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SHORT COMMUNICATION

FAST AND GREEN METHOD FOR ANALYSIS OF SERRA DA ESTRELA CHEESE

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Abstract

Serra da Estrela (SE) cheese is a regional product making part of the gastronomic and socio-cultural heritage of Portugal. It has several aspects that make it unique, namely the manufacturing by the coagulation of raw sheep milk using the thistle flower of *Cynara cardunculus* L., obtaining a final buttery texture with an exclusive typical flavour. The aroma compounds of Serra da Estrela cheese result partially from the action of indigenous microorganisms and enzymes on lactose, lipids and proteins. Although SE production is very ancient, there are few studies concerning its nutritional composition but nowadays consumers demonstrate interest in understanding the global composition of cheese, since it's a product highly appreciated. In the last decades, the use of near infrared spectroscopy (FT-NIR) has become comparable with those of the classical methods with representative advantages such as minimum time sample preparation; it is a green technique (without using toxic reagents) and allows multiparametric determinations. To perform the present study, a total of 24 SE cheese samples were evaluated, originated from 6 representative producers and analysed with a FT-NIR Buchi NIRMaster™ standalone spectrometer with a spectral range of 800–2500 nm and compared with classical methods. SE cheese samples were evaluated in terms of salt, moisture, protein and fat content. In all the studied samples the moisture varied between 42% and 53%, the fat content between 19.6 and 33.3%, the protein content between 18.6 and 26.7% and the salt between 0.7 and 2.2%. The results showed a significant agreement between the pairs of values obtained for the studied parameters.

Keywords: Serra da Estrela cheese, FT-NIR spectroscopy, moisture, protein, fat, salt.

Introduction

Serra da Estrela (SE) is an ewe`s traditional Portuguese cheese, which production is regulated and geographically limited due to its Protected Designation of Origin (PDO) certification, which was obtained in 1985. Serra da Estrela cheese is produced from raw ewe's milk and coagulated using dried thistle flower (*Cynara cardunculus* L.). The cheese characteristics are legally defined, although many extrinsic factors (e.g., climate, nutritional and physiological status, lactation stage) may condition the milk chemical and microbiological characteristics and consequently the final cheese composition (Guiné et al., 2016). Serra da Estrela is the most known and popular Portuguese cheese, appreciated worldwide, being preferentially consumed as a soft cheese, with an average maturation of 30–45 days, although some consumers prefer to consume it as a hard cheese after at least 6 months of storage (Carocho et al., 2015; Carocho et al., 2016, Partidário et al., 1998; Macedo, Malcata, 1996).

The FT-NIR spectroscopy method has recently become widely used in dairy industry and food science laboratory control because it allows multiparametric applications and has the advantage of not requiring chemical reagents and minimizing sample preparation. It operates in most phases of the dairy value chain, starting in farms with fresh milk to assess the product's composition value, before and during processing for process control and in final products to ensure the required specifications (Holroyd, 2017). Non-translucent materials such as cheese are analysed via diffuse reflection. The NIR light penetration is limited by the sample material, interacts with the sample and is refracted and reflected into the sensor. The reflected rays contain the spectral information of the sample (Burns, Ciurczak, 2008). It is well established that fat, moisture and protein composition of cheese can be measured successfully using NIR spectroscopy (Holroyd, 2011).

The classical methods used in the determinations of fat and protein in food have numerous disadvantages, like being time and energy consuming, having limited sample throughput and leading to possible harmful effects on the environment, because of the large amount of chemicals required.

Besides quality control in large scale in milk products' processing, NIR spectroscopy has also been used to detect adulterants in foods (determination of melamine in infant milk powder formula (Scholl et al., 2017)), for the discrimination of cheese adulterations (goat cheeses with cow's milk (Dvorak et al. 2016)) and also in food authentication like the detection of Tyrol cheese (Huck-Pezzei et al., 2014) or Parmigiano Reggiano cheese (Cevoli et al., 2013), among others.

This study intended to compare FT-NIR technique with classical methods in the quantification of major components of SE cheese using a broad-based calibration previously readjusted with samples of SE cheese.

Materials and Methods

The nutritional characteristics of 24 Serra da Estrela cheese samples originated from 6 representative cheese producers of the PDO region were analysed. Samples of cheese of each producer, with approximately 45 days of maturation, were collected in refrigerated boxes and about 1.5 cm of the rind was removed to provide a surface that represented its interior and a slice of about 100 g was placed in a flat-bottom glass cuvette and analysed, in triplicate. Spectra were recorded on

NIRMaster™ spectrophotometer from Buchi NIRSolutions™ (Flawil, Switzerland) equipped with a polarisation interferometer with TeO_2 wedges, an extended range InGaAs detector (temperature controlled) working in diffuse reflectance with a spectral range of 800–2500 nm (resolution: 8 cm^{-1}) combined with NIRWare™ software package, also from Buchi NIRSolutions.™ A blank signal was previously obtained with external reference Spectralon®. The internal background was measured with a gold plate reflector.

The classical methods of analysis were conducted in duplicate, in an external laboratory. Moisture was determined by drying at 105 °C until constant weight. Fat was analysed by the Van Gulik method, total protein was calculated by the Dumas method and salt (NaCl) was calculated through the content in sodium, determined by flame atomic absorption spectroscopy.

The data was analysed using Statistical Package for Social Sciences (SPSS version 25.0). The accuracy and precision of the regression lines obtained were verified by t-test for comparison of the means of paired samples and linear regression. Cook's Distance (Di) was used to detect outliers by the rule that Di>4/n (n: number of samples) is an outlier. Those values were analysed before elimination.

Results and Discussion

Starting with 24 samples of SE cheese, 3 outliers were removed after previous analysis of the adjustment of the regression: 1 in moisture and 2 in protein.

Mean values of major constituents of SE cheese determined by FT-NIR and by classical methods are presented in Table 1. In the samples studied, the moisture varied between 41.7 and 56.4%, the fat content between 16.6 and 35.5%, the protein content between 18.1 and 25.5% and the salt between 0.6 and 3.0% (Table 1).

Table 1

Parameters determined by FT-NIR and classical methods in the samples of SE cheese

Parameters (%)	FT-NIR		Ext Lab	
	x±sd	VR	x±sd	VR
Moisture	48.78±2.92	42.97 52.61	49.02±3.69	41.70 56.40
Fat	24.21±4.49	16.64 33.33	24.40±4.59	17.10 35.50
Protein	20.99±1.60	18.55 25.19	20.39±1.71	18.10 25.50
Salt	1.17±0.34	0.63 2.21	1.79±0.39	1.27 3.00

x±sd – arithmetic mean ± standard deviation; VR – variation range, from minimum to maximum; FT-NIR – near infrared technology; Ext Lab – external laboratory

Fox *et al.* (2017) presented similar contents with mean values of 48.7% of moisture, 27.5% of fat content, 21.3% of total protein and 1.9% of salt. Carocho et al. (2016), Ramos and Juarez (2011) and Associação Portuguesa de Nutrição (2018) obtained similar results.

The relations between classical methods and FT-NIR method for the monitored SE cheese components are shown in Table 2. The regression lines of all measured quantities are shown in Figures 1 and 2. The obtained correlation coefficients for all components of SE cheese were statistically significant ($p<0.001$).

Table 2

Expression of the relationship between FT-NIR and classical methods in moisture, fat, crude protein and salt of SE cheese samples

Parameters	R^2	r	Slope	Intercept	SEP (%)
Moisture	0.639	0.800	0.639	17.79	1.79
Fat	0.934	0.966	0.988	0.47	1.18
Protein	0.913	0.956	1.021	-1.05	0.48
Salt	0.869	0.932	1.077	0.53	0.13

$p<0.001$ for all correlations (r); R^2 – determination coefficient; r – correlation coefficient; FT-NIR – near infrared technology; SEP – standard error of prediction

The reliability and precision of the NIR measurement method was quite satisfactory for protein and fat content as the R^2 resultant of the linear regression was greater than 0.90 (Table 2 and Figure 1).

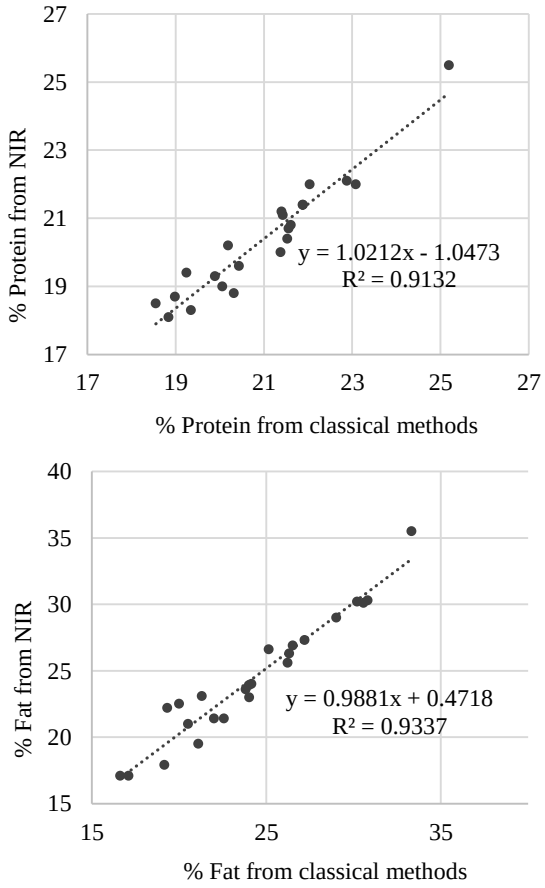

Figure 1. The relationship between classical methods and indirect method (FT-NIR) for SE cheese protein and fat determinations

Similar results were obtained by Adamopoulos et al. (2001) in Greek feta cheeses, with medium values of R^2 =0.952 and 0.956 for protein and fat, respectively.
On the other end, moisture showed a low determination coefficient of 0.64 and a slope and intercept (0.639 and 17.79, respectively) far from the ideal curve y=1x+0 (slope of 1 and intercept of 0), showing a poor correlation between data obtained from NIR and classical analysis. Also, standard error of prediction (SEP) was quite high indicating that the predicted values didn´t quite match the values obtained by the classical methods (Table 2 and Figure 2).

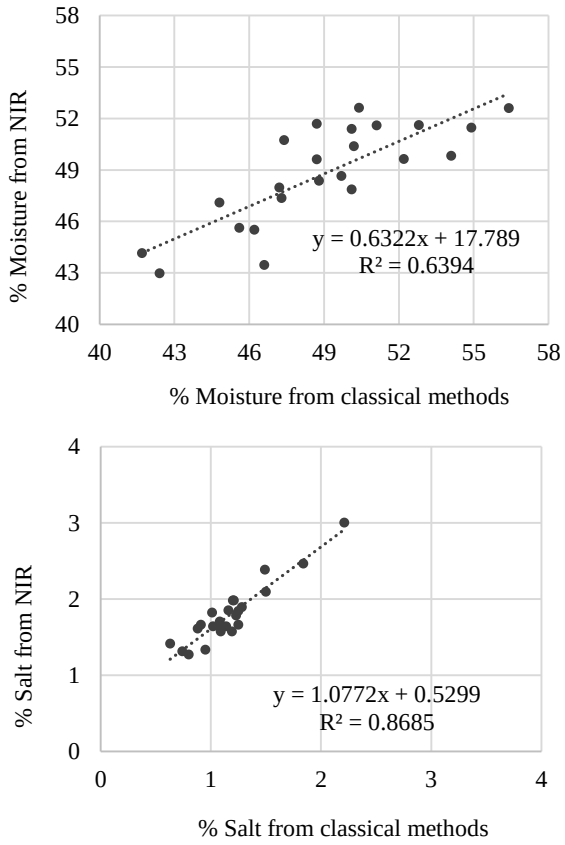

Figure 2. The relationship between classical methods and indirect method (FT-NIR) for SE cheese protein and fat determinations

The salt content regression noticed a good slope and intercept of 1.077 and 0.53, respectively, and a good SEP of 0.13, but at the same time showed a deterioration of the determination coefficient (0.869) indicating a poorer relation between values predicted by NIR and classical analysis (Table 2 and Figure 2).

Conclusions

Broad-based calibration, such as the one that Buchi NIRMaster™ holds and was used in this study, presents some benefits including increased robustness and reliability. However, some parameters such as moisture and salt content of Serra da Estrela cheese samples may need broader adjustment of the FT-NIR calibration model, by increasing classical methods (reference) and NIR sample analysis.

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