Debaryomyces Hansenii in Sausages, Dry-Meat

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Debaryomyces hansenii is a hemiascomycetous yeast of undoubted biotechnological importance. It is a heterogeneous yeast species able to grow under extreme conditions, such as high salt or relatively alkaline pH levels. This yeast has high respiratory and low fermentative activity. It ferments with important variations, depending on the strain and growth conditions used. Briefly, some examples of its beneficial effects are the production of xylitol, lipases, and exopeptidases important in the food industry, and of thermophilic β -glucosidases essential to produce fuel alcohol. Debaryomyces hansenii is, most probably, the most abundant yeast found in sausages and dry-meat products manufactured all around the world.

Keywords: Debaryomyces hansenii ; yeast ; sausages ; dry-meat products ; starter cultures

1. Debaryomyces hansenii Is One of the Most Abundant Yeasts in Sausages and Dry-Meat Products

The usual approach to the study of the microbiome present in dry-meat food and sausages is the isolation and identification of the main populations present in these products. In this way, yeast isolates have been obtained from many different meat products during the last decades. Although their functions and effects in meat were not always defined in detail, there is no doubt that previous studies demonstrate that *Debaryomyces hansenii* is frequently and abundantly found in sausages and dry-meat products [1][2][3][4][5].

The identification of *D. hansenii* as the most common yeast in salami was made as early as 1954 ^[6]. Since then, and especially in the last two decades, the presence of multiple strains of *D. hansenii* in sausages and dry-meat products has been reported. **Table 1** summarizes some of these products and their origins. Many countries where sausages and dry-meat foods have been studied belong to the Mediterranean area ^{[3][7][8][9][10][11][12]}, but dry-cured meat or sausages are prepared and consumed by millions of people worldwide and also in other European countries such as Denmark ^[13], Norway ^[14], Austria ^[2], the United Kingdom ^[1], and Portugal ^[15]. Even in places such as Argentina ^[16] or China ^{[17][18]}, among many others, meat products are manufactured in which it is possible to find *D. hansenii* yeasts, supposedly contributing to the final organoleptic characteristics of the food.

Table 1. A selection of sausages and dry-meat products from which *Debaryomyces hansenii* has been isolated.

Product	Origin	Brief Description	References
"Cacholeira"	Portugal	Traditional Portuguese sausage with delicate flavour obtained from the offal and fat of the pig.	[15]
"Chorizo"	Spain	Traditional Spanish cured meat product made from coarsely chopped pork and pork fat seasoned with garlic, "pimentón", and salt.	[<u>19]</u>
"Jamón ibérico"	Spain	High-quality variety of "jamón" produced in Spain and Portugal.	[<u>8][11]</u>
"Jamón"	Spain	Meat product from pork typical of Spanish cuisine	[<u>20</u>]
"Lacón"	Spain	Cured meat product obtained from the shoulders or front legs of the pig.	[21]
"Salame di senise"	Italy	Traditional dry sausage from the Sinni Valley in the Basilicata region.	<u>[9]</u>
"Salchichón"	Spain	Traditional Spanish cured meat generally made of pig, although other meats can be used.	[<u>5][22]</u>
"Salsicca sarda"	Italy	Traditional fermented dry-cured sausage produced exclusively in Sardinia.	[<u>23</u>]

Product	Origin	Brief Description	References
"Soppressata of Vallo di Diano"	Italy	Traditional Southern Italian dry-fermented sausage.	<u>[24]</u>
"Sucuck"	Turkey	Semi-dry, spicy Middle Eastern sausage with a high fat content traditionally prepared with ground beef and spices.	[<u>10]</u>
Dry-cured Parma ham	Italy	Famous variety of "prosciutto" from the Parma region in Italy.	[<u>25]</u>
Fermented sausage	Norway; Denmark; Italy; United Kingdom; Spain	Diverse kind of fermented meat.	[3][26][13][27] [28]
Greek dry salami	Greece	Traditional Greek dry-cured meat.	[7]
Laowo dry-cured ham	China	Traditional Chinese dry-cured ham obtained from the hind leg of the pig.	[<u>17]</u>
Llama meat sausage	Argentina	Traditional products consumed in the Andrea region of South America.	[<u>16]</u>
Mianning ham	China	Traditional fermented meat product in Meanning, characterized by the use of plump muscle and small legs.	[<u>29]</u>
Panxian ham	China	Famous dry-cured ham in China characterized by strong taste, flavour, aroma, and texture.	[<u>18]</u>
Pork loin	Spain	Cured meat product prepared by removing fat from pork followed by seasoning for six months.	[30][31][32]
Vienna sausage	Austria	Thin, parboiled sausage traditionally made of pork and beef in a casing of sheep's intestine.	[2]

As already mentioned, the very diverse metabolic discrepancies of D. hansenii have been observed in naturally occurring strains isolated from the same meat products. For example, differences in the capacity to assimilate xylose [3][33], in the urease activity [3] or in specific lipolytic and proteolytic enzymatic activities [5], have been reported. However, several groups have obtained similar qualitative results coincident when describing some important physicochemical characteristics in D. hansenii isolates from different origins. For example, a high resistance to NaCl or the sensitivity to relatively high temperatures (37 °C) have been usually observed in D. hansenii strains found in sausages from Italy or Spain [3][5].

From the different studies reporting on yeast populations in sausages and dry-cured meat products, it can be deduced that even the behaviour of the yeast populations during the ripening of the dry-meat products do not seem to be homogeneous. Thus, while a microbiological study of Greek salami reported that yeasts did not significantly increase in the ripened product (after 28 days) [34], the yeast counts in sausages of southern Italy significantly increased during the first days of fermentation, subsequently remaining constant or even decreasing [3]. As a final example and in the case of dry-cured Iberian ham, higher counts of yeast were reached at post-salting or at drying periods and then the number decreased after several months in a cellar [8]. In any case, and independently of the manufacturing process, naturally occurring yeasts are usually found in high numbers indicating that these microorganisms may play an important role in the maturation process [22]. Therefore, it is generally accepted that the yeast populations, including *D. hansenii*, present in artisanal-style products contribute to flavour, colour, and texture development during the ripening of these products. However, the presence of competing bacteria, mostly lactic acid bacteria, and other organisms has made it difficult to reach solid conclusions about the functions and contributions of specific species. That is why most of these studies have focussed their attention on quantifying, identifying, or partially characterizing yeast species during the manufacturing process and/or the maturation period [3][26][35][5]. Very recently some research groups have used "omic" and multidisciplinary approaches to analyse the microorganisms during processing or ripening in traditional Chinese products [17][18]. Bacteria and fungi were identified, for example during panxian ham processing, a traditional Chinese dry-cured ham, and it was concluded that Staphylococcus, Chromohalobacter, and Debaryomyces promoted the production of amino and fatty acids [18], but once again the presence of naturally occurring complex communities made it difficult to reach more specific conclusions.

2. Debaryomyces hansenii as a Starter Culture: Effects on the Final

Characteristics of Meat Products

2.1. Lipolysis

D. hansenii shows lipolytic activity. In 1997 it was shown that a commercial starter of this yeast could hydrolyse a natural fatty substrate like pork fat and release fatty acids. In this case, lipolysis caused by *D. hansenii* was not affected by NaCl (most probably due to the salt-tolerant character of this yeast) and it was still significant at pH 4.7, indicating that this commercially available starter culture may hydrolyse pork fat during the processing of fermented meat products [36].

Later, our group has shown that the lipase activity of several strains isolated from Iberian dry-meat products greatly differed among strains, furthermore it was always higher than the corresponding activity in a control laboratory strain (CBS767) [5].

More recently, we used a selected terroir strain isolated from pork loin, *D. hansenii* Lr1, to inoculate different amounts of yeast either directly onto the meat surface or onto the collagen casing in which each loin piece was stuffed to make the product. Possible changes in the lipid profile of the loins were determined. In all cases, including control samples, loins contained a very low percentage of polyunsaturated fatty acids (PUFAs) and the most abundant were monounsaturated fatty acids (MUFAs). On the other hand, inoculation with the Lr1 strain did not significantly change the fatty acid profiles of any of the treatments applied. Within all the samples, oleic acid (C18:1) constituted around 50%, palmitic acid (C16:0) around 26%, and stearic acid (C18:0) around 10–12% of the total fatty acids. Clear differences could not be found related to the different treatments and only slight, although consistent, effects on the percentage of some fatty acids were observed as, for example, all the treatments showed a decrease in the percentage of t-oleic acid (elaidic acid, C18:1 n9t) when compared to the control samples. In summary, it was shown that inoculation with the *D. hansenii* Lr1 strain did not significantly change the global lipid profile of the loins. Only the amounts of some fatty acids were affected, however changes in the total amounts of saturated fatty acids (SFA), MUFAs, and PUFAs were not significant

However, a different study showed that the generation of free fatty acids during sausage fermentation is affected by yeast inoculation. In this case, the *D. hansenii* P2 strain, previously isolated from naturally fermented sausages "salchichón de Requena [27][37], was inoculated in sausages manufactured with boar back fat or with gilt back fat. It was demonstrated that inoculated sausages had a higher degree of lipolysis and that this was strongly dependent on the ripening time and the conditions. After 63 days of ripening, an increase in the content of free MUFAs, PUFAs, and total free fatty acids was measured in the boar sausages while, in general, lower, or even no significant differences, were observed after 43 days of ripening or when the sausages were manufactured using gilt back fat instead of boar back fat. The authors concluded that environmental conditions affect lipase activity or lipase expression genes, potentially explaining differences between strains or products [38].

2.2. Volatile Compounds

A generally accepted idea is that the generation of volatile compounds by D. hansenii is one of the most important contributions to the ripening process in dry-meat products. It is usually found that the introduction of D. hansenii as a starter culture affects volatile and aromatic compound generation. Among them, esters are essential contributors to the aroma of meat products due to their low detection threshold and sensory notes. The production of these compounds represents a complex scenario and it is strongly dependent on the different isolates and strains and even on the amount of yeast used $\frac{[27][28]}{[28]}$. In fact, production of volatile sulphur compounds from sulphur amino acids greatly varied among D. hansenii strains isolated from different food sources and the generation of, at least, some of these compounds could result from yeast metabolism $\frac{[39]}{[29]}$.

Several groups have used starter cultures containing *Debaryomyces* in combination with other different microorganisms. For example, when dry-fermented sausages were inoculated with *Debaryomyces* spp. plus lactic acid bacteria and staphylococci, it was concluded that the use of this combination of microorganisms had a positive effect on the final flavour and sensory qualities of the product influenced by the generation of ethyl esters [40]. Importantly, the authors found that the amount of yeast used in the starter culture must be optimised, since too many yeasts may mask some positive effects. More recently, a similar approach was followed to study the effects of different starter cultures on volatile compounds of dry-cured foal sausages. In this case, three different starters were used and while two of them contained only bacteria, the third one was prepared with bacteria (*Lactobacillus sakei, Staphylococcus carnosus*, and *Staphylococcus xylosus*) and yeast (*D. hansenii*). Significantly different effects on the volatile compounds or acid taste were found among the different batches, with the batch containing *D. hansenii* showing a high flavour intensity and high levels of compounds derived from carbohydrate fermentation and amino acid catabolism [41]. The use of mixed

starters containing only fungi has been less frequent. When *Penicillium chrysogenum* and *D. hansenii* were inoculated on dry-cured ham they did not remarkably alter the volatile compound profile. Only lower levels for some of the main odouractive volatile compounds were measured but they were not detected by a panel of experts [20].

Evidently, conclusions about the specific contributions of D. hansenii must be analysed from a global point of view, since all these experiments were performed with mixed starter cultures. Due to the difficulties surrounding the proper interpretation of the results obtained in these types of experiments, many other groups have focused on the use of pure D. hansenii starters $\frac{[13][21][22][32]}{[13][21][22][32]}$.

One of the first realistic attempts to understand the specific effect of D. hansenii on aroma formation was performed by Olesen and Stahnke [13] in spiced fermented sausages. It was found that *D. hansenii* had very little effect with the analysis showing only a slight difference between the inoculated sausages and the control, possibly due to the fact that the yeast died out before the ripening process ended. The main reason was that sausages were spiced with garlic, and a fungistatic test of the garlic powder added to the sausages indicated that garlic inhibited the growth of the yeast starter cultures [13]. Later studies showed that D. hansenii contributes to the development of the characteristic flavour of some of these drymeat products $\frac{[21][22][32]}{}$. A research conducted on the dry-fermented sausage "salchichón" and performed with different *D*. hansenii strains indicated that the inoculation of selected isolates may have a positive contribution to the volatile compound generation involved in the flavour development of this meat product. In this study, yeasts were incorporated into the batches and the mixture of each batch was stuffed into regenerated collagen casings. The tested yeast strains promoted the generating of esters, alcohols, and aldehydes, and some volatile compounds derived from lipid oxidation [22]. In addition, when the effect of *D. hansenii* on dry-cured "lacon" and Iberian cured pork loin ("lomo ibérico") was studied, similar approaches were followed: strains were selected from native products and used as starters. Yeasts were spread on the surface of the meat product and their capacity to generate volatile compounds was determined. Figure 1 shows the proliferation of inoculated *D. hansenii* on the surface of pork loin during the ripening period. As expected, quantitative differences were found when both studies were compared. This is not surprising, since both strains and meat products were different. Nonetheless, important qualitative similarities were found. In all cases, and in agreement with previous work in "salchichón". D. hansenii modified the levels of volatile and aromatic compounds by increasing esters and alcohol metabolites in comparison to the non-inoculated samples [21][32]. However, and in contrast with what was reported in the case of "salchichón", a significant decrease in aldehydes was reported in both "lacon" and "lomo ibérico".

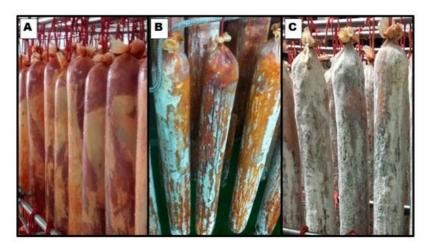


Figure 1. Implantation of inoculated *Debaryomyces hansenii* yeasts on the surface of pork loins after 0 (**A**), 10 (**B**), and 30 (**C**) days of ripening.

In some cases, a different approach was followed, since model minced meats which did not correspond to any specific commercial meat products were used. A first attempt by Olesen and Stahnke [13] indicated that the use of *D. hansenii* as a starter culture had very little effect on the production of volatile compounds in the model minces, although, as previously mentioned, the presence of garlic affected cell viability. More recently, the ability of several *D. hansenii* strains to generate aromas in a fermented sausage model system was evaluated. The performance of seven different strains previously selected on their ability to produce aromatic compounds in a defined culture media [37] were later studied in a meat model system. The presence of each inoculated strain was confirmed in the model system and an increase in volatile compound production was observed in all cases. However, significant differences were found among strains, especially in relation to ester production which was correlated to the lipolytic activity of the strains. Sulphur production was also strongly dependent on the inoculated strain. In summary, it was concluded that: (i) the inoculated *D. hansenii* strains affected the

flavour development of the meat model system; (ii) wide differences do exist among strains, although in all cases volatile compounds increased; (iii) the meat model system is useful to show the ability of *D. hansenii* strains to produce aromatic compounds; and that (iiii) it is necessary to investigate the effects of specific strains in real dry-fermented sausages [27][28].

2.3. Other Physicochemical and Sensory Characteristics Affected by Debaryomyces hansenii

2.3.1. pH

Several studies have shown that changes occur in pH levels during the processing of meat products. The pH level is always lower at the end of the ripening period independently of the presence of yeast. Inoculation with mixed starter cultures (bacteria plus *Debaryomyces*) resulted in the higher acidification of sausages [41]. However, as previously mentioned, the specific contribution of yeast species cannot be easily deduced due to the presence of bacteria in the starter mix [41]. When *D. hansenii* was used as starter it was difficult to find a general conclusion regarding it effect on the final pH of the product, although it is possible to state that the consequences of the yeast activity did not acutely affect pH and that these consequences are strongly dependent on both the yeast strain and product. In some cases, similar pH values to the control were measured at the end of the process, while in some other studies the final pH values slightly higher or lower than non-inoculated products were determined [42][22][27][32]. In conclusion, few and variable changes in pH have been reported in yeast-inoculated meat products.

2.3.2. Water Activity and Moisture

More homogeneous results were obtained by the different researchers when water activity (A_w) or moisture was determined. It has been fully demonstrated that both parameters decrease along the ripening period in both control and inoculated samples. On the other hand, while higher final values of A_w were usually measured and explained as a consequence of yeast growth on the product surface $\frac{[22][32][43]}{[27][43][41]}$, no significant differences in moisture were found when results in control and inoculated products were compared $\frac{[42][27][43][41]}{[42][27][43][41]}$.

2.3.3. Sodium Content

The possible effects of yeast on the sodium levels of meat products has not been frequently studied. While commercial mixed starter cultures did not affect saltiness of dry-cured foal sausages $\frac{[44]}{}$, only in the case of cured pork loins has an effect of *D. hansenii* inoculation on the sodium content of the product been reported $\frac{[32]}{}$. Interestingly, yeast inoculation produced a significant decrease in the sodium content of the product. It is known that *D. hansenii* can accumulate high amounts of sodium from the environment without becoming intoxicated and for this reason it has been defined as a sodium-includer yeast $\frac{[45][46][47]}{}$. This behaviour may explain why inoculated loins contain lower sodium amounts, a characteristic not previously reported.

2.3.4. Colour

Heterogeneous results regarding the consequences of yeast use on this parameter have been reported. On the one hand, the use of D. hansenii to inoculate standard slow dry-cured fermented sausages did not affect colour parameters such as redness, yellowness, or lightness [27]. However, the same research group later reported that in the specific case of sausages produced with entirely male fat, yeast inoculation affected colour parameters, thus lightness values were significantly higher in the inoculated sausages after 43 days of drying [43].

Once again it is important to keep in mind that many of the effects described depend on the meat product and the manufacturing process followed.

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