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Short Communication

Microbiological safety of Thai pandan custard filled products and their ingredients

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Abstract

The aim of this study was to evaluate microbiological quality of Thai pandan custard and their main ingredients used in the product such as pandan leaves, whole egg, pasteurized coconut milk, cornstarch, wheat flour and sugar. The ingredients and Thai pandan custard were collected from some small and medium enterprises in the center of Thailand by sampling 20 samples of high risk ingredients (pandan leaves, whole egg, pasteurized coconut milk), 10 samples of low risk ingredients (cornstarch, wheat flour, sugar) and 20 samples of Thai pandan custard. The samples were tested for four pathogens including *Staphylococcus aureus, Salmonella* spp., *Bacillus cereus* and *Clostridium perfringens*. It was found that *B. cereus* was mostly detected in pandan leaves. This pathogenic microorganism was found to contaminate 80% of the leaves (16 samples) with higher than 3 log CFU/g and 15% (3 samples) with lower than 3 log CFU/g. Besides, *S. aureus* was detected in two samples (10% of the leave sample). *Salmonella* spp. was shown positive in four samples (20% of whole egg samples), while *C. perfringens* was not detected in any samples. Moreover, *B. cereus* was detected in five samples (25%) of the sampling Thai pandan custard after one-day storage at room temperature and the contamination was raised up to 9 samples (45%) after two-day storage with the microbial load of 2.4 to 3.9 log CFU/g. *Salmonella* spp. and *S. aureus* were not detected in any sample. The identification of some isolates of *B. cereus* selected from Thai pandan custard samples by Bureau of Quality and Safety of Food (BQSF), Department of Medical Sciences (DMSc) confirmed that all selected spore-forming bacterial colonies were identified as pathogenic *B. cereus*. The results from this study implied that heating process during Thai pandan custard production was not effective for diminishment of the contaminated *B. cereus* from ingredients which might lead to the safety problem of the product.

Introduction

Thai pandan custard (Sangkhaya baitoey in Thai language), is a Thai traditional dessert made with pandan leaves (*Pandunus amaryllifolius* Roxb.), coconut milk, whole egg, cornstarch, wheat flour and sugar. These ingredients are combined in mixing bowl then poured into a double boiler, placed over high heat and constantly stirred until thickened (Thai Dessert Recipes, 2012). The finished product can be consumed in a variety of ways, e.g., using it as a pastry filling, or as a spread and dip for bakery products. The product is classified as ready-to-eat (RTE) categories and is typically stored, transported and sold at room temperature (25-30°C). According to the high moisture content (high water activity) of this product, which supports the growth of a wide range of bacteria (NZFSA, 2007) and raises food safety concerns, especially pathogenic bacteria such as *Staphylococcus aureus, Bacillus cereus* etc. Many fillings, toppings, and other high-moisture components can support the growth of spoilage organisms including spore formers that survive the cooking process (Cook and Johnson, 2009). There have been outbreaks associated with the consumption of Thai custard bread, in primary school, Bangkok (2013). These outbreaks affected 166 people with over 22 people hospitalized, all via were nausea, vomiting and abdominal pain. It is estimated by Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, laboratory results showed *S. aureus* was also identified in Thai Custard

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Bread (Kulawong *et al*., 2013).

Since Thai pandan custard market has shown to increase as a result of the demand from the ASEAN Economic Community (AEC), thus, this study was to evaluate the microbiological quality of the (RTE) Thai pandan custard filled-product and its fundamental ingredients. Microbiological quality of ingredients was compared to product specification and this product was compared to the Notification of the Ministry of Public Health of Thailand (No.364-2013) Re: Standards for Pathogenic Microorganisms in Food. The following food items in ready-to-eat foods made from cereals or flour basis such as bread with filling, steamed buns, steamed buns with stuffing and mocha cakes (Thai FDA, 2013) are added in appendix 3 of this notification.

Materials and Methods

Thai pandan custard preparation

The production for Thai pandan custard in SMEs produced 50-100 kg. per batch (lot). Weighing ingredients and combined in mixing bowl then poured into a double jacketed boiler, placed steam into double jacketed boiler, constantly stirred until thickened (used 50-60 min. per batch) and hot filling finished product in 5 kg sterile plastic bag. The internal temperature of finished product around 90-92^{o}C and then cool down product for set point in room temperature (30-32^{o}C) about 2-4 hrs. and transportation to filling bun line.

Sample collection

Twenty samples of each high risk ingredients (high moisture content ingredients such as pandan leaves, whole egg and pasteurized coconut milk), ten samples of each low risk ingredients (low moisture content such as cornstarch, wheat flour and sugar). For Thai pandan custard production were collected from some SMEs in the center of Thailand for microbiological quality determination. Each lot of Thai pandan custard which produced from aforementioned high risk and low risk ingredients, were collected and kept in sterile plastic bag for three bags per lot, total twenty lots for this study. Twenty samples were immediately determined for microbiological quality after production. The other twenty samples in sterile plastic bag were kept in room temperature (30-32^{o}C) and determined for microbiological quality after the first and second day of storage time.

Microbiological quality determination

Microbiological quality of each low risk and high risk ingredients for Thai pandan custard production, and Thai pandan custard products (after production and the production which kept in one and two days) were determined for Coagulase positive *Staphylococcus aureus, Salmonella* spp., *Bacillus cereus* and *Clostridium perfringens* using the method recommended by BAM (online), 2001 and 2012. Some suspected isolates of *B. cereus* detected from the Thai pandan custard samples were sent for identification at the Bureau of Quality and Safety of Food (BQSF), Department of Medical Sciences (DMSc).

Results

Microbiological quality of ingredients

The microbiological quality of pandan custard ingredients was shown in Table 1. It was revealed that pandan leaves and whole egg, which were the main ingredients for pandan custard product, were contaminated with pathogenic bacteria. Pandan leaves were contaminated with high number of *Bacillus cereus* (average 3.4 log CFU/g) and were found in the samples in 19 samples (95%; > 3 log CFU/g 16 samples and < 3 log CFU/g 3 samples). Moreover, this ingredient was also contaminated with coagulase *Staphylococcus aureus* for two samples (10%) with the number of 23-93 MPN/g (Data not shown). The whole egg revealed to contaminate with *Salmonella* spp. in four samples (20% of whole egg). *Clostridium perfringens* was not detected in any samples in this study.

Microbiological safety of Thai pandan custard

The microbiological safety of Thai pandan custard production was shown in Table 2, it was revealed that *B. cereus* was still remained in the product after production by heating, while *Salmonella* spp. and *S. aureus* were not detected in any samples. The availability of *B. cereus* in pandan custard was found after production in three samples from 20 batches (15%) and after one day storage at room temperature, this pathogenic strain was detected in five samples from 20 batches (25%) and raised up to nine samples from 20 batches (45%) after two-day storage.

Microbiological safety results showed that 11/20 (55%) batches (samples) of the (RTE) Thai pandan custard was satisfactory according to Standards for Pathogenic Microorganisms in Food (Thai FDA, 2013), following food items in ready-to-eat foods made from cereals or flour basis with *B. cereus* levels detected at no more than 100 CFU/g. However, this study found that 9 batches (samples) of the products sampled had high levels of *B. cereus* with more than 100 CFU/g (>2 log CFU/g), that was shown in Figure 1.

Table 1. Microbiological results of pandan custard ingredients

Name of samples	No. of samples	No. of samples detected in samples			
		S. aureus	*Salmonella* spp.	*B. cereus*	*C. perfringens*
Pandan leaves	20	2 (10%)	0	19 (95%)	0
Pasteurized Coconut milk	20	0	0	0	0
Whole egg	20	0	4 (20%)	0	0
Cornstarch	10	0	0	0	0
Wheat flour	10	0	0	0	0
Sugar	10	0	0	0	0

Table 2. Microbiological safety of Thai pandan custard (n = 20)

Name of samples	No. of samples	No. of samples detected in samples			
		S. aureus	*Salmonella* spp.	*B. cereus*	*C. perfringens*
Pandan custard 0 day[a]	20	0	0	3 (15%)	0
Pandan custard 1 day	20	0	0	5 (25%)	0
Pandan custard 2 days	20	0	0	9 (45%)	0

Note : a = 0 day was after production and cool down (2-4 hrs.) to set point.

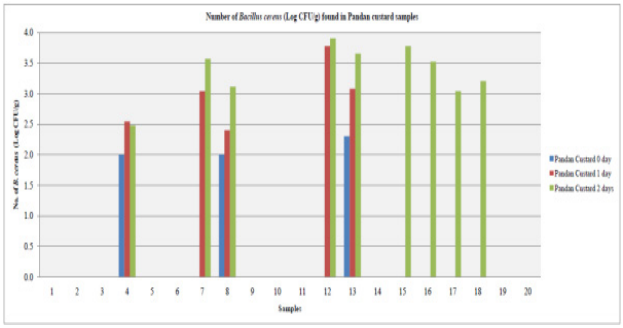

Figure 1 Number of *Bacillus cereus* (Log CFU/g) in Thai pandan custard 20 samples

Discussion

This study was concurred to the report from Microbiological Quality of Bakery Product survey from NZFSA (2007) which reported that 250 collected samples of cream-filled, custard-filled and custard and cream filled bakery products were tested for fecal coliforms, *Escherichia coli, Bacillus cereus,* coagulase-positive staphylococci and *Salmonella* from Jan to Aug 2007. The results informed that 6 samples (2.4%) were unsatisfactory and 3 samples (1.2%) including one cream-filled products and two custard-filled products had a potentially hazardous level of *B. cereus* (> 10^4 CFU/g).

The results of *B. cereus* positive samples in pandan custard product might be from the spore-forming of *B. cereus* which found in pandan leaves. Bacillus spore surviving a heat treatment at 100°C, 10 min corresponding to a bread baking process (Rosenkvist and Hansen, 1994). Cooking the product with high number of spore of *B. cereus* at 100°C or below (internal temperature of product around 90-92°C) can not diminish the whole contaminated spores and will allow some spores to germinate after cooking and storage at room temperature for a long period (Roberts *et al*., 1996). However, only canning can ensure complete destruction of *B. cereus* spore. Other heating processes such as normal cooking or pasteurization are not enough to kill all *B. cereus* spore (EFSA, 2005).

Conclusions

In summary, findings show the heating process during production of (RTE) Thai pandan custard was effective in eliminating *Salmonella* spp. and *S. aureus*. The heating process was not sufficient for eliminating contaminated spore-forming bacteria of *B. cereus* which could lead to food safety problems. For safety product improvement, the SMEs should examine methods of temperature control during cool down, good hygienic practice, could reduce the initial number of spore-forming in raw material and the control of spore germination can be prevented by

control low temperature storage.

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References

European Food Safety Authority (EFSA). 2005. Opinion of the scientific panel on biological hazards on Bacillus cereus and other *Bacillus* spp. in foodstuffs. EFSA Journal 175: 1-48.

Internet: Food and Drug Administration Thailand (Thai FDA) 2013. The standards for pathogenic microorganisms in food. Downloaded from (In Thai) *http://elib.fda.moph.go.th/library/default.asp?page2=detail&ngid=2442&smonth=10&syear=2013* on 31/10/2013.

Internet: New Zealand Food Safety Authority (NZFSA) 2007. Microbiological quality of bakery product. Downloaded from *http://www.foodsafety.govt.nz/elibrary/industry/Microbiological_Quality-Investigates_Relationship.pdf* on 14/9/2013.

Internet: Thai Dessert Recipes. 2012. Pandan coconut custard or dipping sauce. Available at *http://thaidessertsrecipes.blogspot.com/2012/10/pandan-coconut-custard-or-dipping-sauce.html* on 8/10/2012.

Internet: Bacteriological Analytical Manual (BAM) 2001. Clostridium perfringens, Staphylococcus aureus. Available at *http://www.fda.gov/Food/FoodScience Research/ LaboratoryMethods/ucm2006949.htm* on 8/10/2012.

Internet: Bacteriological Analytical Manual (BAM) 2012. Bacillus cereus, Salmonella. Available at *http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods /ucm2006949.htm* on 8/10/2012

Cook, F.K. and Johnson, B.L. 2009. Compound cereal products. Compendium of the Microbiological Spoilage of Foods and Beverages. p. 242-244. New York: Springer.

Roberts, T.A., Baird-Parker, A.C. and Tompkin, R.B. 1996. Microorganism in food 5 microbiological specification of food pathogens. International Commission on Microbiological Specifications for Foods (ICMSF), p. 20-35. Suffolk: Great Britain.

Rosenkvist, H. and Hansen, A. 1994. Contamination profiles and characterization of *Bacillus* species in wheat bread and raw materials for bread. International Journal of Food Microbiology 26: 353-363.

Kulawong, S., Te-chaniyom, T. and Karnjanapiboonwong, A. 2013. Outbreak verification summary. Report of Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health. Thailand: Weekly Epidemiological Surveillance Report 44(22): 343.