"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 microorganism 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 culture media, 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.

Keywords: fermented sausage, flavour, aroma, volatile, yeasts, *D. hansenii*.

Contents

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, Salt initiatives, 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 while 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 70's 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 produces a large variety of products with differences in terms of sensory properties from appearance to flavour (Flores 2011a). The differences in the sensory properties has been described by conventional descriptive analysis (QDA) and are related to appearance, odour, texture, aroma and taste (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, 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 specially at the beginning of the ripening process. Microbial enzymes play an important role 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 on fatty acid autoxidation reactions, Maillard reactions and Strecker degradation reactions. The autooxidation 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. While 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, 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, 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, specially 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 level 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, 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 reach 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 10000 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 Schieberle, 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,). It was remarkable the high impact of compounds: 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. These compounds have been detected in higher proportion in traditional than in industrial dry fermented sausages (Olivares et al., 2010) and 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, 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.

3. Diversity of yeast in dry fermented sausages

Several studies have tried to elucidate the role of yeast as secondary microbiota in fermented meat products. Diverse methodologies based on classical phenotypic characterization, molecular techniques or combinations of both have been used to investigate the yeast microbiota present in meat products (Querol et al., 2008).

The identification by classical methods of yeasts isolated from meat products revealed the occurrence of several genera such as *Debaryomyces*, *Candida*, *Yarrowia*, *Pichia*, *Rhodotorula*, *Cryptococcus* and *Trichosporon* (Romano et al., 2006; Samelis and Sofos, 2003;). Yeast population in fermented meat products seems to change during processing, although variations depending on meat origin and factory environment among others have also been reported. Evaluation of yeast diversity along processing and ripening of sausages in different factories pointed out the presence among others of *Candida* and *Trichosporon* species in raw meat or at the initial ripening stages only, whereas *C. zeylanoides, Y. lipolytica* and *D. hansenii* appeared at later stages of sausage manufacture (Encinas et al., 2000). Meat origin was also found to have influence on yeast species at the beginning of the processing. Comparisons between fresh or frozen beef, pork and lard revealed large variation in yeast species from the genera *Candida*, *Cryptococcus* and *Rhodotorula*. Moreover, different yeast species of additional genera *Debaryomyces*, *Pichia* and *Trichosporon* could be identified at various stages of the fermentation and ripening process (Coppola et al., 2000; Osei Abunyewa et al., 2000). These studies point out to *Debaryomyces hansenii* as the most frequently and abundantly isolated yeast species. Physiological characterization of *D. hansenii* has shown this yeast as highly tolerant to salt and weakly fermentative or aerobic, able to proliferate not only in the surface but also in the interior of dry fermented sausages (Coppola et al., 2000). However, *D. hansenii* is difficult to separate from closely related species using phenotypic characters (Suzuki et al., 2011) and a variety of DNA based methodologies are currently in use to identify and characterize this species (Andrade et al., 2006; Corredor et al., 2003; Martorell et al., 2005; Romano et al., 2006).

Recent utilization of diverse molecular methods has allowed fast and precise identification of *D. hansenii* and other yeasts, in a culture-dependent and culture-independent manner, from dry fermented meat products (**table 2**) (Rantsiou and Cocolin, 2006, 2008). Identification of yeasts from traditional Italian sausages by sequencing of D1/D2 of 26S rDNA revealed *C. parapsilosis*, *S. cerevisiae*, *Sterigmatomyces elviae* and *Pichia triangularis* until day three of the fermentation process, which were subsequently replaced by *C. zeylanoides* in the middle stages. *D. hansenii* was the most abundant species from the beginning till the end of fermentation and was the only yeast species detected by DGGE (Cocolin et al., 2006). Similarly, identification by RFLPs of ITS1-5.8S-ITS2 of yeasts from dry sausages manufactured in Central Italy according to traditional methodologies revealed the occurrence of *Trichosporon brassicae*, *Rhodotorula mucilaginosa* and *D. hansenii* which was also confirmed by DGGE (Aquilanti et al., 2007). Further studies applying DGGE in traditional Italian salamis allowed detection of *Candida psychrophila* and *Saccharomyces barnettii* (Silvestri et al., 2007) as well as *C. zeylanoides* and *P. guilliermondii* (Villani et al., 2007) in addition to *D. hansenii.* Comparison of yeast diversity between traditional style and industrial dry fermented sausages produced in Spain using RFLPs of ITS1-5.8S-ITS2 demonstrated a large variety of yeast species in the former where several species of *Debaryomyces* could be detected (Mendonça et al., 2013). On the contrary, a study by Cano-Garcia et al. (2013) using D1/D2 26S rDNA sequencing confirmed *D. hansenii* as the sole species found at the end of ripening in traditional Spanish dry fermented sausages. Nevertheless, other yeast species different from *D. hansenii* have also shown dominance along the fermentation or ripening process. Yeast identification by D1/D2 of 26S rDNA sequencing of strains isolated along the production line (raw meat, fat, machinery, surfaces, fermentation, ripening and storage) revealed the dominance of *C. zeylanoides* and *C. alimentaria* in most pre-ripening stages whereas *Y. lipolytica* was the only species isolated in the final product (Nielsen et al., 2008). Additional studies by Andrade et al. (2010a) in Iberian pork dry fermented sausages showed *C. zeylanoides* besides to *D. hansenii* as the predominant species. Dry fermented sausages manufactured with meats different from pork have also been explored for yeast diversity. Examination of yeasts along the production, fermentation and ripening of traditional Argentinean llama sausages pointed out the wide diversity in genera and species. This study revealed the co-dominance of *C. zeylanoides* and *D. hansenii* at the final ripening stages, although up to 13 more yeast species could be identified along fermentation and ripening (Mendoza et al., 2014).

Interest has also been paid in the exploration of the strain typing within the dominant yeast species found in the fermentation and ripening processes. Several molecular methods have been applied for this genetic typing. *D. hansenii*, *C. zeylanoides* and *Y. lipolytica* have been explored to evaluate population diversity along the fermentation and ripening processes. Earlier studies using analysis of M13 and RF2 RAPD-PCR revealed a large level of heterogeneity in the patterns obtained for several strains of *Y. lipolytica* isolated from traditional sausages from southern Italy (Gardini et al., 2001). *C. zeylanoides* strain diversity was explored by Mendoza et al. (2014) using M13 RADP-PCR in isolates from two productions of traditional llama meat sausages. The authors found this species along the whole process from the initial day of fermentation to the end of ripening. Moreover, most of the large number of strains analysed grouped in different profiles characteristic of each production, although patterns common to both productions and patterns present along the whole process were also found. Nevertheless, the most extensive studies have been done on *D. hansenii*. In a study by Baruzzi et al. (2006) diversity of *D. hansenii* from a traditional Southern Italian processed sausage was made up to only one strain by RAPD-PCR. On the contrary, a similar study exploring the diversity of *D. hansenii* strains in a traditional Italian sausage demonstrated the concomitance but also replacement of strains along the fermentation process (Cocolin et al., 2006). These results were in agreement with the findings of Mendoza et al. (2014) by analysis of M13 RAPD-PCR of *D. hansenii* from llama traditional sausages. Similarly, Cano-Garcia et al. (2013) demonstrated the large heterogeneity of simultaneously occurring *D. hansenii* strains in finished traditional Spanish dry fermented sausages.

The occurrence of *D. hansenii* as the dominant yeast in a large number of fermentation and ripening processes for production of meat products has led to utilization of *D. hansenii* as starter culture for meat fermentation since 70’s decade (Hammes and Kauf, 1994). Selected strains of this species are now available as starters for fermented meat products. These yeast starters develop on the surface of the fermented meat product contributing to the generation of desired sensory properties (Lücke, 2000). Different approaches to follow up the inoculation and development of the selected *D. hansenii* starters on different meat products have been accomplished by different methods. Restriction analysis of mitochondrial DNA and M13 RAPD-PCR have been used to analyse the effect of the inoculation of diverse strains from *D. hansenii* strains on the aroma of dry fermented sausages (Andrade et al., 2010b, Cano-García et al., 2014b, Corral et al., 2014b, 2015).

4. Potential role of yeast in meat products

Yeast in dry fermented sausages produce a protection against the detrimental effect of oxygen and facilitate the drying process by protecting the sausage against fluctuation in humidity (Lucke, 2000) which will produce changes in sausage appearance. In addition, their proteolytic and lipolytic activities affect flavour development. Their main effects of yeasts are seen by a pH increase, lactate utilization and generation of aroma compounds (Flores et al., 2004). However, the role of yeast in sausage flavour has not been established due to conflicting results. While several authors reported an improvement in appearance, antioxidant effect, production of amino acids and free fatty acids and production of flavour compounds (VOCs) (**table 3**), the sensory characteristics of the sausages was not always enhanced (Cano-García et al., 2014b; Selgas et al., 2003).

4.1. Yeast lipolytic and proteolytic contribution in meat products

The first studies were focused on determining the lipolytic and proteolityc activites in *D. hansenii* yeasts as responsible for the generation of flavour precursors, free amino acids and fatty acids (**table 3**). The presence of lipolytic activity in pork fat emulsion inoculated with cellular extracts of *D. hansenii* and *S. xylosus* (Sorensen and Samuelsen, 1996) was reported and it was further confirmed by the production of free fatty acids in pork fat inoculated with *D. hansenii* yeast (Sorensen, 1997). Lipolysis was highly affected by conditions such as temperature and pH although variations in NaCl concentrations were not significant. In addition to this lipolytic activity, *D. hansenii* proteolytic activity was confirmed by the generation of peptides and free amino acids, in different model systems containing specific substrates as myofibrillar (Rodríguez et al., 1998) and sarcoplasmic proteins (Santos et al., 2001). Moreover, other yeast species, *Saccharomyces cerevisiae*, isolated from Italian salami were found responsible for extensive hydrolysis of myofibrilar proteins in a model system (Chaves-López et al., 2011). Further studies on the proteolytic potential of yeasts were focused on the purification and characterization of the endo and exoproteolytic enzymes from *D.hansenii* yeast strains isolated from traditional dry fermented sausages (Bolumar et al., 2003a, 2003b, 2005 and 2008). Last, the glutaminase enzyme responsible for acid neutralization by ammonium generation was purified from *D. hansenii* yeast (Durá et al., 2002).

Other studies have tried to show the effect of yeast enzymatic content on the sensory properties of dry cured products. For this purpose, yeast extracts from *D. hansenii* were added to the formulation of dry fermented sausages alone or in combination with extracts from other microorganisms (*L. sake*) (Bolumar et al., 2006). The study showed an improvement in the sensory quality of dry fermented sausages that was related to the slight effect of amino acid generation and an enhanced production of VOCs derived from amino acids (branched alcohols and acids) and microbial fermentation (ester compounds). Additional studies performed by inoculating *D. hansenii* yeasts in real dry fermented sausages (**table 4**) showed the activity of the reported enzymes, such as a high myofibrillar degradation and generation of free amino acids in inoculated dry sausages (Durá et al., 2004a). Also, a high lipolytic activity was confirmed by a high generation of free fatty acids in different inoculated dry fermented sausages (Corral et al., 2015; Patrignani et al., 2007).

4.2. Yeast contribution to VOCs generation and aroma in meat models.

In order to understand the effect of *D. hansenii* on sausage aroma and its ability to produce aroma compounds, a key point is to determine yeast ability to produce aroma compounds from model systems to real fermented sausages. The selection of a model system resembling the real sausage fermentation conditions is necessary. In this sense, the first meat models inoculated with *D. hansenii* yeast (**table 3**) did not show an effect on volatile compounds (VOCs) production which was attributed to the low survival of yeast in presence of garlic (Olsen and Stanhke, 2000). However, the ability of *D. hansenii* to metabolize branched amino acids and produce VOCs (branched aldehydes, alcohols and acids) was shown in simple models (Durá et al., 2004b) even though it was affected by the presence of salt, lactate and low pH values. Further studies of yeasts inoculated in model systems resembling the conditions of dry-cured ham processing, generated high proportions of branched aldehydes and alcohols in addition to several sulphur compounds although the production of these VOCs depended on the yeast strain inoculated (Andrade et al., 2009a,b).

These results were in agreement with the production of volatile compounds from different yeast strains (*D. hansenii*) isolated from traditional dry sausages and inoculated in a culture media containing an alcohol and acid compound for ester production (Cano-García et al., 2013). Although not all yeast strains showed the ability to produce volatile compounds, seven yeasts showed a different ability to produce ethyl and methyl esters, sulphur, alcohols, aldehydes and ketones. Even though the production of the ester and sulphur compounds seemed to be a strain trait, no correspondence was found with their molecular profiles. The confirmation of the aroma potential of these isolated yeasts was further studied in a meat model resembling dry fermented sausage conditions (Cano-García et al., 2014a). In this sausage model, three *D. hansenii* yeasts displayed a characteristic aroma potential to produce ester and sulphur compounds in contrast to the other isolated yeasts. But once again, the authors did not find a correspondence between the aroma profile and the molecular patterns of the different yeast.

4.3. Yeast contribution to VOCs generation and aroma in meat products

On the other hand, several studies have shown the effect of different inoculated yeasts (*D. hansenii, C. zeylanoides* and *Y. lipolytica)* on dry cured products on VOCs production but few of them have confirmed the effect on aroma and sensory characteristics (**table 4**). These studies have been done mainly in dry fermented sausages but also in different dry cured products (ham and loins).

The inoculation of yeasts in dry cured hams has shown different effects. High levels of long chain and branched hydrocarbons, furanones, carboxylic acids and esters compounds were reported (Martín et al., 2006). However, yeast inoculation did not produce an impact on flavour, only a low toasted flavour, although the hams had a better overall acceptability. On the other hand, these authors indicated that the effect of curing time was significantly higher than yeast inoculation on VOCs production. In summary, they concluded that the inoculated yeast did not alter the VOCs profile when it was compared to the hams produced with wild fungal population. Recently, Purriños et al., (2013a) inoculated different yeast *D. hansenii, C. deformans* and *C. zeylanoides* on the surface of dry cured ham (Spanish Lacon) and reported a high production of ester compounds except by *C. zeylanoides*. Also, the lowest production of aldehydes was reported in the inoculated batches as a result of an antioxidant effect produced by the inoculated yeast although no sensory results were presented.

In the case of dry cured loin (**table 4**), *D. hansenii* was inoculated on the surface of the loins and its ability to produce VOCs was evaluated at different times of processing (Martin et al., 2003). Again, the effect of the length of the curing process was more noticeable than yeast inoculation on the production of VOCs. The authors reported only a high level of cyclic and aromatic alcohols when the yeast was inoculated.

Regarding dry fermented sausages (**table 4**) there are many studies which have tried to elucidate yeast contribution to aroma. In 2000, Olsen and Stahnke reported an absence of effect on sensory and VOCs production when yeasts were inoculated in dry fermented sausages because they died before the ripening process ended due to a fungistatic effect of the garlic powder used in the manufacture. In agreement, Selgas et al. (2003) reported an absence of effect on the sensory properties of fermented sausages inoculated with different *D. hansenii* and *Y.lipolytica* yeasts in the surface and in the mince although these yeasts showed proteolytic and lypolitic activities. Recently Cano-García (2014b) reported an absence of aroma effect when *D. hansenii* was inoculated in dry fermented sausages. The authors attributed this effect to the interaction of *D. hansenii* with other starter cultures.

In contrast, several studies have reported a significant effect of *D. hansenii* on sausage sensory characteristics, varying from an improvement in texture and appearance (Iucci et al., 2007) to a high preference and acceptability of the inoculated fermented sausages (Corral et al., 2014b, 2015; Flores et al., 2004; Iucci et al., 2007). However, the elucidation of the biochemical changes produced by the inoculated yeasts and their relation to the high consumer preference is essential. In this sense, the identification of the aroma compounds affected can help to understand the sensory changes. The first reported effect on VOCs and aroma by yeast inoculation (*D. hansenii*) in fermented sausages indicated the inhibition of lipid oxidation products (linear aldehydes) and promotion of ethyl ester compounds (Flores et al., 2004). Although, when high levels of yeast were inoculated, the aroma was negatively affected due to acid compounds generation. Yeast antioxidative effect was previously recognized by oxygen consumption and peroxide degradation activity (Lücke, 2000). Further studies inoculating *D. hansenii* and *Y. lipolytica* in dry fermented sausages with different mincing levels, showed a general trend to reduce aldehydes formation. Moreover, despite the volatile profile of sausages depended on the degree of mincing, the sausages inoculated with *D. hansenii* yeast were sensory preferred (Iucci et al., 2007). This study concluded on the necessity of selecting appropriate yeast strains based on the ability to produce VOCs but depending on sausage production process as yeast metabolism was affected by processing factors (raw materials, spices added, starter cultures, degree of mincing). In addition, Andrade et al. (2010a) inoculated different *D. hansenii* strains isolated from dry cured hams in dry fermented sausages (Andrade et al., 2009a,b) to study the effect on VOCs production. They confirmed their previous results such as a high production of branched aldehydes, alcohols and acids which were detected in yeast inoculated sausages although they did not observe an inhibition of the lipid oxidation VOCs. Furthermore, they attributed to the activity of yeast the generation of 3-methylbutanal as it was only detected in the inoculated sausages. On the other hand, these authors reported a high generation of ester compounds in inoculated sausages although this fact was not observed when these yeasts were inoculated into dry cured hams.

In conclusion, the presence of yeasts in fermented meat products seems to be affected by their metabolic capabilities, acid and salt tolerance, proliferation at low temperature as well as their lipolytic and proteolytic activities. However, the main consequence of their proliferation in fermented meat products is the generation of diverse aroma precursors or volatile aroma compounds with a strong influence on the final flavour of the product. Nevertheless, the ability of the yeast to affect the production of VOCs depends on the particular metabolic abilities of the inoculated strain.

5. Yeast as aroma enhancers in reformulated dry fermented sausages.

EU meat processing industry has put in place several strategies to reduce fat and salt in meat products due to the dietetic recommendations (Muguerza et al., 2004). Both fat and salt reduction and the use of substitutes produce many sensory changes in reformulated sausages and especially in terms of flavour (Campagnol et al., 2011; Corral et al., 2013; Muguerza et al., 2004; Olivares et al., 2011). Therefore, several strategies using flavour enhancers (amino acids, yeast extracts, glutamate and ribonucleotides…) have been studied (Campagnol et al., 2011; Gelabert et al., 2003). Nevertheless, inoculation of yeast strains with aroma production potential in reformulated sausages can be a strategy to improve and diversify sausage flavour in order to counteract the effect of the reformulation.

Recently, the isolation of yeast strains from traditional dry fermented sausages and their molecular and physiological characterization (Cano-García et al., 2013, 2014a) provided with several potential aromatic *D. hansenii* strains. These yeast strains were considered potential aroma producers due to their ability to generate sulphur and ester compounds in different media, from culture media (Cano-García et al., 2013) to fermented sausage models (Cano-García et al., 2014a). One of the isolated yeasts produced ester compounds (ethyl acetate, ethyl 2-methylpropanoate and 3-methylbutyl acetate) with high aroma potential determined by the calculation of the odour activity values (OAVs) in the assayed medium. In addition, two other yeast strains were remarkable by their high production ability of aroma sulphur compounds (dimethyl disulphide and dimethyl trisulfide). The volatile production was yeast dependent due to the observed differences in sulphur compounds productions among yeast strains although meat model composition and presence of sulphur amino acids were essential in the production of those aroma compounds (Procopio et al., 2013). The production of these ester and sulphur compounds would contribute with fruity and cured aroma notes in dry fermented sausages. Therefore, two of the selected yeast strains (*D hansenii* P2 and M4) showing a potential aroma production were further inoculated in dry fermented sausages. The results showed that one of the yeast strains had the ability to inhibit lipid oxidation, reduced the formation of aldehydes and increased the production of volatile acid compounds (Cano-García et al., 2014b).

This *D. hansenii* strain with aroma generation potential was further applied to reformulated dry sausages, reduced in fat and salt, to determine its ability to enhance the aroma (Corral et al., 2014b, 2015) (**Figure 1)**. The results showed that the inoculated *D. hansenii* strain was able to compensate the changes in water activity produced by the reformulation while it did not modify the hardness of reduced fat batches nor the decrease in staphylococci growth observed in all reformulated sausages. Moreover, different patterns of consumers were found in terms of acceptability although the sensory characteristics were affected (Corral et al., 2014b). In summary, *D. hansenii* inoculation resulted in an improvement in consumer acceptability in terms of aroma and taste although when both reductions were carried out together it did not improve sausage acceptability. A deeper study of the biochemical reasons for this increase in consumer preference was carried out by Corral et al. (2015). This study confirmed the antioxidant effect of *D. hansenii* together with an increase in lipolysis in the reformulated sausages, which was in agreement with the reported lipolytic activity observed for this yeast strain (Cano-García et al., 2014a). In addition, an increase in VOCs production was observed especially in the generation of compounds derived from amino acid degradation (3-methylbutanoic acid, methional and benzothiazole) and ester activities (ethyl 2-methylpropanoate, ethyl 2-methylbutanoate and ethyl 3-methylbutanoate). A descriptive aroma profile analysis of the sausages confirmed the increase of fruity and cured notes in inoculated reformulated sausages. The results of these studies can be observed in Figure 1 where sausages noticeably reduced in fat and salt differed in flavour acceptance from the ones inoculated with the selected yeast, whilst control sausages appeared in an intermediate position. In addition, the aroma descriptors were preferently related to the yeast inoculated sausages.

Among the different strategies used to enhance aroma in reformulated dry fermented sausages, yeast extracts have also been used to improve the quality of reduced salt fermented sausages (Campagnol et al., 2011). Yeast extract is an ingredient used for flavouring in many foods and it consists of natural components of the yeast, mainly proteins and amino acids without the cell wall. These authors reported that the high free amino acids content of the yeast extract served as precursors of the VOCs identified in the sausages, especially the increase in branched aldehydes, alcohols and several sulphur compounds although they did not find ester compounds attributed to the inhibition of the esterase activity by the low pH. In terms of sensory improvement, they confirmed that the sensory defects produced by KCl substitution were suppressed by the addition of 2 % yeast extract in reformulated sausages by the aroma improvement. In this case the metabolic activity of the yeast is inactivated and commercial yeast extracts only acted as a source of precursors whilst the use of entire alive yeast strains used for aroma enhancement can have different aroma impacts. Nevertheless, as indicated above, the impact on the final aroma characteristics will depend not only on the yeast strain used but also on the processing factors (meat ingredients, technological parameters, presence of starter cultures) that can affect the metabolic activity of the yeast.

6. Conclusions

In conclusion, latest studies have confirmed the aroma potential of yeast *D. hansenii* strains from simple in vitro models to complex sausage models and in the manufacture of dry fermented sausages. Despite the aroma potential of *D. hansenii* observed in dry fermented sausages, the appropriate selection of yeast strains requires a metabolic study of the yeasts together with the effect of meat processing factors. This knowledge will provide new strategies to improve the quality of reformulated dry fermented sausages.

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Figure legends

Figure 1. Flavour acceptance of reformulated sausages (reduced fat RF, reduced salt RS, and reduced fat and salt RF+RS) inoculated with *D. hansenii* yeast (Y). Consumer acceptance (●), aroma profile descriptors (▲) and sausage samples (□) (C:control, RS: salt reduced, RF: fat reduced. RF+RS: salt and fat reduced and the reformulated sausages inoculated with *D. hansenii* yeast; RF+Y, RS+Y and RF+RS+Y) (adapted from Corral et al., 2014 and 2015)

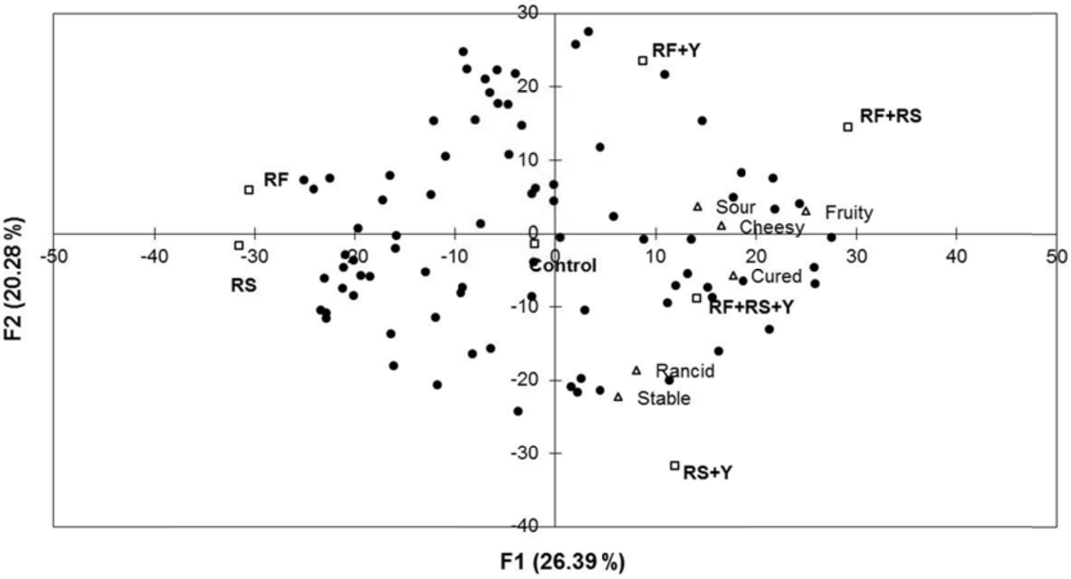

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Table 1. Ester aroma compounds described in traditional and industrial fermented sausages.

Esters	LRI[a]	Traditional sausages	Industrial sausages	Air threshold[b] (ppb)
Propyl acetate	748	fruity, floral[6]	apple, caramel[2];	2700-11000
Ethyl acetate	638	sweet, fruity[6], vegetal [14,15]	synthetic fruity[5], sweet, fruity[,9]	5-5000
Ethyl propanoate	743	fruity[,6]	fruity[5]	9-45
Methyl butanoate	754	fruity[14]		1-43
Ethyl 2-methylpropanoate	789	strawberry, red apple[14,15]	pineapple, strawberry, fruity, caramel[2,3,8,9,10, 16]	0.01-0 .1
Methyl 3-methylbutanoate	804	fruity[14]		-
Ethyl butanoate	825	fruity, caramel[11,]; pineapple[,6]; strawberry[11,14,15]	fruity, caramel[2,3,10,12]; pineapple[5,]; strawberry[9,,13]	0.1-18
Ethyl 3-methylbutanoate	875	fruity, floral[6,,11, 14,15]	sour, pungent[2] fruity[16], floral[3,5,8,10,]	0.01-0.4
Ethyl 2-methylbutanoate	871	fruity[11,14,15]	fruity, floral[3,5,8,10,] strawberry[9,16]	0.01-0.1
Ethyl 3-hydroxybutanoate	990	sweet, fruity[15]		-
Ethyl pentanoate	922	fruity, green[15]	fruity[2,3]; strawberry[9]	1.5-5
Ethyl hexanoate	1026	pear[6]; Flowery, sweet[14,15]	sweet, fruity, cherry[9]	0.3-5
Ethyl benzoate	1225	fruity, herbal, humed[15]	fruity[16]	100-150
Ethyl octanoate	1226	fuity[6]; toasted meat[11]	greasy, green[8];	5-92
Ethyl decanoate	1424	fruity[14,] citric[6]		8-12

[a] LRI: Linear retention indices (calculated for DB-624 column).

[2-16] References: 2. Stahnke (1994), 3. Stahnke (1995), 4. Meynier et al. (1999), 5. Schmidt & Berger (1998a), 6. Schmidt & Berger (1998b), 7. Chevance et al. (2000), 8. Blank et al. (2001), 9. Marco et al. (2007), 10. Söllner & Schieberle (2009), 11. Gianelli et al. (2011), 12. Olivares et al., (2011), 13. Corral et al., (2013), 14. Olivares et al., (2014), 15. Corral et al., (2014a), 16. Corral et al., (2015).

[b] Air thresholds were obtained from Burdock (2002).

Table 2. Main isolated yeast identified by molecular methods in dry cured meat products from different geographical origins.

Predominant Isolated Yeast	Meat product	Country	Reference
D. hansenii, C. kruseiii, W. saturnus and *C. sake*	fermented sausages	Italy	Rantsiou et al., 2005
C. zeylanoides & *D. hansenii*	traditional sausages	Italy	Cocolin et al, 2006
D. hansenii	traditional sausages	Italy	Baruzzi et al., 2006
D. hansenii	dry sausages	Italy	Aquilanti et al., 2007
D. hansenii	traditional salamies	Italy	Silvestri et al., 2007
C. zeylanoides, P. guilliermondii & *D. hansenii*	traditional salamies	Italy	Villani et al., 2007
C. zeylanoides & C. alimentaria	several meat products	Norway	Nielsen et al., 2008
D. hansenii & C. zeylanoides	dry cured Iberian ham	Spain	Andrade et al., 2009a, 2009b
D. hansenii & C. zeylanoides	dry cured meat products	Norway	Asefa et al., 2009
C. zeylanoides & D. hansenii	dry fermented sausages (from Iberian pork)	Spain	Andrade et al., 2010a
D. hansenii & *C. zeylanoides*	dry cured ham (different PDO)	Spain	Andrade et al., 2010b
Debaryomyces	fermented sausages	Spain	Mendonça et al., 2013
D. hansenii & *C. zeylanoides*	dry fermented sausages (from llama)	Argentina	Mendoza et al., 2014
D. hansenii	traditional dry-sausages	Spain	Cano-García et al., 2013
D. hansenii	dry cured ham (Lacon)	Spain	Purriños et al., 2013b
D. hansenii & *C. zeylanoides*	dry cured Iberian ham	Spain	Gallardo et al., 2014
C. zeylanoides	dry cured meat product (beef Pastirma)	Turkey	Ozturk, 2015

Table 3. Effects of *D. hansenii* and other yeast isolated from meat products in different meat models.

Meat model	**Yeast**	**Main effect**	**Affected by**	**Reference**
Pork fat emulsion	cellular extracts of *D. hansenii* and *S. xylosus*	Lipolytic activity	Tª, pH, NaCl	Sorensen & Samuelsen, 1996
Pork fat	*D. hansenii*	Generation of free fatty acids	Tª, pH, NaCl	Sorensen, 1997
Myofibrillar proteins	*D. hansenii*	Generation of peptides and free amino acids		Rodriguez et al., 1998
Myofibrillar proteins	*D. hansenii*	Generation of peptides and free amino acids		Santos et al., 2001
Fermented sausage meat model	*D. hansenii* and *C. utilis*	*C. utilis* generated ester compounds. *D. hansenii* do not have effect on VOCs production		Olsen and Stanhke, 2000
Meat models with meat extract	*D. hansenii*	Generation of branched aldehydes, alcohols and acids	NaCl, lactate, pH	Durá et al., 2004b
Dry cured ham meat model	*D. hansenii* and *C. zeylanoides*	Generation of branched aldehydes and alcohols, and sulphur compounds		Andrade et al., 2009a,b
Culture media (alcohol plus acid compounds)	*D. hansenii* yeast strains	Production of ethyl and methyl esters, sulphur, alcohols, aldehydes and ketones.		Cano-García et al, 2013
Fermented sausage meat model	*D. hansenii* yeast strains	Production of ester and sulphur compounds		Cano-García et al, 2014a

Table 4. Effects of the inoculation of yeast strains in different dry cured meat products.

Meat product	Country	Inoculated Yeast	Main effect	Sensory effect	Reference
Dry-cured ham	Spain	*D. hansenii*	VOCs increase	Improve appearance and texture Low toasted flavour High acceptability	Martin et al., 2006
Dry cured ham (Lacon)	Spain	*D. hansenii,* *C. deformans* *C.zeylanoides*	VOCs increase	-	Purriños et al., 2013a
Dry-cured loin	Spain	*D. hansenii*	VOCs increase	-	Martin et al., 2003
Dry fermented sausages		*D. hansenii* and *C. utilis*	VOCs increase	No effect	Olsesen & Stahnke 2000
	Spain	*D. hansenii,* *Y. lipolytica,* *T- mucoides*	Lipolytic activity	No effect	Selgas et al., 2003
	Spain	*D. hansenii*	VOCs increase, Antioxidant effect	High preference	Flores et al., 2004
	Spain	*D. hansenii*	Proteolytic activity, amino acid release, Ammonia decrease	-	Durá et al., 2004a
	Italy	*D. hansenii,* *Y. lipolytica,*	A_w decrease, Lipolytic activity, Free fatty acid release, Proteolytic activity	-	Patrignani et al., 2007
	Italy	*D. hansenii,* *Y. lipolytica,*	VOCs increase	Improve appearance, Increase consumer acceptance	Iucci et al., 2007
	Spain	*D. hansenii*	VOCs increase		Andrade et al., 2010
	Spain	*D. hansenii*	Antioxidant effect, VOCs production (acid, sulphur, and esters),	no sensory effect	Cano-García et al., 2014b
	Spain	*D. hansenii*	Increase lipolysis and antioxidant effect, increase VOCs derived from amino acids and ester compounds.	Positive sensory effect. High consumer preference. Aroma increased	Corral et al., 2014b, 2015