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Short communication

Tyrosine deposits in brines of salted natural sausage casings of bovine origin

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ABSTRACT

Tyrosine was identified as the major component of water insoluble white particles recovered from salted bovine small intestines intended for sausage casings. The salt sludge taken from the bottom of the transport casks contained more than 2.5% (w/w) of these particles and the wash-off from the salted intestines yielded 0.04% (w/w). Qualitative analysis by confocal Raman microscopy proved tyrosine as the major constituent with an admixture of phenylalanine. The quantitative amino acid analysis revealed 85.5 ± 2.3% tyrosine and 5.0 ± 0.2% phenylalanine. The amino acid analysis of the brine samples showed a typical protein profile with the highest concentrations being observed for glutamate, leucine, alanine, and lysine. Endogenous proteolytic enzymes of the tissue rather than microbial activities are thought to have been responsible for the tyrosine precipitations.

1. Introduction

Natural sausage casings of bovine origin are used for a range of sausage specialties including raw fermented and cooked sausages. For hygienic reasons production and trade of natural casings from animal intestines are regulated in Europe by Council Directive 92/118/EEC and Regulation (EC) 999/2001 and, according to Commission Decision 2003/779/EC, such casings must be accompanied by a health certificate certifying *inter alia* that they have been sanitized e.g. by salting with sodium chloride for 30 days, which is a well-established and accepted preservation procedure in the casings industry (EC., 1993, 2001, 2003). Besides their susceptibility to deterioration by autolytic processes and microbial activities, the main problems associated with natural casings include the possible transmission of pathogenic bacteria, viruses and prions to humans and animals (Anonymous, 2014; Bakker, Houben, Koolmees, Bindrich, & Sprehe, 1999; Wijnker, Koop, & Lipman, 2006). Any deviation from the standard appearance may be indicative of a serious sanitary or quality problem. The sporadic occurrence of puzzling white granular particles in transport casks shipped from South America to Europe gave rise for complaints by the industry (ZVN., 2015). The aim of this study was to identify the composition and the possible origin of these particles.

2. Materials and methods

Two independent samples from different transport casks for natural casings derived from bovine small intestine, both with plenty of white particles (Fig. 1), were provided by a German firm. The availability of samples was limited due to the irregular and infrequent nature of this phenomenon. Sample 1 was a 200 g aliquot of a salt sludge from the bottom of a transport cask, sample 2 a bundle of casings (2.2 kg) from a separate cask shipped several months later. The samples were sealed in polyethylene bags and shipped refrigerated to Kulmbach, where the samples were kept at 5 °C until examination.

The pH value of the brines was determined with a model MP225 pH meter equipped with a puncture-type pH electrode with Xerolyt^{R} polymer electrolyte (Mettler-Toledo). Brines were

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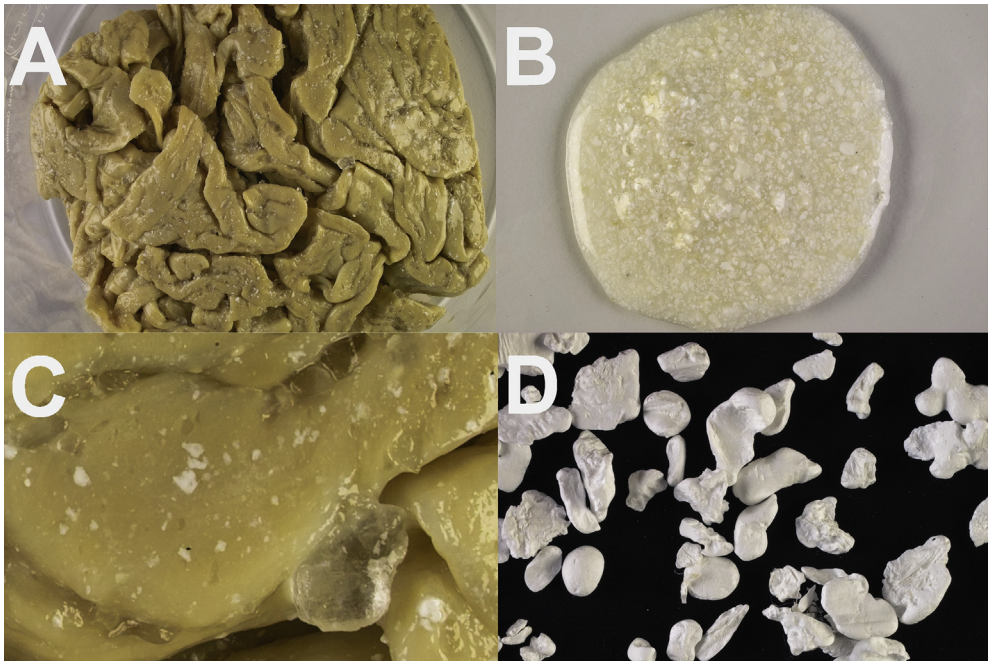

Fig. 1. A, C salted bovine casings with white tyrosine precipitates and translucent salt crystals; B, salt sludge from as transport cask with plenty of white tyrosine particles; D, tyrosine particles purified from the salt sludge.

examined for crystals and microorganisms using phase contrast microscopy (400x) (Leitz, Ortholux II), and purified white particles were examined under a stereo zoom microscope (Motic, SMZ-168). A possible microbiological impact was evaluated by standard microbiological analyses, and by identifying the main microbiota in both samples. Viable counts of aerobic, mesophilic microorganisms from the salt sludge and of a suspension obtained by treating 20 g of casing with 180 mL 0.9% NaCl in a stomacher (AES Laboratories, Mix 1) for 2 min at maximum speed, respectively, were determined in duplicate by plating serial aqueous dilutions on Standard-I nutrient agar (Merck, Darmstadt). Bacterial colonies were counted after 3 days of incubation at 30 °C. Isolates from representative colonies were identified by partial 16S rRNA gene sequence analysis using 616V and 606R as PCR amplification and sequencing primers (Ehrmann, Müller,& Vogel, 2003). The PCR products were purified using the QIAquick PCR purification kit (Qiagen GmbH, Germany) as outlined by the manufacturer and sent for sequence analyses to a commercial sequencing service. Sequencing results were compared to the National Center for Biotechnology Information (NCBI) GenBank data base using the BLAST utility (http://blast.ncbi.nlm.nih.gov; Altschul, Gish, Miller, Myers, & Lipman, 1990). Microbiological standard procedures were used for additional characterization where applicable.

The white particles were physically separated from the brines and casings by repeated wash and sedimentation cycles with deionized water essentially as described previously (Flegel, Bhumiratana, & Srisutipruti, 1981) until a clear solution was obtained with a sediment consisting of the white particles only. The particles were harvested and washed twice with 50 mL of petrolether and 50 mL of acetone. Residual solvent was allowed to evaporate at room temperature and the weight of the air-dried purified particles was determined. The white particles from approximately 2 kg of salted casings were washed off with 1 L of deionized water by agitating them in a polyethylene bag until most of the particles were washed off. The solid particles were transferred with the water into a 2-L glass beaker. An aliquot of the wash water was kept for free amino acid analysis and the sedimented particles were further purified as described above for the salt sludge.

Raman spectra were obtained with a confocal Raman microscope (LabRAM HR, Horiba J. Y., Bensheim, Germany) which was equipped with a 784 nm diode laser (Sacher Lasertechnik, Marburg, Germany), a 600 g/mm grating providing a spectral bandpass of 5 cm^{-1} and a thermo-electrically cooled CCD-detector operating at −70 °C (Synapse 1024x256 FIOP, Horiba). Spectra were recorded in the range of 100 cm^{-1}-2000 cm^{-1} from the dried white powder and from solid L-tyrosine (Merck) using a 50 × long-working distance objective (Nikon, Japan), 22 mW of laser power, 50 s integration time and 3 accumulations per spectrum.

Quantitative amino acid analyses of the purified particles dissolved in hydrochloric acid and of the brines were performed with an automatic amino acid analyzer (ARACUS, membraPure, Germany). Free amino acids in the brines were determined after removing solid particles by centrifugation and protein precipitation with 1/10 volume of cold 10% trichloroacetic acid (TCA).

3. Results and discussion

The pH values of the brines were 5.2 ± 0.1. Microscopy of the liquid phase of the salt sludge and the brine revealed needle-like crystals and, for the salt-sludge, a few rod-shaped bacteria. The purified white particles appeared under the stereo microscope as irregular-shaped, rounded, repelling particles (Fig. 1). The particle diameters ranged from below 1 mm–5 mm with an estimated average of 1–2 mm. From 10 g of salt sludge 0.25 g (2.5%) of purified white particles, and from the casings 0.8 g of clean white air-dry particles (0.04%) were recovered.

The particles did not dissolve in boiling water, sodium hydroxide and common organic solvents, but they readily dissolved in 4 mol L^{-1} hydrochloric acid as well as in 15 mol L^{-1} nitric acid showing a typical xanthoprotein reaction with the latter. This behavior pointed at tyrosine which has very low solubility in water and is known to form visible white precipitates in certain foods (Butz, Blumer, Christian, & Swaisgood, 1974; Arnau, Hugas, García-