Journal of Food Safety and Food Quality 62, Heft 1 (2011), Seiten 1–32

Arch Lebensmittelhyg 62,
20–25 (2011)
DOI 10.2376/0003-925X-62-20

ISSN 0003-925X

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An exploratory investigation on the microbiological quality of *dim sum* (pork dumplings) sold in Chiang Mai, Thailand

Eine explorative Untersuchung über die mikrobiologische Qualität von Dim Sum (mit Schweinefleisch gefüllte Teigtaschen) in Chiang Mai, Thailand

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Summary

An exploratory investigation was conducted to determine the microbiologcal quality of pork dumplings *(dim sum)* sold in Chiang Mai, Thailand. 90 deep frozen industrial samples were collected from 3 brands and another 90 ready-to-eat samples from small enterprises (market samples, street vendors and restaurants).

Although the deep frozen dim sum samples showed on average an internal temperature of only $\bar{x}\pm s$ = –5.5±1.8 °C at the point of selling, their counts of total aerobic mesophilic bacteria ($\bar{x}\pm s$ = 1.38±0.77 log cfu/g) were significantly lower than the APCs (= Aerobic Plate Count) of the small enterprises ($\bar{x}\pm s$ = 3.12±1.83 log cfu/g). The corresponding values were $\bar{x}\pm s$ = 0.71±0.05 resp. $\bar{x}\pm s$ = 1.25±0.70 log cfu/g for lactic acid bacteria, $\bar{x}\pm s$ = 0.8±02.2 resp. $\bar{x}\pm s$ = 24.2±43.2 MPN/g for *Enterobacteriaceae* and $\bar{x}\pm s$ = 0.6±1.7 resp. $\bar{x}\pm s$ = 24.2±43.2 MPN/g for coliforms. *Pseudomonas* spp. were detected in 11 % of the market samples and none of the dumplings passed the detection limit of 10^2 cfu/g for coagulase-positive staphylococci. The percentage of small enterprises which exceeded the official Thai microbial standards for cooked-chilled food (to be reheated before consumption) for the total amount of aerobic bacteriae and coliforms were 12 % and 11 %, respectively.

Storing at room temperature for 6–8 hours raised the APCs of ready-to-eat *dim sum* for two power of ten and reheating of these items to 69 °C reduced the contamination to the initial level.

However, two main conclusions can be drawn from the results of this exploratory investigation. To enhance the hygienic quality of *dim sum* sold in small enterprises in Chiang Mai the personnel training of employees as well as the governmental food inspection should be improved. Concerning the deep frozen industrial pork dumplings it is only necessary to better monitor the temperature of the freezers located in the superstores and eventually adjust when necessary.

Keywords: *dim sum,* pork dumplings, microbiological status, Thailand

Zusammenfassung

Zur Ermittlung der mikrobiologischen Qualität von *Dim Sum* (mit Schweinefleisch gefüllte Teigtaschen) wurde in Chiang Mai/Thailand eine explorative Erhebung durchgeführt. Das Probenkontingent umfasste 90 tiefgefrorene Packungen von drei industriellen Produzenten sowie weitere 90 verzehrsfertige Proben kleinerer Hersteller (Lebensmittelmärkte, Strassenverkauf und Gastronomie).

Obgleich die tiefgefrorenen *Dim Sum*-Proben zum Zeitpunkt des Ankaufs lediglich eine durchschnittliche Kerntemperatur von $\bar{x}\pm s$ = –5.5±1.8 °C aufwiesen, lag ihre mesophile aerobe Gesamtkeimzahl ($\bar{x}\pm s$ = 1.38±0.77 log KbE/g) signifikant unter den korrespondierenden Werten für Kleinbetriebe ($\bar{x}\pm s$ = 3.12±1.83 log KbE/g). Die entsprechenden Daten lauteten $\bar{x}\pm s$ = 0.71±0.05 bzw. $\bar{x}\pm s$ = 1.25±0.70 log KbE/g für Milchsäurebildner, $\bar{x}\pm s$ = 0.8±02.2 bzw. $\bar{x}\pm s$ = 24.2±43.2 MPN/g für *Enterobacteriaceae* und $\bar{x}\pm s$ = 0.6±1.7 bzw. $\bar{x}\pm s$ = 24.2±43.2 MPN/g für Coliforme. *Pseudomonas* spp. wurden bei 11% der Marktproben gefunden, während keine der Teigtaschen die Nachweisgrenze von10^2 KbE/g für koagulase-positive Staphylokokken überschritt. Der Prozentsatz an Kleinbetrieben, welche über den offiziellen mikrobiologischen Grenzwerten Thailands für vorerhitzte, gekühlte Lebensmittel, die vor dem Verzehr wiedererhitzt werden, lagen, belief sich hinsichtlich der aeroben Gesamtkeimzahl und den Coliformen auf 12 % bzw. 11 %. Aufbewahren bei Raumtemperatur für 6–8 Stunden erhöhte die aerobe Gesamtkeimzahl von verzehrsfertigem Dim Sum um zwei Zehnerpotenzen. Wiedererhitzen auf 69 °C reduzierte die Keimbelastung auf das Ausgangsniveau.

Aus der Studie können zwei wesentliche Schlussfolgerungen abgeleitet werden: Einerseits sollten zur Verbesserung des Hygienestatus der von Kleinbetrieben in Chiang Mai verkauften Teigtaschen sowohl die Personalschulung intensiviert als auch die Überwachung verstärkt werden. Andererseits ist es im Hinblick auf das industrielle Angebot tiefgefrorener Ware lediglich notwendig, die Temperatur der Tiefkühltruhen in den Supermärkten ständig zu kontrollieren und gegebenenfalls zu adjustieren.

Schlüsselwörter: Dim Sum, gefüllte Schweinefleisch-Teigtaschen, Keimstatus, Thailand

Introduction

Dim sum originates from the Cantonese cuisine and comes in form of little bite-sized dumplings, buns, rolls and small appetizer-like dishes containing minced meat or seafood fillings (see fig. 3). Part of *dim sum's* popularity is its low price (Newman 1986).

Pork dumplings are one kind of *dim sum* which are well known in and have become part of the Thai culture. Today this hors d'oeuvre is considered by many locals as an indigenous Thai food. The delicacy looks like a parcel or a little bag consisting of a square sheet of wheat dough filled – in the case of pork dumplings – with minced pork mixed with ingredients such as sugar, pepper, minced garlic, mushrooms, spring onions, carrots and coriander leaves. The ingredients for the pastry are flour, salt, sugar and eggs.

Traditionally, pork dumplings are a heat-treated and ready-to-eat meal manufactured by local producers including street vendors and market people. This meal is also prepared and served in restaurants in tiers of bamboo-steamers or small to medium-sized plates. During the last years the food enterprises have begun to produce deep frozen pork dumplings for distribution in South-East Asian supermarkets. During the industrial processing the product is heated until the core temperature reaches 82 °C and than rapidly frozen down to –18 °C. The deep frozen food must be reheated before it is consumed.

This cross-sectional study was designed to characterize the microbiological profile of pork dumplings at the points of sale in Chiang Mai, Thailand. Except for a study on the incidence of *Listeria monocytogenes* in Taiwanese *dim sum* samples (Wong et al. 1990) this seems to be the first report about the bacteriological status of pork dumplings for the South-East Asian region. Here we compare the microbiological profile of industrial products with products from small enterprises. Furthermore, the effect of reheating for consumption on the microbiological load of dumplings was quantified. As indicator bacteria for the microbiological quality and the hygienic status, the number of mesophilic aerobic bacteria, lactic acid bacteria, *Enterobacteriaceae,* coliforms, *Pseudomonas* spp. and coagulase-positive staphylococci were determined.

Material and methods

Sampling design

From December 2006 to April 2007 altogether 180 samples of *dim sum* have been arbitrarily selected in Chiang Mai, Thailand. To represent industrial products 30 packs of deep frozen pork dumplings of each of the three brands on sale were bought in different lots from five superstores. Additionally, 10 parallel samples were selected from each brand to study the effect of reheating. The 90 samples of small enterprises were also divided into three subgroups: 30 samples of ready-to-eat pork dumplings were collected from 30 street vendors, who normally sell 10–20 items in a plastic bag. 10 parallel samples were stored at room temperature for 6–8 hours and reheated to simulate the behaviour of the consumer. The same procedure was applied to 30 samples from 30 vendors in 18 markets as well as to 30 pork dumpling samples from 30 different restaurants. The design of the detailed sampling plan is given in fig. 1. For reheating, the samples were heated to a core temperature of 68.7 ± 8.5 °C for a few minutes.

Microbiological analysis

For the microbiological analysis 25 g of a sample was added to 225 ml of sterile 0.1 % peptone water and homogenized in a stomacher. According to ISO 6887-1 a serial decimal dilution was done.

Total aerobic mesophilic bacteria

As nutrient medium for plating and enumeration of total aerobic mesophilic bacteriae the Plate Count Agar (PCA, Merck®) was used. The duplicated pour plates were incubated at 30°C for 48 hours under aerobic conditions (ISO 4833).

Mesophilic lactic acid bacteria

To enumerate the mesophilic lactic acid bacteria the MRS medium (de Man, Rogosa and Sharpe, Merck®) was employed. Anaerobic conditions were realized by double layer and the incubation lasted for 48 hours at 30 °C (ISO 15214).

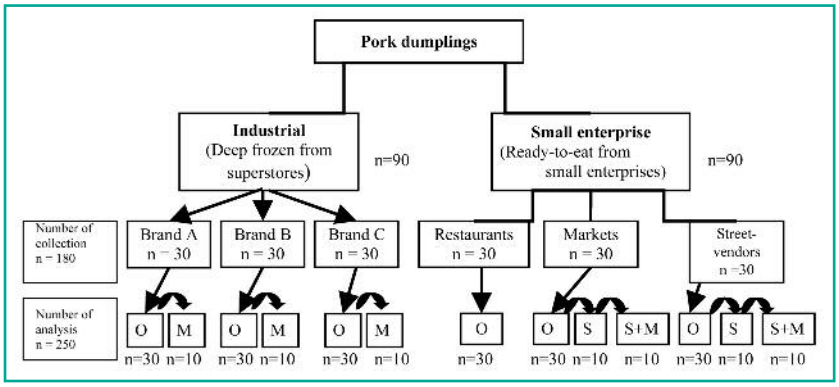

FIGURE 1: *Flow chart of sampling design (microbiological quality of dim sum). O = Original samples, S = parallel samples/stored at room temperature (6–8 h), M = parallel samples/microwave heated.*

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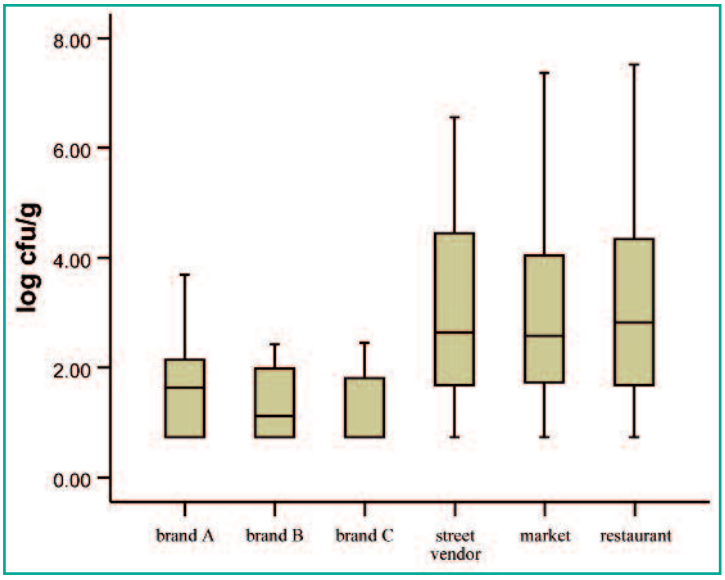

FIGURE 2: *Box plots of total aerobic bacteria counts in dim sum sold in Chiang Mai, Thailand.*

Pseudomonads

CFC selective agar (Merck®) which contains cetrimide, fucidin and cephalotin was used for the enumeration of pseudomonads (ISO 13720). All colonies grown on duplicate spread plates (incubation 25 °C/48 h) were suspected *Pseudomonas* spp. For confirmation 5 colonies were subcultured on Nutrient Agar (Merck®). When they developed a positive oxidase reaction and showed growth only on the surface of Kligler s Double Sugar Iron Agar (Merck®) containing lactose and glucose, the result was judged to be positive for *Pseudomonas* spp.

Coagulase-positive staphylococci

A duplicate spread plate method was performed to count coagulase-positive staphylococci using Baird-Parker Agar (BPA, ISO 688-1). The BPA (Merck®) was prepared by adding sterile egg yolk to a tellurite emulsion (Bacto™, Difco®). After incubation at 37 °C for 48 hours, the coagulase test was applied to black-grey, bright, smooth colonies with clear zones. These typical colonies were transferred to a tube of Brain-Heart Infusion broth (Merck®) and incubated at 37 °C for 24 hours. After that, 0.1 ml of each culture was added to 0.3 ml of rabbit plasma (Bactident®-Coagulase, Merck®) and incubated in a water bath at 37 °C. The coagulase test was regarded as positive, if the volume of the clot occupied more than three quarters of the original volume of the liquid after at least 24 hours.

Enterobacteriaceae and coliforms

The numbers of *Enterobacteriaceae* were determined by the three tubes most probable number (MPN) method (ISO 7402). 3 x 3 tubes containing E. E. broth (Buffered Brilliant Green Bile Glucose broth, Oxoid®) were inoculated with 1.0, 0.1 and 0.01 g of the sample material respectively and incubated at 37 °C for 24 hours. Than a loopful of every broth was streaked on VRBG (Violet Red Bile Glucose Agar, Oxoid®) and incubated 24 hours at 37 °C. Five of the typical pink to red colonies or colourless mucoid colonies were randomly selected for biochemical confirmation after subculturing in Nutrient Agar (NA, Merck®). The number of tubes from which after streaking colonies were produced showing a negative reaction in the oxidase test and a positive fermentation test (Glucose-Agar with bromcresol purple as indicator) was registered as positive for *Enterobactericeae* and used for the calculation of the most probable number.

The three tube MPN was also applied to determine the number of coliforms (ISO 4831). Lauryl Sulphate Tryptose broth (Merck®) was used as selective enrichment medium (incubation 37 °C/48 h) and Brilliant Green Lactose Bile broth (Merck®) as confirmation medium (incubation 37 °C/24 h). The number of tubes which showed gas formation and produced typical colonies on the confirmation medium was rated as positive.

Temperature, pH and sensory analysis

Additional analyses covered temperature measurements in the core of the samples with a food grade thermometer (DeltaTRAK®) and pH value determinations with a food grade pH meter (CyberScan, EUTECH). A simple descriptive sensory analysis was also carried out.

Statistical analysis

The statistical software SPSS 14.0 for Windows was used for statistical analysis. Descriptive statistics were done on the basis of log transformed microbiological counts. Depending on the nature of data Mann Whitney U test, Kruskal-Wallis test, Wilcoxon Signed Rank test or Friedman test were applied for comparisons among different data sets.

FIGURE 3: *Dim sum.*

Results

Microbiological status

Box plots to graphically present measures of central tendency and variability of total bacteria counts are shown in fig. 2, other descriptive statistics of the mesophilic bacteria counts are given in tab. 1 and tab. 2. The medians of these total aerobic bacteria numbers were $x._5$=1.09 log cfu/g for the industrial samples and $x._5$=2.64 log cfu/g for the small enterprise samples, the difference being statistically significant at the $p \leq .05$ level. In 48,9 % of the industrial and 7,8 % of the small enterprise samples $<10^1$ cfu/g have been determined. However, 28.9 % of the small enterprise samples showed an aerobic plate count of 10^4 cfu/g and higher. In addition to that, slightly but not significantly different

TABLE 1: *Descriptive statistics of the microbiological parameters of pork dumplings sold in Chiang Mai, Thailand.*

Parameter	source	n	Arithm. Mean	Median	SD	IQR	Min.	Max.	Range
Aerobic plate count (log cfu/g)	industrial	90	1.38	1.09	0.77	1.28	0.70*	3.66	2.96
	small enterprise	90	3.12	2.64	1.83	2.53	0.70*	7.49	6.79
Lactic acid bacteria (log cfu/g)	industrial	90	0.71	0.70*	0.05	0.00	0.70*	1.18	0.48
	small enterprise	90	1.25	0.70*	0.70	0.07	0.70*	5.27	4.57
Enterobacteriaceae (MPN/g)	industrial	90	0.8	0.2*	2.2	0.0	0.2*	15.0	14.8
	small enterprise	90	24.2	0.2*	43.2	23.7	0.2*	111.0	110.8
Coliforms (MPN/g)	industrial	90	0.6	0.2*	1.7	0.0	0.2*	9.3	9.1
	small enterprise	90	13.8	0.2*	35.0	0.2	0.2*	111.0	110.8
Pseudomonas spp. (log cfu/g)	industrial	90	1.70*	1.70*	0.00	0.00	1.70*	1.70*	0.00
	small enterprise	90	1.83	1.70*	0.40	0.00	1.70*	4.10	2.40
coagulase-positive staphylococci (log cfu/g)	industrial	90	1.70*	1.70*	0.00	0.00	1.70*	1.70*	0.00
	small enterprise	90	1.70*	1.70*	0.00	0.00	1.70*	1.70*	0.00

SD = Standard Deviaton, IQR = Interquartile Range, * half of the detection limit = not detected

arithmetic means and medians existed between the three brands of industrial items as well as between the three sources of small enterprise samples. Small differences between mean and median indicate a symmetrical distribution of the parameters.

As shown in tab. 1 and 2, the lactic acid bacteria counts of industrial pork dumplings have been very low and only one sample passed the line of the detection limit. The arithmetic mean of the small enterprise *dim sum* samples was 1.25 log cfu/g, with 26 of 90 samples showing $>10^1$ cfu/g.

As more than 50 % of the samples showed zero values in MPN determination (tab. 3) – zero values were transformed to a value representing half of the detection limit – a wide gap between the arithmetic mean and the median of the *Enterobacteriaceae* counts was found as shown in tab. 1. When using the median, no significant difference was detected between the three sources of small enterprise samples. However, when comparing on the basis of the mean values the market samples ($\bar{x}$=9.0 MPN/g) differed significantly ($p \leq .05$) from these of the street vendors ($\bar{x}$ =32.2 MPN/g) and the restaurants ($\bar{x}$=31.5 MPN/g).

However, on a slightly lower contamination level the distribution of coliforms was similar to the profile seen in *Enterobacteriaceae* (tab. 1 and 2). Higher arithmetic means of the coliform counts were found in brand C when compared with brand A and B as well as in street vendor and restaurant samples when compared with market samples.

Pseudomonas spp. counts above the detection limit of 10^2 cfu/g were not found in the industrial *dim sum* samples; only in 10 of 90 small enterprise samples *Pseudomonas* was detected (tab. 2) with two of these dumplings even reaching 10^4 cfu/g.

In none of the samples a contamination with coagulase-positive staphylococci could be found using the detection limit of 10^2 cfu/g.

Temperature, pH and sensory analysis

Though legislation demands a maximum temperature of –18 °C (with short deviations up to –15 °C) for deep frozen food, arithmetic mean and standard deviation of the temperature of the deep frozen *dim sum* samples from industrial origin were $\bar{x}\pm s$ = –5.5±1.8 °C. At the point of selling the small enterprise samples showed a temperature of $\bar{x}\pm s$ = 60.7± 10.4 °C with no significant difference between the average temperature of samples from street vendors ($\bar{x}$ =61.2 °C), markets ($\bar{x}$=62.7 °C) and restaurants ($\bar{x}$ =58.3 °C).

The average pH value of the industrial samples was $\bar{x}\pm s$ = 6.76±0.24. The average pH of small enterprise samples was $\bar{x}\pm s$ =7.33±0.64 with the mean of the street vendor samples of $\bar{x}$=7.60 being slightly higher than the means of the market ($\bar{x}$=7.25) and the restaurant samples ($\bar{x}$=7.15).

Concerning the descriptive sensory test only two samples of market enterprises were found to have a deviation from the normal sensory profile: the pork dumplings had turned to rancidity and showed a soft texture.

Storage and reheating experiments

During the reheating of the selected deep frozen dumplings the temperature raised from $\bar{x}\pm s$ = –6.2±1.8 °C to

TABLE 2: *Distribution of microbiological counts in cfu/g for dim sum sold in Chiang Mai, Thailand.*

Microorganism	Number of samples in the following range (cfu/g)							
	<10	$<10^2$	$<10^3$	$<10^4$	$<10^5$	$<10^6$	$<10^7$	$<10^8$
Aerobic plate count (ind.)	44	25	18	3	0	0	0	0
Aerobic plate count (s. e.)	7	23	21	13	11	4	6	5
Lactic acid bacteria (ind.)	89	1	0	0	0	0	0	0
Lactic acid bacteria (s. e.)	64	11	6	4	4	1	0	0
Pseudomonas spp. (ind.)	–	90	0	0	0	0	0	0
Pseudomonas spp. (s. e.)	–	80	6	2	2	0	0	0
Coagulase+ staphylococci (ind.)	–	90	0	0	0	0	0	0
Coagulase+ staphylococci (s. e.)	–	90	0	0	0	0	0	0

ind. = industrial samples (n = 90), s. e. = small enterprise samples (n = 90)

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TABLE 3: *Distribution of microbiological counts in MPN/g for dim sum sold in Chiang Mai, Thailand.*

Microorganism	Number of samples in the following range (MPN/g)			
	<0.3	0.36–9.3	15–110	>110
Enterobacteriaceae (ind.)	82	7	1	0
Enterobacteriaceae (s. e.)	49	17	10	14
Coliforms (ind.)	82	8	0	0
Coliforms (s. e.)	61	16	4	9

ind. = industrial samples (n = 90), s. e. = small enterprise samples (n = 90)

TABLE 4: *Official microbiological Thai standards for cooked-frozen or cooked-chilled foods that have to be reheated before consumption.*

Microorganism	Types of food	
	Chilled food	Frozen food
Total aerobic bacteria (cfu/g)	$<10^6$	$<10^5$
coliforms (MPN/g)	<100	<100
Staphylococcus aureus (cfu/g)	<100	<50
Escherichia coli (MPN/g)	<3	<3
Bacillus cereus (cfu/g)	<100	<50
Clostridium perfringens	not present in 0.01 g	not present in 0.01 g
Vibrio parahaemolyticus	not present in 25 g	not present in 25 g
Salmonella	not present in 25 g	not present in 25 g

60.7±10.4 °C. This treatment caused on average a –0.32 log cfu/g reduction of the mesophilic aerobic plate count. In the cases of lactic acid bacteria, *Enterobacteriaceae,* coliforms, pseudomonads and coagulase-positive staphylococci no particular effect could be observed as the initial contamination was anyhow near or below the detection limit.

The average temperature of the small enterprise samples decreased from $\bar{x}\pm s$ = 61.7±10.3 °C at the moment of purchasing to 20.8±2.1 °C throughout a 6–8 h storage at ambient temperature and raised again to 68.7±8.5 °C during reheating. During the two steps of the experiment the mesophilic aerobic plate count raised from $\bar{x}\pm s$ = 3.54±1.95 log cfu/g to 5.16±1.69 log cfu/g and dropped to 3.81±1.82 log cfu/g due the following heat treatment. For the corresponding results of the other microorganisms the median $x._5$ is much more suited because this parameter is more independent of zero values than the arithmetic mean. Lactic acid bacteria, *Enterobacteriaceae* and coliforms showed a similar behaviour. Starting with $x._5$ under the detection limit a propagation by the factor of hundred happened during storage, followed by a 100-fold decline because of reheating. All the results of the pseudomonads and coagulase-positive staphylococci counts remained under the detection limit except two samples which contained 49 and 170 coagulase-positive staphylococci/g after storage.

Discussion

In this study, a significant difference concerning hygienic indicators was observed between industrial pork dumplings and small enterprise samples. This can be explained as the bacteriostatic effect of freezing of the industrial food immediately after processing. Furthermore, some microorganisms – mainly gram-negative ones – are slowly inactivated or destroyed in a frozen status (Chatopadhy 2000). This has been shown for coliforms (Gunderson and Rose 1948, Oliveira et al. 2009, Reinartz et al. 2010) and *E. coli* (Larkin et al. 1955, Marcy and Adam 1979). It seems to be valid also for micrococci and staphylococci (Prados et al. 2006), but not for moulds (Brown and Hill 1984), lactic acid bacteria (Prados et al. 2006), streptococci (Larkin et al. 1955), *Listeria monocytogenes* (Palumbo and Williams 1991) and EHEC O157:H7 (Doyle and Schoeni 1989).

Another reason for the better hygienic status may be that the industrial production implements advanced hygienic programs and safety assurance systems such as Good Hygienic Practices (GHP) and Hazard Analysis Critical Point (HACCP). The plants which establish these systems are able to minimize the microbiological load due to general hygiene points and suitable critical control points. At the processing line of one of the enterprises from which samples entered this study five critical control points for biological and physical hazards are installed: raw material selection, foreign body elimination, heating to 82 °C core temperature, freezing down to –18 °C within 1.5 hours and metal detection (Chaissowong 2007). Three of these CCPs help to minimize the microbiological contamination. Incoming raw material, especially raw meat may raise the initial bacterial load. Certified suppliers and strict criteria for raw material with low microbiological counts and absence of pathogens could greatly contribute to the hygienic quality and safety of the final product. The thermal treatment with a pasteurizing effect is important for the safety as well as cooling down the dumplings to –18 °C. However, in the superstores the dumplings are offered at temperatures of $\bar{x}\pm s$ = –5.5±1.8 °C. This temperature is low enough to prevent the growth of pathogenic bacteria but enhanced deterioration may occur because of the activity of some bacterial enzymes and the growth of psychrophilic spoilage microorganisms (Sinell 2004).

The high number of total aerobic counts, coliforms and *Enterobacteriaceae* found in small enterprise samples is an indication of insufficient hygienic and sanitary applications along the entire chain of production including farm, slaughter house, production, transport and selling. Especially poor food hygiene at the point of selling contributes to the high counts of microorganisms, including factors such as varying temperatures during storage, inadequate cooking and contaminated equipment. The necessity to improve the hygiene quality of small enterprise *dim sum* becomes evident if the suggested levels for the microbiological standards of the the Food and Drug Administrative of the Ministry of Public Health, Thailand for cooked-frozen or cooked-chilled food that have to be reheated before consumption, i. e. dumplings or pizza, are applied. These standards prescribe total aerobic bacteria counts of less than 5 log cfu/g in the case of frozen pork dumplings and less than 6 log cfu/g in the case of ready-to-eat pork dumplings. All of the industrial samples were found to have APCs below the indicated level. However, 12 % (11/90) of the small enterprise dumplings exceeded the corresponding limit. Concerning the coliforms and their official level < 100 MPN/g, none of the industrial samples had to be rejected but 11 % (10/90) small enterprise items. Contamination with *Staphylococcus aureus* never passed the official limit of 2 log cfu/g.

The laboratory experiments lead to the expected results. Storing at room temperature for 6–8 hours caused a nearly two log-cycles increase of APCs. During reheating to 69 °C

the counts dropped down to the initial level. In two cases coagulase-positive staphylococci were detected after storage. This finding illustrates the risk of breaking the cooling chain. Reheating to 61°C of deep frozen samples produced 0.3 log-cycle reduction only. Besides the relatively low heating temperature this weak effect may also be explained by the inherent nature of the difference between the calculation of the mean and median value of a sample when too many negative results ("zero values") are encountered.

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