"AUTHENTICITY ISSUES IN DAIRY PRODUCTS DEALT BY NIR SPECTROSCOPY"

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NIR AND AUTHENTICATION - OUTLINE

- ✓ **Milk powder:**
  - ➢ **Whey powder addition**
  - ➢ **Addition of vegetable proteins from different sources**
- ✓ **Yogurt:**
  - ➢ **Detection of thickening agents, produced by microbial strains or added as additives**
- ✓ **Cheese:**
  - ➢ **NIR ability in predicting sensory characteristics, which are part of the quality standard for PDO products**
  - ➢ **Study of shelf life of a fresh cheese to classify commercial products on the basis of their age**
  - ➢ **Classification of grated Grana Padano cheese on the basis of supplier and geographical area**

✓ **Milk powder:**

➢ **Whey powder addition**

R. Giangiacomo, F. Braga, C. Galliena. 1992. In: Making Light Work: Advances in Near Infrared Spectroscopy. I. Murray & I.A. Cowe Eds. VCH (UK), pp. 399-407

Addition of whey powder to milk powder to increase the volume of this product is a common deception.

Classical analytical chemical methods to detect the presence of whey powder are time consuming. There is, therefore, the need for a more rapid and easy technique.

Near Infrared Reflectance (NIR) spectroscopy has been used to investigate the possibility of a quantitative method for the determination of whey powder added to milk powder.

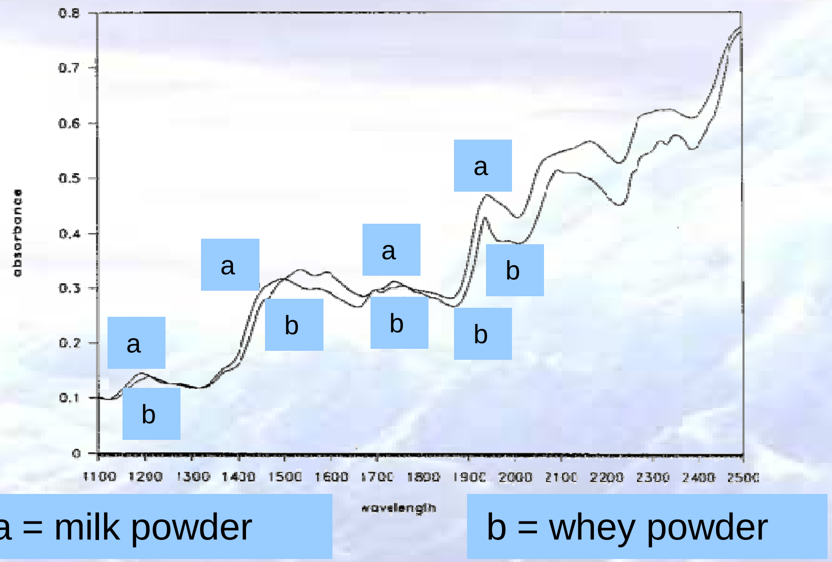
A calibration set of 61 samples was prepared by mixing whey and milk powders, with the whey content ranging from 0 to 30% w/w.

NIR spectra were collected by an InfrAlyzer 500 (Bran+Luebbe, Germany) in reflectance mode.

Wavelength range: 1100 – 2500 nm, 4 nm intervals.

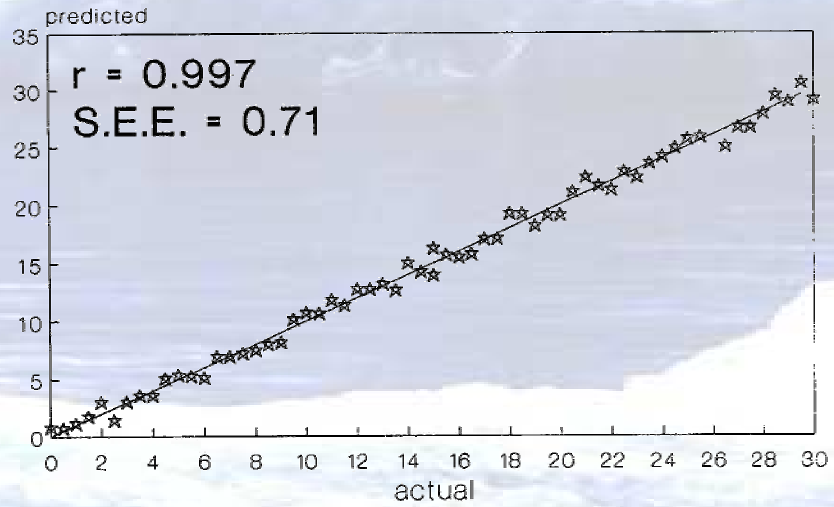
Multiple linear regression and discriminant analysis were used for data processing.

COMPARISON OF WHEY AND MILK POWDER SPECTRA

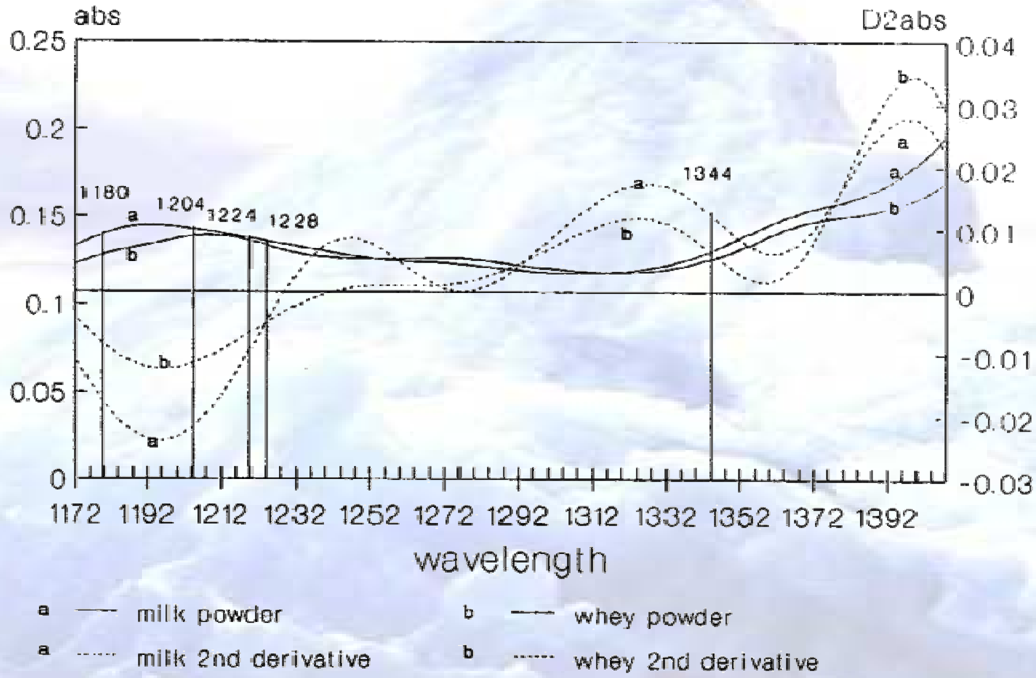


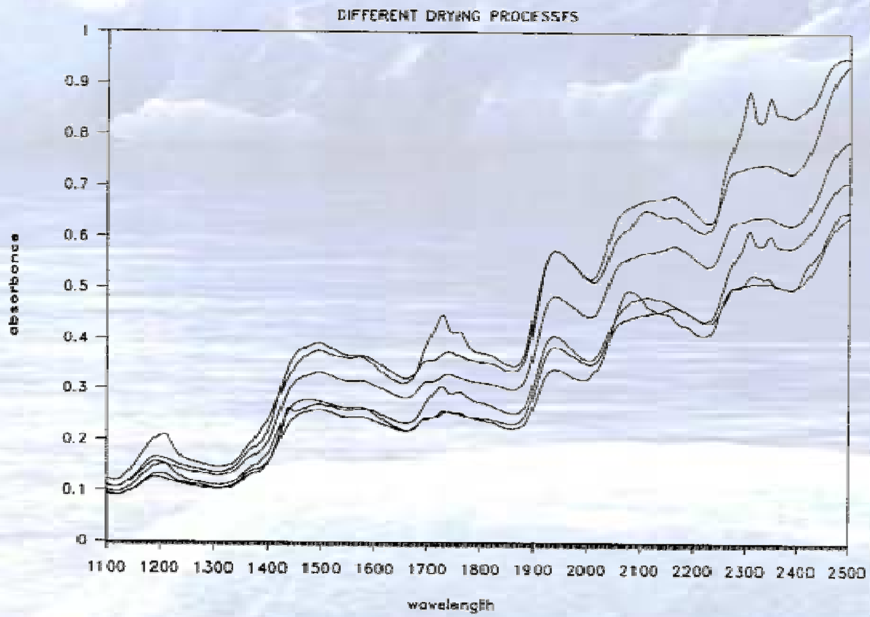
It is possible to observe that the differences are mostly highlighted in the regions 1450-1700 nm and 1950-2300 nm.

Using a calibration set of 61 samples, the best performance from multiple linear regression was obtained using a five wavelength equation.



Second derivative spectra amplify the differences in these wavelength regions





Different drying technologies affect the shapes of spectra of various milk powder types

Considering that the addition of whey is a deception at any concentration, discriminant analysis was applied to a calibration set of 4 pure samples, 31 samples with whey addition ranging from 0.5 to 30% and 6 milk powders dried using different technologies.

The prediction results indicated that just one of pure samples was misclassified as belonging to the added whey class.

The 37 mixtures were correctly classified according to their whey concentration indicating that classification was precise even for samples with low whey addition, while the lower precision in classification of milk powders confirms the importance of the interference effect of different drying technologies.

True Class		Predicted Class	
		M	W
	M	3	1
	1	0	17
	2	0	14
	3	0	6

CONCLUSIONS

NIRS could be a good tool to routinely detect the whey addition to milk powder.

Data demonstrated that it is important to develop a calibration over a wide range of whey percentage because it forces wavelength selection related to whey presence rather than to the drying treatment.

Discriminant analysis appears to be sufficient to reveal the presence of whey powder also at low concentrations.

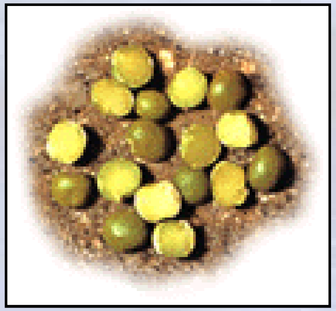
Since, in Italy, the addition of whey is not only a commercial fraud but it is also a criminal offence, positive cases have to be confirmed by officially recognised and legally acceptable analytical methods as cited in EEC regulations.

✓ **Milk powder:**

➢ **Addition of vegetable proteins from different sources**

CATTANEO T.M.P., MARABOLI A., BARZAGHI S., GIANGIACOMO R. (2002) In: Proceedings 10th Int. Conf. on Near Infrared Spectroscopy, Eds. A.M.C. Davies & R.K. Cho. NIR Publications 2002, Chichester, UK. pp.161-166.

MILK PROTEIN REPLACEMENT



on the basis of their cost and their functional properties (yield increase, foaming formation, etc…)

There may be the risk that this fraud is practised in
milk powder, yoghurt and processed cheese

AUTHENTICITY

GENUINITY

QUALITY CONTROL

No official methods are available

Methods usually used:

Electrophoretic procedures

Competitive ELISA

AIM

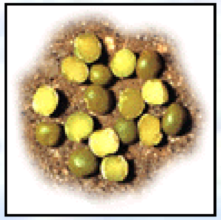
To prove the feasibility of NIR spectroscopy to detect individual vegetable protein isolates (soy, pea and wheat) in milk powder.

Range: 0 – 5% in milk powder

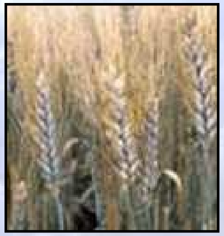
99

224 samples

62



63



Protein isolates were directly added to liquid milk, then spray drying technology was applied.

Control of homogeneity, stability and solubility was made.

NIRS measurements (InfrAlyser 500)

The calibration and prediction sets were prepared by random selection of samples maintaining roughly constant the representation of the addition percentages.

Range 1100 – 2500 nm (intervals: 4 nm; 351 data points)
Reflectance mode; light absorption expressed as log (1/R)

Data pre-treatments (Sesame software):
data normalisation
first derivative of absorbance values

Pre-treated data were processed by using Partial Least Squares Regression PLSR, using the full spectrum (Sesame software).

Cross-validation

SOY

NIR

SEE: 0.193; RMSEPcv: 0.301; RMSEPcal: 0.181; RMSEPpred: 0.229; RMSEPall: 0.191

SEE capillary electrophoresis: 0.77 - SEE ELISA method: 1.10

CONCLUSIONS

NIRS was able to determine more accurately than the other two techniques the percentage of adulteration in the analysed samples

NIRS proved to be an useful tool to detect this kind of adulteration in milk powder with good accuracy

Dedicated calibrations could also help in identifying what type of vegetable protein isolate was added

✓ **Yogurt:**

- ➢ **Detection of thickening agents, produced by microbial strains or added as additives**

GIANGIACOMO R., DELGADILLO I., MEURENS M.. 1998. "Discrimination between naturally produced thickening agents and thickening additives in fermented milks". In Lees M. Eds., "Food Authenticity - Issues and Methodologies", Eurofins Scientific, Nantes, France, pp. 97-101.

PRIORITY

The distinction between naturally produced polysaccharides increasing thickness of fermented milk and substances specifically added to this purpose.

The addition of thickening agents is forbidden in yoghurt, but it is permitted in other fermented milk where they need to be declared on the label.

No official methods nor other suitable analytical procedures exist for this differentiation that are easy to use and at a stage of collaborative studies.

AIM

To investigate the use of spectroscopic techniques for developing a potentially feasible procedure suitable to this purpose.

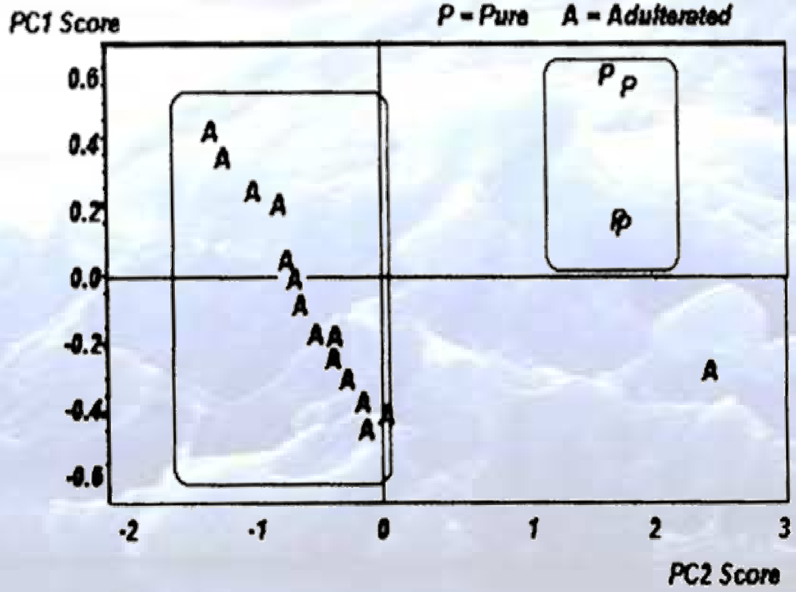
4 skim yoghurts were prepared using combinations of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* ropy and non-ropy.

5 different additives at 3 different levels have been added to the yoghurt obtained from the combination of the two non-ropy strains, obtaining a set of 19 samples.

FREEZE-DRYING

NIR spectral data were recorded in two different labs using two different instruments (InfrAlyser 500 and NIR System 6250) in the wavelength range from 1100 to 2500 nm.
Fresh samples were measured at 4℃ in a cell purposely designed for liquids.
Two spectra were recorded for each sample and each was the average of six replicates.

NIR spectra on fresh samples could not be successfully processed due to the hgigh water content. The very strong water absorption in this region covers the information due to the very limited amounts of additives.



PCA applied to the NIR spectra on the freeze-dried samples gave an optimum number of 3 factors, but only two were sufficient to discriminate between pure yoghurt and samples with added substances.
The score plot PC1 vs. PC2 did also permit to distinguish *Streptococcus thermophilus* ropy and non ropy.

PCA applied to the NIR spectra on the freeze-dried samples, but collected with the second NIR apparatus and processed by a different statistical software, confirmed the possibility to discriminate between *Streptococcus thermophilus* ropy and non ropy in pure samples (Fig. 2).

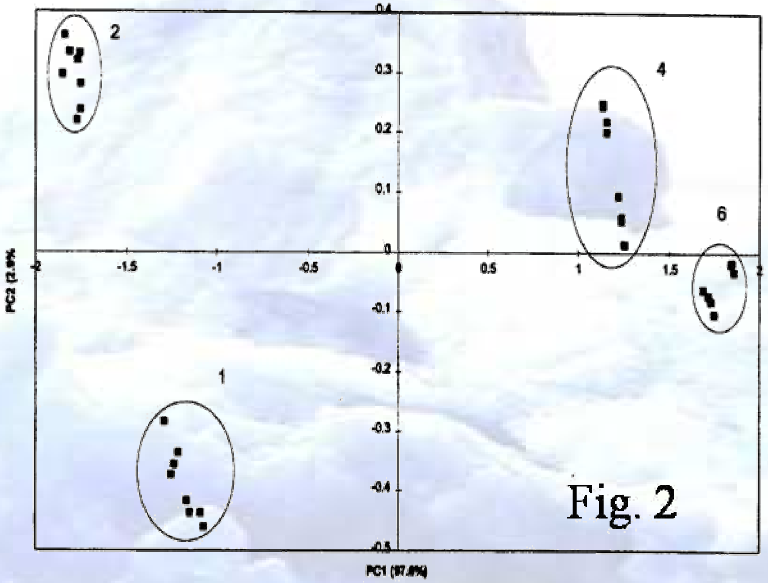


Fig. 2

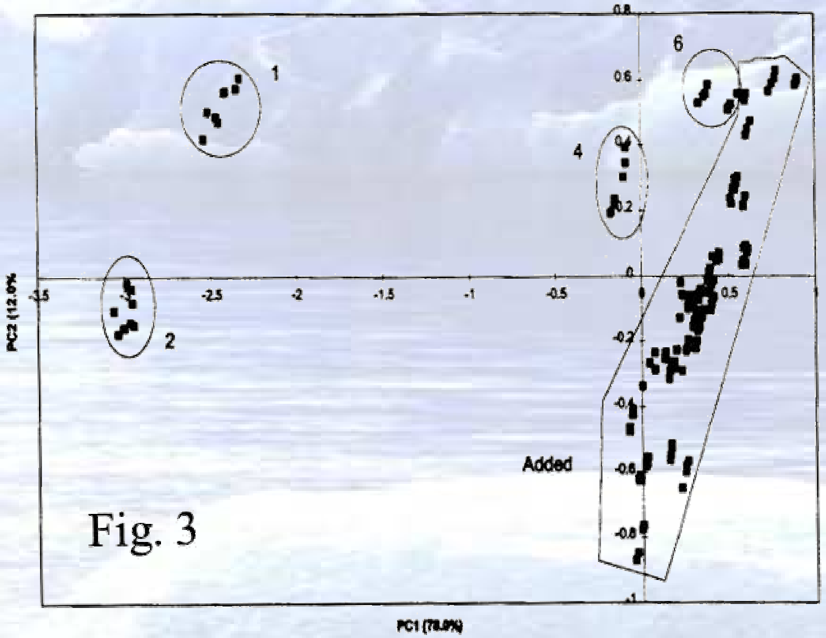


Fig. 3

Fig. 3 shows the score plot for natural samples and yoghurt added with different additives. While it was possible to discriminate between pure and added samples, it seems to be difficult to achieve a discrimination inside the group of added yoghurts, also when the PCA was applied only on the spectra of the samples with additives.

CONCLUSIONS

The use of NIRS shows potential suitable approach to the solution of this authenticity issue.

However, further analysis on much larger data sets should be carried out to validate these preliminary results.

✓ **Cheese:**

➤ **NIR ability in predicting sensory characteristics, which are part of the quality standard for PDO products**

T. CATTANEO , C. TORNIELLI , S. ERINI , E. PANARELLI. 2008. J. Near Infrared Spectrosc., 16, 173-178

Historically, specific types of cheese are made in certain geographic areas. Often they have unique flavour characteristics. Studies have suggested the role of local pastures in determining cheese aroma.

Bitto is a PDO cheese only produced in summer in Valtellina (Lombardy, Italy) from June 1st to September 30th of every year, using local forage and pastures for feeding.

The aim was to study the relationship between sensory scores and NIR data in verifying which attributes can be related to specific absorption bands in the whole NIR region.

This research is part of a larger project, that will be developed over the next two years, with the final aim to create a model able to identify the **high quality and "genuine" Bitto cheese**.

Because flavour perception is multivariate in nature, where multiple compounds produce multiple sensory sensations, it seemed highly appropriate to use multivariate modelling to seek relationship between NIR data and perception.

38 Bitto moulds, with assigned sensory scores

4 samplings each
+
grating

NIRFlex FT-500 (BUCHI, Flavil, CH)

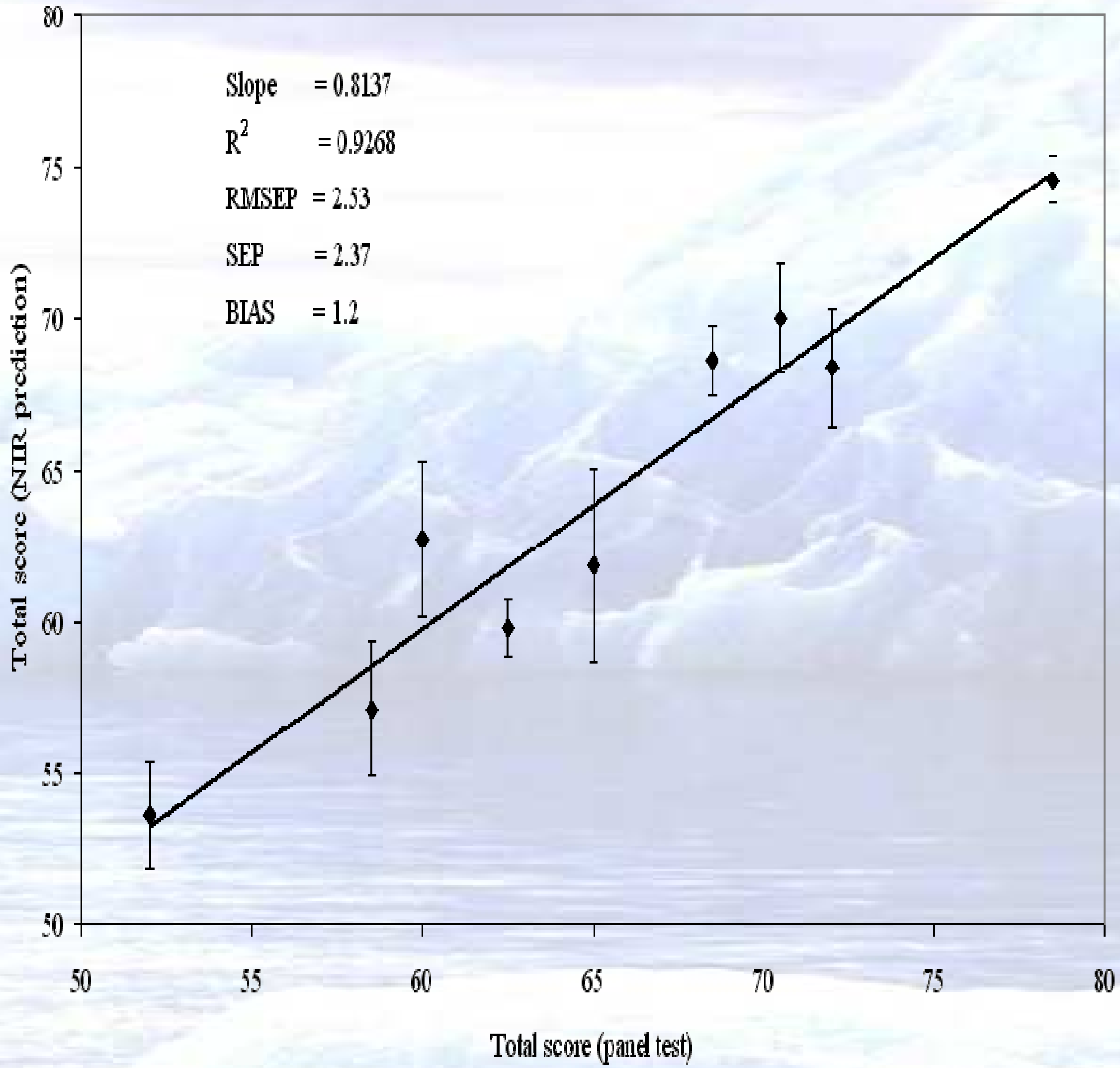
152 spectra

Petri cap (ID: 100mm; sample thickness: 10 mm)

Reflectance mode (resolution = 8 cm-1; 32 scans).

Calibration/Prediction curves for “Total Score” and “Taste and Flavour” attribute were built using a full cross-validation procedure. The models were tested using a test set of 9 samples.

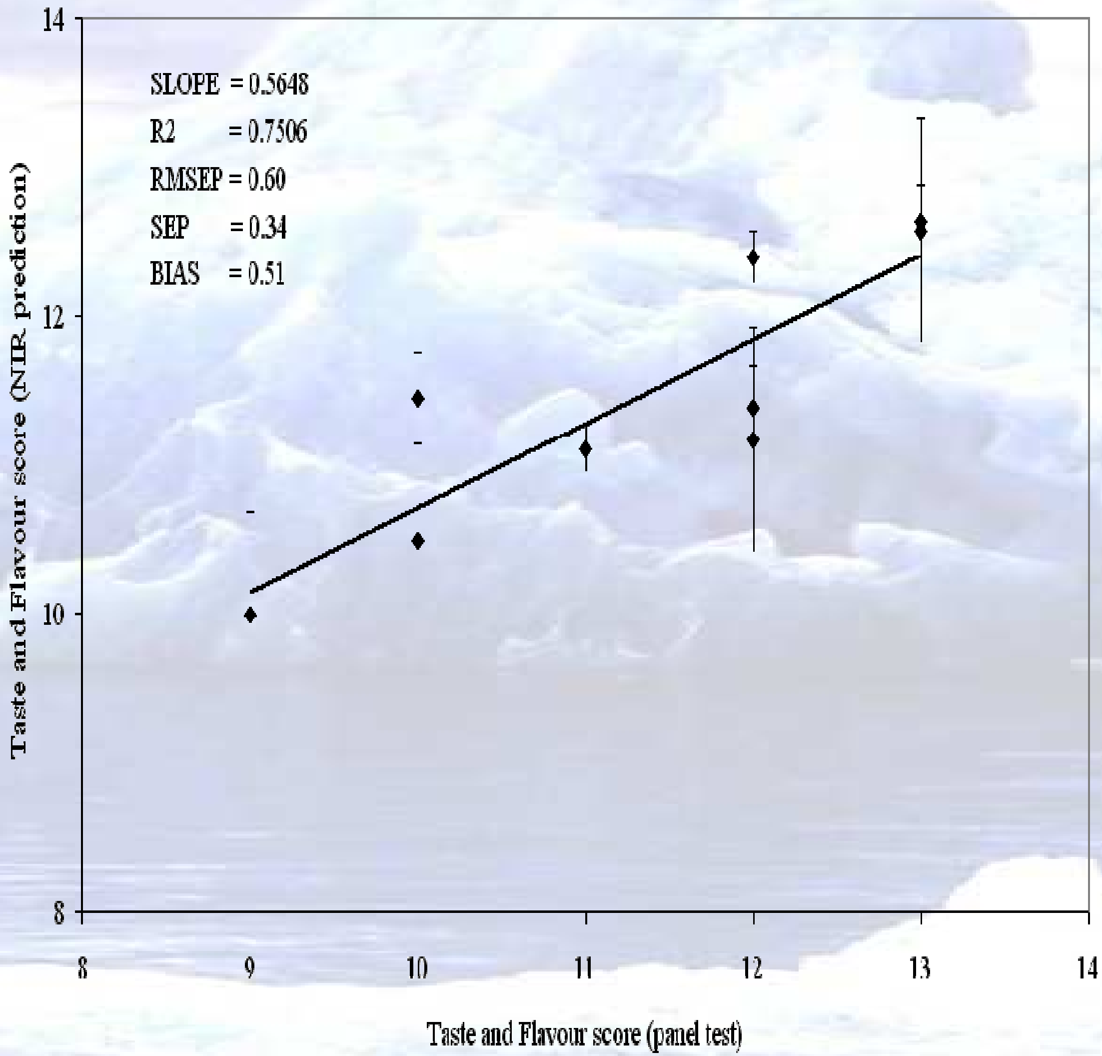
“Total score”: test set. PLSR predicted/measured plot



Results were very satisfactory proving the applicability of NIR technique to predict sensory properties. They were in good agreement with scores assigned by the panel of experts suggesting the possibility to use NIR as objective measurement of cheese sensorial quality. A standard deviation between 0.12 and 3.92 was found to be associated to NIR prediction of Total Score in the range of score values from 50 to 80.

"Taste and Flavour Score": test set. PLSR predicted/measured plot

The obtained predicted values showed an acceptable correspondence with those obtained by the panel of experts (results are reported as the average prediction data of four replicates).
A standard deviation between 0.14 and 1.04 was found to be associated to NIR prediction of Taste and Flavour Score in the range of score values from 8 to 14.



Conclusions

The preliminary results, achieved in this experimental work, proved that FT-NIR can be an useful tool for the prediction of cheese sensory characteristics in terms of Total Score and Taste and Flavour Score.

The total available set of 39 samples was also used to verify the feasability of FT-NIR for samples classification in three "quality" classes.
Satisfactory results were achieved in term of percentage of correct prediction.

The identification of some absorption bands related to the development of taste and flavour have to be confirmed carrying out additional measurements by using analytical techniques, such as GC-MS, HPLC and capillary electrophoresis (CE), able to better identify flavour compounds and/or molecular markers responsible to the final cheese quality.

✓ **Cheese:**

➢ **Classification of grated Grana Padano cheese on the basis of supplier and geographical area**

S. BARZAGHI, K. CREMONESI, A. PERRONE, G. CONTARINI, G. FERRARI and T.M.P. CATTANEO. 2004. In: Proceedings of 11th Int. Conf. on Near Infrared Spectroscopy, Cordoba, Spain.

A.M.C.Davies & A. Garrido-Varo Eds., NIRPublications (UK), pp. 613-616.

Grated cheese fits into the category of convenience-foods and their trade is in continuous increase due to some advantages such as it is simple to use, there is no waste, it is ready to consume, etc..
Italian long ripened hard cheeses, and in particular Grana Padano and Parmigiano Reggiano at different age and deriving from productions of many kinds are used to preparing these products.
Packaging in modified atmosphere or under vacuum is used to preserve the original quality.

Packed grated cheeses should be stored at low temperature (4°C) during the whole shelf-life in order to minimize chemical and microbiological modification phenomena.

AIM

To test the ability of FT-NIRS in discriminating grated Grana Padano cheese deriving from different sources.

The discriminating power of this technique was compared with that obtained by evaluating the indices more commonly used to assess the chemical and microbiological quality of these products.

Seventy-eight samples packed under vacuum were collected from two different Italian companies (A; D), stored at low temperature and analysed at the expiry date.

Some microbiological (coagulase negative staphylococci, enterococci, yeasts and moulds, aerobic and anaerobic sporeforming bacteria and chemical [pH, aw by a CX-3 AQUA LAB apparatus (Decagon Devices, Inc. USA), moisture] indices were determined.

PCA and Linear Discriminant Analysis (LDA) were applied to evaluate the results.

NIR spectroscopy

FT-NIR spectra were collected in the full range from 4000 to 10000 cm^{-1} with a NIRLab N-200 [BÜCHI Italia S.r.l., Assago (MI)] apparatus (64 scans for each spectrum), using Petri's glass plates in transflectance mode, with 4 cm^{-1} resolution (1557 points).

PCA was applied to the whole spectrum, and LDA was carried out using the absorbance values at six selected wavenumbers, chosen on the basis of their relation with the most important constituents. The selected wavenumbers were related with water (5187, 8990 cm^{-1}), protein (5862, 8095 cm^{-1}), and fat (5700, , and 8277 cm^{-1}) absorptions.

LDA was validated by leave one out evaluation set technique.

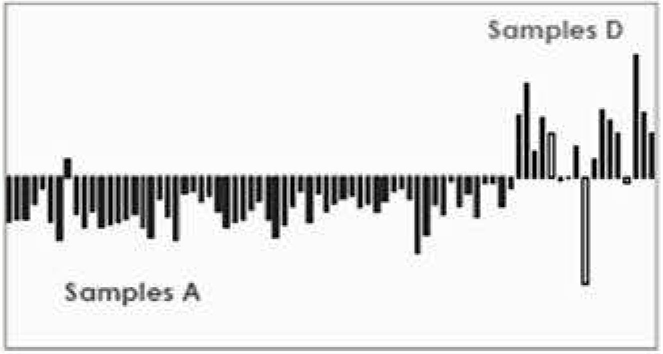


Figure 2 –Histogram for the discriminant scores obtained by LDA carried out on 8 variables and 78 objects.

Table 1 - Prediction of origin of grated Grana Padano cheese samples by LDA by using microbiological and chemical indices.

Category	No. of prediction for category A	No. of prediction for category D	% Correctly predicted
A	59	2	96.72
D	4	13	76.47
Overall			92.31

Satisfactory discrimination among packing companies was obtained using the microbiological and chemical parameters with a correct total prediction of about 92%, also if category A was better predicted than category D.

By NIRS, the value obtained for category D (88.23%) was significantly increased in comparison with that obtained using microbiological and chemical indices.

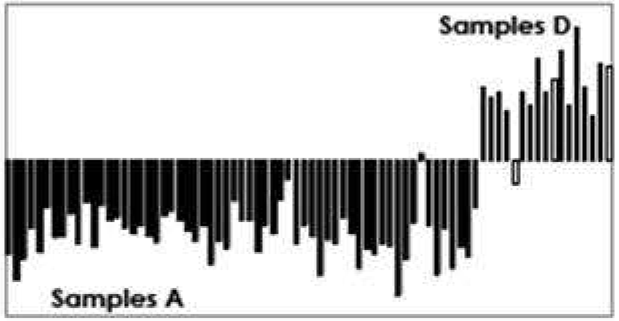


Figure 5 – Histogram for the discriminant scores obtained by LDA carried out on 6 variables and 78 objects

Category	No. of prediction for category A	No. of prediction for category D	% Correctly predicted
A	60	1	98.36
D	2	15	88.23
Overall			96.15

Table 2 - Prediction of origin of grated Grana Padano cheese samples by LDA by using spectroscopic data.

Conclusions

Statistical analysis applied to FT-NIR data was able to classify samples with a good discrimination in two groups, on the basis of the spectra characteristics.

FT-NIRS showed a better total prediction power (percentage of correct prediction = about 96%) than microbiological and chemical indices (percentage of correct prediction = about 92%).

NIR results suggested the potential strong influence of the grating and packaging technology used rather than that of individual parameters related to product characteristics.

The availability of samples derived from several packing companies could improve and confirm these preliminary results. In this way spectral information could be associated with defined technological steps.

GENERAL CONCLUSIONS

NIR spectroscopy started more than 30 years ago with the measurement of agricultural products in laboratories.

Nowadays we can see NIR spectroscopy equipment installed within harvesters and combines.

With low-cost instruments, the technology will go also onto the farm to analyze milk quality on a milking by milking basis.

Robust, relatively cheap portable spectrometers will undoubtedly be available in the near future.

Quality of feed and food products will also benefit from this technology.

With no reagents, simple settings and robust hardware, NIR spectroscopy can potentially offer a complete laboratory within a single instrument.

This feature will become a major advantage in installing new instruments, especially in the developing countries.

Thanks for your attention