Effect of fat and salt reduction on the sensory quality of slow fermented sausages inoculated with \textit{Debaryomyces hansenii} yeast

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\begin{abstract}

The inoculation of a \textit{Debaryomyces hansenii} strain in dry fermented sausages with reduced fat and salt contents was evaluated in terms of chemical, microbial and consumer acceptability. The implantation of the inoculated yeast strain was confirmed by RAPDs of M13 minisatellite. A reduction of 17–20% salt and 10–16% fat content was achieved. These reductions affected the sausage quality by producing an increase in aw, hardness and chewiness values and a decrease of staphylococci growth. However, \textit{D. hansenii} inoculation compensated these changes although it was not able to modify neither the hardness of reduced fat batches nor the staphylococci growth decrease. In terms of sensory acceptability, different preferences patterns of consumers were found. Yeast inoculation improved the aroma and taste quality when fat or salt reductions were carried out in dry fermented sausages.

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\end{abstract}

1. Introduction

The intake of meat products has grown around 10% in industrialized countries in spite of their fat and salt content (WHO/FAO, 2003). However, during the last years a demand for low fat and salt food has grown (Ruusunen & Puolanne, 2005). Therefore, meat products such as dry fermented sausages are being reformulated to adjust their fat and salt content (Aaslyng, Vestergaard, & Koch, 2014; Beriain, Gómez, Petri, Insausti, & Sarriés, 2011). However, these ingredients cannot be reduced without affecting organoleptic and technologic characteristics. On the one hand, fat contributes to nutritional (source of essential fatty acids, liposoluble vitamins and energy), organoleptic (flavour, texture, mouthfeel) and technologic properties (release of moisture) (Olivares, Navarro, Salvador, & Flores, 2010). On the other hand, salt is also involved in organoleptic (flavour, texture) and technologies properties (myofibrillar protein solubilization, aw decrease) (Corral, Salvador, & Flores, 2013).

Different strategies have been studied to reduce fat and salt content in dry fermented sausages, since preservation of product acceptability is a decisive criterion when developing this kind of products (Wirth, 1988). Fat content has been replaced by soy oil (Muguerza, Ansorena, & Astiasarán, 2003), olive oil (Bloukas, Paneras, & Fournitzis, 1997), konjac gel (Ruíz-Capillas, Triki, Herrero, Rodríguez-Salas, & Jiménez-Colmenero, 2012), inulin (Mendoza, García, Casas, & Selgas, 2001), and fibres (Salazar, García, & Selgas, 2009). However, few studies have dealt with fat reduction without replacers and its effect on sensory characteristics. Generally, fat reduction (10%) affected the external appearance and flavour intensity of fermented sausages (Larios, Katsanidis, & Bloukas, 2009) while higher reduction percentages (20%) produced a suitable acceptability (Olivares et al., 2010; Papadima & Bloukas, 1999); in spite of a lowest taste, aroma and hardness (Olivares, Navarro, & Flores, 2011; Olivares et al., 2010) and appearance (Papadima & Bloukas, 1999) perceived by consumers. Regarding salt reduction, different salts have been used as NaCl substitute (Corral et al., 2013; Gimeno, Astiasarán, & Bello, 1999) although they did not achieve sensory acceptable products (Gimeno et al., 1999). Major salt reductions (40–50%) had a negative effect on many sensory characteristics such as hardness, bitterness, aroma and taste acceptability (Campagnol, dos Santos, Wagner, Terra, & Pollonio, 2011; Gelabert, Gou, Guerrero, & Arnau, 2003). With small salt reductions (16%), the best results were found when KCl alone was used even though the aroma acceptability was still affected (Corral et al., 2013).
The effect of fat and salt reductions together in dry fermented sausages has been scarcely studied. A strategy of combining a reduction in the salt content and a simultaneous modification in the lipid fraction using olive or linseed oils resulted in products with lowest sodium content, highest calcium content and a significant supply of omega-3 fatty acids (García-lñiguez de Ciriano, Berasategi, Navarro-Blasco, Astiasarán, & Ansorena, 2013) and highest MUFA content (Beriaín et al., 2011) producing an improved nutritional sausage profile. Both studies indicated a sensory acceptable characteristic of the new formulations although differences in texture and taste were reported by the trained panel.

The use of starter yeasts can be an alternative to improve the sensory characteristics of the dry fermented sausages. Debaryomyces hansenii is the predominant yeast which proliferates in dry fermented sausage environment (Cocolin, Urso, Rantsiou, Cantoni, & Comi, 2006). The growth of D. hansenii in the sausage surface can control water release in low fat sausages thus improving sausage aroma lost by salt reduction as reported by Campagnol et al., (2011) who tried to improve the sensory characteristic by the addition of a yeast extract (Saccharomyces cerevisiae). The mechanisms behind aroma loss due to salt reduction are probably due to the salting out effect that salt produces on volatile compounds (Desmond, 2006) in addition to the effect of salt on the biochemical reactions involved in aroma generation.

Nevertheless, important differences have been observed when different yeasts strains are inoculated in fermented sausages (Andrade, Córdoba, Sánchez, Casado, & Rodríguez, 2009; Olesen & Stahnke, 2000). Recently, Cano-García, Flores, and Belloch (2013) isolated D. hansenii strains from traditional fermented sausages and reported their aroma potential using a meat model system (Cano-García, Rivera-Jiménez, Belloch, & Flores, 2014). Therefore, the aim of this work was to elucidate the effect of fat and salt reduction on the sensory quality of slow fermented sausages. Nevertheless, important differences have been observed when different yeasts strains are inoculated in fermented sausages (Andrade, Córdoba, Sánchez, Casado, & Rodríguez, 2009; Olesen & Stahnke, 2000). Recently, Cano-García, Flores, and Belloch (2013) isolated D. hansenii strains from traditional fermented sausages and reported their aroma potential using a meat model system (Cano-García, Rivera-Jiménez, Belloch, & Flores, 2014). Therefore, the aim of this work was to elucidate the effect of fat and salt reduction on the sensory quality of slow fermented sausages inoculated with D. hansenii, since this yeast could offset the quality defects produced by fat and/or salt reductions.

2. Materials and methods

2.1. Preparation of yeast inoculum

D. hansenii P2 previously isolated from naturally fermented sausages (Cano-García et al., 2013) was used as starter in the production of dry fermented sausages. Yeast was cultivated on GPY medium (2% glucose, 0.5% peptone, 0.5% yeast extract, pH 6.0) and the grown cells washed with sterile saline solution (0.9% NaCl) and centrifuged (7000 rpm for 10 min at 4 °C) to remove the culture medium. The collected cells were prepared to a concentration of 10⁸ c.f.u./ml using dilution plates. The concentrated yeast cells were directly stored at −80 °C until their inoculation in dry fermented sausage batches.

2.2. Dry fermented sausages and sampling

Seven batches of dry fermented sausages were manufactured: a control batch (C) was prepared using 70% pork lean meat and 30% pork back fat and 27 g/kg NaCl content while six batches were manufactured varying salt and/or pork back fat content with or without yeast inoculation (D. hansenii). The reformulated batches were: reduced fat (RF); reduced salt (RS); reduced fat and salt (RF + RS); and the same three batches but inoculated with D. hansenii yeast (RF + Y, RS + Y, RF + RS + Y). Reduced salt batches were 25% salt reduced adding 20.25 g/kg NaCl and 6.75 g/kg KCl. Fat reduced batches were 50% fat reduced adding 85% lean pork meat and 15% back fat. Appropriate volumes of yeast strain D. hansenii P2 suspension were added to the inoculated batches at final concentration of 5 10⁶ c.f.u./g of yeast strain. All fermented sausage batches were produced following the following ingredients: lactose (20 g/kg); dextrin (20 g/kg); sodium caseinate (20 g/kg); glucose (7 g/kg); sodium ascorbate (0.5 g/kg); sodium nitrite (0.15 g/kg); potassium nitrate (0.15 g/kg) and starter culture (0.1 g/kg) SP318 TEXEL SA-301 (Danisco, Cultor, Madrid, Spain) containing Lactobacillus sakei, Pediococcus pentosaceus, Staphylococcus carnosus. The batches were manufactured under the conditions described by Olivares et al. (2010). The meat mixture was kept at 3–5 °C for 24 h and then was stuffed into collagen casings of 9.5 cm diameter (FIBRAN, S.A., Girona, Spain) being the final weight of each sausage approximately 700 g. The sausages were subjected to drying in a controlled drying chamber at 10–14 °C and 70–85% relative humidity (RH) for 61 days. The weight losses and pH were measured during ripening to control the drying process.

From each batch, 300 g of the meat mixture at 0 days and three sausages at 61 days were randomly chosen for microbial and chemical analysis. From each sample, sausage colour was measured, 20 g were taken for microbial analysis and 150 g were minced and used to measure moisture, water activity and pH. The remaining minced sausages from each batch were vacuum packed and frozen at −20 °C till subsequent analysis (fat, protein and ions content). In addition, at 61 days the remaining sausages of each batch were vacuum packaged and stored at 4 °C for sensory and texture analysis. All the results were expressed as means of the three replicates per 100 g of dry matter at each processing time and batch.

2.3. Microbial analysis

Sausages samples (20 g) were aseptically homogenized with sterile saline solution (1/10) in a Stomacher (IUL Instruments, Barcelona, Spain) for 1 min and decimal dilutions were prepared. Lactic acid bacteria population was determined by spread plating on MRS Agar anaerobically (Scharlau Chemie SA, Barcelona, Spain) and staphylococci population by using Mannitol Salt Agar (Scharlau Chemie SA, Barcelona, Spain) both medium were incubated at 37 °C for 2 days. Yeast count was determined in Rose Bengal Agar with chloramphenicol (RBA) (Conda SA, Madrid, Spain) at 28 °C for 3 days.

Ten yeast strains isolated from each batch at the initial and final time of the ripening process were subjected to molecular characterization by minisatellite PCR amplification using the M13 primer as described in Cano-García et al. (2013). The M13 Minisatellite PCR patterns obtained were compared with the originals previously obtained by Cano-García et al. (2013).

2.4. Chemical analysis

The measurement of pH, water activity, colour evaluation (CIE-Lab L*, a*, b*), moisture and fat content was performed as described by Olivares et al. (2010). Nitrogen content was determined by the Kjeldhal method and protein was estimated multiplying the nitrogen content by a factor of 6.25.

Cations (sodium and potassium) and chloride anion were analysed by ion chromatography as described by Corral et al. (2013). The concentration of each ion was determined by calibration curves using a set of standard solutions of Na⁺, K⁺ and Cl⁻ (Fluka, Switzerland, Sigma, St. Louis, MO). All results were expressed as mg/100 g of sample in dry matter.

2.5. Texture profile analysis

Texture profile analysis (TPA) was carried out using TA-XT.plus Texture Analyzer with Texture Exponent software (version 2.0.7.0
Table 1
Effect of salt and fat reduction on pH, $a_w$, and weight losses of dry fermented sausages inoculated with $D.~hansenii$ yeast.

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>$a_w$</th>
<th>Weight losses (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uninoculated</td>
<td>Inoculated</td>
<td>Uninoculated</td>
</tr>
<tr>
<td>C</td>
<td>5.0 Ab</td>
<td>5.0 Ab</td>
<td>0.906 Ab</td>
</tr>
<tr>
<td>RF</td>
<td>4.9 Bb</td>
<td>5.2 Aa</td>
<td>0.914 Ba</td>
</tr>
<tr>
<td>RS</td>
<td>5.1 Ba</td>
<td>5.2 Aa</td>
<td>0.912 Aa</td>
</tr>
<tr>
<td>RF + RS</td>
<td>5.1 Ba</td>
<td>5.3 Aa</td>
<td>0.916 Aa</td>
</tr>
</tbody>
</table>

Different small letters in the same column indicate significant differences at $p < 0.05$. Different capital letters in each row for each parameter indicate significant differences at $p < 0.05$.

3. Results and discussion

3.1. Chemical analysis

The pH, $a_w$, and weight losses are shown in Table 1. The pH showed a reduction in all batches from an initial value of 5.9–6.1 to 4.9–5.3 considered enough to ensure the safety of meat products together with drying and low $a_w$ values (Papadima et al., 1999). In uninoculated batches, RS and RF + RS showed significantly higher pH values than control batch and RF (Table 1) although this has not been previously reported (Corral et al., 2013; Liaros et al., 2009; Olivares et al., 2010). However, the greatest significant differences were found in yeast inoculated batches as all of them presented the highest significant pH probably due to the ability of yeasts to consume organic acids such as lactic acid (Durá, Flores, & Toldrá, 2004).

Water activity ($a_w$) also controlled through the ripening process reached values of 0.90–0.91 thus securing product stability. Few significant differences were observed among batches because the control batch had the lowest $a_w$ (Table 1). The effect of salt reduction in $a_w$ values of dry fermented sausages produced different results, no effect was reported in 16% reduced salt sausages (Corral et al., 2013) while highest $a_w$ values were reported in 50% reduced salt sausages (Olesen, Meyer, & Stahnke, 2004). Moreover fat reduction produced an increase in $a_w$ values (Gómez & Lorenzo, 2013).

Weight losses mainly depend on climatic conditions applied for product ripening (Bloukas et al., 1997). The weight losses in all batches were 39.8–41.6% at the end the process (Table 1). The slow ripening conditions applied during process prevented the effect of fat or/salt reduction on weight loss (Olivares et al. 2010). However, these processing conditions were not able to avoid the differences ($p < 0.05$) found when both reductions were applied together in the inoculated $D.~hansenii$ batch (RF + RS + Y). The

Table 2
Effect of salt and fat reduction on chemical composition of dry fermented sausages inoculated with $D.~hansenii$ yeast.

<table>
<thead>
<tr>
<th>Moisture (%)</th>
<th>Fat (%)</th>
<th>Protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uninoculated</td>
<td>Inoculated</td>
</tr>
<tr>
<td>C</td>
<td>46.0 Ac</td>
<td>46.0 Aa</td>
</tr>
<tr>
<td>RF</td>
<td>46.9 Ab</td>
<td>47.3 Aa</td>
</tr>
<tr>
<td>RS</td>
<td>47.2 Ac</td>
<td>46.3 Aa</td>
</tr>
<tr>
<td>RF + RS</td>
<td>47.6 Aa</td>
<td>46.4 Aa</td>
</tr>
</tbody>
</table>

Different small letters in the same column indicate significant differences at $p < 0.05$. Different capital letters in each row for each parameter indicate significant differences at $p < 0.05$.
highest weight losses produced by fat reduction have been also reported by other authors (Bloukas et al., 1997; Liaros et al., 2009; Papadima & Bloukas, 1999).

Table 2 shows the chemical composition of dry fermented sausages. A fat reduction of 10–16% was achieved. The moisture content was the highest (p < 0.05) in fat reduced batches (Gómez & Lorenzo, 2013). However, this effect was not seen (p > 0.05) in inoculated batches (RF + Y, RS + Y and RF + RS + Y) being in agreement with ω6 values obtained. As expected, the highest (p < 0.05) protein content was found in fat reduced batches due to the highest lean content present as also observed Olivares et al. (2010).

A total sodium reduction of 17–20% was achieved (Table 3). Salt reduced batches both uninoculated and yeast inoculated (RS, RF + RS, RS + Y and RF + RS + Y batches) presented significantly lower Na+ content and higher K+ content than no salt reduced batches (C, RF, RF + Y) (Table 3). By contrast, no significant differences were found for Cl− content among batches since, salt reduced batches were substituted by KCl. Overall, fat or/and salt reduction and D. hansenii inoculation did not produced significant differences in colour parameters (L*, a* and b*) (Corral et al., 2013) (data not shown).

3.2. Microbial analysis

LAB and staphylococci are essential for the ripening process and play an important role in the safety and organoleptic characteristics of dry fermented sausages (Ravety, Vuyst, & Leroy, 2012). The levels of LAB, staphylococci and yeast population were analysed at the beginning and end of the ripening process. At the beginning of the process the mean counts of LAB and staphylococci were 10^6 c.f.u./g of dry fermented sausages (Ravyts, Vuyst, & Leroy, 2012). The levels of yeast (10^7 c.f.u./g) than control batches (10^4 c.f.u./g) and this fact was observed in the inoculated batches (Table 4). At the end of the process, a low yeast growth was detected in the un inoculated batches (10^6 c.f.u./g) although no differences were detected among batches (Table 4).

<table>
<thead>
<tr>
<th>LAB (cfu/g dm)</th>
<th>Staphylococci (cfu/g dm)</th>
<th>Yeast (cfu/g dm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninoculated</td>
<td>Inoculated</td>
<td>Uninoculated</td>
</tr>
<tr>
<td>C</td>
<td>2.7E + 09</td>
<td>2.7E + 09</td>
</tr>
<tr>
<td>RF</td>
<td>2.6E + 09</td>
<td>7.4E + 09</td>
</tr>
<tr>
<td>RS</td>
<td>3.7E + 09</td>
<td>1.2E + 06</td>
</tr>
<tr>
<td>RF + RS</td>
<td>2.8E + 09</td>
<td>4.3E + 06</td>
</tr>
</tbody>
</table>

Different small letters in the same column indicate significant differences at p < 0.05. Different capital letters in each row for each parameter indicate significant differences at p < 0.05.
Panel C, Supplementary Material). However, the percentage increased between 60% and 70% in the uninoculated batches with salt and fat reduction (Fig. 1C, Panels RS and RF, Supplementary Material) and reached 90% in the uninoculated batch with both salt and fat reduction (Fig. 1C, Panel RS + RF, Supplementary Material). The presence of *D. hansenii* strains in the uninoculated sausage batches was probably due to the dispersion of the inoculated yeasts along the 61 days of ripening favoured by air circulation in the drying chamber. Nevertheless, the non-inoculated batches presented lower *D. hansenii* P2 counts than the inoculated ones.

3.3. Texture profile analysis

TPA parameters were analysed in the final product (Table 5). Hardness and consequently chewiness were affected by the different formulations whilst no effect was observed on springiness and cohesiveness. In uninoculated batches, RF, RS and RF + RS showed significant higher hardness and chewiness than control batch. Moreover, RS batch showed the highest hardness and chewiness. On the contrary, in inoculated batches, only RF + Y and RF + RS + Y batches showed significant higher hardness than the control batch. The effect of the inoculated *D. hansenii* yeast was significant in salt reduced batches (RS and RS + Y) as it produced a decrease in the hardness and chewiness. However, springiness and cohesiveness were not affected by neither formulation nor *D. hansenii* inoculation. This increase in hardness and chewiness has been already reported in dry fermented sausage when fat was reduced (Olivares et al., 2010). However, salt effect on fermented sausage texture is contradictory as generally low changes in texture have been reported when KCl was used as unique salt substitute (Gou, Guerrero, Gelabert, & Arnau, 1996) or a decrease in sausage hardness (Gimeno et al. 1999); although any reference has indicated an increase in hardness as observed in our uninoculated sausages.

3.4. Sensory analysis

An Internal Preference Mapping was done with mean scores of overall acceptability and the following supplementary parameters which showed significant differences between batches: pH, *w* sub, weight losses, ions content (Na\(^+\), K\(^+\), Cl\(^-\)), fat, protein and moisture content, microbiological analysis (LAB, Staphylococcus and yeast counts), texture analysis (hardness, chewiness) and consumer liking (appearance, aroma and taste) (Fig. 1). Two principal components were able to explain the 42.8% of the total variance. PC1 accounted for 22.9% of the variance and distinguished samples by the presence of yeast (in the positive part of the axe are placed samples without yeast inoculation and in the negative part of the axe inoculated samples). Taking into account supplementary parameters plotted, C, RF + RS and RF + Y samples were related with moisture, fat and Na\(^+\) and Cl\(^-\) content, staphylococci counts and taste liking; RS and RF samples were related with texture parameters and RS + Y, RF + RS + Y samples were related with pH, protein and potassium content and yeast.

For a better understanding of consumer responses, the preferences for attributes that showed significant differences (taste and overall acceptability) plus the attribute aroma were also analysed by cluster analysis using Euclidean distances (Fig. 2). The attribute aroma was analysed due to the effect of yeast on sausage aroma (Cano-García et al., 2013). The number of consumers in each cluster was different and depended on the analysed attribute, thirty-nine and forty-two consumers for aroma, thirty-four and twenty-seven for taste and fifty and thirty-one for overall acceptability in cluster 1 and 2 respectively. The sausage preference of each cluster was elucidated by one-way ANOVA. Based on aroma, taste and overall acceptability, cluster 1 preferred fat reduced sausages without yeast inoculation while cluster 2 preferred inoculated and fat reduced sausages (Fig. 2).

Salt reduced sausage (RS) was perceived less tasty and the cluster 2 also perceived it less aromatic. Although RS was overall accepted by cluster 2, cluster 1 did not sensory accepted it. Moreover, the yeast inoculation on salt reduced sausages (RS + Y) was accepted better than uninoculated salt reduced sausages by both clusters, even though the sausage aroma and taste did not significantly improve by yeast inoculation. This fact agree with those reported by Corral et al. (2013) and Aaslyng et al. (2014) who pointed out that consumers consider salt reduced sausages an acceptable product although the sensory characteristic are affected. Also, other studies have reported an improvement of sausage aroma and taste using yeast extracts (Campagnol et al., 2011).
Fat reduced sausage was sensory accepted by the two consumer clusters; although, cluster 2 perceived it less tasty and aromatic. However, the yeast inoculation on fat reduction sausage was perceived with more sausage taste and aroma by cluster 2. This result agrees with Olives et al. (2011) who reported a lower aroma in low fat sausages although Liaros et al. (2009) and Papadima and Bloukas (1999) reported no effect of fat reduction on sausage odour or taste. However, when salt and fat reduction was carried out together, the two clusters perceived the sausage the less tasty than the other formulations and cluster 1 even perceived it less aromatic. In this case, the yeast inoculation did not improve the sausage taste or aroma.

Nevertheless, different preferences patterns of consumers were found; the yeast inoculation improved the aroma and taste quality when the fat or salt reductions were carried out in dry fermented sausages. Several authors have studied the effect of *D. hansenii* on sausage aroma reporting an increase in some volatile compounds.

(Andrade et al., 2010) while Olesen and Stahnke (2000) reported few differences between control and yeast inoculated sausages. However, there are not reports about the effect *D. hansenii* inoculation on consumer acceptability and when fat or salt is reduced. In this sense, the addition of yeast extracts produced an increase in the aroma acceptability of sausages (Bolumar et al., 2006; Campagnol et al., 2011) while only Flores, Durá, Marco, and Toldrá (2004) confirmed the beneficial effect of the inoculation of *D. hansenii* in fermented sausages on consumer aroma acceptability. However, they did not confirm the implantation of the inoculated yeast strain. In addition, further studies are necessary to elucidate the biochemical process involved in the improvement of quality and aroma in low salt and fat dry fermented sausages by the inoculation of *D. hansenii* yeast.

4. Conclusion

The inoculation of *D. hansenii* yeast on salt and fat reduced sausages was able to compensate the changes in $a_w$ and texture although it was not able to modify neither the hardness of reduced fat batches nor the decrease in staphylococci growth. In terms of sensory analysis, yeast inoculation improved the aroma and taste quality when fat or salt reductions were done. However, when salt and fat reduction was carried out together, yeast inoculation did not improve sausage taste or aroma. Further studies are necessary to elucidate the biochemical process involved in aroma generation and the interactions with salt and fat reductions.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.foodcont.2014.04.013.

References


