Technical paper

Evaluation of Strawberry Pigments as Pork Sausage Colorant

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The objective of this investigation is to evaluate the possibility of strawberry pigments used in the manufacture of pork sausage. Five treatments of pork sausages (5% fat) with two levels of sodium nitrite (0 and 0.015%), three levels (0.1%, 0.2% and 0.3%) of strawberry pigments were produced. The addition of strawberry pigments resulted in the significant increase of $a^*$ values and $b^*$ values, but the significant reduction of $L^*$ values, VBN values and TBA values ($p < 0.05$). The 0.1% or 0.2% strawberry pigments added samples had better color and sensory qualities (color, flavor, mouthfeel and slice traits) than the controls, equal even better color and sensory qualities than the NaNO₂. Based mainly on the results of overall acceptability during 29 days storage, it could be concluded that strawberry pigments showed a potential for pork sausage manufacture.

Keywords: strawberry pigments, nitrites alternative, pork sausage, quality characteristics

Introduction

The color is one of the important quality characteristics of meat and meat product. Based on it, consumers make a decision of acceptance or rejection (Mancini and Hunt 2005). Nitrite is currently used in the manufacture of cured meat product (Krause et al., 2011) because it plays a multi-functional role: it can produce the characteristic pink color of cured meats (McClure et al., 2011); contribute the characteristic flavor of cured meats (Tsoukalas et al., 2011), and inhibit the growth of microorganism, especially, the growth of Clostridium botulinum, which produces the potential fatal toxin, botulin (Cui et al., 2010). However, nitrite faces a huge challenge used in manufacture meat product because it is reported to form carcinogenic substances such as N-nitrosopyrrolidine and N-nitrosodimethylamine (Honikel 2008). In view of safety and health, natural colorants have become increasingly popular with consumers (Osterlie and Lerfall 2005) and show a potential as nitrite alternative for pork sausage manufacture.

Strawberry (Fragaria × ananassa Duchesne), which has been widely planted in the world, is one of the most commonly consumed berries. It is an important dietary source of fiber and bioactive compounds, both micronutrients and phytochemicals (Tulipani et al., 2008). Strawberries are rich in the content of pigments. These pigments are a group of anthocyanin compounds such as pelargonidin-3-glucoside (Lopes da Silva et al., 1992), and possess the antioxidant activity and bioactivity (Tulipani et al., 2008). Also, strawberry pigments are red and water-soluble (Gil et al., 1997), thus showing a potential in meat product manufacture. However, strawberry pigments face a challenge in product of food because they can be decomposed, which is dependent on temperature, pH, light and other factors (Markaris et al., 1957). To our well known, there has yet been no information published on strawberry pigments used in the produce of meat product. In this paper, our interests are focused on the effects of strawberry pigments on color, volatile basic nitrogen (VBN), thiobarbituric acid (TBA), pH and sensory qualities of pork sausage.

Materials and Methods

Chemicals and materials Ethylene diamine tetraacetic acid and ethanol were purchase from Shantou Xilong Chemical Co., Ltd. China. Boric acid, trichloroacetic acid, magnesium oxide, methyl red, methylene blue, TBA and hydrochloric acid were purchase from Shanghai Sinopharm Chemical Reagent Co. Ltd, China. Sodium tripolyphosphate (56 – 60%, analytical reagent) and sodium hexametaphosphate (≥ 98%, analytical reagent) were purchased from Tianjin
Sausage preparation  The proper amount of frozen pork legs was thawed for 24 h at 2 – 4°C. Lean tissue was trimmed of skin, bone, visible fat and connective tissue. The trimmed lean tissue and fresh pork backfat were mixed with a ratio of 19 to 1, and then ground with a chopper (SYP-MM12, Guangdong of China). To this meat mixture, 2.5% sodium chloride, 0.15% monosodium glutamate, 0.02% ginger powder, 0.02% pepper powder, 5% water, 0.015% compound phosphate (the ratio of sodium tripolyphosphate, sodium pyrophosphate and sodium hexametaphosphate is 2 : 2 : 1) and 0.015% sodium nitrite were added. The mixture was mixed thoroughly for another 5 min. The meat mixture was blended with 90 mL of distilled water for 1 min with a shearer (FA25, Fluko, Germany). pH of the filtrate was measured using a shearer (FA25, Fluko, Germany). pH of the filtrate was measured immediately after 21,000 × g and then placed at 25°C for 30 min. The homogenate was filtered through a filter paper for analysis.

Color measurement  The color of samples was measured using the Hunter scale with an automatic colorimeter WB-2000 IXA (Beijing Kangguang Instrument Co., Ltd., China). The sausage samples were cut to 3.0 cm lengths, and then immediately measured. Six measurements for each of three replicates were expressed as \( L^* \) value, \( a^* \) value, \( b^* \) value, and \( h^* \) value. The overall lightness or darkness was determined by \( L^* \) value (0 = black, 100 = white). Red (positive = \( a^+ \)) and green (negative = \( a^- \)) intensity are represented on the \( a^* \) scale, and yellow (positive = \( b^+ \)) and blue (negative = \( b^- \)) intensity on the \( b^* \) scale. Hue angle (\( h^* \)) is calculated from the following formulas: \( h^* = \tan^{-1}(b^*/a^*) \).

TBA measurement  Thiobarbituric acid reactive substances (TBARS) were performed according to the procedure described by John et al. (2005) with some modifications (in triplicate). Specifically, 5 g meat samples was taken from the sausage samples, and then mixed with 25 mL solution containing 0.375% thiobarbituric acid, 15% trichloro-acetic acid, and 0.25 mol/L HCl. The mixture was heated for 10 min in a boiling water bath (100 ± 1°C) to develop a pink color, cooled in tap water, and then centrifuged (Model CT14RD, Shanghai Techcomp Ltd., China) at 2,010 × g for 25 min. The absorbance of the supernatant was measured spectrophotometrically (model 722E, Spectrum of Shanghai, China) at 532 nm against a blank that contained all the reagents minus the meat. The malonaldehyde (MDA) concentration was calculated using an extinction coefficient of 1.56 × 10^3 M^-1 cm^-1 for the pink TBA-MDA pigment (Sinnhuber and Yu 1958).

The MDA concentration was converted to TBA number (mg MDA/kg meat sample) as follows:

\[
\text{TBA No. (ppm)} = \text{sample A532} \cdot 2.77.
\]

VBN measurement  VBN content was analyzed according to the procedure described in National Standard of China (GB/T5009.44-2003) with some modifications (in triplicate). To 10 g meat sample, 100 mL distilled water was added. The sample was homogenized for 2 – 5 min at 21,000 × g, and then placed at 25°C for 30 min. The homogenate was filtered through a filter paper for analysis.

5 mL filtrate was put into a distillation flask. To the filtrate, 5 mL magnesium oxide suspension (10 g/L) was added. The mixture was distilled using the micro-Kjeldahl distillation apparatus (Auto K9840 Analyzer, Kjeltec, Beijing Changheng Rongchuang Technology Co., Ltd., China). The distillate was collected for 3 min into 10 mL boric acid (20 g/L) containing five-six drops of mixed indicator (2 g/L methyl red indicator alcohol solution and 1 g/L methylene blue indicator aqueous solution). The solution was titrated by 0.01 mol/L HCl. The VBN in the sample in terms of milligrams of VBN per 100 g sausages can be given according to the following formula:

\[
X = 28000(V_1 - V_2)c/m
\]

Here,

\( X \): VBN content of the sample (mg/100 g),
\( V_1 \): the consumption volume of HCl standard solution for titration of the sample (mL),
\( V_2 \): the consumption volume of HCl standard solution for titration of the blank (mL),
\( c \): the actual concentration of HCl standard solution (mol/L)
\( m \): the mass of the sample (g).

pH measurement  pH measurement was performed according to the procedure described by Deng et al. (2002) with some modifications (in triplicate). Meat samples (10 g) were blended with 90 mL of distilled water for 1 min with a shearer (FA25, Fluko, Germany). pH of the filtrate was mea-
measured with a pH meter (model PHS-3C, Shanghai San-Xin Instrumentation Inc., China) equipped with a combination pH electrode calibrated to pH from 0 to 14.

Sensory evaluation A sensory panel consisted of 9 food science and engineering-major graduate student, of Hefei University of Technology. The criteria for the panel were: 4 males and 5 females, age between 18 and 22, normal or superior visual and taste acuity, which have a good grasp of the knowledge of food sensory evaluation and are willing to evaluate the color, flavor, mouthfeel and slice traits of the strawberry pigments-treated pork sausages and also those throughout refrigerated storage. Specifically, these samples included the samples with 0, 0.01%, 0.02%, 0.03% strawberry pigments and 0.015% sodium nitrite respectively and also those stored at 4°C for 1, 8, 15, 22 and 29 days. Before conducting the experiment, the panelists were trained for the development of a sensory memory with respect to each descriptive term throughout refrigerated storage. The method described by Wang and Zhao (2008) was used to prepare the list of descriptive terms, the pork sausage samples being presented in pairs for the panelists to list the differences and similarities. By consensus amongst the panel, the following scales were used: color (10 − 8 = red and bright, 7 − 5 = grey or slight red but still acceptable, 4 − 1 = excessive red and dark, not acceptable); flavor (10 − 8 = flavor, 7 − 5 = moderately off-odor but still acceptable, 4 − 1 = strongly off-odor and not acceptable); mouthfeel (10 − 8 = good, 7 − 5 = moderate, 4 − 1 = poor) and slice traits (10 − 8 = solid and smooth; 7 − 5 = slightly softening along peripheral and slight fissures, 4 − 1 = strongly softening). Also, the overall acceptabilities of pork sausage samples were determined according to color (0.4), flavor (0.2), mouthfeel (0.3) and slice traits (0.1). The pork sausage samples were stored under refrigeration (4 ± 1°C) until the moment of evaluation to the panelists. The testing procedure for the effect of strawberry pigments amount on sensory characteristics of pork sausages was performed in the room where is used for sensory evaluation. Three samples for each treatment were coded with random numbers. The pork sausage samples in polyethylene casing were submitted to each panelist, and then opened and evaluated individually. All the sensory evaluations were carried out at room temperature under natural light.

Statistics analysis The data, expressed as mean ± standard deviation (SD), were analyzed by Excel 2003 (Microsoft official Excel 2003 for Windows). Analysis of variance (ANOVA) was introduced to determine the significance of samples at p < 0.05 level. When a significant effect was obtained, Duncan’s multiple range test was used to compare sample means.

Results and Discussion

Color Figure 1 illustrated the changes in color of pork sausage during storage. As shown in Fig. 1, the addition of strawberry pigments resulted in the significant reductions of $L^*$ values (p < 0.05). The samples that had more strawberry pigments added tended to have lower $L^*$ values (p < 0.05). The changes in $a^*$ values had a contrary tendency, that is, the samples that had more strawberry pigments added tended to have higher $a^*$ values (p < 0.05). The strawberry pigments samples had lower $L^*$ values, but higher $a^*$ values than the controls. Tomato paste was also reported to have similar positive effect on the red color of frankfurters (Deda et al., 2007). As mentioned above, the main chemical components of strawberry pigments are a group of anthocyanin compounds such as pelargonidin-3-glucoside, which usually exhibit red or violet (Gil et al., 1997). Xu et al. (2000) found that these chemical components in strawberry pigments were relatively stable either in acidic media, or in the presence of sodium ion. Therefore, the added strawberry pigments were stable during process, and thus leading to the increase of $a^*$ values of sausage samples. Differed from strawberry pigments, NaNO2 can set free nitric oxide, and then the resulting nitric oxide reacts with myoglobin, thereby affecting the color of pork sausages. The sample that had 0.1% strawberry pigments added had higher $b^*$ values than the control. Moreover, the samples that had more strawberry pigments added tended to have higher $b^*$ values. The addition of NaNO2, or strawberry pigments decreased the $h^*$ values. The effects of strawberry pigments on $h^*$ were related to its level, that is, the sample that had more strawberry pigments added tended to have lower $h^*$ values. Increase in $h^*$ value shows the decrease in the redness of the samples, and/or an increase in the yellowness ($b^*$) of the samples.

Basically, $L^*$ value, $a^*$ value and $b^*$ value decreased during storage, but the differences were not significant (p < 0.05). The results indicated that the strawberry pigments added samples had the lower (p < 0.05) values for lightness, and higher for yellowness and redness in comparison to the controls. Therefore, the addition of strawberry pigments had a positive effect on the red color of pork sausages.

TBA Table 1 showed the changes in TBA of pork sausage during storage. As expected, TBA values of all the samples increased with storage time (p < 0.05). TBA values are indicators of lipid oxidation. The results revealed that lipid oxidation had occurred during storage. It was also observed that the samples that had strawberry pigments added had lower TBA values than the control (p < 0.05), but higher TBA values than the NaNO2 added samples (p < 0.05). The effects of the strawberry pigments level on TBA values were not significant at 1 day storage (p > 0.05), but signifi-
cant when storage time was prolonged (p < 0.05). The more strawberry pigments the lower TBA values at 8 or 15 day storage. The results indicated that the addition of strawberry pigments could prevent lipid against oxidation. Strawberry pigments are a group of anthocyanin compounds (Lopes da Silva et al., 2007), which possess the antioxidant activity (Tulipani et al., 2008), and thus preventing lipid against oxidation during storage.

**VBN and pH** Table 2 illustrated the changes in VBN of pork sausage samples. As expected, it was observed that VBN values of all the sausage samples significantly (p < 0.05) increased when storage time increased. VBN is an index of meat product freshness. The results showed that decomposition of protein by microorganisms occurred during storage. It was found that the strawberry pigments added samples had lower VBN values than the controls (p < 0.05), but higher VBN values than the NaNO2 added samples (p < 0.05). As for the samples that had different levels of strawberry pigments added, the differences of VBN values were not significant at the beginning of storage (p > 0.05), but significant at the final stage of storage (p < 0.05). Kotzekidou et al. (2008) reported that the pigments extract from strawberry could inhibit the growth of the foodborne pathogens such as E. coli O157:H7, S. typhimurium and S. aureus at the lowest concentration (5 mL/100 mL), indicating that the extract from strawberry had an antimicrobial activity. Therefore, it was possible that the added strawberry pigments could also inhibit the growth of microorganism in meat product, and thus protecting protein from decomposition during storage.

Table 3 summarized the changes in pH values of pork sausage samples. The addition of strawberry or NaNO2 did not affect significantly pH values (p > 0.05). The significant reductions were observed in the pH values during 29 days refrigerated storage in all cases (p > 0.05). The results were in agreement with those of several processed meat products (Glass and Doyle 1989), but conflict with those of restructured buffalo meat rolls (Anandh et al., 2011) and those of Chinese sausages with yam powder addition (Tan et al., 2007). The different results reflect that the factors affecting pH are intricate.

**Sensory characteristics** Sensory characteristics of the samples were also evaluated. As illustrated in Fig. 2, the addition of strawberry pigments had significant influences on sensory color, flavor, and overall acceptability (p < 0.05). The addition of 0.1% strawberry pigments increased significantly sensory color, flavor and overall acceptability scores (p < 0.05). Increasing further strawberry pigments decreased significantly sensory color, flavor and overall acceptability scores (p < 0.05) because the 0.3% strawberry pigments samples were excessive red and off-odor. The addition of
strawberry pigments had no significant influences on mouthfeel and slice traits (p > 0.05). The mouthfeel scores and slice traits scores were approximately 7.0 and 8.0, respectively. The samples with 0.1% or 0.2% strawberry pigments addition had better quality characteristics than the controls, equal even better quality characteristics than the NaNO2 added samples. Therefore, the addition of strawberry pigments had the positive influence on quality characteristics of pork sausage. In addition, slice traits had no significant changes (p > 0.05), but sensory color, flavor, mouthfeel and overall acceptability decreased significantly (p < 0.05) during storage. The 0.1% or 0.2% strawberry pigments added samples had still acceptable quality characteristics at 29 days storage.

In conclusion, the pork sausages that had 0.1% or 0.2% strawberry pigments added have more acceptable color than the control. Such samples have acceptable quality characteristics during 29 days storage. Based mainly on the results of overall acceptability, it can be concluded that strawberry pigments can be used in the production of pork sausages.

Acknowledgments The authors thank National Natural Science Foundation of China and Anhui Science & Technology

Table 1. The changes in TBA of pork sausage during storage (n = 3)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Storage days</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
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<tr>
<td></td>
<td>6.35 ± 0.005</td>
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<tr>
<td>C1</td>
<td>6.35 ± 0.005</td>
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<tr>
<td>C2</td>
<td>6.35 ± 0.005</td>
</tr>
<tr>
<td>C3</td>
<td>6.35 ± 0.005</td>
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<tr>
<td>C</td>
<td>6.35 ± 0.005</td>
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<tr>
<td>NaNO2</td>
<td>6.35 ± 0.005</td>
</tr>
</tbody>
</table>

▲ TBA: mg malonaldehyde/kg sample. ▼ C1, C2 and C3: the samples with 0.1%, 0.2% and 0.3% strawberry pigments addition, respectively; C: the controls, and NaNO2: the samples with 0.015% NaNO2 addition. ● Means ± SD within the column having unlike letters (a − d) means that the five treatments are significantly different (p < 0.05) at the same storage time. ■ Means ± SD within the row having unlike letters (r − v) means that the same treatment are significantly different (p < 0.05) at the different storage time.

Table 2. The changes in VBN of pork sausage during storage (n = 3)

<table>
<thead>
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<tr>
<td>C1</td>
<td>6.35 ± 0.005</td>
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<tr>
<td>C2</td>
<td>6.35 ± 0.005</td>
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<tr>
<td>C3</td>
<td>6.35 ± 0.005</td>
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<tr>
<td>C</td>
<td>6.35 ± 0.005</td>
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<tr>
<td>NaNO2</td>
<td>6.35 ± 0.005</td>
</tr>
</tbody>
</table>

▲ VBN: mg/100 g of sample. ▼ C1, C2 and C3: the samples with 0.1%, 0.2% and 0.3% strawberry pigments addition, respectively; C: the controls, and NaNO2: the samples with 0.015% NaNO2 addition. ● Means ± SD within the column having unlike letters (a − d) means that the five treatments are significantly different (p < 0.05) at the same storage time. ■ Means ± SD within the row having unlike letters (r − v) means that the same treatment are significantly different (p < 0.05) at the different storage time.

Table 3. The changes in pH of pork sausage during storage (n = 3)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Storage days</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
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<tr>
<td></td>
<td>6.35 ± 0.005</td>
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<tr>
<td>C1</td>
<td>6.35 ± 0.005</td>
</tr>
<tr>
<td>C2</td>
<td>6.35 ± 0.005</td>
</tr>
<tr>
<td>C3</td>
<td>6.35 ± 0.005</td>
</tr>
<tr>
<td>C</td>
<td>6.35 ± 0.005</td>
</tr>
<tr>
<td>NaNO2</td>
<td>6.35 ± 0.005</td>
</tr>
</tbody>
</table>

▲ C1, C2 and C3: the samples with 0.1%, 0.2% and 0.3% strawberry pigments addition, respectively; C: the controls, and NaNO2: the samples with 0.015% NaNO2 addition. ■ Means ± SD within the row having unlike letters (r − v) means that the same treatment are significantly different (p < 0.05) at the different storage time.


Fig. 2. The changes in sensory qualities of pork sausages during storage

(a): The changes in sensory qualities of pork sausages during 1st day; (b): The changes in sensory qualities of pork sausages during 29th day. ▼ C1, C2 and C3: the samples with 0.1%, 0.2% and 0.3% strawberry pigments addition, respectively; C: the controls, NaNO₂: the samples with 0.015% NaNO₂ addition. ● Means ± SD within Fig 2 (a) or within Fig 2 (b) having unlike letters (a − b) means that the five treatments are significantly different (p < 0.05) at the same storage time. ■ Means ± SD between Fig 2 (a) and Fig 2 (b) having unlike letters (r − s) means that the same treatment are significantly different (p < 0.05) at the different storage time.

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**References**


