Effects of combined starter cultures on quality of fermented sausage during ripening

Huang Lu 1, Huan Yanjun 2

1. School of Food Science and Technology, Jiangnan University, Wuxi, 214122, Jiangsu, China
2. School of Food Science and Technology, Jiangnan University, Wuxi, 214122, Jiangsu, China
E-mail:huangluvipvip@163.com

Abstract: Effect of inoculation with Pediococcus pentosaceus, Staphylococcus carnosus plus Saccharomyces cerevisiae as starter cultures on quality parameters including pH and water activity (Aw), as well as color, texture properties and sensory in fermented sausage were evaluated. Results showed that the pH in samples inoculated with starter cultures and fermented spontaneously was 5.45 and 5.74, respectively. The moisture content in fermented sausage inoculated with starter cultures and fermented spontaneously were 23.4% and 20.6%, respectively. The water activity (Aw) values in fermented sausage inoculated with starter cultures and fermented spontaneously were 0.818 and 0.826, respectively. The protein degradation index (PI) values in fermented sausage inoculated with starter cultures and fermented spontaneously were 14.39% and 9.12%, respectively. The peroxide value (POV) and thiobarbituric acid value (TBAV) in fermented sausage inoculated with starter cultures was reduced by 36.7% and 36.7%, respectively. The levels of L* and a*/b* in sample inoculated with starter cultures were 41.61 and 0.54, respectively, and the L* and a*/b* of the fermented spontaneously were 43.34 and 0.51. The colors were significantly influenced (p < 0.05) by the starter cultures inoculation. The hardness and cohesiveness in sample inoculated with starter cultures were decreased by 15.4% and 12.1%, respectively. And the elasticity, tenderness and chewiness of the sausage were improved. The color, organization appearance, texture and flavor of sensory scores in sample inoculated with starter cultures were higher than that of the control, sample inoculated also exhibited good sensory properties.

Keywords: combined starter cultures; fermented sausage; quality characteristics; ripening stage

1. Introduction

Fermented sausage, also known as raw sausage, which put the ground meat, animal fat, salt, starter cultures and spices blend into the natural casings, under the condition of natural or artificial control, through the microorganism fermentation to produce acid or alcohol, to lower the pH value of meat, and after mature drying (or without mature dry), lowered the Aw of sausage to make a good preservation and the characteristics of the typical flavor of fermented meat products performance [1]. Because of fermented sausages’ beautiful color, unique flavor, stable quality, long shelf life, the advantages of easy to digest absorb nutrition and health care, consumers loved and favored it [2]. In the United States, France, Italy and other countries, it has been recognized as the high-end traditional fermented food [3]. In the process of fermentation, lactic acid bacteria used carbohydrates in the raw material to produce lactic acid and other organic acids to make the pH drop to below 5.3 of sausage finally, and the study of Simion found that the effect of lactic acid bacteria can also drop the Aw accelerately, combined with the low pH, which can inhibit the growth of pathogenic and spoilage bacteria, thus, improving the quality of the sausage and extend the shelf life of products [4]. In the mature process of sausage, microbial starter cultures of exogenous enzymes can promote decomposition of carbohydrates, protein and fat, its product gave the fermented meat products different characteristics, such as unique flavor, color and taste, which is conducive to the maturity of fermented meat products and the formation of fine quality [5]. Moderate oxidation will increase the flavor of the product, but excessive oxidation can lead to corruption. Microbial starter cultures in the process of growth metabolism will produce some antioxidant enzymes, including catalase, superoxide dismutase [6]. Catalase can remove hydrogen peroxide and superoxide dismutase can eliminate free radicals to inhibit lipid oxidation in the process of oxidation [7].

This work will inoculate the mixed three starter cultures into fermented sausage to optimize the technological conditions and get the optimum technology of fermented sausage. Under the optimum technological parameters, analysed the physicochemical properties, microbiological counts and sensory of starter cultures group and the
control group during the ripening of fermented sausage, and compared the differences in quality, which provided
the theoretical foundation for industrialized production.

2. Material and methods

2.1. Sausages manufacturing and samples

Two different batches of pork sausage were manufactured according to traditional techniques, one of them
without starter culture and the other one batch with addition of three starter cultures (The inoculation amount of
P.p, the total inoculation of St.c and Sa.c was also 8 lg cfu/g, the proportion of them was 1:1). Sausage
manufacture was done two different times. The two batches mentioned before were manufactured with the same
ingredients, formulation and technology.

Pork sausage formulation includes lean pork meat (80%) , pork back fat (20%) , NaCl (25 g/kg) , lactose (20
g/kg) , beaten pepper (1 g/kg) , pepper powder (1 g/kg) , ginger powder (1 g/kg) , aginomoto (1 g/kg) , five spice
powder (1 g/kg) , star aniseed powder (1 g/kg). The lean pork meat and the pork back fat were ground through a
10 mm diameter mincing plate and vacuum mixed together with the other ingredients for 3 min. The mix was
maintained at 4 °C for 24 h and then stuffed into natural casings with a diameter of 60 mm and a length of 40 cm.
The sausages were fermented for 2 days at 25 °C and 85% of relative humidity and then transferred into a drying
ripening chamber where they were kept for 26 days at 12 °C and 75% relative humidity. Samples after 0, 2, 7, 14,
21 and 28 days of processing were taken for subsequent analysis.

2.2. Chemical analysis

The pH of sausages was measured using a digital pH meter (model FE20K) equipped with a penetration
probe. Moisture percentage was determined by oven drying (Memmert UFP 600, Schwabach, Germany) at
105 °C until constant weight (ISO, 1997), and calculated as sample (5 g) weight loss. Water activity (Aw) was
determined using a Fast-Lab (Novasina, Switzerland) water activity meter, previously calibrated with sodium
chloride. A portable colorimeter (Konica Minolta CR-400 Osaka, Japan) was used to measure meat color in the
CIELAB space: (lightness, L*; redness, a*; yellowness, b*).

Sausage pieces of 1 × 1 × 2 cm (height×width×length) were compressed at a crosshead speed of 1.5 mm/s
in a texture analyser (TA-XT plus, Stable Micro Systems, Vienna Court, UK). Textural parameters were
measured by compressing to 40% using a compression probe with 25 cm2 of surface contact. Between the first
and second compressions, the probe waited for 5 s. Hardness (kg/cm2), cohesiveness, springiness (kg/cm2) and
chewiness (kg) were obtained using the computer software (Texture Exponent 32, Stable Micro Systems, Vienna
Court, UK).

Lipid oxidation was expressed as t peroxide value (POV) and hiobarbituric acid value (TBAV) (mg
malondialde-hyde kg-1), as determined by Salih [8], using a Unicam UV2600 spectrophotometer.

Non-protein nitrogen (NPN) was determined by Kjeldahl's method (ISO 937, 1978), using a No 323
digestion–distillation unit (Buchi Labortechnik AG Flawil, Switzerland) an automatic Titirino 702 SM equippd
with a No. 6.0233.100 combined pH electrode (Methrom Schweiz, Zofingen, Switzerland). The non-protein
nitrogen was determined after precipitating the proteins with trichloroacetic acid. The proteolysis index was
calculated as g NPN per 100 g TN.

Mince the sausages when they finished fermentation and ripening, then accurately weighted out 1 g sample
into the electronic nose bottle of 40 mL immediately, then blocked and placed it with 1 h at room temperature.
The pure and dry air was used to carrier gas, the gas flow was 1 L/min, and the test time was 300 s.

2.3. Sensory evaluation

A paired comparison test of the color, appearance, texture, aroma and taste of both batches of sausages was
performed at the end of the process. A panel of 10 well-trained tasters assessed the samples. The study consisted
of four sessions (two sessions for each batch). In each session two slices (2 mm thick) were presented to the
tasters at 10-minute intervals. The tasters were asked which sample had a greater intensity of the characteristic
being studied.

2.4. Statistical analyses

All statistical analysis was performed using IBM SPSS Statistics 22 software (IBM, Chicago, IL, USA). After
verification of normal distribution and constant variance of data, significant differences were determined using
one-way analysis of variance (ANOVA). A Duncan's test was performed to compare the mean values for
processing time at a significance level of P<0.05. Correlations between variables were determined by correlation
analyses using the Pearson's linear correlation coefficient.

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3. Results and discussion

3.1. Effect of combined starter cultures on pH, moisture and water activity during the ripening

As was shown in the figure 1, the pH of starter cultures group was lower than the control group in the entire process of fermentation mature, this was because the starter cultures group inoculated microorganism whose metabolism to produce lactic acid to lower the pH, which was same to Essid who studied the variation tendency of pH in the process of fermentation mature of the mutton sausage [9]; In late mature, the pH of each group rose again, the reason was that carbohydrates was consumed in the late mature fermented sausage, nitrogen compounds was used by lactic acid bacteria as energy, and protein was degraded by protease which caused the concentration of alkaline substances increased [10].

Fig 1 Changes of pH value in different fermented sausages during ripening

In figure 2, the moisture of the starter cultures group and the control group was a downward trend. It fell faster in the earlier stage, then it was not smooth until it dropped slowly in the late. the moisture decreased rapidly during 0 to 14 d, because this time of paragraph was mainly free water evaporation, drying rate was relatively quickly; During 14 to 28 d, with the content of free water fewer and fewer, the bound water and immobilized water began to evaporate, the content of bound water and liquid water reduced, at the same time it combined with protein macromolecule organic matter closely to make it evaporated uneasily, then the evaporation rate slowed down [11]. The moisture of starter cultures group was slightly higher than the control group, the lower moisture could affect the taste and tenderness of sausage, so the quality of starter cultures group of the sausage was good.

Fig 2 Changes of moisture in different fermented sausages during ripening

By the figure 3, the Aw of starter cultures group and control group all declined, but its rate of starter cultures group fell faster than the control group, which showed that the Aw dropped as the pH value decreased, both were positively correlated. The drying process of fermented sausage was the moisture lost and the Aw reduced gradually, this was because microbes decomposed the sugar into acids to make the pH value decrease, the degradation of macromolecular reduced the bound water, and it was also a process of producing flavor substances during drying [12]. In general, the Aw of starter cultures group was lower slightly than the control group. High moisture content could ensure the taste of sausage, and at the same time with the low Aw could
extend the shelf life of fermented sausage [13]. This is because the microbes in the starter cultures group closely combine with moisture in the food to make the liquidity of waters in the system inland low and to get a lower Aw.

![Graph showing changes of water activity in different fermented sausages during ripening](image)

**Fig 3** Changes of water activity in different fermented sausages during ripening

### 3.2. Effect of combined starter cultures on instrumental color during the ripening

The evolution of lightness, redness and yellowness during the fermentation and ripening of fermented sausages is shown in figure 4 and 5. It showed that L* value is on the decline from the beginning of fermentation to the end of ripening, and on the first day of the ripening, it reduced obviously, this was because the humidity in the mature room significantly lower than the fermentation chamber during the ripening process, which led to a decrease of the moisture content of fermented sausage, so that the brightness value of the sausage decreased gradually [14]. When evaluating the color of sausage, if just considered the size of a* values was difficult to accurately reflect the color of the sausage, because b* values would cause great influence to redness, a*/b* was usually adopted to compare the color of sausage. The bigger a*/b* value was, the more bright-colored redness of the sausage. a*/b* value was biggest in 7 d of the starter cultures group, whereas, a*/b* value was biggest in 14 d of the control group. And the starter cultures group on the whole is greater than the control group in the whole process, which showed that starter culture benefited for the color.

![Graph showing changes of L* in different fermented sausages during ripening](image)

**Fig 4** Changes of L* in different fermented sausages during ripening
3.3. Effect of combined starter cultures on texture during the ripening

The results of the texture profile analysis (TPA) throughout ripening are presented in Table 1 and 2. The analysis results showed that hardness, cohesiveness, glueyness and chewiness of the starter cultures group was less than the control group, but the elasticity was greater than the control group. These results are in agreement with those reported by Casquete [15] who observed that the combination of the starter culture decreased the hardness of the salchichon samples, probably due to their effect on protein hydrolysis [16]. Generally, the major changes in fermented sausage structure took place during fermentation when the pH declined and the myofibrillar proteins aggregated to form a gel. After fermentation, drying is a major factor affecting binding and rheological properties [17]. Studies have shown that Staphylococcus carnosus could produce protease which promoted degradation of the muscle of plasma protein and myofibril protein, then destroyed the internal integrity of meat, reduced the meat's internal binding force, made the meat organization more loosely, so the hardness decreased. When the strength of internal bond decreased, the cohesiveness reduced. The organization of starter cultures group was more closely, the flexible was higher; Glueyness is the product of hardness and cohesiveness, moreover, hardness and chewiness were extremely significant positive correlation, therefore glueyness and chewiness with starters of the sausage were less than the control group.

<table>
<thead>
<tr>
<th>Day</th>
<th>Hardness (g)</th>
<th>Elasticity (mm)</th>
<th>Cohesiveness</th>
<th>Glueyness (g)</th>
<th>Chewiness (g×mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2092±55a</td>
<td>0.907±0.006d</td>
<td>0.757±0.006e</td>
<td>1511±29a</td>
<td>1366±46a</td>
</tr>
<tr>
<td>2</td>
<td>3497±87b</td>
<td>0.877±0.015c</td>
<td>0.743±0.015e</td>
<td>2696±242ab</td>
<td>2342±131b</td>
</tr>
<tr>
<td>7</td>
<td>5633±122c</td>
<td>0.857±0.029bc</td>
<td>0.623±0.006d</td>
<td>3958±115bc</td>
<td>3877±83c</td>
</tr>
<tr>
<td>14</td>
<td>6331±71c</td>
<td>0.843±0.012ab</td>
<td>0.583±0.006c</td>
<td>4611±292c</td>
<td>4487±234d</td>
</tr>
<tr>
<td>21</td>
<td>11359±955d</td>
<td>0.823±0.006a</td>
<td>0.520±0.010b</td>
<td>6256±676d</td>
<td>5658±105e</td>
</tr>
<tr>
<td>28</td>
<td>17704±1180e</td>
<td>0.817±0.006a</td>
<td>0.457±0.015a</td>
<td>8386±1720e</td>
<td>8345±498f</td>
</tr>
</tbody>
</table>

Table 2: Texture correlation indexes of control group

<table>
<thead>
<tr>
<th>Day</th>
<th>Hardness (g)</th>
<th>Elasticity (mm)</th>
<th>Cohesiveness</th>
<th>Glueyness (g)</th>
<th>Chewiness (g×mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2092±55a</td>
<td>0.907±0.006d</td>
<td>0.757±0.006d</td>
<td>1511±29a</td>
<td>1366±46a</td>
</tr>
<tr>
<td>2</td>
<td>3711±155a</td>
<td>0.853±0.025c</td>
<td>0.717±0.012d</td>
<td>2611±129b</td>
<td>2426±79a</td>
</tr>
<tr>
<td>7</td>
<td>6794±605b</td>
<td>0.763±0.015b</td>
<td>0.657±0.025c</td>
<td>4380±346c</td>
<td>5120±800b</td>
</tr>
<tr>
<td>14</td>
<td>11875±1598c</td>
<td>0.743±0.012b</td>
<td>0.603±0.038b</td>
<td>8609±273d</td>
<td>7577±1118c</td>
</tr>
<tr>
<td>21</td>
<td>15450±923d</td>
<td>0.713±0.006a</td>
<td>0.560±0.026a</td>
<td>9148±467e</td>
<td>7977±694c</td>
</tr>
</tbody>
</table>
3.4. Effect of combined starter cultures on lipid oxidation during the ripening

The POV had showed a rising trend of in the control group, and it reached the highest content in 28 d (Fig. 6), which suggested that the rate of hydrogen peroxide formation was far greater than its degradation rate. However, the POV of starter cultures group reached the highest in 14 d, then it had been in a downward trend, this may be because hydrogen peroxide decomposed into small molecular substances in large quantities at this stage [18].

![Fig 6 Changes of POV in different fermented sausages during ripening](image)

3.5. Effect of combined starter cultures on protein degradation during the ripening

The NPN in all samples gradually increased during the whole processing (Fig 8). This was because the cathepsin effected on the degradation of proteins throughout the whole process of fermented sausage. The degradation of the free amino acids and peptides were constantly accumulated to promote NPN to rise continuously [20]. During the period of fermentation, the NPN of starter cultures group was rising faster than the control group, this might due to starter cultures inoculated which produced protein-degrading enzyme under the appropriate condition. High temperature prompted the activity of cathepsin and oligopeptidase enzyme to make the protein hydrolyze into free amino acids and peptides [21]. In the ripening period, two groups of NPN was a slowly rising trend. On one hand, it might due to the lower temperature which inhibited the activity of cathepsin and microbial; On the other hand, the moisture fell constantly and high concentrations of salt inhibited cathepsin activity, so the degradation of protein slowed.

![Fig 7 Changes of TBAV in different fermented sausages during ripening](image)
As a result, the two groups of TN was decreasing, while the NPN was increasing in the whole process of sausage, the PI of two groups in the process showed an increasing tendency by the formula of \( PI = \frac{NPN}{TN} \), what was more, the starter cultures group was higher than the control group (Fig. 9).

### 3.6. Effect of combined starter cultures on electronic nose during the ripening

The DI value was bigger, the better the distinction was, the farther the distance between the samples and the DI value was greater than 80 percent which was better to distinguish the effect [22]. As was shown in Figure 10 and 11, the DI value of the starter cultures group was 93.4% percent which was more than 80 percent, while the DI value of the control group was 64.2 percent which was less than 80 percent, which meant that the starter cultures could promote the formation of different flavor substances in different stages of sausage.
S1, S4, S5, S8 and S11 was bigger relatively of the starter cultures group (Fig. 12), while S1, S2, S4, S5, S8 and S11 was oppositely bigger of the control group (Fig. 13), in which S1 was on behalf of aromatic compounds, S2 was for nitrogen oxides produced by rotten meat, S4 was for alcohol, ketone, acid, etc produced by microbial fermentation, S5 was represent for pyrazine aroma components with maillard reaction, S8 was gas for environmental detection, S11 was for volatile organic compounds, such as alkanes, aromatic hydrocarbons, aldehydes, etc. The value of S2 in the starter cultures group was smaller significantly than the control group. It suggested that adding starters could delay corruption and prolong the shelf life, even ensure the quality and safety of product.
3.7. Effect of combined starter cultures on sensory evaluation during the ripening

Such as table 3 showed that after adding combined starter cultures of fermented sausages had a high score (80.5 ± 1.6), which was no significant difference (P>0.05) with adding D-sodium erythorbate on sale (80.7 ± 2.5), which illustrated the antioxidant effect of inoculating starter cultures was in agreement with adding antioxidants, that was to say the products of starter cultures group had a good appearance, color, texture and unique flavor of sausage.

<table>
<thead>
<tr>
<th>evaluation items</th>
<th>color ± SE</th>
<th>appearance ± SE</th>
<th>texture ± SE</th>
<th>flavour ± SE</th>
<th>total score ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>starter cultures group</td>
<td>23.5±0.6ab</td>
<td>14.0±0.3b</td>
<td>14.5±0.4a</td>
<td>28.5±0.2b</td>
<td>80.5±1.6a</td>
</tr>
<tr>
<td>add D-sodium erythorbate on sale</td>
<td>23.8±0.3b</td>
<td>13.2±0.5a</td>
<td>14.7±0.8a</td>
<td>29.0±0.9b</td>
<td>80.7±2.5a</td>
</tr>
<tr>
<td>add D-sodium erythorbate on lab</td>
<td>22.5±0.8ab</td>
<td>13.0±0.7a</td>
<td>13.8±0.3a</td>
<td>27.2±0.4b</td>
<td>76.5±2.2a</td>
</tr>
<tr>
<td>control group</td>
<td>21.2±0.2a</td>
<td>11.0±0.1a</td>
<td>13.0±0.2a</td>
<td>20.5±0.3a</td>
<td>65.7±0.8b</td>
</tr>
</tbody>
</table>

[a, b] Different superscript letters within a column indicate significant differences (p<0.05)

4. Conclusions

This study clearly revealed that addition of combined starter cultures could make much difference in the chemical composition of final products. The starter cultures group was found to be the most effective strain in rapid decrease of pH and Aw in final product than the control group. However, the values of moisture, NPN and PI were of starter cultures group higher than the control group to get a variety of flavors. It also showed a significant antioxidant effect on POV and TBAV and texture profile improvement in products. In contrast, the samples inoculated with combined starter cultures significantly improved (p<0.05) the light and red color of sausages. The sensory analyses results revealed that the product inoculated with combined starter cultures was found to be superior in quality attributes. Therefore, the combined starter cultures could be used to produce a high quality dry fermented sausage.

5. References


