ABSTRACT – Reducing salt and fat content in dry-fermented sausages could benefit consumer’s health. This study aimed to evaluate the effects of NaCl and pork fat reduction or replacement on the physical chemical parameters (pH, \(A_w\) and instrumental color) of probiotic fermented sausage. Five batches were manufactured: control (TC), 25% pork fat reduction (T1), 25% pork fat replacement by coconut oil (T2), 25% NaCl reduction (T3), 25% NaCl replacement by KCl (T4). Sausages were inoculated with a commercial starter and a probiotic *Lactobacillus plantarum*. Results were analyzed by ANOVA. Color measures were also submitted to a PCA. Sodium reduction or replacement by KCl (25%) as well as the simple pork fat reduction (25%) did not negatively affect most of the physical chemical parameters of fermented sausages. The addition of coconut oil occasioned color defects on products, suggesting that its use as pork fat substitution is not recommended for probiotic fermented sausages.

KEYWORDS: KCl, coconut oil, pH, color, meat

1. INTRODUCTION

Meat and meat products are a very important in human diet due to their high nutritional value, being especially rich in protein, vitamins and minerals. However, an excessive intake of meat products is not recommended because they usually have high fat and sodium content (Muguerza et al., 2004). The reduction of sodium and fat intake is a public health concern since their excessive consumption is associated with chronic diseases, such as cardiovascular diseases, diabetes mellitus and cancer. Therefore, meat industry has been searching for strategies in recent years addressed to improve the nutritional value of meat products by reducing the content of the unhealthy substances (Nilson et al., 2012). These strategies can include the simple reduction of salt or fat, the substitution of these compounds or both their reduction and substitution.

The most common NaCl substitute used in meat products is potassium chloride (KCl) (Rech, 2010). Several studies report the effect of the addition of KCl as a NaCl substitute on the physical chemical and sensory properties of fermented meat products. Lilic et al. (2008) report the NaCl substitution by KCl in dry-fermentation sausage from 20 to 40%, without significant changes in taste and acceptance of the product. However, the NaCl substitution by KCl at levels above 50% can generate bitter and metallic off-flavour in fermented sausages (Zanardi et al., 2010).
Several animal fat replacers such as vegetable oils, fibers and vegetable proteins have been proposed for meat products (Zhang et al., 2010). Regarding vegetable oils, coconut oil could be an interesting type of oil to be used in healthy foods because its medium-chain fatty acids are easily absorbed in the intestine, without the need of pancreatic lipase enzymes action. Therefore, they do not participate in the cholesterol cycle and are not stored as fat in human body. The consumption of coconut oil is associated to the increase the HDL cholesterol blood level (Liau et al., 2011; Mora-Gallego et al., 2016).

Additionally to the reduction of NaCl and animal fat, the use of probiotics in fermented sausages can improve the safety, the stability and the functional value of these meat products. Few studies have addressed the reduction in salt or fat in probiotic-fermented sausage and the use of coconut oil as an animal fat replacer in meat products. Therefore, the aim of this study was to evaluate the effect of the salt and fat reduction and replacement on the physical chemical characteristics of probiotic dry-fermented sausage.

2. MATERIAL AND METHODS

Five batches of dry fermented sausages were manufactured: control (TC), 25% pork fat reduction (T1), 25% pork fat replacement by coconut oil (T2), 25% NaCl reduction (T3), 25% NaCl replacement by KCl (T4). Control sausages (TC) were formulated as follows: pork (60.0%), beef (20.0%), pork back fat (20.0%), NaCl (3.0%), sugar (sucrose 0.5%; glucose 0.5%), garlic powder (0.3%), white pepper (0.2%), ground nutmeg (0.02%), sodium nitrate (0.005%), sodium nitrite (0.015%), sodium ascorbate (1.0%), commercial starter culture (Bactoferm T-SPX CHR Hansen, Denmark) and probiotic culture (Lactobacillus plantarum 343, from the culture collection of LTPA).

Pork shoulder and lean beef were ground in a conventional meat grinder (JAMAR®, Curitiba, Brazil); pork back fat was cut into cubes (~0.5 cm$^3$). All spices, curing salt and sugars were manually mixed to the meat. The commercial starter and probiotic cultures were added to the batter at the end of mixture (inoculated at ca. 6 and ca. 8 log CFU/g, respectively). Prior to the inoculation, commercial starter culture was reactivated in non-chlorinated water at 37 °C for 30 min. Probiotic strain was grown overnight in MRS broth (Merck,Darmstadt, Germany) at 37 °C, harvested by centrifugation at 5000 rpm for 10 min at 6 °C, washed twice in saline solution (0.9% NaCl). Meat batter was stuffed into 50mm diameter collagen casings. The raw sausages weighed ~100g. All sausages were superficially inoculated with a commercial suspension of Penicillium nalgiovensis (Bactoferm MOLD-600 CHR Hansen, Denmark) immediately after stuffing.

All sausages were ripened for 28 days in a chamber with controlled temperature and relative humidity conditions. Samples were withdrawn at 0, 7, 14, 21 and 28 days of storage to the physical chemical analysis:

- $A_w$: determined using a $A_w$ meter (AQUALAB CX-2, Decagon, U.S.A), in triplicate,
- pH: determined using a pHmeter (HI 99163, Hanna Instruments, Portugal), in triplicate,
- instrumental color: determined using a colorimeter CR-410 (Konica Minolta Inc., Japan) and the color coordinates L*, a*, b*, C* and hue, in triplicate.

Results were analyzed by ANOVA and the means were compared by Tukey’s test (P<0.05), using the STATGRAPHICS Centurion Program (16.1.11, Statpoint Technologies, Warrenton, USA). Instrumental color results were also submitted to a Principal Component Analysis (PCA), carried out with the SAS Program (SAS 9.1; software SAS Institute Inc., Cary, NC).
3. RESULTS AND DISCUSSION

Table 1 shows the time-course of pH and $A_w$ values during ripening, which gradually decreased in all treatments over time (Table 1). The decrease of pH and $A_w$ is attributed to the production of lactic acid by the lactic acid bacteria of starter and probiotic cultures, as a result of carbohydrates fermentation (Olivares et al., 2010), which reaches the isoelectric point of myofibrillar proteins, decreasing the water holding capacity and promoting sausage drying (Terra, 1998).

Among treatments, T2 (25% pork fat replacement by coconut oil) showed lower pH value than T4 (25% NaCl replacement by KCl). T4 (25% NaCl replacement by KCl) showed the lowest $A_w$ at the 28th day. Conversely, T2 (25% pork fat replacement by coconut oil) exhibited the highest $A_w$ value.

Regarding salt content, some studies report higher $A_w$ in NaCl-reduced sausages (Olesen et al., 2004; Roseiro et al., 2008). On the other hand, Corral et al. (2014) found no difference on final $A_w$ of dry-fermented sausages with different NaCl levels (16% reduced and 16% KCl replaced). In the present study, the reduction of 25% NaCl did not affected the $A_w$ of fermented sausage compared to control. However, the replacement of NaCl by KCl decreased $A_w$ values. Similar results were found by Mora-Gallego et al. (2016), in fermented sausages added with KCl. The partial replacement of sodium chloride (NaCl) by potassium chloride (KCl) might influences $A_w$ because potassium ion has higher polarity compared to sodium, which could enable it to bind to a greater number of water molecules, leading to a lower Aw.

The simple reduction of fat did not affect $A_w$ in fermented sausages. Similarly, García et al. (2002) did not find significant differences in $A_w$ in low fat dry-fermented sausages. However, the addition of coconut oil increase $A_w$ in fermented sausage. This result may be related to the fact that coconut oil can cover the meat particles, causing a slower release of water during the drying process (Mora-Gallego et al., 2016). Despite, the higher $A_w$ in T2, at 28th day, $A_w$ values of all treatments were below 0.92, which is considered safe regarding pathogens growth capacity, especially *Listeria monocytogenes* (Ingham et al. 2004).

Table 1. Time-course evolution of pH and $A_w$ in probiotic dry-fermented sausages with different sodium and pork fat contents

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>5.92 ± 0.05&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>5.08 ± 0.06&lt;sup&gt;Da&lt;/sup&gt;</td>
<td>5.38 ± 0.03&lt;sup&gt;Ca&lt;/sup&gt;</td>
<td>5.44 ± 0.06&lt;sup&gt;Bcab&lt;/sup&gt;</td>
<td>5.58 ± 0.08&lt;sup&gt;Bab&lt;/sup&gt;</td>
</tr>
<tr>
<td>T1</td>
<td>5.95 ± 0.01&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>5.02 ± 0.14&lt;sup&gt;Cab&lt;/sup&gt;</td>
<td>5.44 ± 0.18&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>5.58 ± 0.09&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>5.67 ± 0.17&lt;sup&gt;Abab&lt;/sup&gt;</td>
</tr>
<tr>
<td>T2</td>
<td>5.90 ± 0.05&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>4.94 ± 0.01&lt;sup&gt;Eab&lt;/sup&gt;</td>
<td>5.07 ± 0.05&lt;sup&gt;Da&lt;/sup&gt;</td>
<td>5.29 ± 0.04&lt;sup&gt;Ch&lt;/sup&gt;</td>
<td>5.46 ± 0.01&lt;sup&gt;Bb&lt;/sup&gt;</td>
</tr>
<tr>
<td>T3</td>
<td>5.79 ± 0.21&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>4.87 ± 0.02&lt;sup&gt;Cb&lt;/sup&gt;</td>
<td>5.21 ± 0.18&lt;sup&gt;Bca&lt;/sup&gt;</td>
<td>5.48 ± 0.11&lt;sup&gt;Abab&lt;/sup&gt;</td>
<td>5.66 ± 0.08&lt;sup&gt;Aab&lt;/sup&gt;</td>
</tr>
<tr>
<td>T4</td>
<td>5.99 ± 0.01&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>4.88 ± 0.04&lt;sup&gt;Cb&lt;/sup&gt;</td>
<td>5.43 ± 0.24&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>5.64 ± 0.09&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>5.77 ± 0.11&lt;sup&gt;Abab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

TC: Control; T1: 25% pork fat reduction; T2: 25% pork fat replacement by coconut oil; T3: 25% NaCl reduction, T4: 25% NaCl replacement by KCl. Means ± Standard deviation. Different uppercase letters in the same row indicate significant differences among storage time in the same treatment ($P < 0.05$). Different lowercase letters in the same column indicate significant differences among treatments ($P < 0.05$).
Regarding instrumental color, T2 sausages showed the lowest a* and C* values and the highest b* and hue values (Table 2), exhibiting lesser redness and greater yellowness and discoloration than sausages from the other treatments.

Table 2. Instrumental color values in probiotic dry-fermented sausages with different sodium and pork fat contents at the end of processing period (28 days).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TC</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>L*</td>
<td>42.28 ± 1.04a</td>
<td>40.72 ± 0.81ab</td>
<td>40.36 ± 1.49ab</td>
<td>39.47 ± 1.16b</td>
<td>39.43 ± 0.16b</td>
</tr>
<tr>
<td>a*</td>
<td>19.25 ± 1.02a</td>
<td>20.40 ± 1.16a</td>
<td>11.45 ± 0.52b</td>
<td>20.26 ± 0.43a</td>
<td>18.80 ± 0.67a</td>
</tr>
<tr>
<td>b*</td>
<td>6.84 ± 0.27bc</td>
<td>7.47 ± 0.18b</td>
<td>9.59 ± 0.30a</td>
<td>7.15 ± 0.33b</td>
<td>6.25 ± 0.17c</td>
</tr>
<tr>
<td>C*</td>
<td>20.43 ± 1.05a</td>
<td>21.61 ± 1.21a</td>
<td>14.95 ± 0.47b</td>
<td>21.48 ± 0.51a</td>
<td>19.81 ± 0.67a</td>
</tr>
<tr>
<td>h*</td>
<td>19.57 ± 0.31b</td>
<td>19.29 ± 0.53b</td>
<td>19.25 ± 0.82b</td>
<td>18.38 ± 0.44b</td>
<td></td>
</tr>
</tbody>
</table>

TC: Control; T1: 25% pork fat reduction; T2: 25% pork fat replacement by coconut oil; T3: 25% NaCl reduction, T4: 25% NaCl replacement by KCl. Means ± Standard deviation. Different lowercase letters in the same column indicate significant differences among treatments (P < 0.05).

Figure 1 shows that the two main factors of PCA explained 84.67% of the variance, for which the first factor was responsible for 63.37%. The red ellipse in the graphic shows redness (a*) spatial variation, for which T2 exhibited opposite position compared to the other treatments. Similar results were obtained for yellowness (b*), represented by the black ellipse and for h*, blue ellipse. These results showed that the addition of coconut oil negatively affected the color of probiotic-fermented sausage. Bloukas et al. (1997) and Severini et al. (2003) also reported that a direct incorporation of vegetable oil during the manufacture processing created an unacceptable color appearance in fermented sausages. The higher yellowness and discoloration in the coconut oil-added fermented sausage might be related to a higher lipid oxidation caused by the microbial lipases produced by the starter cultures. The lipid oxidation in meat products can reduce myoglobin stability, making it more susceptible to oxidation, which decreases the red color and increases the discoloration of red meat (Kennedy et al., 2004).

Figure 1. Biplot of two principal components computed from the instrumental color coordinates in probiotic-dry-fermented sausages with different sodium and pork fat contents.
4. CONCLUSION

The replacement of pork fat by coconut oil in probiotic-fermented sausage is not recommended since it can cause color defects on product. The reduction of NaCl by 25%, its replacement by KCl or the simple reduction of pork fat by 25% did not generate significant changes in most of the physical chemical parameters of probiotic-fermented sausages. Further studies must be conducted to evaluate the effect of a simultaneous reduction of fat and sodium in the probiotic-fermented sausages properties and sensory attributes. The results of the present study constitute a valuable set of data for helping meat industries interested in reducing salt and fat contents in probiotic dry-fermented sausages.

5. REFERENCES