Effect of Modified Atmosphere Packing and Vacuum Packaging on the Preservation of Chinese Spiced Beef

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Abstract: As one of the most characteristic of Chinese traditional meat products, Chinese spiced beef because of its short shelf life and flavor deterioration problem were seriously affected the development of the modernization and internationalization. In order to solve those problems, this study mainly studied the fresh-keeping effect about Chinese spiced beef in the MAP of different gas components and ratio (vacuum-control, CO₂/N₂ 20:80, CO₂/N₂ 40:60, CO₂/N₂ 60:40, CO₂/N₂ 80:20, O₂/CO₂/N₂ 5:70:25, O₂/CO₂/N₂ 35:40:25) under 25°C with sensory evaluation, total bacterial count, electronic nose, TVBN, TBA, pH, color as the evaluation index and analyzed the difference of the products in the preservation of MAP and vacuum packaging. The results showed that group (O₂/CO₂/N₂ 5:70:25) and (CO₂/N₂ 80:20) could be stored 8-10 days (total bacteria are 4.70-5.08 and 4.67-5.04 log CFU g⁻¹, respectively) and compared with vacuum packaging (total bacteria 6.03-6.69 log CFU g⁻¹) extending at least 30% shelf life. TVBN values (23.42 and 21.98 mg/100 g), TBARS (0.31, 0.37 mg/100 g) and sensory score (5.5) of (O₂/CO₂/N₂ 5:70:25) and (CO₂/N₂ 80:20) were significantly better than the vacuum packaging (30.59, 0.55 mg/100 g, at the end of storage).

Key words: Chinese spiced beef, modified atmosphere packaging, preservation, vacuum packaging, electronic nose

INTRODUCTION

Chinese spiced beef is one of the most representative type of traditional meat products in China. Chinese spiced beef is defined as the product that the high quality fresh beef is mixed with the condiments and the ingredients and cooked in water. Chinese spiced beef has a long history as early as the middle of Qing Dynasty. Liu Lu, a man in Cang Zhou He Bei, invented and developed it. It is still one of China’s favorite meat products. Chinese spiced beef has been possessed in good and unique flavors and it can be changed in a variety of flavors according to the customs of the different regions in China. With the economy developing rapidly, the Chinese people’s living standards improve greatly and the consumption of Chinese spiced beef grow quickly.

The Chinese spiced beef being made up of many small enterprises and informal operations has become a heavy constraint on its modernization, industrialization development in the country at present. The small enterprises have some obvious characteristics for example low yield, short sales radius. Due to this situation, the industrialized and modernized production of the Chinese spiced beef has been quite slow to grow. It turns out that the primary limitation factors of the industrialized and modernized production of the Chinese spiced beef are as follows: first of all, it’s not clear that the craft theoretical of the Chinese spiced beef. Because the Chinese spiced beef can be changed in a variety of flavors according to the customs of the different regions in China, the process parameters have nothing in common with each other. In order to reverse this situation, all regions and departments of China will play an increasingly important role in future. Secondly, short shelf life. There exist rich protein, fat, moisture in the Chinese spiced beef and it is easy to breed microorganisms. Because of its short shelf life has a strong impact on the quality of sale. Thirdly, it is very easy that the flavor of the Chinese spiced beef products deteriorate. In order to prolong the shelf life of the Chinese sauce halogen meat products, the majority of enterprises use second sterilization and as a result of this the flavor of the products is difficult to maintain and easy to deterioration. Short shelf life and difficult to maintain flavor are the factors that they affect the modernization,
industrialization development of the Chinese spiced beef deeply which is also the key and difficult point for the industrialization development. What's more the relation of short shelf life and difficult to maintain flavor is also a see-saw effect. That is to say, it is important to find a equilibrium to harmonize the shelf life and flavor.

In this experiment, studying on the technique of the Chinese spiced beef fresh keeping by modified atmosphere packaging not only did researchers want to achieve a better flavor of the Chinese spiced beef during the storage time but researchers also extended the shelf life as much as possible. Providing theoretical support for the industrialization development of the Chinese spiced beef is the aim of the experiment.

MATERIALS AND METHODS

Sample preparation (Chinese spiced beef production process)

Technological process: Bovine hindquarters (4°C, beef mature 7 days) – Pretreatment (Excluding the fascia and fat, clean)- dry-cured (3.6% salt, 5°C, 3 days) – cook spice – marinating (100°C, 2 h) – cooling – packaging – product.

The product formula (with the raw material of 100 kg as the standard, unit: kg): 6% salt; 0.32% Chinese cinnamon; 0.23% Chinese prickly ash; 0.19% clove; 1.1% onion; 0.26% Amomum villosum; 1.3% Yellow win; 1.4% rock sugar; 0.8% garlic; 0.37% fennel; 0.15% cardamon; 1.1% ginger; 0.08% geranium; 0.4% star anise; 0.12% Angelica dahurica; 1.2% soy sauce; 0.4% Amomum tsao-ko; 0.15% red corde; 0.26% Codonopsis pilosula.

Storage conditions: The Chinese spiced beef were aseptically cut (about 120 g weight each muscle) using sterile cutting boards and knives. Each pack (a total of 148 packs) was prepared in a polystyrene tray (300 mm of thickness) sealed with PE film for gas mixtures for vacuum packaging. A factorial design was used: 7 batches (vacuum-control, CO₂/N₂ 20:80, CO₂/N₂ 40:60, CO₂/N₂ 60:40, O₂/N₂ 80:20, low O₂, and high O₂) x 3 (steaks in each sample point) x 8 sample points (0, 2, 4, 6, 8, 10, 12 and 14 days of storage) = 148 steaks were analyzed.

These packs were separated in 7 batches of 24 packs. Six batches were brought to different packaging atmospheres using a DT-6S packaging machine (Wenzhou, China). The control batch was vacuum packed in a DZ-450 vacuum package machine (Wenzhou, China). The rest of batches were packed in MAP (Modified Atmosphere Packing) but using different gas mixtures: CO₂/N₂ 20:80, CO₂/N₂ 40:60, CO₂/N₂ 60:40, CO₂/N₂ 80:20, O₂/CO₂/N₂ 5:70:25 (low O₂ MAP), O₂/CO₂/N₂ 35:40:25 (high O₂ MAP).

All packs were stored at 25 ±1°C (simulating retail conditions at supermarkets). Twenty one samples (three of each batch) were removed from the chamber at 0, 2, 4, 6, 8, 10, 12 and 14 days of storage for microbial, sensorial, electronic nose analysis and physico-chemical analysis.

Sensory evaluation: Meat samples were evaluated by ten semi-trained panel lists. The attributes which have been studied are the following: red color, off-color and general appearance acceptability using a 9 point Hedonic rating scale. The scale includes the following ranking: 1 = extremely unacceptable, 2 = very much unacceptable, 3 = moderately unacceptable, 4 = slightly unacceptable, 5 = between acceptable and unacceptable, 6 = slightly acceptable, 7 = moderately acceptable, 8 = very much acceptable and 9 = extremely acceptable (Ranganna, 1994).

Microbial analysis: Samples (25 g) taken from each bag were aseptically weighed, homogenized in 225 mL of quarter sterile saline solution for 100 sec in a stomacher (AESAP, 1068, France) at room temperature. Decimal dilutions in quarter sterile saline solution were prepared and aliquots of 0.1 mL of the appropriate dilutions were spread in triplicate on the following media: Plate Count Agar (PCA, Oxoid) incubated at 37°C for 48 h. Results were calculated as the means of Log counts for three determinations.

Flavor: The sensory array was for a period of 45 sec, subjected to the volatiles accumulated in one 100 mL conical flask. Specifically, 10 g of meat were introduced inside a 100 mL volume glass jar and left at room temperature (45°C) for 5 min to enhance desorption of volatile and semi-volatile compounds from the meat into the gas phase (Papadopoulou et al., 2011). Between each sample, the sensor signals were stabilized to the background level in the presence of air for 3 min. Three samples of meat from each package were analyzed on each occasion of sampling. Each sample was analyzed three times by the electronic nose (S/N Per3, 33089, Germany).

TVBN: Total Volatile Basic Nitrogen (TVBN) evaluation of the beef samples was carried out using standard protocols (GB/T, 2003), the TVBN contents were calculated as mg per 100 g of beef samples.

Lipid oxidation: The lipid stability was evaluated using the method proposed by Vyncke (1975). Briefly, a meat sample (10 g) was dispersed in 7.5% trichloroacetic acid (50 mL) and homogenized in an oscillator (HY-5, China) for 30 min. The oscillator was maintained at
room temperature for 30 min and then centrifuged at 1600 rpm for 5 min. The supernatant was filtered through a Whatman No. 1 filter paper. The filtrate (5 mL) was reacted with a 0.02 M TBA (2-Thiobarbituric Acid) solution (5 mL) and incubated in a water bath at 95°C for 50 min then keeping in dark place and cooling for 1 h. The absorbances were measured at 532 and 600 nm, separately.

**pH and color parameters:** Muscle pH was determined using an pH meter fitted with a combined glass electrode in Lab 427 (PXSJ-216, China), previously calibrated at pH 4.0 and 7.0. For each sample, measurements were taken at three locations.

A portable colorimeter (Minolta CR-400 Osaka, Japan) with the following settings was used to measure the meat color in the CIELAB space (Lightness L*, redness a*, yellowness b*) (CIE, 1978). For each sample, measurement of L*, a* and b* values were taken at three locations which included two adjacent locations to the central measurement.

**Statistical analyses:** In order to study the effect of the storage time and packaging system on the meat composition, data were processed by Analysis of Variance (ANOVA). Principal Components Analysis (PCA) was also applied to the data. All statistical procedures were computed using SPSS 13.0.

**RESULTS AND DISCUSSION**

**Sensory evaluation analysis:** Sensory analysis of all the-packagings products is given in the Table 1. Overall score were decreased gradually with the extension of storage time. Organoleptic evaluation of the products revealed a significant differences in the various attributes among control (vacuum-packaging) and hyperoxia group (O2/CO2/N2 35-40:25) having a slight smell at 5 days storage. However, the panelists were not able to detect any difference among the Chinese spiced beef in packagings of MAP O2/CO2/N2 5:70:25 (low O2, MAP) and MAP CO2/N2 80:20. The changes of each index were relatively stable, what's more they still existed relatively good color, smell, taste and appearance after 14 days

| Table 1: Changes on sensory evaluation difference of Chinese spiced beef packaged in different environment during storage |
|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Attributes  | Packaging    | 0           | 2           | 4           | 6           | 8           | 10          | 12          | 14          |                  |
| Color       | Vacuum (control) | 7.5±1.05*  | 6.5±1.05*   | 5.5±1.05*   | 5±1.05*     | 4.83±0.75*  | 3.83±0.41*  | 3.54±0.55*  | 3.54±1.05*  |                  |
|             |              | 4           | 6           | 8           | 10          | 12          | 14          |                  |
| Appearance  | Vacuum (control) | 6.1±1.41*  | 6.0±0.89*   | 5.67±0.82*  | 5.50±0.55*  | 5.00±0.65*  | 4.83±0.41*  | 3.83±0.75*  | 3.83±1.03*  |                  |
| Odor        | Vacuum (control) | 7.1±1.75*  | 6.3±1.82*   | 5.67±0.82*  | 5.50±0.55*  | 5.00±0.65*  | 4.83±0.41*  | 3.83±0.75*  | 3.83±1.03*  |                  |

V = Vacuum (control). * = O2/CO2/N2, 5:70:25 (low O2, MAP). ** = O2/CO2/N2, 35-40:25 (high O2, MAP). *Means within a row with different letters are significantly different (p<0.05); **Means within a column with different letters are significantly different (p<0.05). Significance: NS = Not Significant, *p<0.05; **p<0.01; ***p<0.001.
storage. Therefore, the packagings of MAP O₂/CO₂/N₂ 5:70:25 (low O₂ MAP) and MAP CO₂/N₂ 80:20 have good effects for maintaining flavor of Chinese spiced beef while the control group (vacuum packaging) and high oxygen group (O₂/CO₂/N₂ 35:40:25) only in the short-term (25°C within a week) could maintain good traits of Chinese spiced beef.

**Total plate count analysis:** During circulation, sales and consumption process, the total number of colonies of the Chinese spiced beef should be <800000 cfu g⁻¹ (GB/T 4789.17). Changes in microbial counts are shown in Table 2. In general, microbial counts showed differences (p<0.05) during storage and among packaging conditions at 25°C. Initial Total Viable Counts (TVC) (day 0) were not detected. After 6 days, the high O₂ MAP (O₂/CO₂/N₂ 35:40:25) and the control group vacuum packaging bacteria exceed the standard. However, the packagings of MAP O₂/CO₂/N₂ 5:70:25 (low O₂ MAP) and MAP CO₂/N₂ 80:20 can be stored 8-10 days at 25°C. Thus, modified atmosphere packaging of high carbon dioxide group microorganism change are relatively steady. While the control group, vacuum packaging and low carbon dioxide gas group of microbial changes significantly. Probably it is because that the inclusion of 70-80% carbon dioxide (CO₂) prolongs the shelf life of the Chinese spiced beef significantly by inhibiting bacterial growth (McMillin, 2008).

**Flavor analysis:** According to the measured data of electronic nose by principal component analysis of SPSS, the total contribution rate of the first and second principal components of different packaging of the Chinese spiced beef reached >90%. So, researchers analyzed the first and second principal components in the following. Figure 1 is the degree of variation by electronic nose on different packaging methods, different storage time of the Chinese spiced beef. The number 0 represents the fresh state of Chinese spiced beef. The flavor of O₂/CO₂/N₂ 5:70:25 (low O₂ MAP) and MAP CO₂/N₂ 80:20 were most close to the fresh state of Chinese spiced beef and had a minimum degree of variation with the extension of storage (Fig. 1). Furthermore, combined with sensory evaluation, the beef smelted fragrant at the end of storage. However, the high O₂ MAP (O₂/CO₂/N₂ 35:40:25) and the control group vacuum packaging had significant changes and existed off-flavor. It was find that O₂/CO₂/N₂ 5:70:25 (low O₂ MAP) and MAP CO₂/N₂ 80:20 have the best fresh-keeping effect in the whole process of storage under the condition of normal temperature.

**TVBN analysis:** TVBN is important characteristics for the assessment of quality in Chinese spiced beef products (Cai et al., 2011). And appears as the most common chemical indicators of meat spoilage (Dhaouadi et al., 2007). With the extension of storage time, the TVBN value of all packagings is increasing and reflect the food freshness turning worse (Fig. 2). The control group vacuum packaging and high oxygen group (O₂/CO₂/N₂ 35:40:25) TVBN values increased significantly and reached >30 mg/100 g at the end of the storage time. While the packagings of MAP O₂/CO₂/N₂ 5:70:25 (low O₂ MAP) and MAP CO₂/N₂ 80:20 changed smoothly and not exceeding 25 mg/100 g at 14th day. Therefore, the packaging of high carbon dioxide have a good effect on fresh degree of Chinese spiced beef.

**Lipid oxidation analysis:** Lipid oxidation is a major cause of deterioration in the quality of meat and meat products (Love and Pearson, 1971). Researchers usually use the method of 2-Thiobarbituric Acid (TBA) to assess the lipid oxidation in meat food. When the comparison from the same raw material samples in different stages of the oxidation degree, the TBA test is valuable. The TBARS value is greater and the sample fat oxidation is more serious. Fat oxidation not only influences food flavor, texture, taste changes but can damage the nutritional value of food. The oxidative stability of Chinese spiced beef expressed as the TBARS values increased during storage time (p<0.05) for all seven treatments (Fig. 3). The
Fig. 1: The degree of variation by electronic nose on different packaging methods, different storage time of the Chinese spiced beef; a) The 2nd day; b) The 8th day; c) The 20th day; d) The 14th day; 0 = Fresh state of Chinese spiced beef, V = Vacuum, 1 = O₂/CO₂/N₂ 5:70:25, 2 = O₂/CO₂/N₂ 35:40:25, 3 = CO₂/N₂ 20:80, 4 = CO₂/N₂ 40:60, 5 = CO₂/N₂ 60:40, 6 = CO₂/N₂ 80:20

Fig. 2: Changes on TVBN of Chinese spiced beef packaged in different environment during storage

Fig. 3: Changes on TBARS of Chinese spiced beef packaged in different environment during storage
samples packaged with high O$_2$ MAP (O$_2$/CO$_2$/N$_2$: 35:40:25) and vacuum-packed (control group) had a greater increase on TBARS values along the preservation time. At the end of storage time, Chinese spiced beef from high O$_2$ MAP reached the highest values (0.73 mg/100 g), the vacuum-packed conditions reached 0.54 mg/100 g. While the samples packaged with low O$_2$ MAP (O$_2$/CO$_2$/N$_2$: 35:40:25) and high carbon dioxide group (CO$_2$/N$_2$: 80:20) changed smoothly, they reached values of 3.16 and 3.65 mg/100 g at 14 days of storage, respectively. From the study, researchers found that the TBARS value of low O$_2$ MAP is lower than the high carbon dioxide group, it is possibly that the high carbon dioxide concentration inhibited the breeding of anaerobic bacteria and little oxygen could inhibit the growth of aerobic bacteria and reduced the microbial decomposition of fat oxidation. And the further research is required on the specific reasons.

**pH and color parameters analysis:** The pH value is a fundamental datum to be monitored during storage time (Gault, 1991; Rao et al., 1989). By acting on the pH, it controls several components relating to the meat’s quality such as tenderness, juiciness and color (Gault, 1985; Medyrski et al., 2000; Offer and Knight, 1988). Changes of different packaging methods of Chinese spiced beef pH from the Table 3, the pH of all the packagings changed in a low level. Under vacuum-packaging the pH values of beef decreased during the first 6 days and then increased up to the end of storage. This was probably due to vacuum packaging can inhibit microbial growth and reproduction in the early storage and slow down the oxidation rate of fat. With the increase of storage time, the growth of anaerobic bacteria in meat products become rapid by destruction the muscle tissue resulting in fat, protein oxidation and rising pH (Lorenzo and Gomez, 2012). The pH of Chinese spiced beef in the hypoxia group (O$_2$/CO$_2$/N$_2$: 35:40:25) fell first and rose later, the possible reason is that the CO$_2$ dissolves into the water (Gill, 1996; McMillin et al., 1999). And fat phases of the meat and associates with H$^+$ to form carboxylic acid in the early time (Leygonie et al., 2011) causing a pH decrease. In the end of storage time, the microbial grew and bred.

**Table 3:** Changes on color and pH difference of Chinese spiced beef packaged in different environment during storage

<table>
<thead>
<tr>
<th>Days of storage</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
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<th>14</th>
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- **L**: Vacuum (control) 33.99 ± 1.46
- **1**: 33.99 ± 1.46
- **2**: 33.99 ± 1.46
- **3**: 33.99 ± 1.46
- **4**: 33.99 ± 1.46
- **5**: 33.99 ± 1.46
- **6**: 33.99 ± 1.46

- **Significance**: * NS * NS ** NS

- **NS**: NS NS NS NS NS

- **V**: Vacuum (control) 5.07 ± 0.05
- **1**: 5.07 ± 0.05
- **2**: 5.07 ± 0.05
- **3**: 5.07 ± 0.05
- **4**: 5.07 ± 0.05
- **5**: 5.07 ± 0.05
- **6**: 5.07 ± 0.05

**Note:** * = Not Significant, ** = p<0.05, *** = p<0.01, **** = p<0.001.
rapidly, leading to protein oxidation occurred and causing the pH increase. While pH of other groups declined, this fact might be related to the high percentage of CO₂ in package and the CO₂ dissolved into the beef, reducing the pH.

The color of Chinese spiced beef is considered to be the most important sensory attribute affecting the consumer’s purchasing decision because consumers use discoloration as an indicator of freshness and whole someness (Mancini and Hunt, 2005). Color coordinates \( L^*, a^*, b^* \) are shown in Table 3 for MAP-packaged and vacuum-packaged meat. Lightness \( L^* \), redness \( a^* \) and yellowness \( b^* \) showed significant differences \((p<0.05)\) due to storage time and packaging conditions. The lightness in five groups of Chinese spiced beef changed along with the storage time having significant difference, only the packagings of MAP \( O_2/CO_2/N_2 \), 5:70:25 (low \( O_2 \) MAP) and MAP \( CO_2/N_2 \), 80:20 had no significant change. Redness \( a^* \) is the most important color parameter to evaluate meat oxidation. In accordance with the sensorial results prolonged storage under hypoxia group \( O_2/CO_2/N_2 \), 35:40:25 induces the red color of beef into brown. This change decreases the redness \( a^* \) and makes the meat unacceptable for the consumers while the other groups changed smoothly (Renerre and Bonhomme, 1991; Gatellier et al., 2005). Yellowness \( b^* \): researchers observed that yellowness did not differ between muscles neither during storage time for different packagings of beef. The results reported in this study demonstrated clearly that the hypoxia group \( O_2/CO_2/N_2 \), 35:40:25 of Chinese spiced beef has a poor color-protected effect, probably because of the higher oxygen accelerating the fat oxidation and reproduction of aerobic microbial, then making the color of Chinese spiced beef worse. The color changes of other packagings were not obvious during storage. This can explain that the color of modified atmosphere packaging on Chinese spiced beef preservation does not play a major role.

**CONCLUSION**

How to prolong shelf life and maintain flavor stable? Solving those problems not only for the technology needs but it is relation to the modernization production of Chinese spiced beef. In this experiment, vacuum packaging for the control, researchers studied the difference in the Chinese spiced beef of different modified atmosphere packagings on the maintaining flavor and extending the shelf life. The results show that the \( O_2/CO_2/N_2 \), 5:70:25 (low \( O_2 \) MAP) and \( CO_2/N_2 \), 80:20 group storing at 25°C after 14 days, the total number of bacteria, TBARS, TVBN, electronic nose map which are the main characterization of its shelf-life and flavor stability index of Chinese spiced beef are significantly better than the control group (vacuum packaging) and high \( O_2 \) MAP \( (O_2/CO_2/N_2 \), 35:40:25) and indicated that the \( O_2/CO_2/N_2 \), 5:70:25 (low \( O_2 \) MAP) and \( CO_2/N_2 \), 80:20 packages can prolong the shelf life (according to GB/T 4789.17, the microbial limits of 4.9 orders of magnitude) and be able to maintain the flavor of Chinese spiced beef better (from the degree of variation by electronic nose). From the results it can be seen that with the increase of \( O_2 \) concentration, the microorganism, TVBN numerical of Chinese spiced beef is gradually reduced while with the increase of \( O_2 \) concentration, the degree of lipid oxidation increased and sense evaluation index decreased. Researchers can see that fat oxidation effect of \( O_2/CO_2/N_2 \), 5:70:25 (low \( O_2 \) MAP) packaging was better than the \( CO_2/N_2 \), 80:20, further research is required on the specific reasons. Under the condition of 25°C, the TBARS, TVBN index of the experimental group and the control group appeared significant differences in the early stage of storage. Therefore, in the actual product, comprehensive production cost factors for the short-sales products, you can consider using vacuum packaging to reduce production cost. However, for the sales of a wide range, you can recommend MAP packaging technology to extend product and keep the flavor of the product to reduce the enterprise product safety risk and improve the stability of product quality. What’s more, packaging technology innovations and ingenuity will continue to provide MAP that is consumer oriented, product enhancing, environmentally responsive and cost effective but continued research and development by the scientific and industry sectors will be needed (McMillin, 2008). This study only studies the preservation effect about Chinese spiced beef of different gas composition and proportion of modified atmosphere packaging at 25°C and the low temperature conditions are also to be studied.

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