FLÁVIO FERREIRA SILVA
(ORGANIZADOR)

PRÁTICA E PESQUISA EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS 2

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APRESENTAÇÃO

A obra intitulada “Prática e Pesquisa em Ciência e Tecnologia de Alimentos 2” foi elaborada a partir das publicações da Atena Editora e apresenta uma visão ampla sobre as novidades da área. Esta obra é composta por 15 capítulos bem estruturados e agrupados por assuntos.

Muitos são os problemas a serem solucionados relacionados ao consumo alimentar humano, por isso a prática e a pesquisa de alimentos devem estar bem alinhadas. O desenvolvimento de novos produtos é essencial para melhorar a qualidade de consumo e disponibilizar uma oferta alimentar de qualidade superior para todos os públicos, uma vez que, novas estilos alimentares como o veganismo e outros, vem sendo adotados em uma escala crescente. Não obstante, a otimização dos processos de fabricação e de controle de qualidade alimentar são indispensáveis quando o assunto é a saúde.

Neste sentido, os estudos que são apresentados aqui, alinham-se a estes temas e trazem novas analises que condizem com as necessidades emergentes da prática e pesquisa em ciência e tecnologia de alimentos.

A Atena editora, reconhecendo importância dos trabalhos científicos, oferece uma plataforma consolidada e confiável para a divulgação, propiciando aos autores um meio para exporem e divulgarem seus resultados, enriquecendo o conhecimento acadêmico e popular.

Por fim, esperamos que a leitura deste trabalho seja agradável e que as novas pesquisas possam propiciar a base intelectual ideal para que se desenvolva novas soluções, cuidados e desenvolvimento cientifico acerca destes temas.

Flávio Brah (Flávio Ferreira Silva)

SUMÁRIO

CAPÍTULO 13

USE OF ENERGY DISPERSIVE SPECTROSCOPY AND PRINCIPAL COMPONENT ANALYSIS FOR DETECT PENICILLIN IN POWDERED MILK

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ABSTRACT: The milk and its derivatives are the most consumed food. This study focuses on detection of penicillin residues in powdered milk through Energy Dispersive Spectroscopy. For this simulated samples were considered. The simulated ones take into account veterinary drugs added in milk samples in the following concentration penicillin 4 µg/L (4 ppb). The statistical tool used to discriminate the samples was the principal component analysis (PCA). The PCA discriminated penicillin-contaminated milk powder. Through the vectors in the PCA we also discriminated the samples according to their elemental composition. The methodology proved to be fast and accurate within the maximum residue limits allowed by regulatory agencies.

KEYWORDS: Energy Dispersive Spectroscopy. Milk. Principal Component Analysis. Penicillin.

1 | INTRODUCTION

In the last several years, studies aimed at quality control of milks and their derivatives has been of great interest in the field of food industry and in the scientific researches. The importance

of these studies is due to the fact that the consumer will have a rich food nutritionally and free of adulteration and contaminants. According to the Food and Agriculture Organization of the United Nations, milk is one of the most consumed foods in the world. It not only has its importance in the nutritional level, but also plays an important role in the economy. Global consumption of milk and its derivatives exceeds 6 billion of consumers (LUIZ, 2018)(FAO, 2017). The composition of milk is an important parameter for its quality. Its main components are: proteins, carbohydrates, lipids, minerals and vitamins. Bovine milk contains about 87.1% water, 4.0% fat, 3.3% protein, 4.6% lactose and 0.7% ash. The typical composition of proteins present in bovine milk is casein in 78.3%, whey proteins in 19% and others in 2.7% (LUIZ, 2018)(WALSTRA, 2006)(TAVANTI, 2009). Lactose is the milk carbohydrate, it is a disaccharide formed by monosaccharides: glucose and galactose, which provide 16.8 kJ/g for people (LUIZ, 2019). The minerals found in significant amounts in milk are: calcium (Ca) and phosphorus (P) that are associated with the micelles of casein, chlorine (Cl), potassium (K), sodium (Na), magnesium (Mg). In small quantities: iron (Fe), aluminum (Al), bromine (Br), zinc (Zn) and manganese (Mn) are present. Adulterations in milk have been highly reported in developing countries, such as Pakistan, Brazil, India, and China (LUIZ, 2018)(HANDFORD, 2016). The main method of milk adulteration is to increase milk volume with the addition of water. However, recent studies have been conducted that show very positive results with the development of new equipment and techniques for the detection of water in milk (BRANDÃO, 2017) (NASCIMENTO, 2019) (NASCIMENTO, 2017). Still there are other problems, such as the contamination of milk by residues of veterinary drugs that may be present when the cow is milked in the grace period. In the last years several scientific researchers have been carried out in order to contribute to the analysis of residues of drugs in milk. In 2013, Granella et al. used Liquid Chromatography and Mass Spectrometry (LC/MS) to verify the contamination of organic and conventional milk by chemical residues (GRANELLA, 2013). In 2014, Van Boeckel et al. showed that, between 2000 and 2010, the consumption of antibiotics by the world population has increased by 36% and is related to the appearance of drug-resistant bacteria. Fraction of this increase is due to ingestion of animals or their food derivatives contaminated with antibiotics (VAN BOECKEL, 2014). In 2014, Zhang Y. D et al. examined UHT and pasteurized milks to verify the presence of residues of tetracyclines, sulphonamides, sulfamethazine and quinolones in them, through the ELISA method (ZHANG, 2014). Also in 2014, Luiz et al. detected diclofenac sodium residues in milk samples using Fourier Transform Near Infrared Spectroscopy (FT-NIR) associated with Principal Component Analysis (PCA) (LUIZ, 2014). In 2015, Moharana et al. analyzed by Reversed-Phase High-Performance Liquid Çhromatography (RP-HPLC) the veterinary drug enrofloxacin in cow's milk samples obtained from two cities in India (MOHARANA, 2015). In 2018, Luiz et al. analyzed the veterinary drugs tetracycline, enrofloxacin, penicillin, ceftiofur hydrochloride and diclofenac sodium by means of PCA-associated Fourier transform near-infrared spectroscopy (FT-NIR) to

fast and accurately detect residues of these drugs in milk samples (LUIZ, 2018). In scanning electron microscopy (SEM), a highly energetic and focused electron beam is formed and scans the specimen in a raster scan pattern. Then, different effects can result from the interaction between the electron beam and the electrons in the sample. For example, characteristic x-rays result from electronic transitions in the atoms of a sample by an incident electron beam. The combination of SEM imaging and elemental analysis given by Energy X-ray dispersion spectroscopy (EDS) made it into one of the most powerful tools in scientific research to characterization of samples (GIRÃO, 2017). Spectroscopic techniques combined with chemometric methods allow to analyze, interpret and extract information quickly and accurately with minimal sample preparation (BRANDÃO, 2010). This work deals Energy Dispersive Spectroscopy (EDS) to analyze the elemental composition of a milk powder sample and associated with PCA to detect in this sample traces of veterinary antimicrobial penicillin.

2 I MATERIALS AND METHODS

The analyses FT-NIR were performed in the Process and Products Laboratory (LPP) and in the Materials Spectroscopy Laboratory (LEM), located in the Physics Department of Federal University of Juiz de Fora, Brazil. While the SEM/EDS analyzes were performed at the Laboratory of Instrumentation and Simulation of Applied Scientific Computations (LISComp), located at the Federal Institute of Education, Science and Technology of Rio de Janeiro (IFRJ), Campus Paracambi, Brazil.

2.1 Milk and Drug Samples

To whole powdered milk samples, fortified milk with calcium, iron, zinc and vitamins, marketed internationally was used. To drug samples, the veterinary drug penicillin-reinforced pentabiotic from Zoetis/Pfizer was used. The penicillin used was in powdered form. This medication is widely used in veterinary medicine for various pathologies in dairy cattle: mastitis, diarrhea, pneumonia, meningitis, leptospirosis, arthritis, etc. It was necessary to compact the samples of milk powder and powdered milk + penicillin to make a tablet, in order to obtain a plane surface, so that the emitted X-rays by the samples are not diffuse, reducing their incidence in the detector. For this a compactor and a SPECAC hydraulic press were used. 10 ton of pressure was used for 180 seconds. After the time interval the compactor sample was carefully removed in the form of pellets to be irradiated later.

2.2 Analysis using the SEM/EDS Method

Samples were irradiated with a scanning electron microscope, TM-3000 Hitachi, equipped with EDS System Quantax 70 Bruker. First the samples of pure milk powder

and pure penicillin were irradiated. A Shimadzu AY 220 balance was used to simulate powdered milk contaminated with penicillin within the Maximum Residue Limit (MRL). 0.1 mg of the drug, equivalent to 0.01 mg of potassium penicillin G, was separated to mix in 2.5 kg of powdered milk. Then samples of milk powder contaminated with antimicrobial penicillin inside the MRL were irradiated. Three measurements were performed for each sample, at different points of the sample, for 300 s, with energy of 15.0 kV, magnification of 1000 times.

2.3 Statistical Analysis

The energy spectra were made and analyzed with the software OriginPro®8. The PCA, were conducted with the statistical software The Unscramble® version 9.2. The eigenvalues were calculated with the software BioStat version 5.3.

3 I 3. RESULTS

3.1 Energy Spectrum provide by SEM/EDS

Figure 1, 2 and 3 show the energy spectra obtained by SEM/EDS.

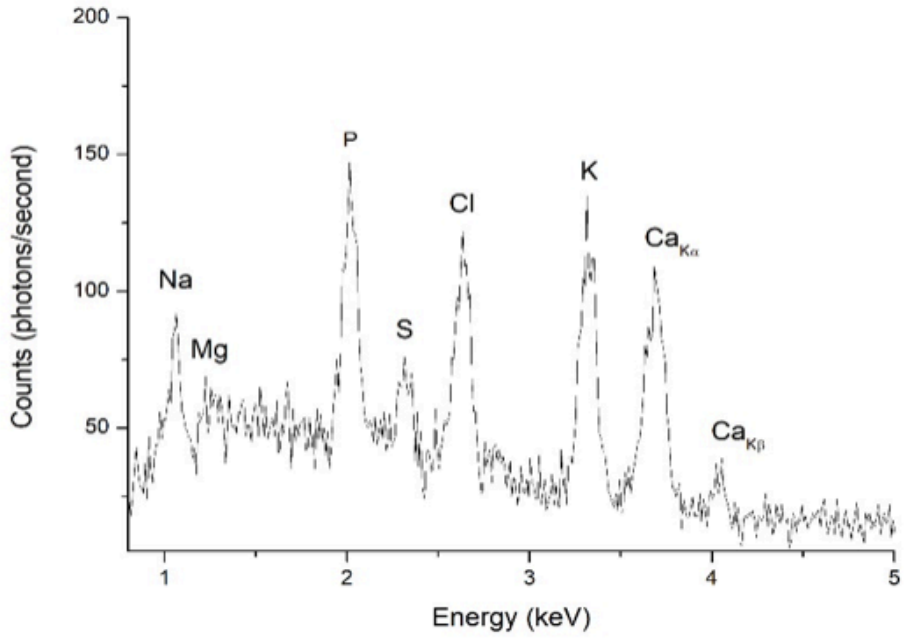

Figure 1 – Spectrum with the elemental composition of the pure milk powder sample.

In Figure 1 we can see some elements that constitute the minerals present in the milk, already mentioned in the introduction of this work, are: sodium, magnesium, phosphorus, chlorine, potassium and calcium.

Figure 2 shows the elemental composition of the pentabiotic veterinary antimicrobial (penicillin G Potassium) sample (line) compared with milk pure sample (filled area).

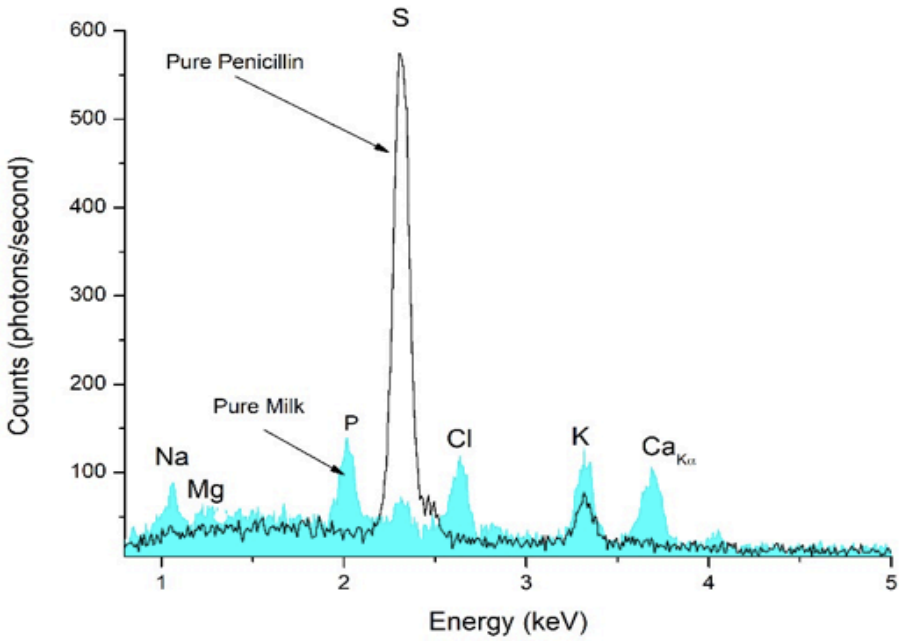

Figure 2 – Spectrum with the elemental composition of the samples of pure milk (filled area) and pure penicillin G potassium (line).

Note, in Figure 2, that the peak relative to sulfur energy (2,31 keV) stands out at a higher intensity (count) than in figure 1. Sulfur and potassium peak refers to the composition of penicillin G Potassium, since its molecular formula is $C_{16}H_{17}KN_2O_4S$.

In Figure 3, the contamination of the milk powder sample with the veterinary medicinal product penicillin G potassium was simulated in order to verify, through the spectrum, the presence of penicillin G.

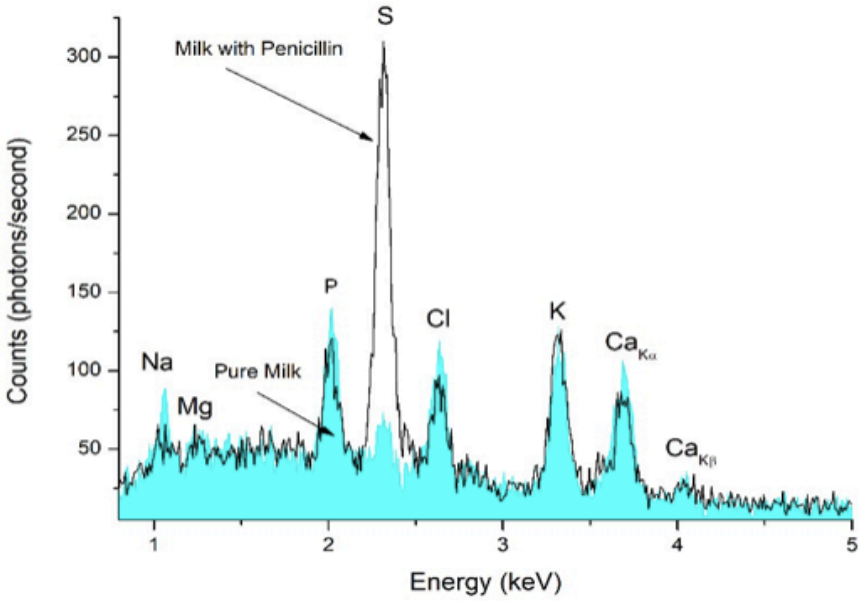

Figure 3 – Spectrum with the elemental composition of the samples of pure milk (filled area) and milk add penicillin G potassium (line).

From Figure 3 note that in the milk sample simulated with the drug (line), the peak for the sulfur element stands out in relation to the same element of the sample of pure milk powder (filled area). This is due to the high presence of the atoms of this element in the pure penicillin G potassium found in figure 2.

The other minerals: manganese, iron and zinc, which are also part of the milk constitution, are not present in the spectrum of Figure 1 because their concentration is very small compared to the other elements, camouflaging their peaks. Then, in an attempt to find the overlapping peaks, the second derivative (Derivative Order 2) in the curve was made so that these peaks became more distinctive. Figure 4 shows the energy spectra (top) and their second derivative (bottom) of pure milk samples.

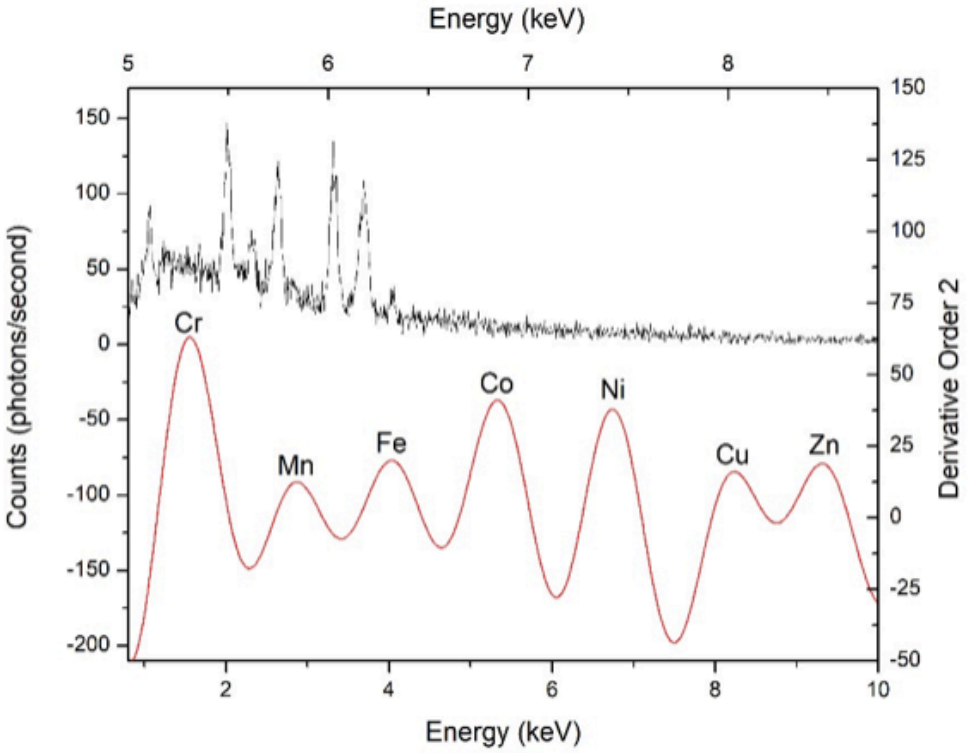

Figure 4 – Energy spectra of pure sample of powdered milk with their respective second derivative.

In Figure 4, the minerals Mn, Fe and Zn present in the milk composition were not found in the spectrum of Figure 1 and in the spectrum (top) of Figure 4. However, minerals appear in the second derivative (bottom). The remaining elements may be a consequence of the beam scattering.

3.2 Principal Components Analysis

The principal component of a set of data (dimensionality) is obtained by means of an analysis that consists in finding the eigenvalues of the covariance matrix. Each eigenvector has a corresponding eigenvalue. The eigenvectors with higher eigenvalues are the principal components and are ordered from the higher to the smaller ones

furnishing the components in significance degree (SAYAD, 2010)(SANTOS, 2012). Table 1 shows the explained and cumulative variances of the principal components of pure and contaminated milk samples within the MRL.

PC	Eingenvalues	Explained Variances (%)	Cumulative Variances
PC1	7.0128	77.9203	77.9203
PC2	0.9308	10.3418	88.2622
PC3	0.4458	4.9536	93.2157
PC4	0.2878	3.1980	96.4137
PC5	0.2005	2.2273	98.6410
PC6	0.0951	1.0568	99.6979
PC7	0.0238	0.2642	99.9620
PC8	0.0031	0.0347	99.9967
PC9	0.0003	0.0033	100.0000

Table 1 – Explained and cumulative variances of pure and contaminated milk samples within the MRL.

Figure 6 shows the score plot with the clustering of milk samples: control (Pure Milk), contaminated with 4 ppb of penicillin G potassium (Penicillin 4 ppb) and pure penicillin G potassium (Pure Penicillin).

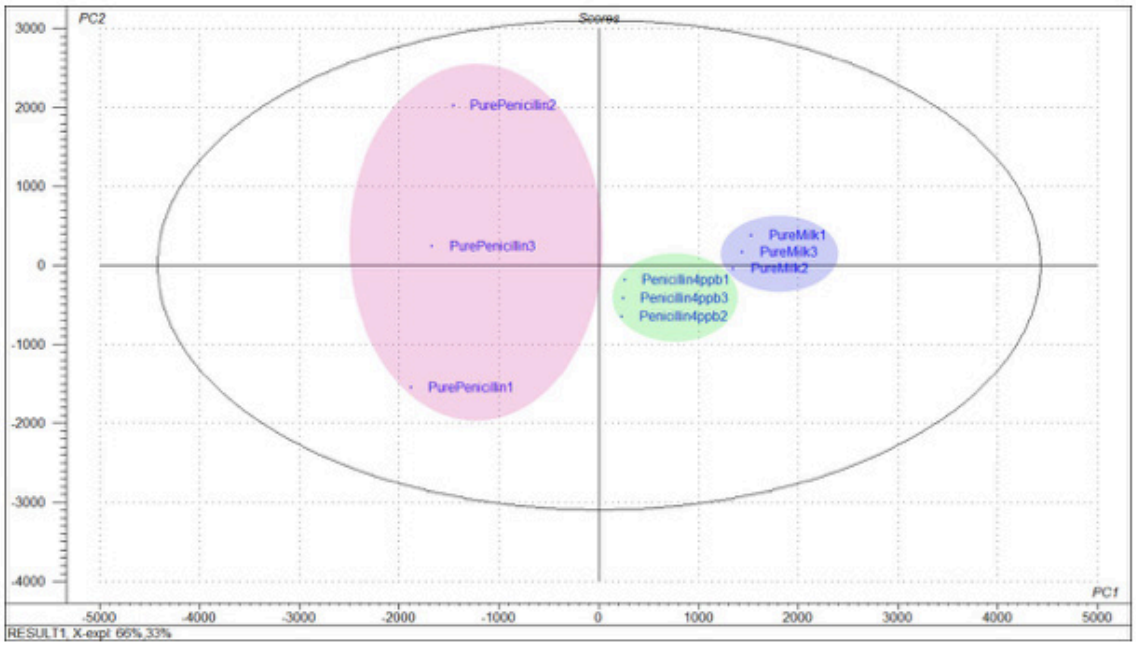

Figure 6 – Hotelling Score plot of statistical analysis (PCA) showing clustering data for control milk samples (Pure Milk), milk with 4 ppb of penicillin (Penicillin 4 ppb) and pure penicillin (Pure Penicillin).

From Figure 6, it is clear the formation of clusters resulting from the high degree of similarity between groups of samples. The PCA accurately discriminated the samples in groups despite the very low concentration of the antimicrobial. From PC1, one can observe that the pure milk and milk contaminated with penicillin have the near

score. Therefore, PC1 represents the degree of milk contamination with antibiotics. In addition, within the cluster itself, each sample has the near scores, showing similarities and belonging to the same group.

The Figure 7 shows the score plot with the average values of milk samples: control (Pure Milk), contaminated with 4 ppb of penicillin G potassium (Penicillin 4 ppb) and pure penicillin G potassium (Pure Penicillin). Note that PCA also discriminated the samples. Where PC1 represents the degree of contamination, because the sample Penicillin 4 ppb is close to the PureMilk sample along PC1.

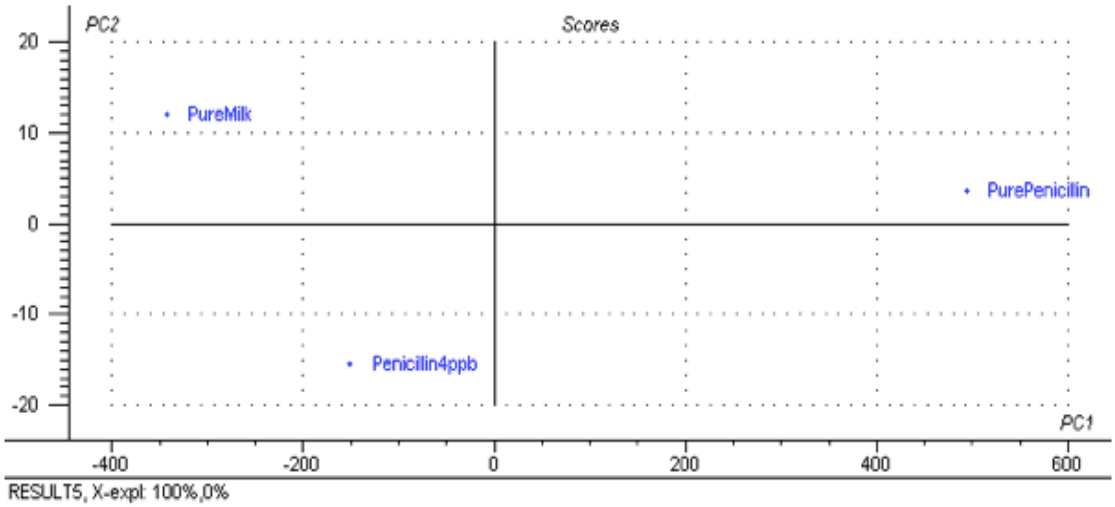

Figure 7 – Score plot of statistical analysis (PCA) with the average values for control milk samples (Pure Milk), milk with 4 ppb of penicillin (Penicillin 4 ppb) and pure penicillin (Pure Penicillin).

The other consideration to be made is through the angles between the vectors in the loading plot. If the angle is close to 0 degree, the correlation is very high and positive; if it is close to 180 degree, the correlation is also high, but negative; if the angle is about 90 degree, the variables are poorly related (BODENMÜLLER, 2010) (SMITH, 2002).

The Figure 8 shows the loading plot referring to chemical composition of samples.

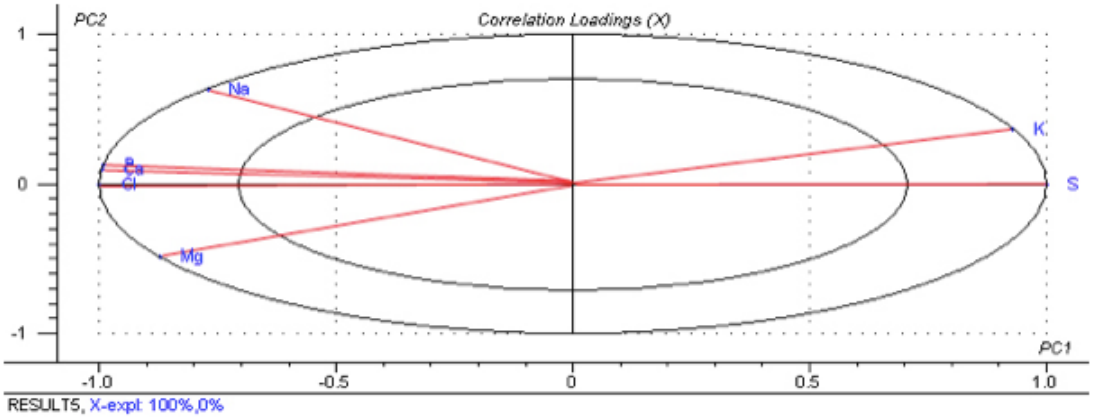

Figure 8 – PCA loading plot, referring to chemical composition of samples.

Analyzing the angles between the vectors of Figure 8, there is a very high and positive correlation between K and S elements. This verification is valid since it is known that these elements constitute the sample of penicillin G potassium. In the opposite quadrants, with a very close angle of 180 degrees, the elements S, Cl, Ca, P and K are present. This is due to the fact that elements K and S constitute the milk sample, but also are present in the drug, that is, a high and negative correlation. At angles of approximately 0 degrees are Ca, P and Cl, these elements are present at higher intensities in pure milk. Potassium is present in milk at high intensity but is also present in penicillin G potassium, so in this case there is a high but negative correlation, being on opposite sides (about 180 degrees).

According to the considerations taken, analyzing Figures 7 and 8, the pure penicillin G potassium sample is associated with the variables K and S, indicating a high degree of similarity with these elements. Thus, even if we did not know that the sample of pure penicillin G potassium was such a drug, the comparison induces us that it would be. The samples of pure milk and milk add penicillin G potassium in 4 ppb are related to the variables Cl, Ca, P and Na indicating that it is possible to milk because these elements are part of the milk.

4 I CONCLUSIONS

The present work presented a fast and accurate methodology for the detection of milk residues in milk using spectroscopic techniques associated to PCA. Through the spectrum provided by the SEM/EDS system it was possible to identify the chemical elements that constituted samples of milk powder, potassium penicillin G and milk contaminated with potassium penicillin G in 4 ppb. The technique detected residues of the drug penicillin potassium in powdered milk. The methodology with SEM / EDS spectroscopy allowed not only to detect the residues of the drug but also the elements that constitute them directly.

5 I ACKNOWLEDGEMENTS

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ÍNDICE REMISSIVO

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