

Effect of Steaming Treatment on Physical Properties and Shelf Life of Hae-Kuen

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Keywords: Physical Properties, Sensory Evaluation, Shelf-life, Shrimp Roll, Steaming Treatment.

Abstract: This research aimed to extend the shelf life of Hae-Kuen, Thai traditional shrimp roll, by effect of steaming temperature (80 and 85°C) and steaming time (5 and 10 minutes) on microbial contents, textural properties and sensory evaluation. Results of the study showed that the microbial contents of steaming Hae-Kuen were decrease as compared with the control sample (without steaming). While the initial microbial content of the control sample was 6.4×10^7 CFU/g, there are steamed at 80°C for 5 and 10 minutes had the microbial levels of 5.3×10^2 and 2.3×10^2 CFU/g, respectively. The microbial contents decreased to 4.7×10^2 and 1.7×10^2 CFU/g as higher steaming temperature (85°C) was used for 5 and 10 minutes, respectively. Being stored at 4°C, microorganisms detected from unsteamed Hae-Kuen increased to the level of 7.7×10^6 CFU/g. which was higher than the Community Product Standards Agency Criteria, at day 3. While, the microbial contents of Hae-Kuen steamed at 80 and 85°C for 5 and 10 minutes were in the range of 10 to 95 CFU/g after storing for 15 days. The texture profile analysis showed that all steamed Hae-Kuen samples had higher values of hardness, springiness, cohesiveness and chewiness when compared to the control sample.

1 INTRODUCTION

Hae-Kuen or shrimp roll is the famous Chinese dish in Thailand. It is made from shrimp and pork fat mixed with corn flour, egg and seasoning, and crushed or pounded until sticky, then wrapped with bean curd skin into long and round pieces. The Hae-Kuen is fried and served with plum sauce. Nowadays, many Thai-Chinese style restaurants classified in the appetizer menu. The restaurants prepared and stored it in refrigerator as uncooked product. Unfortunately, the shelf life of uncooked limited predominantly because of spoilage contamination. Microorganism content of uncooked was higher than 10^6 CFU/g after 3 days which was higher than the Community Product Standards Agency criteria. Spoilage of Hae-Kuen is mostly accompanied with physical properties changes including souring, slimy meat juice exudates and swelling (Purwanto et al, 2017).

It is important to improve Hae-Kuen to meet the Community Product Standards Agency criteria and maintenance of the quality characteristics of Hae-Kuen. Therefore, the aim of this research was to extend the shelf life by steaming treatment. An effect of steaming temperatures at 80 and 85 °C and

steaming times including 5 and 10 minutes on microorganism content and physical properties of Hae-Kuen were evaluated. Sensory test and shelf life were also conducted.

2 MATERIALS AND METHODS

2.1 Preparation of Shrimp Roll, Hae-Kuen

The Hae-Kuen ingredients comprised as following: ground shrimp (80.91%), salt (2.1%), pepper (0.81%), wheat flour (8.09%) and egg (8.09%). The 120 g of mixture were thoroughly mixed and wrapped with bean curd sheet into 5 cm diameter and 15 cm length. Each piece of Hae-Kuen was then packed in a heat resistant nylon laminate bag in vacuum condition.

2.2 Effect of Steaming Treatment

The Hae-Kuen samples were divided into five separated batches, (1) no steaming treatment (control), steaming at (2) 80 °C 5 min, (3) 80 °C 10

min, (4) 85 °C 5 min, and (5) 85 °C 10 min. The samples were steaming in the temperature control batch (MEMMERT Model: WNB series, Germany) at 80 °C and 85 °C which measured at the middle of sample. The sample were incubated for 5 and 10 min and cooled down immediately. The collected samples were kept at 4 °C until analysis.

2.2.1 Microbiological Analysis

After removing the outer plastic casing, a 25-gram of Hae-Kuen samples were aseptically transferred to a sterile plastic bag containing 225 mL of Butterfield's phosphate sterile (0.1% w/v) solution and agitated vigorously for one minute. Tenfold dilution series of the sample solutions were prepared using sterile peptone solution and 1 mL of each dilution was poured plated on selective agar plates in triplicate.

The total viable count (TVC) was determined on plate count agar (PCA) incubated at 35 °C for 48 h and yeast and mold on yeast malt agar (YM) incubated at 35 °C for 72-96 h. Bacterial counts were expressed as colony-forming units per gram of sample (CFU/g). The mean $\pm$ standard deviation of the count will be calculated by using three replicates for each culture time. *S. aureus* were analyzed according to ISO 6888-1:1999/Amd 1:2003 by adding 1 mL of suspension to Baird-Parker agar for 3 plates by separating 0.3, 0.3 and 0.4 mL, respectively (make a duplicate). Spread suspension in each plate and incubated at 37 °C for 22 ± 2 h. Marked the typical colonies which were black or gray color and surrounding with opaque zone. Then continued incubated at 37 °C for 22 ± 2 h, marked all new typical colonies and atypical colonies which were black or gray color without opaque zone, recorded number of all colonies. Selected 5 colonies in each typical and atypical colonies to confirm coagulase test. Selected colonies were inoculated to Brain-heart infusion broth (BHI) and incubated at 37 °C for 24 ± 2 h. 0.1 mL culture was transferred to 0.3 mL of rabbit plasma and incubated at 37 °C for 6 h for clot formation. Detection of *E. coli* in samples was done according to BAM Chap. 4 (2002) by making at least 3 serial dilutions, inoculated 1 mL from each dilution into 3 lauryl tryptose (LST) tubes for 3 tubes MPN, incubated at 35 ± 0.5 °C for 22 h and observed for the presence of gas, the positive tubes were producing gas. Transferred one loop of each suspension to a tube of EC broth, incubated at 44.5 ± 0.5 °C for 24 ± 2 h and observed for the presence of gas, the positive tubes were producing gas. One loop of culture was streaked on Eosin Methylene Blue Agar (EMB) (incubated at 35 ± 0.5 °C for 21 ± 3 h. Characteristics of *E. coli*

colonies were dark purple colonies, with or without a green metallic sheen. Transferred up to 5 suspicious colonies from each EMB plate to PCA slants, incubated at 35 ± 0.5 °C for 21 ± 3 h and use for further testing (IMViC test, Gram test and also re-inoculated back into LST to confirm gas production. *C. perfringens* were tested according to ISO 7937:2004 by adding 1 mL into petri dish, adding SC agar and mixing with rotation overlay 10 mL of Perfringens Agar and incubated at 37 °C for 20 ± 2 h in an anaerobic atmosphere. The colonies were then inoculated into Fluid Thioglycollate Medium at 37 °C for 21 ± 3 h in an anaerobic atmosphere. For confirmation, the culture was inoculated to lactose sulphite medium at 46 °C aerobic atmosphere in a water bath for 21 ± 3 h.

2.2.2 Physical Analysis

Moisture Content of Hae-Kuen sample was determined according to AOAC methods (2002) with the analytical No. 35.1.13.

Water activity was determined by water activity analyzer (AQUALAB Model 4TE, Decagon Devices, Inc., USA).

Texture profile analysis (TPA) was measured by TA.XT-plus Texture Analyzer (Stable Micro System, UK). Each Hae-Kuen was cut into 3 cm diameter x 3 cm length pieces and fried before texture measurement. Five replicates were carried out with an aluminium compression platen (50 mm diameter) with a head speed of strain 5 mm/sec. Two 50 % compression deformations were done with an interval of 5 sec between them. The characteristics of hardness, springiness, cohesiveness, gumminess and chewiness were determined.

2.3 Shelf Life Analysis

The Hae-Kuen samples from the selected condition were stored in refrigerator at 4 ± 1 °C. Samples were taken at 0, 3, 6, 9, 12, and 15 days following the start of incubation. Microbial content was determined in total viable count as described in microbiological analysis.

2.4 Sensory Analysis

Rating for preference tests were performed by 30 trained panellists to compare between (1) unsteamed and (2) steamed Hae-Kuen at 80 °C for 5 min, and (3) steamed Hae-Kuen at 80 °C for 10 min. All sample were fried before serving. The 5 hedonic scale was carried out to evaluate the taste attributes including

appearance, color, aroma, taste and overall preference.

2.5 Statistical Analysis

A completely randomized design (CRD) was used as experimental design. Analyses were done in triplicate. The data was statistical analyzed using one-way analysis of variance (ANOVA) by SPSS program. The Duncan's Multiple Range test at 5% level of significance was applied for all statistical analyses and the statistical significance of differences among mean values was established at ($p < 0.05$). The data were expressed as mean $\pm$ SD (standard deviation).

3 RESULTS AND DISCUSSIONS

3.1 Microbial Profiles of Unsteamed Hae-Kuen

Samples were collected from 3 restaurants and analyzed for pathogenic microorganisms according to the guideline. After 3 days of production, the microbial results observed that the growth of *E. coli* yeast and molds increased as shown in Table 1.

Table 1: Microbial profiles of unsteamed Hae-Kuen.

Microbial	Initial	Day 3
Total Plate Count (TPC) (CFU/g)	$6.37 \times 10^8 \pm 3.27 \times 10^7$	$7.77 \times 10^8 \pm 5.40 \times 10^7$
<i>C. perfringens</i> (Per 0.1 g)	Not Detected	Not Detected
<i>E. coli</i> (MPN/g)	<3.0	8.87 ± 12
<i>S. aureus</i> (Per 0.1 g)	Not Detected	Not Detected
<i>Salmonella</i> spp. (Per 25 g)	Not Detected	Not Detected
Yeasts and Mold (CFU/g)	$1.21 \times 10^3 \pm 7.71 \times 10^2$	$7.42 \times 10^3 \pm 1.0 \times 10^4$

The initial amount of TPC and yeast and mold were found at $6.37 \times 10^8 \pm 3.27 \times 10^7$ CFU/g and $1.21 \times 10^3 \pm 7.71 \times 10^2$ CFU/g, respectively. After 3 days, the amount of TPC and yeast and mold increased to $7.77 \times 10^8 \pm 5.40 \times 10^7$ CFU/g and $7.42 \times 10^3 \pm 1.0 \times 10^4$ CFU/g, respectively. Additionally, *E. coli* was observed at 8.87 ± 12 MPN/g after 3 days. *C. perfringens*, *S. aureus* and *Salmonella* spp. were not detected. Kanatt et al. (2006) also reported that *E. coli* were usually found in shrimps and their products. Therefore, one of the

important characteristics of both the shrimp and shrimp product is that, if processed properties, they receive a cook that is sufficiency to inactivate negative cells of bacteria that are pathogenic to humans (Buchanan, 1991).

3.2 Effect of Steaming Temperature and Steaming Time on Microbial Content

Hae-Kuen samples were then steamed at 80 °C and 85 °C for 10 min. Microbial contents of steamed Hae-Kuen were determined as TPC. The results as shown in Table 2. As time increased, the microbial content decreased in both Hae-Kuen samples.

The Hae-Kuen sample steamed at 80 °C for 5 and 10 minutes had the microbial levels of 53 CFU/g and 23 CFU/g, respectively. The microbial content of Hae-Kuen sample steamed at 85 °C was lower than that of Hae-Kuen sample steamed at 80 °C. The microbial content of Hae-Kuen steamed at 85 °C decreased from 4.45×10^3 CFU/g to 48 CFU/g and 18 CFU/g at 5 and 10 minutes, respectively. Therefore, the steaming temperature and time can decrease microbial contents in Hae-Kuen product. The TPC of steamed Hae-Kuen samples met the Office of Community Product Standards (2003) which state that the survival of microorganisms shall not exceed 1×10^3 CFU/g sample. The previous study found that TPC of chicken drumsticks cooked for 2 min at 70°C reduced from 10^7 CFU/g to < 10 CFU/g (Can and Haran, 2015)

Table 2: Effect of steaming treatment on microbial content of Hae-Kuen.

Time (minutes)	Total Plate Count (CFU/g)	
	80 °C	85 °C
0	$7.15 \times 10^4 \pm 4.95 \times 10^2$	$4.45 \times 10^3 \pm 7.78 \times 10^2$
1	$1.23 \times 10^4 \pm 1.22 \times 10^4$	$5.45 \times 10^3 \pm 1.49 \times 10^3$
2	$1.06 \times 10^4 \pm 1.20 \times 10^3$	$1.07 \times 10^2 \pm 95$
3	$3.90 \times 10^3 \pm 3.90 \times 10^3$	83 ± 4
4	$93 \pm 1.10 \times 10^2$	43 ± 18
5	53 ± 60	48 ± 32
10	23 ± 18	18 ± 11

The heat of steam causes microbial cells to change their condition as follows; resulting in hardening and settling of proteins (protein coagulation) within the cell, resulting in breakage and degradation of genetic material, both ribonucleic acid (RNA) and deoxyribonucleic acid (Deoxyribonucleic Acid,

DNA) as well as destroying the membrane of the microbes causing microbial cells to be destroyed (Office of Community Product Standards, 2003)

3.3 Physical Characteristics of Hae-Kuen Samples

Five Hae-Kuen samples were analyzed for physical characteristics including moisture content, water activity and texture.

3.3.1 Moisture Content

Moisture content of Hae-Kuen samples steamed at 80 °C and 85 °C was not different which ranged from 70.95–71.57% (as presented in Table 3). Control (unsteamed Hae-Kuen) had the highest moisture content at 75.06%. Water in the product was dehydrated by steaming condition. Heat is supplied by steam or heated fluid through a heat exchanger to decrease the moisture in sample (Audibert, 2019).

Table 3: The moisture content and water activity of Hae-Kuen.

Sample	Moisture Content (%)	Water Activity (a_w)
Control	75.06 ± 0.528 ^a	0.9593 ± .00389 ^b
Sample No.1 (80°C, 5 min)	71.57 ± 0.450 ^b	0.9650 ± .00289 ^a
Sample No.2 (80°C, 10 min)	71.14 ± 0.153 ^b	0.9647 ± .00092 ^a
Sample No.3 (85°C, 5 min)	70.95 ± 0.346 ^b	0.9643 ± .00105 ^a
Sample No.4 (85°C, 10 min)	70.91 ± 0.157 ^b	0.9618 ± .00338 ^{ab}

Mean values in the same column with mean in the same row with different lowercase letters are significantly different ($p < 0.05$).

3.3.2 Water Activity (a_w)

The water activity (a_w) of all steamed Hae-Kuen products were approximately at 0.9618-0.9650 as shown in Table 3.

3.3.3 Textural Properties

Textural profiles as shown in Table 4 revealed that all steamed Hae-Kuen samples had significantly higher hardness, springiness, cohesiveness and chewiness values than control (unsteamed Hae-Kuen) ($p < 0.05$).

It was found that Gumminess, Chewiness, Hardness and Springiness values of Hae-Kuen steamed at 85 °C for 5 and 10 min were not different. On the other hand, the values of Hardness, cohesiveness and Gumminess obtained from Hae-

Table 4: Texture profiles of Hae-Kuen samples.

Sample	Hardness (kg)	Springiness (mm/mm)	Cohesiveness (mm/mm)	Gumminess (mm/mm)	Chewiness (kg/mm)
Control	3895± 407.752 ^b	3338± 377.627 ^b	5975± 696.443 ^c	0.651± 0.015 ^a	0.851± 0.038 ^b
Sample No.1 (80°C, 5min)	4490± 910.984 ^b	4589± 1240.371 ^a	7803± 1065.680 ^b	0.592± 0.044 ^{bc}	0.901± 0.021 ^a
Sample No.2 (80°C, 10min)	6331± 736.627 ^a	5702± 768.750 ^a	10,140± 1302.780 ^a	0.623± 0.026 ^{ab}	0.907± 0.013 ^a
Sample No.3 (85°C, 5min)	6098± 1919.689 ^a	4725± 1314.354 ^a	10,064± 2365.910 ^a	0.573± 0.049 ^c	0.894± 0.016 ^a
Sample No.4 (85°C, 10min)	5048± 495.780 ^{ab}	4588± 433.500 ^a	8,791± 404.915 ^{ab}	0.570± 0.032 ^c	0.906± 0.009 ^a

Values are expressed as mean ± standard deviation. Means within a column with different superscript lowercase letters are significantly different ($p < 0.05$).

Kuen steamed at 80 °C for 5 and 10 min were significantly difference ($p < 0.05$). Springiness values were not different among steamed Hae-Kuen samples.

3.4 Shelf Life of Hae-Kuen

Five Hae-Kuen samples were stored at 4 ± 1 °C and taken every 3 days until 15 days for microbial analysis as TPC. The results are presented in Table 5. Unsteamed Hae-Kuen had higher microbial content (7.7×10^6 CFU/g) than the Community Product Standards Agency Criteria (2013), at day 3. On the other hand, the microbial content of Hae-Kuen steamed at 80 and 85 °C for 5 and 10 minutes were in the range of 10 to 95 CFU/g after storing for 15 days.

Table 5: Microbial content of Hae-Kuen samples during storage at 4 °C.

Day	Total Plate Count (CFU/g)			
	Sample No.1 (80°C, 5 min)	Sample No.2 (80°C, 10 min)	Sample No.3 (85°C, 5 min)	Sample No.4 (85°C, 10 min)
0	<10 ^{aa}	<10 ^{aa}	<10 ^{aa}	<10 ^{aa}
3	<10 ^{aa}	<10 ^{aa}	<10 ^{aa}	<10 ^{aa}
6	5.2×10 ^{2ab}	5.2×10 ^{2ab}	8.4×10 ^{2ab}	2.9×10 ^{2ab}
9	3.0×10 ^{2aa}	1.4×10 ^{2aa}	80 ^{aa}	25 ^{aa}
12	1.9×10 ^{2aa}	75 ^{aa}	30 ^{aa}	35 ^{aa}
15	95 ^{aa}	<10 ^{aa}	<10 ^{aa}	45 ^{aa}

The descriptive data of the Hae-Kuen samples indicated that changes occurred in product characteristics during storage. At day 3, the unsteamed Hae-Kuen had slime on the surface and sour taste, while the steamed Hae-Kuen samples were not have. Assessments of the taste of the product varied significantly ($p < 0.01$) with storage time (Can and Haran, 2015). The results of the present study indicate that it can be stored for 42 days at 2°C and present acceptable sensory quality.

3.5 Sensory Profiles

Hae-Kuen steamed at 80°C for 5 and 10 min and the control sample were then tasted for the acceptance in appearance, color, aroma, taste and overall liking. The Hae-Kuen samples were presented to 30 trained panellists. The result of the sensory test is presented in Table 6. The appearance, color, aroma and taste scores were not statistically significant among Hae-Kuen samples.

Table 6: Sensory evaluation.

Sample	Appearance	Color	Aroma	Taste	Overall
Control	4.031± 0.782 ^a	3.906± 0.777 ^a	3.906± 0.837 ^a	3.813± 0.693 ^a	4.063± 0.670 ^a
Sample No.1 (80°C, 5min)	3.687± 0.693 ^a	3.875± 0.707 ^a	3.844± 0.847 ^a	3.750± 0.803 ^a	3.688± 0.896 ^{ab}
Sample No.2 (80°C, 10min)	3.781± 0.706 ^a	3.594± 0.875 ^a	3.531± 0.950 ^a	3.594± 1.011 ^a	3.469± 1.106 ^a

^a Mean values in the same column with mean in the same row with different lowercase letters are significantly different ($p < 0.05$).

The sensory evaluation also revealed that Hae-Kuen steamed at 80 °C for both 5 and 10 min received the overall liking scores similar to the control sample. It indicated that the steamed Hae-Kuen had acceptable sensory quality similar to the traditional Hae-Kuen.

4 CONCLUSIONS

This study indicated that unsteamed Hae-Kuen was an unsafe and risky for consumption without further cooking. The results showed that effect of steaming at 80 °C and 85 °C for 5 and 10 min could decrease microbial content of Hae-Kuen and maintain product properties. For safety improvement of Hae-Kuen product, the processing method should examine. The using of temperature and time for steaming conditions can control good hygienic practice. It is important to prevent cross-contamination during raw material preparation, processing, storage, product distribution

and transportation or cross contamination for extend shelf-life of Hae-Kuen. There are useful to extend the shelf life of product that can apply to production process in restaurant and to reduce the foodborne illnesses.

ACKNOWLEDGEMENTS

We are obliged to a Thai Chinese restaurant that participated in this study. This work is in part supported the presentation expense by King Mongkut's Institute of Technology Ladkrabang, Bangkok.

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