Research Paper
e issn 3026-5339

FAASE.

Frontier Advances in Applied Science and Engineering
Vol. 1, No. 1 (2023) pp 33-46
https://doi.org/10.59535/faase.v1i1.131

Effect of Soaking Time for Eggs with Brown Seaweed Extract (*Sargassum Crassifolium*) for Purebred Chicken Eggs

Azani Saputra [1*], Manjula T [2], Katarzyna Chojnacka [3]

[1] Program Studi Ilmu dan Teknologi Pangan, Universitas Mataram, **Indonesia**.

[2] Department of Zoology, Bharathiar University, Coimbatore, Tamil Nadu, **India**.

[3] Institute of Inorganic Technology and Mineral Fertilizers, Wroclaw University of Technology, **Poland**.

✉ azanisaputra@gmail.com

Open Access

This article contributes to:

Abstract. The purpose of this study was to determine the effect of soaking time for brown seaweed extract (*Sargassum Crassifolium*) as a natural antimicrobial on the quality of consumed chicken eggs. The method offered in this research is experimental design with one factorial Completely Randomized Design consisting of 5 levels with different proportions, namely 0 minutes, 30 minutes, 40 minutes, 50 minutes, 60 minutes, and 70 minutes of soaking time with S. crassifolium extract. with 3 repetitions. The parameters observed were microbiological quality (total microbe, APM coliform, APM *E. coli* and *Salmonella sp*.) And organoleptic quality (appearance and aroma). The observed data were analyzed for variance (Analysis of Variance) with a real level of 5% using CoStat software. If there is a significant difference, a further test of the least significant difference (LSD) is carried out for the total microbial parameters and the Honest Significant Difference (BNJ) for the organoleptic parameters. The long treatment of S. crassifolium extract for 70 minutes was able to reduce the total number of eggshell microbes by 5.5671 Log CFU / shell, coliform value 11.07 APM / shell, *E. coli* value <3.0 APM / shell, and produce free *Salmonella sp*.

Keywords: Antimicrobial, seaweed, soaking time, E. coli, *Salmonella sp*.

1. Introduction

Eggs are one of the animal protein sources that have a delicious taste, are easily digestible, and are highly nutritious. Eggs can be used as a dish, an ingredient in various foods, egg powder, medicine, and so on [1]. Fradinho et al. explains that the high consumption of eggs by the public is considered very reasonable because eggs, when compared to other livestock products such as meat and milk, are more affordable [2]. Eggs come in various types, one of which is commercial chicken eggs [3].

Commercial chicken eggs are eggs produced by commercial laying hens [3], [4]. According to data released by the Directorate General of Livestock in Becker, the production of commercial chicken eggs in Indonesia has been increasing every year in line with the needs of the population and the demand for protein. From 2015 to 2019, the production of commercial chicken eggs in Indonesia increased significantly each year in all provinces. West Nusa Tenggara is one of the provinces contributing to the production of commercial chicken eggs, with a production of 5,242 tons in 2019 [5]. This supports the availability of an economical source of animal protein for the public.

Eggs are one of the food sources that contain essential nutrients required by humans, such as protein, calcium, phosphorus, retinol, α-tocopherol, folate, and vitamin B (Afiyah, 2017). However, eggs also have some weaknesses. One common weakness of eggs is their susceptibility to damage, both natural, chemical, and damage caused by microorganism penetration through the eggshell's pores [6]. Microbiological contamination of eggs by pathogenic microbes is a serious health concern worldwide [7]. Microorganisms that can contaminate eggs include *Salmonella sp*, *Staphylococcus aureus*, and *Escherichia coli*. *Salmonella sp* can contaminate eggs while they are still inside

Article info
Submitted: 2023-08-21
Revised: 2023-10-31
Accepted: 2023-10-31

the hen's oviduct, but more commonly, it occurs after the eggs are laid, especially if the cleanliness of the coop and environment is not adequately maintained [8].

Bojorges et al., microbial contamination of eggs can be reduced by cleaning and packaging the eggs before they are marketed [9]. Washing eggs not only removes dirt but also eliminates harmful bacteria present on the eggshell [10]. One of the disadvantages of chemical disinfectants is their slow activity against some microorganisms, relatively high cost, and the formation of a thin residue (film). Therefore, it is necessary to explore alternative methods that work similarly, one of which is the use of natural antimicrobials. The main advantages of applying natural disinfectants and antimicrobial compounds are their biodegradability, high biological safety, broad spectrum, and non-accumulative nature [11].

Natural antimicrobial compounds can be derived from both animal and plant sources and are generally non-hazardous. Natural plant-based antimicrobial compounds that can be applied include betel leaves, cashew leaves, moringa leaves, and others in food products. According to Wulandari (2013), the use of a 60% concentration extract of betel leaves for 40 minutes results in prolonged egg shelf life and good quality, as seen in the vivid color of the eggshells. Another substance that can be used to inhibit microbial growth on eggs is seaweed or *Sargassum Crassifolium* [12]. *Sargassum seaweed* is a type of seaweed commonly found in the waters of West Nusa Tenggara (NTB) and is often used as the base for local snacks in the Lombok region, such as seaweed dodol and candied seaweed, among others. Mssilou et al. states that *Sargassum sp.* contains tannins, iodine, and phenols that have the potential to act as antimicrobial agents against certain types of pathogenic bacteria [13].

Ethanol-extracted *Sargassum Crassifolium* contains flavonoids, tannins, phenolics, and terpenoids [14]. Azizah et al. reported that *Sargassum sp.* from Jepara waters can inhibit the growth of bacteria such as *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella sp* [15]. According to Abka Khajouei et al. the effect of brown algae extract (*Sargassum sp.*) on the growth of *Escherichia coli* at concentrations above 20% can inhibit *E. coli* activity [16]. Brown algae extract (*Sargassum sp.*) can inhibit *E. coli* at 107 cells/ml, and the concentrations of *Sargassum sp.* extract that can inhibit *E. coli* at 107 cells/ml in accordance with antibiotic standards are 80%, 90%, and 100%, with inhibition diameters of 13 mm (fairly sensitive), 15.7 mm, and 18.6 mm (highly sensitive) [17].

Bacterial cell growth can be disrupted by phenolic components in *Sargassum sp.* extracts. Phenols have the ability to denature proteins and damage cell membranes (Rahayu, 2000). Concludes that ethanol-extracted *Sargassum sp.* has bacteriostatic activity and can inhibit *E. coli* by 7 mm [18]. *Sargassum Crassifolium* is another substance that can be used to inhibit microbial growth on eggs. Based on the above description, it is necessary to conduct research to determine the effect of the duration of egg immersion in brown seaweed extract (Sargassum cassifolium) as a natural antimicrobial on the quality of commercial chicken eggs.

2. Method

The experimental design used in this study is a Completely Randomized Design (CRD) with a single-factor experiment, which is the duration of egg immersion in S. Crassifolium extract, with 5 treatments as follows:

1 Lp0: No immersion (0 minutes)

2 Lp1: 30 minutes

3 Lp2: 40 minutes

4 Lp3: 50 minutes

5 Lp4: 60 minutes

6 Lp5: 70 minutes

Each treatment is repeated 3 times, resulting in a total of 18 experimental units. The data from the observations are analyzed for variance (Analysis of Variance) with a significance level of 5% using Costat software. If there is a significant difference, further tests like Polynomial and Least Significant Difference (LSD) are conducted for the Total Microbial and Organoleptic parameters. Research Tools and Materials: Tools used in this study include measuring cups, laminar air flow, oven, scissors, plastic buckets, stirrer machine, Whatman's filter paper, rotary evaporator, analytical balance, plastic bags, thin cloth, incubator, petri dishes, 1000µL micro pipette with tips, 100µL micro pipette with tips, Bunsen burner, test tubes, test tube racks, Durham tubes. Materials used in this study include S. Crassifolium extract, chicken eggs for consumption, chicken feces, water, buffered peptone water (BPW), 96% ethanol, 1% sodium hypochlorite, sterile distilled water, Plate Count Agar (PCA) medium, Laurryl sulfate tryptose broth (LSTB) medium, BGLB medium, ECB medium, and reconstituted non-fat dry milk sterilized.

2.1 The Preparation of Crude *Sargassum* Extract

Extraction of S. Crassifolium extract is carried out using the maceration method with 96% ethanol. It begins with the collection and drying of seaweed samples. The samples are then crushed and sieved to obtain powdered raw material that passes through a 60-mesh sieve. Extraction is done by mixing S. Crassifolium powder with 96% ethanol. The mixture is then filtered and concentrated to obtain the extract. For experimental egg contamination, chicken feces are collected, homogenized, and tested for the presence of Salmonella typhimurium. Following the test, mixing, filtering, and filtration are performed to prepare the contaminant suspension. The application of S. Crassifolium extract is carried out by immersing eggs in the contaminant suspension for 60 minutes, followed by drying. Each contaminated egg is then dipped into each 60% S. Crassifolium extract solution with different immersion times: 30 minutes, 40 minutes, 50 minutes, 60 minutes, and 70 minutes. Each immersion time is repeated 3 times for observation of the measured parameters.

2.2 Parameters and Observation Procedure:

The parameters observed in this study include the quality of chicken eggs for consumption, which involves microbiological analysis (Total Plate Count, Coliform, E. coli, and *Salmonella sp*), sensory evaluation (aroma and color) on day 0 after immersion in S. Crassifolium extract. This sensory evaluation is conducted with a panel of 20 assessors using scoring tests and chemical analysis (egg pH) afterward. In the microbiological analysis, test procedures include total plate count and coliform tests, as well as *E. coli* testing in accordance with the SNI 2897:2008 standard. For the total plate count, egg samples are immersed in sterile BPW with successive dilutions, followed by incubation in PCA media, and colony counting. Coliform testing includes presumptive and confirmatory tests for coliform bacteria, with a positive result if gas is formed. *E. coli* testing also involves presumptive and confirmatory tests, followed by MPN calculation.

Additionally, sensory analysis (hedonic and scoring) is performed to evaluate changes in the physical and sensory properties of chicken eggs after the washing/disinfection process. Hedonic testing aims to assess the panelists' level of liking for the product, while scoring tests are used to evaluate the sensory quality of the aroma

and appearance of the eggs after the use of S. Crassifolium extract. The scoring for aroma and appearance ranges from 1 to 5 and includes criteria such as a seaweed aroma, dark brown color, or typical egg characteristics. This sensory evaluation is conducted by a panel of 20 assessors after completing the microbiological testing.

3. Result

3.1 The Microbiological Quality

The average of the observation and analysis of the influence of the duration of immersion of brown seaweed (*Sargassum Crassifolium*) on the microbiological quality (Total Microbes, AMP Coliform, AMP *Escherichia coli*, and *Salmonella sp*.) can be seen in Table 1.

Tabel 1 The Average Results of The Observations Duration of Immersion

Duration of Immersion (Minutes)	Average			
	Total Mikroba (Log CFU/Cangkang)	APM *Coliform* (CFU/Cangkang)	APM *E. coli* (CFU/Cangkang)	*Salmonella sp.*
0	8,0024	>1100	11,13	Positive
30	7,7484	>1100	9,93	Positive
40	7,3159	>1100	6,53	Positive
50	6,6944	>1100	3,60	Positive
60	6,0867	>1100	<3,0	Negative
70	5,5671	11,07	<3,0	Negative

Table 2 Significance of the Effect of the Duration of Egg Immersion in Brown Seaweed (*Sargassum Crassifolium*)

Parameter	Significance
Total Microbes	S
APM *Coliform*	S
APM *E. coli*	S

Note: S = Significant (Statistically Different), NS = Non-Significant (Not Statistically Different)

Based on Table 2, it is evident that the duration of egg immersion in brown seaweed (*Sargassum Crassifolium*) extract treatment significantly affects the Total Microbe Test of commercial chicken eggs. Data from the observations and further testing showing significant differences can be subjected to orthogonal polynomial testing (MOP) at a 5% significance level. The results of the orthogonal polynomial method for the effect of the duration of egg immersion in brown seaweed (*Sargassum Crassifolium*) extract on the quality of commercial chicken eggs in microbiological parameters can be found in Table 3.

Table 3 Results of the Orthogonal Polynomial Method (MOP)

Respons	Total Microbes	APM *Coliform*	APM *E.coli*
Linear	S	S	S
Quadratic	NS	S	NS

Based on Table 3, further testing using the orthogonal polynomial method indicates that the duration of egg immersion in *Sargassum Crassifolium* extract significantly affects the total microbe count of chicken eggs and APM *E. coli* in a linear pattern, while in a quadratic pattern, it does not have a significant impact. In terms of APM Coliform, there is a significant effect in both the linear and quadratic patterns.

3.2 Organoleptic Properties

The analysis of the influence of the duration of egg immersion in brown seaweed (*Sargassum Crassifolium*) extract on its organoleptic quality can be found in the appendix. The significance of the effect of the duration of egg immersion in brown

seaweed (*Sargassum Crassifolium*) extract as a natural antimicrobial on the organoleptic quality, appearance, and aroma of commercial chicken eggs can be seen in Table 4.

Table 4 Significance of the Effect of the Duration of Egg Immersion in Brown Seaweed (*Sargassum Crassifolium*)

Parameter	Significance	
	Skoring	Hedonik
Appearance	S	S
Aroma	S	S

Note: S = Significant (Statistically Different), NS = Non-Significant (Not Statistically Different)

Table 4 indicates that the duration of egg immersion in brown seaweed (*Sargassum Crassifolium*) extract significantly influences the scoring and hedonic ratings of the appearance and aroma of commercial chicken eggs. The results that show statistical significance (significantly different) are further tested using the Least Significant Difference (BNJ) test at a 5% significance level. The results of the BNJ test can be seen in Table 5.

Table 5: Average Observation Results and BNJ 5% Test Results for the Effect of the Duration of Egg Immersion in Brown Seaweed

Duration of Immersion (Minutes)	Average			
	Appearance		Aroma	
	Skoring	Hedonik	Skoring	Hedonik
0	$4,8^{a}$	$4,6^{a}$	$4,2^{a}$	$4,4^{a}$
30	$3,9^{b}$	$3,5^{b}$	$3,6^{ab}$	$3,5^{b}$
40	$3,4^{b}$	3^{bc}	$3,4^{b}$	$2,95^{b}$
50	$2,4^{c}$	$2,45^{cd}$	$2,95^{b}$	$2,2^{c}$
60	$2,3^{c}$	$2,05^{d}$	$2,05^{c}$	2^{c}
70	$1,65^{d}$	$1,95^{d}$	$1,85^{c}$	$1,85^{c}$
BNJ (5%)	0,4365	0,4643	0,4591	0,4638

Note: Numbers followed by the same superscript letter in the same column indicate no statistically significant difference at a 5% significance level.

Table 5 shows that the duration of immersion in brown seaweed extract (*Sargassum Crassifolium*) significantly affects the ratings of appearance and aroma of commercial chicken eggs, both in scoring and hedonically. For the appearance in scoring, the 0-minute treatment is significantly different from all other treatments. The 30-minute treatment is not significantly different from the 40-minute treatment. However, the 30-minute treatment is significantly different from the 0-minute, 50-minute, 60-minute, and 70-minute treatments. The 40-minute treatment is significantly different from the 0-minute, 50-minute, 60-minute, and 70-minute treatments. The 50-minute treatment is not significantly different from the 60-minute treatment. However, it is significantly different from the 0-minute, 30-minute, 40-minute, and 70-minute treatments. The 60-minute treatment significantly differs from the 0-minute, 30-minute, 40-minute, and 70-minute treatments.

The 70-minute treatment is significantly different from all other treatments. for the hedonic appearance, the 0-minute treatment significantly differs from all other treatments, as does the 30-minute, 40-minute, and 50-minute treatments. However, the 60-minute and 70-minute treatments are not significantly different. But the 60-minute and 70-minute treatments are significantly different from the 0-minute, 30-minute, 40-minute, and 50-minute treatments.

Regarding the aroma scores, the 0-minute treatment significantly differs from all other treatments. The 30-minute and 40-minute treatments are not significantly different, but they significantly differ from the 0-minute, 50-minute, 60-minute, and 70-minute treatments. Similarly, the 60-minute, 50-minute, and 70-minute treatments are not significantly different from each other but significantly differ from the 0-minute, 30-minute, and 40-minute treatments.

4. Discussion

Based on the observations and analysis of the results, and in relation to relevant theory, the discussion can be as follows :

4.1 Microbiological Properties

The treatment involving the duration of egg immersion in brown seaweed (*Sargassum Crassifolium*) extract as a natural antimicrobial significantly affects the total microbe count in commercial chicken eggs. This influence is depicted graphically in Figure 1.

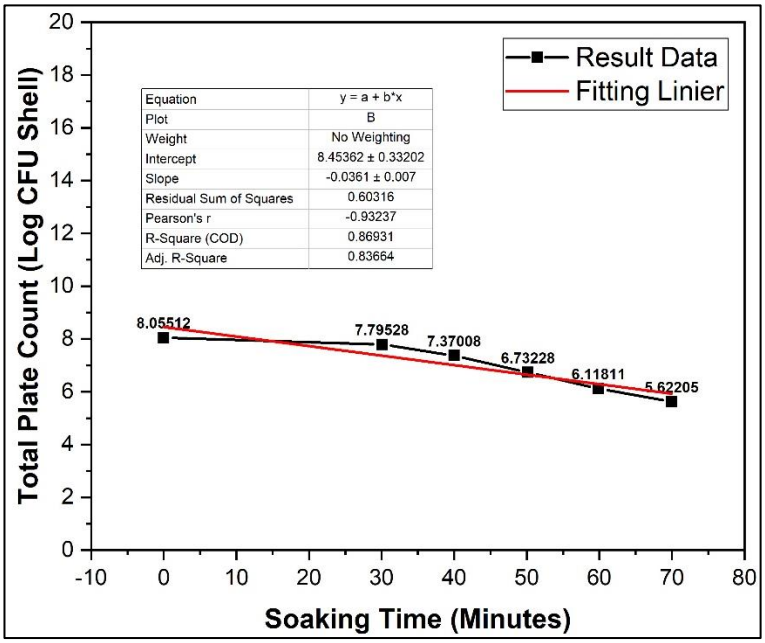

Figure 1 Graph Showing the Influence of Soaking Duration with *Sargassum Crassifolium* Extract on Total Microbes

Based on Figure 1, it can be observed that there is a linear relationship between the duration of egg immersion in brown seaweed extract (*Sargassum Crass-ifolium*) and the total microbe count in commercial chicken eggs. The relationship can be expressed by the equation $y = 0{,}0362x^2 - 0{,}3269x + 8{,}0519$ with a coefficient of determination (R^2) = 0,7772. The value 0.3269 determines the direction of quadratic regression, and the negative value suggests a negative relationship between the duration of egg immersion in *Sargassum Crassifolium* extract and the total microbe count. Meanwhile, the value 0.0362 indicates the direction of linear regression, which is positive. A positive value implies that there is a reduction in total microbes up to a certain duration of immersion in *Sargassum Crassifolium* extract.

The coefficient of determination (R^2) is 0.7772, indicating that 77.72% of the variation in total microbes can be attributed to the duration of immersion in *Sargassum Crassifolium* extract, while 22.28% is influenced by other factors. Additionally, the coefficient of the duration of immersion in *Sargassum Crassifolium* extract and the total microbe count is 0.8815 (as a result of squaring the coefficient of determination, 0.7772), suggesting a strong relationship between the two variables. The average total microbe graph in Figure 1 shows that the total microbe count decreases as the duration of immersion of commercial chicken eggs in *Sargassum Crassifolium* extract increases. This reduction can be attributed to the presence of antibacterial compounds in *Sargassum Crassifolium* that inhibit bacterial growth. Bioactive compounds present in the extract affect the growth inhibition of microbes. A longer immersion duration allows bioactive compounds more time to act on the microbes, leading to their inhibition [18].

Brown seaweed, *Sargassum Crassifolium*, also contains other bioactive compounds, including flavonoids, tannins, and phenols, each with a different mechanism of action. These bioactive compounds are generally bactericidal, meaning they can damage bacterial defense mechanisms, cell membranes, and protein synthesis, ultimately leading to bacterial death. Prolonged immersion allows the bioactive compounds in *Sargassum Crassifolium* more time to suppress the growth of microbes in the eggs [19]. The mechanism of action of antimicrobials includes inhibiting cell wall biosynthesis, increasing cell membrane permeability, and disrupting protein synthesis in bacterial cells, thus inhibiting growth or causing bacterial cell death. Antimicrobials that affect cell wall formation or cell membrane permeability typically work as bactericidal agents, while those that affect protein synthesis function as bacteriostatic agents. This aligns with the research conducted by Taramukai [20], which found that *Sargassum Crassifolium* extract added to bread was able to reduce the total microbe count by 2 log cycles.

4.2 Escherichia coli

The observations and analysis of the effect of egg immersion duration in brown seaweed extract (*Sargassum Crassifolium*) as a natural antimicrobial on the *E. coli* count in commercial chicken eggs can be seen in Table 1. Based on Table 1, the lowest *E. coli* count was found after 60 and 70 minutes of immersion, with values <3.0 CFU/Shell. With increasing immersion duration (30 minutes, 40 minutes, 50 minutes, and 60 minutes), the *E. coli* count decreased progressively. This suggests that the longer the immersion of commercial chicken eggs in brown seaweed extract, the lower the *E. coli* count on the eggshells. This reduction is attributed to the presence of phenols and tannins in *Sargassum Crassifolium*, which exhibit antimicrobial effects, inhibiting the growth of *E. coli.* Longer immersion allows bioactive compounds more time to make contact with the eggs and kill the microbes.

The use of *Sargassum Crassifolium* as an antibacterial agent can create an inhibition zone against *E. coli.* This is because *E. coli* is one of the Gram-negative bacteria. Gram-negative bacteria have a thin peptidoglycan layer in their cell membrane, which has polar properties and is easily penetrated by the polar bioactive compounds found in *Sargassum sp.* [21]. Furthermore, bioactive compounds in *Sargassum sp.* include flavonoids, saponins, tannins, and phenols. Among these compounds, saponin is the dominant one. Saponins are glycosides with sapogenin as their aglycone. According to Pangestuti et al. (2017), the mechanism of saponin action is to disrupt cell permeability, causing intracellular compounds like cytoplasm to leak out, ultimately leading to cell death. Nuria et al. (2009) supported this by explaining that the mechanism of saponin as an antibacterial is to reduce surface tension, leading to increased cell permeability or leakage, causing intracellular compounds to exit, hindering microbial metabolism. Based on the mechanism of saponin, longer immersion provides more time for saponin to reduce surface tension, leading to cell leakage, thus reducing *E. coli* count.

4.3 Coliform

Based on the observations and analysis of the effect of egg immersion duration in brown seaweed extract (*Sargassum Crassifolium*) as a natural antimicrobial on the Coliform count in commercial chicken eggs (Table 1), the lowest Coliform count was observed after 70 minutes of immersion, at 11.07 APM/Shell. This indicates that the longer the immersion of commercial chicken eggs in *Sargassum Crassifolium* extract, the lower the Coliform count. This reduction can be attributed to the presence of phenolic

compounds in *Sargassum Crassifolium*. According to He et al., the growth of bacterial cells can be disrupted by phenolic components in *Sargassum sp* [22].

The mechanism of action of phenolic compounds includes coagulating proteins and lysing bacterial cell membranes. Membrane lysis leads to cell leakage, causing essential intracellular metabolites to exit the cell, disrupting the cell's metabolic processes, denaturing proteins, nucleic acids, and inhibiting nucleic acid and protein synthesis [23]. The decrease in the average Coliform count is a result of the prolonged immersion in *Sargassum Crassifolium* extract. Longer immersion allows the bioactive compounds present in *Sargassum Crassifolium* more time to make contact with the eggs, thus interfering with the growth of Coliform cells [24].

4.4 Salmonella sp.

The observations and analysis of the effect of egg immersion duration in brown seaweed extract (*Sargassum Crassifolium*) as a natural antimicrobial on the *Salmonella sp*. count in commercial chicken eggs can be seen in Table 6.

Table 6 Observations, Enrichment Data for *Salmonella sp*. And Isolation in Commercial Chicken Eggshells

Duration of Immersion (Minutes)	LB	RV	TTB	XLDA	HEA	BSA
0	+	+	+	+	+	-
30	+	+	+	+	+	-
40	+	+	+	+	+	-
50	+	+	+	+	+	-
60	+	-	-	-	-	-
70	+	-	-	-	-	-

Note: *Lactose Broth* (LB), *Rappaport-Vassiliadis* (RV), *Tetrthionate Broth* (TTB), *Xylose Lysine Deoxycholate Agar* (XLDA), *Hektoen Enteric Agar* (HEA), *Bismuth Sulfite Agar* (BSA)

Table 7 presents the observations for the identification of *Salmonella sp*. on the chicken eggshell.

Duration of Immersion (Minutes)	TSIA				LIA				Result
	Agar Miring	Dasar Agar	H2S	Gas	Agar Miring	Dasar Agar	H2S	Gas	
0	Red	yellow	+	-	purple	purple	+	-	+
30	Red	yellow	+	-	purple	purple	+	-	+
40	Red	yellow	+	-	purple	purple	+	-	+
50	Red	yellow	-	-	purple	purple	+	-	+
60	-	-	-	-	-	-	-	-	-
70	-	-	-	-	-	-	-	-	-

Note: *Triple Sugar Iron Agar* (TSIA), *Lysine Iron Agar* (LIA)

Based on the observations in Tables 6 and 7, it can be observed that eggs subjected to 60 and 70 minutes of immersion with brown seaweed extract, S. Crassifolium, obtained negative results. This aligns with Jasumani et al. that S. Crassifolium extract can inhibit and cause the death of *Salmonella sp*. Bacteria [25]. This is because S. Crassifolium contains flavonoid compounds. According to Farobie et al., flavonoid compounds have the ability to inhibit bacterial growth through various mechanisms, including causing damage to the permeability of bacterial walls, microsomes, and lysosomes as a result of the interaction between flavonoids and bacterial DNA [26]. The 60-70 minute immersion period is suitable for eliminating bacteria using the flavonoid compounds abundant in S. Crassifolium extract because prolonged contact with the egg allows the bioactive compounds to interfere with *Salmonella sp*. cells within the egg.

Steroids are one of the compounds found in *Sargassum Crassifolium*. These compounds also have potential as antibacterial agents. Steroid or triterpenoid compounds can inhibit bacterial growth by inhibiting protein synthesis, leading to the accumulation and alteration of the bacterial cell's constituent components. The

properties of these compounds make them easily penetrate the cell walls of both Gram-positive and Gram-negative bacteria [27].

The damage to the bacterial cell wall is caused by antibacterial compounds. The breakdown of the bacterial membrane starts with the H+ ions from phenol compounds and their derivatives, which attack the polar groups (phosphate groups). As a result, phospholipid molecules break down into glycerol, carboxylic acid, and phosphoric acid. This prevents phospholipids from maintaining the shape of the cell membrane, leading to membrane leakage, bacterial inhibition, and even cell death [28].

RV medium, the results indicate that samples subjected to 60 and 70 minutes of immersion did not exhibit a change in color and remained clear. This suggests that this medium yielded negative results, indicating no presence of Salmonella in these treatments. However, samples subjected to 0, 30, 40, and 50 minutes of immersion showed positive results, and the medium changed color from blue to whitish-gray, confirming the presence of growing Salmonella in these treatments. The support of soy peptone in the enrichment process acts as a source of carbon, nitrogen, and amino acids for Salmonella (Oxoid, 1995).

The subsequent selective isolation stage, Xylose Lysine Desoxycholate Agar (XLDA), Hektoen Enteric Agar (HEA), and Bismuth Sulfite Agar (BSA) media were used. On XLDA medium, almost all colonies appeared black or pink with or without a shiny spot, indicating the presence of Salmonella colonies. On HEA medium, Salmonella colonies appeared bluish-green with or without black spots (H2S). Meanwhile, on BSA medium, Salmonella colonies appeared dark or bluish-grey, sometimes metallic, with the area around the colonies turning brown and, with prolonged incubation, becoming black [29].

In this observation, positive results were obtained on the TSIA medium for samples with immersion times of 0 minutes, 30 minutes, 40 minutes, and 50 minutes. This was due to the formation of acid, which was indicated by the yellow color of the butt of the agar. Alkaline formation also occurred, indicated by the red color on the slant of the TSIA agar, and the presence of black color, indicating the production of H_2S, which suggests the presence of *Salmonella sp*. According to Chandrasekaran et al., this is because the sodium thiosulfate content in the agar is reduced by H_2S, which then reacts with iron salt to produce black color [30]. However, for immersion times of 60 minutes and 70 minutes, no gas was formed, and there was no color change in the agar, indicating a negative result or the absence of *Salmonella sp*.

On the LIA medium, immersion times of 0, 30, 40, and 50 minutes gave positive results, as evidenced by the presence of *Salmonella sp*. This was indicated by the appearance of black color on the agar due to the formation of H2S. Additionally, alkaline formation was observed, indicated by the purple color on the agar's butt. However, for immersion times of 60 minutes and 70 minutes, no gas was formed, and there was no color change in the agar, indicating a negative result or the absence of *Salmonella sp*.

4.5 Organoleptic Properties

The treatment of egg immersion with brown seaweed extract (*Sargassum Crassifolium*) as a natural antimicrobial had a significant effect on the appearance of chicken eggs, both in terms of hedonic and scoring evaluations. The relationship between the effect of egg immersion duration with brown seaweed extract (*Sargassum Crassifolium*) on the appearance of chicken eggs, as assessed through scoring and hedonic methods, can be seen in Figure 2.

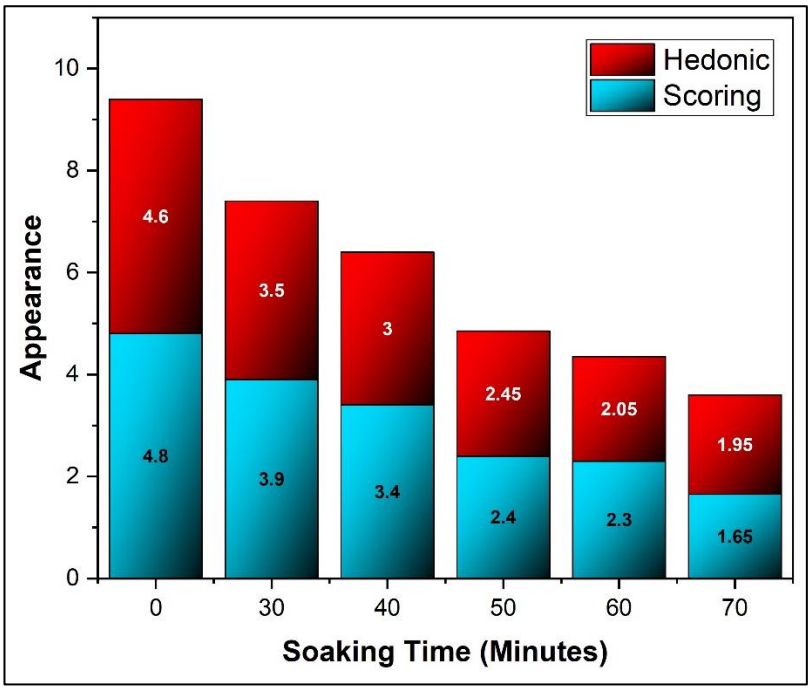

Figure 2 illustrates the impact of immersion duration with *Sargassum Crassifolium* extract on the appearance of commercial broiler eggs using scoring and hedonic methods

Based on Figure 2, the influence of the immersion duration of *Sargassum Crassifolium* extract on the appearance of commercial broiler eggs has a statistically significant effect on both the hedonic and scoring evaluations of the eggs' appearance. According to the hedonic evaluation, the appearance of the eggs from various immersion durations differs significantly, with values ranging from 1.95 to 4.8 (dislike to very much like). The lowest score was obtained with the 70-minute treatment, which scored 1.95 (dislike). This is attributed to the presence of black spots caused by the S. crassifolium extract immersion.

The formation of S. crassifolium extract spots is due to the residue of the extract solution that still adheres to the eggshell. Meanwhile, based on the scoring evaluation, the appearance of broiler eggs varies significantly across different immersion durations, with scores ranging from 1.65 to 4.8 (black to typical brown egg color or no black spots). The lowest score was observed with the 70-minute treatment, scoring 1.95 (somewhat black). Immersing the eggs in *Sargassum Crassifolium* extract results in the presence of black spots on the eggshells.

This is because the longer the immersion duration, the more the compounds from *Sargassum Crassifolium* extract enter through the pores of the eggshell, leading to the formation of black spots on the shell. This is related to Trihadi's research (2016), where tannin compounds, as tanning agents, react with eggshell proteins similar to animal collagen, resulting in a tanning process that forms brown deposits capable of closing the eggshell pores, making it impermeable to gases and air. These black spots on the shell are what many panelists find unappealing

Moving on to aroma, the immersion duration of eggs with *Sargassum Crassifolium* extract, as a natural antimicrobial agent, significantly influences both the hedonic and scoring evaluations of the aroma of broiler eggs. The relationship between the immersion duration of broiler eggs with *Sargassum Crassifolium* extract and the aroma of the eggs, as evaluated through scoring and hedonic methods, can be observed in Figure 3.

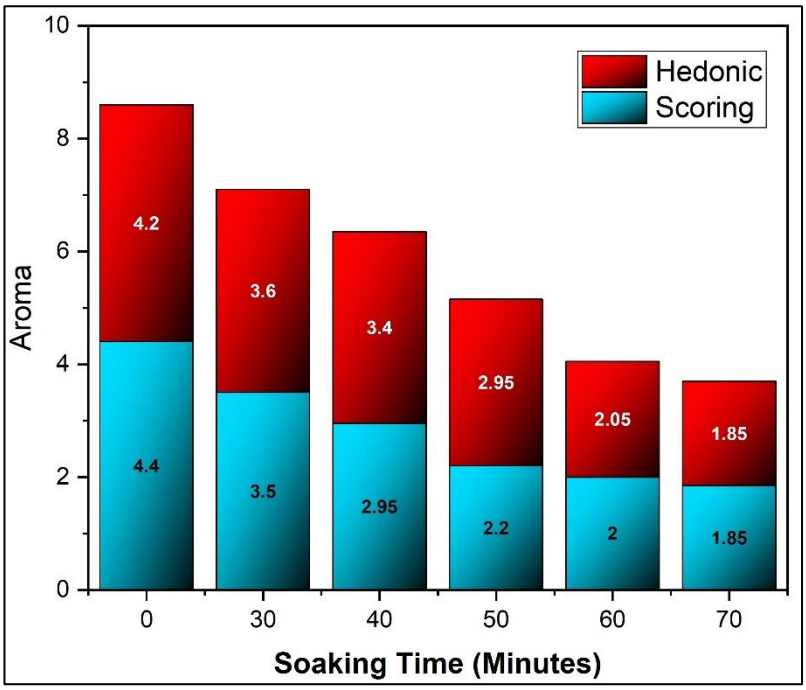

Figure 3: The Influence of Soaking Duration with S. crassifolium Extract on Chicken Egg Aroma as Scored and Hedonically

Based on Figure 3, it is evident that the influence of the duration of soaking with S. crassifolium extract on the aroma parameters of commercial chicken eggs has a significantly different effect on both the scoring and hedonic evaluation of the egg aroma. According to the hedonic test, the aroma obtained from various soaking durations showed a significant difference, with scores ranging from 1.85 to 4.2 (dislike to very much like). The highest score was recorded for the treatment with 0 minutes of soaking, scoring 4.2 (very much like). This is because in this treatment, the aroma of *Sargassum sp.* was not detectable, resulting in a normal egg aroma. The lowest score was observed for the treatment with 70 minutes of soaking, with a score of 1.85 (dislike). This is because the seaweed aroma becomes stronger with longer soaking.

In the scoring of the aroma of the commercial chicken eggs, different soaking durations also yielded significantly different scores, ranging from 1.85 to 4.4 (strong seaweed aroma to distinct egg aroma). The highest score was obtained for the treatment with 0 minutes of soaking, scoring 4.4 (distinct egg aroma), while the lowest score was recorded for the treatment with 70 minutes of soaking, scoring 1.85 (strong seaweed aroma).

The prolonged soaking time of the eggs with *Sargassum Crassifolium* extract causes the aromatic bioactive components to adhere more to the eggshell, resulting in the distinctive egg aroma fading due to the extended exposure. This is attributed to the residue of *Sargassum sp.* extract after soaking, which contains aromatic bioactive components that are the source of the distinctive seaweed aroma in the eggs. The unpleasant aroma of *Sargassum Crassifolium* negatively affects the egg aroma, which should typically have a characteristic egg scent. This phenomenon aligns with the findings of Hugh [31].

5. Conclusion

Based on the analysis and discussion, the following conclusions can be drawn:

a. The duration of soaking in S. crassifolium extract significantly influences both the total microbial count and the organoleptic characteristics, as evaluated through scoring and hedonic assessment, in commercial chicken eggs.

b. The longer soaking duration of commercial chicken eggs with S. Crassifolium extract results in an extended contact time between the extract's antimicrobial properties and the microbes. This extended contact allows the antimicrobial properties to work more effectively
c. Soaking durations of 30 to 70 minutes are effective in significantly reducing the total microbial count on the shells of commercial chicken eggs compared to the treatment with no soaking (0 minutes) in S. crassifolium extract.
d. A soaking duration of 70 minutes with S. crassifolium extract can be recommended as the best treatment, as it achieves a total microbial count of 5.5671 Log CFU/Shell, APM Coliform of 11.07, APM *Escherichia coli* of <3.0, and yields a negative result for *Salmonella sp*. It also results in a slightly darker color and a very distinctive seaweed aroma.

6. Author's Declaration

Author contributions and responsibilities - The authors made major contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation and discussion of results. The authors read and approved the final manuscript.

Funding - This research did not receive external funding.
Availability of data and materials - All data is available from the author.
Competing interests - The authors declare no competing interests.
Did you use generative AI to write this manuscript? - I do not use AI assistance in my manuscript.
Declaration of generative AI and AI-assisted technologies in the writing process - During the preparation of this work the author did not use AI to write, edit, or other things related to the manuscript.

7. References

[1] C. Jiang *et al.*, 'Effects of Breeds on the Content of Functional Nutrition in Eggs', *Animals*, vol. 13, no. 19, Art. no. 19, Jan. 2023, doi: 10.3390/ani13193066.
[2] P. Fradinho, A. Raymundo, I. Sousa, H. Domínguez, and M. D. Torres, 'Edible Brown Seaweed in Gluten-Free Pasta: Technological and Nutritional Evaluation', *Foods*, vol. 8, no. 12, Art. no. 12, Dec. 2019, doi: 10.3390/foods8120622.
[3] J. Fleurence, 'Biostimulant Potential of Seaweed Extracts Derived from Laminaria and Ascophyllum nodosum', in *Biostimulants: Exploring Sources and Applications*, N. Ramawat and V. Bhardwaj, Eds., in Plant Life and Environment Dynamics. , Singapore: Springer Nature, 2022, pp. 31–49. doi: 10.1007/978-981-16-7080-0_2.
[4] F. A. Marufi and M. Saifuddin, 'The Effect of Service and Product Quality on The Purchase Decision of Purebred Chicken Eggs with The Level of Education as a Moderation Variable', *Dinamis : Journal of Islamic Management and Bussiness*, vol. 5, no. 2, Art. no. 2, 2022, doi: 10.24256/dinamis.v5i2.3344.
[5] S. Becker, W. Büscher, and I. Tiemann, 'The British Ixworth: Individual growth and egg production of a purebred dual-purpose chicken', *British Poultry Science*, vol. 0, no. ja, 2023, doi: 10.1080/00071668.2023.2246142.
[6] A. Tanjungsari, S. Andaruisworo, and E. Yuniati, 'The Breeding of Free-range Chickens and Purebred Chickens with Artificial Insemination Technology on Fertility and Hatchability Using Manual Incubator', *IOP Conf. Ser.: Earth Environ. Sci.*, vol. 1168, no. 1, p. 012027, Apr. 2023, doi: 10.1088/1755-1315/1168/1/012027.

[7] A. M. Isa *et al.*, 'Hybrids generated by crossing elite laying chickens exhibited heterosis for clutch and egg quality traits', *Poultry Science*, vol. 99, no. 12, pp. 6332–6340, Dec. 2020, doi: 10.1016/j.psj.2020.08.056.
[8] C. Rizzi, 'Yield Performance, Laying Behaviour Traits and Egg Quality of Purebred and Hybrid Hens Reared under Outdoor Conditions', *Animals*, vol. 10, no. 4, Art. no. 4, Apr. 2020, doi: 10.3390/ani10040584.
[9] H. Bojorges, A. López-Rubio, A. Martínez-Abad, and M. J. Fabra, 'Overview of alginate extraction processes: Impact on alginate molecular structure and techno-functional properties', *Trends in Food Science & Technology*, vol. 140, p. 104142, Oct. 2023, doi: 10.1016/j.tifs.2023.104142.
[10] N. C. Afonso, M. D. Catarino, A. M. S. Silva, and S. M. Cardoso, 'Brown Macroalgae as Valuable Food Ingredients', *Antioxidants*, vol. 8, no. 9, Art. no. 9, Sep. 2019, doi: 10.3390/antiox8090365.
[11] T. Nolte *et al.*, 'Egg Production and Bone Stability of Local Chicken Breeds and Their Crosses Fed with Faba Beans', *Animals*, vol. 10, no. 9, Art. no. 9, Sep. 2020, doi: 10.3390/ani10091480.
[12] J. P. Trigo *et al.*, 'Mild blanching prior to pH-shift processing of Saccharina latissima retains protein extraction yields and amino acid levels of extracts while minimizing iodine content', *Food Chemistry*, vol. 404, p. 134576, Mar. 2023, doi: 10.1016/j.foodchem.2022.134576.
[13] I. Mssillou *et al.*, 'Phytochemical characterization, antioxidant activity, and in vitro investigation of antimicrobial potential of Dittrichia viscosa L. leaf extracts against nosocomial infections', *Acta Ecologica Sinica*, vol. 42, no. 6, pp. 661–669, Dec. 2022, doi: 10.1016/j.chnaes.2021.09.021.
[14] P. S. Birgersson *et al.*, 'Sequential extraction and fractionation of four polysaccharides from cultivated brown algae Saccharina latissima and Alaria esculenta', *Algal Research*, vol. 69, p. 102928, Jan. 2023, doi: 10.1016/j.algal.2022.102928.
[15] R. Azizah *et al.*, 'Kandungan Timbal Pada Air, Sedimen, Dan Rumput Laut Sargassum sp. Di Perairan Jepara, Indonesia', *Jurnal kelautan tropis*, vol. 21, no. 2, pp. 155–156, 2018.
[16] R. Abka Khajouei *et al.*, 'Effect of high voltage electrode discharge on the physicochemical characteristics of alginate extracted from an Iranian brown seaweed (Nizimuddinia zanardini)', *Algal Research*, vol. 56, p. 102326, Jun. 2021, doi: 10.1016/j.algal.2021.102326.
[17] C. Lourenço-Lopes *et al.*, 'Scientific Approaches on Extraction, Purification and Stability for the Commercialization of Fucoxanthin Recovered from Brown Algae', *Foods*, vol. 9, no. 8, Art. no. 8, Aug. 2020, doi: 10.3390/foods9081113.
[18] N. Jerez-Timaure, M. Sánchez-Hidalgo, R. Pulido, and J. Mendoza, 'Effect of Dietary Brown Seaweed (Macrocystis pyrifera) Additive on Meat Quality and Nutrient Composition of Fattening Pigs', *Foods*, vol. 10, no. 8, Art. no. 8, Aug. 2021, doi: 10.3390/foods10081720.
[19] F. E.-Z. A. A. El-Aziz, A. F. Hifney, M. Mohany, S. S. Al-Rejaie, A. Banach, and A. M. Sayed, 'Insecticidal activity of brown seaweed (Sargassum latifolium) extract as potential chitin synthase inhibitors: Toxicokinetic and molecular docking approaches', *South African Journal of Botany*, vol. 160, pp. 645–656, Sep. 2023, doi: 10.1016/j.sajb.2023.07.058.
[20] K. Teramukai, S. Kakui, F. Beppu, M. Hosokawa, and K. Miyashita, 'Effective extraction of carotenoids from brown seaweeds and vegetable leaves with edible oils',

Innovative Food Science & Emerging Technologies, vol. 60, p. 102302, Mar. 2020, doi: 10.1016/j.ifset.2020.102302.

[21] G. Kannan, T. H. Terrill, B. Kouakou, and J. H. Lee, 'Dietary Brown Seaweed Extract Supplementation in Small Ruminants', in *Medicinal Plants: From Farm to Pharmacy*, N. Joshee, S. A. Dhekney, and P. Parajuli, Eds., Cham: Springer International Publishing, 2019, pp. 291–312. doi: 10.1007/978-3-030-31269-5_13.

[22] S. He, M. Zhao, J. Wang, Z. Cheng, B. Yan, and G. Chen, 'Hydrothermal liquefaction of low-lipid algae Nannochloropsis sp. and Sargassum sp.: Effect of feedstock composition and temperature', *Science of The Total Environment*, vol. 712, p. 135677, Apr. 2020, doi: 10.1016/j.scitotenv.2019.135677.

[23] F. Soleymani, M. H. Khani, H. Pahlevanzadeh, and Y. Amini, 'Intensification of strontium (II) ion biosorption on Sargassum sp via response surface methodology', *Sci Rep*, vol. 13, no. 1, Art. no. 1, Apr. 2023, doi: 10.1038/s41598-023-32532-5.

[24] H. Al-Khalaifah and S. Uddin, 'Assessment of Sargassum sp., Spirulina sp., and Gracilaria sp. as Poultry Feed Supplements: Feasibility and Environmental Implications', *Sustainability*, vol. 14, no. 14, Art. no. 14, Jan. 2022, doi: 10.3390/su14148968.

[25] V. Jesumani, H. Du, P. Pei, C. Zheng, K.-L. Cheong, and N. Huang, 'Unravelling property of polysaccharides from Sargassum sp. as an anti-wrinkle and skin whitening property', *International Journal of Biological Macromolecules*, vol. 140, pp. 216–224, Nov. 2019, doi: 10.1016/j.ijbiomac.2019.08.027.

[26] O. Farobie, A. Amrullah, A. Bayu, N. Syaftika, L. Aisya Anis, and E. Hartulistiyoso, 'In-depth study of bio-oil and biochar production from macroalgae Sargassum sp. via slow pyrolysis', *RSC Advances*, vol. 12, no. 16, pp. 9567–9578, 2022, doi: 10.1039/D2RA00702A.

[27] D. A. Devault, F. Massat, A. Baylet, F. Dolique, and P.-J. Lopez, 'Arsenic and chlordecone contamination and decontamination toxicokinetics in Sargassum sp.', *Environ Sci Pollut Res*, vol. 29, no. 1, pp. 6–16, Jan. 2022, doi: 10.1007/s11356-020-12127-7.

[28] M. Francoeur, C. Yacou, C. Jean-Marius, Y. Chérémond, U. Jauregui-Haza, and S. Gaspard, 'Optimization of the synthesis of activated carbon prepared from Sargassum (sp.) and its use for tetracycline, penicillin, caffeine and methylene blue adsorption from contaminated water', *Environmental Technology & Innovation*, vol. 28, p. 102940, Nov. 2022, doi: 10.1016/j.eti.2022.102940.

[29] H. S. Al-Khalaifah, A. Al-Nasser, and T. Surrayai, 'Effects From Dietary Addition of Sargassum sp., Spirulina sp., or Gracilaria sp. Powder on Immune Status in Broiler Chickens', *Frontiers in Veterinary Science*, vol. 9, 2022, Accessed: Oct. 31, 2023. [Online]. Available: https://www.frontiersin.org/articles/10.3389/fvets.2022.928235

[30] K. Chandrasekaran, H. S. George, H. Selvaraj, S. Dharmarajan, and M. Sundaram, 'Anoxic treatment for thiosulfate separation from formate containing dithionite industrial effluent and electrochemical sulfur recovery', *Journal of Environmental Chemical Engineering*, vol. 10, no. 3, p. 107324, 2022.

[31] R. Hugh, 'Classical methods for isolation and identification of glucose nonfermenting Gram-negative rods', in *Glucose nonfermenting gram-negative bacteria in clinical microbiology*, CRC Press, 2020, pp. 1–13.