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Effects of High-pressure Processing Technique on the Quality and Shelf Life of Chinese Style Sausages

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Abstract Fresh Chinese-style sausage is a traditional product in Taiwan. Because it is made from fresh meat, the initial total plate count of packed products can be lowered using non-thermal preservation methods. The high pressure processing (HPP) technique (100-1000 MPa) can be applied to sterilize packed products to reduce microbial contamination. This study applied the HPP technique to fresh Chinese-style sausages and evaluated their physicochemical, microbial and sensory characteristics. The results revealed that when the sausages are processed at a pressure greater than 400 MPa, the *a* value of color increases. Regarding the texture properties, the hardness, cohesiveness, gumminess and chewiness decreases as the pressure rises. Moreover, an increased pressure effectively reduces the initial total plate count. The sensory analysis showed that after HPP, the sausages exhibit significantly high acceptability in texture and overall characteristics. In addition, in a storage treatments conducted at 7 °C, a control group reached 6 log CFU/g in total plate count and swelling was observed after 80 days of treatment. however, sausages that received HPP (at 600 MPa for 10 min) exhibited a significantly lower total plate count of 3 log CFU / g after 90 days of treatment. These results indicate that HPP considerably reduces the initial plate count of the sausages as well as suppresses the growth of the total plate count during the storage period. Therefore, HPP can extend the shelf life of fresh Chinese-style sausages by at least 30%.

Keywords: *meat products, non-thermal preservation process, refrigeration storage, microbial status*

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1. Introduction

High pressure processing (HPP) technique uses water as a pressure-transmitting medium, deactivating the microorganisms and enzymes in food at a high pressure (100-1000 MPa) at room or low temperature [1,2]. HPP deactivates microorganisms by reducing the stability of cell membranes and the comprehensiveness of cell physiological functions [3], such as by inducing phospholipid crystallization on cell membranes to increase the permeability of cell membranes [1], causing protein denaturation to deactivate enzymes and suppress gene expressions [3,4,5,6]. Unlike other conductive processing techniques, HPP can achieve an even effect regardless of the size and shape of products. Compared with conventional thermal processing techniques, HPP at a relatively low pressure exerts a milder effect on the nutrients in and quality of food. However, to reach a higher level of sterilization, higher pressure is required [7]. Higher pressure and longer pressure duration can affect food quality. After being processed under pressure higher than 400 MPa, foods can achieve more satisfactory microbial status. However, such foods may be rejected by consumers because of changes in their appearance, color, and texture. Therefore, the influence of pressure on quality requires investigation [3,6].

Chinese-style sausage is one of the most popular processed pork products in Taiwan. It has a high product value and is considered as a traditional and characteristic Taiwanese food product. The sausage is made by grinding pork, adding minced pork, and filling the mixture into sausage casing. Most of these sausages are vacuum-packed and stored in freezers, while some are vacuum-packed and refrigerated. Sausages are made from fresh meat and thus have a relatively high initial plate count [8]. Moreover, manufacturing procedures such as filling, drying, cooling, and packaging are often accompanied by secondary microbial contamination [9]. These conditions can aggravate the microbial status of sausages when stored in refrigerators. Therefore, the "fresh" refrigerated sausages have a relatively short shelf life.

Several studies in recent years have indicated that administering a pressure of 400-600 MPa to meat products for several minutes at room temperature can deactivate most microorganisms contained within them, thereby improving the safety and shelf life of meat products in the preservation process [3,4,5,6,10,11,12] These studies have verified that by suppressing the growth of (or sterilizing) microorganisms after meat products are packed, HPP can effectively reduce secondary contamination that otherwise occurs during meat product processing. However, the effects of HPP on the sensory qualities of meat products, such as their color, texture, appearance, and flavor, require further consideration. Therefore, this study was carried out to investigate the influences of different HPP conditions (i.e. pressure: 0.1-600 MPa; time: 0-10 min) on physicochemical, microbial and sensory characteristics of fresh Chinese-style sausages. The effect of such technique to enhance the shelf life and provide an alternative nonthermal preservation method for meat products will be in the scope of this investigation.

2. Materials and Methods

2.1. Preparation of Sausages

The sausages were manufactured according to a traditional formula for Chinese-style sausage: 85% Fresh pork ham (Cha I Shan Foods Co., Ltd, Taiwan) and 15% pork back fat (Cha I Shan Foods Co., Ltd, Taiwan) were mixed with 1.5% salt (99.5%, Taiyen Biotech Co., Ltd, Taiwan), 9.0% sugar (99.5%, Taiwan Sugar Co., Taiwan), 0.5% spices (Tomax Enterprise Co., Ltd, Taiwan), 0.2% antioxidant (98%, Gemfont Co., Taiwan) and 0.1% nitrite (4%, Gemfont Co., Taiwan). The tissues were ground with a meat chopper fitted with a plate of 20 mm diameter holes. The ground meat was packaged in plastic bags, 1.0 kg each, and stored at -18°C. Before use, the meats were maintained 20 h / 0°C. The batch was manufactured with 0.2% Potassium sorbate (99.5%, Gemfont Co., Taiwan) and was considered as positive control. The batches were curing at 5°C for 3 days then stuffed into hog casings (Ten Geniuses Ent. Co., Ltd, Taiwan) previously humidified with water. Raw sausages were manually linked 10 cm in length and dried in a drying cabinet at 50°C for 1 h. Following drying, sausages were cooled and vacuum-packaged in plastic bags (NY / LLDPE laminated film, Summit Industrial Co., Ltd, Taiwan) and stored in a refrigerator (7°C). All batches were manufactured in duplicate.

2.2. High Pressure Treatment

Pressure treatments were performed using a 2.0 L capacity high pressure laboratory food processing system QFP 2L-700 (Avure Technologies Inc., Sweden) with temperature control between 10°C and 90°C. The compression fluid used in the sample chamber consisted of 100% water. All samples were individually sealed in vacuum packs and maintained at 7°C until pressure treatment. Samples were pressure treated at 0.1, 200, 400, 600 MPa and using a holding time of 10, 5, 3 min. Following release of pressure all samples were stored at 7°C until required. Positive control (0.2% potassium sorbate, $^{PS+}$) and negative control (without potassium sorbate, $^{PS-}$) samples were no pressure treatment (0.1 MPa).

2.3. Physicochemical Properties of Sausages

Hunter-*L*, *a*, *b* color values of the sausages were measured by color difference meter (Tokyo Denshoku Co., Model TC-1800 MK II, Japan). The mean of ten measurements was taken for each Hunter-*L*, *a*, *b* values.

The pH value of the sausages was measured after homogenization (Interscience Co., Model BagMixer® 400P, France) with distilled water at a ration of 1:10 using a pH meter (Denver Instrument, USA).

The texture profile analyses (TPA) indices of sausages were determined using a texture analyzer (Model TA-XT2 Texture Analysis, England). The conditions of texture analyzer were modified by [16]. Pre-test speed: 3.0 mm / s; test speed: 1.0 mm/s; post-test speed: 3.0 mm / s; distance: 10.0 mm; time: 5.0 s; trigger type: auto; and trigger force, 10 g.

2.4. Microbial Status of Sausages

The total plate count of sausages were determined using 3 M Petrifilm™ (3M Co., USA) Aerobic Count Plate (AOAC OMA: 990.12) [14]. Plates were incubated at 37°C for 48 hour. Results were expressed as $\log_{10}$ CFU / g sausages.

Coliform and *E. coli* of sausages were determined using 3 M Peterifilm™ (3M Co., USA)*E. coli* and Coliform Count Plate (AOAC OMA: 991.14) [15]. Plate were incubated at 37°C for 24 hour. Results were expressed as $\log_{10}$ CFU / g sausages.

2.5. Sensory Analysis

The sensory analysis of sausages was evaluated by 30 untrained assessors selected according to their habits. Samples were labeled with 3-digit random numbers and served in random order to assessor in individual booths. Assessors were instructed to cleanse their palates with water between samples. A hedonic test was carried out using 9 point scales (9 = like extremely and 1 = dislike extremely) in which the assessors evaluated different attributes: appearance, taste, texture, flavor, overall acceptability [16].

2.6. Statistical Analysis

Data were analyzed using SPSS 12.0 for one-way ANOVA. Duncan's new multiple range test was used to resolve the difference among treatment means. A value of $p < 0.05$ was used to indicate significant difference.

3. Results and Discussion

3.1. Color of Sausages

The color of fresh Chinese-style sausages after HPP was analyzed using a colorimeter. The results are shown in Table 1. After HPP treatment, the *L* value of the sausages increased; however, the value decreased as the pressure escalated. The *L* value of sausages obtained after 10 min of processing under 200 MPa and 5 min of processing under 400 MPa was 42.22 and 41.24, respectively; both values were significantly higher than that (39.58) of the control group (0.1 MPa). In similar study, Campus et al. (2008) found that the *L* value of dry-cured loin increased after HPP and their results were consistent with those of the present study. Also, many studies have identified similar results [17,18,19]. HPP induces depolymerization of the myofibrillar proteins, changing the ratio of light reflection and absorption, further contributing to increased brightness [2].

After the sausages received HPP treatment of > 400 MPa, the *a* value increased; the value further rose as the pressure increased. The *a* value of sausages treated for 3 min under 600 MPa rose from 3.74 to 4.40. Bak et al. [20]

specified that HPP significantly enhanced the *a* value of pork longissimus, and that the value increased along with the enhancement of pressure. The result was in line with that of the present test. Jung et al. [21] found that after HPP, the *a* value of beef increased first and decreased later as the pressure increased. The value peaked at 350 MPa, and began to decrease at 500 MPa. Nevertheless, the *a* value obtained at relatively high pressures remained higher than that measured at a relatively low pressure (100 MPa). The changes in the level of redness induced by HPP were mainly attributed to the denaturation of globin, the displacement or release of heme, or the changes of metmyoglobin forms [20,22]. The sausages investigated in the present study lacked oxygen because they were vacuum-packed. Consequently, the myoglobin contained in these sausages mainly existed in the form of hypoxia myoglobin. The control group had a relatively low *a* value. However, after HPP, hypoxia myoglobin was transformed into bis-His coordinated ferrous myoglobin, leading to the enhancement of the *a* value [20,23]. In addition, HPP did not exhibit a significant influence on the *b* value of the sausages, which ranged between 8.12 and 8.77.

Table 1. Effect of HPP Treatment on Color of Chinese-Style Sausage (Mean ± SD, n = 8)

Pressure (MPa)	Time (min)	*L*	*a*	*b*
0.1^{PS+}	-	39.6 ± 0.9^{c}	3.7 ± 0.2^{c}	8.4 ± 0.4^{b}
200^{PS-}	10	42.2 ± 1.1^{a}	3.8 ± 0.1^{c}	8.1 ± 0.4^{b}
400^{PS-}	5	41.2 ± 1.0^{ab}	4.0 ± 0.2^{b}	8.8 ± 0.3^{a}
600^{PS-}	3	40.2 ± 1.2^{bc}	4.4 ± 0.2^{a}	8.1 ± 0.3^{b}

Means with different superscript letters within the same column are significantly different at $p < 0.05$.
$^{PS+}$: 0.2% Potassium sorbate, $^{PS-}$: without Potassium sorbate.

3.2. pH Value of Sausages

The pH value of the sausages after HPP was between 6.70 and 6.74 (Table 2), significantly lower than that measured in the control group (6.90). Variance in the pH value was limited. Souza et al. [24] found that after HPP, the pH value of pork decreased slightly by 0.3-0.5 units, possibly because of the calcium ions released under high pressure.

Table 2. Effect of HPP Treatment on pH Value of Chinese-Style Sausage (Mean ± SD, n = 3)

Pressure (MPa)	Time (min)	pH value
0.1^{PS+}	-	6.90 ± 0.02^{a}
200^{PS-}	10	6.71 ± 0.01^{b}
400^{PS-}	5	6.74 ± 0.00^{b}
600^{PS-}	3	6.70 ± 0.02^{b}

Means with different superscript letters within the same column are significantly different at $p < 0.05$.
$^{PS+}$: 0.2% Potassium sorbate, $^{PS-}$: without Potassium sorbate.

3.3. Texture Profile Analyses

The results of the texture profile analyses of the sausages after HPP are shown in Table 3. The hardness, cohesiveness, gumminess, and chewiness of the sausages decreased as the HPP pressure increased. The effect became obvious when the pressure reached 400 MPa. When sausages received HPP for 3 min at 600 MPa, their hardness decreased (from 3600 to 2428 g), as did their cohesiveness (0.620 to 0.567), gumminess (2156 to 1344 g), and chewiness (1973 to 1179). Sikes et al. [25] determined that the hardness of beef cakes decreased when they received HPP at 400 MPa. Alba et al. [26] processed sliced dried pickled ham at pressures of 400–600 MPa and found that the shear force of ham decreased as the processing pressure and storage time increased. Ma and Ledward [27] indicated that when beef muscle received HPP at 200-600 MPa and was subsequently heated to an internal temperature of 70 °C, the hardness was lower than that observed in the control group, which was heated directly without receiving HPP. Canto et al. [28] reached a similar conclusion in a study on fresh alligator tail meat. Mechanisms reducing the hardness of meat during HPP might be high pressure inducing the meat tissue to disintegrate, or the lysosomal rupturing and releasing proteases, further generating proteolysis in mucles [29].

Table 3. Effect of HPP Treatment on Texture Properties (TPA) of Chinese-Style Sausage (Mean ± SD, n = 3)

Pressure (MPa)	Time (min)	Hardness (g)	Springiness	Cohesiveness	Gumminess (g)	Chewiness (g)
0.1^{PS+}	-	3600 ± 490^{a}	0.865 ± 0.015^{a}	0.620 ± 0.022^{a}	2156 ± 350^{a}	1973 ± 311^{a}
200^{PS-}	10	3234 ± 389^{a}	0.867 ± 0.007^{a}	0.572 ± 0.026^{a}	1820 ± 285^{b}	1809 ± 287^{a}
400^{PS-}	5	2805 ± 324^{b}	0.873 ± 0.012^{a}	0.553 ± 0.052^{b}	1540 ± 245^{bc}	1466 ± 258^{b}
600^{PS-}	3	2428 ± 386^{b}	0.871 ± 0.020^{a}	0.567 ± 0.018^{b}	1344 ± 284^{c}	1179 ± 249^{c}

Means with different superscript letters within the same column are significantly different at $p < 0.05$.
$^{PS+}$: 0.2% Potassium sorbate, $^{PS-}$: without Potassium sorbate.

3.4. Microbial Status of Sausages

Figure 1 presents the initial microbial status, showing that HPP effectively lowers the initial plate count of fresh Chinese-style sausages. Moreover, the suppressing effect significantly increases as pressure rises. Prior to HPP (0.1 MPa), the total initial plate count of the sausages in the positive control group (0.2% potassium sorbate) and the negative control group (without Potassium sorbate) was 3.62 and 3.91 log colony-forming units per milliliter (CFU)/g, respectively, in which the plate count of coliform was 1.54 and 1.39 log CFU / g, respectively. The total initial plate count decreased significantly after 10 min of HPP at 200 MPa, but the plate count of coliform did not decrease. When the pressure was increased to 400 MPa, 5 min of HPP reduced the total initial plate count to 3.15 log CFU / g, and coliform was not detected. When the pressure was increased to 600 MPa, 3 min of HPP reduced the total initial plate count to 2.22 log CFU / g, and coliform was not detected. In addition, no *E .coli* was detected in any treatment group.

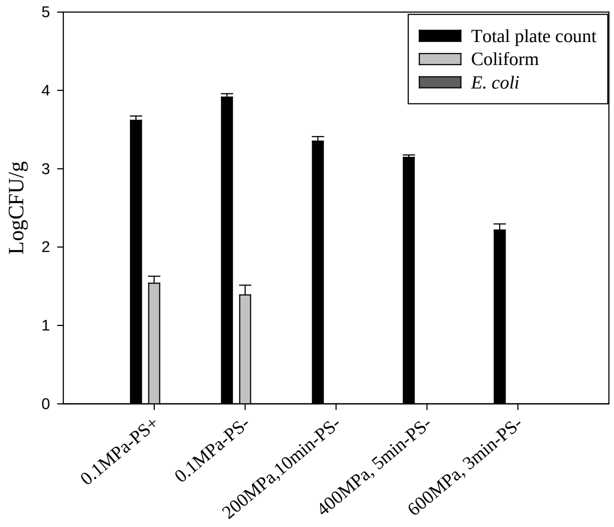

Figure 1. Effect of HPP Treatment on initial microbial status of Chinese-Style Sausage

PS+: 0.2% Potassium sorbate, PS-: without Potassium sorbate.

3.5. Sensory Analysis

This study subsequently adopted sensory analysis to evaluate the influence of HPP on the sensory characteristics of sausages, such as texture and flavor. The results are presented in Table 4 and show that HPP exerted no significant influence on the sausages in terms of taste or flavor, the sensory ratings of which were 6.8-7.1 and 6.8-7.2, respectively. By contrast, the sensory rating of appearance significantly increased. Sausages treated under 200 MPa of pressure exhibited significant differences from those in the control group. After HPP, the sensory rating of sausage texture increased. Specifically, the ratings of sausages treated under 200 MPa for 10 min and treated under 400 MPa for 5 min were 7.1 and 7.2, respectively; both scores are significantly higher than those of the control group. Regarding overall acceptability, the sensory ratings of all the treatment groups that received HPP were significantly higher than those of the control group. The ratings of all the groups were higher than 6 points. Rodríguez-Calleja et al. [30] reported that for chicken breast fillets in modified atmosphere packaging, 5 min of HPP at 300 MPa and 20 °C produced no unfavorable effect regarding the appearance (color), flavor, texture, or overall acceptability of the product. Canto et al. [28] obtained similar results in their study. The researchers processed cod tail meat at a pressure of 200-300 MPa, boiled the meat to an internal temperature of 70 °C, and performed a sensory analysis. The sensory characteristics of the treatment group showed no significant difference with those of the control group (no HPP) in items such as appearance, flavor, tenderness, juiciness, chewiness, and cohesiveness.

Table 4. Effect of HPP Treatment on Sensory evaluation of Chinese-Style Sausage (Mean ±SD, n=30)

Pressure (MPa)	Time (min)	Appearance	Taste	Flavour	Texture	Overall
0.1^{PS+}	-	6.3 ± 0.8^{b}	6.8 ± 0.9^{a}	6.8 ± 1.2^{a}	6.4 ± 1.0^{b}	6.4 ± 0.7^{b}
200^{PS-}	10	6.9 ± 0.9^{a}	7.1 ± 0.9^{a}	7.1 ± 0.9^{a}	7.1 ± 1.2^{a}	6.9 ± 0.6^{a}
400^{PS-}	5	6.8 ± 0.8^{ab}	7.2 ± 0.9^{a}	7.1 ± 0.8^{a}	7.2 ± 1.1^{a}	7.1 ± 0.6^{a}
600^{PS-}	3	6.8 ± 1.1^{ab}	7.1 ± 0.8^{a}	7.2 ± 1.1^{a}	7.0 ± 1.2^{ab}	6.9 ± 1.1^{a}

Means with different superscript letters within the same column are significantly different at $p < 0.05$.
*: 0.2% Potassium sorbate, **: without Potassium sorbate.

3.6. Variations of Microbial Status of Sausages Throughout Shelf life

Table 5 exhibits the variations of the total plate count of vacuum-packed sausages that received HPP and were stored at 7 °C. During the 90 days of storage at 7 °C, the total plate count of all the treatment groups increased. After 90 days of storage, the total plate count of the positive control group rose from 3.62 log CFU / g to 4.18 log CFU / g. The level of increase was relatively small because of the preservatives added. However, for the negative control group, after 80 days of storage, the total plate count exceeded 6 log CFU / g, which is the limit stipulated by the Certified Agricultural Standards of Taiwan, and swelling was observed on the packages. In the treatment groups, sausages first received HPP at 200 MPa for 10 min and were stored at 7 °C. In the initial period of storage (Days 10-30), the total plate count of this group was approximately the same as that in the positive and negative control groups. During Days 40-60 of storage, the total plate count of this group was lower than that of the negative control group, exhibiting effective suppression of the growth of total plate count. However, during Days 70-90 of storage, the total plate count of this group increased rapidly, exhibited an unfavorable suppression effect of total plate count. After 90 days of storage, the total plate count exceeded 6 log CFU / g, and swelling packages were identified. Sausages that received HPP at 400 MPa for 5 min exhibited approximately the same suppression effect in total plate count as that in the positive control group. After 90 days of storage, the total plate count increased from the initial 3.15 log CFU / g to 4.74 log CFU / g. For the sausages that underwent HPP at an increased pressure of 600 MPa for 3 min, the optimal suppression capacity for the total plate count was reached. After 90 days of storage at 7 °C, the total plate count remained 3 log CFU / g, significantly lower than that of the control group. Han et al. [7] verified that treating sliced vacuum-packed cooked ham with HPP reduced the initial total plate count as well as the psychrotroph, lactic acid bacteria, and staphylococci counts; moreover, the sterilization effect for micro- organisms increased with pressure. Garriga et al. [31] indicated that for vacuum-packed dry-cured ham and sliced marinated beef loin, the total plate count as well as the psychrotroph, lactic acid bacteria, yeast, and enterobacteriaceae counts were all significantly lowered after receiving HPP at 600 MPa and 16 °C for 6 min. After 90 days of storage at 4 °C, the plate counts of all treatment groups were lower than those of the control group (no HPP); the counts were all controlled to below 3 log CFU / g, with yeast and enterobacteriaceae controlled to less than 1 log CFU / g. However, the total plate count of dry-cured ham that did not receive HPP exceeded 5 log CFU / g after 60 days of storage, and that of sliced marinated beef loin exceeded 8 log CFU / g after 30 days of storage. Similar results have been obtained in other studies [21,30,32,33]. These findings indicate that HPP can effectively reduce the initial total plate count of fresh Chinese-style sausages and suppress microbial growth in the storage process, thereby improving the microbial status and shelf life of these sausages.

Table 5. Changes of HPP Treatment on Total Plate Count in Chinese-Style Sausage During Storage at 7℃ (Mean ± SD, n = 3)

Pressure	Time	Total plate count (LogCFU / g)									
(MPa)	(min)	0 day	10 days	20 days	30 days	40 days	50 days	60 days	70 days	80 days	90 days
0.1^{PS+}	-	3.6 ± 0.1^{a}	3.8 ± 0.0^{a}	4.1 ± 0.1^{a}	4.4 ± 0.1^{a}	4.2 ± 0.0^{b}	3.8 ± 0.0^{a}	3.6 ± 0.1^{c}	3.6 ± 0.1^{c}	4.0 ± 0.11^{c}	4.2 ± 0.1^{c}
0.1^{PS-}	-	3.7 ± 0.0^{a}	4.0 ± 0.2^{a}	4.0 ± 0.0^{a}	4.0 ± 0.0^{ab}	4.9 ± 0.1^{a}	4.1 ± 0.2^{a}	4.6 ± 0.2^{a}	5.1 ± 0.1^{a}	$>6.00^{a, sp}$	$>6.00^{a, sp}$
200^{PS-}	10	3.4 ± 0.1^{a}	4.2 ± 0.0^{a}	4.2 ± 0.1^{a}	4.0 ± 0.0^{b}	4.0 ± 0.2^{bc}	3.9 ± 0.1^{a}	4.2 ± 0.1^{b}	4.9 ± 0.1^{a}	$5.3 \pm 0.3^{b, sp}$	$>6.00^{a, sp}$
400^{PS-}	5	3.0 ± 0.0^{b}	2.9 ± 0.0^{b}	3.0 ± 0.1^{b}	3.0 ± 0.0^{c}	3.6 ± 0.1^{c}	3.4 ± 0.3^{b}	3.3 ± 0.2^{c}	3.8 ± 0.1^{c}	4.3 ± 0.0^{c}	4.7 ± 0.1^{b}
600^{PS-}	3	2.0 ± 0.1^{c}	2.5 ± 0.1^{b}	2.4 ± 0.1^{c}	2.5 ± 0.1^{d}	2.5 ± 0.0^{d}	2.7 ± 0.2^{c}	2.8 ± 0.0^{d}	3.0 ± 0.1^{d}	2.5 ± 0.1^{d}	3.5 ± 0.1^{d}

Means with different superscript letters within the same column are significantly different at $p < 0.05$.
$^{PS+}$: 0.2% Potassium sorbate, $^{PS-}$: without Potassium sorbate, ^{sp}: swelled packaged.

In conclusion, although HPP can cause changes in color and texture of fresh Chinese-style sausages, the process was determined to exert no unfavorable effect on the sensory characteristics of the cooked sausages.

Furthermore, HPP effectively reduced the initial total plate count and total plate count of the sausages throughout the storage period at 7 °C. The improving in hygiene quality effectively extended the shelf life of the sausages by minimizing the possible secondary contamination during meat product processing.

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