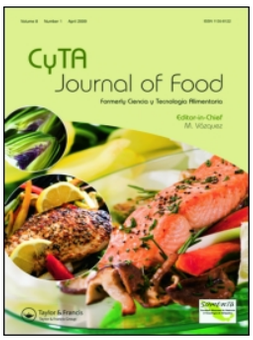

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Determination of preservative residues and microbial contents of commercial Chinese duck neck meat

Zaixiang Lou[a,b], Md Ramim Tanver Rahman[a,b], Jun Zhang[a,b], Kou Xingran[a,b], Smith Gilliard Nkhata[c], Zhu Song[a] and Nabil Qaid M. Al-Hajj[a,b]

[a]State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi, P.R.China; [b]National Engineering Research Center for Functional Food, Jiangnan University, Wuxi, P.R. China; [c]Department of Food Science, Purdue University, West Lafayette, IN, USA

ABSTRACT

Duck neck meat products are traditional, popular snack items in China for its attractive spicy taste, flavor and texture. These products are perishable and different types of chemical preservatives are used extensively. This study determined the levels of preservatives and microbial load in Chinese duck neck meat product by gas chromatography and agar plate methods, respectively. The results showed that Chinese duck neck meat product contains methyl-p-hydroxybenzoate (E-218) in the range 21.15 µg/g–14.5 mg/g, propionic acid (E-280) in the range 26.84–30.24 mg/g and benzoic acid (E-210) in the range not detected – 15.99 µg/g. Sorbic acid (E-200) was not detected. The total plate counts were 1.01–2.03 log cfu/g, and *Lactobacilli* counts were 1.04–1.43 log cfu/g. Refrigerated raw duck neck contained all the tested microorganisms while yeast, mold and *salmonella* were not detected in all of the processed duck neck meat products.

Determinación de los residuos de conservantes y el contenido microbiano de la carne comercial de cuello de pato chino

RESUMEN

Los productos cárnicos de cuello de pato son aperitivos tradicionales y populares en China por su atractivo sabor picante, aroma y textura. Estos productos son muy perecederos y se utilizan diferentes tipos de conservantes químicos de forma extensiva. Este estudio determinó los niveles de conservantes y la carga microbiana en productos cárnicos de cuello de pato chino mediante cromatografía de gas y métodos de placa de agar, respectivamente. Los resultados mostraron que los productos cárnicos de cuello de pato chino contienen metil p-hidroxibenzoato (E-218) en un rango de 21,15 µg/g – 14,5 mg/g, ácido propiónico (E-280) en un rango de 26,84 – 30,24 mg/g y ácido benzoico (E-210) en un rango no detectado – 15,99 µg/g. No se detectó ácido sórbico (E-200). El recuento total por placa fue de 1,01 – 2,03 log cfu/g y los recuentos de Lactobacillus fueron de 1,04 – 1,43 log cfu/g. El cuello de pato crudo refrigerado contenía todos los microorganismos examinados, mientras que no se detectaron levadura, moho ni salmonella en ninguno de los productos cárnicos de cuello de pato procesados.

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Duck; meat; preservative; Chinese duck; duck neck meat; duck meat; methyl-p-hydroxybenzoate; propionic acid; sorbic acid; benzoic acid

PALABRAS CLAVE
pato; carne; conservante; pato chino; carne de cuello de pato; carne de pato; metil p-hidroxibenzoato; ácido propiónico; ácido sórbico; ácido benzoico

1. Introduction

Of the five major regions globally, Asia is the leading producer of duck meat followed by Europe, America, Africa and Australia. Duck meats are a delicacy to many people, while others object to the higher amounts of fat in the carcasses compared to broilers and turkeys. The annual consumption of duck meat per person is at 0.2 kg on a ready-to-cook basis (Maurery, 2003). The international consumption of duck meat is lower as compared to chicken meat. China is the largest producer of duck meat (2,988,408 tonnes annually) followed by France (279,665 tonnes annually) and Malaysia (114,000 tonnes annually) (FAO, 2014).

In China, duck neck meat (often referred to as 'spicy duck neck', Peking duck and Nanjing marinated duck) is very popular among all aged people as traditional food or snack item. In some parts of China, consumers believe that duck meat consumption provides health benefits, such as having a cooling effect on the body. The chain outlets for selling duck neck are increasing day by day. Duck neck products are sold in a vacuum-packed packet (shelf-life of this product is about 6–12 months) or as open cooked dish items which are very often produced at the local level with traditional cooking and chemical preservation methods that do not always follow standard rules and regulations.

Meat and meat products are sources of nutrients for the growth of bacteria, yeasts and molds, and as a result, those products deteriorate very quickly in normal environment. A lot of measures are taken to protect the microbial spoilage such as different processing technology, addition of preservatives, maintaining of storage conditions, etc (Mills, Donnison, & Brightwell, 2014; Nychas, Skandamis, Tassou, & Koutsoumanis, 2008; Ruiz-Capillas & Jimenez-Colmenero, 2008).

Preservatives are used in processed meats for food safety, shelf-life and food technology reasons (Solana & Jiménez-Colmenero, 2009). Chemical preservatives have adverse

CONTACT Zaixiang Lou louzaixiang@126.com State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi 214122, P.R.China

effects on human health. So the acceptable daily intake of every preservative has limited range. Control and regulation of the preservatives are strictly monitored and measured by administrative institute of every country, such as the European Union, FDA, the Food and Agricultural Organization and the World Health Organization of the United Nations through the Codex Alimentarius, and the State Food and Drug Administration – China. Therefore, the identification and quantification of these preservatives and microbial load are important not only for quality assurance/inspection purposes but also for consumer interest and protection.

Several spectrophotometry with UV detection, thin layer chromatography, gas solid chromatography, gas liquid chromatography and high-performance liquid chromatography methods have been developed to analyze for preservatives in meat samples (Choong, 1999; Ruiz-Capillas & Jimenez-Colmenero, 2008; Saad, Bari, Saleh, Ahmad, & Talib, 2005). As for availability, high resolution as well as excellent sensitivity, gas chromatography (GC) analytical techniques were used in our investigation.

Sorbic acid, benzoic acid, sulfites, nitrite, nitrate, the methyl and ethyl esters of 4-hydroxybenzoic acid (parabens) or their salts are organic acids widely used as preservatives, and represent the antimicrobial activity (e.g. mold, yeast, lactobacilli, salmonella, enterobacteriaceae, growth inhibitors) on meat products and permitted by legal authorities within a limit range.

Avoiding the potential health hazards of people as well as evaluating the viable microorganisms count of duck neck products, the content of methyl-p-hydroxybenzoate, sorbic acid, propionic acid, benzoic acid and the total viable counts, lactobacilli, salmonella isolation, enterobacteriaceae, yeast and mold counts in Chinese duck neck meat products were determined in this study.

2. Materials and methods

The content of residues of methyl-p-hydroxy benzoate, sorbic acid, propionic acid and benzoic acid were determined in the duck neck meat samples using GC. Microbial loads were calculated by agar plate count method.

2.1 Sample collection and chemicals

Vacuum-packed duck neck meat products were purchased from local market of Wuxi, China, of four popular manufacturers (A, B, C, D) and 10 samples from each brand. Refrigerated raw duck necks were purchased from local supermarket (Wuxi, China). Samples were used before expiration date (shelf-life 12 months). Chemicals and reagents used were obtained from the following sources: methyl-p-hydroxybenzoate (methyl paraben) E-218 (of purity ≥98.5%), sorbic acid E-200(of purity ≥99%), propionic acid E-280 (of purity ≥99%), benzoic acidE-210(of purity ≥99.5%) – Sinopharma Chemical Reagent Co. Ltd, China. Ultra-pure water was prepared by Milli-Q-plus ultra-pure water system (Milford, MA, USA) throughout the study.

2.2 Sample preparation for GC

Weight of 10 g (accurate to 0.1 g) of duck neck meat without bone was homogenized in a mortar with a pestle with 1 mL H_2SO_4 and 10 mL ethyl acetate and then transferred into a 50 mL plastic tube. Then ultra-sonication (SY-1000, Shanghai Ultraound Co. Ltd, China) was performed for 15 min. The aliquot was centrifuged for 10 min at 4000 rpm (temperature 4°C). An aliquot of 2 mL of supernatant was transferred into a 5 mL glass tube containing 400 mg anhydrous magnesium sulfate and vortexed for 2 min. Finally, the solution was filtered through Xioshi TNL1320 Nylon 13 mm × 0.2 µm (SN/T 3545-2013, 2013).

2.3 Gas chromatography operating conditions

GC operating conditions were as follows: (a) chromatographic column: 30 m × 0.32 mm (i.d.) 0.25 µm film thickness, DB-FFAP silica capillary column; (b) temperature: 70°C to 250°C (18°C/min.; 8 min); (c) injection inlet temp. 240°C; (d) detector temp. 270°C; (e) carrier gas: nitrogen, purity ≥99.99%, 2.0 mL/min; (f) air flow rate 350 mL/min; (g) hydrogen flow rate: 40 mL/min; (h) injection volume: 1 µL

2.4 Sample preparation for microbiological analysis

For preparation of microbial counts, weight of 10 g of duck neck meat sample was homogenized with 90 mL sterile peptone water. Serial 10-fold dilutions were prepared by diluting 1 mL of homogenate in 9 mL of 0.1% peptone water. Appropriate serial dilutions were triplicated. All media were purchased from Beijing Land Bridge Technology Co. Ltd, China. Preparation of samples and serial dilutions were done near flame in a presterilized (by ultraviolet) horizontal laminar flow unit, observing all possible aseptic precautions.

Plate count agar (Beijing Land Bridge Technology Co. Ltd. – product/agar no. CM159) was used to enumerate total viable count. The plates were incubated at 37 ± 1°C for 48 h. MRS agar (CM188), melted and maintained at 44–46°C (added with 1 mL glycerol/100 mL media), was poured gently to prepare plates for the enumeration of Lactobacilli. The plates were incubated at 37 ± 1°C for 48 h and white color colonies were counted. For Salmonella isolation, the homogenate was incubated at 35 C for 24 h. About 1 mL aliquot of pre-enriched homogenate was transferred to 10 mL of tetrathionate broth (Difco) and incubated at 42 C for 24 h. A loopful of the enriched content was streaked on XLT4 agar (CM249) and incubated for 24–48 h at 37°C. Enterobacteriaceae were determined on Violet Red Agar (CM115) with 1% glucose added incubated at 37°C for 24 h. The sterile cooled Potato Dextrose agar medium acidified with 10% sterilized tartaric acid solution (1 mL/100 mL of media) was used for yeast and mold count. The plates were incubated at 25°C for 7 days. Black, white, yellow, red or greenish black colored colonies were counted. Microbial counts were expressed as log cfu per gram of sample (Drosinos & Mataragas, 2009; Pisacane, Callegari, Puglisi, Dallolio, & Rebecchi, 2015).

2.5 Data analysis

The data was subjected to analysis of variance by statistical software IBM SPSS Statistics 20 for mean ± SD. Duncan's multiple range test was carried out to find out the significant difference between mean values of experimental data of the treatments at 5% level of significance.

3. Results

3.1 Amount of the preservatives in duck neck meat product

The amount of methyl-p-hydroxybenzoate, sorbic acid, propionic acid and benzoic acid in duck neck sample was determined by GC (Table 1). The result showed that Chinese duck neck meat product contains methyl-p-hydroxybenzoate E-218 (21.15 µg/g–14.5 mg/g), propionic acid E-280 (26.84–30.24 mg/g) and benzoic acid E-210(0–15.99 µg/g). There are significant differences in the contents of methyl-p-hydroxybenzoate among the four brands. The amount of propionic acid E-280 in brand C is significantly lower than that in other three brands. Sorbic acid was not found in any sample and benzoic acid was detected only in sample D.

3.2 Microbial counts

Counts of the main microbial groups throughout refrigerated raw duck neck meat sample and vacuum-packed duck neck meat sample are shown in Table 2. It is found that the total plate count of Chinese duck neck meat products were 1.01–2.03 log cfu/g, Lactobacilli counts were 1.04–1.43 log cfu/g, and Enterobacteriaceae counts were 0.76–1.01 log cfu/g. There are significant differences in the total plate counts and Lactobacilli counts among the four brands. Yeast and mold were not detected in any sample. Salmonella was also absent in vacuum-packed duck neck meat but found in the raw meat sample.

4. Discussion

Because of unique taste, affordable price and traditional food, duck neck product is very popular in China and many other countries. Preservatives are used in duck neck to extend shelf-life and prevent spoilage and most of the preservatives are synthetic chemicals. The use of synthetic additives has strict limits in all the countries. The excessive use of preservatives is likely to cause harmful effects on consumer health safety.

The amounts and kinds of chemical preservatives used in food are important aspects of food safety, which has become the focus of attention. Thus, the amounts of preservative contents of four kinds of duck neck products were determined. According to the China national standard (GB2760) provisions, in duck neck products, the limits of content of preservatives are as follows: propionic acid, sodium propionate, calcium propionate, 2.5 g/kg, benzoic acid, sodium benzoate, 1.0 g/kg, methyl-p-hydroxy benzoate and its salts (sodium methyl-p-hydroxy benzoate), 0.25 g/kg, sorbic acid and its salts, 0.075 g/kg.

The results showed that the contents of methyl-p-hydroxy benzoate and its salts in brand D significantly exceeded the limits of GB2760. Among all the products, the uses of benzoic acid, sodium benzoate were not excess. The amounts of propionic acid, sodium propionate, calcium propionate were used in excess, even higher than 10 times. Since preservatives and microbial colonies are very important aspects of food safety, numerous studies have been conducted. Saad et al. (2005) investigated 67 foodstuffs (mainly imported), comprising soft drinks, jams, sauces, canned fruits/vegetables, dried vegetables/fruits

Table 1. The amount of preservatives in commercial duck neck meat samples (different brands A, B, C and D).

Tabla 1. Cantidad de conservantes en las muestras de carne comercial de cuello de pato (diferentes marcas A, B, C y D).

	Brand A	Brand B	Brand C	Brand D
Methyl-p -hydroxybenzoate E-218	145 ± 1.3^{C} µg/g	21.15 ± 0.9^{a} µg/g	95.94 ± 2.3^{b} µg/g	14.5 ± 0.8^{d} mg/g
Sorbic acid E-200	ND	ND	ND	ND
Propionic acid E-280	28.55 ± 0.7^{b} mg/g	30.1 ± 2.1^{b} mg/g	26.84 ± 0.8^{a} mg/g	30.24 ± 1.8^{b} mg/g
Benzoic acid E-210	ND	ND	ND	15.99 ± 0.9^{a} µg/g

ND: not detected.
For each parameter, mean values (n = 10 with standard deviation) followed by different letters (a, b, c, d) denote significant differences.

*ND: No detectado.
*Para cada parámetro, los valores promedio (n = 10 con desviación estándar) seguidos de distintas letras (a,b,c,d) denotan diferencias significativas.

Table 2. Microbiological counts of vacuum packed duck neck meat (different brands A, B, C and D) and raw duck neck meat (stored at 4°C).

Tabla 2. Recuentos microbiológicos de carne de cuello de pato envasada al vacío (diferentes marcas A, B, C y D) y carne de cuello de pato cruda (almacenada a 4 °C). Los recuentos microbianos se expresan como log cfu/g.

	Brand A	Brand B	Brand C	Brand D	Raw duck neck
Total plate count	1.01 ± 0.18^{a}	1.54 ± 0.09^{b}	2.03 ± 0.36^{b}	1.78 ± 0.18^{b}	6.19 ± 0.14^{c}
Lactobacilli	ND	1.43 ± 0.06^{b}	1.84 ± 0.10^{c}	1.04 ± 0.09^{a}	2.21 ± 0.30^{c}
Salmonella isolation	ND	ND	ND	ND	1.2 ± 0.04^{a}
Enterobacteriaceae	ND	0.88 ± 0.10^{a}	1.01 ± 0.80^{ab}	0.76 ± 1.01^{ab}	1.98 ± 0.18^{b}
Yeast and mold count	ND	ND	ND	ND	ND

ND: not detected.
For each parameter, mean values followed by different letters (a, b, c) denote significant differences. Microbial counts are expressed as log cfu/g.

*ND: No detectado.
* Para cada parámetro, los valores promedio seguidos de distintas letras (a,b,c) denotan diferencias significativas.

and others. The range of preservatives found were from not detected (nd)-1260, nd-1390, nd-44.8 and nd-221 mg/kg for benzoic acid, sorbic acids, methyl- parabens and propylparabens, respectively. Han et al. (2016) suggested very limited contamination of these seven emerging contaminants (four paraben-type preservatives and three benzophenone-type) associated with some common sea foods.

The total viable counts, *lactobacilli*, *salmonella* isolation, *enterobacteriaceae*, yeast and mold counts of duck neck products were also measured. It was found that these types of microbiological counts of the products are qualified for consumption as per Chinese food regulation standard. It may be because these products were vacuum packaged. For these products, there is a strict sterilization process in the processing, and the packaging technology (vacuum packaged) and materials are also very good, so the microbial counts did not cross the standard limit. Thus, the microbial indicators of packaged duck neck products are satisfactory.

Zhu et al. (2014) reported the quantitative contamination load of Salmonella in raw chicken carcasses at the retail level in six provinces and cities of China within 1595 carcasses over 12 consecutive months. The chilled (55.1%) stored carcasses was significantly higher in prevalence than those frozen (33.5%) and those freshly slaughtered (28.3%) and those unpackaged (45.1%) were more likely to be contaminated with Salmonella than those packaged (37.4%).

Usually, the preservatives are used to extend the shelf-life of food. One of the important role is to inhibit the growth of harmful microorganisms. The results showed that the amount of methyl-p-hydroxybenzoate E-218 in brand B is the lowest, while the Lactobacilli counts were higher in brand B than those in brands A and D. But the Total plate counts and enterobacteriaceae counts were not significantly different to other brands. The amount of propionic acid was lowest in brand C, so the Lactobacilli counts in brand C were the highest among the four brands. All kinds of the preservatives used in brand D are relatively higher among the four brands, while the Lactobacilli counts in brand B was lower than those in brands B and C. Thus, the counts of Lactobacilli have the greatest association with the amount of preservatives. Meanwhile, the counts of microbes are affected by many other factors, such as sterilization situation and performance, packaging materials and conditions, storage conditions and so on. Thus, the microbiological counts might not be well associated with the presence of some preservatives.

5. Conclusion

In conclusion, the microbiological counts and preservative contents of four kinds of typical duck neck meat products in the market were determined. The results showed that methyl p-hydroxy benzoate and its salts are excessively used in one kind of products (brand D). Propionic acid and its salt have significant excessive amount in four kinds of products, which should be concerned. The contents of the other preservatives were satisfactory. At the same time, the total viable counts, lactobacilli, salmonella isolation, enterobacteriaceae, yeast and mold counts of duck neck meat products were satisfactory.

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Conflict of Interest

There is no conflict of interest.

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