A Study on the Application of Natural Antioxidant Lignan in Boiled and Smoked and Boiled Sausages

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Abstract: With fresh carp meat and black fungus as the main raw materials, black fungus and pork sausages with fish flavor and rich nutrition were made through reasonable processing based on the optimal process formula; the color, fat oxidation, odor, and rancidity of the product during storage were investigated after adding different amounts of natural antioxidant lignan; and the comparison with the blank control and analysis were carried out. The results show that the addition of 0.075% lignan had the best antioxidant effect. The TBA value and acid value of lignan-added sausages were significantly lower than those without lignan.

1. Introduction

Every kind of food has a shelf life. It is necessary to add a food additive-antioxidant in order to minimize the occurrence of food deterioration and extend its shelf life. The antioxidant can slow and prevent the oxidation of food [1]. Every kind of food has a shelf life. It is necessary to add a food additive-antioxidant in order to minimize the occurrence of food deterioration and extend its shelf life. The antioxidant can slow and prevent the oxidation of food [1]. Food antioxidants are divided into two categories. One is natural antioxidants and the other is synthetic antioxidants. At present, synthetic antioxidants are widely used because they have many advantages as food additives. However, they are also of toxicity, and will pose a serious health threat to consumers if used beyond a certain standard. Therefore, it is imperative to develop natural food antioxidant additives that are non-toxic and strong anti-oxidant, have no side effects, and can prevent corruption and keeping fresh.

Flaxseed meal contains a physiologically-active substance-lignan. As one of the natural antioxidants, lignan has high antioxidation, health care and bacteriostatic effects; is safe to use; and can scavenge free radicals and inhibit the formation of nitrites, playing an anti-mutation and anti-cancer role. Therefore, it is widely used in meat products.

Boiled and smoked sausages are a kind of meat product, made with meat as the main raw material. The meat after marinated, minced and chopped, is stuffed into casings, and then cooked and smoked to obtain finished sausages [2]. Meat products are rich in fat and Pr, and have a high water activity, so they are prone to be oxidized during storage and transportation, which will result in rancid taste and

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discoloration, making the food lose edible and commercial values. Therefore, developing a solution to improve the shelf life of meat products has become one of the challenges in the current meat industry. Fortunately, the application of a natural food additive-lignan in meat products can prevent and alleviate their deterioration.

2. Materials and Methods

2.1 Test Materials

Pork, fish, fungus, salt, white pepper powder, ginger powder, meat tenderizer, allspice powder, white sugar, monosodium glutamate, chicken essence, soy sauce, Daqu wine and starch: purchased from Hualian Living Supermarket in Jilin City.

Nutmeg powder, carrageenan, sodium erythorbate, lignan (from the early-stage research results), CH₂-COOH, CHCl₃, KI, Na₂S₂O₅, 5H₂O, Na₂SO₄, petroleum ether, soluble starch and K₂Cr₂O₇: all provided by the brewing laboratory of the School of Food Engineering. Casings: purchased from an aquatic product market in Jilin City.

2.2 Test Equipment

ES-B electronic balance: Shanghai Shenghui Electronics Co., Ltd.; CH-201 meat grinder: Everbest Electric Co., Ltd.; 80-type chopper mixer: Hongruimei Industrial Equipment Co., Ltd.; NM-T-10L sausage stuffer: Guangzhou Zhengying Machinery Equipment Co., Ltd.; DYX-500 smoking oven: Yibang Food Machinery Co., Ltd.; TW-300B vacuum packaging machine: Shenyang Xinghuili Packaging Machinery Co., Ltd.; SHSL thermostat electric heating jacket: Shanghai Zengsen Instrument Technology Co., Ltd.; Color difference meter: Shanghai Shanion Creative Electronic Ltd.; 101-A electric blast drying oven: Tianjin Taisite Instrument Co., Ltd.; RE-3000 rotary evaporator: Shanghai Yarong Biochemical Instrument Factory;

2.3 Test Methods

Process Flow

Fungus:
- Soaking → Cleaning → Cutting → Blanching → Cooling → Prepared for use
- Raw fish: Selecting → Removing fish head and viscera → Cleaning → Meat separating → Rinising → Marinating → Cooling → Mincing
- Raw pork: Selecting and processing → Cutting it into blocks → Marinating → Chopping

Lignan:
- Mixing → Stuffing → Poaching → Smoking → Cooling → Packaging → Inspecting → Finished product

Basic recipe: carp meat 25%, black fungus 4%, starch 10%, pork with fat-to-lean ratio of 3:7, salt 2%, nitrite 0.015%, sodium erythorbate 0.1%, white pepper powder 0.2%, Nutmeg powder 0.5%, allspice powder 0.4%, ginger powder 0.15%, carrageenan 0.6%, meat tenderizer 0.5%, soy sauce 6%, sugar 1.8%, monosodium glutamate 0.6% and Daqu wine 0.2% -0.3%

Amount of lignan added: The lignan was added into the sausage products by proportions of 0.01%, 0.025%, 0.05% and 0.075%, respectively. The test group was compared with the control group and the color, fat oxidation, odor and rancidification in the storage period were evaluated with the physical and chemical, as well as sensory evaluation methods. The observation and measurement were conducted once a month to determine the appropriate amount.

Control test: The sausages without lignan were compared with those with lignan.

Determination of Peroxide Value (TBA)

(1) Reagent preparation
- (a) CHCl₃-CH₃COOH mixed solution (volume ratio 40 + 60), (b) Saturated KI solution, (c) 1% starch indicator, (d) 0.02mol/L Na₂S₂O₅, (e) 0.1mol/L NaOH, (f) Petroleum ether treatment: 100ml petroleum ether was subject to rotary evaporation in a water bath below 40℃. The distilling flask was washed by using 30ml CHCl₃-CH₃COOH mixed solution by several times, and then the washing liquid was put into a 250 ml iodine flask. 1.0ml saturated KI solution was accurately added, the flask plug was tightened, the flask was shaken slightly for 0.5min, and placed in the dark for 3min. After the
1.0ml of starch indicator was added, the mixture was mixed well, and no blue appeared. Thus, the petroleum ether would be used for sample preparation.

(2) Sample Preparation

Three portions of sausage were taken by weighing with an electronic balance, and each portion weighed 10g (accurate to 0.001g). The sausage was chopped and placed in a 250ml conical flask. Then, 30ml petroleum ether (treated in advance) was added and the conical flask was shaken well. After the content of the flask was fully mixed, the flask was kept still for more than 12 hours. Next, the sausage was filtered out with a funnel, the filtrate was taken and put in water bath with the temperature below 40°C. Then, the petroleum ether was evaporated to be dry under reduced pressure and the residue was the sample to be tested.

(3) Sample Determination

3ml sample prepared above was taken, and put into an iodine measuring flask. Then, 30ml CHCl₃-CH₃COOH mixed solution was added, and was shaken gently so that it was completely dissolved. 1.0ml saturated KI solution was accurately added, and the plug was plugged tightly, followed by gentle shaking for 0.5min. The measuring flask was placed in a dark place for 3min. Then, the measuring flask was taken out and 100ml water was added into it. Immediately after shaking well, 0.02mol/L Na₂S₂O₃ standard solution was added for titration to so that I₂ was precipitated. The titration was continued until the solution turned light yellow. Then 1ml starch indicator was added, and the titration was continued and the mixture was vigorously shaken until the blue in the solution disappeared [3]. At the same time, a blank test was performed. The volume V of 0.02mol/L Na₂S₂O₃ consumed in the blank test should not exceed 0.1ml.

(4) Calculation

When the peroxide value is denoted by the mass fraction equivalent to I₂ of the peroxide,

\[ X_1 = \frac{(V-V_0) \times C \times 0.1269}{m} \times 100 \]

Where,

- \( X_1 \) —peroxide value, in g/100g
- \( V \) —volume of Na₂S₂O₃ standard solution consumed by the sample, in ml
- \( V_0 \) —volume of Na₂S₂O₃ standard solution consumed in the blank test, in ml
- \( C \) —Na₂S₂O₃ standard solution, in mol/L
- 0.129 —mass of iodine equivalent to 1.0ml Na₂S₂O₃ standard solution \([C(\text{Na}_2\text{S}_2\text{O}_3)]=1.000\text{mol/L}\]
- \( m \) —mass of the sample, in g.

Determination of Acid Value. 15g sausage was taken, chopped into pieces, wrapped with a large piece of filter paper, placed into the extraction cylinder of a Soxhlet extractor. 150ml petroleum ether was added into a flat-bottom distillation flask. The instrument was installed, the water was connected first and then the power and the extraction with reflux was conducted for continuous 4h. The sausage bag was taken out, and the petroleum ether was collected. The remaining liquid was namely the fat sample to be tested. Then, 10g sample to be tested was taken, and put into a 250ml conical flask. Another conical flask was taken, added with 50ml absolute ethanol and 1ml phenolphthalein indicator, shake well, placed into 100°C heat bath until the ethanol was slightly boiling. The conical flask was taken out and the titration was conducted while the ethanol temperature was maintained above 70°C. The solution was poured into the conical flask containing the sample, and the reddish color disappeared. The solution was further heated to slightly boil in a 100°C water bath. In the heating process, the conical flask was shaken continuously, and the titration was conducted using 0.1mol/L KOH standard solution while the solution was still hot until the solution looked reddish, and the redness was kept not fading for 30s. The volume of consumed KOH (V) was recorded [4]. The blank test was necessary for the hot ethanol indicator method, namely:

\[ V_0 = 0 \]

\[ X(\text{mgKOH/g}) = N \times (V-V_0) \times 56.1/m \]

Where: \( X \) —acid value, in mg/g
N—molar concentration of KOH, in mol/L
V—volume of consumed KOH solution, in ml
56.1—millimolar mass of KOH
m—mass of the weighed sample, in g
Color scale: L * (bright degree), a * (red degree) and b * (yellow degree) \[5\] of the sausage slices with lignan and the sausage slices without lignan were measured with a colorimeter, respectively. The measurement was carried out 3 times, and the average was taken.

3 Experimental Results

Table 1. Changes in Sensory Indicators of Sausage during Storage

| Time      | NO.1 Control Group (without lignan)                                      | NO.2~NO.5(with lignan)               |
|-----------|------------------------------------------------------------------------|--------------------------------------|
| Day 0     | The finished product had good color, dry and smooth surface with no wrinkles, delicate and fresh taste, proper saline taste, good flavour, solid and full tissue and even distribution of fungus | Same as the description on the left column |
| 1st month | Glossy, slightly wrinkled, red-and-white color, with inherent odor of sausage | Same as the description on the left column |
| 2nd month | Flat partially yellowing, fungus block softened, with a slight rancidity | Basically normal, with dryer sausage surface, evenly-distributed color, reddish brown meat, and obvious black fungus |
| 3rd month | Flat partially yellowing, fungus block softened, with a slight rancidity | Basically normal, with reddish brown lean, and normal odor |
| 4th month |                                                                                           | Oiling on the surface, with normal odor |
| 5th month |                                                                                           | Fat droplets dissolving out, fat beginning turning yellow, and having a rancidity |

Table 2. TBA Value of Sausage during Storage

| Time      | NO.1 | NO.2 | NO.3 | NO.4 | NO.5 |
|-----------|------|------|------|------|------|
| Day 0     | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 |
| 1st month | 0.37 | 0.27 | 0.26 | 0.24 | 0.24 |
| 2nd month | 0.56 | 0.28 | 0.27 | 0.28 | 0.27 |
| 3rd month | 0.74 | 0.35 | 0.34 | 0.34 | 0.32 |
| 4th month | 0.45 | 0.39 | 0.40 | 0.39 |      |
| 5th month | 0.58 | 0.54 | 0.52 | 0.51 |      |

NO.1 is the sample control group, namely the sausage without lignan, and NO.2 ~ NO.5 are the test groups, in which the sausages were added with 0.01%, 0.025%, 0.05%, and 0.075% lignan, respectively.

Peroxide value is a measure of the rancidity of fats. Generally, the higher the peroxidation value is, the more severe the rancidity will be. This is because some small molecular substances produced by oxidative rancidity have adverse effects on the human body, such as free radicals. Therefore, if the sausage has a high the peroxide value, it will be bad for human’s health. As can be seen from Tables 1 and 2 that the added 0.01% to 0.075% of lignan could prevent and delay the fat oxidation of sausages. The control group had a slight oxidative rancidity in the second month, and by the third month, the fat oxidation was more serious with a bad odor. The peroxide value reached 0.74g / 100g. In contrast, the sausage with lignan still had normal color and odor even in the third month, and its TBA value was between 0.32 and 0.35g / 100g. Till the 5th month, fat droplets dissolved and the fat began turning...
yellow, and had a rancidity. The corresponding TBA value was between 0.51 and 0.58g / 100g. Therefore, the addition of lignan could extend the shelf life of the sausage by at least two months. Therefore, based on comprehensive test results, it is better to add 0.075% lignan in sausages.

Table 3. Changes in Acid Value of Sausages during Storage

|       | Jan     | Feb.    | Mar.    | Apr.    | May     |
|-------|---------|---------|---------|---------|---------|
|       | Control group | Test group | Control group | Test group | Control group | Test group | Control group | Test group |
| m     | 9.92    | 9.96    | 9.97    | 9.82    | 9.46    | 9.95    | 9.52    | 9.85    | 9.68    | 9.78    |
| v     | 2.17    | 2.02    | 3.02    | 2.22    | 4.97    | 3.17    | 5.53    | 4.42    | 6.42    | 5.44    |
| Acid  | 1.23    | 1.14    | 1.68    | 1.27    | 2.95    | 1.79    | 3.26    | 2.52    | 3.72    | 3.12    |

It can be seen from Table 3 that the smaller the acid value of the sausage during storage, the better the quality of the fat, the lower the degree of oxidative rancidity, and the better the freshness. The acid value of the control group is slightly higher than the acid value of the experimental group, which shows that lignan has an antioxidant effect in sausages, and the acid value ranges between 1-4 mg / g. In general, the acid value rises slightly, which will not cause damage to the human body. If the acid value is too high, it will cause gastrointestinal discomfort, diarrhea and damage the liver.

3.1 Study on Color Protection Effect of Sausages during Storage Period

Fig.1 Influence of Storage Time on Bright Degree (L*) Value of Sausages

It can be seen from Figure 1 that the L* value of the test group was lower than that of the control group at the beginning, which might be related to the lignin added in the test group. With the increase of the storage time, the control group had the maximum bright degree value in the third month, and in the following several months, the test group has the maximum bright degree. Subsequently, it value and the L* value decreases in the following months. The experimental group has the highest lightness value in the third month, and then the plateau stabilizes in the following months, indicating that the larger the L* value, the better the gloss of the sausage.
Fig. 2 Influence of Storage Time on Sausage Red Degree (a*) Value

It can be seen from Figure 2 that in the control group, the red degree value was similar to that in the early stage of the test group. With the increase of storage time, the redness degree value significantly decreased, while in the test group, the redness degree value continued to increase, and it started to decrease only after the 4th month, indicating the added lignin in the test group has a synergistic effect of slowing down and stabilizing a*, and has a significant effect on the color protection of the product. Besides, when the value is positive, the larger the value is, the redder the color will be. In the case of long preservation, the value is negative. The larger the absolute value is, the greener the color will be.

Fig. 3 Influence of Storage Time on Sausage Yellow degree (b*) Value

It can be seen from Figure 3 that in the control group the yellow degree value decreased with time, indicating increase of the oxidation degree, while in the test group, the yellow degree value continued to increase. When the yellow degree value is positive, the larger the value is, indicating the more yellow the color will be; when it is negative, the larger the absolute value is, and the bluer the color will be. Therefore, the lignan added in the test group has antioxygenation.

4 Conclusion

In this experiment, natural antioxidant lignan was applied to meat products. The addition of 0.075% lignan in the developed fish black fungus pork sausage, can stabilize the product's color during storage, prevent oxidative rancidity, and extend the shelf life.

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