

Oxidation Level of Vacuum-Packaged Shredded Meat Treated with Different Concentrations of Potassium Sorbate, Sodium Diacetate and Nisin

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Abstract: The preservatives have been common used in food product, drugs and cosmetics to prevent their aging and decay. Shredded meat has been frequently used in Chinese traditional dishes. However, there is little information available for the corrosion protection of preservatives in commercial shredded meat products. The effect of different concentrations of potassium sorbate, sodium diacetate and nisin on the inhibition of oxidation process of shredded meat was studied. Results showed that potassium sorbate (0.05 g/kg) and nisin (0.2 g/kg) showed the greatest antioxidation activity by determining the Aerobic Plate Count (APC), Thiobarbituric Acid-Reactive Substances (TBARS) and Peroxide Value (PV). It could help to provide theoretical basis for developing convenience shredded meat products with long shelf life.

Keywords: Antioxidation activity, nisin, potassium sorbate, sodium diacetate, shredded meat

INTRODUCTION

The ethnic food market has expanded tremendously in Europe, with Chinese/Oriental flavors significantly leading other market segments with a 42% share in 2006, ahead of Mexican/American (27%) and Indian (25%) flavors (Wilson, 2007). Thus, the demand for Chinese food, especially for the Chinese traditional dishes, including fish-flavored shredded pork, shredded pork with sweet bean paste, Kung Pao chicken, smoked tea duck, has been growing continuously all over the world. Chinese dishes could be obtained always in restaurant or cafeteria, however, rarely available for convenience food. Therefore, it is necessary to develop commercial Chinese dishes that are ready to eat. Meanwhile, it is beneficial for the industrialization and internationalization of Chinese cuisine industry.

Fish-flavored shredded pork, a kind of Szechuan cuisine, is one of the most popular dishes in China (Lv and Brown, 2010). Moreover, shredded meat is the main ingredient in Fish-flavored shredded pork. Microbial existence in the shredded meat turns its taste to sour and turns its surface sticky (Kumar *et al.*, 2000). Lipid deterioration easily takes place and both hydrolytic and oxidative rancidities in meat are associated with quality deterioration (Korotkova *et al.*, 2005). All these negative changes limit the marketing process of shredded meat products. By adding preservatives, microbial growth in meat could be inhibited and freshness could also be maintained.

Potassium Sorbate (PS) (Gliemmo *et al.*, 2009) and Sodium Diacetate (SDA) (Grosulescu *et al.*, 2011) within permissible limit are often added to meat products for their bacteriostatic and fungistatic effects, while nisin (Kopermsub *et al.*, 2011) has been used as an antimicrobial substance in food matrix and exhibits strong bactericidal and sporostatic activities against Gram positive bacteria. Prior study showed that PS, SDA and nisin could react with reducing sugars through Maillard reaction during meat product processing, bind with lipids and proteins non-specifically and therefore influence the oxidation level of the product (Sant'Anna *et al.*, 2011). At this point, the concentrations of different preservatives used in shredded meat products and the antiputrefactive role they play in are remained in solution.

Our object was to study the oxidation level of PS, SDA and nisin in commercial shredded meat products and also, the effect of different concentrations of them on the inhibition of oxidation process. The relationship between bacteriostatic ability and antioxidant activity of preservatives was also studied, by the complementary analysis of Aerobic Plate Count (APC), Thiobarbituric Acid-Reactive Substances (TBARS) and Peroxide Value (PV) of the shredded meat.

MATERIALS AND METHODS

Loin sections of pork were purchased from a local market 72 h after slaughter and delivered to laboratory at 4°C. The fresh lean pork, trimmed of fat and

Table 1: List of preservatives added in different batches of shredded meat samples

Batch	Preservatives (/kg meat)
Con	-
PS1	0.03 g PS
PS2	0.05 g PS
PS3	0.07 g PS
SD1	0.01 g SDA
SD2	0.11 g SDA
SD3	0.21 g SDA
N1	0.05 g nisin
N2	0.2 g nisin
N3	0.35 g nisin

connective tissue, was cut into shredded meat of about 0.4×0.6×8 cm with the muscle fibres running longitudinally and frozen (-18°C±1°C) until further use. Meat samples were divided into ten batches (2 kg each) and mixed with starching solution and prepared at 4°C for 30 min. The starching system of shredded meat was composed of water 10, starch 6, cooking wine 0.5, phosphate 0.3%, respectively and different preservatives with different concentrations (Table 1) for each batch. Every component was added at weights of meat samples.

The shredded meat was fried with low temperature 120°C after starching. All the samples were cooked at 120°C for 90s in a fryer to ensure that the core temperature of the sample reached at 74°C. The shredded meat (30 g for each) was vacuum-packaged in retort pouches, labeled and sterilized in boiling water for 10 min, then stored at 4°C for 50 days. At 25 day predetermined time intervals, three randomly chosen packages were taken from each batch to be analyzed for aerobic plate count, thiobarbituric acid-reactive substances, peroxide value and sensory sensory evaluation.

Analysis methods:

Aerobic plate count: Aerobic plate count was carried out according to the method of Yuichiro (Yuichiro *et al.*, 2010). Three 25 g homogenized samples were taken aseptically and each was placed in flask containing 225 mL of 0.9% (w/v) NaCl. The homogenates were serially diluted 10-fold with saline (1.0 mL of homogenate diluted with 9.0 mL of saline) in five replications and poured onto Petri dishes (9 cm diameter). Then, 15-20 mL of plate count agar (Eiken Chemical, Co. Ltd., Tokyo, Japan) at 45-50°C was added and gently mixed. The poured plates were allowed to solidify under a biological clean bench. After incubation at 37°C for 48 h, colonies were counted. Plates were duplicated for each 10-fold dilution. All results for APC were expressed as Colony Forming Unit (CFU)/g.

Thiobarbituric Acid-Reactive Substances (TBARS):

A modified method of Vareltsis *et al.* (2008) was used for measuring thiobarbituric acid-reactive substances. A sample (approximately 2 g) was prepared with 5 mL of

trichloroacetic acid extraction solution (0.6N HCl and 7.5% trichloroacetic acid prepared in double-distilled water) and 1.5 mL of thiobarbituric acid solution (0.02 M thiobarbituric acid in double-distilled water) in tube and mixed. The control sample was prepared by mixing 2 mL of water instead of meat sample. All of the tubes were heated in boiled water for 30 min and 4 mL of solution was taken from the cooled tube. Four mL of chloroform was then added and centrifuged at 3000 r/min for 10 min. The supernatant was collected. Absorbance readings were measured against a blank at 530 nm. Three measurements were carried out for each batch. The TBARS value, expressed as mg of MDA per kg of sample, was calculated using the following equation (Korotkova *et al.*, 2005):

$$\text{TBARS (mg/kg)} = (A_{532}/m) \times 9.48$$

where,

A_{532} : The absorbance (532 nm) of the assay solution

m : The weight of sample

9.48 : A constant derived from the dilution factor and the molar extinction coefficient (152,000 M/cm) of the red, TBA reaction product

Peroxide Value (PV): A modified method of Vareltsis *et al.* (2008). Approximately 2 g of sample was added to 15 mL mixture of chloroform and methanol (2:1, v/v), containing 500 ppm BHT in order to prevent any further peroxidation during the preparation of the samples. The chloroform used must have ethanol as its preservative since other preservatives caused high blank readings. The mixture was homogenized in a centrifuge tube with a Bio homogenizer (IKA T18 basic, IKA-Werke GmbH & Co., Staufen, Germany) at a high speed of 3000 r/min for 30 s. 3 mL of 0.5% NaCl were added to the tubes, which were then vortexed at 1000 r/min for 15s and centrifuged at 4000×g for 5 min at 4°C to separate the sample into two phases. Five mL of the lower phase were collected and transferred to a 16×125 mm glass tube. Five mL of ice cold chloroform: methanol (2:1, v/v) mixture was added to the samples to make a final volume of 10 mL. Ammonium thiocyanate solution was prepared by adding 30 g of the reagent to 100 mL of water. To prepare the ferrous chloride solution, 0.4 g barium chloride was dissolved in 50 mL of water. This solution was added to an equal volume of ferrous sulfate solution which was prepared by dissolving 0.5 g ferrous sulfate in 50 mL of water. The mixture was centrifuged for 5 min at 2000×g and the clear supernatant was used as the ferrous chloride solution. Twenty five µL of each reagent were added to the samples followed by vortexing for 3s. Samples were incubated for 5 min at room temperature. Then absorbance was measured at 500 nm. A standard curve was prepared using cumene hydroperoxide. The lipid hydroperoxides are expressed as µmol per g of lipid.

Sensory evaluation: Sensory evaluation was measured as Mora *et al.* (2011) described. It was performed by a 10-member trained panel that consisted of selected faculty, staff and graduate students who had prior experience with meat product evaluation. Sensory evaluations were performed by panelists in partitioned booths under standard incandescent lighting with a red filter at the university sensory laboratory. The samples were presented monadically in random order in white plastic trays coded with 3-digit numbers. Assessors were asked to score the sensory properties of the vacuum-packaged shredded meat and were provided with distilled water for rinsing between samples. The following sensory attributes were evaluated: color, flavor and general acceptance. Scores were assigned using a 9-point scale as follows: for color 1 = reluster and dislike, 9 = glossy and like very much; for flavor, 1 = too salty or tasteless and abnormal taste, 9 = taste plump. General acceptance with 1 = dislike very much and 9 = like very much.

Statistical analysis: Experiments were replicated three times using different batches of meat. Means and Standard Deviations (SD) were analyzed using the General Linear Models procedure of Statistix 8.1 software package (Analytical Software, St. Paul, MN) for microcomputer. Analysis of Variance (ANOVA) was done to determine the significance of the main effects. Significant differences ($p < 0.05$) between means were identified using Tukey procedures.

RESULTS AND DISCUSSION

Aerobic plate count analysis: The growth of aerobic bacteria on the vacuum-packaged shredded meat samples at 4°C over 50 days was used for the prediction of the total bacteria numbers (Fig. 1). Aerobic plate count in each sample with preservatives showed a slight increase between 25 and 50 days (from 3 to 4.65 log 10 cfu/g). Moreover, minimal inhibitory concentration of Potassium Sorbate (PS), Sodium Diacetate (SDA) and nisin that showed inhibition for aerobic bacterial was 0.03, 0.01 and 0.05 g/kg, respectively. However, increasing levels of PS at higher concentration (0.05 g/kg) did not reveal significant an increased inhibitory, as also observed in SDA and nisin at their high certain levels. The lack of increment on antimicrobial effect by increasing PS level was perhaps due to the vacuum-package (Friedrich *et al.*, 2008) and the heat treatment (Guynot *et al.*, 2004) such as frying and sterilization during shredded meat processing, which could kill most of the bacteria.

Figure 2 shows the effect of the ratio of NaOH to urea (Pretreatment condition: the concentration of NaOH/Urea aqueous solution is 9%, the amount of ethanol is 50 mL, the pre).

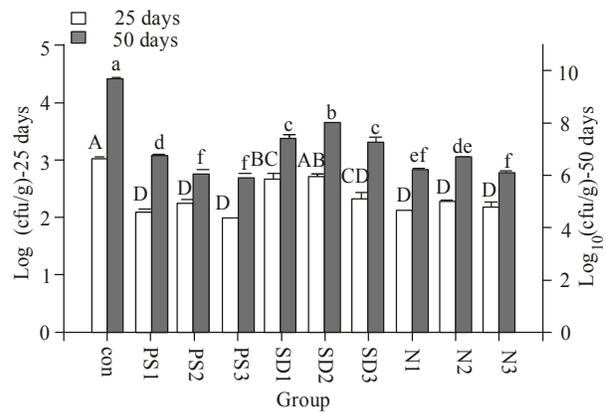


Fig. 1: Microbial growth of vacuum-packaged shredded meat during chilled storage
^{a-b}: Different letters in white columns indicate significant differences ($p < 0.05$); ^{A-B}: Different letters in shadow columns indicate significant differences ($p < 0.05$)

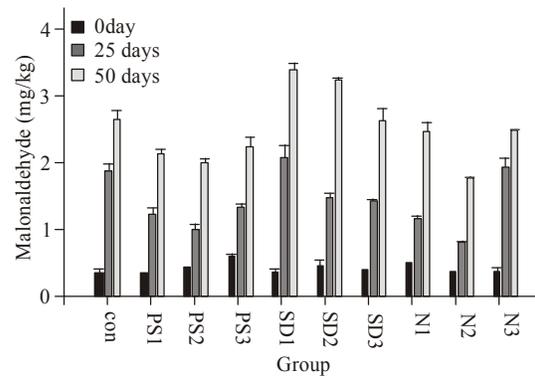


Fig. 2: Changes in TBARS values of vacuum-packaged shredded meat during chilled storage

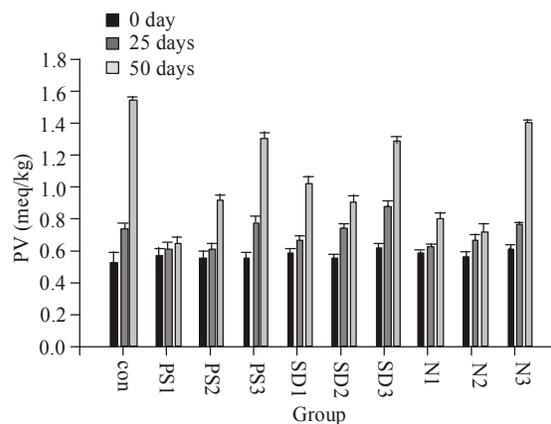


Fig. 3: PV values of vacuum-packaged shredded meat during chilled storage

TBARS analysis: Changes in TBARS of all the samples during chilled storage are shown in Fig. 2. Values of TBARS increased in each sample as the storage time prolonged. The initial value

Table 2: Sensory evaluation of vacuum-packaged shredded meat during chilled storage

		Color	Flavor	General acceptance
25 days	Con	5.02±0.21 ^B	5.08±0.37 ^B	5.20±0.42 ^{AB}
	PS1	5.83±0.68 ^{AB}	5.58±0.42 ^{AB}	5.60±0.19 ^{AB}
	PS2	5.97±0.42 ^A	5.71±0.45 ^{AB}	5.58±0.34 ^{AB}
	PS3	5.93±0.26 ^A	5.83±0.33 ^{AB}	5.75±0.38 ^{AB}
	SD1	5.75±0.82 ^{AB}	5.30±0.27 ^B	5.31±0.22 ^B
	SD2	6.04±0.33 ^A	5.25±0.41 ^B	5.27±0.28 ^B
	SD3	6.08±0.49 ^A	5.58±0.58 ^{AB}	5.50±0.39 ^{AB}
	N1	6.17±0.72 ^A	5.75±0.27 ^{AB}	5.83±0.27 ^A
	N2	5.92±0.43 ^A	5.96±0.26 ^A	5.92±0.36 ^A
	N3	5.89±0.67 ^{AB}	5.67±0.31 ^{AB}	5.61±0.20 ^{AB}
50 days	Con	3.69±0.20 ^B	2.92±0.11 ^C	2.75±0.34 ^C
	PS1	4.06±0.26 ^{AB}	3.67±0.28 ^{AB}	3.79±0.21 ^{AB}
	PS2	4.46±0.69 ^{AB}	4.00±0.35 ^{AB}	3.97±0.39 ^A
	PS3	4.07±0.31 ^{AB}	4.18±0.27 ^A	4.20±0.25 ^A
	SD1	3.88±0.29 ^{AB}	3.35±0.30 ^B	3.49±0.45 ^B
	SD2	4.37±0.54 ^{AB}	3.50±0.43 ^{AB}	3.44±0.19 ^B
	SD3	4.04±0.27 ^{AB}	3.25±0.46 ^{BC}	3.19±0.20 ^{BC}
	N1	4.00±0.55 ^{AB}	3.70±0.35 ^{AB}	3.96±0.38 ^{AB}
	N2	4.46±0.34 ^A	4.25±0.52 ^A	4.31±0.47 ^A
	N3	4.48±0.31 ^A	4.06±0.23 ^A	4.11±0.34 ^A

^{a-b}: Different letters in the same column indicate significant differences ($p < 0.05$)

of TBARS was 37.5 mg/kg meat, suggesting that lipid oxidation occurred during post-mortem handling to some extent, or, on the other hand, the oil used in frying made the samples greasy and be oxidized easily. Thereafter, it increased sharply up to 25 days and gradually increased throughout the storage ($p < 0.05$). The increase in TBARS indicated formation of secondary lipid oxidation products, especially aldehydes (Wang *et al.*, 2005). Therefore, the batches of PS1, PS2, N1 and N2 that showed the lower TBARS value compared with the control indicated that PS and nisin had certain antioxidant inhibition of the secondary reaction products (Mishra *et al.*, 2011). However, increasing the concentration of PS up to 0.07 g/kg of shredded meat (PS3) resulted in no significant reduction in TBARS when compared with PS2. Also, the same trend was observed in vacuum-packaged samples containing nisin. This trend suggested that the antioxidant activity of PS and nisin was not increased when the concentration of them was increased. Additionally, the antioxidant activity of SDA was reduced during 50 days storage might because some of SDA were oxidated.

PV analysis: Marked increase in PV value was observed in most groups throughout the chilled storage up to 50 days ($p < 0.05$) (Fig. 3) and the values of the samples with preservatives were lower than the control. However, no significant change in PV was found in PS1 during the storage ($p > 0.05$). The decreased PV values observed with preservative groups were presumed to be due to the inhibition of peroxide formation (Tanaka *et al.*, 2010) and the decomposition of hydroperoxide (Wang *et al.*, 2011). Moreover, the effect of PS and nisin on inhibiting TBARS formation in the liposome system suggested that lipid peroxides generated in the oxidising liposome system were readily decomposed into malondialdehyde or other secondary products in lipid oxidation process

(Mathenjwa *et al.*, 2012). Nonetheless, with the concentration of PS increasing, the antioxidant activities of it began to reduce. Therefore, appropriate concentrations of PS, SDA and nisin that showed bacteriostasis activity and inhibition for peroxide and secondary reaction products were 0.07, 0.11 and 0.02 g/kg, respectively.

Sensory evaluation: There was no difference between the samples with preservatives and the control in organoleptic attributes at 0 day (data not shown). The shredded meat turned brown after 50 days of storage and was found to be unacceptable (Table 2). This could be due to the growth of spoilage bacteria (Sallam *et al.*, 2007). The addition of PS, SDA and nisin caused significant reduced number of bacterial without any bad taste. Sensory evaluation revealed that group of PS3 and N3 had the best general acceptance. It meant the samples with 0.07 g/kg of PS, 0.11 g/kg of SDA and 0.02 g/kg of nisin showed the most appropriate sensory evaluation values when considered with the APC, PV and TBARS value.

CONCLUSION

The antibacterial activity and antioxidant activity of potassium sorbate, sodium diacetate and nisin were studied by determining the aerobic plate count, thiobarbituric acid-reactive substances and peroxide value in vacuum-packaged pre-cooking shredded meat. All the preservatives showed inhibition for peroxide formation. However, only PS and nisin had the ability to inhibit the formation of secondary lipid oxidation products. PS2 (0.05 g/kg) and N2 (0.2 g/kg) were found to be most effective in preserving pre-cooking shredded meat and showed the best sensory evaluation values. It would be helpful for prolonging shelf life of convenience shredded meat products.

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