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Prevalence of toxigenic *Clostridium perfringens* strains isolated from dried spur pepper in Thailand

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Abstract

The microbiologcal quality of dried spur pepper (*Capsicum annuum* Linn. Var *acuminatu*m Fingerh.), taken from 6 provinces in Thailand, was investigated. High numbers of total mesophiles and high yeast and mold counts were found. One hundred samples contained non proteolytic and proteolytic anaerobic spore-formers at 100% and 83%, respectively, while *Clostridium perfringens* was detected at 86% (as <3 log CFU/g). The medium, positive correlation of non proteolytic anaerobic spore-formers to *C. perfringens* (r=0.439) may be proposed as an index for monitoring *Clostridium* in foods. All 160 isolates had the cpa gene, but not cpe genes representing *C. perfringens* type A, but they were unable to produce enterotoxin. The quality and safety of Thai dried spur pepper did not comply with the National Standard and ICMSF; moreover the presence of *C. perfringens* may limit its acceptability for the EU market. Local and export producers must apply hygienic practices in their processing to produce ingredients that are safe for human consumption.

Introduction

Dried spur pepper may be exposed to a wide range of microbial contamination during pre- and post-harvest, and such contamination may further occur during processing, storage, distribution, sale or use (McKee, 1995; Sagoo *et al*., 2009). Among the important pathogenic bacteria, *Clostridium perfringens* has been reported as a contaminant in dried pepper or chili, chili paste and other chili products. The amount of *C. perfringen*s cells or spores in dried pepper or chili has been reported in many countries such as the United States, Argentina, India, the United Kingdom and Thailand, with a range of 1.0 to 6.0 log CFU/g (Strong *et al*., 1963; Stonsaovapak *et al*., 1996; Banerjee and Sarka, 2003; Aguilera *et al.*, 2005; Sagoo *et al*., 2009). Illnesses caused by *C. perfringens* include nausea, vomiting, abdominal cramps and diarrhea.

Since most patients recover from the symptom within 2–3 days, only a few cases are reported as fatalities (Bureau of Epidemiology, 2015). Based on the toxigenicity, clinical isolates *C. perfringens* have been classified into five types (i.e. A, B, C, D and E) with the representation of gene coding for alpha *(cpa)*, beta *(cpb)*, epsilon *(etx)*, iota *(iA)* and enterotoxin *(cpe)* (Heikinheimo and Korkeala, 2005). All types of the toxigenic strain of *C. perfringens* produce alpha (α) toxin. Of the others, type B strains produce both beta (β) and epsilon (ε) toxins, type C strains produce β toxin, type D strains produce ε toxin and type E strains produce iota (ι) toxin (Hatheway, 1990; Heikinheimo and Korkeala, 2005). In addition, enterotoxin (CPE), the major lethal toxin causing food poisoning symptoms, is almost always associated with C. perfringens type A (McClane, 2001; Heikinheimo and Korkeala, 2005) The most significant food poisoning strain belongs to type A which occurs in humans and animals due to the consumption of contaminated food (Finegold, 1977; Todd, 1978; Shandera *et al*., 1983).

Food poisoning caused by *C. perfringens* may occur when foods are contaminated with cells (10^5–10^7cell/g) or spores (McClane, 1997; Blaschek, 2000). In Thailand, the prevalence of *C. perfringens* has been reported in many types of food particularly in powdered chili, dried spices and chili paste. Basically, dried chili is a common ingredient used in Thai dishes and widely use in many type of food products in the Thai food industry (Stonsaovapak *et al*., 1996; Mahakarnchanakul *et al.,* 2011). *C. perfringens* is considered as one of the significant

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cause of foodborne illnesses in Thailand, although illnesses may be attributed to other food materials such as raw meat more than to dried spur pepper (Bureau of Epidemiology, 2015). Investigation of the current microbiological quality status of dried spur pepper will provide information on food safety and raise the awareness of consumers and food processers, both locally and for export. Thus, this study investigated the prevalence of *C. perfringens* contamination in dried spur pepper from six different provinces which are major production locations in Thailand. The toxinotyping of *C. perfringens* isolates was also examined using multiplex PCR assay to identify which toxigenic type could be the major contaminant in dried spur pepper. The current prevalence data of *C. perfringens* contamination in dried spur pepper of Thailand was the importance of this work. It brings Thai consumer be alert and aware to food safety. Moreover, Thai Bureau of Epidemiology will use it to establish the suitable defensive measure for Thai consumption behavior.

Materials and Methods

Sample collection

One hundred dried pepper samples (500 g of 10 kg/bulk) were collected from local and wholesale markets in six provinces (Bangkok, Nonthaburi, Chiang Mai, Phrae, Sukhothai and Phitsanulok) in Thailand from March to May, 2011 and from August, 2012 to March, 2013. These provinces are major dried spur pepper production and processing sites according to Department of Agriculture Ministry of Agriculture and Cooperatives, Thailand. Then, the microbiological quality of each sample was determined within 24 h.

Investigation of Clostridium perfringens *and other microorganisms in dried spur pepper*

The populations of total mesophiles were determined using plate count agar (Merck, Germany(, incubated at 35°C for 48 h and dichloran rose bengal chloramphenicol (DRBC) agar (Merck, Germany(for yeast and mold counts, incubated at 25°C for 5 days (Downes and Ito, 2001).

Clostridium perfringens in dried spur pepper samples was enumerated using tryptose sulfite cycloserine (TSC) agar (Merck, Germany) with egg yolk emulsion (EYE) according to APHA standard microbiological methods (Downes and Ito, 2001). Typical black colonies (with an opaque zone) were further presumptively tested using selected biochemical tests including Gram staining, the dual or double hemolysis test and the reverse CAMP test (Yeh *et al.*, 1993).

The populations of proteolytic and non proteolytic anaerobic spore-formers were also enumerated according to APHA. Briefly, proteolytic anaerobic spore-formers were analyzed using the plating technique with TSC-EYE agar. Fifty grams of sample in 450 ml of sterile 0.1% peptone water were transferred to a stirred water bath adjusted to 80°C and held for 10 min. Then, inoculated plates were incubated at 37°C for 24 h in an anaerobic jar using 2.5 L AnaeroPack-Anaero (Mitsubishi Gas Chemical Company, Inc., Japan). Typical colonies with a black color (resulting from the reduction of sulfite, which precipitates as iron sulfide) and with or without the opaque zone were enumerated. Non proteolytic anaerobic spore-formers or *C. perfringens* spores were determined using similar procedures as mentioned above but using a different preheating step at 60°C for 30 min (Scott *et al*., 2001).

Toxinotyping of Clostridium perfringens *isolated from dried spur pepper*

One hundred and sixty isolates of non proteolytic anaerobic spore-formers were confirmed as Clostridium perfringens based on the results of complete biochemical testing; Gram staining, a catalase test, aerobic growth, a gelatin hydrolysis test, a litmus milk test, motility and nitrate reduction tests, a urea hydrolysis test and a reverse CAMP test (Yeh *et al.*, 1993; Downes and Ito, 2001). Positive colonies were selected and cultured in 10 ml cooked meat medium (DifcoTM and BBLTM, Becton, Dickinson and Company, MD, USA) at 37°C for 24 h, then the cultures were kept at 4°C for further Multiplex PCR assay.

All 160 tested isolates, including the reference culture ATCC13124, were extracted and total DNA was isolated using multiplex PCR assay as described by Zhou *et al.* (1995). Briefly, two to three colonies of *C. perfringens* grown on a sheep blood agar plate were suspended in 0.5 ml of lysis buffer 1 (lysozyme, Sigma-Aldrich Chemie GmbH, Switzerland) and the mixture was incubated at 37°C for 10 min before centrifugation at 16,000×g (166-0602EDU, Bio-Rad, USA) for 2 min. The pellets were added with 200 µl of lysis buffer 2 (protenase K, Sigma-Aldrich Chemie GmbH, Switzerland) and incubated at 56°C for 60 min. Then, bacterial DNA was harvested by centrifugation at 16,000×g for 2 min, and the supernatant was used as template DNA in the PCR. All bacterial DNA samples were stored at -20°C prior to analysis. An amount of 30 ng of DNA was examined for each PCR reaction. The reference primer containing *cpa, cpb, etx* and *cpe* genes was

purchased from Bio Basic Inc, Canada and used as the reference for toxin identification.

In this study, specific primers corresponding to each toxin were adopted from Meer and Songer (1997) and Heikinheimo and Korkeala (2005). Specificity of CPA (for α toxin, 400 bp), CPB (for β toxin, 196 bp), ETX (for ε toxin, 655 bp) and CPE primer (for enterotoxin, 233 bp) (except for IA, for ι toxin) was confirmed by specific amplification of the toxin genes from 19 bacterial strains with a negative cross reaction (data not shown).

According to the PCR protocol of Meer and Songer (1997) and Heikinheimo and Korkeala (2005), the multiplex PCR was performed to determine the presence of major toxin genes (*cpa, cpb* and *etx*) and the *cpe* gene from *C. perfringens* isolates, obtained from dried spur pepper, in a MultiGene Thermal Cycler (Labnet, USA). The total volume of 25 µl PCR mixture contained 14.05 µl of sterile distilled water, 5 µl of 5× Green Gotag@ Flexi Buffer (Promega, Madison, WI, USA), 2 µl of 25 mM $MgCl_2$ (Promega, Madison, WI, USA), 0.25 µl of 10 µM of primers (CPA, CPB, ETX and CPE), 0.25 µl of 10 mM deoxynucleoside triphosphate (dNTP) mixture (Promega, Madison, WI, USA), 0.2 µl of 5U Go@ Tag DNA polymerase (Promega, Madison, WI, USA), and 1 µl of 30 ng template DNA. The following program was used in this experiment: 5 min at 94°C as a pre-denaturing step, followed by 35 cycles consisting of 1 min at 94°C, 1 min at 53°C, and 1 min at 72°C, then a post-extension step at 72°C for 8 min was required. Ten microliters of the amplified products were electrophoresed in 2% agarose gels (GelMate, Toyobo, Japan) and stained with ethidium bromide (Promega, Madison WI, USA). Five microliters (1 ug/lane) of standard DNA fragments (100 bp+1.5 Kb DNA Ladder, SibEnzyme, Russia) were used as molecular weight markers to indicate the sizes of the amplification products. The products on the gels were observed under UV illumination and photographed using Gel Doc (Bio-Rad, USA).

Results and Discussion

Investigation of Clostridium perfringens and other microorganisms in dried spur pepper

Regarding our microbial investigation of dried spur peppers, the results (Table 1) showed 95% of dried spur pepper samples (N=100) contained a high number of total mesophiles exceeding 4 log CFU/g, with yeast and mold ranging from 2.4 to 5.4 log CFU/g. *C. perfringens* was detected in 86 dried spur pepper samples (86%), ranging from 1 to 3 log CFU/g. Considering the amount of total mesophile counts and yeast and mold counts, 100 samples would comply with the Thai industrial standard of dried chili as only 4% of total samples had high yeast and mold counts above 100 CFU/ g. The median value of *Clostridium perfringens* in dried spur peppers was similar among the five locations, except for the samples from Phitsanulok, which had a lower value due to the high number of negative samples (11/17, 65%). However, the samples did not comply to the safety standards specified for export products to the EU, due to the presence of *C. perfringens*. Non proteolytic and proteolytic anaerobic spore-formers in dried spur pepper samples were detected at 100% and 83%, respectively (Table 1).

According to the Thai Industrial Standards Institute (TISI), the number of total aerobic mesophilic bacteria and total yeasts and molds in dried chili have been limited to not more than 105 and 10^2 CFU/g, respectively (TISI, 1983), while the International Commission on Microbiological Specifications for Foods (ICMSF) has set limits at 10^6 and 10^4 CFU/g, respectively (ICMSF, 2005; Cosano *et al*., 2009). Although our dried spur pepper samples contained total mesophile counts lower than the standard limits of TISI and ICMSF, the count of yeast and mold (>10^4 CFU/g, 47%) was not low enough to satisfy the quality standards of TISI and ICMSF. This result indicated that the quality of dried spur pepper in Thailand needs to be improved. Our investigation also found the moisture content in samples was approximately 11.8 to 19.3% with aw ranging from 0.58 to 0.74 (data not shown), due to some samples containing high moisture levels that may have encouraged the survival of mold and yeast spores. To date, the Commission of the EU has not yet announced limits for the total count and for other microorganisms, except requiring the absence of pathogenic bacteria.

In terms of food safety, in this study our dried pepper samples contained some levels of *C. perfringens* and spore-forming bacteria, but none had *C. perfringens* cell levels higher than 10^3 CFU/g. *C. perfringens* has been reported as the major microorganism of concern in non-heat-treated foods since vegetative cells occasionally cause problems for foodborne diseases, and may become a public health risk. *C. perfringens* could possibly recontaminate heat-treated foods (Brynestad and Granum, 2002). To date, TISI has not set criteria for *C. perfringens* or spore-forming bacteria in such kinds of products, but ICMSF allows a maximum limits of 10^3 CFU of *C. perfringens* per gram of spice (TISI, 1983; ICMSF, 2005).

According to ICMSF (2005), the microbiological

Table 1. Microbiological quality of 100 dried spur pepper samples taken from local and wholesale markets in Thailand

Microorganism	Average of positive sample (log CFU/g)	Range of population (log CFU/g)					Microbiological standard criteria		
		Not detected	1–<2	2–<3	3–<4	≥4	TISI*	ICMSF**	EU***
Total mesophiles	4.5	0%	0%	0%	5%	95%	pass (5%)	pass (100%)	-
Yeast and mold	4.2	0%	0%	4%	49%	47%	fail (100%)	pass (53%)	-
C. perfringens	1.5[a]	14%	72%	14%	0%	0%	-	pass (100%)	pass (14%)
Non proteolytic anaerobic spore-formers	1.7	0%	59%	41%	0%	0%	-	-	-
Proteolytic anaerobic spore-formers	1.9[b]	17%	46%	37%	0%	0%	-	-	-

[a] Only 86 samples were analyzed for C. perfringens positive sample.
[b] Only 83 samples were analyzed for proteolytic anaerobic sporeformer positive sample.
* Microbiological standard criteria as recommended by the Thai Industrial Standards Institute (1983).
** Microbiological standard criteria as recommended by the International Commission on Microbiological Specifications for Foods (2005).
*** Microbiological standard criteria as recommended by the European Commission (2004).
- No criteria mentioned.

quality of Thai dried spur peppers was acceptable since the population of *C. perfringens* was not higher than the limit. However, the presence of *C. perfringens* and both anaerobic spore-formers in dried spur peppers may be considered as a possible health risk. Manufacturing for export products must be concerned with the presence of pathogenic microorganisms since the EU recommends the absence of *C. perfringens* per gram of sample, as well as of other pathogenic bacteria such as *B. cereus* and *Salmonella* (European Commission, 2004). Therefore, the microbiological quality improvement of Thai dried spur pepper is necessary with regard to exporting to the member countries of the EU. Likewise Thai producers should control or decontaminate *C. perfringens* in dried spur pepper which is used as an major ingredient in chili paste and chili-related products. Generally, the prevalence of *C. perfringens* in dried spur pepper could occur in related *Capsicum* spp. and *Piper* spp. products in other countries, for example in the UK. During 2004, the Health Protection agency in the UK determined the microbiological status of dried spices and herbs taken from retail and production premises. The agency investigated 552 samples from *Capsicum* spp.—chili, cayenne and paprika—and found 17 samples (3.1%) were of unsatisfactory quality due to the presence of *C. perfringens* (ranging from 1.0×10^3 to 2.4×10^5 CFU/g). Of the other products from *Piper* spp.—black, white and green peppers and a mixture of red pepper and flower pepper (N=335)—15 samples (4.2%) were of unsatisfactory quality (Sagoo *et al.*, 2009). Although 3.0% of these samples contained high counts of *Bacillus cereus, Escherichia coli* and *C. perfringens*, in terms of using spices and herbs as an addition to ready-to-eat foods, 96% were of acceptable quality and constituted a low potential public health risk.

Our finding is the first intensive report of *C. perfringens* including anaerobic spore-former contamination in Thai dried spur pepper. Proteolytic anaerobic spore-formers (including *C. sporogenes* and *C. botulinum*) and non proteolytic anaerobic spore-formers (including *C. perfringens, C. butyricum, C. pasteurianum* and *C. laramie* and *C. algidicarnis*) have been found in many food products (Scott *et al.*, 2001). Spores may resist heating during cooking or adverse conditions such as drying; therefore, in dried agricultural products, spores will possibly be found more than cells. This simple indicator will assist industry to reduce the cost and time of detection for *C. perfringens*. Thus, the investigation of *C. perfringens* in food can be continuously monitored and closely observed.

Toxinotyping of Clostridium perfringens *isolated from dried spur pepper*

Biochemical properties confirmed that 160 isolates of non proteolytic anaerobic spore-formers with a double hemolytic zone on sheep blood agar were *Clostridium perfringens*. All isolates showed typical characteristics of *C. perfringens*: being Gram positive and rod shaped and having a negative catalase test, aerobic growth, gelatin hydrolysis ability, non-motile ability, reduction of nitrate, production of

Table 2. Occurrence of microorganisms and toxin gene determination of *Clostridia* sp. isolated from dried spur pepper taken from local and wholesale markets in 6 provinces of Thailand

Location	No. of samples	Range (median) log CFU/g					No. (%) of isolates	Biochemical tests[a]	Multiplex PCR[b]
		Total mesophiles	Yeast and mold	*C. perfringens*	Non proteolytic anaerobic spore-formers	Proteolytic anaerobic spore-formers			
Bangkok	36	3.7–6.2 (4.4)	2.4–5.3 (3.8)	0–2.3 (1.7)	0.7–2.6 (2.2)	0–2.5 (1.9)	54 (33.7)	+	*cpa*+
Nonthaburi	10	3.6–4.8 (4.4)	2.7–5.3 (4.7)	0.7–2.2 (1.6)	0.7–2.5 (1.8)	1.3–2.4 (1.7)	20 (12.5)	+	*cpa*+
Chiang Mai	11	3.6–6.1 (4.4)	3.4–5.4 (4.6)	0.7–1.9 (1.7)	0.7–2.4 (1.0)	0–2.4 (1.9)	22 (13.8)	+	*cpa*+
Phrae	15	2.7–6.5 (4.8)	3.3–5.2 (4.2)	1.0–2.3 (1.4)	0.7–2.4 (1.9)	1.4–2.4 (2.1)	30 (18.7)	+	*cpa*+
Sukhothai	11	4.2–4.5 (4.3)	3.4–4.8 (3.8)	1.2–2.3 (1.8)	0.7–2.5 (2.1)	1.4–2.4 (1.9)	22 (13.8)	+	*cpa*+
Phitsanulok	17	4.2–5.6 (4.3)	3.4–4.9 (3.8)	0–1.8 (0)	0.7–2.1 (1.0)	0–2.0 (0)	12 (7.5)	+	*cpa*+
Total	100						160 (100)	+	*cpa*+
C. perfringens ATCC 13124								+	*cpa*+

[a] Biochemical tests: 160 of non proteolytic anaerobic spore-formers isolates were Gram positive and had rod shaped cells, negative catalase test and aerobic growth, hydrolysis of gelatin, clotting of litmus milk test, non-motile ability, reduction of nitrate, production of hydrogen sulfide (H2S), non hydrolysis of urea, double hemolysis colony and positive reverse CAMP test result.

[b] Multiplex PCR for toxin gene: 160 of non proteolytic anaerobic spore-formers isolates possessed the cpa gene (alpha toxin), but not the cpb (beta toxin), etx (epsilon toxin) or cpe genes (enterotoxin toxin).

hydrogen sulfide (H_2S) and a positive reverse CAMP test (Table 2).

Further results from the multiplex PCR revealed that all 160 isolates, which were non proteolytic anaerobic spore-formers, possessed the cpa gene, but not the cpb, etx or cpe genes (Table 2). As shown in Figure 1, the alpha toxin gene was found in some tested sample (lanes 3–7). Further tests showed that the PCR results indicated all 160 isolates from dried spur pepper possessed the alpha toxin gene but there was an absence of the beta epsilon and iota genes. Noticeably, none of the tested isolates possessed the enterotoxin gene as shown in lane 12, as well as the *C. perfringens* reference strain ATCC 13124. In conclusion, all 160 isolates from 100 dried spur pepper samples were represented as toxigenic *C. perfringens* strains as type A and CPE-negative.

C. perfringens is commonly found in agricultural products, animal feeds and various food stuffs such as meat products, spices, chili pastes, sauces and dried food and these food products have been reported as the food vehicles (Duncan, 1976; Gorbach, 1998; McClane, 2001). Our finding is also the first investigation of the occurrence of toxigenic strains of *C. perfringens* in Thai dried spur pepper. All *C. perfringens* isolates (160) from the dried spur pepper samples from six provinces in Thailand were CPE-negative but possessed the cpa gene, which indicates *C. perfringens* type A. Commonly the pathogenicity of *C. perfringens* type A is associated with the production of alpha toxin and enterotoxin (Hatheway, 1990; Stubbings, 1990). Alpha toxin of *C. perfringens* type A causes gas gangrene, cellulitis and septicemia. Gas gangrene and cellulitis are involved in infection and inflammation as well as the more common symptom of bacterial food poisoning, being watery diarrhea and abdominal cramps (Blaschek, 2000). The characteristics of gas gangrene are rapid inflammation at the site of infection, extreme swelling, acute pain and, eventually, necrosis of the infected tissue. In addition the symptoms of cellulitis include tenderness, pain, swelling and redness at the site of infection and septicemia (Jay *et al.*, 2005). Food poisoning is mainly caused by the enterotoxin of *C. perfringens* type A. Thus the absence of CPE in our isolates may imply that the contamination of *C. perfringens* type A in dried spur pepper possess less potential risk to health in terms of food consumption.

C. perfringens food poisoning outbreaks in Thailand have been reported since 1976, though the average number of cases each year has been less than 50, except in 1997 and 1998 (113 and 104 cases, respectively). The food vehicles were the consumption of improperly cooked meat which indicated heavy contamination in unsanitary fresh meat. A recent report of *C. perfringens* food poisoning cases revealed less significant pathogenic foodborne bacteria compared to others, due to the few cases (12 and 4 cases in 2013 and 2014, respectively) (Bureau of Epidemiology Thailand, 2015). Thus, the Bureau of Epidemiology Thailand has undertaken less monitoring of the prevalence of *C. perfringens* in foods compared to other pathogenic bacteria.

The previous study of Heikinheimo and Korkeala

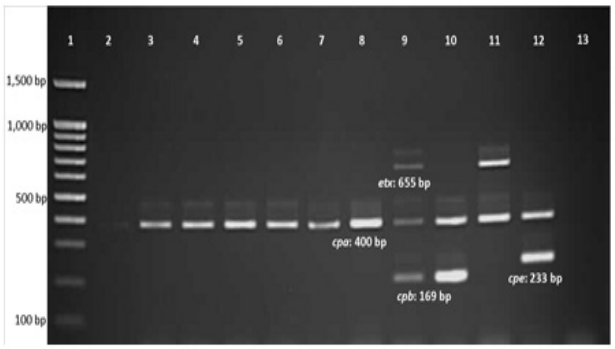

Figure 1. Electrophoretic analysis of *Clostridium perfringens* toxin genes amplified using a multiplex PCR. All toxin genes in *Clostridium perfringens* reference strains were amplified with all primers listed in Meer and Songer (1997) and Heikinheimo and Korkeala (2005). Lane 1, DNA size marker (100-bp+1.5-kb); lane 2, negative *C. sporogenes* ATCC11473; lanes 3–7, tested sample; lane 8, *C. perfringens* type A (alpha toxin); lane 9, *C. perfringens* type B (alpha, beta, and epsilon toxins); lane 10, *C. perfringens* type C (alpha and beta toxins); lane 11, *C. perfringens* type D (alpha and epsilon toxins); lane 12, *C. perfringens* type A (*cpe*-positive) and lane 13, negative control (1 µl of sterile distilled water).

(2005) found that all 118 *C. perfringens* isolates from broiler chicken represented type A and were also CPE-negative. In Japan, Miki and co-workers evaluated Japanese retail meat products for the presence of two genotypes of enterotoxigenic *C. perfringens* (chromosomal and plasmid cpe). Their results demonstrated that approximately 70% of Japanese retail raw meat was contaminated with low numbers of *C. perfringens* bacteria and only 4% were cpe-positive C. perfringens. Most of the cpe-positive *C. perfringens* isolates obtained from Japanese retail meat carried cpe on a plasmid (Miki *et al*., 2008). Similar results from Erol *et al.* (2008) showed 22 *C. perfringens* isolates taken from 180 turkey meat samples, collected from different supermarkets located in Ankara Turkey, over a year were *C. perfringens* type A. Moreover, Ngamwongsatit *et al.* (2016) found that the prevalence of multidrug resistance C. perfringens causes diarrhea in Thailand. A total of 148 samples from 13 farms were PCR-positive for *C. perfringens* toxin genes. Among resistant isolates, 82% were resistant to more than one type of antibiotics such as ceftiofur, enrofloxacin, erythromycin, lincomycin, and tylosin.

Both cells and spores of *C. perfringens* easily contaminate agricultural products or foods (Todd, 1978; Shandera *et al.*, 1983; Beckers, 1986; Bean *et al*., 1996; Olsen *et al*., 2000), particularly, where there are unhygienic processes in the production of dried chili or pepper, and other spices. Although the risk of *C. perfringens* contamination in dried pepper is limited due to number of heating procedures and the level of heating, Thai food mainly consisting of chili, chili paste and any chili products may increase the health risk to Thai consumer. The wide range of exposure to microbial contamination during pre- and post-harvesting, processing, distributing, retailing, utilizing or storage (de Boer *et al*., 1985; McKee, 1995; Zaini *et al*., 2010) needs to be controlled by hygienic practices.

To date, there have been few studies on the prevalence of *C. perfringens* in dried pepper, but our result indicated that dried spur pepper consumption has less potential risk as a source of foodborne pathogens caused by *C. perfringens*. Although the toxigenotyping confirmed most isolates possess toxigenic virulence with the alpha toxin encoding gene, none of them could be able to produce enterotoxin.

Conclusion

Dried spur peppers could be a source of spoilage microorganisms due to high total counts and high yeast and mold counts. Significant pathogens, especially *Clostridium perfringens*, constitute a risk and consequently, food producers must monitor the quality and safety of dried spur peppers used as ingredients in food processing. The application of the appropriate method to reduce the contamination of *C. perfringens* in these foods needs to be closely watched. Correlation between non proteolytic anaerobic spore formers may assist to estimate the level of contamination with *C. perfringens*; nevertheless Multiplex PCR could be use as a proper method for molecular toxinotyping of *C. perfringens,* as it produces accurate results, is less time consuming and is an effective method for typing compared to the conventional method.

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