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Rapid characterization of dry cured ham produced following different PDOs by proton transfer reaction time of flight mass spectrometry (PTR-ToF-MS)

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ABSTRACT

In the present study, the recently developed proton transfer reaction time of flight mass spectrometry (PTR-ToF-MS) technique was used for the rapid characterization of dry cured hams produced according to 4 of the most important Protected Designations of Origin (PDOs): an Iberian one (Dehesa de Extremadura) and three Italian ones (Prosciutto di San Daniele, Prosciutto di Parma and Prosciutto Toscano). In total, the headspace composition and respective concentration for nine Spanish and 37 Italian dry cured ham samples were analyzed by direct injection without any pre-treatment or pre-concentration. Firstly, we show that the rapid PTR-ToF-MS fingerprinting in conjunction with chemometrics (Principal Components Analysis) indicates a good separation of the dry cured ham samples according to their production process and that it is possible to set up, using data mining methods, classification models with a high success rate in cross validation. Secondly, we exploited the higher mass resolution of the new PTR-ToF-MS, as compared with standard quadrupole based versions, for the identification of the exact sum formula of the mass spectrometric peaks providing analytical information on the observed differences. The work indicates that PTR-ToF-MS can be used as a rapid method for the identification of differences among dry cured hams produced following the indications of different PDOs and that it provides information on some of the major volatile compounds and their link with the implemented manufacturing practices such as rearing system, salting and curing process, manufacturing practices that seem to strongly affect the final volatile organic profile and thus the perceived quality of dry cured ham.

1. Introduction

Dry-cured ham is a valuable traditional foodstuff with unique quality traits which are influenced mainly by the characteristics of the raw meat (geographical origin, pigs' breed, feeding regime and rearing system) and by the processing conditions (salting, curing and ripening) [1]. Dry cured ham production is often controlled by a protected designation of origin (PDO) in order to achieve products with high quality sensory characteristics and of reproducible quality [2]. In this paper we consider 4 of the most important PDOs for dry cured ham: *Dehesa de Extremadura* produced in a restricted area in Spain and *Prosciutto di Parma*, *Prosciutto di San Daniele* and *Prosciutto Toscano* produced in central and northern regions of Italy. The geographical origin of dry cured ham is a parameter relevant to their quality characteristics as it defines the implemented processing practices, i.e. type of raw materials, use of spices, addition of nitrates, differences in the type and duration of the curing process. Italian PDOs accept hybrid pigs from various crossing breeds such as Large Withe, Landrace and Duroc-Jersey, whereas Spanish Iberian hams are produced only with Iberian pigs or their direct crossbreeds with Duroc-Jersey [3]. Contrary to the Italian ham's salting process, the addition of small amounts of nitrates is permitted during the Spanish ham production [4]. The use of spices like pepper (added at salting or *sugnatura* phases; in the last a mixture of fat, flour and pepper is used to protect the hams) is permitted in the production of Italian hams, whereas it is banned during the production Spanish Iberian dry cured hams. Finally, the duration of curing process of Italian hams is generally shorter (at least 12 months) than that implemented in the production of Spanish Iberian hams that requires more than 18 months.

One of the most important quality attributes of dry cured hams is their unique flavour produced by a complex mixture of volatile organic compounds (VOCs) that is influenced by the characteristics of the raw materials and the implemented processing practices [5].

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Most VOCs in dry cured ham form during the curing process, and are the result of chemical and biochemical lipid oxidation and of further interaction with proteins, peptides and free amino acids; other VOCs result from Strecker degradation of free amino acids and Maillard reaction with products of the lipid oxidation [6]. The VOCs profile depends also quantitatively and qualitatively on genetic and rearing factors [7,8] that influenced the meat composition as well as on the length of the ripening [9–11]. Therefore the flavour profile as apparent in the VOCs compositions can be used to distinguish differently dry-cured hams in terms of their geographical origin or production process.

Several studies dealing with the volatile compounds profile of different kinds of cured hams have been reported including: Iberian [1,2,10,11] and Serrano Spanish hams [12], Prosciutto di Parma [9,13], Prosciutto di San Daniele [14,15] and Prosciutto Toscano [16] Italian hams. Other researchers investigated the differences in the volatile profile of different kinds of ham are usually based on gas chromatographic (GC) separation preceded by some extraction method as SPME and followed by mass spectrometric identification [17–20]). Other approaches have been proposed to overcome the drawbacks of GC based analysis, namely the time consuming procedure and the need of sample preparation or of a concentration phase [21], a promising possibility being direct injection mass spectrometry and in particular, proton transfer reaction mass spectrometry.

Proton transfer reaction mass spectrometry (PTR-MS) is a novel method that has been successfully applied for the on-line monitoring of VOCs headspace in several model and real food systems as well as the characterization of foods and their production processes [22–28] or origin identification [29,30]. PTR-MS has been described in several review papers [31] and will be not described in detail here. It is based on the protonation of volatiles organic compounds which have a proton affinity higher than that of water and, in its basic version relies on the detection of the product ions by a quadrupole mass spectrometer. To partially overcome the limitations related to the slow and low resolution quadrupole, the coupling of PTR-MS with a time-of-flight (ToF) mass analyzer was recently commercialized [32] offering several advantages including higher mass resolution ($m/\Delta m$ up to 8000) and faster spectra acquisition, see Fabris et al. [27] and Soukoulis et al. [28] for the first applications of PTR-ToF-MS in food science. PTR-ToF-MS is characterized by a high sensitivity with limits down to the low ppt region and a high time resolution (0.1 s) [32].

In this work we studied the volatile compounds profile of dry cured hams produced according to different PDOs, i.e. Italian ham (*Prosciutto di Parma*, *Prosciutto di San Daniele* and *Prosciutto Toscano*) and Spanish Iberian ham (*Dehesa de Extremadura*) aiming (i) at investigating the possibility of using PTR-ToF-MS spectra as fingerprints for their rapid and non invasive classification and (ii) at exploiting the features of PTR-ToF-MS to obtain qualitative and quantitative analytical information on the volatile compounds of the samples considered.

2. Materials and methods

2.1. Ham samples

Forty-six ham samples differing in their geographical origin and production process were selected: nine Spanish Iberian dry-cured hams (PDO *Dehesa de Extremadura*) and 37 Italian ones: 12 from PDO *Prosciutto di Parma*, 12 from PDO *Prosciutto di San Daniele* and 13 from PDO *Prosciutto Toscano*. The Italian hams were produced from heavy pigs with at least nine months of age and with 160 kg of minimum live weight, as fixed by the rules of the PDO Consortia [33]. The animals originated from a specific Italian selection obtained from traditional breeds genetically improved by the Italian Breeders Association. In particular the crossed breeds used were Italian Large White and Italian Landrace. The crossings were reared in the same farm, fed with the same diet, based on standard cereals-soybean meal commercial feeds, and slaughtered in the same abattoir, in three lots, within a period of six weeks. The fresh thighs where distributed in three different processing plants, located in Tuscany, Emilia and Friuli regions, in the hill area of the three different PDOs, by sharing the thighs produced in each slaughtering day between the different PDOs. According to the *Dehesa de Extremadura* PDO, Iberian hams were obtained from heavy Iberian pigs (pure Iberian gilt × 50% Iberian-50% Duroc barrow) with 14 months of age and in the range of 130–160 kg of live weight. These pigs were fattened outdoor for 60 days on grazed feedstuffs and a concentrate feeding ("Campo" Iberian hams, according to DOP *Dehesa de Extremdaura*). The hams were processed by applying the usual temperature and relative humidity values of the traditional processing according to their respective PDO's guidance [18,34]. The ripening duration was 399 days for Parma hams, 413 days for San Daniele hams, 396 days for Toscano hams and 720 days for Iberian hams. All pigs used to produce hams according to the Italian PDOs share the same raw material and production period but, since we decided to follow the indication of the PDOs, it was necessary to use, for the Iberian hams, pigs from a different breed and rearing system. Thus, in this study, the differences among the Italian hams originate only from the production process while the differences between Italian and Iberian ham origin from both raw meat and production process.

2.2. Proton transfer reaction time of flight mass spectrometry (PTR-ToF-MS)

From each ham, a piece of the muscle *biceps femoris* was taken and kept under vacuum at 2 °C. In the moment of the analysis, the external layer of each piece of ham was removed, and 3 meat cubes of 1 cm^3 (3 replicates) were prepared. Each cube was introduced into a 40 ml vial (Supelco, Bellefonte, USA), capped by a PTFE/Silicone septum (Supelco, Bellefonte, USA). To standardise the measurement, all samples were equilibrated at 37 °C for 30 min in a water bath prior to analysis. They were then measured by direct injection of the head space mixture into the PTR-ToF-MS drift tube via a heated (110 °C) peek inlet for 30 s, allowing to take 30 average spectra [27].

Measurements were carried out following the procedure described in previous works for other food samples [27,28] using a commercial PTR-ToF-MS 8000 apparatus (Ionicon Analytik GmbH, Innsbruck, Austria), in its standard configuration (V mode). The sampling time per channel of ToF acquisition is 0.1 ns, amounting to 350,000 channels for a mass spectrum ranging up to m/z 400, with the following conditions in the drift tube: drift voltage 600 V, temperature 110 °C and pressure 2.25 mbar.

2.3. Spectra analysis

The external calibration automatically done by the acquisition program provided a poor mass accuracy, thus internal calibration of ToF spectra was performed off-line [35]. Data pre-processing on ToF spectra was carried out in order to remove the baseline, and noise reduction was achieved by averaging over the 30 consequent ToF spectra corresponding to the same sample, thereby improving the signal-to-noise ratio by about five times. Peak identification and area extraction then followed the procedure described in details by Cappellin et al. [36]. Throughout this paper we report experimental m/z values up to the third decimal, the expected exact m/z values up to the fourth, VOCs concentration is expressed in ppbv (part per billion by volume) and has been calculated from peak areas