Change of *Staphylococcus Aureus* and Salmonella During Storage Period of Food Wastes in China

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Abstract: Food wastes, such as animal feedstuff, may result in risks of disease spreading. Changes of *Staphylococcus aureus* and *Salmonella* in the food wastes during storage period were investigated in this paper. *Staphylococcus aureus* were tested by plate count and coagulase test, and *Salmonella* were tested by plate count and PCR assay. Results showed that both *Staphylococcus aureus* and *Salmonella* existed in the food wastes. After 48 h storage at 20 ℃ and 37 ℃, the amount of *Staphylococcus aureus* decreased from 10^3 - 10^5 CFU/g to 10^2 and 0 CFU/g respectively. The amount of *Salmonella* decreased from 5.5×10^6 - 4.6×10^7 CFU/g to a range between 2.0×10^4 and 2.0×10^5 CFU/g after 48 h storage at 20 ℃, and it was less than 20 CFU/g after 48 h storage at 37 ℃. The two pathogenic bacteria would be restricted by pH decrease, which was caused by the fermentation of the food wastes.

Keywords: Food wastes, *Staphylococcus aureus*, *Salmonella*, pH, Temperature.

1. INTRODUCTION

Food wastes come from hotels, restaurants, canteens and homes, which consist of rice, flour, fruits, vegetables, meat, aquatic products, eggs, bone and other processed food products and their residues. The main components of food wastes include starch, protein, lipids, celluloses, inorganic salts, and high moisture content, which is usually up to 60% to 80% [1]. The microbes would greatly grow and proliferate in the food wastes because of the high content of nutrients, therefore the food wastes become corrupted with terrible odors. Mosquitoes, flies, insects and mice are attracted if food wastes exposed to air or leaked from the containers, which would lead to disease spreading.

Due to the bad dietary habits, food wastes have become a new kind of pollution sources in urban China. A survey by Zhang *et al*. indicated that more than 60 million tons of food wastes were generated in China each year, and more than 1200 tons were generated in Beijing every day [2]. Because of the strict management, human beings have tried to recycle the food wastes to produce biodiesel, biogas, and compost etc [3, 4]. However, large quantity of the food wastes still left without treating or recycling by those normal technologies in China.

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Feeding animals is a good option to reuse the food wastes, which contain much nutritive elements [5, 6]. Some researchers have developed effective technologies which could transform food wastes to animal feedstuff [7], including sterilization [8], improving protein content by bacteria or epiphyte [9, 10]. However, in processing, loading, transporting and storing, the food wastes can be inoculated by bacteria easily, which are prone to become the source of diseases. As a result, the understanding of the status and variation of pathogenic bacteria in the food wastes is necessary.

The state of pathogenic bacteria in the food wastes was often represented by some microbiological characters such as the amount of *Escherichia coli*, coagulase positive *Staphylococcus aureus*, moulds and yeasts, *Salmonella*, and so on [7, 11-13]. The concentration of *Escherichia coli*, the most popular pathogenic bacteria in food wastes, has been widely studied. But *Staphylococcus aureus* and *Salmonella*, which are also major pathogenic bacteria in the food wastes, obtained little attention. Besides, the food wastes show variable hygienic quality depending on the storage time, temperature, the type of wastes as well as the presence of bacteria and moulds [14, 15]. Most researchers investigated the microbiological characters of food wastes, however, ignoring the variation of pathogenic bacteria during storage period. The objective of this work is to study the variation of *Staphylococcus aureus* and *Salmonella* in the food wastes during storage period under different environmental temperatures.

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2. MATERIALS AND METHODS

2.1. Food Wastes

Considering that the pathogenic bacteria would grow vigorously at high temperature, three batches of the food wastes were collected from 12 restaurants as soon as possible after lunch in three days of August 2011 in Beijing, with the sterilized scoops and barrels with barrelhead. The microbial pollution was different for each food wastes collection. In each batch, the samples collected from all the 12 restaurants were mixed together to form the test samples.

2.2. Strains

The *Staphylococcus aureus* CVCC2086 (ATCC6538) and *Salmonella enteritidis* CVCC3377 were purchased from China General Microbiological Culture Collection Center. The *Staphylococcus aureus* CVCC2086 was cultured in 50ml Luria-Bertani (LB) culture medium in a 250ml conical flask at 250 rpm and 37 ℃ in a shaker for 8 - 10 h. The cultured *Staphylococcus aureus* CVCC2086 was used as the inocula of positive control of *Staphylococcus aureus* in the food wastes. The culture and inoculation of *Salmonella enteritidis* CVCC3377 was same as that of *Staphylococcus aureus* CVCC2086.

2.3. Experiment Design

There were 8 samples in each batch of experiments. For each sample, about 300 ml food wastes were loaded in 500 ml conical flask, which was sealed by plastic film. Two samples were sterilized at 121 ℃ for 30 min as the negative controls. Two samples were sterilized at 121 ℃ for 30 min and then inoculated with *Staphylococcus aureus* CVCC2086 with a volume ratio of 1% as the positive controls for *Staphylococcus aureus*. Two samples were sterilized at 121 ℃ for 30 min and then inoculated with *Salmonella enteritidis* CVCC3377 with a volume ratio of 1% as the positive controls for *Salmonella*. The last two samples were left without sterilization and inoculation to simulate the normal food wastes. Each kind of the samples above was separated into two parts, which were stored in incubators of 20 ℃ and 37 ℃ for 48 h respectively. Microbiological characters (coagulase-positive *Staphylococcus aureus* and *Salmonella*) were analyzed in every 12 h. The test arrangement is displayed in detail in Table **1**.

2.4. Analysis

2.4.1. Analysis of pH

The food wastes were diluted with sterile saline in the process of microbiological analysis. The pH of ten-fold dilution of each sample was determined by pH precision test paper.

2.4.2. Counting of Coagulase-Positive Staphylococcus aureus

The amount of coagulase-positive *Staphylococcus aureus* was determined according to National Food Safety Standard, Food microbiological examination: *Staphylococcus aureus* (GB 4789.10-2010) of China. The plate count method of coagulase-positive *Staphylococcus aureus* was performed directly spreading on plates of Baird-Parker agar. The results were reported as CFU/g. Coagulase test was done on rabbit serum [7].

2.4.3. Analysis of Salmonella

According to National Food Safety Standard of China, Food microbiological examination: *Salmonella* (GB 4789.4-2010), the plate count method of *Salmonella* was performed directly spreading on plates of XLD agar. The results were reported as CFU/g. PCR assay was used to identify the suspect colonies, and a

Table 1: Test Sample and Microbiological Analysis Items

Sample Content	Storage Temperature	Analysis Items
Food wastes (DFW)	20 ℃	*Staphylococcus aureus* and *Salmonella*
	37 ℃	*Staphylococcus aureus* and *Salmonella*
Negative control (NC)	20 ℃	*Staphylococcus aureus* and *Salmonella*
	37 ℃	*Staphylococcus aureus* and *Salmonella*
Positive control of *Staphylococcus aureus (*PCA)	20 ℃	*Staphylococcus aureus*
	37 ℃	*Staphylococcus aureus*
Positive control of *Salmonella* (PCB)	20 ℃	*Salmonella*
	37 ℃	*Salmonella*

Salmonella spp. specific 285 bp fragment of the invA gene was used as the target sequence, in which the primers (5' -GTGAAATAATCGCCACGTCGGGCAA-3', and 5' -TCATCGCACCGTCAAAGGAACC-3') were used for PCR detection of *Salmonella spp.* [16, 17]. The PCR was performed in a final volume of 25 μL, which containing 10×PCR buffer, 2.5 mmol/L dNTP, 10 μmol/L each primer, and 2.5 U *Taq* DNA polymerase, and 1 μL sterilized water with a suspect colony as genomic DNA template. The thermal cycling procedure consisted of an initial denaturation step at 94 ℃ for 5 min, 35 cycles consisting of denaturation at 94 ℃ for 30 s, annealing at 60 ℃ for 40 s, and elongation at 72 ℃ for 60 s, and a final extension period at 72 ℃ for 10 min. The PCR products were analyzed by electrophoresis on 2% agarose gel. The suspect colony was validated as *Salmonella* if the target fragment (286 bp) was obtained in the PCR. More than two colonies of each kind of suspect colony were selected to do the validation experiment. The plate count results multiplied by the proportion of positive *Salmonella* in the validation experiment was the final results.

3. RESULTS AND DISCUSSION

3.1. Variation of *Staphylococcus aureus* in Food Wastes

The various amounts of *Staphylococcus aureus* in each sample of food wastes showed that the pollution degree of *Staphylococcus aureus* was different (Table **2**). The amount of *Staphylococcus aureus* in the food wastes generally decreased with the storage time prolonging. In the 1st and 2nd batches, the suspicious colony of *Staphylococcus aureus* decreased, while the coagulase-positive *Staphylococcus aureus* was not detected. In the 3rd batch, the coagulase-positive *Staphylococcus aureus* was detected. The amount of *Staphylococcus aureus* decreased from 4.2×10^4 CFU/g to 1.0×10^2 CFU/g at 20 ℃, and from 1.8×10^4 CFU/g to 0 CFU/g at 37 ℃ after storage of 48 h. A more remarkable decrease at 37 ℃ was observed than that at 20 ℃. For the samples sterilized and inoculated with *Staphylococcus aureus*, the amount of *Staphylococcus aureus* showed insignificant decrease both at 20 ℃ and 37 ℃. Besides, there was no *Staphylococcus aureus* detected in the sterilized samples.

3.2. Variation of *Salmonella* in Food Wastes

Figure **1** shows an example of PCR results for the identification of suspect colonies of *Salmonella*. The amount of *Salmonella* in the food wastes showed the similar tendency as *Staphylococcus aureus*, which generally decreased with the storage time prolonging (Table **3**). At the beginning, the amount of *Salmonella* in the food wastes varied between 4.2×10^6-46×10^6 CFU/g. More *Salmonella* were detected in each sample than *Staphylococcus aureus*, which showed that the food wastes in Beijing might be easier to be polluted by *Salmonella* than *Staphylococcus aureus*. Within the following two days, the amount of *Salmonella* decreased both at temperature of 20 ℃ and 37 ℃. The *Salmonella* in the food wastes storaged at 37 ℃ also showed a more remarkable decrease than at 20 ℃.

Table 2: Variation of *Staphylococcus aureus* in Food Wastes

Temperature	Storage time (h)	*Staphylococcus aureus* in Different Samples (CFU/g)						
		DFW 1	DFW 2	DFW 3	PCA 1	PCA 2	PCA 3	NC 1, 2, 3
20 ℃	0	$<1.0\times10^5$	$<1.0\times10^3$	4.2×10^4	1.0×10^3	1.0×10^3	2.7×10^6	0
	12	$<1.0\times10^3$	$<1.0\times10^2$	2.0×10^2	4.0×10^3	1.0×10^2	2.6×10^6	0
	24	$<1.0\times10^2$	$<1.0\times10^2$	2.0×10^2	1.0×10^3	1.0×10^2	9.2×10^5	0
	36	$<1.0\times10^2$	$<1.0\times10^2$	80	1.0×10^3	1.0×10^3	2.6×10^6	0
	48	$<1.0\times10^2$	<10	1.0×10^2	1.0×10^3	1.0×10^2	4.4×10^5	0
37 ℃	0	$<1.0\times10^5$	$<1.0\times10^3$	1.8×10^4	4.0×10^3	1.0×10^3	2.7×10^6	0
	12	$<1.0\times10^3$	$<1.0\times10^2$	2.0×10^2	5.0×10^3	1.0×10^2	4.0×10^6	0
	24	0	$<1.0\times10^2$	2.0×10^2	1.0×10^3	1.0×10^2	1.4×10^5	0
	36	0	0	0	1.0×10^3	2.0×10^3	1.1×10^4	0
	48	0	0	0	1.0×10^3	1.0×10^2	2.2×10^5	0

Remark: DFW 1, DFW 2 and DFW 3 represent the 1st batch, the 2nd batch and the 3rd batch of the food wastes; PCA 1, PCA 2 and PCA 3 represent the 1st batch, the 2nd batch and the 3rd batch of the positive control of *Staphylococcus aureus*. NC 1, NC 2 and NC 3 represent the 1st batch, the 2nd batch and the 3rd batch of the negative control of *Staphylococcus aureus*.

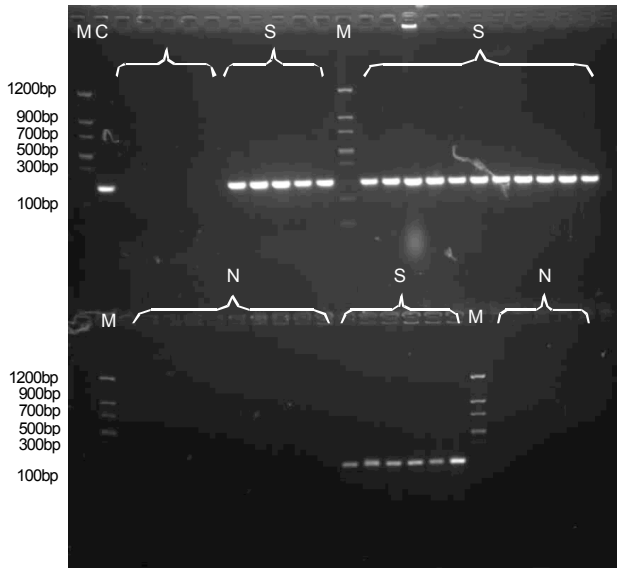

Figure 1: Identification of suspect colonies of *Salmonella* (M is marker, C is positive check, N represents other bacteria, and S represents *Salmonella*).

Table 3: Variation of *Salmonella* in Food Wastes

Temperature	Storage time (h)	*Salmonella* in Different Samples (CFU/g)						
		DFW 1	DFW 2	DFW 3	PCA 1	PCA 2	PCA 3	NC 1, 2, 3
20 ℃	0	5.5×10^{6}	3.3×10^{7}	4.0×10^{7}	5.9×10^{7}	1.0×10^{9}	2.4×10^{9}	0
	12	2.4×10^{7}	1.0×10^{6}	4.4×10^{6}	2.4×10^{9}	1.0×10^{9}	4.0×10^{8}	0
	24	5.0×10^{6}	1.0×10^{5}	1.0×10^{6}	3.5×10^{8}	1.0×10^{9}	3.6×10^{8}	0
	36	5.0×10^{5}	9.5×10^{4}	4.0×10^{5}	5.0×10^{8}	1.0×10^{9}	3.1×10^{8}	0
	48	2.0×10^{5}	2.5×10^{4}	2.0×10^{4}	1.1×10^{9}	1.3×10^{8}	4.0×10^{9}	0
37 ℃	0	4.2×10^{6}	4.6×10^{7}	4.0×10^{7}	2.5×10^{8}	1.0×10^{9}	2.4×10^{9}	0
	12	7.4×10^{4}	2.0×10^{4}	4.0×10^{4}	5.9×10^{7}	2.4×10^{7}	1.6×10^{8}	0
	24	1.0×10^{3}	1.0×10^{3}	4.0×10^{4}	9.2×10^{6}	1.2×10^{7}	4.0×10^{8}	0
	36	1.0×10^{3}	1.0×10^{3}	20	2.0×10^{4}	1.0×10^{3}	1.0×10^{8}	0
	48	<10	<10	20	1.0×10^{4}	1.0×10^{3}	3.1×10^{7}	0

Remark: DFW 1, DFW 2 and DFW 3 represent the 1st batch, the 2nd batch and the 3rd batch of the food wastes; PCB 1, PCB 2 and PCB 3 represent the 1st batch, the 2nd batch and the 3rd batch of the positive control of *Salmonella*. NC 1, NC 2 and NC 3 represent the 1st batch, the 2nd batch and the 3rd batch of the negative control of *Salmonella*.

After storage of 48 h, the amount of *Salmonella* in the food wastes varied between 2.0×10^{4} CFU/g to 2.0×10^{5} CFU/g at 20 ℃, and less than 20 CFU/g at 37 ℃. The amount of *Salmonella* in the samples with sterilization and inoculation of *Salmonella* slightly decreased at 37 ℃, but kept stable or even increase a little at 20 ℃. There was also no *Salmonella* detected in the sterilized samples.

3.3. Reasons for Decrease of *Staphylococcus aureus* and *Salmonella*

The observation that the amount of *Staphylococcus aureus* and *Salmonella* decreased with the storage time in each sample displayed that the food wastes pollution by *Staphylococcus aureus* and *Salmonella* would naturally controlled in the experimental conditions. Table **4** shows that pH of samples without sterilization and inoculation decreased very fast, while pH of samples with sterilization and inoculation slightly decreased, and pH of sterilized samples kept unchangeable. The pH drop of samples without sterilization and inoculation at 37 ℃ decreased faster than at 20 ℃. These results confirmed that the decrease of *Staphylococcus aureus* and *Salmonella* in the food wastes was related with the pH drop. In the samples without sterilization and inoculation, the pH decreased because of the organic acids produced by bacteria, which would cause the killing of the

Table 4: Variation of pH of Food Wastes

Temperature	Sample	pH				
		0 h	12 h	24 h	36 h	48 h
20 ℃	DFW 1, 2, 3	6	4	4	3	3
	PCA/PCB 1, 2, 3	6	5	5	5	5
	NC 1, 2, 3	6	6	6	6	6
37 ℃	DFW 1, 2, 3	6	3	3	2	2
	PCA/PCB 1, 2, 3	6	5	5	5	5
	NC 1, 2, 3	6	6	6	6	6

Staphylococcus aureus and *Salmonella*. High temperature (37 ℃) might be suitable for some bacteria growth and organic acid production, which caused the faster decrease of *Staphylococcus aureus* and *Salmonella*. No detection of *Staphylococcus aureus* and *Salmonella* in the sterilized samples showed that the two kinds might be completely eliminated by thermal treatment. After sterilization, no bacteria producing acids existed in the food wastes and the pH kept stable, meanwhile, the inoculated *Staphylococcus aureus* and *Salmonella* displayed slight variation. These phenomenons suggested that pH drop might be a main reason restricting the growth of the *Staphylococcus aureus* and *Salmonella*g in the food wastes. The developed acidic environment and pH drop have been considered as the determining factors for suppressing both pathogenic and spoilage microorganisms in food fermentation [18]. Wang *et al*. have the similar viewpoint and found that the decrease of pH caused by lactic acid fermentation suppressed the growth of *Staphylococcus aureus* and *Bacillus cereus* in Kitchen wastes which were stored anaerobically at 25±2 ℃ [19]. Ye *et al*. found that accelerating pH drop which caused by inoculation of *Lactobacillus plantarum* BP04 reduced the growth period of enterobacteria in dining-hall food wastes stored at 35℃ [18].

4. CONCLUSION

Staphylococcus aureus and *Salmonella* existed in the food wastes. These two major pathogenic bacteria did not explosively grow in 48-hour storage, and high temperature inhibited their growth more greatly. The pH of the food wastes decreased during 48-hour storage because of the fermentation. The rapider pH decrease at 37 ℃ might result in faster decrease of the amount of both *Staphylococcus aureus* and *Salmonella* than that at 20℃. Consequently, preventing pollution of *Staphylococcus aureus* and *Salmonella* from outer environment may be more important than their growth in the transportation and storage of the food wastes.

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