CHEMOMETRICAL STRATEGIES IN ORDER TO SOLVE DIFFERENT PROBLEMS IN FOOD SCIENCE

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Abstract − The primary objective of the present study is to show how chemometrical strategies can be useful for processing multidimensional complex non-selective data in order to address issues in the food science.

Three different case studies are shown, where different analytical procedures and chemometrical tools were used in order to reach different tasks.

Keywords: Chemometrics, food analysis, non-selective data.

1. INTRODUCTION

Advances in technology and the increasing availability of powerful instrumentation now offer analytical food chemists the possibility of obtaining high amounts of data on each sample analyzed, in a reasonable time frame [1]. Spectroscopic techniques for example may supply, with a single and rapid analysis on a sample, multiple data of homogeneous nature: in fact, a spectrum can be considered as a data vector in which the order of the variables (*e.g.*, absorbance at consecutive wavelengths) has a physical meaning [2].

Univariate methods are still the most used in many cases, although they generally offer only a very limited vision of the global situation.

Chemometrics provides the possibility to extract useful information from big data matrices and the development of new chemometrical methods of analysis continues to advance the state of the art in analytical instrumentation and methodology. Chemometrics is widely applied in the food sector; here, three case studies are presented.

1) Hazelnuts roasting differentiation: European hazelnut (*Corylus avellana* L.) is the most important nut species in the *Betulaceae* family from an economical point of view. There is a general agreement on the fact that, among all varieties cultivated worldwide, hazelnuts from Piedmont (Italy) are among the most valuable, with a particular reference to the *Tonda Gentile Trilobata* (TGT) variety (covered by the PGI designation "*Nocciola Piemonte IGP*", if cultivated in specific areas and according to the disciplinary of production), which is widely recognised as the best-suited hazelnut for industrial transformation into roasted kernels. Roasting is the most important practice for improving preservation of hazelnuts. A common method for hazelnut roasting is a convective heat transfer process, performed in hot-air roasters working either in continuous or batch systems [3]. In contrast to conventional heating mechanisms, in which heat is usually transferred from the surface to the interior, infrared heating was successfully used for dry-roasting and pasteurization of almonds. Infrared (IR) radiation supplies energy in the form of electromagnetic waves, allowing a more rapid heat transfer than convectional conduction mechanisms. IR heating has been found to be more effective compared to conventional heating [4], but requires an initial high capital cost. For the processing industry, it is crucial to know the effect of roasting process and conditions, (*e.g.*, time and temperature) on the hazelnut quality attributes like moisture, colour and rancidity. Different roasting methods in fact were found to yield significant differences in humidity, antioxidant capacity, total phenolic content, protein concentration and other nutritional or technological values.

In this first case study, the effects of hot-air and IR roasting on *Tonda Gentile Trilobata* hazelnut from Piedmont have been investigated in order to evaluate if it is possible to detect the most suitable roasting process depending on the final use of hazelnuts.

Near infrared spectroscopy (NIRS), a rapid and non-destructive technique, has been used for the first time to differentiate *TGT* hazelnut and its processing products (paste and oil) on the basis of the roasting process and conditions.

2) **Porcini mushrooms characterisation**:
Boletus edulis Bull. and allied species (BEAS), known as "porcino mushrooms", represent almost totality of mushrooms placed on the market, both fresh and dehydrated. Due to the globalisation of the mushroom trade, most of the porcini commercially available in Italy or exported by Italy no longer originate there. In most cases, they are collected in Eastern Europe and China, dried on site, and then, after a first selection, imported into Italy. It is not rare to find, among such imported dried boletes, mushrooms of different – less valuable – species. Analyses performed to define macrofungi eligible for sale are mainly based on naked eye inspection by mycologists, aimed at identifying alien species and/or macromorphologic alterations.

One of the species that are most commonly used for adulterating BEAS is *Tylopilus felleus* [5]. In fact, once dried, it is morphologically very similar to BEAS, but very different from an organoleptic point of view. So, BEAS and *Tylopilus felleus* can be easily confused with each other by simple visual inspections, but a taste test would allow to differentiate them, since BEAS are savoury while *T. felleus* is intensely bitter. BEAS derived products may be adulterated even with the purplish-hued Asian species *Boletus violaceofuscus*. It is quite distinct in appearance, especially when fresh, presenting a dark violet cuticula and a conspicuous white reticulation on the stipe. Anyway, identification of small amounts of such a species within dried specimens could be not so straightforward. The present case study describes the development of a rapid, efficient and non-destructive analytical method, based on near infrared spectroscopy (NIRS) coupled with chemometrics, to detect fraudulent additions of lower-quality mushroom species within *Boletus edulis* products.

3) **Yogurt stability evaluation**:
Yogurt is a basic dairy product that has been consumed widely as a part of the diet, even when its beneficial effects were neither fully known nor scientifically proven. According to Codex Alimentarius (Codex Stan 243-2003) yogurt is a product obtained by fermentation of milk by means of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*. Whereas milk has a limited shelf life, yogurt can be kept for up to 20-40 days, under proper storage conditions [6]. Changes in the physical,

chemical and microbiological properties of yogurt determine stability and shelf-life of the product and alterations of these properties cause color, texture and taste deteriorations which are considered important quality criteria by consumers. In the dairy industry, monitoring of products in terms of quality can be performed via physicochemical measurements, generally performed by chromatography-mass spectrometry [7]. However, these methods are time consuming. The increasing demand for fast, reliable and objective techniques to determine stability of dairy products during storage encourages scientists to use non-selective analytical techniques, such as spectroscopic methods that are rapid, non-destructive and require a minimal or no sample preparation. The present case study aimed at investigating the feasibility of UV-VIS spectroscopy with an integrating sphere for diffuse reflectance measurements as a rapid and alternative technique to traditional analytical methods to monitor the stability of yogurt up to 49 days of storage at 4 °C.

2. MATERIALS AND METHODS

1) The effects of hot-air and IR roasting on *Tonda Gentile Trilobata* hazelnut from Piedmont have been investigated in order to evaluate if it is possible to detect the most suitable roasting process depending on the final destination of hazelnuts; in particular, two different conditions for each method (hot-air or IR) have been considered (Normal: low temperature for long time, Fast: high temperature for short time) for a total of four types of roasting. A total of 92 samples were used for data analysis.

Moreover, oils and pastes obtained from hazelnuts roasted following the 4 different processes were analysed. Spectroscopic measurements were made with a FT-NIR spectrometer (Buchi NIRFLEX N-500) in the 4000–10,000 cm^{-1} range with 8 cm^{-1} resolution and 64 scans on the intact hazelnuts, paste and oil. Then Principal Component Analysis (PCA) [8] was applied as a display method in order to extract the useful information from the data.

2) Eighty samples of dried mushrooms were analysed: 44 samples of *Boletus edulis* (the target class to be modelled) and 36 samples belonging to

other species of mushrooms morphologically similar including *Tylopilus felleus* and *Boletus violaceofuscus*. NIR spectra were acquired in the 4000–10,000 cm^{-1} range with 8 cm^{-1} resolution and 128 scans using a FT-NIR spectrometer (Buchi NIRFLEX N-500). The spectra were used to develop reliable and efficient class-models using a novel method, partial least squares density modelling (PLS-DM)[9], and the two most commonly used class-modelling techniques, UNEQ [10] and SIMCA [11]. PLS-DM is a novel class modelling method proposed by Oliveri et al. in 2014 which combines the features of PLS and potential function methods, together with *Q* statistics, to obtain highly efficient class models.

3) Eight samples of yogurts (4 white high-fat, 3 white low-fat and 1 blueberry high-fat) produced in the same day were collected from 4 local dairy farms. Each yogurt was split into eight separate jars, which have been subsequently stored at 4 °C and analysed by UV-VIS spectroscopy once a week until the 49th day of storage.

UV-VIS measurements were performed by placing the yogurt in a 1 cm quartz cuvette in front of the incident light window, and focusing the light reflected from the sample on the detector inside the 60 mm integrating sphere (Agilent, Cary 100, Santa Clara, US). Spectra were acquired in the range 190-900 nm, with spectral bandwidth=4 nm and average time=0.1 s (a method to improve the signal-to-noise ratio) as suggested by a preliminary 2^2 factorial design *(data not shown)*. The spectral region between 800 and 900 nm was removed because not informative, thus, each sample was described by 611 values of reflectance.

PLS [12] was performed as a very effective method for predicting the age of the yogurt samples from their UV-VIS spectra.

3. RESULTS AND DISCUSSION

1) Hazelnuts roasting differentiation

Intact hazelnuts were analysed by NIRS then PCA was applied as a display method on the NIR spectra.

Fig. 1: **Hazelnuts-** PCA score plot of 92 FT-NIR spectra. Hazelnuts roasted with different processes (hot air=A and IR=IR) and conditions (normal or fast=F) are shown.

The score plot on PC1-PC2 (Figure 1) explaining the 87% of the total variance, obtained from the NIR spectra of intact hazelnut after SNV (Standard Normal Variate) and autoscaling as data pre-treatments, shows that it is possible to differentiate hazelnuts on the basis of the roasting process and conditions by NIR spectroscopy.

Fig. 2: **Hazelnut Oils-** PCA score plot of FT-NIR spectra. Oils from hazelnut roasted with different processes (hot air=A and IR=IR) and conditions (normal or fast=F) are shown.

The score plot on PC1-PC2 (Figure 2) obtained from the NIR spectra of hazelnut oils after SNV (Standard Normal Variate) and autoscaling as data pre-treatments, shows that these oils can be differentiated on the basis of the hazelnut roasting process by NIR spectroscopy. Very similar results

were obtained also for the hazelnut pastes (score plot not shown).
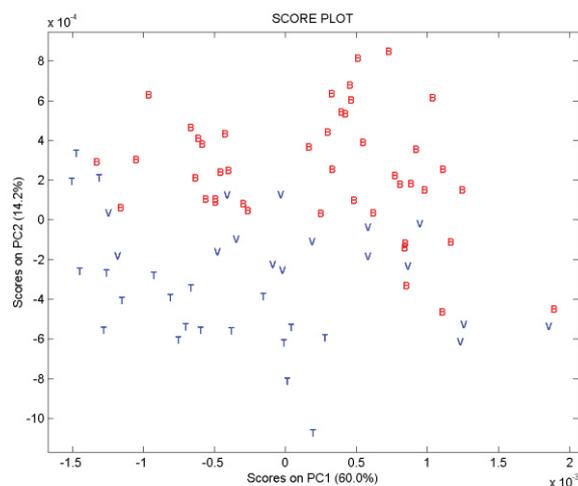
2) *Porcini mushrooms characterisation*

Fig. 3: **Mushrooms** - PCA score plot of FT-NIR spectra of different mushrooms (B=*Boletus edulis* and related species – BEAS, T=*Tylopilus*, V=*Boletus violaceofuscus*).

The score plot on PC1-PC2 explaining the 75% of the total variance shows that it is possible to differentiate BEAS fungi from other similar species by NIR spectroscopy.

As a class-modelling technique, PLS-DM, proved to be the most suitable method for the characterisation of *Boletus edulis* based on NIR spectra. In fact, the PLS-DM model was more balanced in terms of sensitivity and specificity reaching efficiency of 80% on the external test set, if compared with UNEQ and SIMCA models. Such a model can be considered absolutely suitable for screening purposes, considering the nature and complexity of the problem under study.

3) *Yogurt stability evaluation*
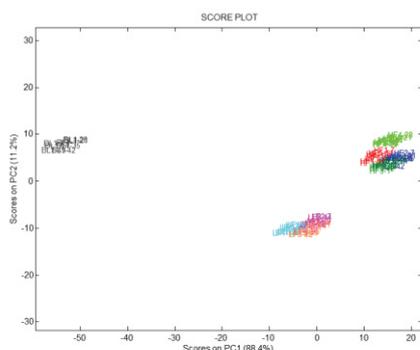
Fig. 4: **Yogurt** - PCA score plot of UV-VIS data: score plot on the plane PC1-PC2. The samples are coded by type, sample number and storage time (*e.g.,*

HF2_14=High Fat yogurt, sample 2, storage time 14 days).

The blueberry sample (BL1) had a negative score on PC1, while the low-fat samples (LF) had negative scores on PC2. Almost no time effect could be detected.
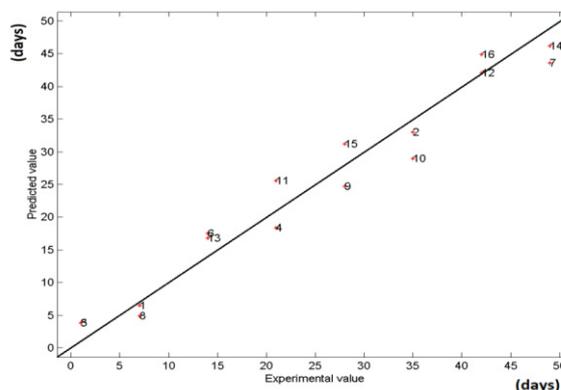

Fig. 5: **Yogurt** - PLS model. Experimental vs. predicted values (days of storage) for the external test set samples (16 samples randomly selected).

PLS was performed on the whole spectra; the optimal complexity of the PLS models, i.e. the number of latent variables (LV), was chosen on the basis of the explained variance in cross validation (5 cancellation groups); the models were calculated at the optimal LV number and the standard error in prediction (SEP) was finally obtained, using an independent external test sets with 16 spectra (2 for each time) randomly selected. Figure 5 shows the experimental versus the predicted response (days of storage, from 0 to 49) for the external test set; the performances of this model were quite satisfactory: SEP: 3.3 (days); bias: -0.1.

4. CONCLUSIONS

The three case studies reported show that chemometrical strategies can be very useful for extracting significant information from multidimensional non-selective data, such as those obtained from spectroscopic techniques, in order to address different issues in the food science, from food characterisation or adulteration to stability evaluation during storage.

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