagulase positive and sulphite-reducing *Clostridium*.

2.6 Sensory evaluation

Acceptance testing was applied in accordance with Instituto Adolfo Lutz (2008) with 60 untrained assessors who evaluated the attributes of color, taste, visual appearance and overall acceptability using a nine-point hedonic scale, which was anchored at the ends with extremely disliked (1) and extremely liked (9). The present study was approved by the Ethics Committee in Research Involving Humans at the State University of Ponta Grossa (UEPG) (protocol n° 256.684), and this analysis was conducted in individual cabins at the Sensory Analysis Laboratory of the Food Engineering Department, at UEPG. The samples were cut in 2 cm cubes at temperature below 10 °C, coded with random three-digit numbers, and served monadically in an incomplete block design during three different sessions. The assessors were given water and/or toast between assessments to cleanse the palate.

2.7 Statistical analysis

The results were expressed as mean \pm standard deviation. The correlation between the physicochemical, rheological, microbiological, and sensory variables was analyzed using the Spearman's method (Granato et al., 2014b).

Hierarchical Cluster Analysis was conducted on autoscaled data based on the physicochemical, rheological, and sensory analyses, instrumental color, and technological properties, totaling 34 variables.

The parameters used to establish the clusters were Euclidean distances and the Ward hierarchical agglomerative method (Zielinski et al., 2014b). Dendrograms were built for the samples and for the variables. The homogeneity of variance between the groups obtained by HCA was verified by Levene's test, and the statistical differences were determined by one-way ANOVA or the Welch test, followed by the Fisher's test or the Kruskal-Wallis ANOVA test for parametric and non-parametric data, respectively.

Principal Component Analysis was conducted in two stages. The first PCA was done according to the following physicochemical and instrumental results: retail price, moisture, protein, fat, sodium, L^* , a^* , b^* , C, h, syneresis, freezing and heating losses, WHC, hardness, and chewiness. The other PCA was done to include sensory data related to color, taste, and visual and overall acceptance. For both PCAs, the results of each parameter were adopted as columns and the samples as rows. Two principal components (PC1 and PC2) were used for data projection, and eigenvalues were obtained to determine the amount of variance explained by each principal component. Autoscaling was used as a pre-treatment of the results to equalize the statistical importance of all variables (Granato et al., 2014a). Statistical analysis was conducted using the Statistica 7.0 software (Statsoft Inc., Tulsa, USA) and Excel 2010 (Microsoft, Redmond, WA, USA).

3 Results and discussion

All of the samples met the microbiological criteria established by Brazilian legislation (Brasil, 2002).

The results of the chemical composition of the samples are presented in Table 1. The moisture content ranged from 74.66 to 78.71%. The average protein content was 14.21%, but it was observed that one sample showed results below the legal requirements (11.57% \pm 0.51). Two samples showed results above the level allowed for the water/protein ratio (5.73 \pm 0.36 and 6.48 \pm 0.28), indicating a higher addition of water to the product. Two samples had a positive result for the presence of starch, presumably indicating economic fraud. Overall, eight of the eleven samples tested met all the physicochemical standards established by legislation.

The results of fat, which were between 0.89 and 3.57%, confirmed that cooked ham can be considered a low fat meat product when compared to *apresuntado* and sausages. *Apresuntado* is a Brazilian meat product similar to cooked ham, which is made from whole or cut shoulder muscles with or without minced pork meat. Products made with minced cuts are known worldwide as "reconstituted cured-cooked meat

products", name adopted in this paper. Baggio & Bragagnolo (2008) compared three types of products: cooked ham, reconstituted cured-cooked meat products and sausage, which are sold in Brazil, and concluded that the cooked ham had the lowest fat content, with results ranging from 2.1 to 3.7%, while the reconstituted cured-cooked meat products showed results between 3.9 and 8.0%, and Tuscan-style sausage had lipid content ranging from 13.4 to 29.0%.

The sodium content of the samples ranged from 0.79 to 1.35%, and these values were compared to those shown on the product labels using the t test for single samples. Two samples showed significantly different results from the values reported on the label: sample number 2 had higher values than those reported (p = 0.023) and sample number 9 showed lower sodium content than the values reported (p = 0.008). Cooked hams produced and sold in Brazil have lower sodium content than cooked hams produced in other countries Casiraghi et al. (2007) found average levels of sodium of 2.2% in cooked hams produced in Denmark and France, while in the Czech Republic, Válková et al. (2007) found values between 2.16 to 3.48%.

The physicochemical, color, mass loss analysis, technological quality analysis, and sensory evaluation were treated statistically by HCA. The dendrogram shown in Figure 1 proves the correlation between the variables, and Figure 2 shows the dendrogram of the samples.

The L^* value and lipid content parameters showed positive correlation by Spearman analysis ($\rho=0.90$; p< 0.001), also showed strong correlation, as shown in Figure 2. The lipid content may have been negatively correlated to the a^* parameter ($\rho=-0.93$, p<0.001). These results were similar to those found by Tomović et al. (2013) when evaluating cooked hams with 20% injection. These authors found a positive correlation between L^* and lipids (r=0.49, p<0.05) and a negative correlation between a^* and lipids (r=-0.58, p<0.05) by Pearson's analysis, confirming that increasing the lipid content increases the lightness of the product, but the characteristic pink color, which is derived from myoglobin, decreases. Lightness (L^*) is considered one of the major quality parameters of meat products, and, along with

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Table 1.	PHYSICOCHEHIICA	i resums umea	ロエるけんしに	ommerciai sa	annoies of cooked har	11.

Sample	Moisture (%)	Ash (%)	Protein (%)	Water/Protein Ratio	Fat (%)	Sodium (g.kg ⁻¹)	Starch
1	74.66±0.11	3.90±0.01	11.53±0.51	6.48±0.28	0.89±0.01	1.09±0.11	positive
2	76.01±0.70	3.97±0.01	14.05±1.72	5.44±0.65	3.57±0.16	1.35 ± 0.14	negative
3	78.18 ± 0.14	3.57±0.01	14.61±0.21	5.35±0.08	2.22±0.39	0.96 ± 0.11	negative
4	77.74±0.16	3.39 ± 0.03	15.13±0.04	5.14 ± 0.02	1.80 ± 0.08	0.85 ± 0.13	negative
5	78.17±0.75	3.52±0.19	14.52 ± 0.32	5.38±0.17	1.43±0.29	1.04 ± 0.15	negative
6	77.12±0.36	3.88 ± 0.01	14.42±0.15	5.35±0.05	2.06±0.15	1.19 ± 0.07	negative
7	77.19±0.29	3.73 ± 0.04	14.45±0.29	5.35±0.12	2.32 ± 0.04	1.05±0.09	negative
8	78.71±0.15	3.09 ± 0.03	13.77±0.86	5.73±0.36	2.94 ± 0.04	0.79 ± 0.19	negative
9	78.70 ± 0.09	3.57 ± 0.03	14.97±1.80	5.31±0.62	1.56±0.18	1.03 ± 0.04	negative
10	75.30±0.36	4.10 ± 0.12	14.44 ± 0.40	5.22±0.14	1.29 ± 0.10	1.25±0.19	positive
11	77.56±0.31	3.70 ± 0.02	14.41±1.03	5.40 ± 0.37	2.41±0.11	1.12±0.18	negative
Standard ¹	VNE	VNE	min 14%	max. 5.35	VNE	VNE	negative
p- value ²	< 0.001	< 0.001	< 0.001	0.003	< 0.001	0.002	

¹Brasil (2000); VNE - value not established by legislation; ²one-way ANOVA or Welch-ANOVA test.

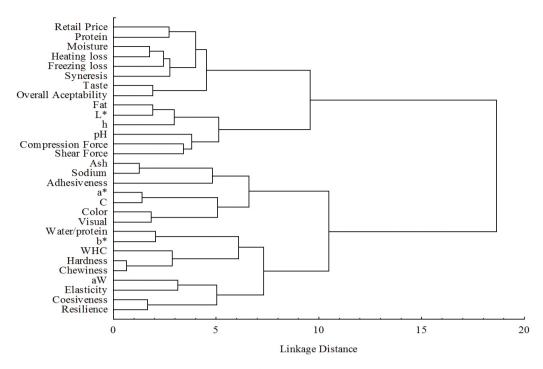


Figure 1. Hierarchical Cluster Analysis applied to the studied variables.

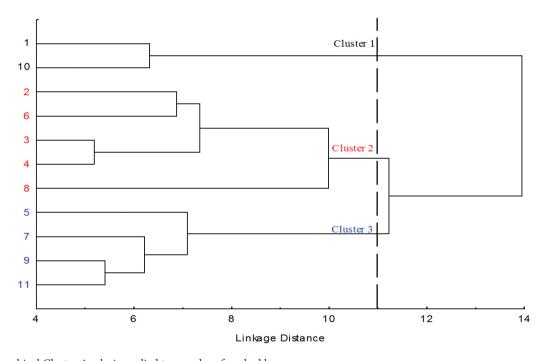


Figure 2. Hierarchical Cluster Analysis applied to samples of cooked ham.

the a^* value, it contributes the most to the pink color intensity, (Brewer et al., 2001). The a^* value is the most sensitive value generated by instrumental color, and it assesses the stability of meat products (García-Esteban et al., 2003).

Hardness was positively correlated with CRA ($\rho=0.71$; p=0.02). The myofibrillar proteins that are extracted create a dense protein network and retain water. The use of ingredients that incorporate water, such as starch, increase the protein

network (Tornberg, 2005). Prestes et al. (2013) reported an increase in WHC and in the compression force of samples of ham produced with ingredients that act as gelling agents. In the present study, WHC was negatively correlated to freezing loss ($\rho=$ -0.71; p=0.02) and heating loss ($\rho=$ -0.63; p=0.04). It was observed that the greater the product ability to retain water in the protein matrix, the lower the water loss during processing stages.

As shown in Figure 2, the samples were categorized into three groups according to the similarity of their physicochemical characteristics. Table 2 shows the results of ANOVA for the groups obtained by HCA and demonstrates a significant difference (p <0.05) in moisture, chromaticity, syneresis, freezing and heating losses, and sensory acceptance.

Group 1 was named "lower price and quality" and showed significantly lower results for moisture (p = 0.011), syneresis loss (p = 0.010), freezing loss (p = 0.030) and heating loss (p = 0.006). The samples in this group showed an unexpected positive result for the presence of starch. Since the addition of starch in ham is forbidden by Brazilian law, this result constitute a clear violation, which was probably intended to retain more water in the product to increase its industrial output to improve the performance of the product during processing and manufacturing and to improve stability during shelf life.

Pedroso & Demiate (2008) demonstrated that the addition of starch in turkey ham showed a significant negative effect on heating loss. These authors investigated the single and combined use of cassava starch and carrageenan in turkey ham

and concluded that there were improvements in the processing characteristics of the product, such as reduced losses and increased water holding capacity. Prestes et al. (2013) reported that the addition of 2% of modified starch in turkey ham reduced heating and freezing losses and that adding a mixture of 2% modified starch, 2% hydrolyzed collagen, and 0.3% guar gum resulted in the lowest syneresis.

Group 2, designated "intermediate price and quality", had the highest lipid content (p = 0.06) and the lowest chromaticity (C) value (p = 0.009) and the highest negative correlation (ρ = -0.90, p <0.001) between these two variables, according to Spearman analysis. Groups 2 and 3 ("high price and quality") showed higher values of syneresis, freezing and heating losses, moisture content, and their acceptance scores in the sensory evaluation were significantly higher than those of group 1 (p = 0.021), which may demonstrate a greater acceptance for products with higher moisture content.

The PCA in Figure 3 shows a graphical representation of the samples according to the physicochemical and rheological analyses. Together, the first principal components (PC1, 42,19%

Table 2. Average values and standard deviations of Clusters 1, 2, and 3 of the parameters of the cooked hams studied.

	Cluster 1 – Low value, low quality	Cluster 2 – Intermediate value, high	Cluster 3 – (High value, high quality	PSD*	<i>p</i> -Levene	<i>p</i> -Anova/ Welch test
	(n=2)	quality (n=5)	(n=4)			
Price (R\$/kg)	11.45	15.63	18.02	3.459	0.726	0.072
Moisture (%)	74.98^{b}	77.55^{a}	77.91^{a}	1.354	0.419	0.011*
Ash (%)	4.00	3.58	3.63	0.288	0.122	0.213
Protein (%)	12.99	14.39	14.59	0.964	0.601	0.426
Water/Protein	5.85	5.40	5.36	0.368	0.772	0.969
Fat (%)	1.09	2.52	1.93	0.771	0.153	0.060
aW	0.98	0.98	0.98	0.005	0.159	0.582
pН	6.48	6.49	6.46	0.091	0.342	0.924
Sodium (g.kg ⁻¹)	1.17	1.03	1.06	0.164	0.567	0.484
L^*	63.48	66.77	64.72	2.128	0.611	0.119
a^*	13.74	11.95	13.12	1.133	0.258	0.097
b^*	9.75	8.60	8.81	0.757	0.378	0.196
C	16.86 ^a	14.76 ^b	15.83 ^a	1.000	0.587	0.009*
h	35.32	35.93	34.02	3.454	0.786	0.751
WHC (%)	99.51	97.70	98.56	1.262	0.062	0.063
Syneresis (%)	1.17^{b}	5.01 ^a	6.56 ^a	2.391	0.247	0.010*
FL (%)	2.50^{b}	11.21a	10.52^{a}	4.476	0.200	0.030*
HL (%)	5.44 ^b	17.22a	15.21 ^a	5.296	0.538	0.006*
Hardness (N)	14.77	11.05	13.36	2.362	0.150	0.110
Adhesiveness ¹	-0.10	-0.16	-0.63	0.366	0.063	0.098
Elasticity ²	0.93	0.90	0.95	0.036	0.054	0.055
Cohesiveness ¹	0.87	0.87	0.88	0.009	0.070	0.202
Chewiness ³	11.83	8.70	11.19	2.025	0.254	0.063
Resilience ¹	0.55	0.55	0.58	0.022	0.171	0.141
CF (N)	33.76	50.01	38.93	10.632	0.363	0.108
SF (N)	10.23	17.68	11.03	5.754	0.302	0.056
Color ⁴	7.40	7.01	7.51	0.365	0.270	0.097
Taste ⁴	6.79	7.30	7.24	0.300	0.129	0.102
Visual ⁴	7.16	7.07	7.48	0.291	0.304	0.088
Acceptability ⁴	6.81 ^b	7.19^{a}	7.42^{a}	0.283	0.320	0.021*

^{*}PSD: pooled standard deviation; pLevene: analysis of homogeneity of variance; pANOVA: analysis of variance; abDifferent letters in the same row mean significant differences; WHC – water holding capacity; FL: freezing loss; HL: heating loss; CF: compression force; SF: shear force; ¹: no unit; ²: mm; ³: N.mm; ⁴: 1 to 9 score.

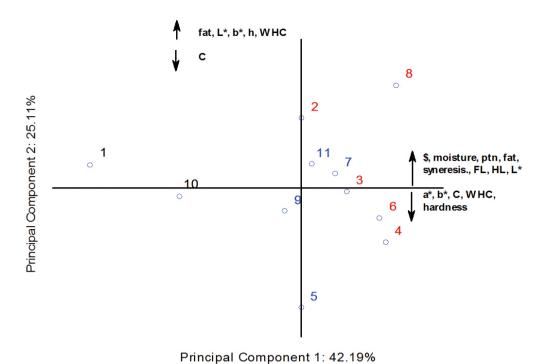


Figure 3. PCA applied to the physicochemical and rheological results of the commercial cooked ham samples evaluated.

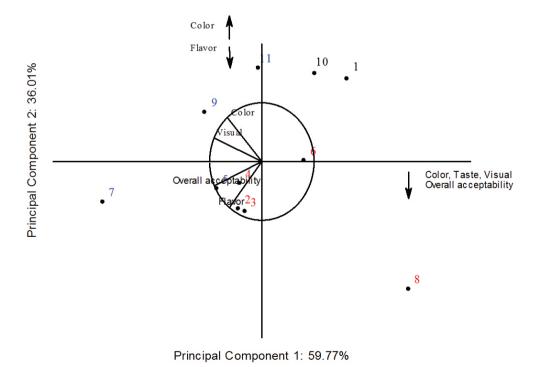


Figure 4. PCA applied to sensory results of the commercial cooked ham samples evaluated and projection of the attributes.

of explained variance) and the second principal component (PC2, 25,11% of explained variance) explained 67.3% of data variance.

The right area of PC1 included the samples with higher retail price, moisture, protein, and fat content, higher syneresis, freezing and heating losses, and higher lightness. The left area

included the samples with high chromaticity, highest WHC, and greatest hardness. As for the PC2, the upper area included the samples with higher fat, lightness, hue, and higher WHC; the samples with higher chromaticity were located in the lower area.

The PCA shown in Figure 4 refers to the results of the sensory analysis, with 95.8% of explained variance, and it also

shows the graphical position of the samples and the projection of the analyzed attributes.

The samples with higher flavor and overall acceptability scores were grouped in the 3rd quadrant and corresponded to some of the samples belonging to groups 2 and 3 of HCA. The samples that were most accepted by consumers regarding these attributes showed high values of moisture, protein, fat, and lightness (samples 2, 3, 4, 5, and 7). On the opposite side, in the 1st quadrant, the samples were placed in group 1 of the HCA, which had lower scores in the sensory evaluation.

4 Conclusion

The evaluation of the commercial cooked hams showed that eight of the eleven samples met all requirements established by law. The presence of starch was detected in 2 samples, which constitutes a violation of law. All microbiological analysis results were satisfactory according to the law criteria.

The use of cluster analysis allowed the unsupervised classification of the samples into three groups, which showed significant differences in terms of moisture content, chromaticity, syneresis, and heating and freezing losses. The agglomeration of these groups could be confirmed by the use of PCA, which indicated that the samples that were most accepted by the consumers in terms of flavor and overall acceptability were those that showed the highest values of lightness, moisture, protein, and fat content.

Despite the low number of samples that were analyzed, this study evaluated more than 90% of the brands of cooked hams that are marketed in Brazil. It was possible to demonstrate that multivariate techniques were effective in assessing the quality of the hams and also in indicating the physicochemical parameters associated with the perception of product quality. The results obtained using these tools can be very useful for the processing industries to monitor the quality of their products and develop new products to meet consumer needs.

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