Use of celery extract and starter culture (Staphylococcus carnosus) as an alternative sources of sodium nitrite in cooked ham: influence upon colour

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Abstract
The effect of a new source of nitrites from vegetable origin was evaluated. A celery extract (CE) and holding time on the colour, reflectance spectrum, pH and residual nitrite in Medellín-type cooked ham (MCH), as well as effects of the cooking and cooling processes was studied. The CE was incorporated as a powder at concentrations of 0.2, 0.3 and 0.4%. The holding time was set at 12, 18 and 24 h. The control, to which only nitrite was added, showed a significantly higher residual nitrite content than the other treatments used. Two colour zones were identified in all MCH containing celery extract, the peripheral zone differing significantly from the control values for the colour coordinates of lightness (L*), red-green (a*) and yellow-blue (b*). The reflectance spectrum for the external zone differed between the different experimental treatments and the control, especially for yellow and red wavelengths.

Keywords: Cooked ham; nitrite substitutes; celery extract; colour; reflectance spectroscopy

Introduction
At present, nitrite is added directly as an ingredient for curing meats, although many attempts have been made to replace (Shahidi et al., 1994; Shahidi et al., 1997; Pegg and Shahidi, 2000) or reduce it (Alesón-Carbonell et al., 2003; Fernández-Ginés et al., 2003; Jafari and Emam-Djomeh, 2007; Fernández-López et al., 2009; Zarringhalami et al., 2009). In this respect, it has been found that plants and their extracts can be used as indirect sources of nitrate (Shahid-Umar and Iqbal, 2007; Shahid-Umar et al., 2007; Parks et al., 2008) in the production of meat products (Sebranek and Bacus, 2007). Sindelar et al. (2007) mentioned that certain plants are capable of storing nitrate in their structures, and that if added to meat products along with suitable starter cultures (nitrate-reductants), the nitrites necessary for transforming meat into a cured product may be generated in the matrix. The most widely used plant extract in this field is celery extract (CE) since, unlike other vegetables; celery is highly compatible with cured meats since it has a low pigment concentration (carotenoids, anthocyanins or chlorophyll). From a sensorial point of view, Sebranek and Bacus (2007), mention that celery extracts do not significantly modify the taste of the products. Moreover, in many meat formulations, especially cooked and emulsified meat products, celery is used as spice (Pérez-Alvarez et al., 2002).

In general, the colour of meat products can be affected by several factors, some of them affected the ultrastructure. Thus ultra-structural changes can affect meat product appearance. Several technological factors and ingredients usually used in meat processing can affects directly or indirectly the meat appearance (meat colour) for example freezing and thawing techniques or addition of functional ingredients (Pérez-Alvarez and Fernández-López, 2009).
According to Mendoza et al. (2009), the final colour of cooked ham depends on the formulation, microstructure imparted to the ham during fabrication (i.e., due to curing, roasting, cooking, smoking, and cooling processes), and storage conditions among others. Such differences in appearance are expected to be specific for each ham type. The specific colour characteristics of a specific cooked ham type can be used initially as a fingerprint. Thus colour coordinates and reflectance spectra could be regarded as useful tools not only to objectively check in situ the quality specifications during fabrication but also to judge the final product quality. Therefore, the substitution of nitrite by natural sources that contain nitrate requires, from a technological and scientific point of view, more information before new products can be launched.

Pérez-Alvarez (2006) describes the necessity to establish specific colour descriptors that specifically describe cooked meat products. Thus colour characteristics of cooked ham must be clearly specific. The objective of this study was to determine the influence of using CE and different holding times on the colour characteristics, reflectance spectra, pH and residual nitrite levels in a Medellin-type cooked ham made according to the Colombian industrial elaboration process.

Materials and Methods

Raw material and Formulation: In this study was elaborated a Medellin-type cooked ham. The control formula was as follows: 53% lean pork meat, 29% water (ice), 6% wheat starch, 3.8% mixture of spices and commercial flavourings (w/w), 2.5% soy protein (w/w), 2.5% sodium lactate (w/w), 2.2% iodised salt (w/w), 0.5% sodium polyphosphates (w/w), 0.5% refined carrageenin (w/w), 500 mg/kg sodium ascorbate, 350 mg/kg sodium nitrite, all approved for use in foods. None of the natural or artificial colorants usually used in this type of product was used since it was intended that any effect on the resulting colour would be the effect of the factors under study.

Using the control formula (0% CE and 24 h holding time) as a base, two different concentrations (0.2% and 0.4% CE) plus an intermediate value (0.3% CE) with two different holding times (12 h and 24 h) with a central time (18 h) were used to evaluate the behaviour of the response variables. In this case the complete experimental layout is depicted in Table 1. The central point was set to verify whether the interaction was statistically significant or, in other words, to establish whether there was a non linear relationship between concentrations and holding times in the response surface. The starter culture, Staphylococcus carnosus (culture CS 299), was used at 0.02% weight of the formula in all cases where CE (Vegetable Juice Powder Natural NA) was used. Both were provided by CHR Hansen Colombia S.A.

Mixing process: The products were prepared in the pilot plant of Industria de Alimentos Zenú S.A.S., Medellín, Colombia, using the formula described below. The lean pork meat (kept at 2 ± 1 °C) was reduced in a grinder PT 98 (Mainca,

<table>
<thead>
<tr>
<th>Treatment (Cmb)</th>
<th>% CE</th>
<th>Resting Time (h)</th>
<th>Batch 1</th>
<th>Batch 2</th>
<th>Batch 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>24</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td>12</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>0.3</td>
<td>18</td>
<td>7</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>0.4</td>
<td>12</td>
<td>16</td>
<td>17</td>
<td>18</td>
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<tr>
<td>5</td>
<td>0.2</td>
<td>24</td>
<td>19</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>6</td>
<td>0.4</td>
<td>24</td>
<td>22</td>
<td>23</td>
<td>24</td>
</tr>
</tbody>
</table>

Cmb: Combinations
Barcelona Spain) using 5 mm discs to favour the distribution of the brine within the meat pieces. The brine was previously prepared in an industrial liquidiser CLE 12/90 (Colcocinas, Medellín, Colombia), adding the ingredients in an order that favoured their total homogenisation. Both the ground meat and the brine were then placed in a 100 L tumbler, Model TV 100, (Talsa, Medellín, Colombia) for an hour at 16 rpm to facilitate the incorporation of the brine in the meat and the extraction of the protein necessary to bind the pieces. The holding time of 12 h and 24 h plus 18 h for the central point (a factor in the experimental design, where 24 h was used for the control level) was counted from the moment tumbling had finished. This took place in a refrigerated chamber at 4º C and was intended to favour the chemical and biochemical reactions that influence the sensorial impact of the final product. After the different holding times, the mixture was stuffed in a chamber Vemag Roby TYP 134 (Germany) into plastic casings with an oxygen permeability of 20 cm$^3$/ m$^2$/ 24h at 1 atm and then placed in 10x10 cm stainless steel moulds. The samples were then placed in a smoke chamber (Ahumadero Talsa, 1995, Medellín, Colombia) with saturated steam for 2 hours and 40 minutes at 80º C, until the product reached a temperature of 70 ± 2 ºC at its geometrical centre (PT 100 sensor). The cooked hams were then placed in a cool chamber at 4º C for 24 h before cutting and slicing Trief NR 104740 (Trief, Germany) according to the needs of each analysis carried out. The meat was vacuum packaged by a Tiromat Compact 320 (CFS, Germany).

**Residual nitrites concentration:** The nitrite content of the CE and the final product was determined according to AOAC method (AOAC, 2006). Residual nitrite determination was reported in mg/kg of the sample.

**Colour analysis:** The CIELAB colour space was chosen to measure colour, as described by Cassens et al. (1995). This provided the coordinates lightness ($L^*$), redness ($a^*$), yellowness ($b^*$) and the psychophysical magnitudes Chroma ($C_{ab}$) and hue ($h_{ab}$). In addition, the reflection spectrum between 400 nm and 700 nm were obtained at 10 nm intervals. An integrated sphere spectrophotometer was used Model SP 62 (measuring geometry d / 8), circular low reflectance glass and computer incorporating the software X-RiteColour Master QA II (Missouri, USA). The data were obtained with $D_65$ illuminant and an observer angle of 10º (Honikel, 1998). Infinite solid was obtained according to Sánchez-Zapata et al. (2011).

**Experimental design and statistical:** The two factors were CE% and holding time, each evaluated at two levels, including a central point of the combination of both factors. Three replicates were made for each of the treatments and for each replicate a block with a different batch of meat to determine the effect that this might have on the results. Also three replicates were carried out in the central point of the statistical design (Table 1). The 24 assays were carried out randomly. All the samples were stored and the evaluations were carried out immediately after packaging. In the colour analysis, an additional factor was included, corresponding to the slicing or cutting zone (central or peripheral). A classical analysis of variance (ANOVA) was applied to determine any differences between the treatments. A Dunnett’s test was carried out to establish specific differences between the control and the treatments. A confidence level of 95% was used in all cases to establish the level of significance. Statistical analysis was made using R statistical software (R 2008).

**Results and Discussion**

**Residual nitrites:** Table 2 shows the analytical results for the residual nitrite recorded in the final products. In this table can be observed that different treatments of celery extract and resting time (Combinations: Cmb 2 to 6), had no statistically significant effect on the residual nitrite content ($p>0.05$). The low levels were found when CE is added. These results suggest that new studies are needed to determine the impact of this method of producing Colombian hams on the role of the starter culture in reducing nitrates to nitrites. In combinations 2 to 6, the concentration of 40-50 mg/kg, that are considered sufficient for the
technological and microbiological effects sought in most products (USDA, 1995), was not reached. According to Sebranek and Bacus (2007), it is not possible to measure the quantity of nitrite produced when natural sources are used as nitrate source in a meat system. Since the nitrites rapidly react with meat’s components. Residual nitrite levels for all treatments are shown in Table 2. The type and concentration of starter culture used in this study (Staphylococcus carnosus) was the same as that used by Sindelar et al. (2007) in studies on sausages and cooked ham.

Table 2: Mean values of residual nitrite levels in Medellin-type cooked ham (MCH).

<table>
<thead>
<tr>
<th>Treatment (Cmb)</th>
<th>% EC</th>
<th>Resting Time (h)</th>
<th>Nitrates (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>24</td>
<td>136.52 a</td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td>12</td>
<td>12.78 b</td>
</tr>
<tr>
<td>3</td>
<td>0.3</td>
<td>18</td>
<td>10.33 b</td>
</tr>
<tr>
<td>4</td>
<td>0.4</td>
<td>12</td>
<td>12.62 b</td>
</tr>
<tr>
<td>5</td>
<td>0.2</td>
<td>24</td>
<td>11.10 b</td>
</tr>
<tr>
<td>6</td>
<td>0.4</td>
<td>24</td>
<td>12.06 b</td>
</tr>
</tbody>
</table>

Cmb: Combinations Values followed by the same letter within the same column are not significantly different (p>0.05) according to Dunnett’s Test.

**Colour analysis:** Colour stability is one of the most important characteristics of cured meat products, and it is the primary quality attribute seen by the consumer. From a consumer’s point of view, cured meat discolouration can be defined as divergence from the consumer-defined ideal to something less desirable, for example, pink-reddish to grey (Illescas-Hernández et al., 1993). Since the chroma value of the colour indicates how much a specified colour differs from grey, a faded red or pink colour may be considered as non-suitable for human consumption. Valous et al. (2009) described how in cooked ham, the morphological features of pores and fat-connective tissue may contribute to characterizing the appearance of sliced ham surfaces. In general, cooked hams look similar, with non-homogeneous colour surfaces. For example, the internal surface colour visibly differed between the central area and the peripheral area of the ham slices from the treatments 2, 3, 4, 5 and 6, while the control showed no such colour change (Figure 1). These observations might be explained because the ham surface was exposed to temperatures much above those considered optimal for the correct development of the microbiological culture during the heating process, especially in the treatments where CE was used. As mentioned above, new studies might point to the need to change the manufacturing process in order to favour the role of the starter culture and so correct the colour differences observed. Note that measurement of nitrites made in a homogenous sample and not in the differently coloured zones.

Table 3 shows the colour parameters studied. Lightness is considered to be the main parameter governing the quality of meat products (García-Esteban et al., 2003) and the best predictor of visual pink colour intensity (Brewer et al., 2001). Colour stability is very important for cooked cured meat products, and a less stable cured meat product or discoulouration may result in a substantial financial losses to the retailer and other segments of the industry. It must be taken into account that this serious defect occurs during the storage of cured meat products, during which time the desirable pink-reddish colour usually changes to grey.

The lightness values ($L^*$) increased in the treatments 2, 3, 4, 5 and 6 with respect to the control, although no statistically significant differences existed between the treatments in which CE was used as regards ($L^*$) in the external zone ($P>0.05$). However, there was a statistically significant difference ($P>0.05$) between these treatments and the control, the external zone being lighter in the treatments. For the same coordinate, there were no statistically significant differences ($P>0.05$) between the treatments. According to Sammel and Claus (2003), $L^*$ values significantly increased with higher end-point cooking temperatures and longer storage periods colour is also lighter, because denatured muscle proteins scatter more light. Valous et al. (2009) reported average $L^*$ value in cooked ham surfaces of 69.0 ± 4.4, while our results pointed to lower values. The colour coordinate $a^*$ is the most sensitive colour measurement parameter, characterizing redness and colour stability (García-Esteban et al., 2003).
Figure 1: Illustrative images of Medellinean-type cooked ham (a) control samples, (b) samples added with celery extract (Treatments: 2, 3, 4, 5, and 6).

Table 3: Mean values for CIELAB colour parameters, lightness (L*), redness (a*), yellowness (b*), chroma (C*<sb>ab</sb>) and hue (h<sub>ab</sub>) in central and peripheral zones of Medellín-type cooked ham (MCH) produced using different concentrations of celery extract (CE).

<table>
<thead>
<tr>
<th>Treatment (Cmb)</th>
<th>Zone</th>
<th>CIELAB Color Parameters</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>L*</td>
<td>a*</td>
<td>b*</td>
<td>C*&lt;sub&gt;ab&lt;/sub&gt;</td>
<td>h&lt;sub&gt;ab&lt;/sub&gt;</td>
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<tr>
<td>1</td>
<td>Peripheral</td>
<td>62.91 a</td>
<td>6.44 a</td>
<td>8.20 a</td>
<td>10.43 a</td>
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<td>52.16 a</td>
</tr>
<tr>
<td></td>
<td>Peripheral</td>
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<td>1.45 b</td>
<td>10.56 b</td>
<td>10.67 a</td>
<td>82.13 b</td>
</tr>
<tr>
<td>2</td>
<td>Central</td>
<td>63.08 a</td>
<td>5.88 a</td>
<td>7.36 c</td>
<td>9.43 b</td>
<td>51.44 a</td>
</tr>
<tr>
<td></td>
<td>Peripheral</td>
<td>64.93 b</td>
<td>1.68 b</td>
<td>10.73 b</td>
<td>10.86 a</td>
<td>81.05 b</td>
</tr>
<tr>
<td>3</td>
<td>Central</td>
<td>63.01 a</td>
<td>5.99 a</td>
<td>7.67 a</td>
<td>9.749 b</td>
<td>52.03 a</td>
</tr>
<tr>
<td></td>
<td>Peripheral</td>
<td>65.48 b</td>
<td>1.53 b</td>
<td>10.91 b</td>
<td>11.02 a</td>
<td>81.98 b</td>
</tr>
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<td>Central</td>
<td>62.71 a</td>
<td>5.87 a</td>
<td>7.42 c</td>
<td>9.47 b</td>
<td>51.68 a</td>
</tr>
<tr>
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<td>Peripheral</td>
<td>65.81 b</td>
<td>2.17 b</td>
<td>10.26 b</td>
<td>10.52 a</td>
<td>77.80 b</td>
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<td>Central</td>
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<td>5.54 a</td>
<td>7.45 a</td>
<td>9.30 b</td>
<td>53.43 a</td>
</tr>
<tr>
<td></td>
<td>Peripheral</td>
<td>65.67 b</td>
<td>1.50 b</td>
<td>10.84 b</td>
<td>10.95 a</td>
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</tr>
<tr>
<td>6</td>
<td>Central</td>
<td>63.42 a</td>
<td>6.08 a</td>
<td>7.47 a</td>
<td>9.64 b</td>
<td>50.82 a</td>
</tr>
</tbody>
</table>

Values followed by the same letter within the same column are not significantly different (p>0.05) according to Dunnett’s Test.
Figure 2: Reflectance spectra of central area (a) and peripheral area (b) of Medellin-type cooked ham (MCH) with different celery extract (CE) concentrations added [Treatments (Cmb: Combination): Cmb 1: control; Cmb 2: 0.2% CE; Cmb 3: 0.3% CE; Cmb 4: 0.4% CE; Cmb 5: 0.2% CE; and Cmb 6: 0.4% CE].

Figure 3: Reflectance spectra of peripheral area of Medellin-type cooked ham (MCH) considered only the samples with different celery extract (CE) concentrations added [Treatments (Cmb: Combination): Cmb 2: 0.2% CE; Cmb 3: 0.3% CE; Cmb 4: 0.4% CE; Cmb 5: 0.2% CE; and Cmb 6: 0.4% CE].
A deeper red appearance is probably suggestive of added artificial colouring and in cooked ham greater redness is usually correlated with lower acceptability.

It can be assumed that consumers prefer products with a lighter colour, with less red colouring, i.e. with a higher value of \( L^* \) and a lower \( a^* \). The parameter \( a^* \) was lower in all the treatments to which CE had been added, while the final product showed higher green components in the external zone. In agreement with Sammel and Claus (2003), lower values indicate less pinkness. Statistically significant differences in \( a^* \) were observed between the treatments 2-6 and the control but not in the central zone.

As regards the yellow-blue coordinate \((b^*)\), this was higher in the treatments 2-6 than in the control, yellow components increasing in the external zone to a statistically significant extent \((P<0.05)\). As regards the centre, only the pairs of treatments 1, 2, and 1, 4, showed statistically significant differences \((P<0.05)\), both influenced by the holding time. In the central zone, significant differences \((P<0.05)\) were found between the treatments and the control, in which the colour was more vivid since the grey colour component decreased. Miltenburg et al. (1992), reported that \( C_{ab}^* \) is also related to the quantity of pigments, but Pérez-Alvarez and Fernández-López (2000) mentioned that discolouration in cooked cured meat products means decreased \( C_{ab}^* \) values. Hue is the attribute of a colour perception denoted by blue, green, yellow, red, purple, etc. (Wyszecki and Styles, 1982), and is related with the state of meat pigments (Pérez-Alvarez and Fernández-López, 2000). In the external zone, the hue parameter \((h_{ab})\) showed statistically significant \((P<0.05)\) difference between the control and the treatments 2-6, being lower (tendency towards red) in the former than in the latter (tendency towards yellow). No statistically significant \((P<0.05)\) differences were observed between any of the treatments (all presented reddish hue).

**Reflectance spectra:** Another tool for evaluating the meat (raw materials) (Sánchez-Zapata et al., 2010), processing (Prieto et al., 2009) and shelf life (Pérez-Alvarez et al., 1997) of these products is to use reflectance spectra (infrared and visible). Reflectance spectra have also been used to evaluate the effect of spices (Fernández-López et al., 2002), colourants, antioxidants, ingredients and other functional ingredients (Fernández-Ginés, 2005), on meat products. The reflectance spectra generated for the central zone (Figure 2a) do not show significant differences \((P>0.05)\) between the treatments for any of the wavelengths. The reflectance spectra obtained for the peripheral zone (Figure 2b) show significant differences \((P>0.05)\) between the treatments and the control, mainly between 580-600 nm (yellow) and 650-700 nm (red). For the same analysis and excluding the control (Figure 3), it can be seen that no statistically significant differences exist \((P<0.05)\), so that it can be affirmed that treatments 2-6 differ from the control in the peripheral zone. The results of this analysis are coherent with those obtained for residual nitrite and the colour coordinates \((L^* a^* b^*)\). The spectral differences may reflect the greater development of nitrosylhemochrome in the central zones of treatments 2 and 6 than in the peripheral zone, perhaps due to deterioration of the culture on the ham surface as a result of exposure to high temperatures during the heating step.

**Conclusion**

In conclusion, the production process used led to the appearance of two colour zones in the MHC produced with all the treatments containing CE, especially for the coordinates \(L^* a^*\) and \(b^*\), as corroborated by the reflectance spectra. New studies are needed to determine the impact of the processing method on the role of the starter culture, to throw light on the way in which nitrosopigments are obtained on the whole ham surface and to ascertain the most suitable way of obtaining a uniform colour (closer to that obtained with the control here) when CE is used.

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**References**


