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FT-NIR coupled to chemometric method to discriminate antimicrobial and antiparasitic residues in milk

FT-NIR associado a método quimiométrico para discriminar resíduos de antimicrobianos e antiparasitário no leite

Abstract

Introduction: Milk is one of the most consumed foods by humans. Proteins, vitamins, fat, carbohydrates and minerals are part of its composition and play important roles in human nutrition. The practice of adulteration in milk is old and is still present today in several countries, including Brazil. In order to obtain a higher profit, some suppliers usually add to the milk: water, starch, citrate, urea, caustic soda, sodium chloride, sucrose, whey, melamine and other components. However, there is still another problem, that of contamination of milk by veterinary medicines. These can cause damage to the health of the consumer and damage to the production of its derivatives. ***Objective:*** The present work proposes a methodology that allows to quickly detect the presence of residues of veterinary medicines in milk, within the maximum residue limit of each drug. ***Methods:*** The use of spectroscopy in the near infrared by Fourier transform associated with the analysis of principal components was used. Infrared spectroscopy has been used not only for the authenticity of dairy products, but to determine their quality. ***Results:*** It was possible to detect residues of penicillin, oxytetracycline and enrofloxacin, and also of the antiparasitic ivermectin in the milk samples. ***Conclusion:*** The methodology fastly and accurately detected the residues of the analyzed drugs, even in very low concentrations. Thus, it is an option to other existing ones, already used for this purpose.

Keywords: Antibiotic. Drugs. Chemometrics. Ivermectin. Infrared.

Resumo

Introdução: O leite é um dos alimentos mais consumidos pelos seres humanos. Proteínas, vitaminas, gordura, carboidratos e sais minerais fazem parte de sua composição e desempenham importantes funções para a nutrição humana. A prática de adulteração no leite é antiga e ainda se faz presente nos dias de hoje em diversos países, inclusive no Brasil. A fim de obter lucro maior, alguns fornecedores costumam adicionar ao leite: água, amido, citrato, ureia, soda cáustica, cloreto de sódio, sacarose, soro do leite, melamina e outros componentes. No entanto, ainda há outro problema, o da contaminação do leite por medicamentos veterinários. Estes podem causar danos à saúde do consumidor e prejuízos para a produção de seus derivados. ***Objetivo:*** O presente trabalho propõe uma metodologia que permite detectar de maneira rápida a presença de resíduos de medicamentos veterinários em leites, dentro do limite máximo de resíduos de cada droga. ***Métodos:*** Fez-se o uso da espectroscopia no infravermelho próximo por transformada de Fourier associada à análise de componentes principais. A espectroscopia no infravermelho tem sido utilizada não somente para a autenticidade de laticínios, mas para determinar sua qualidade. ***Resultados:*** Conseguiu-se detectar resíduos de penicilina, oxitetraciclina e enrofloxacino, e também do antiparasitário ivermectina nas amostras de leites. ***Conclusão:*** A metodologia detectou de maneira rápida e precisa os resíduos das

drogas analisadas, mesmo em concentrações muito baixas. Assim, é uma opção a outras existentes, já utilizadas para tal objetivo.

Palavras-chave: Antibiótico. Medicamento. Quimiometria. Ivermectina. Infravermelho.

INTRODUCTION

In mammals, milk is the primary source of food, being essential for the future health of individuals. In humans, it continues to be part of the diet even in adulthood, either through its direct ingestion, or through its derivatives.

The most commercialized milk in Brazil is that derived from cows, which has several components, among which the following stand out: proteins (3.3%), fat (4.0%), lactose (4.3%), in addition to vitamins and mineral salts.[1] The main proteins found in milk are casein (78%) and whey protein (19%), commonly known as whey protein, and others, totaling 2.7%.[2]

Whey protein is widely used by athletes for the purpose of gaining muscle mass, but for decades, it was the part of milk dispensed by the food industry. Only from the 70s, scientists began to study its properties. Not only athletes, but also practitioners of physical activities, physically active people and people with diseases use this protein to enjoy its benefits. Recent studies support the theory that milk proteins, including whey proteins, in addition to their high biological value, have bioactive peptides (PBAs), which act as antimicrobial, antihypertensive agents, regulators of immune function, growth factors, etc. The components of whey proteins and peptides, with their respective amino acid residues, are: beta-lactoglobulin (BLG) (162 amino acids), alpha-lactoalbumin (ALA) (123 amino acids), bovine serum albumin (BSA) (582 amino acids), immunoglobulins (Ig's), glyco-macropeptides (GMP) (64 amino acids).[3,4] The mineral salts found in significant quantities in milk are: calcium (Ca) and phosphorus (P), which are associated with micelle structures of casein, chlorine (Cl), potassium (K), sodium (Na) and magnesium (Mg). Already in small quantities are iron (Fe), aluminum (Al), bromine (Br), zinc (Zn) and manganese (Mn).[5,6] Lactose, the main carbohydrate in milk, consists of two monosaccharides, glucose and galactose. It carries important nutritional functions, such as providing up to 16.8 kJ/g of energy to a person.[7]

Dairy products such as milk, consumed worldwide, have high nutritional value. However, some suppliers usually add adulterants such as water, starch, citrate, urea, lye, sodium chloride, sucrose, whey, melamine, among others, in order to obtain a higher profit. Recently, adulterations of this nature have still been reported, especially in developing countries, such as Pakistan, Brazil, India and China.[8] The most common is the addition of water in milk in order to increase its volume. However, research has shown positive results in the development of new equipment and techniques to detect adulteration in milk with water.[9-11]

Another factor, also very important, that can lead to a public health problem and that is linked to milk quality, is the use of veterinary drugs in dairy cows. In this case, the waiting period, the period for eliminating the veterinary medicine in milk, after the last application must be respected. The presence of these drugs above the maximum residue limits (MRLs) is worrying, as they can have harmful consequences for human health, such as allergic reactions and intoxication.[12]

Milk contaminated by chemical substances, such as medicines, for example, is considered adulterated and unsuitable for consumption.[13] An important group of drugs that may be present in milk are mainly antimicrobials and anti-inflammatory drugs, but results of analyzes released by the Programa de Análise de Resíduos de Medicamentos Veterinários em Alimentos de Origem Animal (PAMVet) also show the presence of antiparasitic agents in milk.[14] The results found by PAMVet showed the appearance of doramectin in UHT milk samples above the MRL, while ivermectin residues were also found, however below the MRL.[14] However, these drugs should not administered to lactating cows. The adverse effects of these drugs are, in general, mild and transient.

Studies show that the inappropriate use of anti-inflammatory drugs can cause an additional effect, such as the appearance of renal tumors in guinea pigs, after long-term exposure to phenylbultazone.[15] Tetracyclines are among the most used antimicrobials in cattle. In 2014, Zhang et al.[16] conducted a study in China, warning

that veterinary medicines have been widely used in the treatment of dairy cattle, to prevent and control diseases or to promote the growth of these animals, causing the presence of residues of these drugs in milk UHT marketed in that country. The indiscriminate and excessive use of these drugs, in addition to causing serious allergic reactions, is the main cause of antimicrobial resistance.[16]

For monitoring, the controlling agencies use a variety of reliable and accurate analytical methods to detect residues of veterinary drugs in milk, such as high performance liquid chromatography (HPLC), gas chromatography coupled with mass spectrometry (GC-MS), and kits for detecting inhibitors (antibiotic residues) in milk. These methods, although reliable and accurate, have some disadvantages, such as: slowness and nonspecificity for some drugs (tetracycline), high cost, complexity, training and small amount of samples to be analyzed for a period of time.[17] Spectroscopic techniques combined with chemometric methods allow to analyze, interpret and extract information quickly and accurately, with minimal sample preparation.[18]

Fourier transform near infrared spectroscopy (FT-NIR) has been the method increasingly used to determine authenticity and adulteration in food. It is an analytical, non-destructive technique, based on the interaction between this electromagnetic wave and matter, which allows to quickly and accurately analyze several types of samples.[19,20] Principal component analysis (PCA) is a mathematical algorithm that allows to reduce the dimensionality of a set of data variables in a new set of variables, called main components. This increases the interpretability of the data without losing information from the primary data.[21]

Infrared spectroscopy combined with chemometrics, an interdisciplinary field that uses methods such as multivariate statistics, mathematics and computer science to extract information from chemical systems, allows analyzing the sample without a lengthy preparation method. In 2014, FT-NIR associated with PCA was used to detect the presence of sodium diclofenac residues in milk in simulated contamination percentages.[22] Recently, in 2020, Pereira et al.[23] presented a review with the most recent applications of spectroscopy in the near and medium infrared to assess the quality of dairy products.

Based on this scenario, this work presents a methodology based on the use of the FT-NIR technique combined with the PCA that allows to discriminate pure milk from milks with residues of veterinary drugs, from the classes of antimicrobials and antiparasitic, all within the MRL.

METHOD

The analysis of the physical-chemical characteristics of milk, as well as those of FT-NIR spectroscopy were performed at the Products and Processes Laboratory (LPP) and at the Materials Spectrometry Laboratory (LEM), located in the Physics Department of the Federal University of Juiz de Fora, Minas Gerais, Brazil.

Samples

To simulate the contamination of milk with veterinary drugs, those of frequent use in cattle were chosen, due to the most common pathologies. The chosen classes were the 10% injectable enrofloxacin Baytril® antimicrobials, which contains 10 g of enrofloxacin in 100 mL of vehicle (grace period, 3 days); terramycin/LA Zoetis/Pfizer® for injection, which has 20 g of oxytetracycline in 100 g of vehicle (grace period, 4 days); pentabiotic reinforced with penicillins from Zoetis / Pfizer (grace period, 8 to 10 milkings). Invermectin OF was chosen as an antiparasitic, which contains 1 g of ivermectin in 100 mL of vehicle (grace period of 35 days for slaughter and should not be administered in dairy cattle).

In this work, two different samples of homogenized whole type "A" pasteurized milk purchased in the region of Juiz de Fora-MG, Brazil, were used. These milks were known to be free of veterinary medicines. Then, two portions were reserved, one for the standard that would serve as a control, and the other for a self-controlled sample with the drugs used here. Therefore, they were subjected to a preliminary analysis to verify whether their physical and chemical characteristics are within the specifications of Normative Instruction No. 76, of November 26, 2018 (IN76).

In this step, the milk samples were taken to the LPP and analyzed by Delta Instruments® Lactoscope equipment, to determine fat, protein, lactose and total solids. To determine the melting point of milk, the digital electronic cryoscope ITR MK 540 Flex was used; the density was measured by means of the H15 Brazil Q 15° C lactodensimeter, while to obtain the titratable acidity value, the Dornic test was used. The pH was obtained with the bench pH meter AT355. Each analysis was performed in triplicate. After verifying that the physical-chemical characteristics of the milks used were in accordance with IN76, the self-controlled portion was manipulated, preparing samples with percentages of the active ingredients in the milk. That is, in order for the medication to stay within the MRL, the simulation was done according to the active principle of each drug and not in relation to its volume, since most drugs are constituted by the active principles plus the excipients. For this purpose, each drug (penicillin G, oxytetracycline, enrofloxacin and ivermectin) was first diluted in distilled water, and finally, part of this dilution was added to pure milk, a self-controlled sample, in order to achieve the concentration of the active principle in milk.[24]

Analysis using FT-NIR

Qualitative investigations of the samples were performed with Bruker's Multi-Purpose FT-NIR Analyzer, operating in reflectance mode in the range of 13,500 to 3,700 cm^{-1} of wave numbers with a Te-InGaAs detector and 4 cm^{-1} resolution. The samples were placed in borosilicate cuvettes 8 mm thick. Each analysis was performed in triplicate with 32 scans. OPUS® software version 5.5 was used for data acquisition.

Statistical analysis

The reflectance spectra, as well as their respective first order derivatives, were built with OriginPro® software. The eigenvalues were calculated using the BioEstat software version 5.3. Principal component analyzes were conducted using The Unscrambler® software version 9.2. Adobe PhotoShop CS6 was used only to highlight the clusters on the PCA.

RESULTS AND DISCUSSION

Characterization of milk

Tables 1 and 2 show the results found in the physical-chemical analyzes of the milk samples used to subsequently simulate the contamination, with antimicrobials and antiparasitic, in order to verify whether they were within the reference limits.

Table 1. Results of the physical-chemical characteristics of the samples of type "A" whole pasteurized milk that was used to simulate contamination with antimicrobials within the MRL, Juiz de Fora-MG, Brazil, 2019.

Analyze	Values found	Reference values[a,b]
Cryoscopy	(-0,536 ± 0,001) ºH	(-0,555 a -0,530) ºH
Acidity	(17,3 ± 0,6) ºDornic	(14 a 18) ºDornic
Density	(1,031 ± 0,001) g/mL	(1,028 a 1,034) g/mL
pH at 25 ºC	(6,72 ± 0,01)	(6,60 a 6,80)
Fat	(3,65 ± 0,01) %	≥ 3,00
Protein	(3,14 ± 0,01) %	≥ 2,90
Lactose	(4,50 ± 0,01) %	≥ 4,30
Solids	(11,29 ± 0,01) %	≥ 8,40

Source: LUIZ, 2018. [a]IN76; [b]FAO/TCP/KEN/6611

Table 2. Results of the physical-chemical characteristics of the samples of pasteurized type "A" whole milk that was used to simulate the contamination with the antiparasitic within the MRL, Juiz de Fora-MG, Brazil, 2019.

Analyze	Values found	Reference values[a,b]
Cryoscopy	(-0,540 ± 0,001) ºH	(-0,555 a -0,530) ºH
Acidity	(16,8 ± 0,6) ºDornic	(14 a 18) ºDornic
Density	(1,030 ± 0,001) g/mL	(1,028 a 1,034) g/mL
pH at 25 ºC	(6,71 ± 0,02)	(6,60 a 6,80)
Fat	(3,94 ± 0,01) %	≥ 3,00
Protein	(3,07 ± 0,01) %	≥ 2,90
Lactose	(4,46 ± 0,01) %	≥ 4,30
Solids	(11,47 ± 0,01) %	≥ 8,40

[a]IN76; [b]FAO/TCP/KEN/6611

Comparing the values found for the milk samples with the reference values adopted in IN76, it was found that all samples are within specifications, thus meeting the legislation.

Vibrational spectra

Figures 1 and 2 refer to the reflectance spectra for the mean values of pure milk, pure antimicrobials and milk + antimicrobials (within the MRLs), obtained by FT-NIR spectroscopy.

Figure 1. Reflectance spectrum of samples of pure milk (control) and contaminated with antimicrobials: enrofloxacin, penicillin G and oxytetracycline (terramycin), within the MRLs.

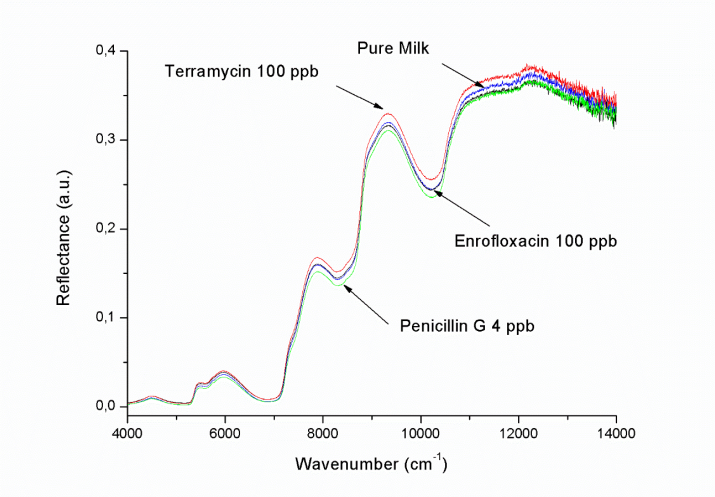


Figure 2. First derivative of the reflectance spectrum of pure milks (control), and contaminated with antimicrobials: enrofloxacin, penicillin G and oxytetracycline (terramycin), within the MRLs.

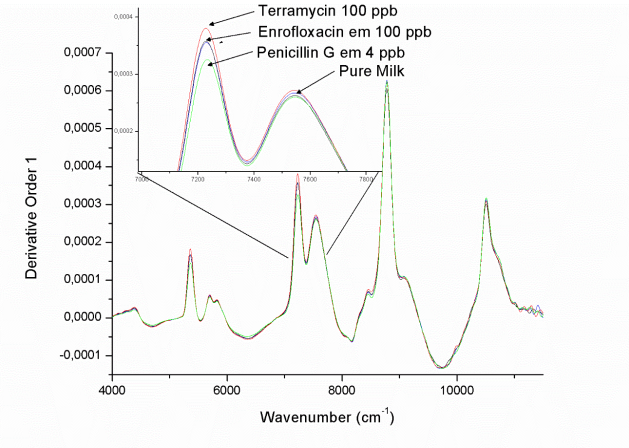
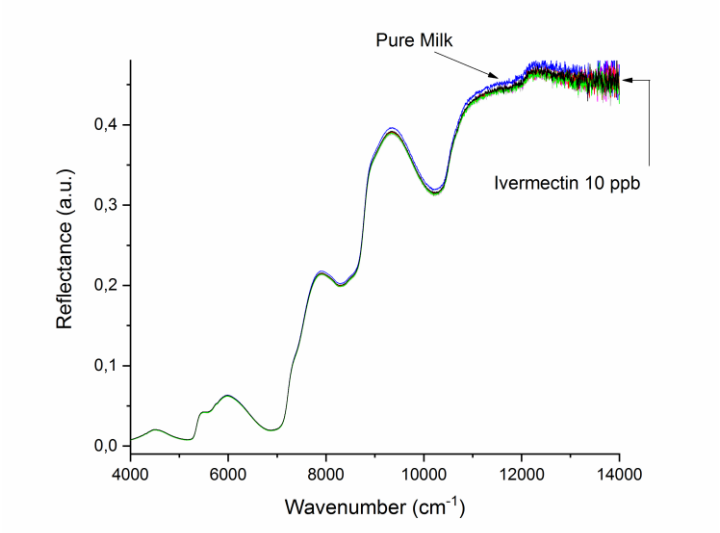


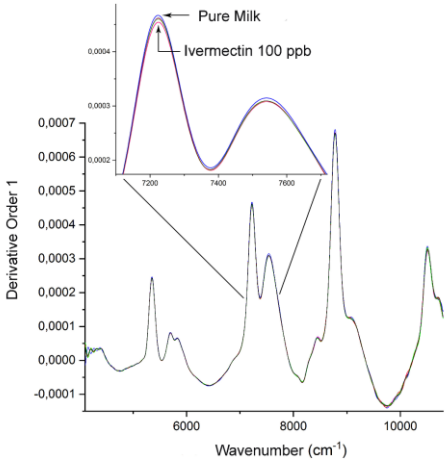
Figure 3 shows the FT-NIR reflectance spectrum for the average values of pure milk and milk + antiparasitic, with a reduction in drug concentration of 100 parts per billion (ppb), 50 ppb, 40 ppb, 30 ppb, 20 ppb up to achieve at the 10 ppb MRL.

Figure 3. Spectrum of reflectance of pure milk samples (control) and contaminated with the antiparasitic ivermectin in drug concentrations in milk: 100 ppb, 50 ppb, 40 ppb, 30 ppb, 20 ppb and 10 ppb, the latter the MRL for this medicine.



The figure 4 refers to the first derivative of the spectrum of Figure 3 with an enlargement of the spectrum of Figure 3, between the values of wave numbers 7100 cm^{-1} to 7800 cm^{-1}, in order to increase the observation zone in this spectral range.

Figure 4. Reflectance spectrum with magnification in the range between 7100 cm^{-1} and 7800 cm^{-1} of samples of pure milk (control) and contaminated with the antiparasitic ivermectin in drug concentrations in milk: 100 ppb, 50 ppb, 40 ppb, 30 ppb , 20 ppb and 10 ppb, the latter the MRL for this medicine

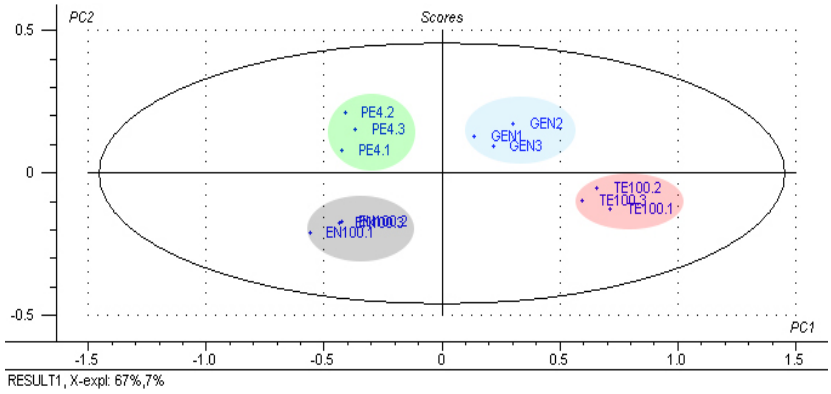


In figures 1, 2, 3 and 4, within the range of number of waves used, it makes the discrimination between the samples analyzed, directly by a visual analysis of the spectrum, not very efficient, because the concentrations of veterinary drugs are very small, in the order of ppb (mcg/L or µg/L). It can be seen in Figure 4 that, even expanding a given region of interest, the analysis via this spectrum can only clearly distinguish the sample of pure milk, control (upper line) from the milk sample added to ivermectin in 100 ppb (lower line). Samples with lower and closer concentrations were between these two lines.

Principal component analysis

The figure 5 represents the PCA hotelling scores graph, showing the clustering data for samples of pure milk (control), milk with: 100 ppb of enrofloxacin; 100 ppb of oxytetracycline; and milk with 4 ppb of penicillin G.

Figure 5. Hotelling scores plot of the statistical analysis (PCA) showing the data of clustering for samples of pure milk (control), GEN1, GEN2 and GEN3; milk with 100 ppb of enrofloxacin, EN100.1, EN100.2 and EN100.3; 100 ppb of oxytetracycline, TE100.1, TE100.2 and TE100.3; and milk with 4 ppb of penicillin G, PE4.1, PE4.2 and PE4.3.

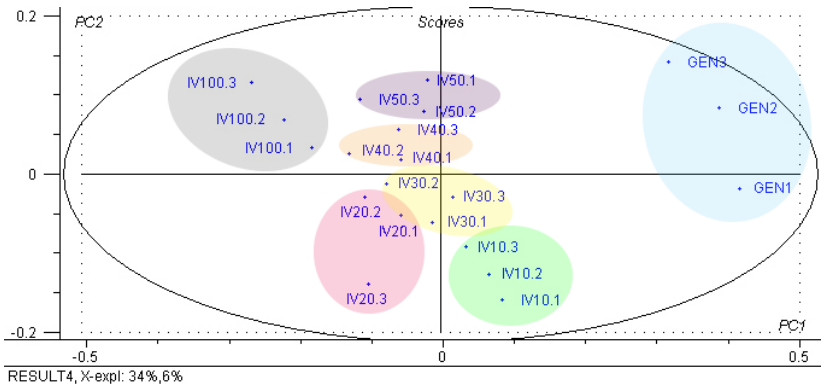


Analyzing figure 5, the formation of clusters (clusters) resulting from the high degree of similarity between the groups of the samples is clearly observed. Four groups are presented, one in each quadrant. Group 1 refers to samples of pure milk (control) and is located in the first quadrant. Group 2, a cluster located in the second quadrant, represents a group of samples contaminated with 4 ppb of penicillin G. The third quadrant contains elements from group 3 which is related to milk samples contaminated with 100 ppb of enrofloxacin, while the fourth quadrant is occupied by group 4, containing milk samples contaminated with 100 ppb of oxytetracycline. Among the elements of each group, none is very distant from each other, discarding the presence of outliers, that is, isolated samples that can be doubtful. The PCA accurately discriminated samples in groups, despite the fact that the concentrations of antimicrobials in milk are very low. From PC1, it can be seen that penicillin G and enrofloxacin have the same score in contrast to oxytetracycline and pure milk. Therefore, PC1 represents the degree of contamination of milk with antibiotics.

The data group containing pure milk is close to the center of the axis. Contaminated clusters have different distances and different positions from the center. This is related to the fact that the drug concentrations are different. For example, samples with 100 ppb of medication (groups 3 and 4) have a similar position. From the above reasoning, it appears that PC2 is related to the similarity of milk.

Figure 6 presents the PCA hotelling scores graph, showing data on possible clustering for samples of pure milk (control) and milk with: 100 ppb; 50 ppb; 40 ppb; 30 ppb; 20 ppb and 10 ppb ivermectin.

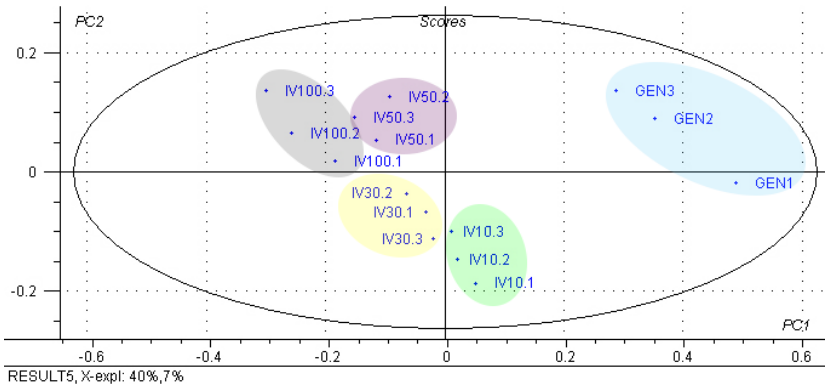
Figure 6. Hotelling scores plot of the statistical analysis (PCA) showing the data of clustering for samples of pure milk (control), GEN1, GEN2 and GEN3; milk added the antiparasitic ivermectin in: 100 ppb, IV100.1, IV100.2 and IV100.3; 50 ppb, IV50.1, IV50.2 and IV50.3; 40 ppb, IV40.1, IV40.2 and IV40.3, 30 ppb, IV30.1, IV30.2 and IV30.3, 20 ppb, IV20.1, IV20.2 and IV20.3 and 10 ppb, IV10. 1, IV10.2 and IV10.3, the latter the MRL for this medicinal product.



In figure 6, it is clear the discrimination between samples of pure milk from those contaminated, especially the one with the highest concentration of the drug, 100 ppb. It is observed that in PC1, vertical axis, the samples with the lowest concentration, 10 ppb (IV10.1, IV10.2 and IV10.3), are closer to the samples of pure milk (GEN1, GEN2 and GEN3), control. This is because these samples are the most similar to those of pure milk, due to the concentration of the drug in the supposedly contaminated sample being the smallest. As already mentioned, the opposite also occurs with those contaminated with a higher concentration of medication, 100 ppb, where they are farther from the samples of pure milk, in relation to PC1. Drug concentrations below 50 ppb until they reach the MRL (10 ppb) appear to form a large cluster, but within the same, it is possible to check other small ones, corresponding to each concentration of the drug in milk (50 ppb, 40 ppb, 30 ppb, 20 ppb and 10 ppb). This is due to the difference between the concentrations, which are very

small, 10 ppb. Figure 7 also refers to the simulation of contamination of pure milk with ivermectin, but now increasing the difference between its concentrations.

Figure 7. Hotelling scores plot of statistical analysis (PCA) showing the data of clustering for samples of pure milk, control, GEN1, GEN2 and GEN3; milk added the antiparasitic ivermectin in: 100 µg/L (100 ppb), IV100.1, IV100.2 and IV100.3; 50 µg/L (50 ppb), IV50.1, IV50.2 and IV50.3; 30 µg/L (30 ppb), IV30.1, IV30.2 and IV30.3 and 10 µg/L (10 ppb), IV10.1, IV10.2 and IV10.3, the latter the MRL for this medicine.



There is again a cluster formation. In PC1, the samples of pure milk are located in the group on the right, well away from the largest group, in the center, referring to contaminated milk. Samples with 100 ppb are more distant when compared to pure milk, control, indicating that there is no strong similarity relationship between them. Samples with lower drug concentrations, 10 ppb (MRL), are the closest to pure milk, indicating a strong relationship between them. The samples with intermediate values, 30 ppb and 50 ppb, are very close, in relation to PC1, which is to be expected, because the difference in concentration is small.

It is observed, however, that the midpoint of the 30 ppb samples is about -0.05 on the horizontal axis of PC1, while for the 50 ppb samples it would be approximately -0.15, on the same axis. As the pure milk samples are located in positive points, around 0.4, the 30 ppb samples would be closer to the control samples, pure milk. This is a fact, as they have less drug concentration when compared to 50 ppb.

CONCLUSION

The FT-NIR technique associated with PCA stood out for being fast, done in a few seconds, and also easy to operate. PCA analysis was essential to discriminate the control sample, of pure milk, of milk contaminated with veterinary drugs: antimicrobial and antiparasitic, even in low concentrations, 10 ppb and 4 ppb, respectively. This shows that the methodology used here can be studied to be used as a complementary technique to the other existing ones, already used for detecting residues of veterinary drugs in milk.

When proposing new analysis techniques, new possibilities for similar researches appear, which contributes to the increase in inspection, collaborating for inspection and offering alternatives for analysis laboratories.

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REFERENCES

1. Pontes Netto D, Lopes MO, Oliveira MCS, Nunes MP, Machinski Junior M, Bosquiroli S L, Benatto A, Benini A, Bombardelli ALC, Vedovello Filho D, Machado E, Belmonte IV, Alberton M, Pedroso PP, Scucato ES. Levantamento dos principais fármacos utilizados no rebanho leiteiro do Estado do Paraná. Acta Scientiarum. Animal Sciences 2005;27(1):145-151. DOI: 10.4025/actascianimsci.v27i1.1260

2. Tavanti VK, Bassi lG, Ferreira GCC, et al. Composição e a capacidade de coagulação de leites de vacas holandesas e girolandas. Revista do Instituto de Laticínio Candido Tostes 2009;370(64):5-9. [acesso em 09 de novembro de 2020] Disponível em: https://www.revistadoilct.com.br/rilct/article/view/92:

3. Almeida CC. Avaliação de Suplementos Nutricionais à Base de Whey Protein. Niterói. Dissertação [Mestrado em Medicina Veterinária] - Universidade Federal Fluminense; 2014.

4. Haraguchi FK, Abreu WC, PAULA H. Proteínas do Soro do Leite: Composição, propriedades nutricionais, aplicações no esporte e benefícios para a saúde humana. Rev. Nutr [online] 2006;19(4):479-488. DOI: 10.1590/S1415-52732006000400007.

5. Neves VA, Souza KAFD. Análise de alimentos: pesquisa dos componentes do leite. Experimentos de Bioquímica. UNESP. [acesso em 09 de novembro de 2020] Disponível em: http://www.fcfar.unesp.br/alimentos/bioquimica/analise_leite/analise_leite.htm

6. Morzelle MC. Composição Química do Leite. Notas de Aula, ESALQ/USP; 2016. [acesso em 09 de novembro de 2020] Disponivel em: https://edisciplinas.usp.br/pluginfile.php/1810879/mod_resource/content/1/Aula%202.pdf

7. Pereira MCS, Brumano LP, Kamiyama CM, Pereira JPF, Rodarte MP, Pinto MAO. Lácteos com baixo teor de lactose: uma necessidade para portadores de má digestão da lactose e um nicho de mercado. Revista do Instituto de Laticínios Cândido Tostes 2012;67(389):57-65. DOI: 10.5935/2238-6416.20120079

8. Handford CE, Campbell K, Elliott CT. Impacts of milk fraud on food safety and nutrition with special emphasis on developing countries. Comprehensive Reviews in Food Science and Food Safety 2016;15(1):130-142. DOI: 10.1111/1541-4337.12181

9. Brandão MP, Neto MG, Anjos VC, Bell MJV. Detection of adulteration of goat milk powder with bovine milk powder by front-face and time resolved fluorescence. Food Control 2017;81:168-172. DOI: 10.1016/j.foodcont.2017.06.008

10. Nascimento WWG, Souza MPO, Valente ACMM, Anjos VC, Furtado MAM, Bell M. J. V. Results from portable and of low-cost equipment developed for detection of milk adulterations. Food Sci. Technol 2017; 37(spe):38-74. DOI: 10.1590/1678-457x.06617

11. Nascimento WWG, Souza MPO, Valente ACMM, Anjos VC, Furtado MAM, Bell M. J. V. Resultados a partir de equipamento portátil e de baixo custo desenvolvido para detecção de adulterações em leite. Inovação em Ciência e Tecnologia de Alimentos 2019;2:274-281. DOI: 10.22533/at.ed.99719091031

12. Brito MAVP, Lange CC. Resíduos de antibióticos no leite. Embrapa; 2005. Comunicado Técnico 44. [acesso em 09 de novembro de 2020] Disponível em: https://ainfo.cnptia.embrapa.br/digital/bitstream/item/65413/1/COT-44-Residuos-de-antibioticos.pdf

13. Forsythe SJ. Microbiologia da Segurança Alimentar. Porto Alegre: Artmed; 2002.

14. Agência Nacional de Vigilância Sanitária-ANVISA, Limites Máximos de Resíduos de Medicamentos Veterinários em Alimentos de Origem Animal: Documento de base para discussão regulatória, Brasilia; 2018. [acesso em 09 de novembro de 2020] Disponível em: http://terraviva.com.br/site/2018/outubro/3010med1.pdf

15. Gallo P, Fabbrocino S, Dowling G, Salini M, Fiori M, Perrata G, Serpe L. Confirmatory analysis of non-steroidal anti-inflammatory drugs in bovine milk by high-performance liquid chromatography with fluorescence detection. Journal of Chromatography 2010;1217(17):2832-2839. DOI: 10.1016/j.chroma.2010.02.047

16. Zhang YD, Zheng N, Han, RW, Zheng BQ, Yu ZN, Li, SL, Zheg, SS. Occurrence of tetracyclines, sulfonamides, sulfamethazine and Quinolones in Pasteurized Milk and UHT milk in China's market. Food Control 2014;36:238 – 242. DOI: 10.1016/j.foodcont.2013.08.012

17. Sivakesava S, Irudayara J. Rapid Determination of Tetracycline in Milk by FT-MIR and FT-NIR Spectroscopy. Journal of Dairy Science 2002;85(3):487-493. DOI: 10.3168/jds.S0022-0302(02)74099-X

18. Brandão MCMP, Carmo AP, Bell MJ, Anjos VC. Characterization of Milk by Infrared Spectroscopy. Revista do Instituto de Laticínios Cândido Tostes 2010;65(373):30-33. [acesso em 09 de novembro de 2020] Disponível em: https://www.revistadoilct.com.br/rilct/article/viewFile/121/125

19. Leite AIN, Pereira CG, Andrade J, Vicentini NM, Bell MJV, Anjos V. FTIR-ATR spectroscopy as a tool for the rapid detection of adulterations in butter cheeses. LWT 2019;109:63-69. DOI: 10.1016/j.lwt.2019.04.017

20. Pereira CG, Leite AIN, Andrade J, Bell MJV, Anjos V. Evaluation of butter oil adulteration with soybean oil by FT-MIR and FT-NIR spectroscopies and multivariate analyses. *LWT* 2019;107:1-8. DOI: https://doi.org/10.1016/j.lwt.2019.02.072

21. Jolliffe IT, Cadima J. Principal Component Analysis: a review and recent developments. Philosophical Transactions Series A, Mathematical, Physical and Engineering Sciences 2016;374,(2065). DOI: https://doi.org/10.1098/rsta.2015.0202

22. Luiz LC, Bell MJV, Rocha RA, Mendes TO, Anjos VC. Análise de Resíduos de Diclofenaco Sódico Veterinário em Leite por Espectroscopia no Infravermelho Próximo. Rev. Bras. Ciências Saúde 2014;18(3),219-224. DOI:10.4034/RBCS.2014.18.03.05

23. Pereira CG, Luiz LC, Bell MJV, Anjos V. Near and Mid Infrared Spectroscopy to assess Milk Products Quality: A Review of Recent Applications. *Journal of* Dairy Research & Technology 2020; 3:014. DOI:10.24966/DRT-9315/100014

24. Luiz LC, Bell MJV, Rocha RA, Leal NL, Anjos VC. Detection of Veterinary Antimicrobial Residues in Milk through Near-Infrared Absorption Spectroscopy. Journal of Spectroscopy 2018;ID5152832,1-6. DOI: 10.1155/2018/5152832.

Contributors

Luiz, LC worked in all stages, from the conception of the study to the final review; Bell MJV and Anjos VC worked on analyzing and interpreting the data, as well as reviewing the text.

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