บทความวิจัย

คุณภาพด้านจุลชีววิทยาเพื่อการประเมินจุดควบคุมวิกฤติของการผลิตอาหารปริมาณมากในโรงอาหารมหาวิทยาลัย: กรณีศึกษายำไข่ต้ม

อำพร แจ่มผล[1*] อัญชนีย์ อุทัยพัฒนาชีพ[1] วราภา มหากาญจนกุล[2] และ ทัศนีย์ ลิ้มสุวรรณ[1]

บทคัดย่อ

การวิเคราะห์จุลชีววิทยาของการผลิตยำไข่ต้มปริมาณมากสำหรับการจำหน่ายในโรงอาหารมหาวิทยาลัย ผลจากการทดลองใช้เป็นข้อมูลพื้นฐานในการวิเคราะห์ ระบุอันตรายและใช้ในการประเมินจุดวิกฤติที่ต้องควบคุม ยำไข่ต้มประกอบด้วยส่วนผสมที่ผ่านความร้อนและไม่ผ่านความร้อน ส่วนผสมที่ผ่านความร้อนได้แก่ ไข่ หมูบด และกุ้งแห้ง ผลจากการวิเคราะห์พบว่าส่วนผสมเหล่านี้หลังผ่านการให้ความร้อนตรวจไม่พบจำนวนจุลินทรีย์ทั้งหมดและจุลินทรีย์ที่ก่อโรค (*Salmonella, Staphylococcus aureus* และ *Vibrio parahaemolyticus*) สำหรับส่วนผสมที่ไม่ผ่านความร้อนได้แก่ ขึ้นฉ่าย ต้นหอม ผักชี พริกขี้หนู ผักกาดหอม ทำการวิเคราะห์จุลินทรีย์ทั้งหมดก่อนและหลังล้างพบว่าไม่มีความแตกต่างทางสถิติที่ระดับนัยสำคัญ 0.05 ของจำนวนจุลินทรีย์ทั้งหมดในผักสดก่อนล้างและหลังล้าง นอกจากนี้จำนวนจุลินทรีย์ทั้งหมดหลังการล้างยังพบในปริมาณสูงและพบปริมาณสารตกค้างจากยาฆ่าแมลงในผักชีอยู่ในระดับไม่ปลอดภัย ยำไข่ต้มที่ปรุงเสร็จเมื่อตั้งที่อุณหภูมิห้องเพื่อรอการจำหน่ายที่เวลา 0, 2, 4, 6 และ 8 ชั่วโมง พบจำนวนจุลินทรีย์ทั้งหมดเพิ่มขึ้นตามระยะเวลาที่ตั้งรอการบริการจาก 6.0×10^4 cfu/g เป็น 4.5×10^4, 1.0×10^5, 2.4×10^5 และ 1.7×10^6 cfu/g ตามลำดับ ดังนั้นการพบจำนวนจุลินทรีย์ทั้งหมดในยำไข่ต้มที่อุณหภูมิห้องที่เวลา 0 ชั่วโมงเป็นผลจากส่วนผสมที่ไม่ผ่านความร้อน หลังจากตั้งยำไข่ต้มไว้ที่อุณหภูมิห้องเป็นเวลา 4 ชั่วโมงจำนวนจุลินทรีย์ทั้งหมดเพิ่มขึ้นอย่างมีนัยสำคัญ ($p < 0.05$) และในชั่วโมงที่ 8 พบจำนวนจุลินทรีย์สูงกว่าเกณฑ์สำหรับอาหารพร้อมบริโภค การประเมินจุดควบคุมวิกฤติด้วยการใช้แผนภูมิการตัดสินใจ (decision tree) พบว่าจุดควบคุมวิกฤติของการผลิตยำไข่ต้มในปริมาณมาก คือ ขั้นตอนการล้างผักสด การให้ความร้อนในส่วนผสมที่ต้องทำให้สุก และระยะเวลาที่อาหารตั้งไว้ที่อุณหภูมิห้องก่อนบริโภค

คำสำคัญ: คุณภาพด้านจุลชีววิทยา อันตรายทางชีวภาพ สารตกค้างของยาฆ่าแมลง การประเมินจุดควบคุมวิกฤติยำไข่ต้ม

[1]ภาควิชาคหกรรมศาสตร์ คณะเกษตร มหาวิทยาลัยเกษตรศาสตร์

[2]ภาควิชาวิทยาศาสตร์และเทคโนโลยีการอาหาร คณะอุตสาหกรรมเกษตร มหาวิทยาลัยเกษตรศาสตร์

*ผู้นิพนธ์ประสานงาน, e-mail: agramt@ku.ac.th

Microbiological Quality for Assessment of Critical Control Points of Mass production in the University Canteen : Case Study of Spicy Boiled Egg Salad

Amporn Jamphon[1*], Anchanee Utaipatanacheep[1], Warapa Mahakarnchanakul[2] and Tasanee Limsuwan[1]

ABSTRACT

Analysis of microbiology was undertaken for the mass production of spicy boiled egg salad "Yam Kai Tom" in the university canteen and these results provided basic information for hazard analysis and the identification of critical control points. Spicy boiled egg salad is composed of cooked and uncooked ingredients mixed together. For the cooked ingredients; total aerobic plate count (TPC) and pathogen counts (*Salmonella, Staphylococcus aureus* and *Vibrio parahaemolyticus*) were not detected in eggs, minced pork, and dried shrimps after the heating process. For the uncooked ingredients; the TPC in vegetables (chinese celery, spring onion, coriander, hot chili and lettuce) remained at a high level after washing and the pesticide residue in coriander was at unsafe level. The TPC in the vegetables were not significant before and after the washing process. Spicy boiled egg salad held at ambient temperature for 0, 2, 4, 6 and 8 hours showed an increase in TPC from 6.0×10^4 cfu/g to 4.5×10^4, 1.0×10^5, 2.4×10^5 and 1.7×10^6 cfu/g respectively. Thus, the TPC in spicy boiled egg salad held at ambient temperature for 0 hour there was a considerable resulting from the uncooked ingredients. The TPC was significant increase ($p < 0.05$) after the product was held for 4 hours. The TPC at 8 hour holding time was over the microbiological quality criteria of ready-to-eat food. The identification of the critical control points (CCPs) was conducted by using a decision tree. The critical control points (CCPs) for the spicy boiled egg salad production were indicated at the process of washing the raw vegetables, heating the cooked ingredients, and holding time at ambient temperature before serving.

Keywords: microbiological quality, biological hazard, pesticide residue, assessment of critical control points, spicy boiled egg salad

[1]Department of Home Economics, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand
[2]Department of Food Science & Technology, Faculty of Agro-industry, Kasetsart University, Bangkok 10900, Thailand
[*]Corresponding author, E-mail: agramt@ku.ac.th

Introduction

Spicy salad is a popular Thai-style dish with a combination of sweet, spicy, salty and sour tastes. The production of spicy boiled egg salad calls for the use of cooked and uncooked ingredients mixed together and normally it is preferred to be served fresh after cooking and mixing. Thai spicy salads are not reheated after preparation which is one of the major causes of food poisoning in Thailand. The presence of bacteria in ready-to-eat food indicated either post-cooking or post-preparing contamination of the dish. For the mass production it is commonly precooked, premixed in advance and kept for several hours in order to increase customer choice. The food poisoning cases in Thailand were reported 119,392 cases in 2012 and 131,870 cases in 2013 [3, 4]. The risk factors for food-borne diseases indicated that the majority of outbreaks result from incomplete cooking processes and faulty food handling practices. The hands of food handlers can be pivotal as vectors in the spread of food-borne disease due to poor personal hygiene or cross-contamination [18]. Food handlers that were nasal carriers of *Staphylococcus aureus* or hand carriers of enterobacteria can constitute a potential risk to the food contamination [23]. At the same time, a long time and inadequate temperature holding during the distribution of meals were also a contributory factor [1]. The pesticides have been reported to cause toxin effect on humans, such as headaches and nausea to chronic effects like cancers. The pesticides residues have been found in various fruits and vegetables [2, 6].

The Hazard Analysis and Critical Control Points (HACCP) system, which is science-based and systematic, identifies specific hazards and measures for their control to ensure the safety of food. HACCP system is a tool to assess hazards and establish control systems that focus on prevention rather than relying mainly on end-product testing [9]. In general, HACCP aims to eliminate factors that result in food-borne diseases in humans from the production, handling, treatment, transportation and storage of food. HACCP system has been applied in food industry and foodservice [8, 33]. HACCP system in food industry differs from the HACCP system in foodservice due to the fact that foodservice contains hazards mainly due to the time and process involved from the preparation of foods before serving. Therefore, for HACCP application in foodservice, the first task is to list the food served in the menu and find the hazard location and list them on the flow chart for the food preparation and then determine CCPs [21, 24]. The canteens at University, which is prepared and cooked ready-to-eat food for students and staffs. These foods have been implicated in the transmission of food-borne disease. Thus, the objective of this study was to determine the microbiological quality the mass production of Thai spicy salad by using spicy boiled egg salad as a study sample at canteens of Kasetsart University campus in Bangkok. These results provided basic information for hazard analysis and identify the critical control points and microbiological status of spicy boiled egg salad and recommend critical control points to improve its quality.

Materials and Methods

One of the canteens at Kasetsart University campus in Bangkok was chosen to observe and collect the samples of spicy boiled egg salad preparation and this canteen processed approximately 300 meals daily for students and staffs.

Sample collection

Samples were collected in December 2012, January 2013 and February 2013. Samples of foods (100 grams) were aseptically collected and placed in sterile containers at different stages of preparation process (raw material receiving, preparing, cooking, mixing cooked and uncooked ingredients and serving) stored at 4°C in an icebox and then taken to the laboratory at the Institute of Food Research and Product Development (IFRPD), Kasetsart University within 2 hours of collection for microbiological analysis. The microbiological analysis of ingredients varied depending on the types of ingredients as follows; fresh eggs were analyzed for Total Plate Count (TPC) and *Salmonella.* Minced pork was analyzed for TPC, *Salmonella* and *S. aureus.* Dried shrimps were analyzed for TPC, *Salmonella, S. aureus* and *Vibrio parahaemolyticus.* Vegetables (chinese celery, spring onion, coriander, hot chili, lettuce) were analyzed for TPC, *Escherichia coli* and *S. aureus.* Cooked eggs, cooked minced pork and cooked dried shrimp were analyzed for TPC, *Salmonella* and *S. aureus.* Spicy boiled egg salad held at ambient temperature for 0, 2, 4, 6 and 8 hours were analyzed for TPC, *Salmonella* and *S. aureus.*

The 500 grams of the vegetables used were taken to analyze for pesticide residues in the phosphates and carbamates group by using the GT- test kit (The 3rd award on Inventor's Day 1997 from The National Research Board of Thailand, Patent No. 8446, Thailand) [27]. Temperature and time concerned with food preparation or holding were recorded. The thermometer was washed, cleaned with 70% alcohol and dried before use each time.

Microbiological analysis

Twenty-five grams of samples were taken at random and stored in stomacher bags. Then, 225 ml of 0.1% peptone solution was added into the bag. The sample mixture was homogenized in a stomacher for 2 minutes and serial diluted [35] for microbial analysis. Triplicate measurements were made in each sample taken. Total plate counts were determined by surface spreading homogenate dilution (1.0 ml.) on standard plate count agar and incubated at 35°C for 48 hours [28]. The Most Probable Number (MPN) of *E. coli* was determined according to the U.S. Food and Drug Administration method [29]. The serial dilutions of a sample were inoculated into a series of triple sets containing of lauryl sulfate tryptose broth (LST, Merck,

Germany) at 35°C for 24 hours. All positive tubes which have gas production were sub-cultured onto a tube of EC broth and incubated at 45.5°C for 24 hours. Each gassing EC tube was identified. A loop suspension was streak onto an eosin methylene blue agar plate (L-EMB, Merck, Germany) and incubated at 35°C for 18-24 hours. *V. parahaemolyticus* were analyzed using phosphate buffered saline (PBS) dilution water (incubated overnight at 35°C). A loopful suspension was streaked onto thiosulfate citrate bile salt sucrose agar (TCBS, Difco, USA). The suspect colonies were confirmed by using API 20E [30]. *S. aureus* were determined on Baird-Parker agar plates (Merck, Germany) (incubated at 35°C for 45-48 hours). The suspect colonies were confirmed using coagulate test [31]. *Salmonella* were analyzed using lactose broth and Rappaport-Vassiliadis Broth with soya (RVS, Oxoid, UK) medium and incubated at 42°C for 24 hours respectively. The positive cultures were streaked onto xylose lysine deoxycholate agar (XLD, Oxoid, UK) and incubated 24 hours at 37°C [19]. Microbiological results were compared with the microbiological quality criteria of ready-to-eat food (Uncooked and Cooked Foods) of the Department of Medical Science, Ministry of Public Health, Thailand [10]. These criteria are summarized in Table 1.

Table 1 Microbiological quality criteria of ready-to-eat food.

Type of Foods	Safe level	
Ready-to-eat foods		
- Uncooked foods ready to eat immediately Clean vegetables or fruits such as salad, papaya salad, etc.	MPN *E. coli* (/gram)	Less than 10
	Salmonella (/25 grams)	Not found
- Cooked foods that are cooked dishes, noodles, Thai rice noodles, chili sauce, sausage, cold meat, cooked squid, biscuit, candied fruit, etc.	Total microbe (/gram)	Less than 1×10^6 cfu/g
	MPN *E. coli* (/gram)	Less than 3
	S. aureus (/gram)	Less than 100 cfu/g
	V. parahaemolyticus (/25 grams)	Not found
	Salmonella (/25 grams)	Not found

Source: Department of Medical Science, Ministry of Public Health, Thailand [10]

Pesticide residue analysis

Pesticide residue was analyzed by using the GT-test kit to detect the pesticides in phosphates and carbamates groups. This test kit is useful in filtering out unsafe food for the consumers by Food and Drug Administration, Department of Health, and Department of Medical Science, Ministry of Public Health, Thailand. This semi-fast testing method tests based on the cholinesterase Inhibition technique. The pesticides residues from samples were extracted with dichloromethane, and then removed the liquid extraction by evaporation. The pesticides residues in samples were detected by acetylcholine and cholinesterase enzyme. If sample tube has less color than control tube, the result was not detected. If sample tube has greater color than control tube, the result was detected as safe to be consumed. In case of sample tube has greater color than critical tube, the result was detected as unsafe level.

The safety levels are made from the toxicity of one or the combined chemical that hampers the performance of the Enzyme-chlorine-esterase by at least 50%. Effectiveness of GT-test kit for toxic residues in vegetables tests are a sensitivity value of 92.3%, specificity of 85.1% and accuracy of 87.1%.

Hazard analysis and identification of critical control points in the spicy boiled egg salad production

Sources and potential types of biological hazard contamination of raw materials and ingredients during the production process were identified using the modified technique of Loken [17] based on microbiological criteria to foods and ingredients [18]. Critical control points were identified by using the Decision Tree technique [30] focusing on biological hazards.

Statistical analysis

Data obtained were computed for the analysis of variance (ANOVA) and the difference between averages values were compared by Duncan's multiple range tests (DMRT) using a computer program.

Results and Discussion

Preparation and cooking process of spicy boiled egg salad

The preparation of spicy boiled egg salad was observed and the process diagram is shown in Figure 1. The process consisted of the steps of raw material receiving and preparing (washing and cutting), cooking, mixing the cooked and uncooked ingredients and serving.

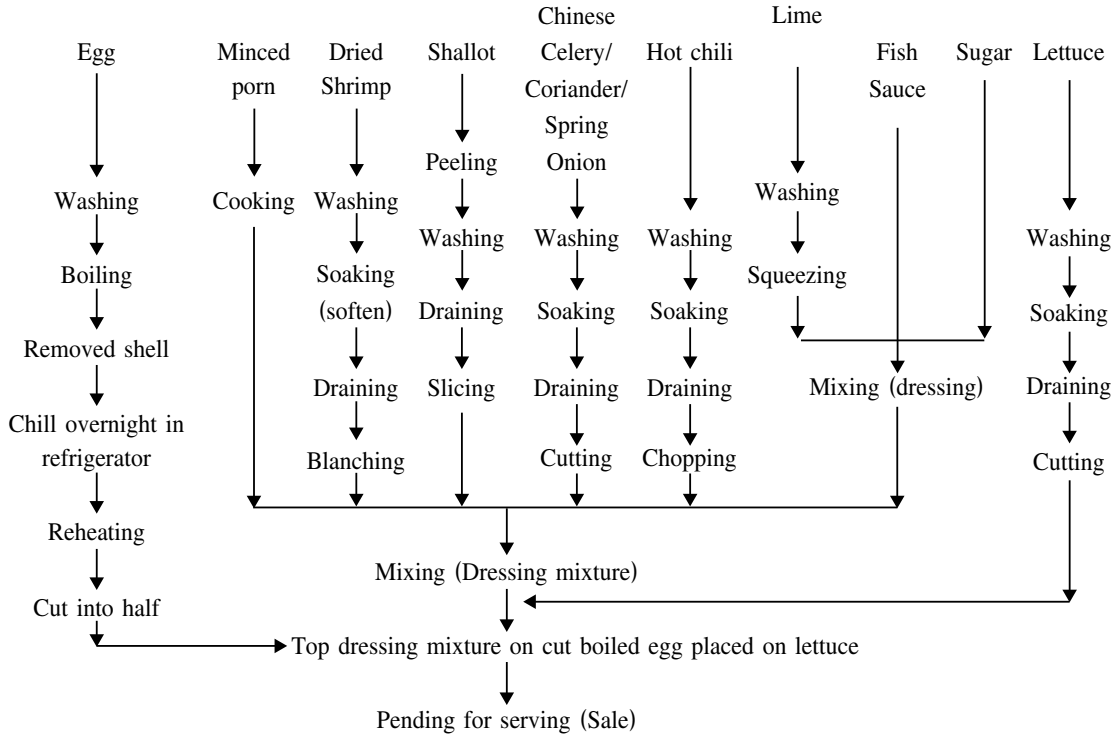

Figure 1 Process diagram of spicy boiled egg salad production.

Preparation of raw materials; egg, minced pork, dried shrimp, spring onion, hot chili, shallot, chinese celery, coriander, and lettuce were as follows: 1. Eggs were boiled (at 98°C for 10 minutes) and removed shells before keeping them overnight in a refrigerator. Before serving, these eggs were reheated at 90°C for 10 minutes with internal temperature at 75°C and cut in half. 2. Minced Pork was cooked until the internal temperature reached 74°C for 2 minutes. 3. Dried shrimp was washed and soaked to soften, and then blanched in boiling water at 98°C for 1 minute. 4. Vegetables (spring onion, chinese celery, coriander and lettuce) were washed and then cut into pieces and kept in a refrigerator. 5. Shallots were peeled, washed and chopped. Hot chili was washed twice, soaked in water for 1 hour and chopped.

Dressing was prepared by squeezing the limes and mixing the lime juice with fish sauce and sugar. The dressing was mixed with the cooked meat products (cooked minced pork and blanched dried shrimps), then the fresh vegetables (sliced shallot, chopped hot chili, chinese celery, spring onion and coriander) were added and poured on top of the cooked eggs.

Microbiological analysis of raw and cooked meat products

Ingredients for spicy boiled egg salad consisted of cooked eggs, cooked minced pork, blanched dried shrimp, and fresh vegetables including shallot, chinese celery, spring onion, coriander, hot chili, lime, and lettuce, were mixed together and flavored with fish sauce and sugar.

The bacterial counts of raw and cooked meat products are presented in Table 2. TPC is shown for all raw samples. *Salmonella* was found in minced pork, but not found in raw eggs and dried shrimp. *Vibrio parahaemolyticus* was not detectable in any raw samples. All the cooked meat products had showed no detectable number of bacteria for total plate count, *Salmonella* or *S. aureus* counts. These results were similar to those of Martins and Germano [20] who reported that the number of microorganisms in meat lasagna production was significantly reduced ($p < 0.001$) after cooking. These results reaffirm the wisdom of the recommended precaution to heat foods to reach an internal temperature of at least 74°C (165°F) for 15 seconds in order to eliminate the number of microorganisms [11].

Table 2 Microbiological analysis of raw meat and meat products in spicy boiled egg salad.

Raw materials	Average microorganism number			
	TPC (cfu/g)	*Salmonella* (/25 g)	*S. aureus* (cfu/g)	*V. parahaemolyticus* (/25 g)
Egg				
Raw egg	8.1×10^2	ND	N/A	N/A
Boiled egg	none	ND	none	N/A
Boiled egg kept overnight in refrigerator and reheated	none	ND	none	N/A
Minced pork				
Raw minced pork	7.7×10^7	Detected	none	N/A
Cooked minced pork	none	ND	none	N/A
Dried shrimp				
Raw dried shrimp	5.4×10^3	ND	none	ND
Cooked dried shrimp	none	ND	none	ND

ND = Not detectable in 25 g, N/A = Not analyzed, none = Not detectable at dilution 1 : 10

Microbiological quality and pesticide residue of raw vegetables, before and after washing

All the vegetables in this study were washed twice with running tap water and then soaked in water. The washing process tended to reduce the number of microorganisms but the difference between before and after washing was not significant among each type of vegetables (Table 3). Moreover the TPC in the washed vegetables were over the level of the microbiological quality standard of ready-to-eat food of the Department of Medical Science, Ministry of Public Health [10]. The small vegetables with a short stem like coriander and lettuce showed higher microbiological counts than hot chili, spring onion, and chinese celery. This could be because the shorter stems make the leaves closer to the ground causing a higher risk of microbiological contamination. Moreover the characteristics of the edible part may also be a factor [7]. The characteristics of leaves of coriander and lettuce tended to hold foreign matter better than those of hot chili, spring onion and chinese celery.

Unsafe levels of Pesticide residue were found in chinese celery, spring onion, coriander, hot chili and lettuce before washing. The water washing process reduced the pesticide residue to a safe level in all the vegetables except for the coriander. This could be due to the farm production practice and the characteristics of the vegetables and the edible part as mentioned earlier for the risk of microbiological contamination. Coriander was among the types of vegetables showing higher risk of pesticide contamination in the previous reports [12].

The higher TPC values and unsafe level of pesticide residue measured in all raw vegetable samples demonstrated the need for good quality control of raw materials and an efficient washing process in the food service operation. Sanitizing agents are suggested for use in the washing step to decrease the number of microorganisms [21]. Some chemicals are claimed to successfully reduce pesticide residue in vegetables as well [5, 16, 19, 26]. The efficiency of a sanitizing agent in reducing microorganisms depends on the type of vegetable and the areas in which the microorganisms live, such as on rough leaves or stems, which can be more difficult to clean than areas with smoother surfaces. In addition, physical methods such as the use of shear force, Jacuzzi washer, ultrasound, high pressure (HP), high-intensity field pulses (HEFP), ultraviolet radiation (UV), radio frequency (RF), and ionizing radiation showed an inhibitory effect on bacterial growth [13]. However, these methods have not been implemented in the university canteen. Also specific cleaning and sanitation procedure, prevention of cross-contamination were recognized.

Table 3 Microbiological analysis and pesticide residue level of raw vegetables before and after washing.

Raw materials	Average microorganism number			pesticide residues level*
	TPC (cfu/g)	*E. coli* (MPN/g)	*S. aureus* (cfu/g)	
Chinese Celery				
Before washing	$2.8 \times 10^{6(a)}$	<3	none	Unsafe
After washing	$3.0 \times 10^{7(a)}$	<3	none	Safe
Spring onion				
Before washing	$2.6 \times 10^{7(a)}$	<3	none	Unsafe
After washing	$2.6 \times 10^{7(a)}$	<3	none	Safe
Coriander				
Before washing	$1.1 \times 10^{8(a)}$	<3	20	Unsafe
After washing	$1.4 \times 10^{7(a)}$	<3	none	Unsafe
Chili				
Before washing	$3.4 \times 10^{7(a)}$	<3	none	Unsafe
After washing	$5.9 \times 10^{6(a)}$	<3	none	Safe
Lettuce				
Before washing	$1.9 \times 10^{8(a)}$	36	none	Unsafe
After washing	$8.2 \times 10^{6(a)}$	13	none	Safe

Note: Average microorganism number of before and after washing in column with the same letters (a) are not significantly different ($p \leq 0.05$).

*Safe = the sample tube has greater color than control tube.

*Unsafe = the sample tube has greater color than critical tube.

Microbiological analysis in ready-to-eat spicy boiled egg salad

Salmonella and *S. aureus* were not detected in ready-to-eat spicy boiled egg salad and TPC was reported at 6.0×10^4 cfu/g, which is considered at the safe level (less than 1.0×10^6 cfu/g) (Table 4). Thus, the TPC did not result from the cooked ingredients of the spicy boiled egg salad; the raw vegetables were, probably, one of the contributors for the increase of the TPC in the final product. The measurement of microorganisms in the final product is an important means to ensure that hygiene and sanitation standard of ingredients that are not submitted to further thermal treatment during the production, and information to the consumers. On the other hand, it is necessary to evaluate the ingredients supplier's quality assurance.

Table 4 Total plate counts and food borne pathogens count in ready-to-eat spicy boiled egg salad held at ambient temperature for different lengths of time.

Sample	Microbiological analysis		
	TPC (cfu/g)	*Salmonella*/25 g	*S. aureus* (cfu/g)
Spicy boiled egg salad held at ambient temperature			
1. For 0 hour	$6.0 \times 10^{4(c)}$	ND	none
2. For 2 hours	$4.5 \times 10^{4(c)}$	ND	none
3. For 4 hours	$1.0 \times 10^{5(c)}$	ND	none
4. For 6 hours	$2.4 \times 10^{5(b)}$	ND	none
5. For 8 hours	$1.7 \times 10^{6(a)}$	ND	none

Note: Average microorganism numbers in column with the same letters (c) are not significantly different ($p \leq 0.05$), Average microorganism numbers in column with the different letters (a, b, c) are significantly different ($p \leq 0.05$), ND = Not detectable at dilution 1 : 10

The longer holding time, higher TPC for ready-to-eat spicy boiled egg salad was showed in Table 4. The TPC counts in spicy boiled egg salad samples held for 0, 2 and 4 hours at ambient temperature were not significantly different. The increase in TPC was significantly higher at 95% confidence after holding for 4 hours; and the TPC at the 8 hour-holding time was over the safety level suggested by the Department of Medical Science, Ministry of Public Health [10]. The environmental air in the distribution area could be a factor as suggested by Celina *et al.* [8] who reported a high TPC in the environmental air measured from the distribution area in a traditional Brazilian seafood restaurant.

Hazard analysis and identification of critical control points

The overall production process of spicy boiled eggs salad consisted of 5 major steps as discussed earlier. The results of microbiology and pesticide residues showed the needs of improving best practices in good hygiene practice (GHP). The potential hazards in each step were identified and possible Critical Control Points (CCP) was evaluated by using a CCP decision tree. And the results of microbiology at all stages provide basic information to identify and monitor the CCPs in the preparation of this food. A summary of the CCPs is presented in Table 5.

Table 5 Hazard analysis and critical control point of spicy boiled egg salad production.

Processes or Steps		Hazard	Question (Decision tree)				
			Q 1	Q 2	Q 3	Q 4	CCP
1. Raw material receiving							
1.1 Egg	Biological	*Salmonella*	Y	N	Y	Y	N
1.2 Pork	Biological	*Salmonella, S. aureus*	Y	N	Y	Y	N
1.3 Dried shrimp	Biological	*Salmonella, S. aureus* *V. parahaemolyticus*	Y	N	Y	Y	N
1.4 Fresh vegetables (shallot, chinese celery, spring onion, lettuce, chili and coriander)	Biological	TPC, *E. coli, S. aureus*	Y	N	Y	Y	N
	Physical	Soil, sand	Y	N	Y	Y	N
	Chemical	Pesticide residues	Y	N	Y	Y	N
2. Preparation							
2.1 washing (shallot, chinese celery, spring onion, lettuce, chili and coriander)	Biological	*E. coli* from water and handlers *S. aureus* from handlers	Y	N	Y	N	Y
	Chemical	Pesticide residues	Y	N	Y	N	Y
2.2 cutting/slicing (shallot, chinese celery, spring onion, lettuce, chili and coriander)	Biological	- *S. aureus* from handlers - Contamination of pathogens from utensils or equipments	Y	N	N	-	N
3. Cooking (Boil egg, cook minced pork and blanch dried shrimp)	Biological	Survival of pathogens	Y	Y	-	-	Y
4. Holding time before serve	Biological	- Contamination of pathogens from air - Growth of *E. coli* from vegetables	Y	N	Y	N	Y

Note: Y = Yes, N = No

Q.1 Do control measures exist at this step or subsequent step for the identified hazard? No - No CCP, Yes - Q 2

Q.2 Does this step eliminates or reduces the likely occurrence of a hazard to an acceptable level? No - Q 3, Yes - CCP

Q.3 Could contamination with identified hazard(s) occur in excess of acceptable level(s) or could these increases to unacceptable level(s)? No - No CCP, Yes - Q 4

Q.4 Will a subsequent step eliminate identified hazard(s) or reduce likely occurrence to an acceptable level? No - CCP, Yes - No CCP

Three CCP were identified as follows:

1. Washing process of the vegetables

The washing step could reduce the number of *E. coli* in lettuce and *S. aureus* in coriander, the microbe numbers were still above the microbiological quality criteria of ready-to-eat food (uncooked and cooked Foods) of the Department of Medical Science, Ministry of Public Health, Thailand [10]. This step was considered CCP because these vegetables were not reheated after preparation. This indicated a need for a good quality raw material acquisition and an efficient washing technique. Therefore, disinfectants (sanitizing agents) are suggested to use in washing step to decrease the number of microorganisms such as calcium hypochlorite, chlorine dioxide, hydrogen peroxide and lactic acid, chlorine and peroxyacetic acid, sodium hypochlorite that can reduce microorganisms on surface of vegetables by 2-3 log units [13]. Work by Leawchawalit *et al.* [16] suggested that ozone (0.35 ppm), sodium hypochlorite (HC, 25 ppm) and chlorinated trisodium phosphate (CTSP, 25 ppm) reduced TPC by 3.0, 3.2, and 4.1 log cycles in 15, 10 and 5 minutes for carrots, water convolvulus and lettuce respectively, which is at the safe level of microbiological quality criteria of the ministry of public health, Thailand. Chantapanyasin [5] found that soaking contaminated tomatoes in tap water (pH 4) combined with sodium hypochlorite or sodium chlorite, at concentration of 100 and 50 ppm respectively, completely destroyed *E. coli* 3 $\log_{10}$ cfu/g. within 10 minutes, without any effect on color, odor and texture of fruit. Mahakarnchanakul *et al.* [19] found that sodium chlorite solution (50 ppm at pH 4 with acetic acid at $30 \pm 2^{\circ}C$) could destroy total aerobic bacteria on lettuce and white cabbage within 30 minutes, and completely eliminated *E. coli* on lettuce, coriander and peppermint within 15 minutes. Sodium hypochlorite solution (200 ppm at pH 4 with acetic acid at $30 \pm 2^{\circ}C$) completely kills total aerobic bacteria on lettuce and *E. coli* contaminated on white cabbage and coriander within 15 minutes.

Furthermore, the unsafe levels of pesticide residue were found in all raw vegetable samples. In order to help consumers reducing their risk, certified products which have official certify symbols on the products, such as Q-Mark, food safety label, organic vegetable are recommended.

2. Cooking process of the meat products

Cooking was the only heat process for meat products (eggs, minced pork and dried shrimp) used in spicy boiled egg salad preparation. Cooking must ensure the pathogenic microorganisms do not survive to reduce the risk. In this study, TPC, *Salmonella* and *S. aureus* were not detected in all the cooked meat products. The heating step was identified by using decision tree as one of the critical control points. They should consider cooking times and temperature. Temperature and time of heating were essential factors. These must be carefully

implemented to ensure that a sufficiently high end-point temperature can be achieved. The control measure were thoroughly cooked until the internal temperature at least 74°C (165°F) for 15 seconds in order to eliminate the number of microorganisms [11].

3. Holding time for serving

The TPC of samples of ready-to-serve spicy boiled egg salad held for 0, 2, 4, and 6 hours showed bacteria numbers within the microbiological quality criteria of ready-to-eat food. The ready-to-serve spicy boiled egg salad held for 8 hours showed bacteria numbers over the microbiological quality criteria. Also holding time for ready-to-eat spicy boiled egg salad was shown to be one of the critical control points because of the possibility of cross-contamination and the growth of *E. coli* from fresh vegetables which was a biological hazard. Also spicy boiled egg salad should be served as soon as possible after mixing. The temperature and time were monitored at least every hour. Spicy boiled egg salad samples should held at ambient temperature (21-60°C) for less than 2 hours [11].

Also the application of HACCP system in food production will help food managers to identify and control problems in their operation and reduce the incidence of food borne illness.

Conclusion

The production of spicy boiled egg salad makes use of cooked and uncooked ingredients. For cooked ingredients; analyzed for total aerobic plate count and pathogens (*Salmonella and Staphylococcus aureus*) was not found in egg, minced pork, and dried shrimp after the heating process. For uncooked ingredients (chinese celery, spring onion, coriander, chili and lettuce) the TPC showed no significant in ingredients before and after the washing process. The TPC in washed vegetables were over the level of the microbiological quality standard and the insecticide residue in coriander was at the unsafe level.

Spicy boiled egg salad held at ambient temperature for 0, 2, 4, 6 and 8 hours showed an increase in TPC from 6.0×10^4 to 4.5×10^4, 1.0×10^5, 2.4×10^5 and 1.7×10^6 cfu/g respectively. The increase of TPC was significant ($p < 0.05$) after holding for 4 hours. The TPC at 8 hour-holding time was beyond the safety level. The critical control points for the spicy boiled egg salad production were indicated at the process of washing fresh vegetables, heating ingredients, and holding time before serving.

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ได้รับบทความวันที่ 26 มกราคม 2558
ยอมรับตีพิมพ์วันที่ 10 มีนาคม 2558