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Analytical Methods

Monitoring the authenticity of Brazilian UHT milk: A chemometric approach

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ABSTRACT

In this work, chemometric methods are reported as potential tools for monitoring the authenticity of Brazilian ultra-high temperature (UHT) milk processed in industrial plants located in different regions of the country. A total of 100 samples were submitted to the qualitative analysis of adulterants such as starch, chlorine, formol, hydrogen peroxide and urine. Except for starch, all the samples reported, at least, the presence of one adulterant. The use of chemometric methodologies such as the Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) enabled the verification of the occurrence of certain adulterations in specific regions. The proposed multivariate approaches may allow the sanitary agency authorities to optimise materials, human and financial resources, as they associate the occurrence of adulterations to the geographical location of the industrial plants.

1. Introduction

Food adulteration has been practiced since biblical times but has become more sophisticated in the recent past. Foods or ingredients most likely to be targets for adulteration include those which are of high-value and which undergo a number of processing steps before they appear in the market (Karouri & Baedemaeker, 2007). As a matter of fact, the authenticity of food has become a worldwide problem, making it more necessary to detect the introduction of certain deceitfully labelled and low quality products, either for economical reasons or for public health matters (Veloso, Teixeira, Ferreira, & Ferreira, 2002).

Dairy products are of particular interest, because they are a group of foods that play an important role in feeding the population and are essential for certain groups of consumers as women, children and the elderly; in fact, milk is a fairly expensive raw material, and from an economic point of view it could, therefore, be attractive to modify its composition and replace part of it with other dairy or non-dairy ingredients (De La Fuente & Juarez, 2005).

Dairy products have high nutritional value and are consumed all over the world. From 1997 to 2007, consumption of liquid milk increased by nearly 18 million to 112 million tons, corresponding to an average growth of 1.7% per year. A large part of this growth can be attributed to the UHT procedures and to government programs promoting milk consumption (International Dairy Federation, 2008). Animal products, such as dairy products, are rich in highly nutritional proteins and, therefore, more valued in the market. That is the reason why they are so vulnerable to several types of adulterations aiming at maximising producers' profits.

Chemometrics is an interdisciplinary research field that involves multivariate statistics, mathematical modelling and computing, especially applied to chemical data. Some of its main areas include the design and optimisation of experimental procedures and the extraction of the maximum amount of chemical information from analytical data (Gemperline, 2006). Chemometric methods have been a useful tool in evaluating the quality and identity control of processing parameters for dairy products (Faye, Konuspayeva, Messad, & Loiseau, 2008; Kasemsumran, Thanapase, & Kiatssonthon 2007; Rodriguez-Nogales & Vasquéz, 2007; Sacco et al., 2009; Sola-Lanarranga & Navarro-Blasco, 2009; Watkins & Wijesundera, 2006). However, its practical use towards the management of the activities that should be prioritized and the consequent optimisation of financial resources is scarce. The purpose of this work was to evaluate the use of chemometric techniques for monitoring the authenticity and quality of Brazilian UHT milk in order to generate data to maximise financial and material resources for Health Agencies.

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2. Material and methods

2.1. Sampling

A total of 100 samples of Brazilian UHT milk processed in industrial plants located in different states – Paraná and Rio Grande do Sul (South region – S), São Paulo, Rio de Janeiro and Minas Gerais (Southeast region – SE) and Goiás (Mid-west region – CO), which are the main producer areas in Brazil, were used in this research. After collection, the samples were transported to the laboratory on ice maintaining sterile condition.

2.2. Physicochemical analysis

Samples were submitted to qualitative analysis regarding the presence of adulterants such as starch, formol, chlorine and urine. The analyses were performed according to Brazilian official protocols (Brasil, 2006), using a positive and a negative control.

The identification of chlorine was based on the formation of free iodine from potassium iodide by the action of free chlorine or hypochlorite. The identification of hydrogen peroxide was based on the transformation of guaiacol to leuco form (coloured compound), by the action of milk peroxidase on hydrogen peroxide, releasing oxygen. The identification of starch was based on the formation of a blue adsorption compound came from the chemical reaction with iodine. In order to identify formaldehyde (reagent consisting of potassium iodide, mercuric chloride and potassium hydroxide) in the milk samples, the Nessler reagent was used. The formaldehyde (strongly reducing agent) in the presence of iodine and mercury (oxidising agents) and alkaline conditions cause a redox reaction, which can be observed by a purple–violet complex that is formed. If the formaldehyde is in a high proportion, a grey colour is formed in the milk (Brasil., 2006).

2.3. Chemometric techniques

The Hierarchical Cluster Analysis (HCA)'s primary goal is to display the data in such a way as to emphasise their natural clusters and patterns in a two-dimensional space. The results, qualitative in nature, are usually presented as a dendrogram, allowing the visualisation of clusters and correlations among samples or variables. In HCA, the Euclidean distances between samples or variables are calculated and transformed into a similarity matrix whose elements are similarity indexes ranging from 0 to 1; a smaller distance means a larger index and therefore, a larger similarity (Granato, Castro, & Katayama, 2010). Principal Component Analysis (PCA), on the other hand, is based on the correlation among variables. It maps samples through scores and variables by the loadings in a new space defined by the principal components. The PCs are a simple linear combination of original variables. The scores vectors describe the relationship between the samples and allow checking if they are similar or dissimilar, typical or outlier, while the loadings vectors describe the importance of each variable (Ferreira, Morgano, Queiroz, & Mantovani, 2000; Granato, Castro, and Katayama, 2010).

The experimental results were organised in a matrix format based on 100 rows (samples) and 4 columns (adulterants) as positive and negative frequencies for each adulterant, where the following criterion was adopted: 1 (absence of the adulterant) and 0 (presence of the adulterant). Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) (Arvanitoyannis, 2006), implemented in the software Pirouette 2.2 (Infometrix, Seattle, WA, USA), were the chemometric methods used to analyse the results. The raw data was analysed directly, without any preprocessing. For HCA, sample similarities were calculated on the basis of the Euclidian distance and the complete linkage method was adopted to establish clusters. As the presence of starch was not observed in the milk samples, this adulterant was not included in the chemometric evaluation.

3. Results and discussion

Fig. 1 shows the percentage of conformity found in several commercial brands of UHT milk regarding the presence of adulterants. The highest values of non-conformities were found for urine (55%) and formaldehyde (44%), followed by hydrogen peroxide (30%) and chlorine (12%). The presence of starch was not verified in any of the analysed samples. The high rates of positive results for urine – added to disguise the addition of water – can be related to its immediate availability for the producer, while starch, although it is also a low cost substance, is a raw plant food and would have to be acquired. This suggests that the addition of water is still a very common practice among Brazilian producers.

The addition of water to milk reduces significantly its nutritional value. It alters the ratio of the constituents from milk and reduces industrial performance, increasing the risks of microbial contamination and consequently causing economical losses. The positive results for peroxide and formol suggest that chemical treatment is still practiced by some rural producers. This can be related to the difficulties that some regions face regarding the availability of enough electrical power to enable the immediate cooling of the milk, forcing them to this practice for milk preservation. Once Brazilian legislation (Brasil., 2002) prohibits the use of chemical substances for milk preservation, this product is considered adulterated. The low index of chlorine found in the analysed samples indicates that the rising step of the equipments used in the raw milk handling, during the sanitation process, might have been done inappropriately.

Similar results were found in other researches, suggesting that dishonest practices with milk are still being conducted, especially in countries under development. Presence of water in milk destined to the production of fermented milk is reported in Sudan (Adam, 2009) and (El Zubeir, Abdalla, & El Owni, 2005), as well as in raw milk destined to the production of pasteurised milk in South Africa (El Zubier, Gabriechise, & Johson, 2008). In a broader study involving several regions of India, Arora (2004) reported the incidence of 30.5% of adulterants in fluid milk. The main non-conformities found were water addition, corresponding to 11.7%, neutralizers in general, 9.2% and sugar, 2.3%. The authors also reported that 3.8% of the samples contained more than one adulter-

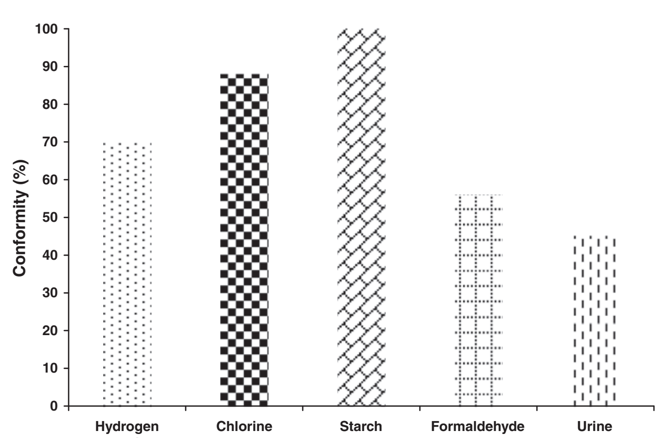

Fig. 1. Percentage of conformity in UHT milk samples regarding the presence adulterants.

Table 1
Loadings for the first four varimax rotated principal components (PCs).

Compounds	1	2	3	4
Formaldehyde	**0.58**	**0.67**	−0.45	−0.15
Chlorine	0.22	0.14	0.17	**0.95**
Urine	**0.64**	−**0.73**	−0.25	0.005
Hydrogen peroxide	0.46	0.10	**0.84**	−0.27
Variance (%)	47.80	29.77	16.23	6.16
Cumulative variance (%)	47.80	77.57	93.83	100.00

ant. Ruegg (2003) considers the chemical adulteration of milk a potential threat to humans. The existence of adulteration in dairy products submitted to UHT treatment is reported with the raw material, that is, raw milk, being the farmers the main responsible. However, one can not discard the possibility of dishonest practices inside the industrial unit during the processing of dairy products.

Table 1 show the results from Principal Component Analysis. Four dimensions are necessary for the total explanation of data variability. The two first principal components (PC1 and PC2, respectively) explain together up to 77.57% of the variability and are associated with the variables urine and formaldehyde, respectively, while the third and fourth factors explain 21.94% and 8.32%, and are associated with hydrogen peroxide and chlorine, respectively. It is suggested that there is predominance on the use of formaldehyde to reconstitute acidity, allied to the classical addition of urine to disguise the addition of water. However, there is a number of adulterated samples with the presence of peroxide as well, or even the mixture of the two adulterants.

Table 2 shows the results of HCA on incidence of adulterants in UHT milk processed in different regions. With a similarity index of 0.487, six clusters can be observed: cluster 1 (11 samples – 10 S and 1 CO) reports the presence of formalin in 100% of the samples and peroxide in all the samples from Rio Grande do Sul. Only one sample from RCO that is positive both for formalin and peroxide was included in this cluster. In cluster 2 (21 samples – 12 SE and 8 CO), 100% of the samples were positive for peroxide hydrogen and none for formol. In cluster 3 (32 samples – 10 S, 3 SE, 9 CO, 10 SE), the results is the opposite of what happens in cluster 2. Samples in cluster 4 (5 samples SE), 100% are positive for formol and urine. Cluster 5 included only one sample from São Paulo state, which differs from the others as it presents positive results for both formol and chlorine. In cluster 6 (30 samples SE), 100% of the samples are positive for urine.

The authenticity of any processed dairy product must be uniform, regardless of the region where it is processed. However, it is necessary to consider the reality of each region and their respective problems, as well as the producers' level of knowledge on obtaining milk and on adopting quality systems in the milk processing in industrial units. Indeed, regulatory agencies may use groupings to establish food authenticity in terms of geographical region or biological source, or to detect adulteration of foods that have specific identities or legislated standards (Kozac & Scaman, 2008). These directions also are valid for food products consumed routinely, as is the case of the UHT milk for Brazilian people.

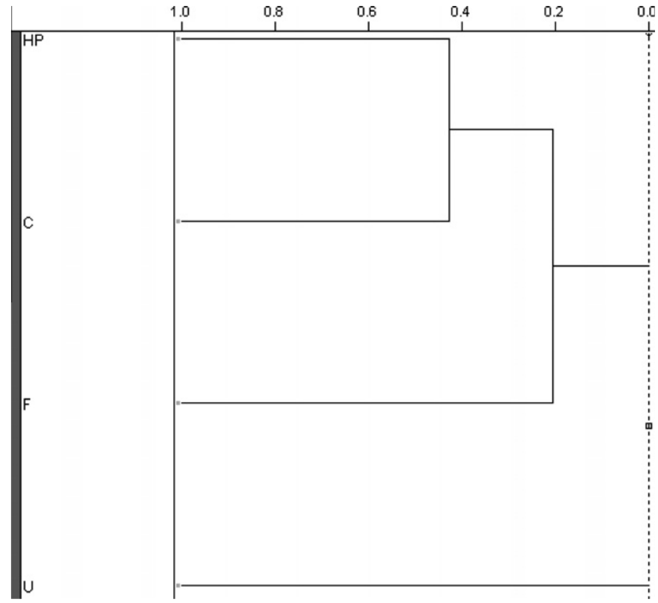

Fig. 2. Dendrogram (HCA) for variables: HP = hydrogen peroxide, F = formaldehyde, C = chlorine, U = urine.

The main reason for adulteration is to increase profits, which directly violates consumers' rights, who put their trust on the production chain of the milk they decide to buy. Authenticity is an important food quality criterion and analytical methods to guarantee it are being demanded by the food producers (Alonso-Sales et al., 2004).

There is room for prioritizing and optimising the activities of sanitary agency authorities in all these Brazilian states. Programs involving the quality and authenticity of UHT milk sold in the South region of Brazil should prioritize the analysis of chlorine and formaldehyde. Additionally, there should be periodic audits to check the observation of the standard operational sanitation procedures (PPHO) used, once the presence of chlorine indicates a deficient rinsing of the equipment used in milking, as the substance is used in the processing plants of UHT milk. The current legislation for dairy plants makes the elaboration of standard operational sanitation procedures obligatory for all surfaces that have contact with the food. It also reports the need of previous training of personnel involved in the operations, routinely monitoring and evaluating the activities before and after operations (Brazil, 2003).

Peroxide hydrogen and urine analysis should be prioritized in the Southeast region. For samples from the plants located in the Mid-West, peroxide and formol analysis should be prioritized, as there are higher incidence rates of positive results for these substances. This report is extremely valuable, as it allows the concentration of efforts for the analysis of adulterants of higher incidence for certain areas, optimising the use of the financial and material resources available.

Table 2
Results of HCA on incidence of adulterants in Brazilian UHT Milk processed in different areas.

Cluster	Sampling	Origin	Characteristics
1	11	10 S, 1 CO	72.7% positive for chlorine in 72.7%. 100% of samples tested positive for formaldehyde and peroxide
2	21	12 SE, 8 CO	100% positive for peroxide and 60% for chlorine
3	32	10 S, 13 SE, 9 CO	100% positive for formaldehyde and 0% for peroxide
4	5	5 SE	100% positive for both formaldehyde and urine
5	1	1 SE	Only one sample of the Southeast region is present. It differs from others as it is positive for both formaldehyde and chlorine
6	30	1 SE	100% of samples tested positive for urine

Fig. 2 shows the dendrogram for variables obtained through HCA. It can be observed that the substance used for the reconstitution of the milk density – urine – is isolated, separated from the substances for milk conservation – hydrogen peroxide and formol. It can be noticed that there is a similarity between hydrogen peroxide and chlorine, indicating that the samples that are positive for the first are frequently positive for the second as well, suggesting product adulteration problems and milking good practices, especially in the sanitation process. This confirms the results obtained in the analysis of the main components previously reported.

4. Conclusion

The use of chemometric methods has been shown as a potential additional alternative for monitoring the authenticity of UHT milk, making it possible to verify milk adulteration in certain geographical regions of Brazil. This makes it possible for Health Agencies to optimise material, human and financial resources, as it associates the occurrences of adulteration to the geographical location of the industrial plants.

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