Investigation of microbiological and chemical contaminants contained in "Hoi-dong", Thai-fermented green mussel

Yuttana Sudjaroen
Department of Applied Science, Faculty of Science and Technology, Suan Sunandha Rajabhat University, Dusit, Bangkok 10300, Thailand, yuttana.su@ssru.ac.th

Pimporn Thongmuang
Department of Aesthetic Health Science, College of Allied Health Sciences, Suan Sunandha Rajabhat University, Samut Songkhram 75000, Thailand

Kanittada Thongkao
Department of Applied Science, Faculty of Science and Technology, Suan Sunandha Rajabhat University, Dusit, Bangkok 10300, Thailand

Abstract

Green mussels (Perna viridis) are commonly preserved as "Hoi-dong", traditional fermented mussel, in Thai Gulf area. Presently, Hoi-dong is reported as functional food and health promoting benefits. Therefore, fishery products are commonly concerning on microbials and toxic metal contaminants from environments and/or during food processing. Food additives, such as preservatives and colorants are also concerned by intentionally added within Hoi-dong for prolong shelf-life and attractive to consumers, respectively. This study was purposed to investigate microbiological and chemical contaminants contained Hoi-dong, which was purchased from fresh markets locating in Samut Songkhram, Thailand; and evaluate its quality. Salinity and acidity were measured by food pH meter and salinity checker, respectively. Microbiological contaminants were evaluated according to Bacteriological Analytical Manuals (BAM). Pb and Hg were determined according to AOAC Official Method. Food preservatives including benzoic acid and sorbic acid, and synthetic food colorants were determined according to Codex Committee on Food Additives (CCFA). Hoi-dong (n =16) was processed through optimal salinity (NaCl = 3.5-4.4%) and acidity (pH = 3.98-4.74). All samples were met within standard ranges for microbiological criteria of food quality and were safe from toxic metals including Pb and Hg. However, five samples were contaminated with food preservative, benzoic acid (1,200-3,089 mg/kg) and eight samples were detected for synthetic food colorants i.e., Sunset Yellow FCF, Tartrazine and Ponceau 4R. The find was concerned on food preservative and synthetic food colorant uses in traditional fermented food and public relation is need to conduct with among government officers and traditional manufacturers.

Keywords: *Hoi-dong, Thai-fermented mussel, Perna viridis, food contamination, food additives.*

INTRODUCTION

The consumption of fish and fishery products are globally demand, and Thailand is one of most exporters who is exporting these products [1]. Due to consumption of fish and fishery products, seafood is trend to concern on its safety from foodborne contamination [2]. Common pathogenic bacteria contaminated in seafood products are Vibrio, Salmonella, Shigella, Escherichia coli and Clostridium botulinum [3-5]. Common Thai seafood including, pacific white shrimp, oysters, blood cockles and seabass from fresh markets located in Bangkok had high prevalence of Vibrio and

Salmonella isolated along with rate of fecal coliforms and E. coli contaminants [6]. In addition, awareness of chemical contaminants in seafood especially toxic metals i.e., mercury (Hg), cadmium (Cd), arsenic (As), and lead (Pb), are also considered as risk assessment on this food safety [7].

Green mussels (Perna viridis), in Thai namely “Hoi-ma-laeng-poo” are traditionally cultivated in the aquaculture sector near estuaries and had exposed with various contaminants exposures [8, 9]. Samut Songkhram and other provinces located in coastal area along Gulf of Thailand are commonly preserved green mussels as “Hoi-dong”, traditional fermented mussel, which is a darkish orange semi-solid appearance with sour (pH 3.1-6.1) and salty taste [10, 11]. Presently, Hoi-dong is reported as functional food and health promoting benefits, such as cholesterol-lowering, and immunomodulatory effects are affected from lactic acid bacteria occurred during fermentation process [12, 13]. However, there are some concerns dealing with food additives added to Hoi-dong during food processing. Benzoic acid is naturally present in fruits and commonly used as preservative in foods and beverages. This preservative is authorized in the European Union on the condition that the maximum use rules are respected [14, 15]. Sorbic acid is also widely used in the food, beverage, cosmetic, and other industries and is widely used to inhibit the growth of spoilage microbials to extend the shelf life and reduce the possibility of microbial toxin poisoning [16]. Benzoic acid, sorbic acid and its salts are common antimicrobials, therefore excessive use of this preservatives may cause adverse effects to consumers. Hence, the control of their use on food processing is necessary [16]. Color additives are used to correct or restore color of food when color has been lost during processing and storage, food color improvement, changing of food color or color intensity and covering the low food quality. However, utilizing of food colorants are controlled according to the Codex Alimentarius Commission (CAC) [17]. Synthetic food colorants more are favorite in food industry rather than natural colors due to its stability and simply to use. Therefore, adverse effects such as behavioral disorders, hyperactivity, attention impairments and teratogenicity are concerned especially in children [18]. Due to color of fermented mussels can be fade during fermentation, packaging or storage, synthetic food colors may add to Hoi-dong. Recently, benzoic acid and its salt and sorbic acid and its salt are allowed to use as preservatives in Thai food according to the Ministry of Public Health (MOPH) Notification No. 391 (2018). However, maximum limit (ML) for sorbates in processed meat product at 1,500 mg/ml and not allowed ML for benzoate [19]. In Thailand, synthetic food colorants including Sunset Yellow FCF, Azorubine, Tartrazine, Ponceau 4R, Brilliant blue FCF are contained in food at low level and there are not harmful with children. However, some of Thai food are not allowed to added colorant, the use of synthetic food colorants is need to control strictly [20]. According to the Thai Agricultural Standard for traditional Thai fermented fishery product, a satisfactory product is control by specific criteria such as, physical properties, salt content, allowed food additives and acceptable criteria on contaminants [21, 22]. This study was purposed to investigate microbiological and chemical contaminants contained Hoi-dong, which was purchased from fresh markets locating in Samut Songkhram, Thailand; and evaluate its quality status as food safety product for consumers. The finding may useful for surveillance the quality of traditional fermented

food and prevent the risk of foodborne diseases on public health concerns.

MATERIALS AND METHODS

Sample Collection

Hoi-dong samples (n = 16) were collected from one manufacturer and fifteen retailers in Muang district, Samut Songkhram, Thailand. Each sample collection (400 g to 1 kg) was triplicated, then pooled and transferred to sterile plastic bag. Twenty-five grams of Hoi-dong was homogenized in sterile buffered peptone water (Himedia, Mumbai, India) and solution was transported at 4 □C to microbiological laboratory, Faculty of Science and Technology, Suan Sunandha Rajabhat University. The remaining of sample was prepared to determine toxic metals, food preservatives and synthetic food colors according by Standard methods notified from Ministry of Public health, Thailand.

Fermentation Status

Determination of pH and %NaCl was used to monitor ability on food preservation during fermentation process, which is due to lactic acid production from probiotics and NaCl concentration for growth inhibition of pathogenic and spoilage microbials. Acidity and salinity of Hoi-dong were determined by food pH meter (HI981036, Hanna, Romania) and salinity checker (SO-303, Tanita, Japan). Data was represented as pH and %NaCl, respectively.

Microbiological Contaminant Evaluation

Microbiological contaminants were evaluated according to Bacteriological Analytical Manuals (BAM). One milliliter of sample solution was ten-fold diluted with trypticase soy broth (Himedia, Mumbai, India) and serial dilutions were 10-3 to 10-5. Sample dilutions were poured into selective media including phenol red egg yolk polymyxin (PREYP), tryptose sulfite cycloserine (TSC), eosin methylene blue (EMB), xylose-lysine deoxycholate (XLD) and mannitol salt agar (Himedia, Mumbai, India), which were used for isolation of Bacillus cereus, Clostridium perfringens, E. coli, Salmonella spp. and Staphylococcus aureus. Thiosulfate citrate bile salt sucrose agar (TCBS) and 0-10% NaCl in nutrient broth were isolated and identified Vibrio species. Enumeration of E. coli and fecal coliforms was determined according from lactose fermentation and represented as most probable number (MPN). There was used to indicate recent fecal contamination or unsanitary food processing. Serial dilutions of inoculated samples were incubated and score was enumerated from lactose fermented colonies [23].

Evaluation of Toxic Metals and Food Additives

Toxic metal determination

Homogenized sample (0.5 g) in PTFE container was extracted with 1.0 ml of H2O2 (30% w/w) and 7.0 ml of HNO3 65% (w/w). Sample was digested and microwave digestion system (MWD series, METASH, China) at 180 □C for 15 min. After sample was cooled, its volume was adjusted to 25 ml by deionized water [24]. Pb and Hg determination were analyzed by graphite furnace atomic absorption spectrophotometry, GFAAS (iCE 3000 Series, Thermo Fisher Scientific, USA). Methods were referenced from AOAC and EPA, respectively [25, 26], and the limit of detection (LOD) was 0.005 mg/kg. All analyses were performed in triplicate, and the mean values were used to analyze the data. Results are given in mg/kg wet weight.

Determination of Food Preservatives

Hoi-dong was homogenized and 5 g of sample was weighed. Sample was extracted for 15 min by extract solution (20% v/v) with Carrez I and Carrez II solutions (1% v/v for each). Sample mixture was filtered with Whatman No.1 and sample clarification kit with membrane filter (Type nylon 0.45 µm 13 mm). Filtered sample (20 l) was injected through high-performance liquid chromatography, HPLC (1100 Series, Agilent, USA). Mobile phases (75:25) were 0.01 M ammonium acetate buffer (pH 4.5-4.6) and methanol with isocratic solvent, flow rate: 1 ml/min, column: Bondapak C18, injection volume: 20 µl, run time: 12 min). Benzoic acid and sorbic acid were determined by UV detector at 235 nm, and measured according by retention time and calibration curve [27].

Determination of Food Colorants

Homogenized sample (10 g) was extracted with ethanolic-ammonia (25 ml) at room temperature for overnight and filtered. After three-time extraction, solvent was removed under water bath and sample was acidified with acetic acid before analysis. Polyamide powder was dissolved with distilled water and filled to column (2-3 cm.). Column was cleaned with 10 ml of 1% acetic acid for twice-time. Prepared sample (20 ml) was added with 2-3 drops of glacial acetic acid, and filled into polyamide column. Loaded polyamide column was cleaned twice-time with distilled water and acetone, respectively. Colorant fraction was eluted with 20 ml of 50% methanol/ammonium hydroxide (95: 5) for twice-time. Solvent of eluent was evaporated with water bath for 45 C, and dissolved with 10 ml of distilled water, and filtered with membrane filter (0.45 µm 13 mm). Synthetic food colorants contained in Hoi-dong, were determined by HPLC (1100 Series, Agilent, USA). Optimal condition of HPLC system including injection volume: 20 l: mobile phase: 0.005 M TBAH buffer (pH 4.5-4.6): acetonitrile (55:45), flow rate: 1.0 ml/min (isocratic), column: Hypersil BDS C18, wavelength for detector: 254 nm. for Sunset Yellow FCF, Azorubine, Tartrazine, Ponceau 4R, and 630 nm. for Brilliant blue FCF. Approximate Retention time were 5.8, 7.6, 8.6, 10.2, and 6.6 min for Sunset Yellow FCF, Azorubine, Tartrazine, Ponceau 4R and Brilliant blue FCF, respectively [7].

Statistical Analysis

The results were compared and judged with criteria of General Standard for Food Additives and contaminants from Ministry of Public Health, Thailand, and this criteria was corresponded to CODEX [22]. Data were analyzed and represented by descriptive statistic.

RESULTS AND DISCUSSION

We found that all of samples (n = 16) were met within standard ranges for microbiological criteria of food quality (Table 1). In addition, all of samples were safe from toxic metals, which were implied that green mussel cultivated in this area were not affected from environmental problem. However, some of samples was contaminated with food preservatives, such as benzoic acid and synthetic food colorants i.e., Sunset Yellow FCF, Tartrazine and Ponceau 4R (Table 2). The finding was represented adding of food preservatives and synthetic food colorants. It means that contamination was occurred during food processing rather than from environment. In addition, overuse of chemical preservatives can inhibit pathogens and may also reduce lactic acid bacteria as probiotics. Therefore, the salinity and acidity of Hoi-dong were within

NaCl = 3.5-4.4% and pH = 3.98-4.74 as reference range [13].

Table 1 Microbiological contamination of Hoi-dong (n = 16) compared with standard criteria of food quality [23] *

Microbial tests	Unit	Results	Standard criteria
E. coli	MPN per g	Less than 3	Less than 3
Salmonella spp.	CFU per 25 g	Not detected	Not detected
S. aureus	CFU per g	Less than 10	Not more than 100
B. cereus	CFU per g	90	Not more than 1000
C. perfringens	CFU per g	Less than 10	Not more than 1000
V. cholerae	CFU per 25 g	Not detected	Not detected

* All samples were met to standard microbiological criteria; MPN = most probable number;

CFU = colonies forming unit

Table 2 Chemical contamination of Hoi-dong (n = 16) compared with standard criteria of food quality [22, 27]

Chemical tests	Unit	Results	Standard criteria
Toxic metals			
Pb	mg/kg	Not detected	Not detected
Hg (total)	mg/kg	Less than 0.05	Not more than 0.5
Preservatives			
Benzoic acid	mg/kg	1,200-3,089 [b]	Not allowed
Sorbic acid	mg/kg	Not detected	Not more than 1,000
Synthetic colors [a]	mg/kg	Detected	Reference 22 and 27

a Synthetic colors included Sunset Yellow FCF, Azorubine, Tartrazine, Ponceau 4R and Brilliant blue FCF were investigated. Only Sunset Yellow FCF, Tartrazine and Ponceau 4R were detected from eight of suspected samples; b this positive value was ranged from five of suspected samples

Hoi-dong is fermented green mussels and distributed as traditional food in Samut Songkhram. The variety of quality and taste are depended on flavoring that ingredient and their amount during fermented process i.e., salt, fish source, soy source, vinegar, etc. Hence, its optimal salinity and acidity are necessary for promoting lactic acid bacteria, which is play role on inhibiting growth of pathogenic and spoilage microbials [11, 13, 14]. Therefore, food preservatives and colorants are intentionally adding within Hoi-dong for prolong its self-life and attractiveness of this food. These chemical additives may concern for adverse health effects [16, 17, 19, 20]. In addition, Pb and Hg are commonly suspect

contaminants in seafood and seafood products and monitoring of this contamination is still required [6-9]. This study had reported benzoic acid and synthetic food colorants as contaminants contained in Hoi-dong. The food hygiene and safety on fermentation and food processing were need to control and surveillance by public relation among government officers and traditional manufacturers.

CONCLUSION

In this study, Hoi-dong, fermented green mussels (n = 16) was processed through optimal salinity and acidity. There were met within standard ranges for microbiological criteria of food quality and were safe from toxic metals including Pb and Hg. However, was contaminated with food preservatives, such as benzoic acid and synthetic food colorants i.e., Sunset Yellow FCF, Tartrazine and Ponceau 4R. The find was concerned on food preservative and synthetic food colorant uses in traditional fermented food and public relation is need to conduct with among government officers and traditional manufacturers.

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