Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.jzus.zju.edu.cn; www.springerlink.com
E-mail: jzus@zju.edu.cn

Systematic study of the quality and safety of chilled pork from wet markets, supermarkets, and online markets in China*#

Dong-wen HU[§1], Chen-xing LIU[§1], Hong-bo ZHAO[1], Da-xi REN[†‡2], Xiao-dong ZHENG[1], Wei CHEN[†‡1]

[1]*Department of Food Science and Nutrition, National Engineering Laboratory of Intelligent Food Technology and Equipment, Zhejiang Key Laboratory for Agro-Food Processing, Zhejiang University, Hangzhou 310058, China*

[2]*Institute of Dairy Science, College of Animal Sciences, Zhejiang University, Hangzhou 310058, China*

[†]E-mail: dxren@zju.edu.cn; zjuchenwei@zju.edu.cn

Received May 8, 2018; Revision accepted Aug. 10, 2018; Crosschecked Dec. 5, 2018

Abstract: Background: With increasing media coverage of food safety incidents, such as that of clenbuterol residues in pork, food safety has become a major public health concern in China. Rapidly developing online markets attract increasing numbers of Chinese consumers to purchase food on the Internet. However, the quality and safety of food sold online are uncertain and are less reported on. Objective: This research aimed to systematically study the quality and safety of chilled pork from wet markets, supermarkets, and online markets in China. Results: The chilled pork samples from online markets were fresher than those from wet markets and supermarkets based on the surface redness (*a** value). Chilled pork contained high levels of nutritional elements, especially the magnesium and phosphorus levels in samples from online markets. The levels of heavy metal element residues and veterinary drug residues in all chilled pork samples were within the standards limits. In addition, huge differences existed in the quality and freshness of the chilled pork samples from online markets according to principal component analysis (PCA). Conclusions: Most chilled pork sold in Chinese markets was qualified and safe. It is necessary to establish an effective online market supervision system for chilled pork.

Key words: Chilled pork; Quality; Safety; Online markets; Principal component analysis (PCA)
https://doi.org/10.1631/jzus.B1800273 **CLC number:** S872

1 Introduction

Pork meat is widely consumed around the world, especially in Europe and Southeast Asia. China, the largest consumer of pork in the world, accounts for approximately half of global pork consumption. In 2017, pork consumption in China reached 54.8 million tons, twice as much as that in the European Union (20.8 million tons) and five times as much as that in the United States (9.5 million tons) (United States Department of Agriculture and Foreign Agricultural Service, 2018). Pork consumption per capita in China grew steadily between 1975 and 2012 (Verbeke and Liu, 2014). Pork not only possesses a pleasant favor and taste but also contributes to the daily intake of protein, fat, and important micronutrients, such as essential trace elements (de Smet and Vossen, 2016). Because of the high levels of protein and fat and the contamination by microorganisms, pork is easily corrupted during storage, transportation, and sales, resulting in low quality or even safety problems. On the other hand, the residue of harmful substances has become a big challenge to the safety of pork, including

‡ Corresponding authors
§ The two authors contributed equally to this work
* Project supported by the National Key Technology R&D Program of China (No. 2016YFD0401201)
# Electronic supplementary materials: The online version of this article (https://doi.org/10.1631/jzus.B1800273) contains supplementary materials, which are available to authorized users
ORCID: Wei CHEN, https://orcid.org/0000-0002-2373-2437
© Zhejiang University and Springer-Verlag GmbH Germany, part of Springer Nature 2019

heavy metal elements and veterinary drugs. In 2011, clenbuterol, a kind of β-agonist class veterinary drug, which is banned in China, was found in fresh pork produced by Shuanghui Group, the largest Chinese meat producer. It has been reported that β-agonists cause adverse effects to human health, such as cardiovascular and central nervous diseases (Shao et al., 2009). Nevertheless, these detrimental compounds are still used illegally as feed additives for their functions in enhancing growth and reducing the body fat of pigs (Wang et al., 2010). Therefore, the safety of pork is receiving more and more attention from consumers. With the increasing exposure of food safety incidents, media coverage left consumers with a high level of uncertainty and wariness regarding the safety of food. Food safety has become a major public health concern in China.

Chinese consumers are used to purchasing pork from wet markets and nearby supermarkets. As an important part of traditional sale channels, wet markets provide fresh and cheap food for consumers, and so still function well in most cities. The majority of outdoor wet markets have been moved to indoor operations in cities (Bai et al., 2008). Moreover, the urban wet markets usually possess large scale and considerable numbers of consumer groups. In wet markets, pork is put on the table in a big hunk at room temperature, and sellers will cut off a part of it and sell according to consumers' request, which makes pork exposed to the potential environmental hazards and cross-contamination. Therefore, supermarkets have become the other choice for consumers to buy pork. Supermarkets, where the pork is under the scrutiny of the supermarket's food merchandising department, are considered as a safer and better source than wet markets, especially the multinational supermarket chains (Qing et al., 2014). In supermarkets, pork is cut into small pieces, packed, and stored in the refrigerator for sale. With the wide diffusion of internet technologies into businesses and households, ecommerce industry and online markets are growing rapidly. More and more Chinese consumers prefer to shop on the Internet because of its convenience. The total sales of online shopping in China reached up to 308 billion dollars in 2013, accounting for 7.9% of total sales (Ding and Lu, 2015). However, research regarding the quality and safety of food sold in online markets is limited. The food in online markets, whose sources are more varied intricate than the traditional sale channels, is difficult to regulate and supervise.

The objective of this research was to systematically study the quality and safety of chilled pork from wet markets, supermarkets, and online markets in China. The information obtained from our study might roughly reflect the current situation of the Chinese pork market. Hangzhou, the city where we chose to sample, is one of the most renowned and prosperous cities in China and the location of the headquarters of Alibaba, the world's largest retailer. Hangzhou possesses a developed economy and logistics industry. The chilled pork samples from wet markets and supermarkets were purchased in Hangzhou, while samples from online markets were purchased on the Internet and sent to Hangzhou by logistics.

2 Materials and methods

2.1 Chilled pork samples

A total of 143 different chilled pork (*longissimus lumborum* muscle) samples were collected from wet markets, supermarkets, and online markets. Among them, 57 and 33 samples were purchased from wet markets and supermarkets, respectively, in six main districts in Hangzhou, Zhejiang, China (Fig. 1). The wet markets where we chose to sample possess large scale and stable consumer groups. The supermarkets where we chose to sample mostly belong to multinational retail corporations, including Walmart, Tesco, Carrefour, etc. At least three chilled pork samples (approximately 400 g/each sample) from three different disperse stalls were purchased at each wet market or supermarket. Then the samples were immediately transferred into sterile bags and put into a bubble chamber with ice packs. The refrigerated samples were transported to the laboratory within 1 h and were immediately measured for color, pH, and total volatile basic-nitrogen (TVB-N). The remaining chilled pork samples were stored at −20 °C for further analysis.

All 53 chilled pork samples were purchased at online markets located in 12 different provinces in China, including Anhui (1 sample), Beijing (4 samples), Guangdong (1 sample), Hainan (4 samples), Hebei (3 samples), Henan (1 sample), Jiangsu (3 samples),

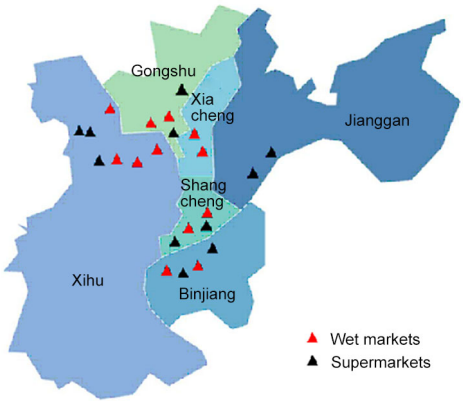

Fig. 1 Map of six main districts in Hangzhou with locations of sampled wet markets and supermarkets

Shandong (5 samples), Shanghai (17 samples), Sichuan (2 samples), Tianjin (1 sample), Zhejiang (11 samples). These samples were transported to the laboratory in cold chain with modified atmosphere packaging and then immediately measured for color, pH, and TVB-N. The remaining chilled pork samples were stored at −20 °C for further analysis.

2.2 Determination of color, pH, and TVB-N

The surface color value of chilled pork samples was measured by CIE $L^*a^*b^*$ system using a Minolta chromameter (CR-10 Plus, Minolta, Japan), with measurements standardized with black and white calibration plates. The pH of samples was measured at different sites on the slice of chilled pork using a pH meter (PH-STAR, Matthäus, Germany). The TVB-N content of samples was determined according to Chinese National Standard GB 5009.228-2016 (NHC, 2016). Briefly, 10.0 g of the chilled pork sample was homogenized with 100 mL of distilled water and filtered through filter paper. Ten milliliters of the filtrate were added to 5 mL of magnesia (10 g/L) and then distilled for 5 min using a Kjeldahl distillation unit. The distillate was absorbed by 10 mL of boric acid (20 g/L) and titrated with 0.01 mol/L hydrochloric acid. The TVB-N (mg/kg) content was calculated by the following equation:

$$\text{TVB-N} = \frac{(V_1 - V_2) \times c \times 14}{m \times 10/100} \times 1000,$$

where V_1 is the titration volume of the tested sample (mL), V_2 is the titration volume of blank (mL), c is the actual concentration of hydrochloric acid (mol/L), and m is the weight of the chilled pork sample (g).

2.3 Determination of element contents

The element contents of samples were determined according to Chinese National Standard GB 5009.268-2016 (NHC and SAMR, 2016) with slight modifications. In order to determine the contents of elements, the chilled pork samples first underwent a digestion procedure. In brief, approximately 10.0 g of the sample was homogenized using a tissue homogenizer. The homogenate (1.0 g) was placed in a quartz pressure reaction vessel and 5 mL of nitric acid was added. Then the vessel was removed to a microwave oven (MarsXpress, CEM, USA) for digestion. The digestion procedure was conducted by applying a program as follows: 0–5 min, 20–120 °C; 5–10 min, 120 °C; 10–15 min, 120–150 °C; 15–25 min, 150 °C; 25–30 min, 150–180 °C; 30–45 min, 180 °C. After the vessel had cooled, the digest was transferred to a 50-mL volumetric flask and diluted with distilled water. The element contents (Ca, Fe, Zn, Mg, Cu, and P) in samples were determined by inductively coupled plasma optical emission spectrometry (Optima 8000, Perkin Elmer, USA). The other element contents (Pb, As, Hg, Cd, and Cr) were determined by inductively coupled plasma mass spectrometry (ELAN 9000, Perkin Elmer, USA). The limit of detection was as follows: Ca, 0.2 mg/kg; Fe, 1.0 mg/kg; Zn, 0.5 mg/kg; Mg, 5.0 mg/kg; Cu, 0.2 mg/kg; P, 1.0 mg/kg; Pb, 0.02 μg/kg; As, 1.0 μg/kg; Hg, 1.0 μg/kg; Cd, 0.1 μg/kg; Cr 10 μg/kg. The intra-assay coefficients of variation (CVs) of element content determination ranged from 0.53% to 3.73%, whereas the inter-assay CVs ranged from 3.67% to 6.94% (Table S1).

2.4 Determination of veterinary drug residues

2.4.1 Tetracyclines

The residues of tetracyclines (tetracycline, oxytetracycline, and chlortetracycline) were detected by high-performance liquid chromatography (HPLC; Agilent 1260, Agilent, USA) and quantified by commercial standards according to Chinese National Standard GB/T 21317-2007 (SAMR, 2007) with slight modifications. Briefly, a chilled pork sample was homogenized using a tissue homogenizer. Five grams of the homogenate was weighed into a 50-mL plastic tube, added 40 mL of ethylenediaminetetraacetic acid

(EDTA)-McIlvaine buffer solution, and then extracted by ultrasound for 30 min. The extraction was centrifuged at 3000 r/min for 5 min. The collected supernatant was diluted to 50 mL and purified by solid-phase extraction cartridge. Finally, the extraction was obtained with an injector, filtered through a 0.22-μm membrane filter, and injected into a HPLC system equipped with Inertsil C18-3 column (4.6 mm× 250.0 mm, 5 μm). The mobile phase, consisting of methanol (A), acetonitrile (B), and distilled water (C) with 10 mmol/L trifluoroacetic acid, was pumped at 1.5 mL/min into HPLC system with the following gradient elution program: 0–5 min, 1%–6% A, 4%–24% B; 5–9 min, 6%–7% A, 24%–28% B; 9–12 min, 7%–0% A, 28%–35% B; 12–15 min, 0% A, 35% B. The total running time was 15 min. The absorbance was detected at 350 nm. The column temperature was maintained at 30 °C and the injection volume was 100 μL.

2.4.2 β-Agonists

The residues of β-agonists (clenbuterol, ractopamine, and salbutamol) were detected by the liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS; Agilent 6460, Agilent, USA) according to Bulletin 1025-18-2008 of the Ministry of Agriculture of China (MARA, 2008a) with slight modifications. Two grams of the homogenized samples were weighed into a 50-mL plastic tube and 10 mL of sodium acetate buffer solution and 50 μL of β-glucuronidase/arylsulfatase were added. Then the solution was incubated at 37 °C for 12 h. After that, the solution was acidified to pH 1.0 with perchloric acid and centrifuged at 5000 r/min for 10 min. The supernatant was transferred to another tube and adjusted to pH 4.0 with sodium hydroxide. Next, the solution was centrifuged at 5000 r/min for 10 min and purified by solid-phase extraction cartridge. Finally, the extraction was filtered through a 0.22-μm membrane filter and injected into a LC-MS/MS system equipped with Waters Atlantics C18 column (2.1 mm× 150.0 mm, 5 μm). The mobile phase, consisting of acetonitrile (A) with 0.1% formic acid and distilled water (B) with 0.1% formic acid, was pumped at 0.2 mL/min into the LC-MS/MS system with the following gradient elution program: 0–2 min, 4% A; 2–8 min, 4%–80% A; 8–21 min, 80%–23% A; 21–22 min, 23%–95% A; 22–25 min, 95% A; 25–25.5 min, 95%–4% A. The total running time was 25.5 min. The column temperature was maintained at 30 °C and the injection volume was 20 μL.

The mass spectrometer was operated in positive electrospray ionization mode (ESI+). The following parameters were optimal: capillary voltage, 3.2 kV; cone voltage, 35 V; multiplier voltage, 650 V; desolvation gas flow rate, 600 L/h; cone gas flow rate, 50 L/h; source temperature, 110 °C; desolvation temperature, 350 °C. The radio frequency (RF) lens was set at 0.5 V. Ion energies 1 and 2 were both held at 0.5 V. The quantitative ion-pairs were as follows: clenbuterol, 277.1>203.3 *m*/*z*; ractopamine, 302.3> 164.3 *m*/*z*; salbutamol, 240.2>148.2 *m*/*z*. Detection was carried out in multiple reaction monitoring (MRM) mode. Argon was used as the collision gas.

2.4.3 Sulfonamides

The residues of sulfonamides (total) were detected by LC-MS/MS (Agilent 6460, Agilent, USA) according to Bulletin 1025-23-2008 of the Ministry of Agriculture of China (MARA, 2008b) with slight modifications. Five grams of the homogenized sample were weighed into a 50-mL plastic tube and 30 mL of ethyl acetate was added. The mixture was vortexed for 2 min and centrifuged for 10 min at 5000 r/min. Hydrochloric acid (0.1 mol/L) was added to the collected supernatant. Then the mixture was evaporated at 45 °C to remove the ethyl acetate. The remaining mixture was purified by solid-phase extraction cartridge. Finally, the extraction was filtered through a 0.22-μm membrane filter and injected into a LC-MS/MS system equipped with Waters Atlantics C18 column (2.1 mm×150.0 mm, 5 μm). The mobile phase, consisting of acetonitrile (A) with 0.1% formic acid and distilled water (B) with 0.1% formic acid, was pumped at 0.2 mL/min into the LC-MS/MS system with the following gradient elution program: 0–5 min, 10%–25% A; 5–20 min, 25%–55% A; 20–30 min, 55%–10% A. The total running time was 30 min. The column temperature was maintained at room temperature and the injection volume was 10 μL.

The mass spectrometer was operated in ESI+. The following parameters were optimal: capillary voltage, 3 V; desolvation gas flow rate, 440 L/h; source temperature, 80 °C; desolvation temperature, 300 °C. Detection was carried out in MRM mode. Argon was used as the collision gas. The intra-assay

CVs of the veterinary drug residue determination ranged from 2.02% to 4.60%, whereas the inter-assay CVs ranged from 3.18% to 9.17% (Table S2).

2.5 Statistical analysis

Data were expressed as mean±standard deviation (SD) from at least three independent experiments. One-way analysis of variance (ANOVA) with the multiple range significant difference (Duncan) test (P<0.05) was carried out using SPSS 22.0 to determine the significant differences among the chilled pork from three different sources. Principal component analysis (PCA) was carried out using the Unscrambler X 10.3 to reduce the dimensional space and visualize the data trends. For PCA, the input data were standardized (1/standard deviation) and mean centered (mean=0).

3 Results and discussion

3.1 Color, pH, and TVB-N

Color (L^* value, a^* value, b^* value), pH, and TVB-N of chilled pork samples from three different sources were presented in Fig. 2. Color is one of the important quality traits of chilled pork for consumers in the purchase decision. As we can see from Fig. 2, L^* value and b^* value showed no difference in chilled pork samples from three different sources, while the a^* value of chilled pork from online markets was significantly higher than that from offline markets (wet markets and supermarkets). The difference of a^* value was related to the accumulation of metmyoglobin on the chilled pork surface. After pigs were slaughtered, oxygen diffused to a deep level in meat and oxidized the myoglobin to oxymyoglobin in the first few hours. Afterwards, the cherry red oxymyoglobin was further oxidized to grey-brown metmyoglobin, and thus the a^* value of meat increased in the first 24 h and declined gradually during storage under refrigerated conditions (O'Sullivan et al., 2002; Estévez et al., 2003). The higher a^* value of chilled pork from online markets than that from offline markets indicated a better freshness of pork samples procured from online markets. The pH value of meat gradually increased during storage due to the decomposition of protein and accumulation of alkaline substances (ammonia and amine compounds). No significant difference was observed in pH values among the chilled pork samples from three different sources. As for the TVB-N value, one of the important reference indices for evaluating meat freshness, the chilled pork samples from supermarkets and online markets showed significantly lower TVB-N values than those from wet markets. Similar to pH value, the TVB-N value increased gradually during storage under refrigerated conditions (Huang et al., 2017). The TVB-N value remained below 150 mg/kg, which suggested that the meat was still fresh (Huang et al., 2014). Most of the chilled pork samples (132/143) in this investigation were fresh according to this criterion. Collectively, our results suggested that the chilled pork samples from wet markets, supermarkets, and online markets were mostly fresh and acceptable. Furthermore, the chilled pork samples from online markets were the freshest, which might be attributed to the cold chain system and the modified atmosphere packaging.

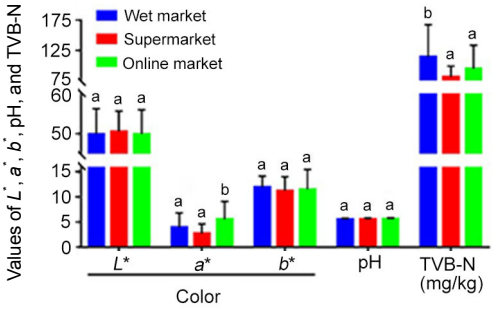

Fig. 2 Physicochemical parameters of chilled pork samples from three different sources

Data are expressed as mean±standard deviation. Different lowercase letters in the same parameter indicate significant differences (P<0.05) obtained by ANOVA and Duncan test. TVB-N, total volatile basic-nitrogen

3.2 Nutritional elements

The contents of nutritional elements (Ca, Fe, Zn, Mg, Cu, and P) in chilled pork samples from three different sources were presented in Table 1. As an important kind of red meat, pork provides nutritional elements for the human diet in an organic and well-absorbable form (de Smet and Vossen, 2016). Pork is a good carrier of calcium. The Ca levels of chilled pork samples from wet markets, supermarkets, and online markets were (0.23–330.60), (0.57–85.42), and (1.51–787.84) mg/kg, respectively. There was no

detectable variation in Ca levels among the chilled pork samples from the three different sources. Zhu et al. (2007) compared the levels of Ca between boar pork and conventional pork, and no significant difference was observed. The dietary composition and rearing regime also showed no effect on the Ca level of pork, indicating that the level of Ca might be a stable value in pork (Zhao et al., 2016). Similarly, no significant difference in Fe levels was observed among chilled pork samples from the three different sources. The total Fe level of pork was not influenced by the rearing system and storage time (Karwowska and Dolatowski, 2013). Zn levels in chilled pork samples (10.25–51.44 mg/kg) were in the range reported by Jablonska et al. (2013) in 24 selected meats (4.3–87.3 mg/kg). Meat contributed to 26% of the dietary zinc intake in the Polish diet (Jablonska et al., 2013). As shown in Table 1, the chilled pork samples from wet markets possessed a higher Zn level than those from supermarkets. Observed levels of Mg in chilled pork samples ranged from 203.3 to 385.0 mg/kg, and the samples from online markets showed the highest Mg level. Our results for Zn and Mg levels in chilled pork samples were slightly higher than those reported by Djinovic-Stojanovic et al. (2017) in samples collected from Serbian markets. No significant difference in Cu level was observed among the chilled pork samples from the three different sources. The Cu levels of pork samples obtained in our study (e.g. (2.89±1.82) mg/kg in samples from supermarkets) were almost five- to ten-fold higher than those from Swedish supermarkets and butcheries ((0.41±0.23) mg/kg) (Gerber et al., 2009). As a growth promoter, Cu was incorporated into feed at a concentration exceeding the physiological requirements of pigs. However, the excessive dietary Cu cannot be absorbed by the pigs (Zhao et al., 2016). The efficiency of dietary Cu absorption was related to the genetic variation (Andrée et al., 2010). Regarding P levels, the chilled pork samples from online markets ((2.58±0.33) mg/kg) showed significantly higher values than those from wet markets ((2.30±0.24) mg/kg) and supermarkets ((2.41±0.21) mg/kg).

3.3 Heavy metal elements

The contents of heavy metal elements (Pb, As, Hg, Cd, and Cr) in chilled pork samples from three different sources were presented in Table 2. The maximum residue limits of heavy metal elements in pork were based on the Chinese National Standard GB 2762-2017 (NHC, 2017). Apart from the nutritional elements, the heavy metal element residues in meat have received increasing attention in recent years. The heavy metal elements were accumulated and concentrated in the organs and tissues of animals from feed, pasture, polluted air or water (Andrée et al., 2010). Owing to the reduction of Pb emissions, the Pb level in food decreased significantly (Pilarczyk, 2014). As shown in Table 2, the Pb level of chilled pork samples from online markets ((11.27±12.74) μg/kg) was nearly half of those from wet markets ((22.82±20.48) μg/kg) and supermarkets ((25.55±21.29) μg/kg). The levels of Pb observed in our study were similar to the previous study reported by Gerber et al. (2009) in pork loin ((18.0±1.0) μg/kg). As for As and Hg levels, these heavy metal elements were mostly found in seafood, while in meat they were often detected at marginal levels (Andrée et al., 2010). Expectedly, the As levels of all chilled pork samples were lower than the standard limit (0.5 mg/kg), and the samples from wet markets showed the lowest As levels. There was

Table 1 Contents of nutritional elements in chilled pork samples from three different sources

Source	Ca (mg/kg)		Fe (mg/kg)		Zn (mg/kg)	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
Wet market	39.81±43.62[a]	0.23–330.60	7.20±3.57[a]	3.59–16.21	21.38±9.04[b]	12.58–51.44
Supermarket	40.62±27.23[a]	0.57–85.42	6.97±4.33[a]	3.51–22.33	16.02±3.23[a]	10.25–25.49
Online market	66.15±124.45[a]	1.51–787.84	6.36±2.49[a]	2.87–12.57	19.08±7.22[ab]	11.73–47.43
Source	Mg (mg/kg)		Cu (mg/kg)		P (g/kg)	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
Wet market	281.72±27.73[ab]	217.70–331.40	3.18±1.51[a]	0.94–7.32	2.30±0.24[a]	1.97–3.71
Supermarket	274.55±23.41[a]	228.60–314.80	2.89±1.82[a]	0.47–11.13	2.41±0.21[a]	2.18–3.05
Online market	294.19±38.70[b]	203.30–385.00	3.68±2.31[a]	0.55–8.47	2.58±0.33[b]	1.68–3.25

[a, b] Means in the same line with different lowercase letters are significantly different (P<0.05) according to ANOVA and Duncan test. SD: standard deviation. The measurements of chilled pork samples were based on wet weight

no detectable variation among the Hg levels of chilled pork samples from the three different sources. The Hg levels of 44 samples (14 samples from wet markets, 15 samples from supermarkets, 15 samples from online markets) were below the limit of detection (1.0 μg/kg). Cd, a human carcinogen (Group 1), is efficiently retained in the liver and kidney after absorption by the human body and has a long biological half-life (10–30 years). For the general non-smoking population, food is the main source of Cd exposure (Tomović et al., 2011). The Cd levels in chilled pork samples were extremely low, ranging from 0.10 to 2.25 μg/kg. The Cd levels in samples from online markets ((0.47±0.39) μg/kg) were higher than those from wet markets ((0.21±0.31) μg/kg) and supermarkets ((0.18±0.19) μg/kg), but much lower than those reported by López-Alonso et al. (2007) in pork samples obtained from north-west Spain ((9.0±7.0) μg/kg). No significant difference in Cr levels was observed among chilled pork samples from the three different sources. The heavy metal element levels of all chilled pork samples were in line with the Chinese National Standard, indicating that the chilled pork sold in Chinese markets was qualified and safe in heavy metal element residues and contaminants.

3.4 Veterinary drugs

The veterinary drug residues of chilled pork samples from the three different sources were presented in Table 3, including three groups: tetracyclines (oxytetracycline, chlortetracycline, and tetracycline), sulfonamides (total), and β-agonists (clenbuterol, ractopamine, and salbutamol). The maximum residue limits of veterinary drug in pork were based on the Chinese Agricultural Industry Standard NY/T 632-2002 (MARA, 2002). The levels of detected veterinary drugs in chilled pork samples were all below the limit of detection, indicating that all samples were in line with the industry standard in the veterinary drug residues. Tian et al. (2016) developed a novel ultra-HPLC-MS/MS approach for detection of 46 veterinary drugs and analyzed 30 pork samples

Table 2 Contents of heavy metal elements in chilled pork samples from three different sources

Source	Pb (μg/kg)		As (μg/kg)		Hg (μg/kg)	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
Wet market	22.82±20.48[b]	2.70–138.00	7.58±6.72[a]	ND–31.70	1.61±1.78[a]	ND–7.50
Supermarket	25.55±21.29[b]	7.00–102.20	14.05±8.24[b]	4.40–43.30	1.48±2.10[a]	ND–6.20
Online market	11.27±12.74[a]	0.10–80.60	11.49±4.91[b]	4.50–31.40	1.38±2.28[a]	ND–13.00
Limit	≤200		≤500		≤50	

Source	Cd (μg/kg)		Cr (μg/kg)	
	Mean±SD	Range	Mean±SD	Range
Wet market	0.21±0.31[a]	ND–2.25	46.16±57.17[a]	ND–335.90
Supermarket	0.18±0.19[a]	ND–0.69	72.01±31.78[a]	ND–130.40
Online market	0.47±0.39[b]	ND–1.85	77.82±58.27[a]	ND–182.20
Limit	≤100		≤1000	

[a, b] Means in the same line with different lowercase letters are significantly different (P<0.05) according to ANOVA and Duncan test. ND: not detected, <1.0 μg/kg for As, <1.0 μg/kg for Hg, <0.1 μg/kg for Cd, <10.0 μg/kg for Cr. The measurements of chilled pork samples were based on wet weight

Table 3 Contents of veterinary drugs in chilled pork samples from three different sources

Source	Oxytetracycline (μg/kg)	Chlortetracycline (μg/kg)	Tetracycline (μg/kg)	Sulfonamides (μg/kg)
Wet market	ND (<50)	ND (<50)	ND (<50)	ND (<0.5)
Supermarket	ND (<50)	ND (<50)	ND (<50)	ND (<0.5)
Online market	ND (<50)	ND (<50)	ND (<50)	ND (<0.5)
Limit	≤100	≤100	≤100	≤100

Source	Clenbuterol (μg/kg)	Ractopamine (μg/kg)	Salbutamol (μg/kg)
Wet market	ND (<0.5)	ND (<0.5)	ND (<0.5)
Supermarket	ND (<0.5)	ND (<0.5)	ND (<0.5)
Online market	ND (<0.5)	ND (<0.5)	ND (<0.5)
Limit	Negative	Negative	Negative

ND: not detected. The measurements of chilled pork samples were based on wet weight

and 20 beef samples obtained from Beijing local markets using this method. No veterinary drug residue was detected among the 50 commercial samples, except enrofloxacin in one pork sample and sulfadimethoxine in one beef sample. Regarding the veterinary drug residues of pork from two provinces in Vietnam, Hung Yen and Nghe An, tetracyclines were not present in pork samples, but 50% (9/18) and 5% (1/18) samples were positive for sulfonamides and β-agonists, respectively (Tuyet-Hanh et al., 2017).

3.5 Principal component analysis

PCA was applied to individual color, pH, TVB-N, and elements of chilled pork samples for reducing the dimensional space built on the dissimilarities among samples from different sources. To visualize the data trends and discriminate the efficiency of detected parameters, a scatter plot of chilled pork samples was obtained using the first two principal components from PCA (Fig. 3). In PCA graphics, points, which are geometrically close to each other, possess similar properties. As shown in Fig. 3, the chilled pork samples from online markets were highly dispersive, while those from wet markets and supermarkets were relatively concentrated on the left side of the graphic, indicating that the chilled pork from online markets possessed huge differences in quality and freshness. These chilled pork samples were purchased from online markets that were located all over China. The breed and feed of pig or sanitary conditions of abattoirs might vary greatly, which could result in the huge differences of the pork. Therefore, the safety and quality of chilled pork sold in online markets needed to be paid more attention.

The influence of different parameters that described the chilled pork samples from the three different sources can be evaluated from the scatter plot (Fig. 3). Most samples from wet markets located on the left side of the graphic showed high TVB-N value. In wet markets, the chilled pork was sold on a cutting board at room temperature. The cutting board, knife, hands of seller, or air might contaminate the pork, leading to the multiplication of microorganism and the increase of TVB-N value. Several chilled pork samples from wet markets showed high Zn and Fe levels. A large number of chilled pork samples from online markets were located on the lower-right side of the graphic, indicating the high levels of Ca, Mg, P, and Cd, which was consistent with the results given by Duncan's test.

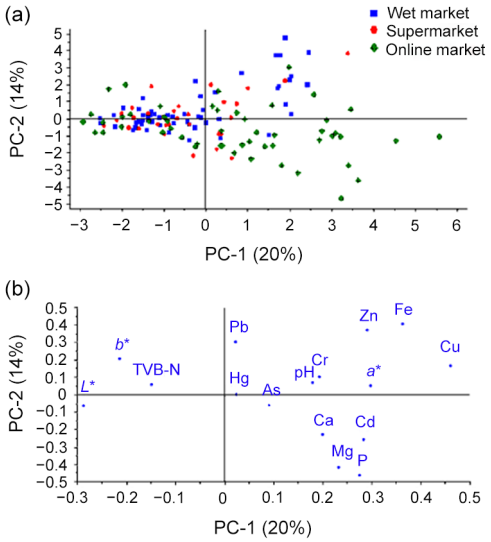

Fig. 3 PC-1 vs. PC-2 scatter plot of the chilled pork samples from the three different sources

(a) Scores plot of PCA on individual color (L^*, a^*, b^*), pH, TVB-N, and elements of chilled pork samples from three different sources (57 samples from wet markets, 33 samples from supermarkets, 53 samples from online markets); (b) Loadings plot. PCA, principal component analysis; TVB-N, total volatile basic-nitrogen

4 Conclusions

A total of 143 different chilled pork samples from wet markets, supermarkets, and online markets were evaluated in our study. Levels of color (L^*, a^*, b^*), pH, TVB-N, nutritional elements, heavy metal elements, and veterinary drug residues in chilled pork samples were determined and assessed by comparing with the levels in standards and data in the literature. The results showed that all chilled pork samples were in line with standards in heavy metal element residues and veterinary drug residues, indicating that the chilled pork sold in Chinese markets was qualified and safe. In addition, chilled pork samples from online markets were fresher than those from wet markets and supermarkets. However, huge differences existed in the quality and freshness of chilled pork samples from online markets according to PCA analysis. Therefore, it is necessary to establish an effective online market supervision system for chilled pork.

Contributors

Dong-wen HU and Chen-xing LIU performed the experimental research and data analysis, wrote and edited the manuscript. Hong-bo ZHAO performed the determination of element content and veterinary drug residues. Da-xi REN, Xiao-dong ZHENG, and Wei CHEN contributed to the study design, data analysis, writing and editing of the manuscript. All authors read and approved the final manuscript and, therefore, had full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Dong-wen HU, Chen-xing LIU, Hong-bo ZHAO, Da-xi REN, Xiao-dong ZHENG, and Wei CHEN declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Andrée S, Jira W, Schwind KH, et al., 2010. Chemical safety of meat and meat products. *Meat Sci*, 86(1):38-48. https://doi.org/10.1016/j.meatsci.2010.04.020

Bai J, Wahl TI, McCluskey JJ, 2008. Consumer choice of retail food store formats in Qingdao, China. *J Int Food Agribus Mark*, 20(2):89-109. https://doi.org/10.1080/08974430802186217

de Smet S, Vossen E, 2016. Meat: the balance between nutrition and health. A review. *Meat Sci*, 120:145-156. https://doi.org//10.1016/j.meatsci.2016.04.008

Ding Y, Lu H, 2015. The interactions between online shopping and personal activity travel behavior: an analysis with a GPS-based activity travel diary. *Transportation*, 44(2): 311-324. https://doi.org/10.1007/s11116-015-9639-5

Djinovic-Stojanovic JM, Nikolic DM, Vranic DV, et al., 2017. Zinc and magnesium in different types of meat and meat products from the Serbian market. *J Food Compos Anal*, 59:50-54. https://doi.org/10.1016/j.jfca.2017.02.009

Estévez M, Morcuende D, Cava R, 2003. Oxidative and colour changes in meat from three lines of free-range reared Iberian pigs slaughtered at 90 kg live weight and from industrial pig during refrigerated storage. *Meat Sci*, 65(3): 1139-1146. https://doi.org/10.1016/S0309-1740(02)00343-1

Gerber N, Brogioli R, Hattendorf B, et al., 2009. Variability of selected trace elements of different meat cuts determined by ICP-MS and DRC-ICPMS. *Animal*, 3(1):166-172. https://doi.org/10.1017/S1751731108003212

Huang SR, Liu B, Ge D, et al., 2017. Effect of combined treatment with supercritical CO_2 and rosemary on microbiological and physicochemical properties of ground pork stored at 4 °C. *Meat Sci*, 125:114-120. https://doi.org/10.1016/j.meatsci.2016.11.022

Huang XW, Zou XB, Zhao JW, et al., 2014. Sensing the quality parameters of Chinese traditional Yao-meat by using a colorimetric sensor combined with genetic algorithm partial least squares regression. *Meat Sci*, 98(2):203-210. https://doi.org/10.1016/j.meatsci.2014.05.033

Jablonska E, Gromadzinska J, Klos A, et al., 2013. Selenium, zinc and copper in the Polish diet. *J Food Compos Anal*, 31(2):259-265. https://doi.org/10.1016/j.jfca.2013.05.016

Karwowska M, Dolatowski ZJ, 2013. Comparison of lipid and protein oxidation, total iron content and fatty acid profile of conventional and organic pork. *Int J Food Sci Tech*, 48(10):2200-2206. https://doi.org/10.1111/ijfs.12205

López-Alonso M, Miranda M, Castillo C, et al., 2007. Toxic and essential metals in liver, kidney and muscle of pigs at slaughter in Galicia, north-west Spain. *Food Addit Contam*, 24(9):943-954. https://doi.org/10.1080/02652030701216719

MARA (Ministry of Agriculture and Rural Affairs of the People's Republic of China), 2002. Chilled Pork, NY/T 632-2002. MARA.

MARA, 2008a. Determination of β-agonists Residues in Animal Derived Food by Liquid Chromatography-tandem Mass Spectrometry, Bulletin 1025-18-2008. MARA.

MARA, 2008b. Determination of Sulfonamides Residues in Edible Tissues of Animal Liquid Chromatography-tandem Mass Spectrometry, Bulletin 1025-23-2008. MARA.

NHC (National Health Commission of the People's Republic of China), 2016. Determination of Volatile Basic Nitrogen in Food, GB 5009.228-2016. NHC.

NHC, 2017. Maximum Levels of Contaminants in Food, GB 2762-2017. NHC.

NHC, SAMR (State Administration for Market Regulation), 2016. Determination of Multi-elements in Food, GB 5009.268-2016. NHC, SAMR.

O'Sullivan MG, Byrne DV, Stagsted J, et al., 2002. Sensory colour assessment of fresh meat from pigs supplemented with iron and vitamin E. *Meat Sci*, 60(3):253-265. https://doi.org/10.1016/S0309-1740(01)00131-0

Pilarczyk R, 2014. Concentrations of toxic and nutritional essential elements in meat from different beef breeds reared under intensive production systems. *Biol Trace Elem Res*, 158(1):36-44. https://doi.org/10.1007/s12011-014-9913-y

Qing P, Xi AQ, Hu WY, 2014. Consumer preference for meat in China: a case study of Beijing. *Emerg Mark Financ Tr*, 50(2):135-143. https://doi.org/10.2753/REE1540-496X5002S209

SAMR, 2007. Determination of Tetracyclines Residues in Food of Animal Origin—LC-MS/MS Method and HPLC Method, GB/T 21317-2007. SAMR.

Shao B, Jia XF, Zhang J, et al., 2009. Multi-residual analysis of 16 β-agonists in pig liver, kidney and muscle by ultra performance liquid chromatography tandem mass spectrometry. *Food Chem*, 114(3):1115-1121. https://doi.org/10.1016/j.foodchem.2008.10.063

Tian YP, Jia JH, He JJ, et al., 2016. Simultaneous detection of

46 veterinary drug residues in animal meat by UHPLC. *Chromatographia*, 79(7-8):457-471. https://doi.org/10.1007/s10337-016-3041-0

Tomović VM, Petrović LS, Tomović MS, et al., 2011. Cadmium concentrations in the liver of 10 different pig genetic lines from Vojvodina, Serbia. *Food Addit Contam: Part B Surveill*, 4(3):180-184. https://doi.org/10.1080/19393210.2011.589035

Tuyet-Hanh TT, Sinh DX, Phuc PD, et al., 2017. Exposure assessment of chemical hazards in pork meat, liver, and kidney, and health impact implication in Hung Yen and Nghe An provinces, Vietnam. *Int J Public Health*, 62(Suppl 1):75-82. https://doi.org/10.1007/s00038-016-0912-y

United States Department of Agriculture, Foreign Agricultural Service, 2018. Livestock and Poultry: World Markets and Trade. https://apps.fas.usda.gov/psdonline/circulars/livestock_poultry.Pdf [Accessed on Apr. 10, 2018]

Verbeke W, Liu RD, 2014. The impacts of information about the risks and benefits of pork consumption on Chinese consumers' perceptions towards, and intention to eat, pork. *Meat Sci*, 98(4):766-772. https://doi.org/10.1016/j.meatsci.2014.07.023

Wang WY, Zhang YL, Wang JY, et al., 2010. Determination of β-agonists in pig feed, pig urine and pig liver using capillary electrophoresis with electrochemical detection. *Meat Sci*, 85(2):302-305. https://doi.org/10.1016/j.meatsci.2010.01.018

Zhao Y, Wang DH, Yang SM, 2016. Effect of organic and conventional rearing system on the mineral content of pork. *Meat Sci*, 118:103-107. https://doi.org/10.1016/j.meatsci.2016.03.030

Zhu HQ, Wang QK, Yin SP, 2007. The comparison analysis of the boar meat and pork in nourishment composition. *Acta Agric Boreali-Occid Sin*, 16(3):54-56 (in Chinese). https://doi.org/10.3969/j.issn.1004-1389.2007.03.014

List of electronic supplementary materials

Table S1 Intra-assay and inter-assay coefficients of variation (CVs) of element content determination

Table S2 Intra-assay and inter-assay coefficients of variation (CVs) of veterinary drug residue determination

中文概要

题　目：市售冷鲜肉的品质与安全的系统性调研——基于农贸市场、大型超市和电商平台 3 种主要销售渠道

目　的：近年来，食品安全事件层出不穷。食品安全已成为公众关注的热点问题。快速发展的电商平台吸引了越来越多的中国消费者在线上购买食品。但网购得到的食品的品质参差不齐，而且鲜有研究或报道。因此，本课题旨在对农贸市场、大型超市和电商平台售卖的冷鲜肉的品质与安全进行系统性的调研。

方　法：从浙江省杭州市 6 大主城区的大型农贸市场和大型超市分别采得 57 和 33 个冷鲜肉样；从各大电商平台采得 53 个冷鲜肉样，共计 143 个肉样。测定各样品的肉色、pH 值、挥发性盐基氮值、钙、铁、锌、镁、铜、磷、铅、砷、汞、镉、铬元素含量、四环素类（四环素、土霉素、金霉素）、磺胺类（总量）、β-受体激动剂类（克伦特罗、莱克多巴胺、沙丁胺醇）兽药残留等指标。

结　论：购自农贸市场、大型超市、电商平台 3 种不同销售渠道的所有冷鲜肉的重金属残留和兽药残留量均符合国家标准。购自电商平台的冷鲜肉比购自农贸市场和大型超市的肉新鲜一些。主成分分析结果表明，购自电商平台的猪肉个体间差异明显。建议尽快建立有效的电商平台冷鲜肉监管体系。

关键词：冷鲜肉；品质；安全；电商平台；主成分分析