International Journal of Food Microbiology 279 (2018) 26–32

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Sausage fermentation and starter cultures in the era of molecular biology methods

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ARTICLE INFO

Keywords:
Lactic acid bacteria
Coagulase negative cocci
Starter cultures
Bioprotection
Ecology
Metataxonomics
Metagenomics

ABSTRACT

Fermented sausages have a long tradition originating from Europe and they constitute a significant part of the Mediterranean diet. This kind of products has a specific microbiota that is typical of the region or area where they are produced. Therefore, in order to protect the traditional aspect of these products, it is essential to understand the microbial ecology during fermentation by studying the dynamic changes that occur and to select autochthonous starter cultures that can be used in the production. In this paper we summarize the state of the art concerning the selection and use of starter cultures and ecology aspects of naturally fermented sausages. We pay particular attention to the application of bacteriocinogenic strains as they could provide an additional tool in the prevention of foodborne pathogens as well as enhancing the competitiveness of the starter organisms. Microbial ecology of fermented sausages has been determined by traditional microbiological methods, but the introduction in food microbiology of new molecular techniques complements the studies carried out so far and allows scientists to overcome the limitations of traditional methods. Next Generation Sequencing (NGS) techniques represent a change in the way microbiologists address ecology and diversity in foods. Indeed the application of metataxonomics and metagenomics will permit a detailed understanding of microbial ecology. A thorough knowledge of the mechanisms behind the biological processes will enhance meat fermentation control and modulation to obtain products with desired organoleptic properties.

1. Introduction

In Europe, dry fermented sausages have a long tradition originating from Mediterranean countries during Roman times. Processing conditions, as well as ingredients and additives, vary among the different types of fermented sausages (Gardini et al., 2001). In fact, 'typical' foods of any region or area have their own peculiar characteristics that are deeply rooted in tradition and linked to the territory and which arise from the use of local ingredients and specific production techniques (Aquilanti et al., 2007; Casaburi et al., 2007). The production process begins with small pieces of meat and fat that are minced; salt and spices and in some cases sugar, herbs and/or other ingredients are then added. The homogenised mixture is then stuffed into casings, and undergoes fermentation and drying. European legislation, under Reg. EC 1333/2008 (and subsequent modifications), allows the use of nitrate and nitrite as preservatives, unless subject to other regulations for protected denomination of origin (PDO) products (Aquilanti et al., 2016). The qualitative characteristics of fermented sausages are known to be largely dependent on the quality of the ingredients and raw materials, the specific conditions of the processing and ripening, and the composition of the microbial population (Aquilanti et al., 2007). Pathogenic and spoilage bacteria are inhibited; consequently, the final product has an increased shelf-life (Hugas and Monfort, 1997).

Meat fermentations are complex microbial ecosystems in which bacteria, yeasts and molds coexist. Considerable microbial diversity is observed during the fermentation process and is evidenced by the presence of several species belonging to different genera, but also strains of the same species. Through fermentation, highly perishable raw materials, such as meat and fat, are transformed in microbiologically stable final products, characterized by a defined sensory profile, enhanced due to sodium chloride supplementation and to the drying process (Cocolin et al., 2011). Changes that occur during fermentation and drying influence the aroma development in fermented sausages (Flores et al., 2004).

Many typical fermented meat products are still produced with traditional technologies without selected starters. However, in the modern sausage production, the use of starter cultures is becoming more frequent to guarantee safety and to standardize product properties, for example consistent flavor and colour and shorter ripening time (Cocolin et al., 2001).

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https://doi.org/10.1016/j.ijfoodmicro.2018.04.038
Received 18 February 2018; Received in revised form 23 April 2018; Accepted 24 April 2018
Available online 25 April 2018

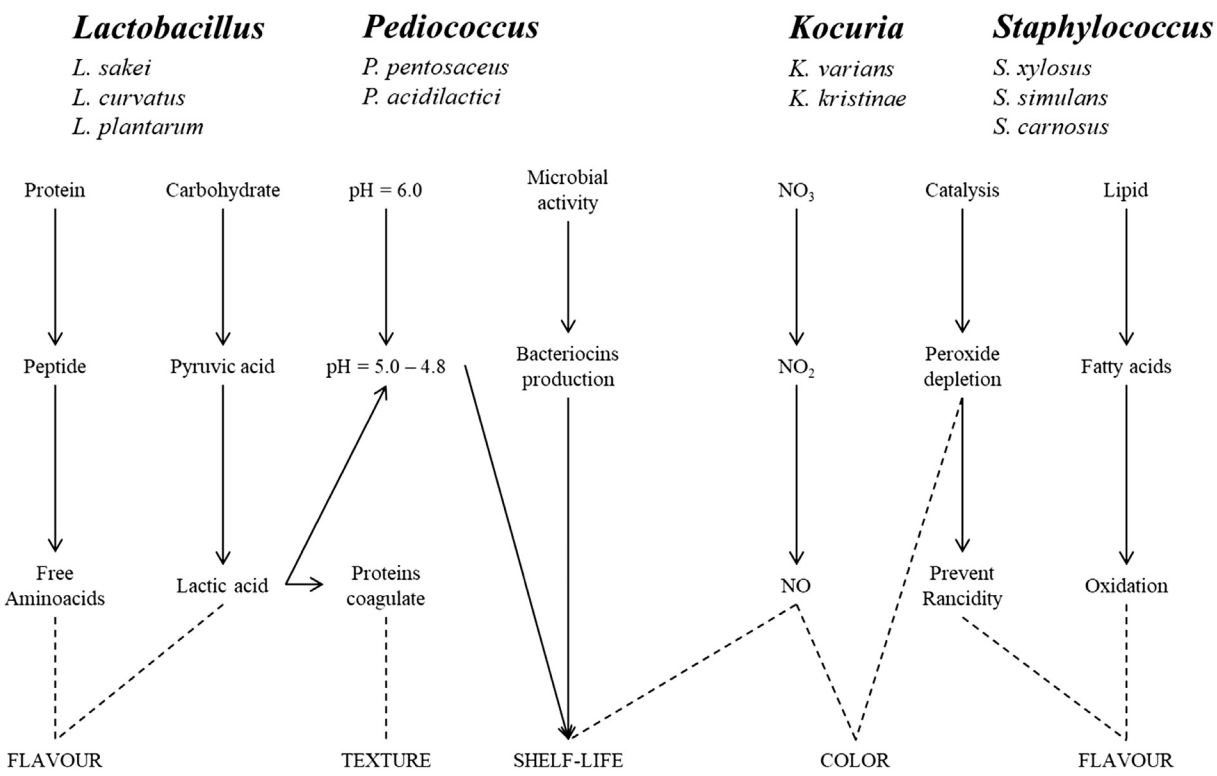

Fig. 1. Summary of biochemical activities performed by principal microbial groups in fermented sausages. The most frequently isolated species of each group are nominated.

2. Starter cultures

Starter cultures are preparations that contain actively growing or resting forms of microorganisms that with their metabolic activity (Fig. 1) impart desired effects during fermentation (Hammes and Hertel, 1998). Industrialized production of starter cultures is a consequence of the gradual shift in sausage production from small local producers to large-scale processing plants and the increasing awareness of the risks for consumer health, in view of overall process efficiency (Magistà et al., 2017). The introduction of starter cultures has become essential in order to shorten the ripening period, ensure colour development, enhance the flavor and improve product safety, given that industrial production of fermented sausages is increasing (Lücke, 1986). In fact, a starter culture should be capable of conducting the fermentation, colonizing the product and dominating over other microorganisms from the beginning to the end of the process (Cocolin et al., 2006).

On the other hand, the use of commercially available starters, mainly constituted of lactic acid bacteria and coagulase negative cocci, may result in a loss of peculiar organoleptic characteristics found in spontaneously fermented sausages with an impoverishment of flavor and aroma. For this reason, in several European countries, the artisanal sausages that are manufactured by relying on an unknown 'factory biota' are preferred by the consumer (Samelis et al., 1994). The quality of such artisanal, spontaneously fermented sausages possess distinctive characteristics and are often superior if compared to controlled fermentations, inoculated with industrial starters. The principal differences between traditional and industrial fermented product are summarized in the Table 1.

This is due to the technology used, the properties of the raw material (Moretti et al., 2004) and the specific composition of the microbiota (Leroy et al., 2006). Nonetheless, Sunesen and Stahnke (2003) reported that sausages produced with commercial molds show more consistent flavor, taste, drying rate, and a more uniform appearance with respect to artisanal fermented sausages.

Table 1
Main differences between traditional and industrial fermented food products. (Adapted and modified from El Sheikha and Montet, 2016.)

Traditional fermented products	Industrial fermented products
Small-scale	Large-scale
Manual	Automated
Intensive to time	Time-sensitive
Possible exposure to contaminants	Minimal exposure to contaminants
Varying quality	Constant quality
Complex sensory attributes	Less complex sensory attributes
Attention to organoleptic characteristic of the product	Safety driven operation
Shorter shelf-life	Longer shelf-life
Large undefined microbial diversity	Reduced microbial diversity
Limited use of selected microbial cultures	Extensive use of microbial cultures

The microbial ecology of fermented sausages has become of increasing interest over the last few decades given that different genera, species, and even strains, have been shown to significantly affect the sensory traits of fermented sausages (Rantsiou and Cocolin, 2006). Production of artisanal sausages largely depends on the skill and experience of the meat manufacturer and may be considered an art rather than a process fully based on scientific and technological understanding. Meat fermentation is, in fact, a complex biological phenomenon accelerated by the desirable action of certain microbes in the presence of a variety of synergistically acting or competing species. A great variability in the quality of the products is due to traditional practices and variation in the microorganisms involved in the process.

De Vuyst (2000) underlines that it is of primordial importance to investigate and analyze the influence of the environment on the performance of a starter culture before using it in a selected product.

In order to protect the traditional aspects of these products and to select autochthonous starter cultures to be used, it is essential to