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Assessment of Trentingrana cheese ageing by proton transfer reaction-mass spectrometry and chemometrics

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Abstract

Proton transfer reaction-mass spectrometry (PTR-MS) data have been analysed by chemometric techniques to monitor cheese ageing by means of on-line direct head-space gas analysis. Twenty cheese loaves of Trentingrana, a trademarked cheese produced in northern Italy, of different origin and ripening degree, were sampled over the whole Trentingrana production area. An increase of the spectral intensity with ripening has been observed for most of the PTR-MS peaks and a univariate analysis identified 16 mass peaks that were significantly different for ripened and young cheeses, respectively. Moreover, the usefulness of different discriminant analyses and class modelling techniques have been investigated. Discriminant Partial Least Squares analysis, while indicating average behaviour and possible outliers, was not able to correctly classify all samples. Soft class modelling performed better and allowed a 100% correct classification. Partial least square calibration predicted the ageing time of each loaf with reasonable accuracy with a maximum cross-validation error of 3.5 months.

Keywords: Grana cheese; Cheese ripening; Proton transfer reaction-mass spectrometry; Head-space analysis; Chemometry; Volatile organic compounds

1. Introduction

Grana Padano is one of the best known Italian hard cheeses and its long tradition and typicality is well recognised and certified by a protect denomination of origin (EC, 1996). Within the consortium of Grana Padano, a more specific geographic identification with its own trademark is recognised as "Trentingrana" (TG) referring to loaves produced in the territory of Trento province (northern Italy). TG, belonging to the family of Grana Padano, is produced with partly skimmed, raw cows' milk acidified with a mixture of Lactobacillus and coagulated by the addition of bovine liquid rennet but, having a more restrictive production protocol, the use of lysozyme is not allowed. Further difference is the prohibition to use silage for the cows' feeding (Carini & Lodi, 1997). Even if the production of TG is relatively small (about 4000 ton year^{-1}; consortia personal communication) when compared with that of the direct competitors (about 125 000 ton year^{-1} for Grana Padano; and about 113 000 ton year^{-1} for Parmigiano; http://www.granapadano.com, www.crpa.it), it is relevant for the local economy.

Cheese flavour compounds result from the action of microorganisms and enzymes on the carbohydrates, fats and proteins of the milk and curd and the principal biochemical pathways for their formation have been reported by many authors (Kristoffersen, 1973; Manning, 1979; Engels & Visser, 1994; Fox, Singh, & McSweeney, 1995; Fox & Wallace, 1997; McSweeney, 1997) and reviewed by McSweeney and Sousa (2000). The volatile components of dairy products have been widely studied (Aston & Dulley, 1982; Law, 1984; Manning, Ridout, &

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Price, 1984; Yvon & Rijnen, 2001; Adda, 1986; Liu, Holland, & Crow, 2004) and more than 600 volatile compounds have been identified in cheese (Maarse & Visscher, 1989; Stratton, Hutkins, & Taylor, 1991). In particular, the volatile organic compounds (VOCs) present in Parmigiano Reggiano and Grana Padano have been extensively studied by chromatographic techniques (Bottazzi & Battistotti, 1974; Dumont, Roger, & Adda, 1974; Meinhart & Schreier, 1986; Barbieri et al., 1994; Moio & Addeo, 1998). An alternative approach to the expensive and time-consuming chromatographic techniques is the use of direct headspace-mass spectrometry for analysis of VOCs (Marcos Lorenzo, Perez Pavon, Fernandez Laespada, Garcýa Pinto, & Moreno Corsero, 2002).

Along this line we characterised TG cheese by means of a direct headspace analysis using proton transfer reaction-mass spectrometry (PTR-MS). As indicated by Boscaini, Van Ruth, Biasioli, Gasperi, and Märk (2003) when analysing similar cheese samples, the choice of PTR-MS has several advantages because of a number of interesting characteristics: (i) PTR-MS is fast, time-dependent variations of the headspace can be monitored with a sub-seconds time-resolution and the headspace of a sample can be measured in a few minutes; (ii) samples are not subjected to any previous treatments thus reducing the risk of artefacts; (iii) mass spectral intensity can be transformed into absolute headspace concentrations in principle without any calibration with external standards; (iv) PTR-MS has a detection limit in the ppt range (Lindinger, Hansel, & Jordan, 1998) and a high dynamic range of several orders of magnitude. Probably, the main drawback of a direct PTR-MS analysis is that, lacking of a chromatographic separation and of an exhaustive database on pure compounds fragmentation, the interpretation of PTR-MS spectra in the case of complex mixtures, remains a difficult issue. However, for the majority of the compounds reported in the paper the fragmentation pattern acquired in standard condition (the same we used) can be found in Buhr, van Ruth, and Delahunty (2002) and a further interpretation can be based on the work of Boscaini et al. (2003) on similar samples. Tentative peak attribution has been assisted by peaks correlation, isotopic ratios, and by further comparison between headspace evolution of cheese and pure compounds. The possibility to follow on line the decrease of compounds in the headspace of liquid solutions allows sometimes the separation of isobars (Karl, Yeretzian, Jordan, & Lindinger, 2003; Pollien, Jordan, Lindinger, & Yeretzian, 2003; Yeretzian, Jordan, & Lindinger, 2003). This has been used, e.g., to estimate acetaldehyde in cheese based on the intensity of the signal at $m/z = 45$ (Aprea et al., 2003).

We studied Trentingrana samples of different ages from different origins in order to demonstrate the possibility to characterize certain features of TG cheese, i.e., in particular for age prediction by PTR-MS analysis. Different chemometric methods have been applied to the PTR-MS fingerprint data and their usefulness for age prediction has been evaluated. In a recent work (Biasioli et al., 2006) correlation among PTR-MS spectral fingerprinting and odour and flavour sensory profile of TG has been investigated.

2. Materials and methods

2.1. Cheese samples

Twenty cheese loaves of TG, aged between 8 and 28 months, were obtained from the main cheese-factories located in Trentino province (Italy) and certified by the "Trentingrana" consortium (the actual sampling took a period of 3 months). Each cheese factory provided two samples: a "ripened" one (not less than 18 months) and a "young" one (less than 15 months) (Table 1). All the cheese factories followed the same protocol (Battistotti & Corradini, 1993) for the cheese production, the only relevant declared difference was that for two factories

Table 1
The factories of the Trentingrana consortium where the analysed cheeses have been sampled

Cheese making factory code	Age of the loaves (months) and production period				Production: loaves year^{-1}	Management system[a]
	Young		Ripened			
A[b]	(9)	Aug'01	(21)	Aug'00	6292	M,T
B[b]	(11)	Sep'01	(22)	Oct'00	6292	M,T
C	(8)	Sep'01	(25)	May'00	5226	T
D	(11)	Jul'01	(22)	Aug'00	5260	M,T
E	(12)	Jul'01	(23)	Aug'00	6480	M,T
F	(11)	Jul'01	(28)	Mar'00	6622	T
G	(14)	May'01	(23)	Aug'00	9182	T
H	(11)	Aug'01	(23)	Aug'00	3218	T
I	(9)	Aug'01	(19)	Oct'00	8750	M
J	(12)	Jul'01	(18)	Jan'01	6745	M

[a]T = traditional management system: small farms (<20 cows), milk production <25 L day^{-1}cow^{-1}, feeding with local hay and low level of concentrates. M = modern management system: medium (20–40 cows) or large (>40 cows) farms, milk production of 25–40 L day^{-1}cow^{-1}, feeding with high level of concentrates.

[b]A and B refers to the same cheese factory that provided four loaves in two different sampling.