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Yeast strains as potential aroma enhancers in dry fermented sausages

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ABSTRACT

Actual healthy trends produce changes in the sensory characteristics of dry fermented sausages therefore, new strategies are needed to enhance their aroma. In particular, a reduction in the aroma characteristics was observed in reduced fat and salt dry sausages. In terms of aroma enhancing, generally coagulase-negative cocci were selected as the most important group from the endogenous microbiota in the production of flavour compounds. Among the volatile compounds analysed in dry sausages, ester compounds contribute to fruity aroma notes associated with high acceptance of traditional dry sausages. However, the origin of ester compounds in traditional dry sausages can be due to other microorganisms as lactic acid bacteria, yeast and moulds. Yeast contribution in dry fermented sausages was investigated with opposite results attributed to low yeast survival or low activity during processing. Generally, they affect sausage colour and flavour by their oxygen-scavenging and lipolytic activities in addition to, their ability to catabolize fermentation products such as lactate increasing the pH and contributing to less tangy and more aromatic sausages. Recently, the isolation and characterization of yeast from traditional dry fermented sausages made possible the selection of those with ability to produce aroma active compounds. Molecular methods were used for genetic typing of the isolated yeasts whereas their ability to produce aroma compounds was tested in different systems such as in culture media, in model systems and finally on dry fermented sausages. The results revealed that the appropriate selection of yeast strains with aroma potential may be used to improve the sensory characteristics of reformulated fermented sausages.

1. Introduction

Dry cured meat products constitute a wide group of products from hams to sausages which have been consumed for a long time (Toldrá and Flores, 2014). Among them, dry fermented sausages are widely consumed worldwide due to its characteristic flavour (Flores and Olivares, 2015). Moreover, the actual healthy trends for meat products with reduced fat and salt contents (European Commission, 2009) may produce a significant effect on high quality products such as traditional dry sausages. The reformulation following healthy trends may represent an added value to the production and consumption of traditional dry sausages. However, flavour, the most important characteristic for consumers, can be affected (Ruusunen and Puolanne, 2005).

In the last decade many studies have tried to reformulate dry fermented sausages in terms of fat and salt reduction maintaining the sensory characteristics although little attention has been focused on traditional dry fermented products (Olivares et al., 2011). These traditional fermented products are characterized by an indigenous microbiota that produces regional specific flavours (Talon et al., 2007). Many studies have been focused on this microbiota (Baruzzi et al., 2006; Fontana et al., 2005) as they are involved in hygienic and sensory properties, such as lactic acid bacteria involved in acidification whilst coagulase-negative staphylococci (CNS) group in the development of colour and flavour (Ravyts et al., 2012; Talon et al., 2007). On the other hand, the potential role of yeast in traditional sausages has not been established yet and it is necessary to look for new strategies to improve and diversify traditional dry sausage flavour (Ravyts et al., 2012; Talon et al., 2007) in order to counteract the effect of the reformulation. Nevertheless, the importance of yeasts in the manufacture of meat products has been recognized since the 70s with the use of *Debaryomyces hansenii* as starter culture for sausage manufacture (Hammes and Kauf, 1994). Therefore, further studies on yeast diversity from fermented dry products might reveal new yeasts with increased abilities for aroma production.

2. Flavour of dry fermented sausages

The large differences in processing produce a large variety of products with differences in terms of sensory properties from appearance to flavour (Flores, 2011a). The differences in the sensory properties have been described by Quantitative Descriptive Analysis (QDA) and are related to appearance, odour, texture, aroma and taste

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(Benito et al., 2004; González-Fernández et al., 2006; Iaccarino et al., 2006; Valencia et al., 2006).

Dry fermented sausage flavour is affected by many processing factors such as different raw materials, starter cultures, smoking, etc. (Leroy et al., 2006) and it is different from the one originated through thermal meat treatment (Calkins and Hodgen, 2007; Flores, 2011b; Mottram, 1998) because of different biochemical processes involved (Flores and Toldrá, 2011; Flores and Olivares, 2015). Moreover, starter culture metabolism is affected by the raw material used as well as by the processing factors mentioned (Leroy et al., 2006). The main pathways involved in flavour development are: carbohydrate metabolism, degradation of free amino acids and fatty acid to volatile compounds, and addition of spices (Flores and Olivares, 2015).

The fermentation of carbohydrates is mainly performed by lactic acid bacteria that dominates the fermentation process and produces lactic acid and other aroma compounds such as diacetyl, acetaldehyde, ethanol, acetic, and propionic acids among others (Ravyts et al., 2012).

The enzymatic generation of amino acids and free fatty acids comes from proteolysis and lipolysis reactions (Toldrá et al., 2001). In general, muscle endogenous enzymes are responsible for these reactions especially at the beginning of the ripening process. Microbial enzymes play an important role in generating free amino acids and fatty acids (Flores and Toldrá, 2011). The further degradation of these precursors to aroma compounds is essentially mediated by chemical and microbial reactions (Flores and Olivares, 2015). The chemical degradation reactions consist of fatty acid auto-oxidation reactions, Maillard reactions and Strecker degradation reactions. The auto-oxidation of polyunsaturated fatty acids generates compounds such as aldehydes, alcohols, alkanes, esters and carboxylic acids that evoke specific aroma notes to the dry fermented sausages as a result of their low odour threshold values (Shahidi et al., 1986). In Maillard reactions, an amino compound reacts with the carbonyl group of a reducing sugar in the presence of heat being the amino acids the nitrogen source. Whilst these reactions have been deeply studied in cooked meat flavour (Calkins and Hodgen, 2007) little is known about their role in dry fermented sausages mainly due to the low temperatures that do not favour them. Nevertheless, many volatile compounds produced from these reactions, such as pyrazines, furans, etc., have been described as potent odorants in dry fermented sausages (Flores and Olivares, 2015).

Microorganisms contribute to the generation of flavour compounds by the microbial degradation of amino acids and fatty acids. Degradation reactions are transamination and further decarboxylation of amino acids (branched, aromatic and linear) which result in their respective aldehydes, alcohols or acids that impart aroma notes to the sausages (Ordoñez and de La Hoz, 2007).

The contribution of spices in dry fermented sausages is towards flavour in addition to their impact on appearance as well as taste. In dry fermented sausages, the most widely used spices are garlic, black pepper, paprika, onion, mustard, nutmeg, oregano, and aniseed among others (Chi and Wu, 2007).

2.1. Flavour of traditional vs industrial dry fermented sausages

The classification of dry fermented sausages based on the manufacturing processes, traditional and industrial, produces singular attributes in the sausages, especially in flavour. These traditional dry fermented sausages are highly appreciated by consumer due to their singular flavour (Conter et al., 2008; Olivares et al., 2014). Generally, European consumer's satisfaction with pork meat products is due to its taste, easy to prepare and consume and good price/quality relationship (Resano et al., 2011). Although, sensory differences have been appreciated by consumers between industrial and traditional fermented sausages, it has been described that consumers have different expectations due to cultural and experience levels that influence perception of typical food quality (Iaccarino et al., 2006).

Traditional or naturally dry fermented sausages are manufactured without the use of starter cultures but autochthonous microbiota is responsible for the sensory characteristics. Generally these sausages reach pH values higher than 5 and are considered as low acid sausages (Montel et al., 1998; Talon et al., 2007). Due to the long ripening times applied, both nitrite and nitrate are used as curing agents although the processing temperature must be kept low to control the growth of pathogenic bacteria. In contrast, industrial sausages are produced with the use of starter cultures that increase the safety and quality of the final product. Moreover, industrial sausages are characterized by a fast fermentation process that reaches low pH values (lower than 5) that may affect the colour and flavour due to the excessive acid taste (Flores, 2011b). In this case, the use of nitrite as exclusive curing agent imparts an aroma character different from those manufactured with nitrate such as traditional dry sausages (Marco et al., 2006). In this sense, it has been proposed that the development of indigenous starters from traditional dry fermented sausages may help to diversify sausage flavour (Talon et al., 2007).

Recently, it has been indicated that only 3% of the 10,000 volatiles expected in foods contribute to the aroma (Dunkel et al., 2014). In the case of dry fermented sausage, hundreds of volatile organic compounds (VOCs) have been identified but only few of them contribute to the aroma (Flores and Olivares, 2015). The use of olfactometry techniques has allowed the elucidation of these aroma compounds (Stahnke, 1994). In the last years this technique has evolved to unveil the most important odorants by the use of specific olfactometry techniques and the calculation of odour activity values (De Roos, 2007).

In this sense, several aldehydes, acids, sulphur and ester compounds (acetic, 3-methyl-butanoic and butanoic acids, 3-methylbutanal and phenylacetaldehyde and the esters; ethyl butanoate, ethyl 2-methylpropanoate) together with other compounds derived from the smoking process and spices were described as the most potent odorants in dry fermented sausages (Marco et al., 2007; Söllner and Schierberle, 2009). However the composition of these aroma compounds may vary due to differences in processing parameters (smoking process, etc.) and raw materials (meat, spices …) used.

In contrast, the aroma of traditional dry fermented sausages was studied and many VOCs compounds were selected for their aroma impact (Corral et al., 2014a; Gianelli et al., 2011; Olivares et al., 2014; Schmidt and Berger, 1998a,b). The high impact of compounds was remarkable: ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, 4-methylphenol, ethyl benzoate, benzothiazole, 2,4-decadienal (E,E), methyleugenol and γ-nanolactone (Corral et al., 2014a). When traditional and industrial dry fermented sausages were compared in terms of flavour, several ester compounds were reported as responsible for the characteristic "fruity" aroma detected in traditional dry sausages. Ester compounds were detected in both types of sausages, traditional and industrial (Table 1) and it was characteristic the low threshold values imparted by ethyl branched ester compounds together with ethyl butanoate. The highest ester compounds present in traditional dry fermented sausages (Olivares et al., 2010) may be the reason for its special fruity aroma character.

The origin of ester compounds in dry fermented sausages has been mainly attributed to CNS together with the production of branched aldehydes and methyl ketones (Montel et al., 1998). CNS produces aroma compounds form branched chain amino acids generating branched aldehydes that can be converted to ester compounds imparting fruity notes as well as, through the beta-oxidation pathways CNS produces methyl ketones. However, other microorganisms, such as lactic acid bacteria, yeast and moulds, have been suggested as contributors to ester compounds (Tjener and Stanhke, 2007) and, therefore, further research on yeast ability to produce ester compounds during dry fermented sausage manufacturing is essential to understand their role in flavour development.