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Proton transfer reaction time-of-flight mass spectrometry: A high-throughput and innovative method to study the influence of dairy system and cow characteristics on the volatile compound fingerprint of cheeses

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ABSTRACT

The aim of this work was to study the effect of dairy system and individual cow-related factors on the volatile fingerprint of a large number of individual model cheeses analyzed by proton transfer reaction time-of-flight mass spectrometry (PTR-ToF-MS). A total of 1,075 model cheeses were produced using milk samples collected from individual Brown Swiss cows reared in 72 herds located in mountainous areas of Trento province (Italy). The herds belonged to 5 main dairy systems ranging from traditional to modern and the cows presented different daily milk yields (24.6 ± 7.9 kg × d^{-1}), stages of lactation (199 ± 138 d in milk), and parities (2.7 ± 1.8). The PTR-ToF-MS revealed 619 peaks, of which the 240 most intense were analyzed, and 61 of these were tentatively attributed to relevant volatile organic compounds on the basis of their fragmentation patterns and data from the literature. Principal component analysis was used to convert the multiple responses characterizing the PTR-ToF-MS spectra into 5 synthetic variables representing 62% of the total information. These principal components were related to groups of volatile compounds tentatively attributed to different peaks and used to investigate the relationship of the volatile compound profile obtained by PTR-ToF-MS to animal and farm characteristics. Lactation stage is related to 4 principal components which brought together 52.9% of the total variance and 57.9% of the area of analyzed peaks. In particular, 2 principal components were positively related to peaks tentatively attributed to aldehydes and ketones and negatively related to alcohols, esters, and acids, which displayed a linear increase during lactation. The second principal component was affected by dairy system; it was higher in the modern system in which cows received total mixed rations. The third principal component was positively related to daily milk production. In summary, we report the first application of this innovative, high-throughput technique to study the effects of dairy system and individual animal factors on volatile organic compounds of model cheeses. Individual cheesemaking procedures together with this spectrometric technique open new avenues for genetic selection of dairy species with respect to both milk and cheese quality.

Key words: volatile compound fingerprint, cheese smell, proton transfer reaction time of flight mass spectrometry (PTR-ToF-MS), new phenotypes, dairy system

INTRODUCTION

Flavor is an important driver of cheese purchase and consumption (Drake et al., 2008; Childs and Drake, 2009). Food quality assessment now requires low-cost, rapid, nondestructive testing techniques that can be applied to small samples (Rodriguez-Saona et al., 2006). Analytical tools that enable qualitative fingerprinting of a wide range of dairy products are particularly attractive, and among these there is growing interest in direct injection spectrometric techniques, particularly proton transfer reaction time of flight mass spectrometry (**PTR-ToF-MS**; Biasioli et al., 2011). The PTR-ToF-MS is based on the ionization of volatile organic compounds (**VOC**) with a proton affinity higher than water upon reaction with hydronium ions (H_3O^+) and subsequent detection by a high-resolution time of flight mass analyzer. In particular, this apparatus is composed of a hollow cathode discharge ion source in which H_3O^+ reagent ions are produced from water vapor used as reagent gas; a drift tube where the VOC to be analyzed are continuously injected and ionized

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by proton-transfer reaction with H_3O^+ ions; and a quadruple mass analyzer where the ions are detected. Unlike other techniques with better identification capability, such as GC-MS, PTR-ToF-MS allows direct injection of the headspace without extraction or preconcentration steps, and has a shorter analysis time (only a few seconds per sample) and greater sensitivity. As in other headspace gas chromatographic analyses, it is also nondestructive, does not require chemicals, and permits on-line monitoring of VOC, whereas the resulting spectral information can provide a very detailed description of samples, which is useful for characterizing food quality and typicality (Biasioli et al., 2011; Cappellin et al., 2012a). For example, PTR-ToF-MS has applications in food science and technology, dairy products in particular, for monitoring VOC production in cheese during ripening (Aprea et al., 2007; Fabris et al., 2010; Soukoulis et al., 2010), correlating the cheese volatile profile with the sensory characterization of cheese flavor and odor (Biasioli et al., 2006), and for investigating the geographical origin and the typicality of cheese (Galle et al., 2011).

Cow management and breed, animal genetics, season, and technological factors influence milk characteristics and, as a consequence, cheese quality and sensory properties (Coulon et al., 2004; Bittante et al., 2011a,b). Studies have been carried out on the influence of dairy cow feeding on the sensory properties of cheese (Martin and Coulon, 1995b; Cornu et al., 2009), but little is known about the effects of individual animal-related factors on cheese flavor.

This work was part of a large research project (Cowplus Project) aimed at developing new phenotypes of dairy cow depicting the relationships between animal characteristics, milk quality, cheesemaking aptitude, and cheese sensory traits. In the frame of this project, Cipolat-Gotet et al. (2013) proposed a method to produce model cheeses from individual milk samples that has proven to be particularly useful in determining the cheesemaking aptitude of milk and in allowing for the study of both individual phenotypic sources of variation (DIM, parity, milk yield) and environmental (dairy system) factors on a large scale. The individual model cheese-manufacturing process was also used to estimate genetic parameters of the cheesemaking properties of milk and of daily cheese production through direct measurement (Bittante et al., 2013) or Fourier-transform infrared spectrometry prediction (Ferragina et al., 2013, 2015; Bittante et al., 2014). Cipolat-Gotet et al. (2013) applied the model cheesemaking procedure to 1,264 cows from 85 herds to study cheese yield as well as the recovery of individual milk components in the curd and also to quantify the ratios between the curd contents of fat, protein, DM, and energy versus the content of the corresponding nutrient in the processed milk. Those authors found a significant effect of herd and individual animal factors.

As part of the same Cowplus Project, Bergamaschi et al. (2015) showed that this individual cheesemaking procedure could also be used for qualitative studies of individual cheeses. In a pilot study on a sample of 150 individual model cheeses obtained from 30 herds, they studied the VOC profile of ripened cheeses using solid-phase microextraction GC-MS (**SPME/GC-MS**) and characterized the VOC profile of ripened cheeses: 55 compounds were identified and an exploitable variability according to dairy system and individual animal characteristics was observed. Moreover, SPME/GC-MS analysis of selected cheeses offers the possibility to support PTR-ToF-MS fingerprint analysis in a large number of samples (Cappellin et al., 2012a).

The aims of the current work were (1) to study the potential of PTR-ToF-MS for rapid characterization of cheeses on the basis of their volatile fingerprint, and (2) to analyze the effects of dairy system and individual cow characteristics on the volatile compounds of cheese using a large number of individual model cheeses.

MATERIALS AND METHODS

Field Data

To carry out this study, also part of the Cowplus Project, a total of 1,075 Italian Brown Swiss cows reared in 72 herds located in Trento Province (Italy) were selected. Fifteen cows were chosen randomly from each herd and sampled once on the same day: the herds were sampled year round to cover all seasons and rearing conditions. The sampled cows presented different milk yields (24.6 ± 7.9 kg $\times$ d^{-1}), stages of lactation (199 ± 138 DIM), and parities (2.7 ± 1.8). The sampling procedure is described in detail by Cipolat-Gotet et al. (2012) and Cecchinato et al. (2013). The production environments, which varied in terms of production level, destination of milk, modernization of structures, and management, are discussed by Sturaro et al. (2013). The selected dairy farms of this Alpine area (825 ± 334 m above sea level) used different dairy management strategies, with variations in facilities, feedstuff distribution, use of silages, and transhumance to temporary summer Alpine pastures.

Traditional dairy farms were those dairy systems utilizing small barns, tied animals milked at the stall, calving concentrated mainly in autumn, transhumance of cows and heifers to Alpine pastures for the summer, and feed mainly composed of hay and compound feed. These were either with or without automatic feeders at the manger (traditional with automatic feeders, 9 farms,