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Bacteriological evaluation of dried prawn powder and pickled fish available from some local markets in Yangon

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Bacteriological analysis and the assessment of the effect of heat treatment on decontamination of bacterial pathogens were done on 20 samples of dried prawn powder and pickled fish collected from local markets in Yangon from July 2007 to March 2008. Coliform count, thermotolerant coliform count, viable bacterial count and detection of contaminated pathogenic bacteria were carried out according to standard microbiological techniques. Coliform count of 46 to 540 most probable number (MPN)/100 ml and thermotolerant /faecal coliform count of 23 to 70 MPN/100ml were detected in all tested dried prawn powder samples. Coliform count of 240 to >2400 MPN/100 ml and thermolerant coliform count of 17 to >2400 MPN/100 ml were detected in all tested pickled fish samples. All dried prawn powder and pickled fish samples had viable bacterial counts of 10^3 colony forming unit (CFU)/ml to uncountable numbers (>10^5 CFU/ml). Coliform, thermotolerant coliform and viable bacterial counts were again detected after heating the dried prawn powder samples up to 70-80˚C for 3 minutes and steaming the pickled fish samples at 90^{o}C for 15 minutes. After heat treatment, in all tested samples, the coliform count was reduced to <2 to 8 MPN/100ml and thermotolerant coliform count was reduced to <2 MPN/100 ml. Bacterial pathogens such as Enteropathogenic *Escherichia coli*, *Klebsiella spp*., *Citrobacter frundii*, *Proteus spp.* and *Staphylococcus spp.* were isolated from the 60% of dried prawn powder samples and 85% of pickled fish samples. After heat treatment, the pathogenic bacteria were not isolated in all contaminated dried prawn powder samples and in 88.2% of contaminated pickled fish samples. This study highlighted the poor microbiological quality of dried prawn powder and pickled fish available in local markets in Yangon and advantage of heat treatment in decontamination of pathogenic bacteria in food.

INTRODUCTION

Food plays a major role in transmission of diseases and food-borne illness is one of the major health problems. The food-borne illness is characterized by disturbance in the gastrointestinal tract including abdominal pain, diarrhoea and sometimes vomiting. Common food-borne diseases are diarrhoea, dysentery, cholera, typhoid, hepatitis and food poisoning [1]. Common bacterial pathogens which have been identified as the cause of food-borne diseases include *Bacillus cereus, Brucella species, Campylobacter jejuni, Clostridium botulinum, Clostridium perfringens,* enteropathogenic *Escherichia coli* (EPEC), enteroinvasive *Escherichia coli* (EIEC), enterotoxigenic *Escherichia coli* (ETEC), enterohemorrhagic *Escherichia coli* (EHEC), *Listeria monocytogenes, Salmonella* species, *Shigella species, Staphylococcus aureus, Vibrio cholerae*, *Vibrio parahaemolyticus* and *Yersinia enterocolitica* [2].

Current statistics for food-borne illnesses in various indrustrialized countries showed that up to 60% of cases were due to poor food

handling techniques in food service establishments. In Myanmar, the notifiable food-borne diseases include diarrhoea, dysentery, typhoid and paratyphoid fevers. It has been estimated up to 70% of diarrhoea cases were due to contaminated food [3].

Microbiological quality of ready-to-eat food is determined by: viable colony count and detection of indicator organisms in a particular food. Viable colony count is a count of bacteria which includes those that occur naturally in most food and those present through contamination. The count increases significantly over time in response to poor temperature control of a product. It indicates the quality and potential keeping quality (freshness) of the product.

Indicator bacteria are usually present in faeces of human and other warm-blooded animals. Detection of these organisms demonstrates the contamination of faecal matter and the possibility of presence of intestinal pathogens in food. Coliforms mainly consist of microorganisms of faecal origin and those containing in soil and vegetation. Thermotolerant (Faecal) coliforms include mainly the genera *Escherichia*, *Enterobacter*, *Citrobacter* and *Klebsiella*. Measurement of coliform count, faecal coliform count, total bacterial count and detection of pathogenic microorganisms are necessary in determining the safety of food [4].

Dried prawn powder is widely used among Myanmar people and an ingredient in preparation of various kinds of salads (Letthoke). Most of the sellers use ready-made dried prawn powder sold by vendors of local markets where poor standard of environmental and personal hygiene exists. Pickled fish is a favorite side dish in Myanmar meals. It is a kind of fermented salted fish and usually sold by vendors and road side food stalls in markets. Most people used to prepare it as it is or after washing with water and eat as salad after mixing with onion, chilli, garlic, oil and fish sauce. The profile of pathogenic bacterial contamination in dried prawn powder and pickled fish needs to be explored as they can act as one of the vehicles for transmission of food-borne diseases.

Bacteria are killed if exposed to temperatures above 62.8˚C for long enough [5]. As heat can kill majority of microorganisms, heated samples are needed to be tested to determine whether heating can reduce the contaminated microorganisms. Thus microbiological analysis of dried prawn powder and pickled fish sold in local markets in Yangon and the assessment of the effect of heat treatment on decontamination of bacterial pathogens may provide valuable data in formulating strategies to prevent the occurrence food-borne illnesses.

MATERIALS AND METHODS

Twenty samples of dried prawn powder and pickled fish were collected from the local markets in South Okkalapa Township, North Okkalapa Township, Latha Township, Dagon Township and Yankin Township from July 2007 to March 2008. They were collected in sterile plastic bags and transported to Bacteriology Research Division, Department of Medical Research (LM) to perform laboratory procedures. Each sample was thoroughly mixed, homogenized and divided into 2 parts to perform laboratory tests in two sets for heated and unheated samples.

Laboratory tests for unheated samples:

Determination of viable bacterial count

A 20-gram sample was mixed with 180 ml of sterile phosphate buffered saline and then homogenized. A ten-fold serial dilution in normal saline was done by using 1 ml of homogenized suspension. Viable bacterial count was determined by surface spread method [6].

Detection of indicator bacteria

Coliform and thermotolerant/faecal coliform counts were determined by multiple tube method according to the WHO guidelines for drinking water quality, 1997 [7]. The most probable number (MPN) of bacteria present

in the samples was statistically interpreted by Mc Crady Table.

Isolation of pathogenic bacteria in unheated samples

An approximately 60 ml of homogenized samples was centrifuged at 0˚C for 15 min and primary isolation was done on Nutrient agar, MacConkey agar, Salmonella-Shigella agar, Manitol Salt agar and Thiosulphate Citrate Bile Salt Sucrose agar. Simultaneously, Selinite F broth and alkaline peptone water were used for secondary isolation. Suspected colonies were inoculated onto short set of biochemical reaction for identification using Triple Sugar Iron agar, Lysine Iron agar, Urea agar and Sulphide Indole Motility agar [8].

Laboratory tests for heated samples

Another set of dried prawn powder and pickled fish samples were treated by heating the dried prawn powder samples at 70-80˚C for 3 min and steaming the pickled fish samples at 90^{o}C for 15 min. Viable bacterial count, coliform, faecal coliform count and detection of pathogenic bacteria were performed by the same laboratory procedure as described above.

RESULTS

Total viable bacterial count

Total viable counts of unheated and heated 20 dried prawn powder samples and pickled fish samples are shown in Table 1. It was found that all unheated samples had total viable bacterial count in uncountable numbers (>10^5 CFU/ml). Heating reduced the count 10^3 to10^4 lower than the count in unheated samples.

Coliform and thermotolerant coliform count of unheated and heated samples

Coliform count and faecal coliform count from 20 unheated and heated samples, expressed in MPN/100 ml are shown in Table 2 and 3. In dried prawn samples, coliform bacteria ranging from 22 to >2400 MPN/100 ml were detected in all unheated samples and thermotolerant coliforms ranging from <2 to 70 MPN/100 ml were detected in 80% (18/20) of unheated samples. After heating at 70-80˚C for 3 min, the coliform counts were reduced to <2-8 MPN/ml and thermotolerant coliform counts were reduced to <2-7 MPN/100ml.

Table 1. Total viable bacterial count CFU/ ml of dried prawn powder and pickled fish samples

Township	Sam-ple	Dried prawn samples		Pickled fish samples	
		Unheated	Heated	Unheated	Heated
South Okkalapa	1	UC	5×10^2	UC	5×10^3
	2	7×10^4	1.5×10	UC	6×10^2
	3	3×10^4	2×10^2	UC	7×10^2
	4	5×10^5	1×10^3	UC	9×10^2
North Okkalapa	5	UC	7×10^2	UC	1×10^2
	6	UC	1×10^2	UC	6×10^2
	7	UC	2×10^3	UC	3×10^2
	8	6×10^4	1×10^2	UC	2×10^3
Latha	9	UC	1×10	UC	1.5×10^3
	10	7×10^4	3×10^2	UC	1×10^2
	11	4×10^4	2×10^2	UC	9×10^2
	12	7×10^4	1×10^2	UC	4×10^5
Yankin	13	UC	1.5×10	UC	1×10^3
	14	UC	5×10^3	UC	5×10^3
	15	UC	5×10^2	UC	5×10^2
	16	UC	7×10^4	UC	7×10^4
Dagon	17	UC	2×10^3	UC	2×10^3
	18	UC	7×10^2	UC	3×10^2
	19	2×10^4	1×10^2	UC	1×10^2
	20	3×10^5	1×10^3	UC	1×10^3

UC= Uncountable (>10^5CFU/ml)

In pickled fish samples, coliforms and thermotolerant coliforms were detected in high count in all unheated samples. After steaming, the coliform count reduced to <2-240 MPN/100 ml and faecal coliform count reduced to <2-23 MPN/100 ml.

Isolation of contaminated pathogenic bacteria

Among 20 samples of unheated dried prawn powder samples, the pathogenic bacteria were isolated from 60% (12/20) of tested samples; *Escherichia coli* (4/20), *Escherichia coli* and *Citrobacter freundii* (1/20),

Table 2. Coliform and thermotolerant coliform counts of tested unheated and heated dried prawn powder samples (n=20)

Township	Sample	Coliforms MPN/100ml		Thermotolerant coliforms MPN / 100ml	
		Unheated	Heated	Unheated	Heated
South Okkalapa	1	46	<2	2	<2
	2	240	8	70	4
	3	110	8	6	<2
	4	140	4	12	<2
North Okkalapa	5	350	<2	2	<2
	6	350	4	24	<2
	7	220	4	7	<2
	8	130	8	9	<2
Latha	9	>1800	8	23	7
	10	540	<2	33	<2
	11	540	8	4	<2
	12	920	8	<2	<2
Yankin	13	540	<2	23	<2
	14	130	<2	7	<2
	15	350	4	4	<2
	16	79	<2	4	<2
Dagon	17	33	<2	<2	<2
	18	22	<2	<2	<2
	19	49	<2	2	<2
	20	94	<2	2	<2

Table 3. Coliform and hermotolerant coli form counts of tested unheated and heated pickled fish samples (n=20)

Township	Sample	Coliforms MPN/100gm		Thermotolerant coliforms MPN / 100gm	
		Unheated	Heated	Unheated	Heated
South Okkalapa	1	>2400	350	>2400	13
	2	>2400	7	220	<2
	3	>2400	9	350	<2
	4	>2400	3	170	<2
North Okkalapa	5	>2400	<2	23	<2
	6	>2400	2	44	<2
	7	>2400	<2	23	<2
	8	>2400	3	37	<2
Latha	9	>2400	240	>2400	23
	10	>2400	4	26	<2
	11	>2400	2	70	<2
	12	240	2	22	<2
Yankin	13	1600	9	23	<2
	14	>2400	34	240	11
	15	540	<2	11	<2
	16	1600	7	23	<2
Dagon	17	920	5	17	<2
	18	350	3	14	<2
	19	>2400	240	110	11
	20	1600	9	26	<2

Escherichia coli and *Staphylococcus albus* (1/20), *Klebsiella* spp. (4/20), *Proteus mirabilis* (1/20) and *Staphylococcus aureus* (1/20).

The pathogenic bacteria were not isolated in all contaminated dried prawn powder after heating the samples at 70-80˚C for 3 min. Among 20 samples of unheated pickled fish samples, pathogenic bacteria were isolated from 85% (17/20) of tested samples; *Escherichia coli* (6/20), *Citrobacter freundii* (2/20), *Escherichia coli* and *Citrobacter diversus* (1/20), *Klebsiella* spp. (3/20), *Proteus mirabilis* (2/20), *Serratia* spp. (1/20), *Staphylococcus aureus* (1/20) and *Baccillus* spp. (1/20). Some 88.2% (15/17) of pickled fish samples which showed pathogenic bacterial contamination were found to be free of pathogenic bacteria after steaming at 90˚C for 15 min. One *Bacillus* spp. contaminated sample and one *Klebsiealla* spp. contaminated sample were found to be still contaminated with these bacteria after steaming.

Serotyping of isolated E. coli strains

Serotyping was done on 6 *E. coli* strains isolated from dried prawn powder samples and 7 *E. coli* strains isolated from pickled fish samples. In those of dried prawn samples, 2 isolates were ETEC (serotype O8 K25 and O25K+) and one isolate was EPEC (serotype O86 K61). In those of pickled fish samples, one isolate was EPEC (serotype O1 K51), two isolates were EIEC (seotype O124 K72 and O114 K90) and two isolates were ETEC (serotype O6 K15, 0159 K+).

DISCUSSION

Microbiological analysis of food consists of determining coliform and thermotolerant/ faecal coliform count, total viable count and isolation of pathogenic bacteria from the representative samples. Satisfactory microbiological quality means when food is free from pathogens and the indicator bacteria are within the permissible range.

In the present study, coliform count of 46 to 540 MPN/100 ml and thermotolerant coliform count of 23 to 70 MPN/100ml were detected in all tested dried prawn powder samples. Coliform count of 240 to >2400 MPN/100 ml and thertolerant coliform count of 17 to >2400 MPN/100 ml were detected in all tested pickled fish samples. All dried prawn powder and pickled fish samples had viable bacterial count of 10^3 CFU/ml to uncountable numbers (>10^5 CFU/ml).

The average level of bacterial count which can give rise to illness is about >10^5 CFU/ml. Some virulent bacteria eg. *Salmonella* spp. causing typhoid can cause disease when 10 CFU/ml of organisms are ingested. It showed that dried prawn powder and pickled fish can be a potential source of food-borne illness if they are contaminated with infectious dose of pathogenic bacteria.

Bacteria were killed if exposed to temperatures above 62.8˚C for long enough. Non-spore forming bacteria like enterobacterieae, salmonella, shigella, staphylococcus spp. were killed at 62.8˚C for 3 min [5]. After heating the dried prawn powder samples up to 70-80˚C for 3 min, the coliform count reduced to <2 to 8 MPN/100ml and thermotolerant coliform count reduced to <2-7 MPN/100 ml. After steaming the pickled fish at 90˚C for 15 min, the coliform counts were reduced to <2-240 MPN/100 ml and faecal coliform count reduced to <2-23 MPN/100 ml.

All unheated samples had total viable bacterial count in uncountable numbers (>10^5 CFU/ml) to (>10^3-10^4 CFU/ml). Heating of the samples reduced the total viable bacterial count to 10^3-10^4 lower than the count in unheated samples. According to the microbiological limits for various food determined by the New Zealand Standard 1998, recommended total standard plate count is 10^4 to 10^5 CFU/ml and thermotolerant coliform count is zero for ready-to-eat food. Thus, heating the dried prawn powder at 70-80˚C for 3 min and steaming the pickled fish at 90˚C for 15 min can reduce the bacterial count and make the food quality to a fairly safe level.

This finding is comparable with a study carried out on bacteriological profiles of mohinga (rice noodles with fish soup) which showed significant reduction of the number of bacteria contaminated in mohinga noodles after mixing with hot fish soup [9]. However, there is risk of bacteria recontamination of samples even after heating. Thus, proper processing, handling and storage of food before consuming play a major role in prevention of food-borne diseases. The pathogenic bacteria were isolated from 60% (12/20) of dried prawn samples and 85% of pickled fish samples.

Mensah *et al.* described that the container in which the food was served was also important and the use of paper and leaves increased the risk of contamination [10]. Khin Nwe Oo *et al.* reported that enteric bacteria were isolated from flies, cooked children's foods, drinking water, currency notes and vegetables [11].

In this study, all the samples were taken from local markets with poor hygienic condition where food is sold without covers; the microorganisms can be contaminated from improper handling, uncleaned utensils and containers, contaminated water, dusts, insects and exposure to flies.

Serotyping of isolated *Escherichia coli* strains showed O8 K25, O25 K+, O86 K61, O1 K51, O124 K72, O159 K+, O114 K90 and O6 K15 strains which related to two EPEC, two EIEC and four ETEC. These pathogenic types of *E. coli* can cause serious type of diarrhoea in children and adults.

All dried prawn powder samples and 88.5% of pickled fish samples which showed pathogenic bacterial contamination in unheated samples were found to be free of pathogenic bacteria after heat treatment.

One *Bacillus* spp. contaminated sample and one *Klebsiella* spp. contaminated sample were found to be still contaminated with these bacteria after steaming at 90˚C for

15 min. Although heating at 62.8˚C for long enough can kill non-spore forming bacteria, spore forming bacteria like *Bacillus* spp. can resist the steaming temperature. One sample which showed *Klebsiella* spp. contamination even after steaming was found to have heavy initial bacterial load, thus steaming cannot kill all the pathogenic bacteria. This study highlighted the poor microbiological quality of dried prawn powder and pickled fish available in markets in Yangon and the findings contribute the scientific information on the benefits of proper heating of food before consuming.

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