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Dynamics of the yeast flora in artisanal country style and industrial dry cured sausage (yeast in fermented sausage)

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ABSTRACT

Yeast can act as an adjunct in the sausage-making process as a way to prevent or reduce excessive acidification during aging of products. Two kinds of process were studied: industrial and artisanal country style. Three hundred and fifty three yeast strains were isolated, characterized and identified by biochemical and molecular techniques. Evolution of pH, Aw, weight loss, bacterial growth and proteolytic and lipolytic activity was studied. Final pH in artisanal country style product was higher than in the industrial sausage. There was little difference noted between final weights of products but it was observed a lower yeast count in artisanal country style sausage. No relevant difference was observed in center or surface yeast count in both products. The biochemical assay identified six yeast genera and the molecular test confirmed four different genera, and further analysis showed predominance of the genera *Debaryomyces*. The relations between this four genera and isolation point (center or surface of sausage) were established. The presence of yeast in the center/surface of ART sausage was more prevalent than in the same places of industrial sausage.

1. Introduction

Dry-cured sausages are a popular meat product enjoyed by millions of consumers worldwide. Their acceptance by consumers relies mainly on sensory quality. Aroma is one of the most important parameters for product quality, and this is affected by raw materials, processing techniques and fermentation quality (Dirinck, Van Opstaele, & Vandendriessche, 1997; Sánchez-Peña, Luna, Gárcia-Gomez, & Aparicio, 2005).

The production of dry sausage was defined as an art based on ancient experience and the customs of each region. However, the most important aspect of modern fermented sausage manufacture, is to produce a product to the required standards (safety, consumer acceptability, shelf-life) in the minimum time so as to reduce holding periods which constitute a cost factor to production. Then for this purpose every day new technologies are development.

In the artisanal country style (ART) process, natural meat fermentation is allowed to occur over extended time periods, bringing about a gradual decrease in pH and drying-out of the product. This process has been progressively replaced by industrial rapid curing methods that use controlled drying chambers and starter cultures to guarantee the safety and quality of the final products (Coventry & Hickey, 1991).

There are reports in the literature which demonstrate that some species of yeasts contribute to flavor and texture development during the curing of various products (Arboles & Julia, 1999; Boissonnet et al., 1994; Deiana et al., 1990; Miteva, Kirova, Gadjeva, & Radeva, 1986; Viljoen & Greyling, 1995). Some studies have shown the influence of yeast strains on the development of the characteristic flavor of dry-cured meat products (Durá, Flores, & Toldrá, 2004; Flores, Durá, Marco, & Toldrá, 2004; Jessen, 1995; Martín, Córdoba, Aranda, Córdoba, & Asensio, 2006). Furthermore, differences in flavor development associated with particular yeast species and biotypes growing on ham have been recently reported (Andrade, Córdoba, Sánchez, Casado, & Rodríguez, 2009).

Yeasts are usually found in high numbers in dry-cured products, especially fermented sausages; even they are not added in methods of production. These high levels suggested that this micro-organism may play an important role in the maturation process (Andrade, Córdoba, Casado, Córdoba, & Rodríguez, 2010). Despite the desirable effect that yeast may have on meat products, no special attention has been given to studying, identifying or quantifying the yeast species present in dry-cured fermented sausage.

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The contribution of yeast to flavor and aroma in a variety of products is directly attributed to the ability of some species to ferment different sugars and as a result produce ethanol, acetaldehyde, ethyl acetate and other compounds. Amino acid degradation leads to generation of volatile molecules during the processing, contributing to the typical flavor of dry-cured sausages (Durá et al., 2004). Yeast species also play a synergistic role by metabolizing the lactic acid present in fermented products, which causes a shift in pH toward neutrality and thus produces a sweeter end product.

The composition of yeast flora is an important determining factor for the quality and sensory characteristics of meat products. In this study, we endeavored to investigate yeast growth during the manufacturing process of ART and industrial style (IND) Spanish dry-cured sausage (Salchichón).

2. Materials and methods

2.1. Sausage manufacture and sampling

The formulation of the two batches of sausages included lean pork and pork fat (1:1), 2.8% (w/w) NaCl, 3.0% (w/w) lactose, 1.0% (w/w) sodium caseinate, 0.5% (w/w) sodium ascorbate, 0.02% (w/w) spices, 0.030% (w/w) KNO_3 (only for batch 1, ART) or 0.015% (w/w) $NaNO_2$ (only for batch 2, IND). Batch 2 also contained 0.01% of lactic acid starter MC-156 (*Lactobacillus sake*, *Pediococcus pentosaceus*, *Staphylococcus carnosus*, *Staphylococcus xylosus* – from Textel, Europe Rhone Poulenc, Madrid, Spain).

The lean meat and fat were chilled at 4 °C for two days and kept at −5 °C overnight. They were then minced through a 6 mm mincer. The meat components and remaining ingredients were mixed under vacuum (Fatosa Mixer, Model AV80, 60 mm Hg) for two in both directions. Afterwardthe mixture was stuffed into 75/80 mm diameter collagen casing (Fibran). The final weight of each sausage was approximately 500 g.

The ART products were kept at 20–22 °C and 75% RH for 6 h and, then at 85–90 % RH and the same temperature for three days to promote product fermentation. The IND products were kept at 24–26 °C and 75% RH for 6 hand then at 85–90 % RH and the same temperature for three days for fermentation. The ART and IND sausages were dried at 12–15 °C and 70–75 % RH for 50 days.

Samples were taken for analysis at different stages during the processing: raw minced meat (0 day), after the initial pH drop/final fermentation (3rd day), at the beginning of curing (7th day), during curing (10/20/30 days) and at the end of curing (50th day). Four samples were taken each day for each treatment.

2.2. Microbiological analyses

For each sausage, samples of 25 g were taken aseptically from the inner part and 25 g were taken aseptically from the surface. The samples were homogenized with peptone water 0.1% (ADSA Micro, Barcelona, Spain) (w/v) in a blender (Stomacher Model 400) for 3 min. Serial decimal dilutions were made using the same medium, then plated in duplicate for yeast count in Rose Bengal chloramphenicol agar (ADSA Micro) and incubated at 27 °C for 48 h. Five representative yeast isolates were sub-cultured on yeast Malt Extract Agar (YM, ADSA Micro) and incubated for 48 h at 27 °C for control of purity by colony morphology and microscopy. The pure cultures were stored at 4 °C on Malt Extract Agar (MA, ADSA Micro) during the period of investigations.

Tributyrin Agar, Gelatin and Milk Agar were used as the medium to estimate lipolytic and proteolytic activity of yeast isolates (Besançon et al., 1992; Boissonnet et al., 1994).

2.3. Characterization and identification of isolates

2.3.1. Biochemical tests

The representative yeast isolates were classified according to the identification keys described by Van der Walt and Yarrow (1984) and by Barnett, Payne, and Yarrow (2000). The tests used for the genus and species determinations were: pseudomycelium or true mycelium formation on Corn Meal Agar (CMA, Difco), ascopore formation, carbohydrate fermentation and nitrate assimilation, formation of extracellular amyloidal compounds (starch test), production of ammonia from Urea (Urease test), assimilation of carbon compounds, resistance to 0.01 and 0.1% cycloheximide and growth on vitamin-free medium. Colony appearance, pigment and extracellular polysaccharide formations were noted by streaking onto MA.

2.3.2. Molecular characterization

2.3.2.1. PCR and analysis of rDNA. DNA was extracted and prepared according to the method used by Querol, Barrio, and Rámon (1992). The primers used to amplify the ITS region (ITS1-5′TCCGTAGGTGAACCTGCGG3′ and ITS4-5′TCCTCCGCTTATTGATATG C3′) were described by White, Bruns, Lee, and Taylor (1990). The separation of the fragments was performed by electrophoresis on 3% agarose gel in TAE buffer. The marker used was 100 bp DNA ladder (Biolabs, Inc), and bands were visualized in a Vilber Lourmat ultraviolet light transilluminator after gel had been stained with ethidium bromide.

2.3.2.2. Amplification and sequencing of mitochondrial DNA (mtDNA). The mitochondrial genes G_1C_1 A_1T_1 were amplified and sequenced according to Querol et al. (1992), with the following restriction enzymes: *Cfo*I, *Hae*III, *Hinf*I. Restriction enzymes were used according to the provider's recommendations (Boehringer Mannheim). The separation of the fragments was performed by electrophoresis on 1% agarose gel in TBE buffer. The marker used was DNAλ digested with PstI (Boehringer Mannheim). The bands were visualized in a Vilber Lourmat ultraviolet light transilluminator after gel had been stained with ethidium bromide.

2.3.3. Analytical methods

The pH of sausage was determined in each sample obtained by blending 10 g of sample with 30 mL of distilled water and measured in an Orion Research pH meter (Expandable Ion Analyzer EA920, Boston, USA).

Ten sausages were randomly picked from each batch, and the weight loss was measured by weighing the selected sausages each day throughout the processing period (day 0 to day 50). Water activity was measured with a Humtat-RC Novasina apparatus.

3. Results

3.1. Evolution of yeast flora and pH during sausage processing

The evolution of yeast flora during the processing of artisanal country style (ART) and industrial (IND) sausages is reflected in Fig. 1. The viable yeast count increased from an initial count of 3.43 log UFC/g to 5.03 on surface for ART samples and increased from 3.54 log UFC/g to 4.71 for IND samples, during the first seven days. A slight decrease in the viable count occurred between seven and 10 days, once again increasing during the next period. The viable yeast count between ART and IND sausages for surface count (continuous line) was very similar and corresponded to other reports in the literature (Banks & Board, 1987; Boissonnett et al., 1994; Fung & Liang, 1990; Molina, Silla, & Flores, 1990; Smith & Palumbo, 1973). The viable yeast count (point line), in the inner