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Research Article

Production of red-pink pigment from *Salinicoccus sp.* as natural coloring agent in high-fat-containing foods

Qinke Yu[1], Pachara Tangudomkan[2], Cheunjit Prakitchaiwattana[2,3] and Wen-Chen Huang[1*]

[1] *The Bachelor of Science in Biotechnology (International Program), Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand*
[2] *Department of Food Technology, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand*
[3] *The Development of Foods and Food Additives from Innovative Microbial Fermentation Research Group, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*
* *Corresponding author: elliott0810@gmail.com*

Abstract - This study investigated the potential of red-pink pigments produced by *Salinicoccus sp*. (82-1) strains, isolated from salty fermented foods, as an alternative source of natural food colorants with antioxidant and antimicrobial properties. This research aimed to optimize the cultivation of *Salinicoccus sp*. in a medium derived from salty fermented food and milk, extract the pigments produced, evaluate their bioactive properties (antioxidant and antimicrobial), and finally apply them to food models with high fat content. The optimal cultivation medium consists of 1% peptone water supplemented with 5% milk. This medium yielded a cell count of 8.8 log CFU/ml in a 3 L bioreactor, corresponding to 28.75 g of wet cells, and it yielded a total of 15.65 g of crude pigments. The extracted pigment exhibited significant free radical scavenging activity (51.98% by the DPPH method) and antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*, with clear zones of 12.5 mm and 15.4 mm, respectively. When applied to food models at a concentration of 6.67%, the pigment provided coloration with no antimicrobial activity similar to commercial synthetic (CS) coloring agents. Furthermore, it effectively inhibited rancidity in the food models by reducing the peroxide value significantly compared to the CS coloring agent. In summary, the pigment derived from *Salinicoccus sp*. has potential for use as a

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natural food colorant with added functional benefits, offering an alternative to synthetic colorants while providing antioxidant and antimicrobial properties.

Keywords: Red-pink pigment, microbial pigment, pigment production, halophilic bacteria

1. Introduction

The food coloring industry is undergoing a significant change due to increasing consumer health concerns and a preference for natural additives. The increasing presence of products labeled "no artificial colors" is evidence of this change. Natural food coloring agents are considered safer for human consumption compared to synthetic dyes. The demand for natural and sustainable food additives has increased, reflecting consumer preferences for healthier and environmentally friendly products (Martins et al., 2016).

Natural colorants, derived from plants and microorganisms, offer a viable alternative to synthetic dyes, addressing health and food safety concerns. Microbial-derived pigments, in particular, are promising due to the unique advantages microorganisms offer. These advantages include short life cycles, low sensitivity to seasonal and climatic changes, scalability, and the ability to produce a wide range of colors and shades depending on the species. Microbial pigments have already found applications in both food and feed products (Pankaj et al., 2016). Notably, carotenoids produced by microorganisms have been commercialized and widely used.

Beyond their role as coloring agents, microbial pigments exhibit bioactivity, such as antimicrobial and antioxidant properties, making them attractive for various biotechnological applications. These applications range from functional food production to the development of new drugs and biomedical therapies (Pankaj et al., 2016; Nigam, 2016). To further enhance the use of microbial pigments in the food industry, ongoing research focuses on identifying new microbial sources, utilizing low-cost substrates, and optimizing production processes for economic viability (Khanduri & Nautiyal, 2024). One such promising pigment-producing microorganism, *Salinicoccus sp*. (82-1), generates carotenoids, which mainly consist of lycopene, lutein, and β-carotene (Sricharoen et al., 2022). Carotenoids are precursors of vitamin A and have recognized health benefits (Cooperstone & Schwartz, 2016). Additionally, carotenoids are lipid-based compounds, which means they are less insoluble in water but more soluble in lipids. The fats and oils can react with oxygen, leading to the formation of free radicals and subsequent oxidative degradation. Thus, the bioactivity and stability of bacterial pigments containing carotenoids are of great importance. Despite their potential, the application of the red-pink pigment as a food colorant and preservative is still limited. In the development of bio-pigment production from microorganisms, many agri-food by-products such as fruit pomace, seeds, peels, corn steep liquor, molasses, whey, bran, etc. have been utilized as possible fermentable substrates (Noby et al., 2023). Overall, investigating microbial pigments, especially those from species like *Salinicoccus sp*., represents a promising alternative in the development of natural, sustainable, and health-promoting food colorants. This approach aligns with the increasing consumer demand for natural products and provides potential advantages for both food safety and environmental sustainability (Ashenafi et al., 2023; Rao & Rao, 2007).

This study proposed the development of a process for producing red pigments from *Salinicoccus sp.* The steps of work

included (i) optimization of cultivation process, (ii) conduction of bioactivity assay of the pigment, and (iii) application in food model as coloring agent with bioactivity.

2. Materials and methods

2.1 Bacteria and starter preparation

Salinicoccus sp. (82-1) was isolated from Pla-ra, a traditional fermented fish product from Thailand's northeastern region, obtained from the collection of the Department of Food Technology, Faculty of Science, Chulalongkorn University. Glycerol stock prepared from the master stock was activated by streaking onto nutrient agar at 37°C for 48 hr. The same generation of glycerol stock was used throughout this study. The isolates were cultivated in nutrient broth (NB) (Himedia, India) containing 3% sodium chloride (NaCl) (QReC, New Zealand) under orbital shaking at 150 rpm at room temperature until optical density (OD_{600}) reached 0.46 (6-7 log CFU). This fresh culture was used as a starter culture.

2.2 Cultural medium optimization

To evaluate the optimal growth medium for *Salinicoccus sp*. (82-1), the experimental design involved screening nine different media derived from fish sauce, soy sauce, and milk, each at concentrations of 1%, 2.5%, and 5%. All media were standardized to contain 3% NaCl. A starter culture of 0.1 ml was added to 25 ml of each prepared medium to achieve a final cell concentration of 6 log CFU/mL. The cultures were incubated at 35°C with shaking at 150 rpm for 72 hours (Sricharoen et al., 2022). Bacterial growth was monitored by viable population counts at both 0 and 72 hours. Following the initial screening, the best-performing based medium was selected for further optimization with the addition of peptone (Himedia, India) or nutrient broth (NB) at 1% and 5% concentrations. The best-performing medium formulation for the optimal growth of *Salinicoccus sp*. (82-1).

The viable population was determined by total plate count (TPC) method. Culture was 10-fold serially diluted with 0.85% saline water. 0.1 ml from each dilution was spread onto NA (Himedia, India) plates supplemented with 3% NaCl and incubated at 35 °C for 48 hours. Colonies were then counted and reported as CFU/mL.

2.3 Cells production in bioreactor

The optimal media selected from the previous step was used for cultivation of *Salinicoccus sp*. (82-1) in Bioreactor. Three liters of the sterile media were prepared in a 5-liter glass fermenter (BEMT, BEMT-T-5L, Thailand). Subsequently, 10% of the starter culture was introduced into the bioreactor and cultivated at 30°C with an agitation speed of 120 rpm and aeration rate of 1.0 vvm. Total plate count (TPC) assessments were performed at 12 hours and every 24 hours for a duration of 96 hours. The cells were harvested by centrifugation (Thermo Fisher, Legend XTR, Germany) at 10,000 rpm and 4°C under aseptic conditions. The cell pellet was washed twice with a normal saline solution before being stored at -21 °C for 24 hours prior to pigment extraction.

2.4 Pigment extraction

Pigment extraction process was modified from Sricharoen et al. (2022). The frozen cell pellet was thawed before extraction. Sodium Hydroxide (NaOH) (LOBA Chemie, India) solution was added to the cells at a ratio of 20 mg cells to 500 ml of NaOH solution. Subsequently, sonicating (ULTRAsonik) with ice water at 40 Hz for one hour. Citric acid (QReC, New Zealand) was applied to adjust the pH to 7. Then, washed twice with absolute ethanol (Merck, Germany) (the concentration reached 70% of the total solution volume) for phase separation. Ethanol-rich phase containing carotenoids was collected and underwent vacuum evaporation (Buchi Rotavapor, R-100, V-100, I-100, Switzerland).

2.5 Test of some bioactivities of crude pigment

DPPH assay (2,2-diphenyl-1-picrylhydrazyl assay) was used to determine the antioxidant activity of crude pigment using the method from (Shimada et al., 1992). The DPPH solution was prepared by dissolving 0.004 g of DPPH in 100 ml of methanol (Merck, Germany) and stored in the dark. The DPPH assay is widely used to evaluate antioxidant activity. The crude pigment, commercial colorant (Best Odour, Thailand), and one blank group were tested. 0.25 ml of samples were added into the 0.75 ml DPPH solution in a ratio of 1:4. 0.25 ml of methanol was added in place of a sample for the blank group. They were then allowed to react in the dark for 30 minutes. A spectrophotometer (Eppendorf, BioSpectrometer Basic, Thailand) set to a wavelength of 517 nm was used to test the samples. Color changes were observed, and percent scavenging capacities were calculated for crude pigment and commercial colorants as $[(A_0-A_1)/A_0] \times 100$ (A_0 = absorbance without extract; A_1 = absorbance with crude pigment or commercial colorant).

Antimicrobial activity was determined by a spot-on-lawn assay modified from (Sricharoen et al., 2022). *Escherichia coli* and *Staphylococcus aureus* were used to test the antimicrobial capacity of the crude pigment. One loop of each pathogen was introduced into nutrient broth (NB) and incubated for 24 hours at 37°C with shaking at 150 rpm. Then, 0.1 ml of the prepared cultures were spread on each nutrient agar (NA) plate. A control was prepared using diluted tetracycline. 0.1 mL of crude pigment and 0.1 mL of diluted tetracycline (T.C. Pharma-Chem, Thailand) were spotted on each bacterial lawn. Inhibitory zones (mm) were measured after 48 hours of incubation at 35°C.

2.6 Application of crude pigment in food models

Three experimental groups were used to evaluate the effect of crude pigments on high-fat whipping cream (Anchor, New Zealand) and homemade mayonnaise. In the first group, the 6.67% concentration of crude pigments was directly applied to the whipping cream and mayonnaise. The second group was used as a comparison, with a 4% concentration of commercial colorants applied to each food sample until the color matched that achieved with the extracted pigments in the first group. In the control group, no pigments were added to the samples. All samples were stored at the recommended temperature of 4◎. The testing period spanned 7 days, during which the samples were monitored for changes in quality attributes such as microbial quality, color stability, and rancidity.

Microbial quality was assessed through Total plate count (TPC) and total yeast and mold count in milk were determined using procedures described in Chapters 3 and 18 of the FDA Bacteriological Analytical Manual (BAM) (FDA, 1998)

Rancidity was assessed through the determination of peroxide value, a primary oxidation product formed in the sample, which was detected through the peroxide value (PV) test, following the AOAC method. Four reagents were prepared. Acetic acid (LOBA Chemie, India) and chloroform (VWR, USA) were mixed in a ratio of 3:2 to make the acetic acid-chloroform solution. A saturated potassium iodide solution was prepared. Potassium iodide (KI) (Univar, Australia) was dissolved in water. Heating was applied until the solution reached saturation, as indicated by the inability of additional KI to dissolve. 0.001 M sodium thiosulfate (Univar, Australia) standard solution and 1% starch solution were prepared as well. 3 ml of acetic acid-chloroform solution was mixed with 0.5 g of samples until dissolved.

0.05 ml of saturated KI solution and 3 ml of water were then added. A 0.05 ml aliquot of 1% starch solution was subsequently introduced as an indicator. Titration was then performed using a 0.001 M sodium thiosulfate solution until a color change was observed. The volume of the sodium thiosulfate solution used (S) was recorded. Peroxide values were then calculated using the formula S * 0.001 M * 1000 / g, with the unit of milliequivalent peroxide/kg.

2.7 Statistical analysis

All experiments were conducted with 2 replications. An analysis of variance (ANOVA) was employed in this study using IBM-SPSS software (SPSS Inc., Chicago, IL, USA). Probability at $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1 Medium optimization

To find the optimal source of medium, thirteen different candidate media were evaluated, derived from fish sauce, soy sauce, and milk. (Table 1) illustrates that 5% milk concentration exhibited the most pronounced growth of *Salinicoccus sp*. (82-1) over the observed period and was selected for further optimization. Despite previous studies suggesting that microbial-derived pigments can be isolated from salty environmental sources, including salty fermented foods like Thai fermented fish (Pla-ra) (Det-udom et al., 2022) and soy sauce (Pankaj et al., 2016), the composition and nutrient content of milk differ from the rest. Milk is rich in nutrients such as vitamins A, B1, and B2, as well as calcium and iron; particularly, it contains a high amount of glutamate (Glu) (Ceballos et al., 2009) which is reported as a key TCA intermediate for carotenoid synthesizing bacteria (Raita et al., 2023). Thus, the presence of additional nutrients in milk may contribute to its enhanced microbial growth. Thus, a 5% milk-based medium was selected for further optimization.

Table 1. Growth of *Salinicoccus sp*. (82-1) in different media derived from milk, soy sauce, and fish sauce in log CFU/ml. (n = 28)

Media composition	0 hr	72hr
Control (NB & 3% Nacl)	5.6	7.5
Fish Sauce 1%	5.6	0
Fish Sauce 2.5%	5.6	0
Fish Sauce 5%	5.6	0
Fish Sauce 10%	5.6	0
Fish Sauce 20%	5.6	0
Soy Sauce 1%	5.6	0
Soy Sauce 2.5%	5.6	0
Soy Sauce 5%	5.6	0
Soy Sauce 10%	5.6	0
Soy Sauce 20%	5.6	0
Milk 1%	5.6	6.2
Milk 2.5%	5.6	6.4
Milk 5%	5.6	7.5

Media containing 5% milk was enriched with various contents of peptone and nutrient broth (NB). (Figure 1) depicts a significant increase in cell population relative to a non-enriched medium. In addition, after the log phase, a slight increase in the population of *Salinicoccus sp*. (82-1) after reaching the stationary phase was observed. This increase could be attributed to the activation of a self-protection mechanism by the bacteria in response to limited nutrition. The growth of *Salinicoccus sp*. (82-1) increased significantly at 96 hours. Multiple comparison tests were conducted using IBM-SPSS statistics. Two subsets were identified where the mean of the second subset significantly differs from the first. Both NB 1% and 5% fell into the same group as the control, indicating a closer association with the control group. Moreover, there was no significant difference between the 5% and 1% NB concentrations. Hence, the 5% milk with 1% NB condition was selected for scaling up in the bioreactor due to its lower material usage.

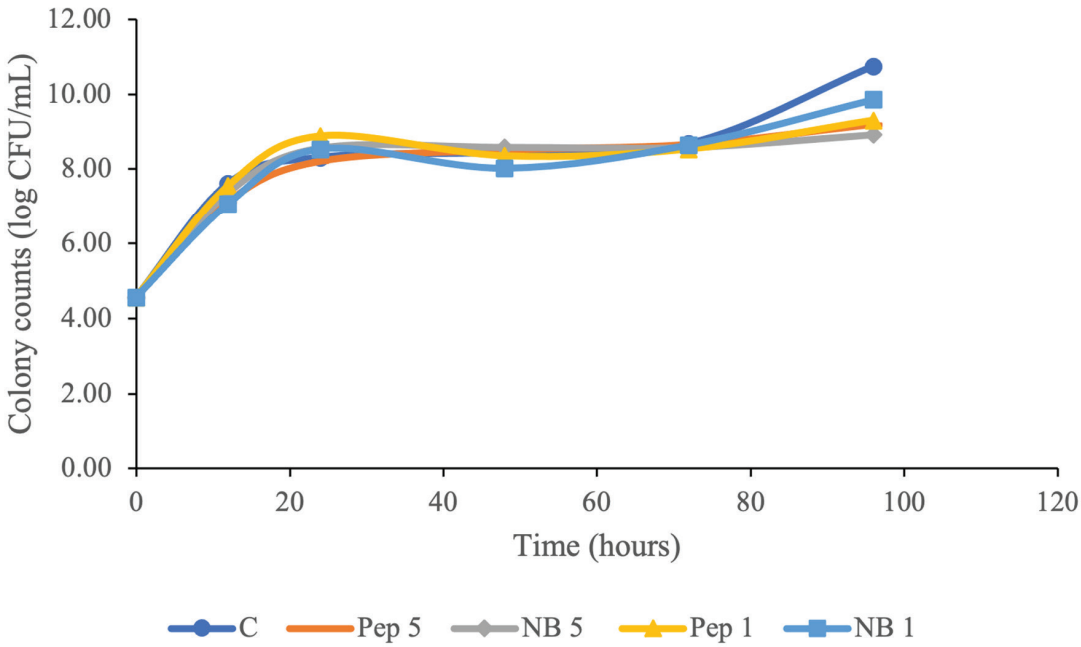

Figure 1. Growth of *Salinicoccus sp*. (82-1) in medium derived from milk with different enriched media (C for control ; Pep for peptone ; NB for nutrient broth) (n = 10)

3.2 Bioreactor, pigment extraction and crude pigment concentration

The selected media was then added to the 3 liters of bioreactor to increase the production of pigments. After six days of inoculation, the data were then collected. In (Figure 2), the growth curve of *Salinicoccus sp*. (82-1) is displayed with a horizontal axis of time in hours and an axis of colony counts in log CFU/ml. The count of *Salinicoccus sp*. (82-1) displayed a noticeable increase during the first 24 hours, which could be interpreted as the lag phase and the log phase. The stationary phase could be started from 24 hours to 72 hours as the colony count remained stable, and there was a slight decrease after 72 hours, which can be identified as the death phase. Microbial carotenoid is the secondary metabolite that is synthesized during the stationary phase of growth (López et al., 2023); hence, the culture at 72 hours was considered to be in the mid-

stationary phase, and the cell was harvested for further pigment extraction. From the bioreactor experiment, the collection of 28.75 g of wet cells yielded a total of 15.65 g of concentrated crude pigment. This red-pinkish crude pigment, as observed in (Figure 2), appears as a viscous red solution. According to the report of Sricharoen et al. (2022), these crude pigments have been identified as xanthophylls, which are oxygenated carotenoids. Examples of such carotenoids include lycopene, lutein, and β-carotene.

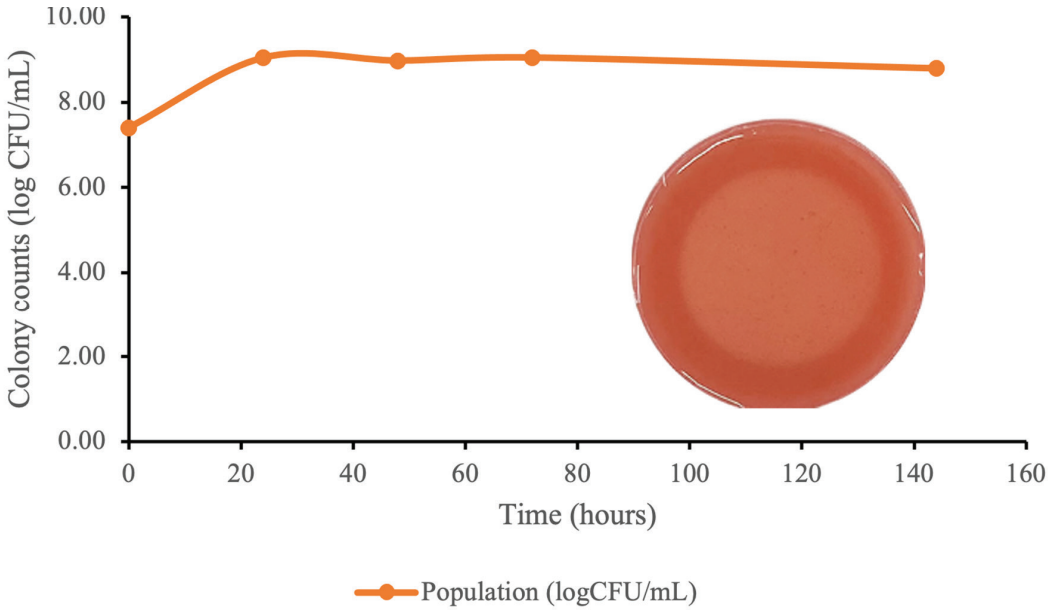

Figure 2. The growth profile of *Salinicoccus sp*. (82-1) in a 3 L bioreactor and the crude pigment obtained. (n = 2)

3.3 Biological activities of crude pigment

Spot-on lawn assay and DPPH radical scavenging analysis were applied for antimicrobial and antioxidant activities, respectively. Inhibitory effects of pigment extracts on food pathogens such as gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* were investigated. Tetracycline (30 μg) was used as the standard antibiotic. The results in (Table 1) demonstrated that the crude pigments extracted from *Salinicoccus sp*. (82-1) inhibited both *S. aureus* and *E. coli* with an inhibition zone of 15.39 ± 0.34 and 12.45 ± 0.00 mm, respectively. Carotenoids are reported for their antimicrobial activities. However, based on inhibition zones observed in this assay were significantly large. This inhibitory effect is possibly due to bacteriocio produced by bacteria along with carotenoid synthesis that could also inhibit the growth of specific pathogens. According to Juturu et al. (2018), there are many bacteriocins produced by various bacteria, such as Nisin from *Lactococcus lactis* subsp. *Lactis*. Bacteriocin production is well investigated for the *Staphylococcaceae* family, which is the same family as *Salinicoccus*, such as Epidermin from *Staphylococcus epidermidis*. (Sandiford & Upton, 2012). *Staphylococcus epidermidis* produces epidermin as bacteriocin through a biosynthetic pathway involving gene clusters for peptides such as precursor peptides (epiA) and transport proteins (epiE, epiF). These genes are transcribed into mRNA and translated into precursor peptides, which then undergo post-translational modifications to form the

epidermin (Peschel et al., 1996). Although the research on the bacteriocin genes of *Salinicoccus sp*. is limited, the bacterium's halophilic nature suggests potential for similar bacteriocin production. The bacteriocin production and mechanism of antimicrobial capacity of *Salinicoccus sp*. should be further investigated.

Lycopene and lutein have been detected in the crude pigment of *Salinicoccus sp.* (82-1) based on the report by Sricharoen et al. (2022) . Lycopene has been documented to reduce the risk of chronic diseases (Palozza et al., 2011), such as pancreatic cancer, due to its ability to affect cell viability and inhibit cell proliferation promoted by reactive oxygen species (ROS) (Jeong et al., 2019). Similarly, high amounts of lutein can increase ROS levels and exhibit anticancer activity (Eom et al., 2023). As modulators of ROS, both lycopene and lutein can damage living cells at high concentrations (Palozza et al., 2011). These two compounds are the primary components of the crude pigment. On the other hand, DPPH radical scavenging analysis was conducted for antioxidant activity of pigment extracts, with the results shown in (Table 1). This was reported as a percentage (a higher percentage indicates more efficient at reducing the DPPH radicals, meaning much stronger antioxidant activity). and a lower percentage vice versa) with 37.59 ± 0.14 and 63.26 ± 0.46 for the synthetic coloring agent (Figure 4) and the crude pigment sample, respectively, which indicated that the crude pigments were effective at scavenging free radicals over the synthetic ones. The antioxidant capacity of carotenoids is well documented. Lycopene has the highest ability to scavenge singlet oxygen (Di Mascio et al., 1989), significantly reducing ROS and thus preventing lipid peroxidation. This capability is due to its chemical structure as a tetraterpene hydrocarbon with a polyene chain consisting of 11 conjugated double bonds, ROS, and unconjugated double bonds, making it highly reactive towards oxygen and free radicals (Kelkel et al., 2011). However, the application and antioxidant capacity of bacteriocins from *Salinicoccus* species, as well as those from related families, are limited. The results indicate that the carotenoids present in the crude pigments can be utilized as an alternative source of natural pigments (Sricharoen et al., 2022). These pigments offer biopreservative benefits due to their antimicrobial and antioxidant properties, making them valuable for applications that require both natural coloration and preservation (Uppal et al., 2021; Roman et al., 2022)

Table 2. Antimicrobial activity by Spot-on lawn assay and antioxidant activity by DPPH assay of crude pigment from *Salinicoccus sp*. (82-1) (n =)

Strain for production of Crude pigment	Antimicrobial activity Inhibition zone (mm)		DPPH analysis (% of Inhibition) *p < 0.01
	E. coli	S. aureus	
Salinicoccus sp. (82-1)	12. 45 ± 0.00	15.39 ± 0.34	63.26 ± 0.46*

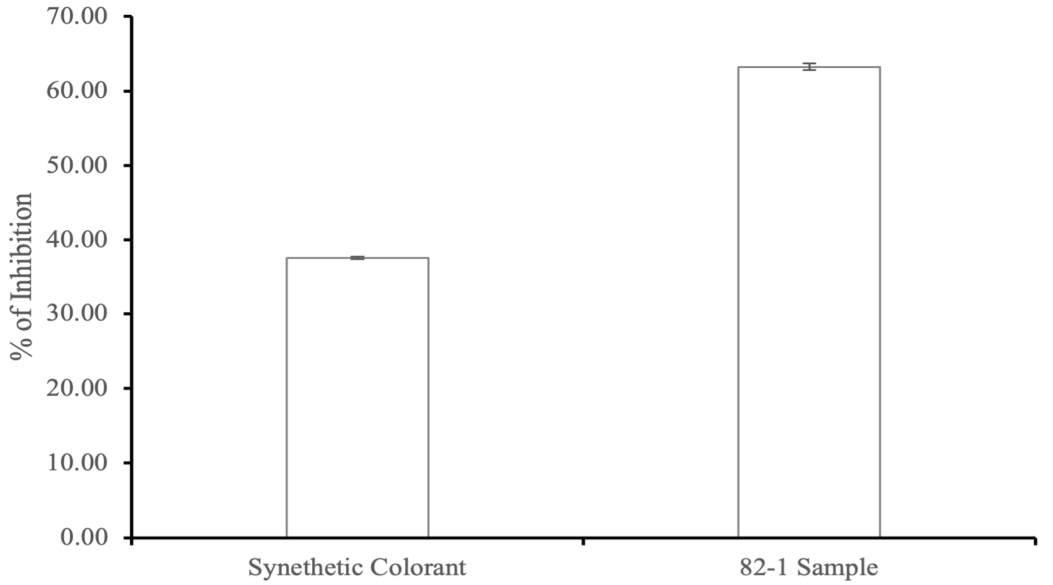

Figure 3. DPPH assay in synthetic colorant and crude pigment of *Salinicoccus sp*. (82-1)

3.4 Application in food models

The peroxide value, an indicator to measure the level of rancidity by measuring the degree of oxidation of the substance, was applied. A higher peroxide value indicates higher rancidity (Othón-Díaz et al., 2023). When applied crude pigment to both mayonnaise and whipping cream, compared to synthetic food coloring agents, the ones with crude pigments have a relatively lower peroxide value than the ones that contain synthetic colorants (Synthetic Sample) when stored at 4◎ (Table 2). These results indicate that the crude pigment obtained from *Salinococcus* could possess antioxidant properties. The pigments act as natural antioxidants by scavenging free radicals and neutralizing the reactive molecules by donating hydrogen atoms or electrons to inhibit the oxidation process (Othón-Díaz et al., 2023). Natural antioxidants often comprise a mixture of bioactive compounds, such as polyphenols, flavonoids, and carotenoids, primarily extracted from *Salinococcus*, with lycopene and lutein as key components, as demonstrated in this study. These compounds work synergistically, enhancing their overall antioxidant effect. In contrast, synthetic antioxidants are typically single compounds that lack this synergistic interaction. Consequently, the combined action of natural compounds often results in stronger antioxidant properties compared to synthetic antioxidants. Furthermore, from a health perspective, studies have shown that when both natural and synthetic antioxidants are consumed, only a fraction of the natural antioxidants is absorbed and utilized as free-radical scavengers in vivo (Jan Pokorný, 2007), highlighting the significance of natural antioxidants.

Table 3. Peroxide value changes in food model during storage under room temperature reported in Meq/kg

Food samples	Day 0	Day 1	Day 3	Day 5
Mayonnaise with 82-1 crude pigment	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.15 ± 0.02
Whipping cream with 82-1 crude pigment	0.00 ± 0.00	0.00 ± 0.00	0.13 ± 0.05	0.23 ± 0.03
Mayonnaise with synthetic coloring agent	0.00 ± 0.00	0.05 ± 0.00	0.37 ± 0.05	1.4 ± 0.03
Whipping cream with Synthetic coloring agent	0.00 ± 0.00	0.00 ± 0.00	0.42 ± 0.03	0.55 ± 0.05
Mayonnaise blank	0.00 ± 0.00	0.00 ± 0.00	0.36 ± 0.06	0.95 ± 0.12
Cream blank	0.00 ± 0.00	0.00 ± 0.00	0.29 ± 0.08	0.59 ± 0.06

The effect of antimicrobial activities of the pigment in food models was evaluated through microbial quality (TPC and YMC) of food changed during storage. (Figures 4) and 5 reveal that the antimicrobial effects of the crude pigment and a synthetic colorant in food models were not significantly different. This suggests that the crude pigment did not show a significant inhibitory effect on microorganisms present in the food. In contrast, (Table 2) shows that the crude pigment exhibited significant inhibitory effects on *Staphylococcus* and *E. coli*, representing gram-positive and gram-negative bacteria, respectively, indicating that the pigment does have antimicrobial properties. The lack of antimicrobial activity in the food models likely results from an insufficient amount of pigment being used and/or the pigment having no inhibitory effect on the native flora present in foods, whose properties are different from those of *Staphylococcus* and *E. coli* used as tested strains. Consequently, further research is necessary to determine the effective dose required to achieve antimicrobial effects in food applications.

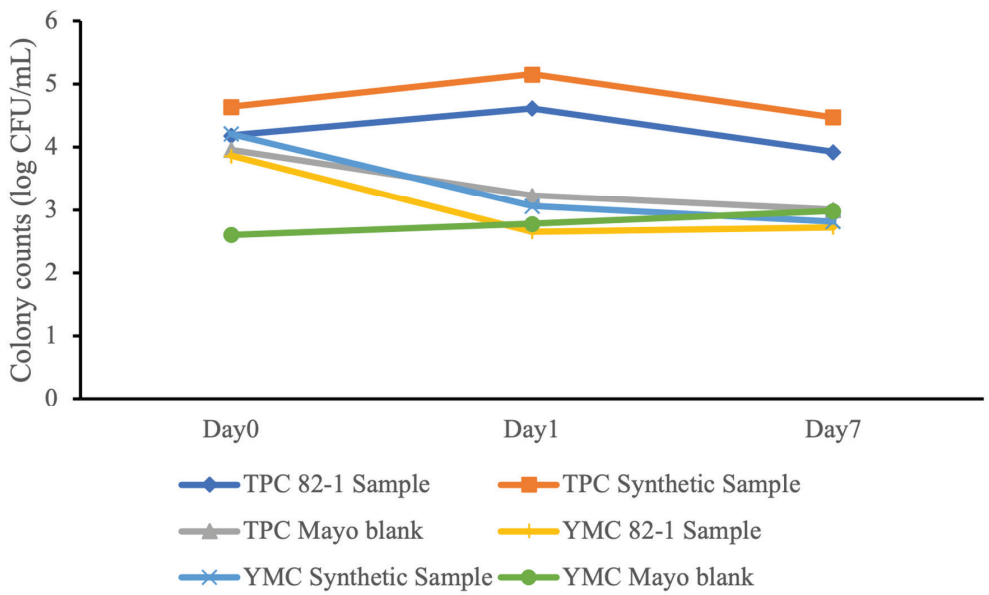

Figure 4. Total plate count (TPC) and yeast & mold count (YMC) in mayonnaise during storage under refrigerated temperature

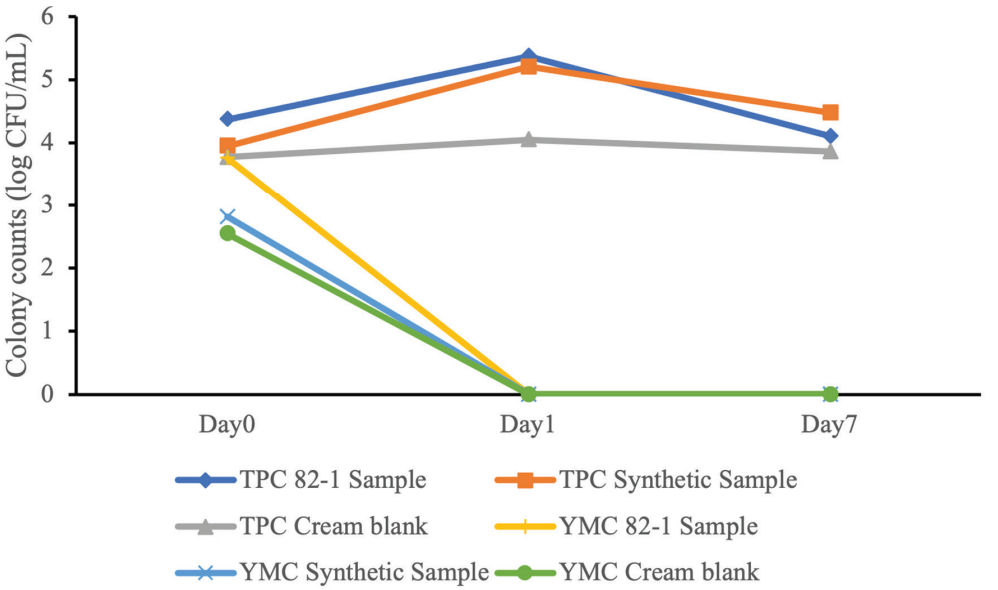

Figure 5. Total plate count (TPC) and yeast & mold count (YMC) in whipping cream during storage under refrigerated temperature

(Figure 6) illustrates the stability of crude pigments and synthetic colorants when incorporated into mayonnaise and whipping cream. The crude pigment extract demonstrated relatively high stability compared to synthetic pigment, especially in mayonnaise, over the observation period since the carotenoid group is lipid-base, which interacted better with the crude pigment extract. The results implied that the crude pigments interacted with mayonnaise more favorably than whipping cream due to its fat content or emulsifying agents that help the crude pigment maintain and prevent separation (Kotake-Nara et al., 2022).

The mayonnaise in control samples thickens over time and forms a yellowish color, which might be attributed to the natural oxidative process or microbial activity. The control groups in whipping cream also exhibited thickening over time, indicating that the aging process might be due to the changes in fat structure. However, the color remained stable, which made it less challenging for further studies on coloring in comparison to mayonnaise.

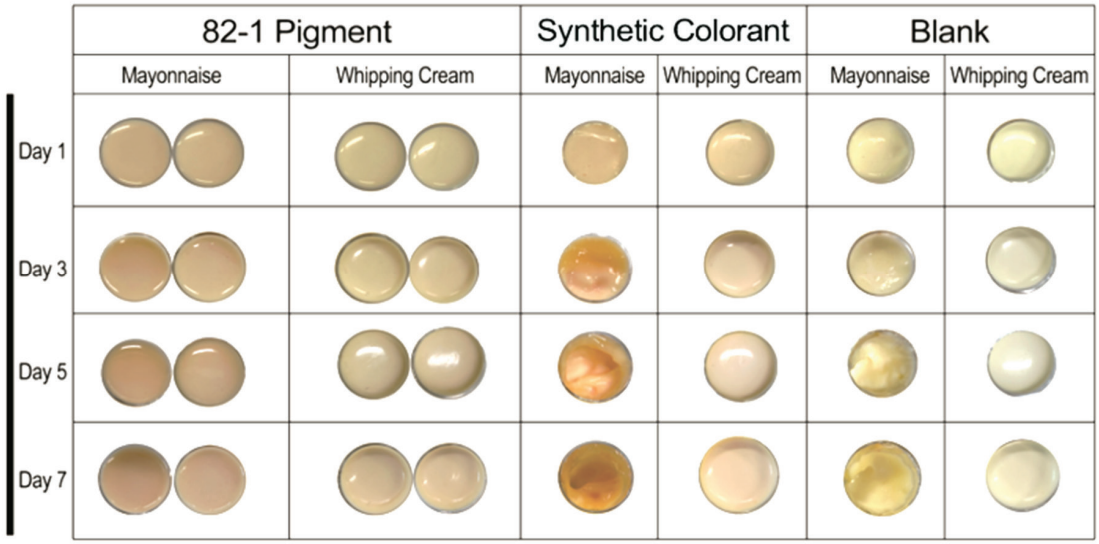

Figure 6. Color appearance and stability in food model storage under refrigerated temperature for seven days.

4. Conclusion

The optimized cultivation medium for maximizing the production of pigments was developed using 5% milk enriched with 1% nutrient broth. The crude pigment extracted from this medium showed significant free radical scavenging activity along with antimicrobial effects against *Escherichia coli* and *Staphylococcus aureus*. When incorporated into high-fat food models, the pigment effectively mitigated rancidity by lowering peroxide values in comparison to those treated with synthetic coloring agents. In addition, it exhibited color stability during storage. However, it did not display antimicrobial activity on any of the food models. Future efforts will focus on determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) to further explore its antimicrobial potential in food models as well as CIElab, and sensory evaluation should be further investigated.

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