Food Chemistry 192 (2016) 736–744

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Correlation between volatile profiles of Italian fermented sausages and their size and starter culture

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ARTICLE INFO

Article history:
Received 4 April 2015
Received in revised form 22 June 2015
Accepted 14 July 2015
Available online 16 July 2015

Keywords:
Fermented meat
Sausage size
Aroma profile
Linear discriminant analysis (LDA)
Starter cultures

ABSTRACT

The aroma profiles of 10 traditional Italian fermented sausages were evaluated. The volatile organic compounds (VOCs) obtained by solid-phase microextraction and gas chromatograph–mass spectrometry were analysed using principal component analysis (PCA) and linear discriminant analysis (LDA). PCA allowed an acceptable separation but some sausage typologies were not well separated. On the other hand, the supervised approach of LDA allowed a clear grouping of the samples in relation to sausage size and starter culture. In spite of the extreme variability of the volatile profiles of the sausage typologies, this work showed the influence of diameter on VOC profile. The differences observed can be related to the effects that some fundamental physicochemical characteristics (such as water loss kinetics and oxygen availability) have on the results of ripening processes. Differences in VOC profiles were also observed due to the lactic acid bacteria used as starter cultures, with differences mainly attributable to compounds deriving from pyruvate metabolism.

1. Introduction

European countries are the major producers and consumers of cured meats, even if these products are widespread around the world (Lachowicz, Zochowska-Kujawska, & Sobczak, 2012). Among cured meats, fermented sausages, which originated in the Mediterranean area during Roman times, have great importance (Leroy, Geyzen, Janssens, De Vuyst, & Scholliers, 2013). They are a heterogeneous group of products, with great differences in relation to raw material (type of lean meat and fat), ingredients (salt concentration, nitrate/nitrite, spices and herbs, additives), size (diameter, weight, type of casing) and ripening conditions (temperature, relative humidity, use of moulds and/or smoke) (Leroy et al., 2013; Toldrá, 2006).

In Italy the impact on the food industry of fermented sausages manufacture in 2013 was very important; the total production of cured meats was 1,180,000 tonnes, with an economic value of about €7900 million and, within this production, 109,000 tonnes (€925 million) were represented by fermented sausages (ASSICA, 2013).

Because of historical, cultural and traditional practices (often different from one city to another), Italian traditional cured meats are characterised by a high variability, as evidenced by the European Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI) recognitions (European Commission, 2014). In spite of their roots in tradition, the processes for Italian fermented sausages production have been modified through the centuries, by adapting to improved technological standards and modifications in the perception of food (Leroy et al., 2013). These changes have undergone an exponential acceleration in the last decades due to the risks associated with novel pathogens or the market requests for products with lower salt/fat content or functional properties (Toldrá & Reig, 2011). Nowadays, the use of starter cultures and rigorous conditions of temperature and relative humidity during ripening are commonly applied by Italian producers. This could result in a standardisation of the traditional features of different products, even if the wild ripening microbiota maintains a crucial role on the final characteristics and on flavour formation (Ravyts, De Vuyst, & Leroy, 2012).

Volatile organic compounds (VOCs) are formed essentially through the metabolism of lipids and proteins, as well as being end-products of the lactic fermentation, mainly through oxidative transformations (Leroy et al., 2013; Ordóñez, Hierro, Bruna, & de la

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http://dx.doi.org/10.1016/j.foodchem.2015.07.062
0308-8146/

Hoz, 1999). The action of enzymes (endogenous or microbial) during maturation is responsible for the typical sausage aroma profile. The metabolism of lactic acid (derived from sugar fermentation) leads to the production of molecules such as acetic, propionic and butyric acids, acetaldehyde, diacetyl and acetoin. The products with major impact originating from the metabolism of amino acids liberated by proteases and peptidases are branched aldehydes, acids and alcohols. The free fatty acids deriving from lipids can be the substrate for the production of carbonyl compounds (aldehydes and methyl ketones), and also hydrocarbons and alcohols (Carballo, 2012; Demeyer, 2004; Ordóñez et al., 1999).

These processes determine the characteristic VOC profiles of fermented sausages and depend on complex interactions among several factors, including raw material properties, mincing degree of meat batter, sausage dimension, type of casing, ripening process length, relative humidity and temperature, use of starter cultures, and characteristics of the wild microbiota (Incze, 2010; Janssens, Myter, DeVuyst, & Leroy, 2012; Leroy et al., 2013; Ravyts et al., 2010; Toldrá, 2006).

In the last decades the fermented meat industry was subjected to a high degree of innovation, determined by the challenge of new safety concerns (including emerging pathogens and salt reduction) and by an improvement of process efficiency (Leroy et al., 2013). In spite of the risk of product standardisation due to these innovative practices, the industry focuses its attention on the distinctiveness of the aroma profiles of "typical" products to promote their recognisability and uniqueness as a fundamental key to develop market strategies.

The objective of this work was to study the aroma profiles of 10 different industrially produced Italian fermented sausages, in order to highlight the presence of volatile molecules responsible for flavour formation through a solid-phase microextraction/gas chromatography–mass spectrometry (SPME/GC–MS) analysis. The same products were analysed in a previous study (Tabanelli et al., 2014) in relation to some physicochemical and microbiological attributes, as well as in relation to their biogenic amine content. In this work, some statistical exploratory tools – principal component analysis (PCA) and linear discriminant analysis (LDA) – have been used to correlate the differences observed in VOCs profiles to the size of the tested sausages and to the type of starter culture used for their manufacture. The main aim of this work was to better understand the effects on the aroma profile of important physicochemical features (such as water loss and oxygen availability), highly influenced by sausage diameter.

2. Materials and methods

2.1. Fermented sausages

Ten types of fermented sausages were considered in this trial. All of them were produced by CLAI, a company located in Imola (Italy). The samples (six salamis for each typology) were taken directly from the factory at the end of ripening in May 2014. The six samples for each sausage typology were taken from two different batches produced on different days and for each batch three samples were taken from a different position in the ripening chamber. The sausage typologies were grouped in relation to their diameter. The small sausages (diameter less than 50 mm) were Cacciatore (CCT), Salsiccia passita (PST) and Salame Aquilano (AQL); the medium sausages (diameter between 50 and 100 mm) were Felino type salami (FLN), Salame Romagnolo (RMG) and Salame Napoli (NPL); the large sausages (diameter greater than 100 mm) were: Milano type MLN), Lombardo type (LMB), low fat sausage (LFS) and Sicilian type sausage (SCL).

Two types of starter cultures were used: starter culture A contained *Lactobacillus sakei* and a mixture of *Staphylococcus xylosus* strains, while starter culture B contained *Pediococcus pentosaceus* and the same mixture of *S. xylosus* strains. The choice of the starter lactic acid bacteria depended on previous experiences and trials of the sausage producer. The external mould growth was induced in all the sausages by inoculating with a selected *Penicillium nalgiovense* strain. All starter cultures were provided by Chr. Hansen Italia (Parma, Italy). All the sausages contained nitrates and nitrites, according to European legislation (DIRECTIVE 2006/52/EC, 2006) and spices (black pepper, garlic).

The main characteristics of these sausages are summarised in Table 1.

2.2. Aroma profile analysis

Volatile organic compounds of the ten different typologies of fermented sausages were analysed using solid phase microextraction coupled with gas chromatography–mass spectrometry (SPME/GC–MS), as previously reported by Tabanelli, Montanari, Grazia, Lanciotti, and Gardini (2013). In particular, samples (3 g) were placed in 10-mL sterilised vials, sealed by PTFE/silicon septa and heated for 10 min at 45 °C. After that a fused silica SPME fibre covered with 75 μm Carboxen/polydimethylsiloxane (CAR/PDMS StableFlex) (Supelco, Steinheim, Germany) was introduced into the headspace for 40 min. Adsorbed molecules were desorbed in the gas-chromatograph for 10 min. For peak detection, an Agilent Hewlett–Packard 6890 GC gas-chromatograph equipped with a 5970 MSD MS detector (Hewlett–Packard, Geneva, Switzerland) and a Varian (50 m × 320 μm × 1.2 μm) fused silica capillary column were used. The conditions were as follows: injection temperature, 250 °C; detector temperature, 250 °C; carrier gas (He) flow rate, 1 mL/min. It is the ion source temperature. The MS acquisition parameters were: Low Mass: 30; High Mass: 300 and Threshold : 100; 5.1 scan/sec. The oven temperature was programmed as follows: 50 °C for 1 min, from 50 °C to 65 °C at 4.5 °C/min, from 65 °C to 230 °C at 10 °C/min, then holding for 25 min. Volatile peak identification was carried out by computer matching of mass spectral data with compounds contained in the NIST 98 and Wiley 6 mass spectral databases.

For each type of fermented sausage, the volatile profile composition was expressed as relative percentage of each single peak area with respect to the total peak area. Data reported are the means of six different sausages.

2.2. Statistical analysis

Data for each sausage typology are the mean of six different samples. ANOVA, principal component analysis (PCA) and linear discriminant analysis (LDA) were carried out in the statistical environment R (R Development Core Team, Vienna, Austria).

ANOVA was carried out by fitting the classical one-way model:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}, \quad i = 1,2,3;\ j = 1,\ldots J_i$$

$$\varepsilon_{ij} \sim N(0,\sigma^2)$$

where μ is the overall mean compound concentrations and τ_i denotes the deviation of the response from μ when the i-th group is considered. The aim of this step was simply to point out the differences in the compound concentrations with respect to the three sausage diameter sizes. Therefore the following hypothesis system was tested:

$$\begin{cases} H_0 : \tau_1 = \tau_2 = \tau_3 \\ H_1 : \text{At least two of the } \tau_i\text{'s are different} \end{cases}$$

If the null hypothesis was rejected, post hoc tests were performed to distinguish the different τ_i's. To correct the p-values to allow pairwise comparisons, Tukey's procedure was applied.