British Journal of Applied Science & Technology
17(1): 1-8, 2016, Article no.BJAST.27740
ISSN: 2231-0843, NLM ID: 101664541

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Contribution of Catalase Positive Cocci on Flavour Formation in Fermented Sausages

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJAST/2016/27740
Editor(s):
(1) Ming-Chih Shih, Department of Health and Nutrition Science, Chinese Culture University, Taiwan.
Reviewers:
(1) Yiannis Kourkoutas, Democritus University of Thrace, Greece.
(2) Marcelo Teixeira Leite, Federal University of Paraíba, Brazil.
(3) Falodun Olutayo Israel, University of Ibadan, Ibadan, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/15690

Mini-review Article

Received 16th June 2016
Accepted 27th July 2016
Published 6th August 2016

ABSTRACT

Flavour is an essential quality of food and formed by large number of volatile (alcohol, ketones, aldehydes, esters and terpenes etc.) and non-volatile compounds (amino acids and peptides etc.). In fermented sausage, flavour develops from biochemical reactions and is influenced by several variables such as formulation, process conditions and starter culture. Microorganisms involved in fermentation play an important role in the formation of aroma. Lactic acid bacteria and catalase-positive cocci are the most important starter cultures for fermented sausage. Lactic acid bacteria are mainly responsible for lactic acid production in sausage. Catalase-positive cocci have an impact on catalase activity, colour stability, prevention of rancidity and flavour formation. These microorganisms enhance flavour through proteolytic and lipolytic activity. This study reviews the functions of catalase-positive cocci and their effects on flavour in fermented sausages.

Keywords: Catalase-positive cocci; fermented sausage; flavour; branched chained amino acid; volatile compounds.

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1. INTRODUCTION

Fermentation is one of the oldest methods for extending the shelf life of food products. Biochemical and physical reactions that take place during fermentation change the product's initial properties. Fermented sausages are produced from a mixture of meat, fat, curing agents (nitrate/nitrite), spices and sugars through lactic acid fermentation [1]. Lactic acid bacteria and catalase-positive cocci are the most important microorganisms in the technological and sensorial properties of dry or semi-dry fermented sausages [2-5]. Lactic acid bacteria play a major role in acidification [6]. Therefore, they improve the product's safety and stability. Gram-positive catalase-positive cocci play an important role in colour stability, prevention of rancidity and flavour formation [7-9].

Catalase-positive cocci grow slowly or not at all during ripening, and the growth occurs mainly on the surface of unsmoked sausages. Because of this, high levels of catalase-positive cocci (10^6–10^7cfu/g) are added to sausage mixture. At this point, the specific enzyme activity of the strain is particularly important [10]. Slow ripening in fermented meat products provides an advantageous environment for catalase-positive cocci. These microorganisms are generally active until lactic acid formation results in low pH levels [11]. Adding small amounts of sugar at low ripening temperature causes slow acid formation, which encourages the growth of these microorganisms. Moreover, in fermented sausages produced using nitrate and low levels of sugar, such as traditional Italian fermented sausages, catalase-positive cocci may be the dominant flora [12].

The typical flavour of dry fermented sausages is the result of a careful balance between volatile (alcohol, ketones, aldehydes, esters, terpenes, aliphatic hydrocarbons, aromatic hydracarbons and furans) and non-volatile compounds (amino acids, peptides, sugars and nucleotides), which come from the basic ingredients (meat, spices, nitrites and other additives) or generated by the breakdown of carbohydrates, lipids and proteins through the action of microbial and endogenous meat enzymes during ripening [13]. This study reviews the functions of catalase-positive cocci and their effects on flavour in fermented sausages.

2. THE ROLE OF CATALASE-POSITIVE COCCI IN FERMENTED SAUSAGES

The catalase-positive cocci identified from various types of traditional fermented sausage are given in Table 1. *Staphylococcus xylosus* is the most frequently isolated catalase-positive cocci in dry fermented sausages [14-16]. Strains of *Kocuria* and coagulase-negative *Staphylococcus* are used as starter cultures. These species grow in the presence of 10% NaCl, produce catalase and reduce nitrate to nitrite. However, they exhibit slow growth under anaerobic conditions. In sausages which are ripened normally and slowly, there are fewer catalase-positive cocci in the core than the periphery after a few days of ripening, due to

Table 1. Catalase positive cocci strains identified from different traditional fermented sausages

Strains	Fermented sausage	References
Staphylococcus xylosus	Fermented Italian sausages	[17-20]
	Chorizo	[21]
	Slovak fermented sausage	[22]
	Fermented sausage (Basilicata region)	[23]
	Greek fermented sausage	[24,25]
	Sucuk (Turkish fermented sausage)	[16]
	Salami (Italian fermented sausages)	[26]
S. saprophyticus	Naples type salami	[27]
	Iberian type sausage	[28]
	Sucuk	[16]
	Artisanal Argentinean sausage	[29]
S. carnosus	Spanish dry-cured sausage	[30]
	Sucuk	[16]
S. succinus	Salami	[31]
S. equorum	Sucuk	[16]

oxygen sensitivity. *K. varians* in particular needs oxygen and it is less competitive in fermented sausage mixture in comparison to *S. carnosus* [10].

Catalase-positive cocci play an important role in product colour, stabilization, the prevention of rancidity and the emergence of volatile compounds that are effective in flavour. Effects of catalase positive cocci as starter culture on fermented meat products are given in Fig. 1. They are effective in the formation of nitrosomyoglobin, the compound responsible for the characteristic red colour of fermented sausages, by reducing nitrate into nitrite through nitrate reductase activity. Catalase-positive cocci utilise available free oxygen in the sausage after filling and provide necessary reductive conditions for colour stability. Catalase activity of these cocci decomposes hydrogen peroxide and thus the negative effects of peroxide on the colour of cured meat products are avoided [32,13]. In the case of high amounts of O_2 and insufficient enzyme activity for peroxide decomposition, H_2O_2 causes colour defects and early rancidity [13]. Catalase and superoxide dismutases of catalase-positive cocci significantly affect the sensorial properties of fermented sausage through antioxidant activity on lipid oxidation [33,34].

Nitrate reductase is an intracellular enzyme located on the cytoplasmic membrane. This enzyme reduces nitrate to nitrite. However, rapid drops in pH prevent microbial nitrate reduction and colour defects occur as a consequence. Thus, it is reported that the pH should not be decreased below 5.4 until a sufficient amount of nitrate has reduced to nitrite [13]. Several strains of staphylococci such as *S. carnosus*, *S. xylosus*, *S. equorum* and *S. lentus* have nitrate reductase activity [21,34-40].

The development of flavour is influenced by several variables such as product formulations, processing conditions, and starter cultures [13]. Olesen et al. [41] reported that sausages cured with nitrate have higher volatile compounds derived from lipid autooxidation, such as 1-pentanol, 1-hexanol and 2-heptanone, than sausages cured with nitrite. These differences are caused by antioxidative properties of nitrite. Stahnke [42] showed that glucose levels affect the amount of volatile compounds when *S. carnosus* and *S. xylosus* are used as starter cultures. In a study on *S. xylosus* and *S. carnosus* the amount of diacetyl and acetoin increased with glucose content, but ketones and sulphides level decreased for *S. carnosus*. On the contrary, glucose blocks tricarboxylic acid cycle and has negative effects on the growth rate of *S. carnosus* [42] and *S. xylosus* [43].

Staphylococcus and *Kocuria* contribute to the development of colour and flavour by decomposing free amino acids and inhibiting the oxidation of unsaturated free fatty acids [44]. Different species of *Staphylococcus* produce different aroma compounds in different amounts [6,45]. The volatile compounds currently recognized as products of staphylococci are primarily amino acid catabolites, pyruvate metabolites, and methyl ketones from β-oxidation of fatty acids [7,9,46].

During meat fermentation, several volatile compounds are generated by the action of endogenous meat enzymes as well as the proteolytic and lypolytic activity of catalase-positive cocci. Many researchers have focused on proteolytic and lipolytic activity of catalase-positive cocci in sausage fermentation [15,40,47-50]. Because of their effects on colour, texture and flavour, catalase-positive cocci are commercially used as starter cultures with lactic acid bacteria to ferment meat. Among catalase-positive cocci, *S. xylosus* and *S. carnosus* are commonly used as starter cultures. *S. xylosus* and *S. carnosus* have several enzymes that contribute to protecting fermented sausages from the detrimental effects of oxygen. The antioxidative properties of *Staphylococcus* strains are responsible for a decrease in levels of volatile compounds derived from lipid oxidation reactions and prevent a reduction in sausage quality [33,51]. Also, the enzymes involved in the β-oxidation of fatty acids, which enhance the cured aroma in fermented sausages by increasing the concentration of methyl ketones, are purified from *S. Carnosus* [51]. On the other hand, *Staphylococcus* strains used as starter cultures have lipolytic activity [52], but it is also argued that lipase activity is limited under fermented sausage conditions [51].

For flavour development, proteolysis is the most important biochemical change. It involves the generation of amino acids, peptides, amines and aldehydes, which are precursors of volatile compounds [53-55]. It has been reported that *S. xylosus, S. equorum*, *S. carnosus*, *S. simulans*, *S. warneri* and *S. cohnii* strains that were isolated from dry cured meat products had proteolytic activity on sarcoplasmic and/or myofibrillar proteins [40,53,56].

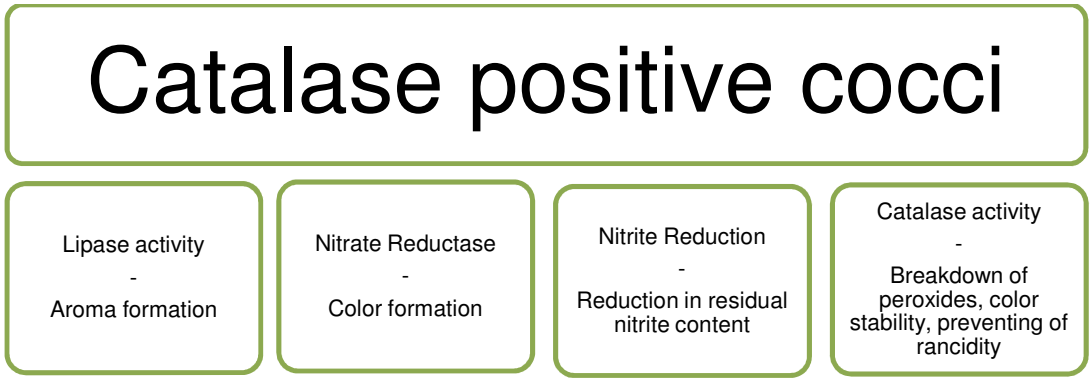

Fig. 1. Effects of catalase positive cocci used as starter culture on fermented meat products [8]

Aroma compounds originating from the degradation of amino acids are important for sausage flavour, especially the breakdown of leucine, isoleucine and valine into branched-chain methyl aldeydes and acids [7,57]. The initial step is the deamination of leucine catalyzed by branched-chain acid transferases, amino acid oxidases and dehydrogenases [58]. In one study, Stahnke [42] showed that the cell counts of *S. carnosus* and *S. xylosus* were correlated with methyl-branched aldehydes and ketones. Many factors can influence amino acid metabolism in these strains. Adding free amino acids to fermentation media did not affect total transaminase activity or volatile compounds. However, adding α-ketoglutarate increased the levels of methyl-branched alcohols, aldehydes, ethanol, 2-phenyl ethanol, phenylacetaldehyde, benzaldeyhde, diacetyl, 4-methyl, 2-pentanone and etyhl esters. α-ketoglutarate increased transaminase activity. The limiting factor in the transamination of branched-chain amino acids (BCAAs) was the amount of amino group acceptors [59].

The decomposition of BCAAs leucine, valine and isoleucine into methyl-branched aldehydes, acids and alcohols by staphylococci strains has been studied by Masson et al. [60] Larrouture et al. [61], Beck et al. [62], Olesen and Stahnke [63] and Olesen et al. [64]. It was shown that *S. carnosus* [6,7,60] and *S. xylosus* [62] have an effect on branched aldehydes (3-methyl butanal, 2-methyl butanal) and acids (3-methyl butanoic acid and 2-methyl butanoic acid) from amino acid degradation.

Leucine catabolism of *S. xylosus* [64] and *S. carnosus* [60, 61] were inhibited by nitrate. The formation of 3-methyl butanoic acid was significantly reduced by the presence of nitrate [60,61,64]. However, Stahnke [42] showed that nitrate influences the production of 3-methyl butanoic acid from leucine catabolism. Another study reported that nitrite content increased the level of free amino acids while nitrate decreased in dry fermented sausages [65]. In contrast, Olesen et al. [64] determined that nitrite decreased the levels of volatile compounds through BCAA degradation.

The volatile profile of dry-fermented sausage consists of a wide variety of compounds such as aromatic and aliphatic hydrocarbons, aldehydes, alcohols, esters, sulphur containing compounds and furanes, etc. [6,48,66-68] and also most of those volatile compounds effect sausage flavour [57]. Aroma compounds generated from the degradation of amino acids play a major role in sausage flavour [42], especially the degradation of BCAAs into branched-chain alcohols, aldehydes, acids and esters [61,62].

It is thought that there is a correlation between amino acid and volatile compounds profiles. Waade and Stahnke [65] showed that amounts of valine, isoleucine and leucine are inversely correlated with 2-methyl propanal, 2-methyl butanal and 3-methyl butanal, respectively.

Masson et al. [60] showed that the use of nitrate has a strong effect on the formation of 3-methyl butanal in the presence of *S. carnosus*. However, it was also reported that adding nitrate decreased 3-methyl butanol and 3-methyl butanoic acid formation in the same strain. In another study on *S. xylosus* as a starter culture, nitrite reduced the formation of 3-methyl butanal and 2- methyl propionic acid by inhibiting amino acid degradation [48].

Olesen et al. [41] indicated that sausages with added nitrate have higher volatile compounds that originate from BCAA degradation than

sausages with added nitrite. That may be caused by the metabolic activity of the staphylococci strains that were used. The inhibition effect of nitrite on the degradation of BCAAs was also reported by Demeyer et al. [69] and Olesen et al. [64].

Some studies reported a correlation between catalase-positive cocci and acetoine formation. Montel et al. [7] characterized *S. saprophyticus* strains by their acetoin production capacity. Ravyts et al. [70] observed *S. sciuri*, *S. succinus* and *S. xylosus* strains as acetoine producers in southern European sausage.

In fermented sausages, another factor for aroma formation is the rate of fermentation. Researchers have reached different conclusions. According to the results of Tjener et al. [71] rapidly-acidified sausages showed high levels of ketones, sulphides and methyl-branched acids. However, there were high levels of methly-branched alcohols, aldehydes and esters, methional and phenylacetaldehyde in slowly-acidified sausages in the same study. Ravyts et al. [70] showed that the acidification rate was depended on the catalase-positive cocci species. They reported that due to the survival rate of catalase-positive cocci in fermentation conditions, there is a volatile profile.

3. CONCLUSION

Many factors affect the volatile compounds generated by catalase-positive cocci in sausage fermentation. Studies strongly agree that volatile compounds result from the degradation of amino acid products by catalase-positive cocci. Flavour can be enhanced by regulating enzyme activities. Further studies on optimizing enzyme activity of catalase-positive cocci would lead to a better understanding of flavour formation during sausage fermentation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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