Determination of free amino acids in Bitto cheese: a preliminary approach

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Introduction

Bitto cheese is a semi-hard cheese very appreciated and known among cheese produced in Valtellina (Lombardy, Italy). It is a cheese of Protected Denomination of Origin (PDO) only produced in summer, from whole raw milk and local cow breeds. Sometimes, up to 10% of ovine milk can be added.[1] The Denomination of Origin for Bitto cheese[2] was created in, and it is guaranteed by the "Consorzio Tutela dei formaggi Valtellina Casera e Bitto", since 1995. The quality of PDO cheese is more and more required to be guaranteed to satisfy the consumer demands of safe and authentic products, and the continued differentiation of food ingredients and products complicates the assurance of authenticity.

Important parameters for the evaluation of cheese characteristics, are some degradation products of proteolysis, peptides and peptones as well as free amino acid (FAA) pattern.[3,4]

The uniqueness and the type of a cheese arise from a balance of fermentation and enzymatic processes, and these characteristics can be represented by considering the free amino acid pattern, especially in long ripening cheeses, such as Grana Padano and Parmigiano-Reggiano.[5] The specificity of these indices was also demonstrated for Montasio cheese ripening, in which the presence of gamma-aminobutyric acid (GABA) and alpha-aminobutyric acid (AABA), within certain limits, is related with some aspects of cheese-type.[6]

The analysis most commonly involved in the identification of authenticity characteristics requires the use of methods that more and more frequently involve the combination of different analytical techniques.[7,8] The availability of a reliable and rapid technique to assess food quality and authenticity, such as near infrared (NIR) spectroscopy is, therefore, required.

The aim of the present work was to verify the ability of NIR spectroscopy to predict Bitto cheese quality on the basis of the quantitative determination of chemical compounds identified as typical for its production, particularly with respect to selected free amino acids (FAA), related to the product origin and ripening.

Materials and methods

Thirty-nine Bitto moulds, aged from 70 to 120 days, were analysed by HPLC (Thermo Scientific, Rodano, Milan, Italy) for FAA content by using a RP-HPLC procedure[9] (pre-column derivatisation with OPA; C18 column – 150 mm × 4.6 mm; Flow: 0.9 mL min^{-1}; Column Temperature:

40°C; Loop: 20 µL; Internal Standard: Glucosaminic Acid; External Standard: 21 FAA standard solution (Fluka, Buchs-Switzerland); Fluorescence Detector: λ_{ex}:340 nm, λ_{em}:420 nm). Samples were homogenised and protein precipitation was carried out before analysis. Peak identification and data processing were carried out by using the ChromQuest Software v. 4.2.34 (Thermo Scientific).

On the same samples NIR spectra were collected after grating, by using a NIRFlex FT-500 (BUCHI, Flavil, Switzerland) in the whole NIR range from 1000 to 2500 nm. Spectra were recorded in reflectance mode, sampling moulds four times each (resolution = 8 cm^{-1}; 32 scans), and using Petri caps (ID: 100 mm; sample thickness: 10 mm). To identify the amino acids mainly affected by cheese ripening, principal component analysis (PCA) of the amino acid data was performed (Unscrambler 9.2, Camo Inc., OSLO, Norway).

Before data processing, mean-centring was applied as pre-treatment, ensuring that all results would be interpretable in terms of variation around the mean. In order to reduce varying light scatter effects, the enhanced multiplicative scatter correction (EMSC) method was applied. PLS2 (11 LV) and Cross-Validation using the Leave One Out technique (CV-LOO) were applied in order to estimate the ability of NIRS in predicting the content of some characteristic FAAs in Bitto samples. PLS1 (11 LV) and CV-LOO were used to determine the total FAA content on the same samples.

Results and discussion

Examples of the collected Bitto cheese spectra are shown in Figure 1.

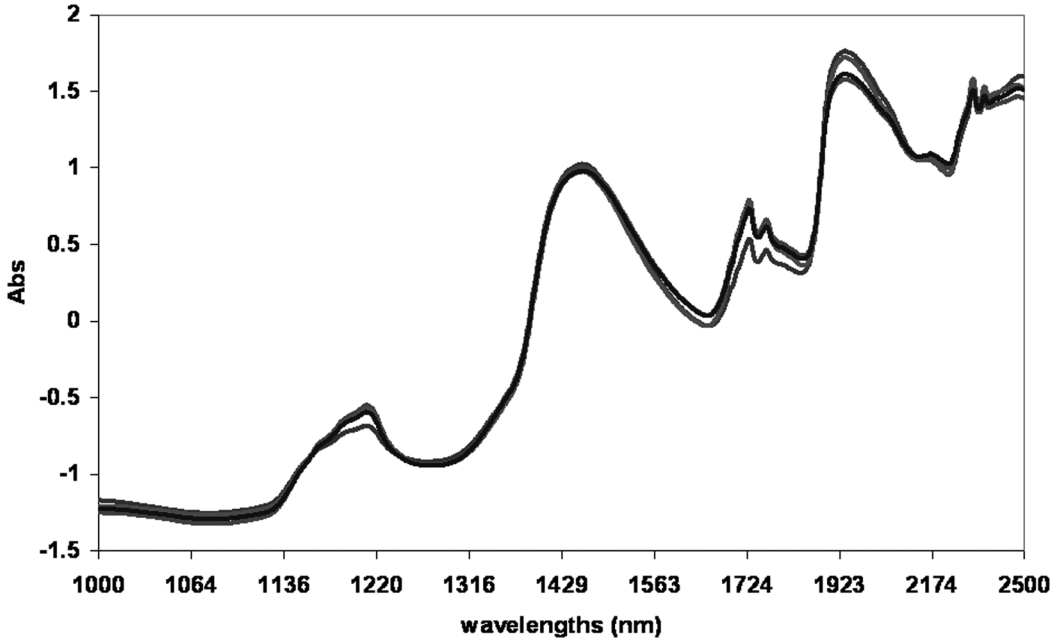

Figure 1. Examples of FT-NIR spectra of Bitto moulds.

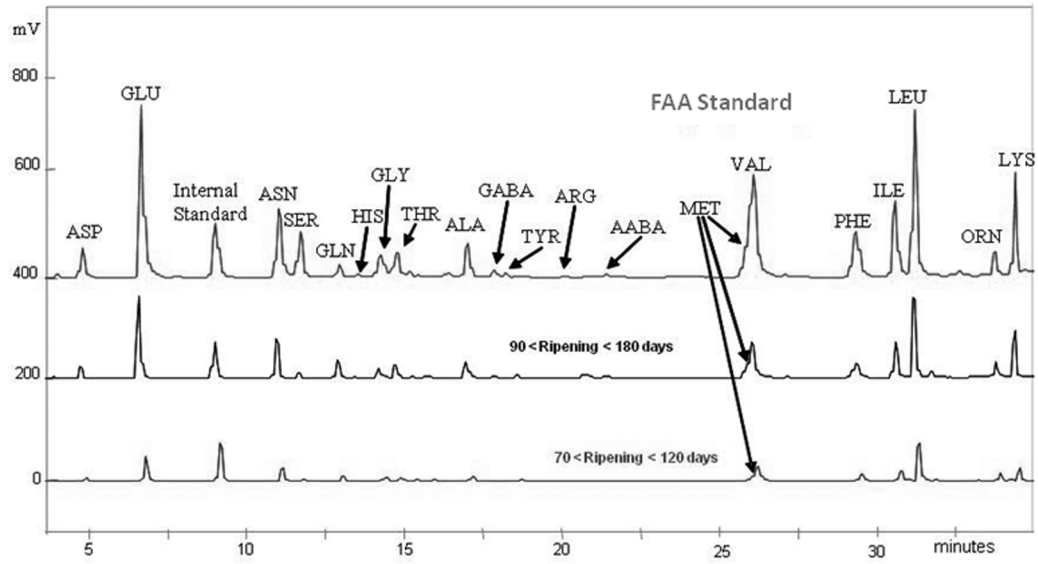

Figure 2. Examples of HPLC analysis of FAA content in Bitto cheese.

The 1210 nm peak is related to the second overtone C–H stretch. The peaks at 1440 and 1920 nm are related to the first overtone O–H stretch. The peaks at 1715–1750 nm are related to first overtone C–H stretch. The 2294 nm band may be related to N–H stretch, as well as the 2132 and 2242 bands, and that at 2336 nm is related to C–H stretch and deformation.[10] The PCA of the FAA in Bitto samples allowed the selection of six FAAs [Asparagine (ASN), Alanine (ALA), Lysine (LYS), Isoleucine (ILE), Leucine (LEU) and Valine (VAL)] all positively correlated with cheese ripening: the higher their concentration, the longer the ripening period (results not shown). In Figure 2 an example of a typical Bitto HPLC chromatogram during ripening is reported, in comparison with the FAA standard chromatogram.

Table 1. PLS calibration and validation results. Total and selected FAA concentration (ppm) in Bitto samples, correlation coefficients in calibration and *CV* (*R*, *R*val), root mean square error in cross-validation (*RMSECV*) for each parameter; (see text for FAA acronyms).

Compound	min	max	mean	R	Rval	RMSECV
Total FAA	2500	19200	7150	0.96	0.83	2035
ASN	5	1100	190	0.94	0.81	145
ALA	74	473	175	0.94	0.82	53
LYS	310	2533	790	0.90	0.67	300
ILE	75	908	245	0.93	0.78	110
LEU	420	2050	1070	0.94	0.84	237
VAL	145	1200	545	0.94	0.82	151

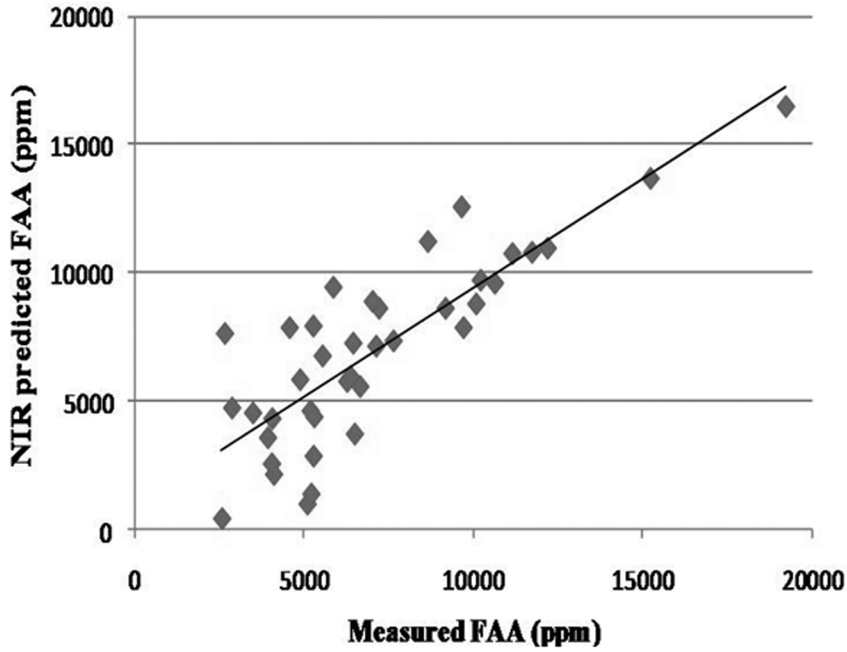

Figure 3. NIR predicted (cross-validation) vs measured concentration of total FAA in Bitto cheese. (see Table 1 for statistics).

Table 1 shows an overview of the selected FAA statistics expressed as the minimum, maximum and the average values (ppm), measured using the reference method and the statistical figures obtained by FT-NIR prediction.

The PLS model for the total FAA content was able to explain 26% of the spectral variance (*Y*) and 92% of the analytical variance (*X*) by using 11 latent variables. In Figure 3, the NIR prediction performance by cross-calibration for this parameter is shown.

The FT-NIR coefficient of variation (*CV%*) was calculated in order to compare NIR spectroscopy with HPLC results. NIR spectroscopy showed *CV%* mean values (10–12%) about double those of HPLC (5–6%). These preliminary results were very satisfactory, proving the applicability of NIR spectroscopy to predict FAA content in Bitto cheese. They were in good agreement with results obtained by HPLC, suggesting the possibility of using NIR spectroscopy as a fast tool for assessing Bitto quality on the basis of the FAA content, as a quality marker.

Conclusions

In general, acceptable *RMSECV* values were found, suggesting the possibility of evaluating the quality of Bitto cheese in terms of the presence of typical nitrogen compounds, by using a rapid technique. A second and a third collection of samples have already been made, in order to

confirm these results. Implementation of the calibration curve and an external validation will also be made next year to verify these findings, and the accuracy and precision of the proposed NIR application.

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References

1. G. Cavallotto and P. Cerlesi, in: *Il Bitto*, Ed by S. Carini. La Moderna Press, Lodi, Italy, p. 4 (1988).
2. D.M. 18/08/2006, *Gazzetta Ufficiale* n. 202, 31/08/2006.
3. M. Yvon and L. Rijnen, *Int. Dairy J.* **11,** 185 (2001).
4. P.F. Fox, *J. Dairy Sci.* **72,** 1379 (1989).
5. Y. Ardö and H. Meisel, *IDF Bull,* **261,** 10 (1991).
6. G. Bertacco, O. Boschelle, A. Giomo, and G. Lercker, *Sci. Tecn. Lat. Cas.* **45,** 147 (1994).
7. J.H. Lee, R. Diono, G.Y. Kim and D.B. Min, *J. Agric. Food Chem,* **51,** 1136 (2003).
8. J. Otte, M. Zakora, K.R. Kristiansen and K.B. Qvist, *Lait,* **77,** 241 (1997).
9. P. Resmini, L. Pellegrino, C. Pazzaglia and J.A. Hogenboom, *Sci. Tecn. Lat. Cas.* **36,** 557 (1985).
10. I. Murray and P.C. Williams, in: *Near-Infrared Technology in the Agricultural and Food Industries*, Ed by P. Williams and K. Norris. AACC, Inc., St Paul, Minnesota, USA, p. 17 (1987).