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ScienceDirect
Meat Science 80 (2008) 345–354
MEAT SCIENCE
www.elsevier.com/locate/meatsci

Effect of processing methods and starter culture (*Staphylococcus xylosus* and *Pediococcus pentosaceus*) on proteolytic changes in Turkish sausages (sucuk) during ripening and storage

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Received 31 July 2007; received in revised form 18 December 2007; accepted 19 December 2007

Abstract

Proteolytic changes in Turkish sausages (sucuk) produced by two methods (heat processing and traditional) were determined during processing and storage for 90 days. The sausages were produced with or without starter culture in both methods. A mixture of *Staphylococcus xylosus* and *Pediococcus pentosaceus* was used as starter culture for their acidic and proteolytic characteristics.

The major changes in proteolytic characteristics of sucuk took place during the fermentation stage, with an increase in non-protein nitrogen (NPN) and a decrease in protein solubility. Proteolytic activity was observed in both starter-inoculated and non-inoculated (control) sausages during processing. Moreover, a slight increase in proteolytic activity was detected during storage in both starter-inoculated and control traditional sausages, and also in heat-processed sausages due to some heat-resistant proteolytic enzymes. Protein solubility was significantly affected by processing time and heat treatment. Sarcoplasmic and myofibrillar proteins were also affected by starter addition, fermentation, drying and heat processing. During fermentation, starter-inoculated and control sausages showed intense proteolysis in both the traditional and heat processing methods. After heating, intensive degradation of both sarcoplasmic and myofibrillar proteins due to denaturation was observed in heat-processed samples.

Keywords: Turkish sausage (sucuk); Starter culture; Proteolysis; Traditional method; Heat processing method; Processing and storage days

1. Introduction

Turkish sausage (sucuk) is a semi-dried meat product mostly produced by traditional methods, which mainly include formulation with or without starter addition, fermentation and ripening/drying stages (Soyer, Ertaş, & Üzümcüoğlu, 2005). The characteristics of the final sausage are the result of complex biochemical, microbiological, physical and sensorial changes occurring in the meat formulation during ripening under defined conditions of temperature and relative humidity (RH). In the past decade, heat processing has been incorporated into the production stage and results in a shorter processing time and a safer product. The sausages are generally heated to an internal temperature of 45–70 °C after a short fermentation period. However, compared with traditional sausages, heat-processed sausages have insufficient flavour and taste, since the heating results in inadvertent destruction of desirable microbial flora (Erçoşkun, 2006). Previous studies on heat processing of sucuk sausages have mainly focused on changes in colour and sensory properties and pathogen destruction under different time–temperature conditions (Erçoşkun, 2006; Filiz, 1996; Tayar, 1989; Vural, 1993).

Turkish fermented sausages can be manufactured using microbial strains belonging to the genera *Lactobacillus* (*L. plantarum*, *L. pentosus*, *L. curvatus* and *L. sake*), *Pediococcus* (*P. pentosaceus* and *P. acidilactici*), *Micrococcus* (*Korucia varians*) and *Debaryomyces* (*D. hansenii*) as starter

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doi:10.1016/j.meatsci.2007.12.022

cultures (TSE, 2002). The commercial starter cultures commonly used in sucuk production are selected according to their technological activities such as fermentative, proteolytic or lipolytic characteristics. However, they are not always suitable for sucuk production and may result in losses of some desirable sensory characteristics. While environmental factors such as temperature, relative humidity, pH, raw meat and other ingredients interact to limit the number of strains that are sufficiently competitive to dominate the process, appropriate starter cultures have to be selected, according to product characteristics (Rebecchi, Crivori, Sarra, & Cocconcelli, 1998).

Several biochemical and physical changes occur during the ripening of fermented sausages that determine the flavour and odour of the end product. These changes are mainly acidification as a result of fermentation, pH decrease, changes in initial microflora, reduction of nitrates to nitrites and formation of nitrosomyoglobin, solubilisation and gelification of myofibrillar and sarcoplasmic proteins, proteolytic, lipolytic and oxidative phenomena, and dehydration (Casaburi et al., 2007; Ordóñez, Hierro, & Bruna, 1999). Proteolysis is one of the main degradation mechanisms affecting proteins during the ripening of fermented sausages (Diaz, Fernandez, Garcia de Fernando, de la Hoz, & Ordóñez, 1993). Proteolysis of dry cured meat products has been attributed mainly either to endogenous enzymes (Toldrá, 1998; Verplaetse, 1994) or to exogenous enzymes originating from microorganisms (Diaz, Fernandez, Garcia de Fernando, de La Hoz, & Ordoñez, 1997; Mauriello, Casaburi, & Villani, 2002; Sanz et al., 1999).

The objective of this study was to determine the proteolytic changes during processing and storage in Turkish sausages (sucuk) produced with or without the addition of a specific starter culture, a mixture of *Staphylococcus xylosus* and *Pediococcus pentosaceus*. Two different production methods were compared, traditional and heat processing.

2. Materials and methods

2.1. Sausage production

Traditional and heat processing sausage production methods were compared in three trials, which meant that sausage production was performed three times. Right and left forequarters obtained from three 2-year-old cows (each of which represented one trial) that had been reared in the same rearing system were used 24 h post-slaughter for sausage production. Visible fat and connective tissue was trimmed off the meat. The sausage formulation included 80% lean meat, 20% adipose tissue separated from the tail of sheep, 1.6% NaCl, 1.2% garlic, 0.5% sucrose, 0.5% hot red pepper, 0.6% sweet red pepper, 0.6% black pepper, 0.8% cumin, 0.004% $NaNO_3$ and 0.01% $NaNO_2$. The meat and frozen adipose tissue were minced using a medium-scale grinder through a 12 mm mesh plate (Ari Torna Co., İstanbul, Turkey) and mixed with the other ingredients in a mixer (Yuneka Metal Co., Bursa, Turkey). After mixing, the sausage mixture was divided into four batches. Two of these were inoculated with starter culture and the others were non-inoculated, to act as control. The starter culture was composed of a mixture of *S. xylosus* and *P. pentosaceus* (TSPX –100, CHR Peyma Hansen). Freeze–dried starter culture was added to the sausage mixture at a concentration of 10^7 cfu/g. The traditional and heat processing methods each included one starter culture-inoculated batch and one non-inoculated control batch.

The batches were allowed to stand overnight at 4 °C, and then stuffed into 36 mm diameter collagen casings using a hydraulic filling machine (Yuneka Metal Co., Bursa, Turkey). Sausages were hung on stainless steel hangers and allowed to equilibrate at 20 °C and 70% RH for 6–8 h. They were then placed in a ripening chamber (Biogen Co., Ankara, Turkey) equipped with a process control system. Traditionally processed sausages were subjected to the following conditions of RH and temperature: fermentation for 3 days at 25 °C and 85–90% RH, followed by drying for 3 days at 22–24 °C and 80–85% RH, for 2 days at 18 °C and 70–75% RH, and for 1 day at 5 °C and 65% RH. The sausages were vacuum packaged and stored at 4 °C for 90 days. Total processing time was 9 days for the traditional method.

Heat-processed sausages were exposed to fermentation for 3 days as described above for traditional processing and dried for 1 day at 22–24 °C and 80–85% RH and removed from the chamber. They were then heat processed at 68 °C until 15 min after the core temperature had reached 68 °C. After the heat treatment, the sausages were cooled to room temperature by spraying with cold water for 10 min. The sausages were dried at 16 °C for 12 h, vacuum packaged and stored at 4 °C for 90 days. Total processing time was 5 days for the heat processing method.

2.2. Sampling

Samples were taken before stuffing (at day 0) and during processing (on days 1, 2, 4, 7 and 9) for the traditional method and on days 0, 1, 2, 4 and after heat treatment (day 5) for the heat processing method. Samples were also taken on days 30, 60 and 90 of storage. On each sampling occasion, three sausages from each batch were taken for microbiological, water activity (a_w), pH and moisture analyses. The remainder of the sausages were vacuum packaged and stored at −65 °C for further protein analysis.

2.3. Microbiological analyses

Two 10 g slices from each sausage were weighed aseptically, transferred to sterile plastic pouches, diluted with 90 mL of sterile saline diluent containing 1% peptone and homogenised for 2 min using a Stomacher 400 (Seward, London, UK). Total viable counts were determined on plate count agar (PCA) (Merck, Darmstadt, Germany) and micrococci–staphylococci counts on Baird Parker Agar (BPA) (Merck, Darmstadt, Germany) supplemented