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The pleasure to attendance in

The 1st KPRU International Student Research Conference On Science, Mathematics, And Technology

June 28, 2023

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50th Anniversary KPRU

THE 1ST KPRU INTERNATIONAL STUDENT RESEARCH CONFERENCE ON SCIENCE MATHEMATICS AND TECHNOLOGY

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Message of the Dean

The 1^{st} KPRU International Student Research Conference on Science Mathematics and Technology on June 23, 2023, at the Faculty of Science and Technology in Kamphaeng Phet, Thailand marked several exiting milestones for our organization, it was the first international conference after the covid-19 pandemic, saw a dramatic increases in students and involvement. There were 10 oral presentations and 16 poster presentations from 5 different universities from 3 countries.

These highlights are important to mention because they demonstrate our contribution to the field of science and technology in higher education. The supportive and collaborative nature of the conference also builds on our mission to support students in the context of research and presentations.

The contribution by the authors of the following papers reflect their dedication to higher education in various setting and contexts.

I would like to thank the reviewers, Assoc. Prof. Dr. Katekan Dajanta, Asst. Prof. Dr. Danchai Kreungngern, Asst. Prof. Dr. Wijitra Liaotrakul, and Assoc. Prof. Wachira Singkong for all their hard work on this document. I would like to thank all the authors who presented their research at the conference and ultimately for print in this edition of our journal.

As we continue to grow as an organization, your participation will be increasingly important to carrying out the work we are charged with from our mission.

With many thanks,
Assoc. Prof. Dr. Preecha Panya
Dean of the Faculty of Science and Technology
Kamphaeng Phet Rajabhat University

Contents

Antibacterial Activities of Palmitic and Lauric Acids from Palm Kernel Oil for the Development of Food-Grade Disinfectants

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Abstract

Palmitic and lauric acids, derived from palm kernel oil, exhibit antibacterial activities that are less harmful to humans. This study aimed to evaluate the antibacterial activities and optimal concentrations of palmitic and lauric acids against *S. aureus* and *E. coli* using the agar well diffusion method, with the goal of developing a food-grade disinfectant. The results demonstrated that a ratio of 87.5:175 mg/ml of palmitic acid to lauric acid was the optimal mix for inhibiting the growth of *Staphylococcus aureus* and *Escherichia coli*. The average size of the inhibition zone was 10.40±0.57, 10.59±1.08, 10.57±0.40, 8.67±0.98, 6.38±0.29, and 10.98±0.59 mm. The MIC ratios (200, 62.5, 87.5 mg/ml) and MBC ratios (50, 62.5, 87.5 mg/ml) of palmitic acid combined with lauric acid were 800, 125, 175 mg/ml and 200, 250, 175 mg/ml, respectively. The study found that the combination of palmitic and lauric acids effectively inhibited *S. aureus* and *E. coli*. Furthermore, these acids could be used to disinfect food and reduce the reliance on chemical disinfectants in food manufacturing cleaning processes.

Keywords : Palmitic acid, Lauric acid, Disinfectant, *S. aureus*, *E. coli*

Introduction

The cleaning method is an essential part of food safety and sanitation control in food processing plants. The use of chemical disinfectants was widely applied and increased of use in food

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industry section [1]. Nowadays, we expose chemical contaminants of disinfectants that remain on food contact surface and have a negative impact on our long-term health. Then, the food grade disinfectants can help reduce using chemical disinfectant. Many researchers focus on fatty acid disinfectants, fatty acids (FA) there are 2 types of disinfecting properties: Palmitic acid (PA) and Lauric acid (LA). Palmitic acid (PA) and lauric acid (LA) are the main components of palm kernel oil (PKO), and PA and LA have antibacterial activity against pathogens such as *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). Palm oil is a raw material used in the food and chemical industries for biofuel production due to the demand for vegetable oils in food and food products and the relatively low cost of palm oil. [2-7]. PA and LA is a product of the fatty acid synthase (FAS) complex, which is important for ensuing desaturation [8], PA increases cellular toxicity and the rate of cell damage due to genes responsible for fatty acid desaturation; damage to DNA, RNA, and proteins by reactive oxygen species (ROS), which are by-products of prokaryotes and eukaryotic cells, where cells are electron transport and metal catalyzed oxidation by mitochondria [9-10]. Fatty acids are separated from fat by the action of lipase and become free fatty acids, which have great biological activity potential [11].

The biological activity of free fatty acids plays a role in host defense against opportunistic microorganisms or pathogenic microorganisms in humans, and it is important to inhibit growth or destroy bacteria rapidly, as reported by [12], who found that gram-positive bacteria are more sensitive than gram-negative bacteria, and many fatty acids, such as palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, and -linolenic acid, can act as surfactants (anions) and have antibacterial and antifungal properties. These elements may be factors that affect fatty acids. It has been shown that palmitic acid has antibacterial activity [13-14], then LA can be used as a cleaning agent and one of the natural products without chemical residues and this study aimed to detect the antibacterial activity of palmitic and lauric acid to inhibit *E. coli* and *S. aureus*.

Research Objectives

To evaluate the antibacterial activities and proper concentrations of commercial palmitic and lauric acids against *S. aureus* and *E. coli*.

To develop the food grade disinfectant of palmitic and lauric acid to reduction the concentration of *E. coli* and *S. aureus*.

Table 1: The concentration ratio of palmitic acid (A) combination with lauric acid (B) by using the experimental plan of 62 Factorial designs in Completely Randomized Design (CRD).

Factor	Independent variable	Level of factor (g/ml)					
Palmitic acid	A	0	0.2	0.4	0.6	0.8	1.0
Lauric acid	B	1.0	0.8	0.6	0.4	0.2	0.0

Factor	Ratio of fatty acid (g/ml)					
	A_1	A_2	A_3	A_4	A_5	A_6
B_1	0.0:1.0	0.2:1.0	0.4:1.0	0.6:1.0	0.8:1.0	1.0:1.0
B_2	0.0:0.8	0.2:0.8	0.4:0.8	0.6:0.8	0.8:0.8	1.0:0.8
B_3	0.0:0.6.8	0.2:0.6	0.4:0.6	0.6:0.6	0.8:0.6	1.0:0.6
B_4	0.0:0.4.8	0.2:0.4	0.4:0.4	0.6:0.4	0.8:0.4	1.0:0.4
B_5	0.0:0.2.8	0.2:0.2	0.4:0.2	0.6:0.2	0.8:0.2	1.0:0.2
B_6	$0.0:0.0^{Con*}$	0.2:0.0	0.4:0.0	0.6:0.0	0.8:0.0	1.0:0.0

A: Palmitic acid (mg/ml); An: Palmitic acid concentration (g/ml); B: Lauric acid; Bn: Lauric acid concentration (g/ml); n: run of sample; Con*: negative control is DMSO (non-combine of palmitic acid and lauric acid there is only DMSO for negative control).

Research Methodology

1) Materials

The palmitic acid and lauric acid from palm kernel oil were brought from the World Chemical Fareast Co., Ltd. They were prepared by dissolving one gram of fatty acid in one milliliter of dimethyl sulfoxide (DMSO) (RCI Labscan, Bangkok, Thailand) to a final concentration of one g/ml (w/v) (Table 1)

2) Determination of growth curve of *Escherichia coli* and *Staphylococcus aureus*

2.1) Microorganism Preparation

The two cultures of *E. coli* and *S. aureus* were kindly supported from Food Science and Technology laboratory, Division of Food Science and Technology, Agro-Industry, Chiang Mai University.

The streaking plate method was used to cultivate a single colony of purified *E. coli* and *S. aureus* and kept to stock cultures by using Mueller-Hinton broth (MHB) (R211443; Becton, Dickinson and Company Sparks, MD 21152 USA) and incubated for 18 h at 37 °C. Using the half-fold serial dilution method, the suspension was adjusted to a concentration that was a mix of MHB and 0.85 percent NaCl and then added to a 96 well microplate. Next, 20 µL of each microbial suspension was added to each well and incubated for 24 h at 37 °C. The turbidity of the suspension was adjusted using the optical density method using a microplate reader (EZ Read 2000, Biochrom, Holliston, MA, USA), and the UV-Vis absorbance was measured at 630 nm to obtain the initial cell at O.D. = 0.1 to achieve the 10^6 colony-forming units (CFU). To confirm the concentration of bacteria, the spread plate method was used to quantify the amount of *E. coli* and *S. aureus* by counting colony forming units (CFU) [15].

2.2) Preparation of the bacterial growth curve

Purified *E. coli* and *S. aureus* were grown in the MHB medium for 18 h at 37 °C. The concentration was adjusted by mixing MHB and 0.85 percent NaCl in a 96-well plate using the half-fold serial dilution method with the bacterial concentration set at 1 percent in each well. Then, the samples were incubated at 37 °C for 0, 2, 4, 6, 8, and 10 h. Bacterial samples were collected every two hours to measure the turbidity of the cells using the optical density method and the UV-Vis absorbance at 630 nm in accordance with the number of bacteria confirmed by the spread plate method at hours 6, 8, and 10 (modification method from [15].

3) Antibacterial Activity

3.1) Antimicrobial activity test of palmitic and lauric acid compare with commercial disinfectants by agar disc diffusion.

Swabbing *E. coli* or *S. aureus* on Mueller-Hinton agar (MHA) (CM0337, Oxoid Ltd., Wade Road, Basingstoke, Hants, RG24 8PW, UK) from the starter culture (MHB and 0.85 percent NaCl), then waiting for 15 min and dropping 10 µL of PA combined with LA at various concentrations, as shown in Table 1 and DMSO with 10 µL for negative control on MHA, then putting all three substances Amoxycillin (0.03 mg/mL) (CT0223B, Oxoid Ltd., Wade Road, Basingstoke, Hants, RG24 8PW, UK), Chloramphenicol (30 µg) (CT0013B, Oxoid Ltd., Wade Road, Basingstoke, Hants, RG24 8PW, UK), and Tetracycline (30 µg) (CT0054B, Oxoid Ltd., Wade Road, Basingstoke, Hants, RG24 8PW, UK) of disc on the surface for positive control. After incubation at 37 °C for 20 h, the diameter of the inhibition zone was measured and the qualification ratio of fatty acids in the high inhibition zone was calculated, then agar disc diffusion

method it is a preliminary screening for antimicrobial activity which qualitatively the result whether the pathogen is susceptible to the test, and the experiment was repeated in three replications [16].

3.2) Minimum Inhibitory Concentration (MIC)

The purified *E. coli* and *S. aureus* strains were grown in MHB for 6 h at 37 °C, then adjusted using the McFarland standard no.5, the cultures turbidity was adjusted to 10^8 CFU/mL using sterile 0.85 percent NaCl (R20410, Lenexa, Kansas 66215, UK). Three-dimensional swabs were obtained over the entire surface of the MHA medium using a sterile cotton bud dipped in the suspension. Allow the surface to dry for 3-5 minutes. A sterile corker borer was used to press wells on agar with a diameter of 4 mm. Each of formulation of fatty acid concentration was added at 20 µL and diluted the concentration of each formulation by 0.5-fold dilution method. The MIC results were determined by examining the inhibition zone of the tested strain and measuring the diameter size of the tests was carried out in triplicate. The MIC of fatty acid was the lowest fatty acid content that did not allow culture to growth and the experiment was repeated in three replications [17].

3.3) Minimum Bactericidal Concentration (MBC)

The streak plate method was used to calculate the MIC results for MBC values by picking the colony from the MIC inhibition zone with the lowest concentration, at least three concentrations, streaking on the MHA, incubating at 37 °C for 24 h, and monitoring and recording bacterial growth. Fatty acids that destroy bacteria do not allow bacteria to thrive on MHA. The tests were carried out in triplicate, and the MBC of fatty acid was calculated using the lowest fatty acid content at which 99.9% or more of the initial inoculum was destroyed and the experiment was repeated in three replications [17].

4) Statistical Analysis

The experimental plan of Factorial designs in Completely Randomized Design (CRD) was designed for varying the concentration ratio of PA (A) combination with LA (B). All measurements were performed at least in triplicates. The means and standard deviations (± SD) were calculated. Significant differences were determined by analysis of variance (ANOVA) and Duncan's multiple range tests at 95% confidence intervals using SPSS software version 17.0. (IBM, U.S.A.)

Research Methodology

1) Growth curve of *E. coli* and *S. aureus*

The growth curves of *E. coli* and *S. aureus* were investigated. The growth curves of *E. coli* and *S. aureus* at 10 h show that the bacteria entered the stationary phase, as shown in Figure 1 and 2. Colonies of both bacteria were graphed at 10 h and the bacterial population was constant. This indicates that the bacteria on longer multiply, or the spawn rate, is equal to the mortality rate of the bacteria growing into the stationary phase because the waste produced by the bacteria inhibits bacterial growth. At 8 h, the bacteria were in the log phase with approximately 10^8 and 10^9 log CFU/ml, respectively, as shown in Figure 3 and 4.

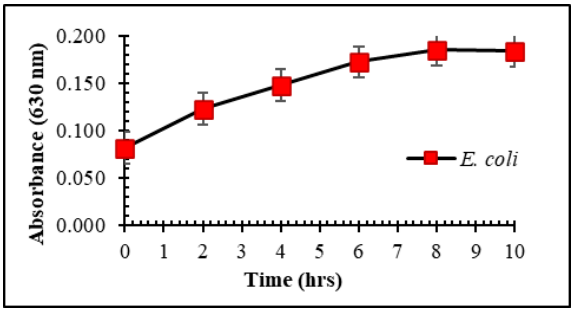

Figure 1: Growth curve of *E. coli* at 0, 2, 4, 6, 8 and 10 hrs.

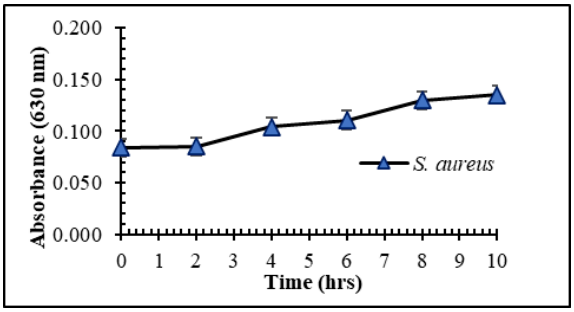

Figure 3: Growth curve of *S. aureus* at 0, 2, 4, 6, 8 and 10 hrs.

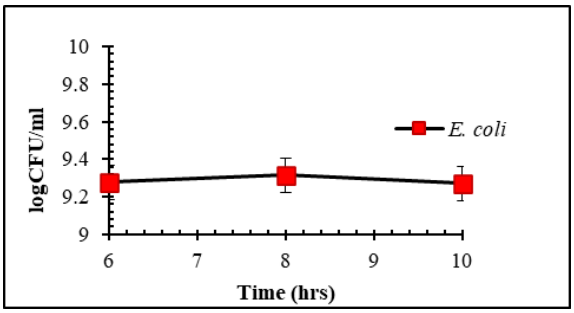

Figure 2: Number of *E. coli* colonies from spread plate technique at 6, 8 and 10 hrs.

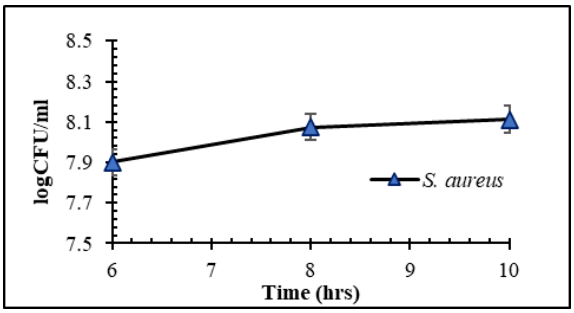

Figure 4: Number of *S. aureus* colonies from spread plate technique at 6, 8 and 10 hrs.

2) Antibacterial Activity

2.1) Antimicrobial activity test of palmitic and lauric acids compared with commercial disinfectants using the agar disc diffusion method

Table 2: Sensitivity analysis of commercial antibiotics.

Strains	Inhibition zone diameter (mm)		
	Amoxycillin (0.03 mg/mL)	Chloramphenicol (30 µg)	Tetracycline (30 µg)
E. coli	24.73 ± 0.35^{cB}	30.37 ± 0.90^{aA}	28.33 ± 1.29^{bA}
S. aureus	40.37 ± 1.06^{aA}	22.03 ± 0.31^{cB}	23.27 ± 0.60^{bB}

a, b, c ... in horizontal letters; then, A and B vertical letters show a statistically significant difference ($p < 0.05$) by Duncan's test from SPSS software version 17.0

Table 3: Sensitivity analysis of palmitic and lauric acids ratio.

Factor	Inhibition zone (mm)					
	E. coli					
	A_1	A_2	A_3	A_4	A_5	A_6
B_1	7.32 ± 0.20^{ghijk}	6.41 ± 0.20^{lm}	10.57 ± 0.40^{a}	8.38 ± 2.40^{ab}	6.64 ± 0.47^{klm}	6.72 ± 0.47^{ijklm}
B_2	10.40 ± 0.57^{a}	10.59 ± 1.08^{a}	8.64 ± 0.39^{b}	8.89 ± 0.38^{b}	8.46 ± 0.35^{bc}	7.89 ± 0.84^{cde}
B_3	8.71 ± 0.40^{b}	7.69 ± 0.27^{def}	7.14 ± 0.52^{ghijk}	7.80 ± 0.48^{cdef}	7.36 ± 0.49^{ghij}	7.31 ± 0.46^{ghijk}
B_4	8.28 ± 0.25^{bcd}	7.41 ± 0.28^{ghi}	7.42 ± 0.47^{ghi}	7.37 ± 0.46^{ghij}	7.43 ± 0.59^{ghi}	7.63 ± 0.85^{defg}
B_5	8.26 ± 0.58^{bcd}	6.97 ± 0.51^{ghijkl}	6.68 ± 0.58^{jklm}	6.33 ± 0.36^{lm}	6.39 ± 0.39^{lm}	6.40 ± 0.32^{lm}
B_6	0.00^{Con}	6.83 ± 0.19^{hijkl}	7.29 ± 0.57^{hijk}	6.88 ± 0.32^{hijkl}	6.96 ± 0.55^{ghijkl}	6.11 ± 0.03^{m}
Factor	Inhibition zone (mm)					
	S. aureus					
	A_1	A_2	A_3	A_4	A_5	A_6
B_1	13.92 ± 1.51^{a}	11.73 ± 0.51^{cde}	10.98 ± 0.59^{fgh}	6.26 ± 0.09^{n}	6.62 ± 0.22^{mn}	11.37 ± 0.68^{de}
B_2	8.67 ± 0.98^{k}	6.38 ± 0.29^{n}	6.92 ± 0.73^{mn}	6.67 ± 0.36^{mn}	7.60 ± 1.06^{l}	10.91 ± 0.66^{fgh}
B_3	13.54 ± 0.65^{ab}	11.63 ± 0.46^{cdef}	11.30 ± 0.75^{defg}	11.44 ± 1.04^{def}	12.17 ± 0.06^{c}	11.21 ± 0.85^{efg}
B_4	12.97 ± 0.77^{b}	11.97 ± 0.54^{cd}	11.44 ± 0.44^{def}	10.42 ± 0.37^{hi}	10.88 ± 0.76^{fgh}	9.40 ± 0.78^{j}
B_5	10.34 ± 0.64^{hi}	10.61 ± 0.67^{gh}	10.98 ± 0.97^{fgh}	9.91 ± 0.80^{ij}	9.49 ± 0.87^{j}	9.22 ± 0.55^{jk}
B_6	0.00^{Con}	6.32 ± 0.18^{n}	6.48 ± 0.29^{mn}	7.51 ± 0.71^{l}	7.13 ± 0.61^{lm}	6.37 ± 0.30^{n}

a, b, c ... in letters show a statistically significant difference ($p < 0.05$) by Duncan's test from SPSS software version 17.0; An, palmitic acid concentration (g/ml); Bn, lauric acid concentration (g/ml); n: run of sample; Con*, negative control is DMSO.

The effects of PA and LA were tested at different ratios using the disc-diffusion method. It

Table 4: MIC and MBC values of palmitic acid in combination with lauric acid.

Formula	*E. coli*		*S. aureus*	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
A_1B_2	200	800	50	200
A_2B_2	62.5	125	62.5	250
A_3B_1	87.5	175	87.5	175

negative control is DMSO; AnBn: combination of palmitic and lauric acid ratios; n: run of sample.

inhibited the growth of *E. coli* and *S. aureus*, as shown in Table 3. In addition, when comparing the inhibitory activity between PA and LA with commercial antibiotics including Amoxycillin (0.03 mg/mL), Chloramphenicol (30 µg) and Tetracycline (30 µg) for inhibition of *E. coli* and *S. aureus* (Table 2).

The optimum ratio for inhibiting *E. coli* was A3B1, A1B2, and A2B2; the PA to LA ratios were 0.4:1.0, 0:0.8, and 0.2:0.8 g/ml and the optimal ratio for inhibition of *S. aureus* was A1B1 and A1B3; and the ratio between PA and LA was 0:1.0 and 0:0.6 g/ml as shown in Table 3. This is the most effective ratio of the inhibition zone for *E. coli* and *S. aureus* for studying the effects of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) because from the result finding for optimum growth ratio due to *E. coli* to be stronger and can increase resistant to antibiotic, than *S. aureus*. Therefore, a disinfectant formula that has a high in inhibition zone with *E. coli* is used to select the highest optimum ratio for inhibition bacteria.

2.2) Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of palmitic acid combined with lauric acid

The study results in effect of MIC and MBC on inhibition of *E. coli* and *S. aureus* were showed in Table 4, it was found that the optimal ratio of PA and LA to inhibit and destroy both of *E. coli* and *S. aureus* was A3B1 due to the highest inhibition ability affect to *E. coli* and *S. aureus* compared to A1B2 and A2B2, and giving the diameter of MIC of A3B1 , A1B2, and A2B2 with concentration raged between 50-800 mg/ml at 8.85±0.43, 9.30±0.67, 8.42±0.67, 9.58±0.49, 8.13±0.25 mm., and 11.37±0.56, 13.15±0.68, 13.22±0.45, 14.25±1.26, 14.09±0.91 mm., respectively, as shown in Table 5.

Table 5: Diameter of MIC of palmitic combination with lauric acid.

Diameter of MIC (mm)					
E. coli					
A_1B_2		A_2B_2		A_3B_1	
Concentration (mg/ml)	MIC	Concentration (mg/ml)	MIC	Concentration (mg/ml)	MIC
800	12.22 ± 0.98^{a}	1000	10.83 ± 1.61^{a}	1400	8.85 ± 0.43^{ab}
400	10.42 ± 0.65^{b}	500	9.07 ± 0.28^{b}	700	9.30 ± 0.67^{a}
200	8.72 ± 1.50^{c}	250	9.00 ± 0.45^{b}	350	8.42 ± 0.67^{bc}
100	NA	125	8.33 ± 0.69^{c}	175	9.58 ± 0.49^{a}
50	NA	62.5	6.23 ± 0.09^{d}	87.5	8.13 ± 0.25^{c}
25	NA	31.25	NA	43.75	NA
S. aureus					
A_1B_2		A_2B_2		A_3B_1	
Concentration (mg/ml)	MIC	Concentration (mg/ml)	MIC	Concentration (mg/ml)	MIC
800	14.85 ± 0.46^{ab}	1000	10.23 ± 0.40^{c}	1400	11.37 ± 0.56^{c}
400	11.50 ± 0.87^{c}	500	13.10 ± 0.62^{b}	700	13.15 ± 0.68^{b}
200	15.33 ± 0.49^{a}	250	12.97 ± 1.09^{bc}	350	13.22 ± 0.45^{b}
100	12.35 ± 0.32^{b}	125	14.18 ± 0.34^{a}	175	14.25 ± 1.26^{a}
50	10.32 ± 1.32^{d}	62.5	13.92 ± 1.85^{ab}	87.5	14.09 ± 0.91^{a}
25	NA	31.25	NA	43.75	NA

Values with different superscripts between rows differ significantly at $P<0.05$ by Duncan's test from SPSS software version 17.0; NA: no activity; negative control: DMSO; AnBn: combination of palmitic and lauric acid ratios; n: run of sample.

Discussion

1) Growth curve of *E. coli* and *S. aureus*

From figure 1-4, where the log-phase is when bacteria multiply the most, then have a constant rate of division of chemical components of cells and processes as well as the same physiological

properties. Bacteria cells intensely increased in size, specially the first hour and from 3 to 6 h, have cells size increasing and at 10 h can see of dead cells and the growth curve of *E. coli* at a constant temperature [18,28], it was observed that there was an increase in growth over an increased period of time, and at 6 hours *E. coli* ranged between 10^6 to 10^7 logCFU [19], which is consistent with the results of our experiments. The growth curves of *E. coli* and *S. aureus* measured by UV-Vis absorbance at 620 nm showed that both bacteria grew similarly. It is an exponential increasing (log phase) during, and the first 6 to 8 hours are stable stationary phase, log phase for *E. coli* appears to be longer than that measured for *S. aureus* [15], but log phase of *E. coli* this research are shorter than *S. aureus* because the condition experimental are difference. Therefore, the optimal time of inoculation to test the efficacy of the antimicrobial palmitic acid and lauric acid was between 8 h, since the bacteria were in the log phase. In this result is conducted an experiment of growth curve at 10 h because both bacteria are growth to log-phase prior to the stationary phase at 8 h.

The log-phase of growth curve higher to stationary phase and the log-phase prior to the stationary phase the bacteria are higher in activity and resistance to conditions than the other phases due to due to the number of bacteria at stationary phase are relatively constant and the number of bacteria that grown is same as the number of bacteria that die, which was effect from lack of nutrients or accretion of toxic metabolic products [20,30], then were chosen at log-phase prior to the stationary phase for the three formula of food grade disinfectant tests in antimicrobial.

2) Antibacterial Activity

PA and LA were found to be highly inhibited compared to commercial antibiotics, and from the result found that *E. coli* can be resistant to antibiotic and disinfectant than *S. aureus* and our result agrees with findings of similar studies by Bantawa, K. et al., (2019), Acsa, I. et al., (2021) and Sheikh, A. A. et al., (2012) because there is a difference in mode of reaction of Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) to disinfectants, then affect to *E coli* were more resistant to the disinfectants compared to *S. aureus* and Gram-positive bacteria tend to be more responsive to disinfectant compared to Gram-negative bacteria due to intrinsic factors and difference in cell structure between Gram-positives and Gram-negatives, and found that the cell wall of Gram-positive bacteria does not action as barrier for disinfectants which difference to Gram negative [21-22,29]. According to other research found that PA and LA are Saturated Fatty Acids (SFAs) that can act as antimicrobial agents (as shown in Table 2-3) [23-24]. Both types of bacteria are sensitive to fatty acids due to Gram-negative and Gram-positive bacteria are inhibited differently by different types of fatty acids due to the permeability of the outer membrane of Gram-negative bacteria, which acts as a barrier for Gram-negative bacteria [24]. When comparing the antibacterial effects of PA and LA, and then

LA inhibited *E. coli* and *S. aureus* better than PA, and the combination of PA and LA promoted the inhibitory effect of both types of bacteria. The results in table 3 found that the most appropriate ratio of PA and LA to inhibit *S. aureus* and *E. coli* was 0:1.0 and 0:0.6 g/ml, respectively. The reactive oxygen species (ROS) from fatty acid synthase (FAS) complex of PA and LA increases cellular toxicity and the rate of cell damage due to genes responsible for fatty acid desaturation; damage to DNA, RNA, and proteins [8-10], and then effect to Inhibit gram-negative and gram-positive bacteria. The phenomenon can be explained caused by Palm Kernel Oil (PKO) was active against certain strains of microorganisms, including *E. coli* and *S. aureus* [26], and Palm Oil (PO), Red Palm Oil (RPO), and PKO have antibacterial activity against gram-positive bacteria such as S. aureus because they contains a variety of fatty acids that can inhibit bacteria, including PA and LA contained in PO, RPO, and PKO [5]. When tested for the antibacterial activity of PKO using the disc diffusion method, moderately sensitive and highly sensitive antibacterial activity of *E. coli* and *S. aureus*, respectively, showed that PKO was more effective in inhibiting the bacteria [2] and LA affect to growth of *S. aureus*, and *E. coli* which LA at 5%, 10%, 15%, and 20% dissolved in n-hexane as a solvent based on the disc diffusion method was determined by measuring the diameter inhibition zone to inhibit the growth of bacteria such as S. aureus with diameters of 40, 37, 28, and 25 mm, respectively, and *E. coli* with diameters of 41, 36, 28, and 26 mm, respectively, showing that LA had the best inhibitory effect on *E. coli* and *S. aureus* [7] and consistent this research.

And the MIC of bacteria was 87.5 mg/ml (Table 4) due to the high LA concentration ratio and the ability to inhibit bacteria better than PA. From the Table 5, It was found that the ratio of A3B1 has the best MBC were 87.5 and 175 mg/ml, then compared the results MIC test of other research found that PO, RPO and PKO with PA constituents were 42.93%, 41.96% and 9.46%, respectively, and the composition of LA was 0.20%, 0.23% and 45.24%, respectively, the effect of MIC from PO, RPO and PKO are less than 4.50, 4.50 and 1.13 mm, respectively [5], It has less antibacterial effect than using PA and LA directly, then from study result concluded that *E. coli* was inhibited by the PKO because of contains a main fatty acids such as PA and LA and the inhibition zone was 5 mm [5,27]. Therefore, PA and LA from this study were used to inhibit and destroy *E. coli,* and *S. aureus* was found to be more efficient than PO, RPO, or PKO.

Conclusion

In conclusion, palmitic acid (PA) and lauric acid (LA) were more effective than palm oil (PO), refined palm oil (RPO), and palm kernel oil (PKO) at inhibiting and disinfecting *E. coli* and *S. aureus*. The

efficacy of lauric acid enhances that of palmitic acid, thereby affecting the inhibition and disinfection effects on bacteria. The combination of these fatty acids proved more effective than using palmitic acid alone. Therefore, with the increasing demand to reduce the use of chemical cleaning agents in the food industry, it is necessary to develop food-grade disinfectants capable of inhibiting *E. coli* and *S. aureus*.

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Evaluation of the Population's Health Risk from Aflatoxin in Dried Chillies in Ban Palad Community, Li City, Lamphun Province, Thailand

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Abstract

The occurrence of aflatoxin, a potent carcinogenic toxin produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, in food is a significant public health concern worldwide. Dried chilli is among the most commonly contaminated food items, particularly in tropical and subtropical regions. In Thailand, the consumption of dried chilli is prevalent, and its contamination with aflatoxin has been reported in several regions. One such region is Ban Palad community, located in Li City, Lamphun Province, where the majority of the population relies on agriculture and small-scale food processing for their livelihoods. Despite the potential health risks of aflatoxin exposure through contaminated chilli consumption, no studies have been conducted to assess the exposure and health risks in this community. Therefore, the present study aimed to assess the levels of aflatoxin contamination in dried chilli consumed by the population in Ban Palad community and to estimate the associated health risks. The results of the survey showed that 52% of respondents and 48% of respondents, respectively, had known and unknown mycotoxins. There were 84.67% and 15.33%, respectively, of people who were aware that dried chilli can develop mold when maintained in a moist or humid environment under known and unknowing factors. The percentage of individuals known to have liver cancer caused by the mycotoxin in dried chilli peppers was 16.67% and 83.33%, respectively. This study's findings will provide valuable information for policy-makers and local com-

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munities to develop strategies to reduce aflatoxin contamination and mitigate the associated health risks. In conclusion, the risk assessment of aflatoxin in dried chilli from Risk Ranger program indicates that there is a moderate level of risk associated with consumption. Consumers should be aware of the potential hazards and take necessary precautions to reduce the risk of exposure.

Keywords : Aflatoxin, risk assessment, exposure, Risk Ranger program

Introduction

Aflatoxin, a potent carcinogen generated by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, is found in food and is a major global public health concern. One of the most frequently contaminated foods is dried chilli, especially in tropical and subtropical areas [1]. The United Nations Food and Agriculture Organization has recognized mold toxins in food as a significant global food processing safety problem. The most common strain is *Aspergillus flavus*, which produces aflatoxins (AFs) [2]. Aflatoxin B1 contaminants found in food are the main dangerous substances that have an effect on public health. Aflatoxin chronic exposure can occur at any age, including in the fetus, and increases with repeated consumption. Aflatoxin exposure at high levels makes acute poisoning fatal [3]. When consumed in high-risk foods, aflatoxins, which are made *Aspergillus flavus* and *Aspergillus paraciticus* and classified as human carcinogens, can withstand heat up to 268 degrees Celsius. Therefore, the poison cannot be rendered harmless by normal cooking [4]. In order to protect consumer health, the amount of aflatoxin contamination in food cannot go above the limits defined by the Codex Alimentarius Commission. Each country thus uses it as a trade barrier and a negotiation tactic when purchasing agricultural products. As a result, the product's market value decreases. The World Commerce Organization has established guidelines for negotiations when problems with global commerce arise. Risk Ranger Program is an effective tool for defining the hazard risk assessment to decide the strategies to control the hazards. The program operates aligned with the information related to the consumption data, frequency of consumption, proportion of population consuming the product and Probability that a serving of raw product is contaminated, these are advantages for assessment the risk to contribute in various size of population [5]. In Thailand, it is unclear how much consumption of chilli paste affects the risk of cancer brought on by exposure to aflatoxin-containing chilli paste that is frequently consumed in Northern of Thailand, a variety of chilli pastes in both homemade and store-bought. Therefore, the objective of this study is to assess the risk involved with consuming chilli paste that can be contaminated with aflatoxin and to investigate the likelihood of chilli paste consumers are exposed to aflatoxin. By offering guidelines to lessen the likelihood of contamination in dried chilli raw materials and food safety management standards for dried chilli manufacture at

both the home and commercial levels, the risk of harm to consumers will be lowered.

Research Objectives

To ascertain the possible dangers of consuming foods that include dried chilli that has been contaminated with aflatoxins.

To identify the most effective techniques for reducing the risk of exposure to aflatoxin contamination in dried chilli.

To introduce the development of a toxin-free dried chilli production technique that fits with the demand for production.

Research Methodology

1 Materials

The purpose of this study is to assess the possibility of aflatoxin exposure among chilli paste inhabitants of Lamphun Province by randomly spreading the area and selecting certain Ban Pha Lat sample populations. Aflatoxin is a fungal toxin that is contaminated in dried chilli. There were 150 volunteers in total. Find out how to manage the threat of aflatoxin in dried chilli. After data collection and before conducting behavioral and dried chilli eating frequency questionnaires, volunteers will be divided into two groups.

1.1 Primary producers (farmers who grow chillies for their own consumption) must continuously planted and processed dried chillies for domestic consumption by farmers who grow them for their own purpose (Preliminary Producer Representative).

1.2 Customers who buy dried peppers to consume (consumer representatives) by gathering information on distribution points, storage locations, and dried chilli product specifics.

2. Methods

This study has been certified of research human ethics by Associated Medical Sciences, Chaing Mai University, Chiangmai, Thailand.

2.1 Research prevention strategies for aflatoxin in dried chilli.

The populations of this study were Participatory Action Research: PAR random by using purposive sampling [6]. Information gathering: to carry out this study volunteer in Ban Palad Community, Li City, Lamphun Province. The subject criteria are people age 18 and above with the consumption of dried chilli, extensive surveys and interviews were conducted. They were divided into two groups. 1) Farmers who grow chilli for their own consumption (Preliminary producer representative): there must be continuous planting and processing of dried chilli for household consumption. By gathering data on the processes used by farmers to produce dried chilli, the growing season, drying techniques like sun drying or smoking, storage, and processing used by farmers in the area, and summarizing all the data to get an overall picture. Integration of production and processing processes (to be studied in the risk assessment process to suggest ways to improve the production management process of dried chilli from the actual situation in Ban Palad community. 2) Consumers that purchase dried chilli for consumption (consumer representatives) by gathering data on distribution hubs, storage facilities, and dried chilli product details.

2.2 The questionnaire study in the population

The questionnaire was divided into 3 parts, which were multiple-choice questions as follows:

Part 1: Ask about the general information of the respondents, including sex, age, education level, occupation, and earnings.

Part 2: Inquired about consumer behavior towards dried chilli products. Ask about the qualities and origins of dried chilli peppers that are commonly consumed (processing, storage).

Part 3: The frequency of consumption of foods containing dried chilli was the subject of Part 3's investigation. This is done in order to research how the locals make dried chilli. Including knowledge, management and practise tests.

2.3 The probabilistic risk of dietary exposure

The probabilistic risk of dietary exposure of assessment of health risk from aflatoxin in dried chilli of population. The risk estimation of aflatoxin in dried chilli were analyzed using the Risk Ranger program to determine the likelihood of daily illness [5]. According to the consumer concerned, the combined forecast of illness per day per consumer of interest total predicted illnesses/annum in population of interest. The risk rating (0 to 100). Exposure to the food will depend on how much is consumed by the population of interest, how frequently they consume the food and the size of the exposed population. Probability of exposure to an infectious dose will depend on: 11 questions

Question 1: Hazard severity

Question 2: Susceptibility of the population in which you are interested

Question 3: Frequency of consumption

Question 4: Proportion of population consuming the product

Question 5: Size of consuming population

Question 6: Probability that a serving of raw product is contaminated

Question 7: Effect of processing

Question 8: Potential for recontamination after processing

Question 9: How effective is post-processing control?

Question 10: What level of increase is needed to cause illness

Question 11: Effect of meal preparation

Figure 1: The questionnaire surveying of this research in Ban Palad Community, Li City, Lamphun Province, Thailand

Results

1. Research prevention strategies for aflatoxin in dried chilli.

The questionnaire surveying of this research, the evaluation of the population's health risk from aflatoxin in dried chilli, was conducted in Ban Palad Community, Li City, Lamphun Province, as shown in figure 1.

In Table 1: General Information of the Population, it was presented that the gender of males was 37% and that of females was 63%. The average age range between 18–35, 36–60, and more than 60 was 5.33%, 66.67%, and 28%, respectively. The occupations of the subjects are farmer merchant, government employee, and others: 75.33%, 5.33%, 10.00%, and 9.33%, respectively.

2. The questionnaire study in the population

Based on Table 2, the consumption habits of dried chilli were analyzed and separated into five factors. The first factor was cultivation, which showed that the highest percentage of dried chilli was cultivated during April-June, accounting for 62.75%. The second factor was processing and storage methods, which revealed that smoking or grilling over fire was the most common processing method, accounting for 66.67%, while storing dried chilli in plastic bags was the most popular storage method, accounting for 72.55%. The third factor was retailing, which showed that neighborhood stores were the most common retailers for dried chilli, accounting for 5.88%. The fourth factor was the keeping period, which showed that dried chilli was usually kept for six months, accounting for 50.98%.

The knowledge of mycotoxin in foods is presented in Table 3. The percentages of people who had known and unknown mycotoxins were 52% and 48%, respectively. The percentage of people who recognized that mold can grow up on the dried chilli when kept in a humid or wet condition with known and unknown conditions was 84.67% and 15.33%, respectively. The percentage of individuals known to have liver cancer caused by the mycotoxin in dried chilli was 16.67% and 83.33%, respectively.

3. The preliminary risk assessment of exposure to aflatoxin in dried chilli

The probability of illness per day per consumer of interest was 2.35E-6, and total predicted illnesses per annum in the population of interest was 7.51E-4. The risk rating (0 to 100) was 67. The probabilistic risk of dietary exposure to dried chilli was shown in Table 4.

Table 1: The concentration ratio of palmitic acid (A) combination with lauric acid (B) by using the experimental plan of 62 Factorial designs in Completely Randomized Design (CRD).

Variables	Frequency (n)	Percent (%)
Sex		
Male	55	37.00
Female	95	63.00
Age		
18-35	8	5.33
36-60	100	66.67
> 60	42	28.00
Caregiver educational level		
Illiterate	4	2.67
Grade 1–6	114	76.00
Grade 7–9	9	6.00
Grade 10–12	13	8.67
Tertiary education	4	2.67
Earnings per month		
< 10,000 Baht.	105	70.00
10,000 – 14,999 Baht.	35	23.33
15,000 – 19,999 Baht.	7	4.67
> 20,000 Baht.	3	2.00
Marital status		
Married	138	92.00
Single	2	1.33
Divorced/and or separated	10	6.67
Occupation		
Farmer	113	75.33
Merchant	8	5.33
Government employee	15	10.00
Other (such as day laborer, etc.)	14	9.33

Table 2: Consumption habits of dried chilli

Variables	Frequency (n)	Percent (%)
Grown ourselves	51	34.00
cultivate		
April-June	32	62.75
July-September	17	33.33
October-December	3	5.88
Processing		
Sun exposure	18	35.29
Smoked (grilled over the fire)	34	66.67
Drying	0	0.00
storage methods		
plastic bag	37	72.55
Bottles/jars	15	29.41
baskets/sacks	0	0.00
available		
Merchant	0	0.00
Factory/Company	0	0.00
Neighborhood	3	5.88
Keeping		
1 month	13	25.49
3 month	4	7.84
6 month	26	50.98
1 year	4	7.84
>1 year	5	9.80

According to the risk criteria in Aflatoxin in dried chilli, the hazard severity is considered severe as it can cause death to most susceptibles. The susceptibility of the population is general, which includes all members of the population. The frequency of consumption is 18 liters per year, which is equivalent to 4.87 kilograms per year, and the proportion of consumers is most at 75%. The size of the population is 1.17 million. The probability of raw product contamination is 3.03%, but the effect of processing usually eliminates hazards by 99%. There is no possibility of recontamination,

Table 3: knowledge of mycotoxins

Variables	Frequency (n)	Percent (%)
number of persons knowledge of mycotoxins		
known	78	52.00
Unknown	72	48.00
number of persons knowledge of Mold can grow on dried chilli when they are kept in humid environments.		
known	127	84.67
Unknown	23	15.33
number of individuals known that the mycotoxins in dried chilli can cause liver cancer in humans.		
known	25	16.67
Unknown	125	83.33

and post-process control is controlled. The increase to infective dose is 5.17 illnesses per 66,511,700, and further cooking before eating is effective in reducing the hazard by 99%. Based on these risk criteria, the predicted annual illness in the population is 2.35E-6, and the probability of illness per day per consumer of interest (Pinf x Pexp) is 7.51E-4. The risk ranking on a scale of 0 to 100 is 67, which indicates a moderate level of risk [6,7]. Therefore, it is recommended that consumers take precautions when consuming dried chilli to minimize the risk of exposure to aflatoxins.

Discussion

1. Regarding consumer behavior in this study, community stores were the most common place for purchasing dried chilli, accounting for 80.81%, followed by fresh markets (12.12%) and shopping malls (7.07%). The period of consumption was found to be more than four weeks, 2-3 weeks, 2-3 days, and 1 week, with a percentage of 78.79, 21.21, 10.10, and 9.09, respectively. The quantities of dried chilli consumed were about one kilogramme, 500 grammes, and 250 grammes, accounting for 50.51, 20.20, and 8.08%, respectively.

2. According to a number of studies on mycotoxins in foods, the general population's knowledge and awareness of these harmful substances remain low, which corresponds to the research work of Viang et al., 2014 [8] which found that agriculture has been placed chilli on the roof or at sidewalk of street. From research work of Liu and Wu. 2010 [9], Risk Assessment Global burden of Aflatoxin-

Table 4: The probabilistic risk of dietary exposure of dried chilli

Risk criteria	Aflatoxin in dried chilli
Dose and severity	
1.Hazard severity	SEVERE hazard-causes death to most victims
2.Susceptibility	GENERAL-all members of the population
Probability of exposure	
3.Frequency of consumption 18 liters per year	4.87 kilogram per year
4.Proportion consuming	Most 75%
5.Size of population	1.17 million
Probability of contamination	
6.Probability of raw product contaminated	3.03 %
7.Effect of processing	Us usually eliminates hazads (99 %)
8.Possibility of recontamination	No
9.Post-process control	Controlled
10.Increase to infective dose	5.17 illnesses per 66,511,700
11.Further cooking before eating	Effective in reducing 99% hazard
Predicted annual illness in the population considered	2.35E-6
Probability of illness per day per consumer of interest (Pinf x Pexp)	7.51E-4
Risk ranking (0-100)	67

Induced Hepatocellular carcinoma (HCC) found that agricultural regions in Africa and Asia are located in climatic zones favorable to the propagation of A. flavus and A. parasiticus. Inappropriate field practices and poor drying/storage conditions make crops vulnerable to A. flavus and A. parasiticus. Fungal infection and aflatoxin accumulation increase exposure to aflatoxin even in the same country. The risk of aflatoxin-induced HCC can vary significantly. Rural populations generally have higher levels of aflatoxin exposure than urban residents in developing countries. This is because urban populations tend to consume more variety of food than rural populations. And there may be foods that control contaminants better. Only 52% of participants in our study were aware of mycotoxins in foods, while 48% were unaware. However, there was a higher awareness of mold contamination in crops, with 84.67% of people recognizing that mold can grow on dried chilli in wet or wet

conditions with suggested from research of Jalili, Jinap, 2011 [10]. In general, spices are susceptible to fungal contamination since they are usually produced under non-sanitary conditions, especially during drying. For example, chilli is usually sun-dried for 3–7 days, and its final moisture content will nevertheless be around 30%. These conditions are conducive to the growth of mycotoxigenic fungi and subsequent mycotoxin production. Furthermore, the high incidence and levels of aflatoxins (AFs) and ochratoxin A (OTA) contamination in dried chilli samples are frequent by countries located in tropical and sub-tropical areas. Such environments offer desirable conditions for mold growth and mycotoxin production.

This study found that AFs in samples purchased from open markets were significantly higher ($p < 0.05$) than the corresponding levels detected in the samples obtained from supermarkets; this may be due to the fact that dried chilli sold in the open market is usually stored and/or displayed in improper conditions and is accordingly exposed to dust and other sources of environmental pollution. Long storage and improper storage conditions can lead to increased mold growth and mycotoxin production. This is an important finding, as dried chilli are a common food item in many cultures and can contain high levels of mycotoxins if not properly stored and processed. Another key finding of the study was that only 16.67% of individuals who were aware of mycotoxins in dried chilli knew that these substances can cause liver cancer, while 83.33% were unaware of this health risk. This is concerning, as liver cancer is a serious and potentially fatal disease that can be caused by prolonged exposure to mycotoxins [11] and its contamination was involved in liver diseases and proved to be hepatotoxic. Aflatoxin B1 was believed to have a role in causing hepatic and extrahepatic carcinogenesis in humans by causing single and double DNA breaks. The bioactivation of Aflatoxin B1 will produce its epoxide metabolite, which will bind to DNA molecules and eventually cause a neoplastic transformation of the cells. Another study confirmed the carcinogenic effect of AFB1, which was indicated in 2002 to belong to Group 1 of the cancerogens because it induces the formation of DNA adducts that contribute to liver cancer [12].

3. The preliminary risk assessment of exposure to aflatoxin in dried chilli

According to a recent study, the risk of exposure to aflatoxins through consumption of dried chilli is moderate. The study found that the increase in infective dose is 5.17 illnesses per 66,511,700, and that further cooking before eating is effective in reducing the hazard by 99%. Based on these risk criteria, the predicted annual illness in the population is 2.35E-6, and the probability of illness per day per consumer of interest (Pinf x Pexp) is 7.51E-4. The risk ranking on a scale of 0 to 100 is 67, which indicates a moderate level of risk [7]. Therefore, it is recommended that consumers take precautions when consuming dried chilli to minimize the risk of exposure to aflatoxins. These precautions may

include thoroughly washing the chilli before use, cooking the chilli at a high temperature for an extended period, and avoiding the consumption of moldy or discolored chilli. It is important to note that aflatoxins are a type of mycotoxin produced by certain strains of fungi, and can have harmful effects on human health. Aflatoxins have been linked to liver cancer, hepatitis, and other health problems [13].

Conclusion

Overall, these results highlight the need for greater education and awareness about mycotoxins and their health risks, particularly among populations that consume high levels of dried chilli and other potentially contaminated foods. By increasing knowledge and promoting safe food storage and processing practices, we can help reduce the incidence of mycotoxin-related illnesses and improve public health. In conclusion, the risk of exposure to aflatoxins through consumption of dried chilli is moderate, and consumers should take precautions to minimize their risk of exposure. By taking these precautions, consumers can enjoy the flavor and benefits of dried chilli while minimizing their risk of exposure to harmful toxins.

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Food Safety Blockchain Technology for Monitoring the CCPs in UHT Milk Production

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Abstract

Food safety management systems increasingly utilize blockchain technology for verification and archival purposes. In the domain of milk production, the application of this technology offers an efficient approach to mitigate risk threats. This study aimed to develop a food safety blockchain for UHT milk production by incorporating HACCP-compatible risk assessments to identify risk parameters and mitigate microbial hazards. Over a year, it was found that 86.25% of a population of 1,000,000 consumed UHT milk. The Exponential and Modified Beta Poisson Models predicted potential illnesses caused by *E. coli* and *S. aureus* to be 1.02E+01 and 2.99E-01, and 3.59E-03 and 5.47E-04 persons per year, per 1,000,000 people, respectively. Using the recorded data, a functional blockchain technology system was created for the UHT milk supply chain. This novel blockchain system, the latest in Thailand, offers an alternative for enhancing food safety in various production sectors.

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Keywords : Food Safety, Risk Assessment, Blockchain Technology, Milk Production

Introduction

Milk is a good source of nutrition for people at all ages [1]. Maintaining milk at high temperatures for a short amount of time is the main goal of dairy preservation. This results in the least harmful chemical, physical, and sensory alterations by killing microorganisms and inhibiting enzyme activity while also time [2]. This procedure increases the milk's shelf life without affecting its nutritional value. UHT processing uses a continuous flow of milk, which results in less chemical change [3]. Food risk management attempts to safeguard public health by minimizing food risks through the selection and implementation of appropriate controls. Food safety management systems, such as HACCP, which are frequently created on the basis of qualitative data, aim to protect people's health by limiting food risks as effectively as possible through the selection and implementation of appropriate procedures. Quantitative risk assessment can be used to provide more quantitative insight into food safety issues. A better knowledge of the elements affecting food safety is provided by quantitative risk assessment. There are four steps in it: identification of hazards, evaluation of exposure to hazards, characterization of hazards, and characterization of risks [4]. Food supply chain management has become more significant as a result of the rapid population growth and concomitant rise in food consumption. However, food supply chains around the world lose close to one third of their annual production. Food supply chain operations can now benefit from food safety, transparency, and traceability thanks to the growing usage of digital technology like blockchain [5, 6]. Therefore, it is essential to analyze each step of the food supply chain in order to increase its effectiveness and sustainability [7]. To verify the quality of raw milk, storage conditions, technology, animal welfare, and the environment, milk supply chains based on blockchain technology must be traced [8]. The milk industry uses blockchain technology to reduce the likelihood of milk spoilage and other hazards throughout the supply chain. Reduce risks by using blockchain technology to gather quality and hygiene data along the whole supply chain. Each piece of information, including the time, location, and distribution, were recorded. Each product group's milk transactions are all kept track on [9]. Throughout the supply chain, records of food-related data, milk quality, milk temperature, and moisture were maintained. For instance, a number of norms and laws have been established regarding temperature, humidity, expiration date, hygienic conditions, and other factors [10, 11]. It can use risk assessment concepts to gather data and do analysis food hazard related factors affecting food safety in order to create mathematical models and apply it in conjunction with blockchain technology to food safety.

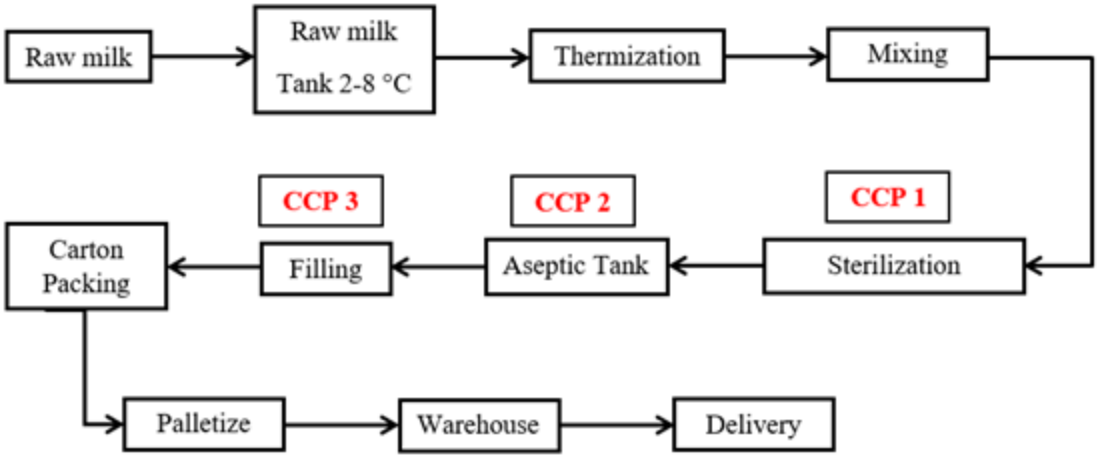


Figure 1: Process flow diagram of UHT milk

Research Objectives

To apply the food safety risk assessment concept for identifying the parameters for enhancing the CCPs of UHT milk production

To develop the food safety blockchain as a new tool for complying the HACCP system in UHT milk production

Research Methodology

1 Materials

Chiangmai Fresh Milk Co., Ltd., located in Chiang Mai Province, Thailand, kindly supplied the raw milk and UHT milk for this study. The samples of both raw milk and UHT milk were stored at temperatures of 0-8 °C and room temperature respectively, inside covered containers before transportation to the Faculty of Agro-Industry at Chiangmai University.

2. Identification of Parameters for Hazard Control of UHT Milk Production

The factory utilized in this research is Chiangmai Fresh Milk Co., Ltd. The UHT milk production process is controlled according to the HACCP standard, with critical control points (CCP) defined at three stages: 1. Sterilization, 2. Aseptic Tank, and 3. Filling. Figure 1 illustrates the chart of the production process and quality control for UHT milk.

3. Physico-chemical and Microbiological Analysis for Hazard Control and Quality Con-

trol

3.1 Physical Analysis

Color analysis for all samples was performed using a Minolta colorimeter (Konica Minolta, CR-400 Series), measuring L*, a*, and b* values on the Hunter scale.

3.2 Chemical Analysis

The determination of pH, total soluble solids (TSS), fat content, and protein content followed the AOAC 2000 guidelines.

3.3 Microbiological Analysis

The total plate count was performed following the FDA BAM analysis standards using 3M Petrifilm TM Aerobic Count Plates. The samples were applied to the plates with a micropipette and incubated for 48 hours at 35±1 °C. The colonies were counted twice upon completion of the process. Similarly, raw milk and UHT milk samples were tested for pathogenic bacteria according to the FDA BAM analysis standards. The criteria for sample selection included critical control points (CCPs) and food safety risk assessment. Pathogen counts for *Escherichia coli* and *Staphylococcus aureus* were carried out using 3M Petrifilm TM count plates following similar procedures.

3.4 Statistical Analysis

All measurements were performed in triplicate. The mean and standard deviation (SD) were computed for the data. Analysis of variance (ANOVA) and Duncan's multiple range tests were used to identify significant differences, with analysis conducted using SPSS software version 17.0.

4. Microbial Risk Assessment

Data on *E. coli* and *S. aureus* exposure to UHT milk were collected. This information, combined with data from literature, internet sources, and experimental data, was used to establish the dose-response model. Laboratory research data from the Faculty of Agro-Industry, Chiang Mai University, were also calculated in this study.

The probabilistic models of *E. coli* and *S. aureus* contamination in processed UHT milk samples were adequately explained by statistical models. The outcomes showed a statistical distribution

of the amount and prevalence of *E. coli* and *S. aureus* in the final product. Microsoft Excel TM was used to sample 10,000 times to ensure that the output distributions' tails would eventually converge (Microsoft Corp., Redmond, WA, USA). To compute the intake dosage distributions, the data were employed.

4.1 Dose Response relationships

The measured relationship between the quantity of exposure (dose) and the frequency of occurrence of this effect in the exposed population of hosts (response) for a given concentration is represented by the dose-response correlation. In the dose-response relationship, the disease end-point used in this study was illness. The probability of ingesting number j is Pexp(j). The number of organisms may cause infection with a probability of infection (Pinf), and the infection may result in symptoms and develop into an illness with a probability of illness (Pill). Assuming that pathogenic organisms are distributed randomly within the food, the probability of exposure (Pexp) can be explained by Equation 1 [12].

$$P_{\text{exp}}(j) = \frac{\mu^j}{j!}e^{-\mu} \tag{1}$$

where μ is the average number of organisms ingested per piece, j is the total number of ingested organisms, duration > 0 indicates infection, and the length of the infection event is regarded as the most important factor in determining the disease. The length of the infection reflects the balance between host defenses and pathogen growth, which may vary depending on dose [13].

4.2 Exponential dose response model

The exponential dose-response model assumes a Poisson distribution with a mean of dose (λ) [14].

$$P(inf/\lambda) = 1 - exp(-\lambda_P) \tag{2}$$

where λ_P is the predicted number of infections caused by this dosage and the equation for $P(inf/\lambda)$ denotes the Poisson probability of at least one infection. Equation 2 can be rewritten as follows:

$$P(inf/\lambda) = 1 - exp(-r_D) \tag{3}$$

where r is equal to the previous probability p, but the Poisson mean λ is substituted by the

actual dosage D received, which contradicts the underlying principle. Experimental data for the Thai dosage-response model were not available in our study. Alternatively, [15] used epidemiological and dietary survey data to construct a mathematical model to derive the R value for the exponential and dose-response models. The dose-response relationship of the pathogen may be characterized by the exponential model in Equation 4, based on the notion of a purposely cautious dose-response relationship:

$$P_{inf} = 1 - e^{-RN} \tag{4}$$

where P is the likelihood of a negative health consequence and N (or D) denotes the quantity of biological agents ingested (CFU). Equation 4 can be inferred and translated into Equation 5 using the following formula:

$$P_{inf} = 1 - e^{-RN} \tag{5}$$

where R is a constant specific to each pathogen, which aids in defining the dose-response curve. R is defined as follows:

$$ID_{50} = \frac{ln0.5}{-r} \tag{6}$$

where ID_{50} denotes the ingested dosage required to infect half of the exposed population or the median infectious dose.

4.3 Modified Beta Poisson model

Furumoto & Mickey, 1967 originally explained the modified beta poisson model [16], Haas, 1983 [17] used it for microbial dose-response calculations and applied further by Teunis et al., 1999 [18]. Pathogen-host interactions can be used to characterize the pathogen-host survival probability using a fixed value based on the results of the exponential model. The modified beta poisson model accounts for variability in pathogen-host interactions. If the pathogen-host survival probability is represented by the beta probability distribution, the probability of infection $(Pinf)$ may be expressed as follows:

$$P_{inf} = 1 - [1 + \frac{d}{ID50}(2^{1/\alpha} - 1)]^{-\alpha} \tag{7}$$

where d denotes the dose, α is a measure of the model's proximity to the Poisson distribution (exponential) or pathogen infectivity (slope parameter), and $ID50$ is the dose that infects half of the exposed population or the median infective dose. Given a dosage response function, the $ID50$ was calculated as follows:

$$ID_{50} = \frac{\beta}{(2^{1/\alpha} - 1)} \tag{8}$$

Where $\alpha$is shape parameter and β is scale parameter when $\beta \geq 1$and $\beta > \alpha$.

5. Application of Blockchain Technology in UHT Milk Production

Blockchain technology, a form of Distributed Ledger Technology (DLT), ensures data integrity by prohibiting alteration or modification of recorded data. It utilizes encryption principles and distributed computing capabilities to establish trust [19].

Blockchain technology allows for secure data storage in a distributed computing environment, reducing the risk of hacker attacks and identity theft. It provides access to the same data group for each user, simplifying verification and identity confirmation processes. The implementation of "self-sovereign identity" theory with blockchain technology enables organizations to compile personnel data without intermediaries. This not only reduces paperwork but also increases efficiency in verification processes. The integration of IoT and blockchain technology advances food safety traceability, permitting real-time audits and ensuring HACCP compliance from production inception to consumption.

Figure 2 illustrates the transition from a centralized database to a blockchain system for UHT milk production. Risk management data, including physical, chemical, and microbiological analysis data, are recorded at various stages of production such as homogenization/sterilization, aseptic tank filling, and carton packing/finished product.

The results of the microbiological risk assessment, encompassing information on pathogenic microorganisms in food and the likelihood of *E. coli* causing human illness, were included in the risk assessment data and made accessible to all departments involved in the production of UHT milk.

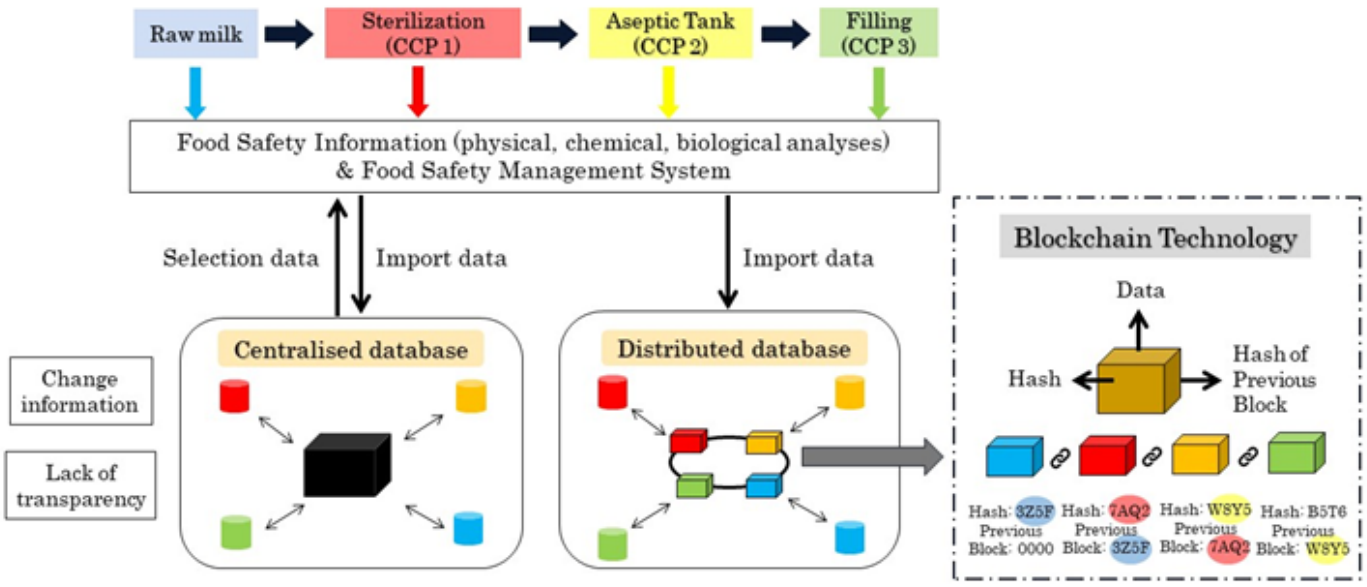


Figure 2: Application of Blockchain Technology to UHT milk production

Results

This research paper's Results section mainly focuses on the quality control methods of milk processing, specifically UHT (Ultra-high temperature processing) milk, and the monitoring of *E. coli* and *S. aureus* in UHT milk.

1. Hazard Control Analysis

Discusses the methods employed to control food-related illnesses in the processing of milk, implementing critical checks at various stages. These checks include sterilization, aseptic tank, and package integrity. The checks are measured against control limits to ensure they remain within safe parameters.

Food-related illnesses are becoming more prevalent. However, continuous conflicts over standards in food quality and safety inhibit international trade were to achieve acceptable quality standards. To ensure that the approved standards are maintained, milk quality must be monitored and managed using tests that have received international approval. There are rules and regulations for milk and milk products [20].

Quality assurance is required before the consumption of processed food can be achieved through the implementation of planned and systematic activities at each stage of the quality system. HACCP systems for food processing use critical checks at each stage of food processing to assess

the risk of physical, chemical, or microbiological hazards. There was a critical control point, control measures and monitoring system as follows:

CCP 1: Sterilization

This was found that harmful germs survived. This was as a result that the Ultra-high-temperature processing temperature and time was dependent of the specified value. Control measures, such as temperature control and sterilization time, were created. UHT milk that has heated for one to eight seconds, typically at 135-154 ℃, is the critical limit. The sterilization operator reads the data from the monitor at the pasteurized, tetra therm aseptic, and storage area every 30 minutes for sterilization batches as part of the monitoring system (Figure 3).

CCP 2: Aseptic Tank

This was found that harmful germs survived. This was as a result that the pasteurization's temperature and time was dependent of the specified value. Control measures, such as temperature control and pasteurization time, were created. End cluster valve at 110–120 ℃ and agitator at 95–105 ℃ for 30–40 minutes are the critical limits. A pasteurizer operator reads data from a monitor at the end cluster valve (110–120 ℃) and an agitator on top (95–105 ℃) for every sterilized batch every 30 minutes as a part of the monitoring system (Figures 4 and 5).

CCP 3: Filling (Package Integrity)

Within the constraints of the filler's sterile zone, aseptic packaging systems fill sterile product into sterile packages. From the point where sterilized packaging enters the sterile zone to the point where the sealed package was evacuated, there is an aseptic zone/sterile zone. Tetra Pak, Inc. was employed as a carton packing device and hydrogen peroxide sterilant for dairy goods.

Microorganisms, dust, and other pollutants contaminate products as a result of brazing and fields between packaging and incomplete products. In order to examine the coordination and welding between the package and the product at the top of the box, bottom of the box, pull test, and ink injection, control procedures were devised. the links to the packages below. It has a monitoring system, and every 30 minutes, the controller takes a sample of the product to verify.

The TS (left and right jaw) at the top and bottom of the box did not exhibit any leaks, and the connections were sound, according to Table 1's examination of the box for leaks. Erythrosin red ink was injected directly down the air channel without deviating, and after that, the box seam connection was finished without leaks or fractures.

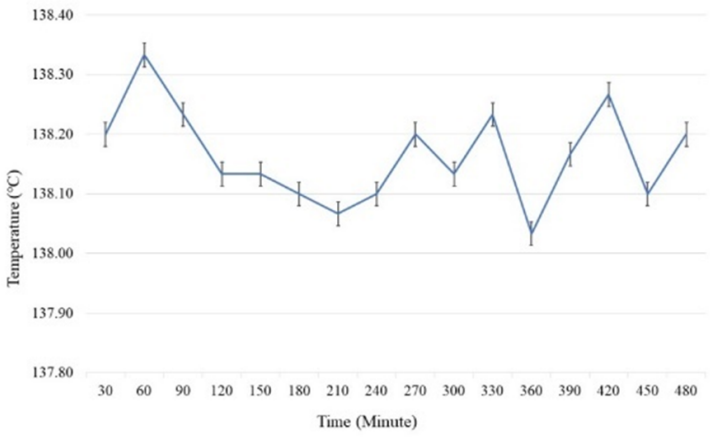


Figure 3: Monitoring of the sterilization temperature at 135 °C every 30 minutes.

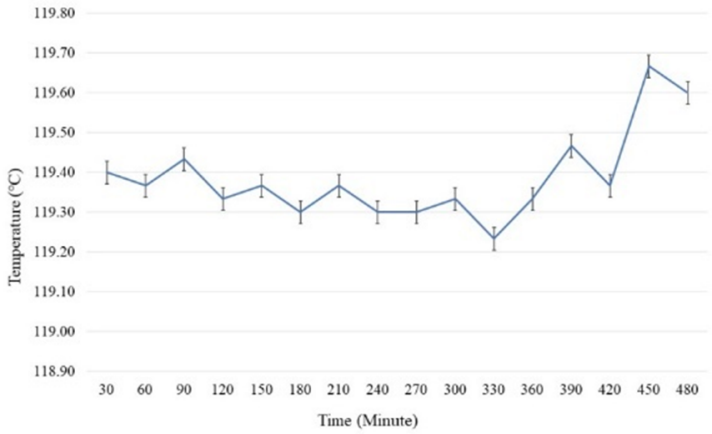


Figure 4: Temperature monitoring at the end of cluster valve at 110-120 °C every 30 min.

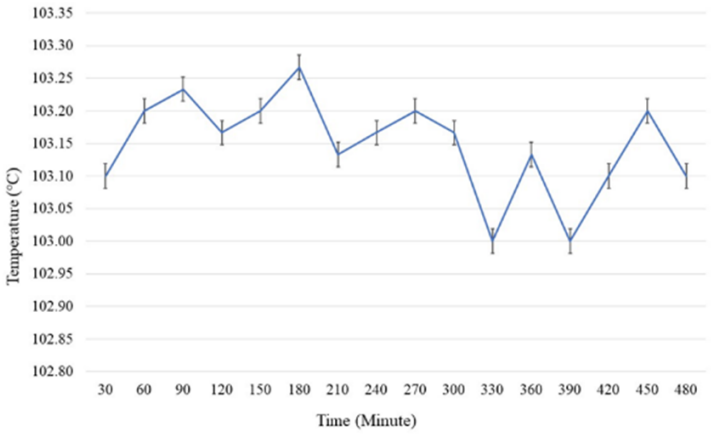


Figure 5: Temperature monitoring at the agitator's top at 95-105 °C every 30 minutes.

Table 1: Statistical analytical results of physical-chemical analyses of raw milk and UHT milk

Parameter	Color (Hunter scale)			pH	TSS	Fat	Protein
	L*	a*	b*		(°Brix)	(%)	(%)
Raw Milk	85.29 ± 0.96^{c}	-3.38 ± 0.21^{b}	5.51 ± 0.63^{b}	6.74 ± 0.03^{a}	9.88 ± 0.96^{d}	3.79 ± 0.05^{a}	3.05 ± 0.01^{b}
UHT Milk (CCP1)	91.64 ± 1.43^{b}	-2.81 ± 0.48^{a}	6.93 ± 0.96^{a}	6.53 ± 0.02^{b}	12.70 ± 0.09^{c}	3.57 ± 0.03^{bc}	3.04 ± 0.06^{a}
UHT Milk (CCP2)	92.30 ± 0.70^{ab}	-2.99 ± 0.32^{a}	7.27 ± 0.72^{a}	6.53 ± 0.01^{b}	12.90 ± 0.15^{a}	3.56 ± 0.01^{c}	3.05 ± 0.03^{b}
UHT Milk (CCP3)	92.47 ± 0.86^{ab}	-3.05 ± 0.39^{ab}	7.48 ± 0.42^{a}	6.54 ± 0.05^{b}	12.73 ± 0.13^{bc}	3.57 ± 0.02^{bc}	3.06 ± 0.05^{b}

Values in a column followed by different letters indicate significant differences. (P<0.05)

2. UHT Milk Quality

The research delves into the physical, chemical, and microbiological aspects of UHT milk quality. This includes comparing raw milk and UHT milk samples in terms of their components, such as fat, protein, and pH levels. The comparison also extends to their color using the Hunter scale. It concludes that there's no significant difference between raw milk and UHT milk in these aspects. The section also demonstrates the effectiveness of sterilization in eliminating bacteria.

The data in Table 1 show that the average values for color (a*, b*, and L* on the Hunter scale), pH, TSS, fat, protein, and color in samples of raw milk were 85.29±0.96, -3.38±0.21, 5.51±0.63, 6.74±0.03, 9.88±0.23, 3.79±0.05, and 3.05±0.01, respectively. For raw cow milk, the TAS 6003-2010 Thai Agricultural Standard was set for the quality and safety of products. It indicates that the protein content should be at least 3 percent by weight, the pH should be between 6.6 and 6.8, and the fat content should be at least 3.35 percent by weight.

Thermoresistant spores in milk are not totally inactivated by pasteurization procedures, despite the fact that possible pathogenic germs are successfully eradicated. The elimination of all bacteria is accomplished through sterilization. Physical and chemical testing were done to make sure the end product (UHT milk) complied with the requirements for quality and safety. The data provided in Table 1 demonstrates that there was no significant difference in the mean values of color (a*, b*, and L* on the Hunter scale), pH, TSS, fat, and protein in UHT milk samples (CCP 1-3). The 2013 Notification (No. 352) from the Ministry of Public Health guided the conduct of this study.

Table 2: Statistical analytical results of microbiological analysis of raw milk and UHT milk

Parameter	Raw Milk				UHT Milk (CCP1)				UHT Milk (CCP2)				UHT Milk (CCP3)			
	Count (CFU/ml)															
Dilution	10^1	10^2	10^3	10^4	10^1	10^2	10^3	10^4	10^1	10^2	10^3	10^4	10^1	10^2	10^3	10^4
Total Plate Count	TNTC	TNTC	$8.3x10^4$	ND	ND				ND				ND			
E. coli	$1.12x10^2$	ND	ND	ND	ND				ND				ND			
S. aureus	TNTC	TNTC	ND	ND	ND				ND				ND			

TNTC = too numerous to count and ND = not detected

As a result, Table 1's physical-chemical analysis of raw milk and UHT milk demonstrates that sterilized raw milk provides UHT milk that completed all necessary processing. Comparing BE 2013 to the Thai Agricultural Standard (TAS 6003-2010) and the Ministry of Public Health's Notification (No. 352) for raw cow milk.

The data shown in Table 2 illustrates that the mean values of total plate counts, total coliform counts, *Escherichia coli* counts, and *Staphylococcus aureus* counts (cfu/ml) in raw milk samples were total plate counts at dilutions 10^1 and 10^2 were too numerous to count, at dilutions 10^3 and 10^4 were $8.3x10^3$ cfu/ml and not detected, respectively. Total coliform counts at dilutions 10^1, 10^2, 10^3, and 10^4 were not detected, *E. coli* found at dilution 10^1 was $1.12 x10^2$ cfu/ml and at other dilutions was not detected, and *S. aureus* found at dilutions 10^1 and 10^2 was too numerous to count and at other dilutions was not detected. For raw cow milk, all results meet the Thai Agricultural Standard (TAS 6003-2010).

Accordingly, the results of the microbiological investigation of raw milk and UHT milk (Table 2) demonstrated that the production of UHT milk was prevented by microorganisms, as evidenced by the lack of total plate count, total coliform count, *E. coli* count, and *S. aureus* count in UHT milk. UHT milk effectively destroys bacteria during production, but it also signifies the creation of crucial control points in a successful HACCP system. It may effectively remove the main microorganisms that are harmful to milk.

3. Probabilistic Distribution of Exposure to *E. coli* and *S. aureus* in UHT Milk

Describes the probabilistic distribution of exposure to *E. coli* and *S. aureus* in UHT milk. The research makes an assumption that these bacteria are randomly distributed in all processes, and

transfer rates were calculated using a given formula. The study then uses dose-response models to predict the risk of illness based on the presence of these bacteria.

To simplify the probabilistic model, we assumed that *E. coli* and *S. aureus* were randomly distributed in all the processes, including raw milk, processing, and consumption. *E. coli* and *S. aureus* were not found in UHT milk products collected during processing. To establish the concentrations of *E. coli* and *S. aureus* in the UHT milk samples, the transfer rates of *E. coli* and *S. aureus* to UHT milk were determined. The transfer rates were calculated based on the number of microorganisms present on surfaces that were recovered by a single contact plate, using the following formula: % transfer rate = (Ns/Nf) × 100%, where Nf = CFU recovered from food and Ns = CFU on surfaces recovered by contact plate [21].

From the dose-response relationships, the imputed daily dose can be computed based on the daily and annual risks. It was assumed that *E. coli* and *S. aureus* infections were restricted to the Thai population (66,159,679 individuals).

The total amount of UHT milk consumed by the sub population of concern was approximately 49 g/day, and the levels of *E. coli* and *S. aureus* in UHT milk were assumed to be 50 and 100 mean CFU/g, respectively (from our laboratory). Thus, the levels of *E. coli* and *S. aureus* ingested when these products are consumed were calculated by multiplying the level of *E. coli* and *S. aureus* in the UHT milk by the serving size, N *E. coli* = 50 × 49 g = 2,450 CFU/serving/day, N *S. aureus* = 100 × 49 g = 4,900 CFU/serving/day. The total number of servings per day of UHT milk products consumed by the Thai population with a high level of *E. coli* was 49 × 50 ≈ 2,450 serving/day and *S. aureus* was 49 × 100 ≈ 4,900 serving/day.

An exponential dose-response curve was constructed from the concept of a purposefully conservative dose-response relationship for the Thai population. The Beta-Poisson curve assumes that the R value is not a coefficient constant, but is a beta probability distribution [22]. The parameter values used to predict the dose-response curves are listed in Table 3.

Table 3: Summary of parameters used in Exponential and Beta Poisson dose response models

Data set	1	2	3	4
Agent	*E. coli* O157:H7	*E. coli* O157:H7	*Staphylococcus aureus*	*Staphylococcus aureus*
Best fit model	Exponential	Beta-Poisson	Exponential	Beta-Poisson
Optimized parameter(s)	R = 2.18E-04	α = 1.55E-01 β= 2.11E+06	R = 7.64E-08	α=1.30E-01 β=2.3E+09
LD50/ID50	3.18E+03	-	9.08E+06	-
Host type	pig	human outbreaks	human	-
Agent strain	EHEC O157:H7,	*E. coli* O157:H7	-	-
Route	Oral (in food)	Oral (in milk)	subcutaneous	-
# Of doses	3	3	6	-
Dose units	CFU	-	CFU/cm^2	-
shedding in feces	shedding in feces	-	infection	-
Reference	[23]	[24]	[25]	Predicted

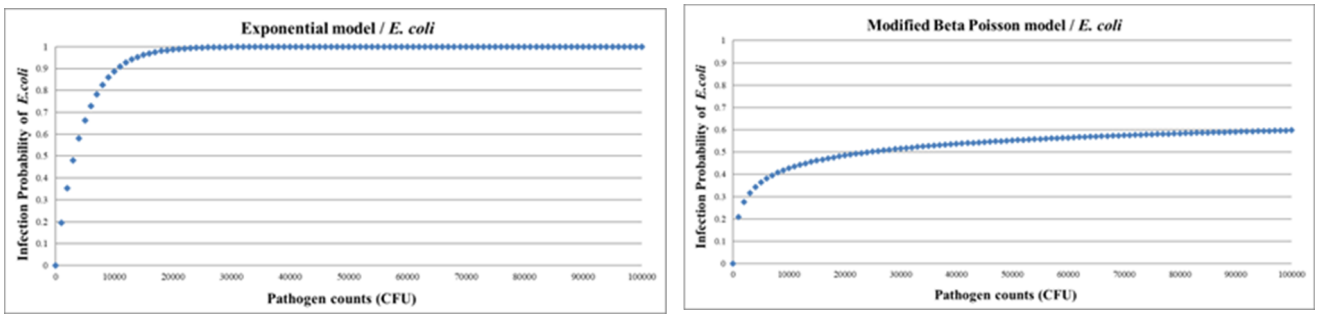
3.1 Dose Response Models

The dose-response models used in this study were the Exponential and Beta-Poisson models. The R values were 2.18E-04 and 7.64E-08 of datasets 1 and 3 were used to calculate the dose-response curves of the Exponential model for *E. coli* and *S. aureus*, respectively. The alpha (α) values of dataset 2 (*E. coli*) and dataset 4 (*S. aureus*) for the Beta-Poisson models are 1.55E-01 and 1.30E-01, respectively, and the beta (β) values for datasets 2 and 3 are 2.11E+06 and 2.3E+09, respectively.

The R, α and β values in datasets 1, 2, and 3 were obtained from [23, 24, 25] and predicted from dataset 3, respectively. The results for these formulas are presented in Table 3.

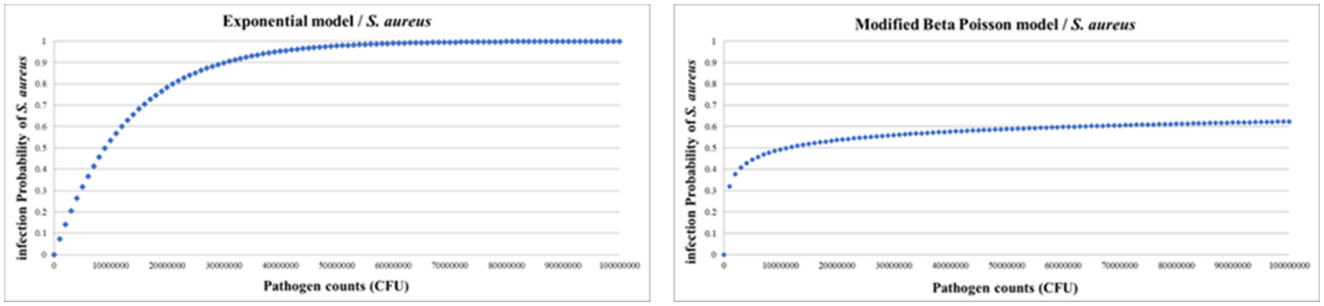
Figures 6 (a, b) to 7 (a, b) illustrate the predicted models' probability of illness from infection based on datasets 1, 2, 3, and 4. The risk estimate was obtained using the dose-response parameters. It was calculated how several *E. coli* and *S. aureus* disease cases there seem to be each year in Thailand.

The section continues to explain the dose-response models - Exponential and Beta Poisson models - used to calculate the risk of illness from *E. coli* and *S. aureus*. The paper also predicts



(a) (b)

Figure 6: Probability of *E. coli* infection predicted by Exponential model (a) and Modified Beta Poisson model (b)



(a) (b)

Figure 7: Probability of *S. aureus* infection predicted by Exponential model (a) and Modified Beta Poisson model (b)

how many disease cases from these bacteria could occur in Thailand per year. The research finally discusses the use of blockchain technology in the production of UHT milk. The technology is touted as a solution to challenges in data traceability and transparency in milk production. The use of smart contracts, automation through the Internet of Things, and consumer access via mobile applications are presented as potential advancements.

4. Model of Blockchain Technology System for UHT Milk Production

Figure 8 illustrates that blockchain technology has been applied to the production of UHT milk. Information can really be edited or modified using the distributed database system for use by blockchain. Data gathering helps manage and store important products and supply chains. Through this, it is possible to overcome the challenges to data traceability and transparency in the production of UHT milk. All participants in the UHT milk process, including employees, supply chain representatives, and stakeholders, have required to follow by the guidelines of the hazard analysis and critical

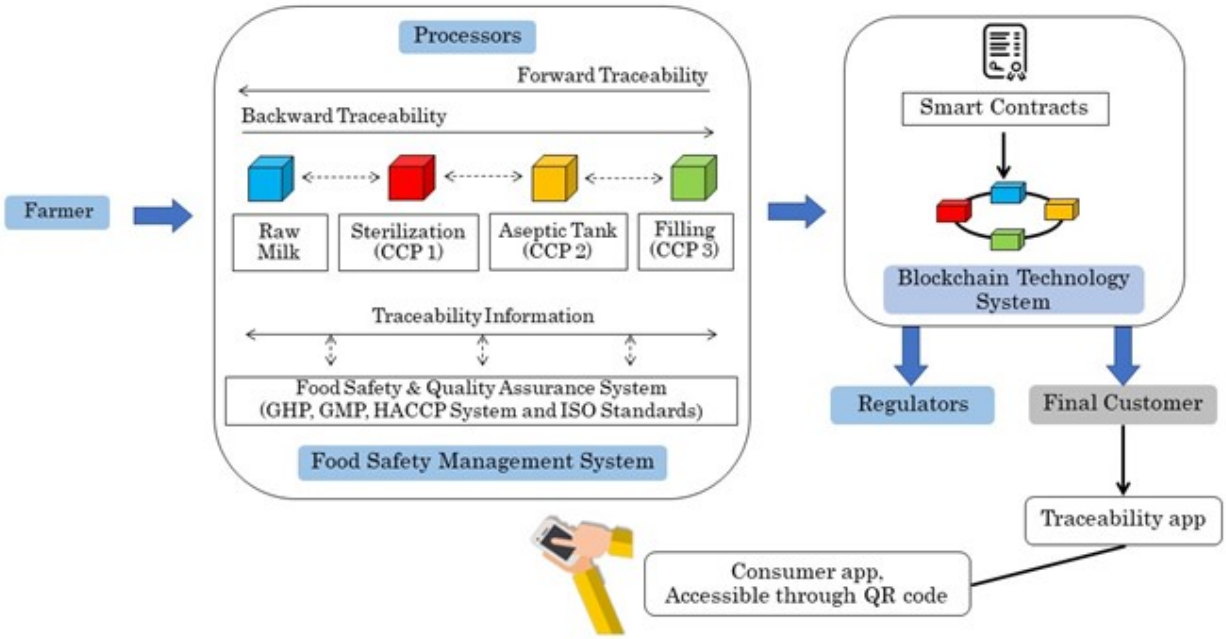


Figure 8: Model of Blockchain Technology System for UHT Milk Production

control point (HACCP) system. The Internet of Things can automate it. RFID tags, sensors, and other devices can be used for automation to update data based on the system.

It is important to use blockchain technology in smart contracts to conduct thorough, recorded administrative audits that could hinder the overall performance of the UHT milk process. Smart contracts should also be encrypted to prevent fraud, theft, and other management concerns. Blockchain networks are deploying smart contracts. Each linked node receives these contracts. Recent modifications to the local database could lead the computer code's conditions to behave in accordance with the relevant stream or notification.

The UHT milk process is completed by consumers, who can access the consumer app using an Android or iOS device. According to the information given, this can be accessed by scanning a QR code located on the UHT milk product. to improve trust and transparency, as well as to help consumers choose products.

In summary, this research provides significant information on the safety measures, quality assurance techniques, and risk assessment of bacterial contamination in the production of UHT milk. Moreover, it explores the potential of technological advancements in maintaining food safety standards.

Discussion

1. Hazard Control Analysis

Based on the determination of the critical control point, control measures, and monitoring system, it was discovered that the control measures at CCP1-3 (CCP 1: Sterilization, CCP 2: Aseptic Tank, and CCP 3: Filling (Package Integrity) were within the required criteria during the UHT milk production process every 30 minutes, indicating that the critical control point was defined in the HACCP system can truly control the hazards that may occur in the production process. UHT processing uses continuous flow, which renders less chemical change to the product in comparison to retort processing. Minimum processing times and temperatures are determined by the inactivation of thermophilic bacterial spores [3]. Ideal time-temperature profiles inactivate bacterial endospores and limit chemical changes with minimal decrease in nutritional and sensory quality [26]. The major challenge in UHT milk production is sufficient heat treatment with minimal flavor change. Direct heating imparts less flavor change but requires more energy in comparison to indirect heating. Total microbial lethality at constant time and temperature varies between direct and indirect heating systems [27].

2. UHT Milk Quality

The Ministry of Public Health's notification (No. 352) B.E. 2013 Re: Other Milk Products, stating that there was no contamination with pathogenic bacteria, is consistent with the average values of total plate count, total coliform count, *E. coli* count, and *S. aureus* count (cfu/ml) not being found in UHT milk. The total plate counts, total coliform counts, *E. coli* counts, or *S. aureus* counts (cfu/ml) should not be included in a 0.1 ml sample, though. All findings were within control limits as per the HACCP plan and the Ministry of Public Health (No. 352) B.E. 2013 Re: Other Milk Products. Product characteristics such as pH, water activity, viscosity, composition, and dissolved oxygen dictate the processing conditions necessary to achieve commercial sterility [28].

And as shown in Table 2, the results of the analysis of microbial contamination show that the microorganisms analyzed are all bacteria. Coliform bacteria, *E. coli* and *S. aureus*. The results of the analysis can be discussed as follows: Analysis of raw milk found *E. coli* and *S. aureus* which are consistent with Bryan, 1983 [29]. Raw milk was often implicated in outbreaks of staphylococcal intoxication before rapid cooling of milk and pasteurization became accepted practices. Several workers isolated *E. coli* from milk and stated that it might cause a potential risk, particularly for children [30], comparable to what was reported by Salman and Hamad, 2011 [31] reported that about 32% of the raw bulk milk was *E. coli* positive in Khartoum State. After UHT milk, there was no

growth of *E. coli* in the samples obtained from factory (Table 2). This result was in agreement with that reported by SSMO, 2007 [32] for pasteurized milk of *E. coli* which was zero, and this stated that UHT milk should be free from pathogenic microorganisms, with a total bacterial count of not more than 10 cfu/ml and free from *E. coli*. Higher results were obtained by El-Asuoty, 2006 [33].

The results of this study showed that, no growth of *S. aureus* in samples (Table 2). The results are in disagreement with Laszlo, 2003 [34] reported count of more than 10^3 cfu/ml. While Lílian, et al. (2011) found that 30% of the samples were contaminated with *Staphylococcus aureus*. This study revealed that the Total Plate Count, Total Coliforms, *S. aureus* and *E. coli*, are absent (Table 2). This result agrees with [35] after the application of HACCP. The lower bacterial counts might be due to quality of raw milk, good manufacturing practices, and efficient storage conditions. The present study revealed proper pasteurization due to the reduction of microorganisms, which agreed with Dumalisile et al., 2005 [36].

3. Probabilistic Distribution of Exposure to *E. coli* and *S. aureus* in UHT Milk

An exponential dose-response curve was constructed from the concept of a purposefully conservative dose-response relationship for the Thai population. The ID50 or ND50 and alpha values for the Beta-Poisson model were calculated from the value of R using Equation 8. The Beta-Poisson curve assumes that the R value is not a constant, but is a beta probability distribution [37]. The ID50 and alpha values of this study were lower than the study of Crockett et al., 1996, where the ID50 was 2.54E+13 and the alpha values were 9.47E+2 [38].

4. Model of Blockchain Technology System for UHT Milk Production

Figure 8 shows the application of blockchain technology in UHT milk demonstrates the storage process that enables short-time and reliable traceability of food safety-related data. In those study of Steiner et al., 2016; Tian, 2016; Kamath, 2018; Lin et al., 2018 [39, 40], it was determined that: Blockchain Technology is a rapidly evolving technology and with wide use, it appears to be changing many areas in the public and private areas of the food and agricultural industry. Traceability of block chain technology to improve safety / Quality of the global food supply chain and international distribution, blockchain technology. It has the ability to keep records unchanged and a traceable transaction history, which has great potential for increased efficiency, transparency and traceability [41].

Security management is the creation of an information database. That is beneficial to the management of the factory and will be information for factory executives to make decisions in various fields. It is a reflection of the overall risks. Risk management will give management a better under-

standing and realize the importance of information system management and manage the resources efficiently and appropriately. This makes the management confident in the management and making decisions in areas such as strategy formulation future event planning, etc.

There are extremely few persons that use computer technology in the food industry. And we can demonstrate that computer technology can manage security data. This is advantageous to both manufacturers and consumers Farmers and producers can properly explain raw ingredients and manufacturing processes. Meanwhile, consumers or buyers may correctly verify the product's origin as well as the supply chain. Scan a QR code on the package to obtain information. You can also learn about the certification of international quality and food safety standards. Blockchain Technology that has been generally acknowledged for data accuracy in various organizations such as finance, procurement, and supply chain, etc., to boost customer and consumer confidence in accessing information.

Conclusion

Hazard Characterization, it was found that the risk was low. The probability of illness per person per year from the consumption of UHT milk. The Exponential Model and the Modified Beta Poisson Model caused by *E. coli* were 1.02E+01 and 2.99E-01 and *S. aureus* was 3.59E-03 and 5.47E-04 person per year, per 1,000,000 people, respectively, based on this study, that also examines the perceived hazard of *E. coli* and *S. aureus* illness related to the consumption of UHT milk products. The CCPs and risk parameters at each steps of UHT milk production had been approached.

Using blockchain technology, it's also able to rapidly and securely validate every piece of information related with a food item. Similar to the above, in the instance of an outbreak of a foodborne illness, the history of a particular food item can be examined to identify the heart of the problem, allowing it to be rapidly recalled. Food products with blockchain-based information dissemination can enhance consumer confidence in a food product with blockchain-based information attached to it, ultimately preventing consumers from falling for counterfeit food products. This is because the information stored in the blockchain platform cannot be altered.

Finally, despite the fact that utilizing blockchain-based dairy supply chain systems seems to provide a variety of advantages, there are also a number of difficulties in their implementation. Blockchain is a recent technology, and business owners now face higher administrative costs. The noncommercial and fragmented dairy farming industry in developing nations is another significant

limitation. The participation of these farmers in a blockchain-based dairy supply chain system does not come without difficulties. Therefore, in-depth research is required in order to apply blockchain technology to the dairy industry. Furthermore, these techniques can be applied to other related food industries in the future.

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Quality of Black-Boned Chicken (*Gallus Domesticus*) Carcass and Development to Black-Boned Chicken Soup

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Abstract

Black-boned chickens are one of the native fowl with distinctive characteristics. It was found that it has high protein and low-fat content. Additionally, it had melanin which is a natural antioxidant compound. Chicken soup is a popular functional food that contains carnosine and anserine. These substances are the most prevalent histidine-containing dipeptides not found in plants. Thus, this study aimed to examine the quality of black-boned chicken carcasses and evaluate the biological properties of melanin, carnosine, and anserine were evaluated in various part of black-boned chicken: breast meat, thigh meat, and femur bone as well as the soups derived from each part. The chicken breast meat and its soup contained more carnosine and anserine than the those found thigh meat and femur bone soups ($P<0.05$). Although, the melanin content showed different amount in raw samples. The chicken soups were not significantly different ($P\geq0.05$). The anserine content in chicken muscles were found to be higher compared to that of carnosine. The biological properties of carnosine and anserine extracts from chicken muscle increased proportionally with the amount of these peptides in the muscle. Meanwhile, the femur bone had fewer peptides than the muscle. The femur bone exhibited higher antioxidant activities than the chicken thigh soup, as measured by ABTS and FRAP assays. Hence, the antioxidant activities corresponding with the dipeptides except femur bone.

Keywords : Black-boned chicken, Soup, Functional foods, Carnosine, Anserine, Melanin

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Introduction

The expansion of the chicken meat in Thailand can be attributed to the rising demand from both local and international markets [1]. The Thai poultry industry predominantly relies on the broiler genotype owing to its superior growth performance, which leads to reduced production costs. In addition, Thailand offers alternative genotypes for meat consumption, including Thai indigenous and the black-boned chicken, which are commercially available.

Thai indigenous chickens are a sought-after meat for culinary purposes due to their distinctive texture, favorable taste, and low-fat content, despite their high cost and slow growth rate [2]. The black-boned chicken, despite its distinct physical characteristics, is associated with a relatively high price point. However, it is widely believed that the breed's purported health benefits have resulted in increased consumer interest when compared to other chicken breeds.

Black-boned chicken also contains melanin that represents the black color of skin, flesh, and bone [3]. It acts as an antioxidant and is also associated with the immune system function in the body [4]. This chicken has higher amounts of carnosine and anserine than conventional chickens about two times [5].

Moreover, carnosine content was higher in breast meat than thigh meat. The breast meat contained higher anserine content compared with thigh meat. The sex effect on anserine was not consistent between breast and thigh meat [6].

The amount of melanin pigment in the tissues of different organs of black-boned silky chickens was significantly different ($P<0.05$). The melanin pigment in femur bone was the highest at 21.3 mg/g of tissue followed by ovarian and testicular tissue, trachea, muscle tissue. and skin respectively [7].

Our world has changed in various ways recently, including lifestyle adaptation [8]. Consumers are more conscious of their food choices due to time limitations, especially students and working age who do not have much time to cook [9]. Therefore, functional food is an alternative that can satisfy this consumer group.

Functional foods containing bioactive peptides have been found in plants and animals [10]. In particular, chicken is a good source of bioactive peptides. It is not only rich in protein but also has high bioactive peptide content [11]. Thus, making it highly desirable to consumers. The important bioactive peptides include carnosine and anserine [12].

The soup of chicken has been long one of the most popular functional foods. Because it contains carnosine and anserine, which are significant dipeptides [13]. Carnosine is one of the anti-aging nutrients and can also help restore tiredness during exercise by reducing the buildup of lactic acid in the muscles [14]. Anserine can help reduce stress and fatigue [15], stimulate brain activity, increase concentration, improve learning and memory capacity [16].

Research Objectives

1. To examine the quality of black-boned chicken carcasses.

2. To evaluate the biological properties of melanin, carnosine, and anserine in a black-boned chicken parts and soups derived from each part.

Research Methodology

1. Materials

Black-boned roosters (royal project breed) averaging 1.6 kg and four weeks of maturation were obtained from the royal project farm in Chiang Mai, Thailand. Samples were taken to reduce the residual blood by soaking in water at 25±0.5 °C for 10 min before further processing (adapted from [17]).

2. Methods

2.1 Sample preparation

Raw chickens consisted of three parts: breast meat, thigh meat, and femur bone were studied separately. The breast and thigh meats were cut into 1x1x1 cm^3, and femur bones were reduced to small pieces about 1-2 cm.

2.2 Stewing Process

Each treatment contained the chicken part-to-water ratio of 1:1. The contents were filled in a pressure-resistant glass bottle. Subsequently, autoclaved at 115 °C and 10 psi for 2 h (HVA-85, Hirayama, Saitama, Japan). Then, separated meat and soup by filter paper no.4 (Whatman). The extracts were kept under -20 °C with light protection for further analysis.

2.3 Proximate analysis

Determined the amount of moisture, fat, and ash in raw samples and soups [18]. Kjeltec 2300 Analyzer (Foss Tecator, Hoganas, Sweden) was used to determine crude protein according to the Kjeldahl technique. The derived protein amount was multiplied by an overall nitrogen value of 6.25.

2.4 Physical and chemical properties

2.4.1 pH

The pH of the raw materials and their soup samples were measured potentiometric (Lab284, Metrohm, Switzerland). The raw materials were mixed with water in a ratio of 1:5 at a temperature of 25 ºC. The pH of the soup samples was directly measured.

2.4.2 Color

The color parameters of the raw chicken parts and their soup samples were monitored using a colorimeter (ColorQuest XE, HunterLab, Reston, VA). Reported in terms of the CIE system and displayed in the form of values L^* (100 to 0 represents lightness to darkness), a^* (where red has a positive value and green has a negative value), and b^* (a positive value indicates yellow, whereas a negative value illustrates blue).

2.5 Carnosine and anserine

Each part of the raw chicken samples was homogenized with 0.01 N HCl, then centrifuged at 10,000 rpm for 20 min (3-30KS, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). The collected clear upper solution was stirred with acetonitrile and preserved at the refrigerator temperature for 20 min. Subsequently, the solution was centrifuged at the same speed for 10 min. The HPLC system was made on an Agilent (Santa Clara, California, USA). The operation was performed according to [19]. A Restek C18 column (4.6 ×250 mm 5μm) was used. Twenty microliters of each sample were injected into the process. The mobile phase consisted of two parts: a 25:75 mixture of 0.65 mM ammonium acetate (pH 5.5) and acetonitrile. The other was a 70:30 mixture of 4.55 mM ammonium acetate (pH 5.5) and acetonitrile. A gradient system was employed in this operation (percentage ranging of the second solvent from 0% (v) to 100% (v)) at a flow rate of 0.8 mL/min for 16 min. A diode array detector was used to measure at 214 nm of absorbance. Standard carnosine and anserine were purchased from Sigma-Aldrich (Sigma Co. St. Louis, MO, USA).

2.6 Melanin content

For melanin isolation, approximately 20 g of muscle and bone samples were randomly collected from each sample using a slightly modified method described by [20]. The extracted

melanin samples were stored at -20 °C for further determination of melanin content compared to the synthetic melanin linear standard curve (Sigma No. M8631, USA). A suspension of synthetic melanin was prepared for the standard curve by preparing 0.01 g of synthetic melanin in 100 mL of 0.1 M NaOH at a concentration of 100 ppm. The melanin suspension was diluted to obtain a calibration curve by 0, 5, 10, 20, 25, and 30 mg/mL. The absorbance was read at 490 nm with a microplate reader (Spark, Tecan, Switzerland) illustrated in milligrams per milliliters. Then, the melanin content was converted to milligrams per gram.

2.7 Antioxidant activities

2.7.1 DPPH (2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay)

DPPH radical scavenging capacity was tested according to [21]. The 0.06 mmol DPPH solution in 99.5% methanol was mixed with black-boned chicken soup at 1:39 %v/v and left for 30 min with light protection. The absorbance was detected at a wavelength of 517 nm, compared to the Trolox concentration curve standardized. Reported as micromole Trolox equivalent per gram of black-boned chicken parts and soups.

2.7.2 FRAP (Ferric reducing antioxidant power assay)

Measurement of the ability of the sample to reduce ferrous ions was based on the method of [22]. Freshly mixed acetate buffer (300 mM, pH 3.6), TPTZ solution (10 mM TPTZ in 40 mM of HCl), and $FeCl_3 \cdot 6H_2O$ solution (20 mM) in a 10:1:1 ratio were used to prepare the FRAP solution. The solution was incubated at 37 °C before using for 4 min. Then, 100 μL of the sample was mixed with 2800 μL of the FRAP solution and incubated at 37 °C for 30 min. The absorbance was read at 593 nm, the Trolox concentration curve was standard. The values were reported as micromole Trolox equivalent per gram of black-boned chicken parts and soups.

2.7.3 ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging activity assay)

A modified method of ABTS radical scavenging activity was described by [23]. The ABTS solution was made by incubating 7.4 mM ABTS and 2.6 mM potassium persulfate for 12 h. Before ABTS solution was used, it was diluted with distilled water until the absorbance value was achieved of 0.700±0.020. The sample was used at 100 μL and the diluted ABTS 2800 μL. The mixture was incubated for 6 min in the dark at 37 °C. The absorbance was detected at 734 nm, compared with the Trolox standard. Exhibited data in micromole Trolox equivalent per gram sample of black-boned chicken parts and soups.

Table 1: Macronutrients of black-boned chicken parts.

Chicken parts	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Breast meat	74.68 ± 0.28^{a}	23.44 ± 0.19^{a}	0.48 ± 0.03^{c}	0.90 ± 0.04^{b}
Thigh meat	73.36 ± 0.10^{b}	20.49 ± 0.17^{b}	2.11 ± 0.06^{b}	0.89 ± 0.03^{b}
Femur bone	46.18 ± 0.13^{c}	17.67 ± 0.14^{c}	13.15 ± 0.11^{a}	22.43 ± 0.24^{a}

Data are presented as mean ± SD, the alphabet in each column indicates a significant difference (P<0.05).

Table 2: Macronutrients of black-boned chicken soups.

Chicken parts	Moisture (%)^{ns}	Protein (%)	Fat (%)	Ash (%)^{ns}
Breast meat	94.61 ± 0.78	2.38 ± 0.03^{c}	0.10 ± 0.02^{b}	0.57 ± 0.07
Thigh meat	95.01 ± 1.05	3.42 ± 0.25^{b}	0.13 ± 0.03^{b}	0.56 ± 0.14
Femur bone	94.70 ± 1.02	3.89 ± 0.61^{a}	0.27 ± 0.04^{a}	0.62 ± 0.09

Data are presented as mean ± SD, the alphabet in each column indicates a significant difference (P<0.05), ns indicates no significant difference (P≥0.05)

3. Statistical analysis

The experiment was planned to have completely randomized design (CRD) with three repetitions. Data are shown as mean ± standard deviation. All investigations were carried out using the SPSS statistical program (17.0, SPSS Inc., California). Compared mean using analysis of variance (ANOVA) via Duncan's least significant test that significance was determined at P<0.05.

Results

1. Proximate analysis

The chemical composition of the raw samples is shown in Table 1. Breast meat contained the highest protein content (23.44%), whereas the fat content was the lowest (0.48%). The moisture content of the meat parts was significantly different compared to the bone (P<0.05). The muscle parts showed significant differences in protein and moisture content compared to the bone (P<0.05). Meanwhile, the femur bone was found as the greatest fat and ash contents due to it rich in bone marrow and minerals, respectively [24]. This result showed that breast meat can be used as a raw

Table 3: Physical chemical properties of black-boned chicken parts.

Chicken parts	pH	Color		
		L^*	a^*	b^*
Breast meat	5.94 ± 0.03^c	38.87 ± 0.96^a	0.80 ± 0.26^c	1.97 ± 0.66^b
Thigh meat	6.37 ± 0.01^b	36.26 ± 0.97^b	2.31 ± 0.42^b	3.68 ± 0.36^a
Femur bone	7.10 ± 0.04^a	35.22 ± 0.86^c	4.44 ± 0.93^a	3.41 ± 0.65^a

Data are presented as mean ± SD, the alphabet in each column indicates a significant difference ($P<0.05$).

material to produce high-protein, low-fat chicken soups.

The basic composition of the black-boned chicken soups is shown in Table 2. There were no statistically significant differences in moisture and ash contents among the different chicken parts ($P \geq 0.05$). The protein content of the femur bone soup was found to be the highest at 3.89%, while the thigh meat and breast meat soups showed protein contents of 3.42% and 2.38%, respectively. The chicken soup derived from the femur bone had the highest fat content compared to soup derived from breast meat and thigh meat.

2. Physical and chemical properties

The physical and chemical parameters of untreated chicken parts are shown in Table 3. The pH level is an important indicator of meat quality [25]. Each piece of fresh chicken had a varied pH value ($P<0.05$). The pH of the muscles was lower than that of bones. Furthermore, it was observed that the pH of the breast muscle was lower in comparison to that of the thigh muscle.

The distinguishing characteristic of black-bone chicken is the black color of the muscles and bones. Considering that the various parts of a black-boned chicken have distinct hues due to the accumulation of black pigments and the chemical composition of each part. The present investigation resulted in statistically significant differences ($P<0.05$) between the breast and thigh muscles. The breast muscle had the highest levels of brightness (L^*) and the lowest levels of redness (a^*) and yellowness (b^*). Compared to the bone, the muscles had a higher L^* value but a lower a^* value. The tissue of the thigh was more yellowness than that of the breast.

As shown in Table 4, this study observed the increase in pH after stewing compared to raw chicken. The pH of each chicken part was significantly different ($P<0.05$). The results indicated that the pH levels of the muscle soups were significantly lower than those of the bone soup ($P<0.05$).

Table 4: Physical chemical properties of black-bone chicken soups.

Chicken parts	pH	Color		
		$L*$	$a*$	$b*$
Breast meat	6.37±0.01^{c}	25.58±0.20^{c}	-1.50±0.35^{b}	0.50±0.04^{a}
Thigh meat	6.57±0.01^{b}	27.67±0.28^{b}	-1.35±0.08^{b}	-0.06±0.23^{b}
Femur bone	7.12±0.06^{a}	29.73±0.05^{a}	-1.00±0.04^{a}	-0.60±0.03^{c}

Data are presented as mean ± SD, the alphabet in each column indicates a significant difference (P<0.05).

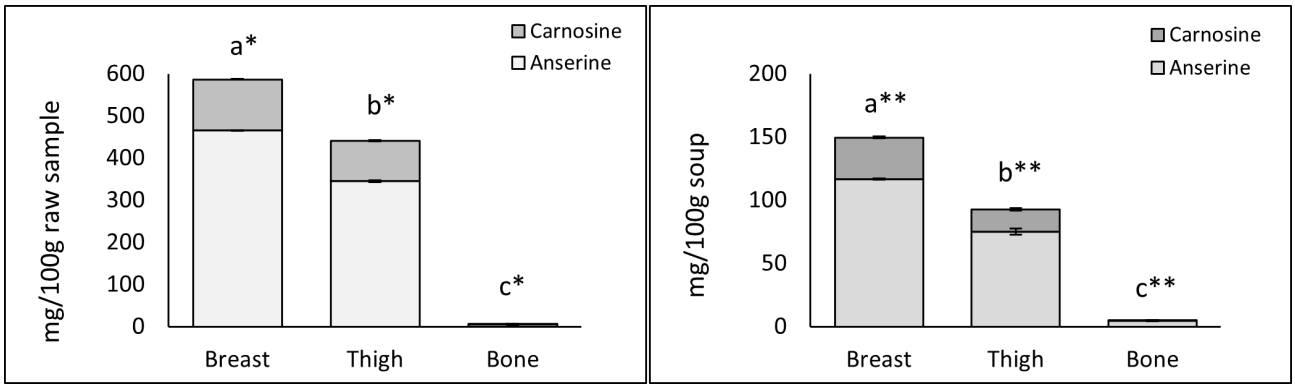

Figure 1: Carnosine and anserine contents in a) black-boned chicken parts and b) black-boned chicken soups, a small letter in each column indicates a significant difference in chicken type (P<0.05), and *, ** indicates a significant difference in each sample type (P<0.05).

Following stewing, a notable reduction in the brightness (L^*) of the broth derived from each chicken part was observed, with statistical significance (P<0.05). The femur bone soup exhibited the highest brightness, whereas the thigh and breast muscle samples displayed lower levels of brightness in descending order.

The study found that there was no significant difference in the redness of chicken soup made from breast and thigh muscles. The femur bone soup exhibited the highest level of redness. This study revealed that the b^* values, which indicate yellowness, of chicken soup varied significantly (P<0.05) among different parts. Specifically, the breast muscles exhibited the highest degree of yellowing, followed by the thigh muscles and femur bone.

3. Carnosine and Anserine

Carnosine and anserine were found in the greatest numbers in raw breast tissue, followed

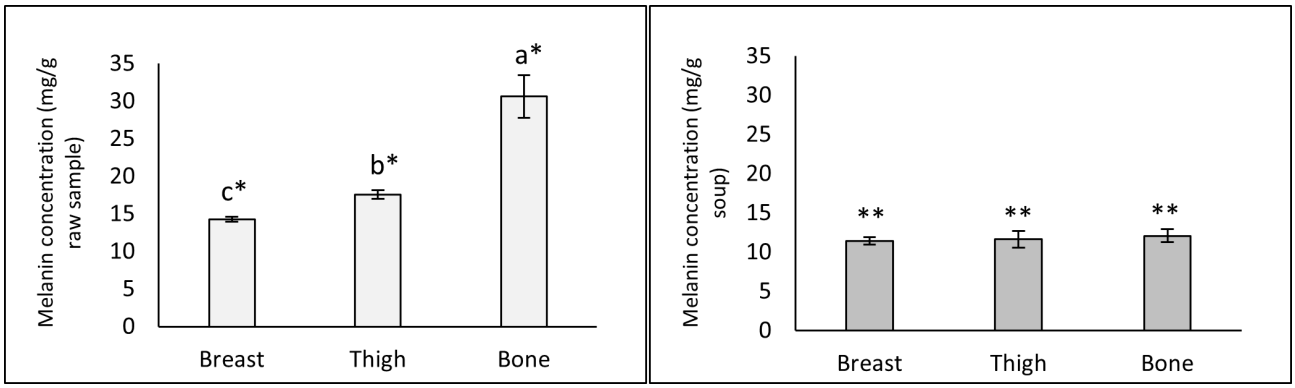

Figure 2: Melanin contents in a) black-boned chicken parts and b) black-boned chicken soups, small letter in each column indicates a significant difference in chicken type ($P<0.05$), and *, ** indicates a significant difference in each sample type ($P<0.05$).

by thigh tissue and femur bone, respectively (Figure 1a). This work found that these dipeptides were found at low levels in raw femur bone. After stewing chicken broth using breast muscle, it had the highest histidine-dipeptides compared to thigh and femur bone soup (Figure 1b). In addition, the anserine content in chicken breast broth was 1.35 and 58.1 times higher than that in thigh tissue and femur bone, respectively.

The ratios of anserine and carnosine contents in the black-boned chicken broth made from breast muscle, thigh muscle, and femur bone were 3.57, 4.24, and 24.15, respectively. Compared to similar raw meat samples, processing caused carnosine losses of 73%, 82%, and 88% in the cases of breast, thigh, and bone, respectively. At the same time, anserine was found to be lost by 75%, 78%, and 43% for the meats (breast and thigh), and bone, respectively.

4. Melanin

The findings revealed that the amount of melanin accumulated in various chicken parts varied (Figure 2a). After stewing, there were no significant differences ($P\geq0.05$) in the chicken broth from each part (Figure 2b). In contrast, there were significant decrease ($P\geq0.05$) from 14.29 to 11.4 mg/g for breast soup, 17.57 to 11.63 mg/g in thigh soup, and 30.63 to 12.08 mg/g in femur bone soup. Due to the absence of the black pigment from melanin, the color values obtained were consistent with the melanin content.

5. Antioxidant activities

The antioxidant activities of the raw black-boned chicken samples were determined using the DPPH, ABTS, and FRAP methods (Table 5). The breast and thigh meats exhibited significantly higher antioxidant activity in the DPPH assay compared to the femur bone ($P<0.05$).

Table 5: Antioxidant activities of black-boned chicken.

Chicken parts	DPPH (μmol Trolox eq/g)	ABTS (μmol Trolox eq/g)	FRAP (μmol Trolox eq/g)
Breast	0.390 ± 0.014^{a}	0.364 ± 0.030^{b}	0.230 ± 0.024^{a}
Thigh	0.374 ± 0.018^{a}	0.338 ± 0.039^{b}	0.174 ± 0.021^{b}
Femur bone	0.289 ± 0.004^{b}	0.440 ± 0.029^{a}	0.219 ± 0.027^{a}

Data are presented as mean ± SD, small letter in each column indicates a significant difference in chicken part ($P<0.05$).

Table 6: Antioxidant activities of black-bone chicken soup.

Chicken parts	DPPH (μmol Trolox eq/g)	ABTS (μmol Trolox eq/g)	FRAP (μmol Trolox eq/g)
Breast	0.524 ± 0.030^{a}	0.854 ± 0.046	0.254 ± 0.023^{a}
Thigh	0.501 ± 0.054^{a}	0.844 ± 0.101	0.217 ± 0.021^{b}
Femur bone	0.434 ± 0.034^{b}	0.910 ± 0.025	0.241 ± 0.024^{ab}

Data are presented as mean ± SD, small letter in each column indicates a significant difference in chicken part ($P<0.05$), ns indicates no significant difference in chicken part ($P \geq 0.05$).

The ABTS antioxidant activity test indicated that the femur bone had the highest antioxidant activity. This activity allowed it to scavenge $ABTS^{\cdot+}$ free radicals more effectively than the breast and thigh meats. Furthermore, the FRAP assay revealed the electron-donating capacity. This study revealed that the FRAP values of breast muscle and femur bone were comparatively higher than those of thigh muscle.

Discussion

1. Proximate analysis

The chemical composition of chickens varied due to growth and fat accumulation [26]. Muscle components exhibited high protein content due to their prevalence in meat, and characteristics and respective distribution within the tissue contributed to the meat's leanness and overall quality [27].

Furthermore, the femur bone exhibits a high concentration of minerals. Calcium was a crucial constituent of bone structure. Also, iron content, which is a component within the bone [24].

Change in protein might be due to the possible degradation of myofibrils and connective tissue. This resulting in a decrease in protein content [28]. The femur bone soup contained the fattest. Comparable to chicken breast and thigh broth. Because the bone is composed of bone marrow, which includes fat and fatty acids [29]. These results are consistent with [30] and [2].

2. Physical and chemical properties

The physical properties have a significant impact on the visual quality of meat. In normal conditions, the pH of a freshly slaughtered chicken carcass typically fell within the range of 5.30-6.50 [31]. After dressing, chicken meats exhibited a normal pH range between 6.26 and 6.30. This study pH of the carcass could be related to lactic acid buildup induced by the anaerobic respiration process. The observed phenomenon may be attributed to the highest accumulation of lactic acid in the breast muscles, consequently leading to the lowest pH in the meat.

Black-boned chickens are characterized by the dark coloration of their bones and other anatomical structures. The presence of melanin pigment throughout the chicken's body is responsible for its relatively low levels of redness and yellowness in comparison to other chickens. Additionally, chicken meat is classified as white muscle, accumulating myoglobin in smaller quantities than red muscle [32]. Red muscle functions by storing and absorbing oxygen from the capillaries, delivering it to various cells that require oxygen for the oxidation process. The breast muscle exhibited brighter than the thigh muscle. A lower proportion of red and intermediate fibers in comparison to white fibers was observed in the thigh muscle. The results of this study are consistent with previous research by [33].

The color of chicken meat typically correlates with a pH range ranging from 5.70 to 6.30 [34]. Chicken meat that has not been contaminated will be vibrant yellowish-white in color and have a pH between 5.70 and 6.50. Several factors, such as pH value, influence the color of chicken meat: the lower the pH value, the paler the color of the meat.

After stewing, the change of protein occurs due to the degradation of the protein and the shortening of the amino acid chain. It might increase the peptide that has a lower molecular weight than fresh samples. Moreover, this phenomenon could be attributed to the hydrolysis of proteins, which may lead to the separation of globin from the heme group, thereby inhibiting the formation of the dark color. So, the chicken soups were lighter yellow color [35]. The results align with [36], which demonstrated that protein hydrolysis led to a lighter yellow color in chicken soup, particularly

when combined with mushroom flavoring.

Furthermore, it can be observed that after heat treatment, melanin was not extracted from the raw samples into the broths. Consequently, this phenomenon contributed to a reduction in the overall brightness of the soups when compared to the raw samples.

3. Carnosine and Anserine

Different chicken parts contained varying levels of carnosine and anserine. Anserine is mainly found in poultry meat, whereas carnosine is more common in beef and pork [37]. The anserine to carnosine ratio of poultry meat was higher than that of pork and cattle. This finding is corresponded to the research of [38].

These outcomes are consistent with the findings of [39], that reported carnosine loss in beef and turkey meat due to boiling processes. Furthermore, heat treatments, which remove proteins from untreated samples, can lead to protein denaturation through aggregation. Additionally, sterilization processes could cause the pyrolysis and decomposition of certain amino acids [40]. Under these circumstances, not only the essential amino acids but also the crucial dipeptides may be lost.

The quantities of carnosine and anserine were found to be influenced by factors such as muscle fiber type, genotype, sex, age, and mating [41, 42]. Those substances were generally found in muscles, which help regulate body pH. Therefore, play an essential role in maintaining the balance of the anaerobic glycolysis [39]. Notably, the breast muscle exhibited a higher accumulation of these dipeptides compared to the thigh muscle. Conversely, due to the relatively small amount of muscle in the femur bone, the presence of these crucial substances was the lowest.

4. Melanin

The femur bone exhibited the highest concentration of melanin due to the presence of genes that influence the production of dark pigments. Consequently, melanin from this region diffused throughout the chicken, resulting in lower melanin levels in the thigh and breast muscles.

This study's findings align with previous research on Silky Fowl (black chicken) tissues, which also reported consistent melanin content [3]. Notably, the periosteum of the femur displayed the highest melanin concentration compared to other organs. Furthermore, the melanin pigment levels in this chicken's muscle tissues exhibited statistically significant differences [7].

The stewing conditions might limit the ability of melanin extraction from the cell. A high basic solution is required for melanin to be removed from cells [43]. In this study, the sample preparation maintained a weakly acidic to weak base acidity, thus preventing further melanin extraction.

According to [44], melanin could break down because of the high temperature since 114.49 °C. This process is attributed to the complex structure of melanin, which comprises eight repeating indole-5,6-quinone units that can lose one unit at high temperatures. This reaction could form a complex web of hydrogen bonds with naturally occurring complementary peptide-type structures. Changes were made to the physiological environment. The complicated structure of melanolipoprotein had the potential to degenerate, resulting in depigmentation.

The antioxidant properties of melanin include reducing power and ferrous ion binding capacity (Fe^{2+}) [20]. Melanin was not the only factor in the system that prevents free radicals from melanin. However, it was the outcome of several mechanisms combined in chicken meat.

5. Antioxidant activities

The free radical scavenging properties using DPPH and ABTS methods. These methods are considered easy, convenient, and fast due to the relative stability of DPPH and ABTS as free radicals. It measures the ability of the test substance to scavenge free radicals based on the hydrogen atom principle.

The results obtained from the ABTS method contrasted with those obtained from the DPPH and FRAP methods when analyzing raw samples. It is noteworthy that raw femur bone may exhibit a high concentration of unsaturated fatty acids [2]. This observation suggests that the presence of unsaturated fatty acids led to the depletion of intra-bone antioxidants due to the inhibition of polyunsaturated fat oxidation. Consequently, this phenomenon may contribute to the reduced free radical scavenging capacity observed in the bone when compared to the breast and thigh muscles. These findings are consistent with prior research presented in [45].

The assessment of antioxidant activity in chicken broths from individual parts using the DPPH method indicated a proton donor ability that aligned with the results of the ABTS and FRAP methods. Particularly, the ABTS results of the soup samples exhibited higher activity than the DPPH results. This discrepancy can be attributed to the hydrophobic nature of the free radicals generated in the DPPH method, resulting in a higher specificity of radicals in the DPPH assay for proton donation reactions when compared to the ABTS assay.

When considering the antioxidant activity assessed by FRAP, it may imply that the primary constituents in chicken broth derived from breast meat had a notable electron transport potential. Prior research had indicated that the imidazole group on the histidine residue of peptides could effectively bind metal ions, inhibiting residual oxygen activity and scavenging hydroxyl free radicals [46, 47].

In addition, the DPPH and FRAP results exhibited correlations with the carnosine and anserine content in both breast and thigh soups. However, the femur bone presented an anomalous observation: it displayed a high antioxidant activity according to the FRAP assays, despite containing minimal amounts of carnosine and anserine. The femur bone might contain other proteins or peptides, such as collagen, which undergo hydrolysis, resulting in the production of peptides with significantly higher antioxidant activity than carnosine and anserine, as suggested in previous research [48].

Moreover, proteins may undergo conversion into low molecular mass peptides through stewing, accompanied by Maillard reactions. This process led to the formation of brown compounds with hydroxyl and pyrrole groups, capable of donating electrons to create stable molecules, effectively terminating the free radical chain reaction [49, 50].

Conclusion

Differences in chemical composition, bioactive components, and biological activities were observed among various parts of black-boned chicken. Breast meat exhibited a high protein content and low-fat content. The breast muscle contained a high concentration of carnosine and anserine, while the femur bone showed the highest melanin concentration. Chicken soup derived from the breast muscle exhibited the highest carnosine and anserine content among all the soups. Melanin concentrations exhibited no significant variations among soups prepared from various chicken parts. Furthermore, the antioxidant activities of both the raw samples and their soup samples were associated with carnosine and anserine, except for the femur bone.

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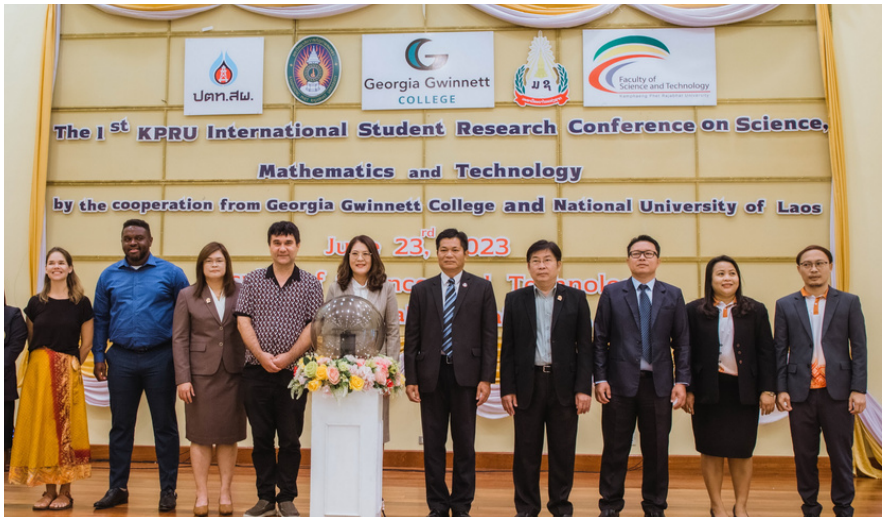
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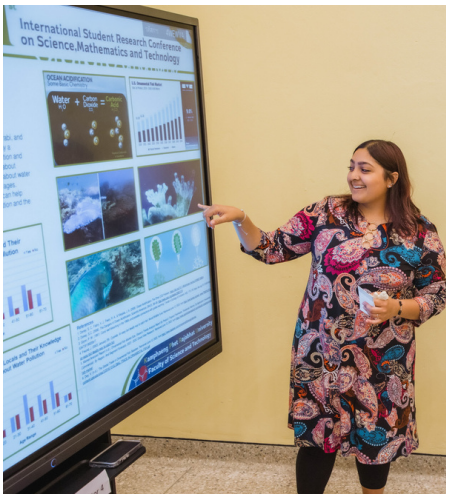
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