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Effect of wine addition on microbiological characteristics, volatile molecule profiles and biogenic amine contents in fermented sausages

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ABSTRACT

The aim was to evaluate the effect of wine addition during manufacturing of dry fermented sausages, in terms of safety aspects (biogenic amine accumulation), aroma profile and sensory characteristics. Three batches of salami were produced: without wine addition and with 7.5% or 15% (v/w) of white wine. The fermented sausages showed characteristics that can increase product diversification. Some of the sensory features (i.e. increased salty perception) can represent an important strategy because of the trend to reduce salt intake for health reasons. The presence of wine immediately reduced the pH and is a source of ethanol, which can have an inhibitory effect against undesirable microflora. The microbiological results observed regarding *Enterobacteriaceae* and enterococci were encouraging. The addition of wine did not negatively affect the ripening time or increase the presence of biogenic amines. The samples containing wine showed reduced concentrations of putrescine.

1. Introduction

Fermented sausages are obtained through fermentation of a meat batter composed of lean and fat meat, and other ingredients (curing agents etc.), stuffed in natural or synthetic casings, and, then, subjected to a ripening process under controlled temperature and relative humidity. The type of meat cuts and their degree of mincing, as well as the ratio between lean and fat can vary depending on local traditions and product characteristics (Feiner, 2006; Toldrá, 2006). In addition to meat, many other ingredients can be added to the batter, including spices (black and red peppers, fennel, nutmeg, cumin, etc.), curing agents (nitrate and nitrite), sodium chloride, sugars (to favor lactic fermentation) and starter cultures (lactic acid bacteria, micrococci, staphylococci and fungi) (Hammes, Haller, & Gänzle, 2003; Toldrá, 2006).

Sometimes, especially in the Mediterranean area, wine can be added to influence directly the aroma profile of the final product, or as a vehicle to introduce other aromatic substances, such as garlic, previously soaked in wine. Usually, wine is added in low amounts (1% v/w or less) (Casaburi et al., 2008; Spaziani, del Torre, & Stecchini, 2009). However, some exception in which wine is added in higher proportions is known. Rason, Laguet, Berge, Dufour, and Lebecque (2007) studied fermented sausages produced in the Massif Central (France) using up to 50 mL of wine per kg of meat batter. In addition, in a typical Italian product (*Salama da sugo*) this content is up to 15% (v/w) (Gardini et al., 2013) and was presented to the European Commission for IGP approval (Gazzetta Ufficiale della Repubblica Italiana, 2012). The addition of amount of wine is important, not only in relation to its effect on the flavor profile, but also for other parameters. Firstly, wine has a low pH (3.0–3.6) due to the presence of organic acids such as tartaric, malic, succinic, lactic and acetic acids (Volschenk, Van Vuuren, & Viljoen-bloom, 2006). As a consequence, high wine concentrations can be responsible for a significant lowering of the meat batter pH, with a relevant contribution to the inhibition of undesirable microbial growth. In addition, ethanol (present in wine at 10–13% v/v) can reach concentrations to which some microorganisms are sensitive. In particular, the activity of molds can be affected by ethanol, limiting their role during ripening (Dao & Dantigny, 2011). The effects of wine on fermented sausage aroma profiles are well known; in particular, wine contributes to the presence of esters and higher alcohols (Gardini et al., 2013). Although the literature does not report information about the production of salami in the presence of high wine concentrations, several local commodities produced using wine during mixing can be found. In addition to Salama da sugo, there are some other examples of salami with wine produced in different regions of Italy, such as Tuscany (http://www.terraditoscana.com/default.aspx?lpg=cucina_prodotti&obj=salumi_salamevino), Trentino (http://www.fratellicorra.it/component/option,com_k2/Itemid,126/id,26/view,item/) and Piedmont (http://www.albain.com/cn/salumi.asp). In addition to its antimicrobial properties the presence of high amounts of wine can increase product differentiation, allowing these fermented foods to reach a wider market. In fact, in recent years there has been an increasing demand for new varieties of food products that are similar to traditional

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ones for safety but are differentiated by sensory, nutritional and rheological characteristics (Lanciotti et al., 2004).

Among the sensorial features, the aroma profile plays a crucial role in the characterization of the different types of fermented sausages. Volatile compounds are formed through the metabolism of lipids and proteins as well as the end-products of the lactic fermentation (Ordóñez, Hierro, Bruna, & de la Hoz, 1999). The aroma profile is influenced by the type and ratio of ingredients, by the starter cultures and by the microbial flora selected during ripening (Tabanelli et al., 2012). In addition, during ripening toxic and undesirable substances can be produced. Among them, biogenic amines, which derive from the microbial decarboxylation of amino acids, can be found (EFSA, 2011); tyramine, cadaverine and putrescine are the most important amines in sausages, though histamine can also be produced (Suzzi & Gardini, 2003).

This study was conducted to evaluate the effect of the addition of high wine amounts during the manufacture of dry fermented sausages on biogenic amine accumulation, aroma profile and sensory characteristics of the sausages. With this purpose three batches of salami were produced; in the first batch wine was not added, while in the second and third batches 7.5% (v/w) and 15% (v/w), respectively, of white wine were added during the preparation of the meat batter.

2. Materials and methods

2.1. Sausage manufacture

Sausages were produced in a local factory using lean pork (73% w/w), pork fat (27% w/w), NaCl (2.3% w/w), sodium ascorbate (0.05% w/w), dextrose (0.2% w/w), KNO_3 (0.015% w/w), $NaNO_2$ (0.010% w/w) and spices (black pepper and garlic). After chopping and mixing, the mixture was divided into three batches (A, B and C) of about 30 kg each. Starter cultures belonging to the species *Pediococcus pentosaceus* and *Staphylococcus xylosus* (Startec TCSS 1/150, Tec-Al, Traversetolo, Parma, Italy) were added to all the batches with an initial concentration for each species of about 6 log CFU/g.

In batches B and C 7.5% (v/w) and 15% (v/w) of white wine were added to the meat batter, respectively. No wine was added in batch A, which was used to produce the control samples. White wine, provided by a local winery, was produced from the Sauvignon grape and was characterized by 10.5% ethanol (v/v), 6.70 g/l total acidity (as tartaric acid) and pH 3.03.

Mixtures were stuffed into synthetic casings (Texda Textildarm GmbH, Osnabrück, Germany) with 65 mm diameter.

Thirty sausages of approximately 1000 g were produced for each batch. Sausages were placed in a drying chamber at 23 °C and 90% relative humidity (RH) for 48 h. Thereafter, sausages were kept in the ripening chamber at 13 °C and 80–70% RH for 58 days.

2.2. Microbiological and physicochemical analyses

Microbiological analyses were performed at time zero (meat mixture prior to stuffing) and after 1, 2, 3, 7, 14, 30 and 60 days of ripening. For this purpose, 20 g of sausage (without casing) was aseptically removed and homogenized for 2 min with 180 mL of 0.9% (w/v) NaCl physiological solution using a Stomacher (Laboratory Blender Seward, London, U.K.). The solution was then used to prepare decimal dilutions. *Enterobacteriaceae* were counted on VRBGA (Oxoid, Basingstoke, UK) incubated for 24 h at 37 °C; enterococci on Slanetz and Bartley medium (Oxoid) after 48 h at 45 °C; lactic acid bacteria (LAB) on MRS agar (Oxoid) at 30 °C for 96 h under anaerobic conditions; *Micrococcaceae* and *Staphylococcaceae* were enumerated on Baird–Parker agar with Egg Yolk Tellurite Emulsion (Oxoid) incubated at 30 °C for 72 h; the results are given as Coagulase Negative Cocci (CNC) if the colonies do no form halos and Coagulase Positive Cocci (CPC) if the colonies form halos. Yeasts were counted on Sabouraud Dextrose Agar (Oxoid, Basingstoke, UK) added with 200 mg/L of chloramphenicol incubated at 28 °C for 48 h.

For each batch and for each sampling time 3 sausages were analyzed. The microbiological data are means of 3 repetitions and 3 replicates.

During ripening, the weight loss, pH and a_w were measured using a scale (Acculab, United States), a pH-meter Basic 20 (Crison Instruments, Barcelona, Spain) and an Aqualab CX3-TE (Labo-Scientifica, Parma, Italy), respectively.

2.3. Detection of biogenic amines

For the detection of biogenic amines for each batch three sausage samples were extracted with trichloracetic acid following the method reported by Coloretti, Chiavari, Armaforte, Carri, and Castagnetti (2008) after 7, 24, 48 and 60 days from casing.

Analysis was carried out with a Waters HPLC (Milan, Italy), equipped with a Waters 1525 binary pump, a dual wavelength absorbance detector Water 2487 set at 250 nm, and a Symmetry C18 column. Solvent A was HPLC grade water (Carlo Erba reagents, Milan, Italy), and solvent B was HPLC grade methanol (Carlo Erba reagents). An elution gradient was programmed for solvent B as follows: 50% for 0.5 min, from 50 to 15% in 6.5 min followed by 5 min at 15% then from 15 to 50% methanol in 2 min, followed by 2 min in 50% methanol. A flow rate of 0.8 mL/min was employed, and 20 μL of sample was injected. Breeze 3.30 SPA software (Waters) was used for data acquisition and processing on a personal computer. All of the biogenic amines were tentatively identified by comparison of retention time and co-elution with commercial standard compounds (Sigma, St. Louis, MO). For biogenic amine quantification, calibration curves covered the range 0.5–100 μg/mL for each amine standard solution using peak area versus analyte concentration to quantify the biogenic amine. The linear range was assessed using seven different concentrations that were injected four times.

2.4. Volatile molecule profiles

Volatile compounds of the three different batches of fermented sausages (A: no wine; B: 7.5% of wine; C: 15% of wine) were monitored after 7, 24, 48 and 60 days from casings using gas-chromatographic–mass spectrometry coupled with solid phase microextraction (GC–MS–SPME). Samples (3 g) were placed in 10 mL sterilized vials, sealed by PTFE/silicon septa and heated for 10 min at 45 °C and volatiles were 23adsorbed for 40 min on a fused silica fiber covered by 75 mm Carboxen Polydimethyl Siloxane (CAR/PDMS StableFlex) (Supelco, Steinheim, Germany). 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 MS detector 5970 MSD (Hewlett–Packard, Geneva, Switzerland) and a Varian Chrompack CP Wax 52 CB capillary column (50 m × 320 μm × 1.2 μm) fused silica capillary column (Chrompack, Middelburg, The Netherlands) as stationary phase were used.

The conditions were as follows: injection temperature, 250 °C; detector temperature, 220 °C; and carrier gas (He) flow rate, 1 mL/min. The oven temperature was programmed as follows: 50 °C for 1 min; from 50 °C to 65 °C, at 4.5 °C/min; and 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 those of compounds contained in the Agilent Hewlett–Packard NIST 98 and Wiley vers. 6 mass spectral databases. For each sample, the GC–SPME results are expressed as the mean of three different sausages.

2.5. Sensory analysis

A panel of seven assessors for descriptive analysis for sausage trained according to Chiavari, Coloretti, Ferri, and Nanni (2007) was used for the sensory analysis. The evaluation form was modified by introducing wine odor and aroma. Sausages were evaluated, in two