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INCREASING THE DURABILITY AND FUNCTIONALITY OF NATURALLY COLORED *Se'I* TUNA RAISE BY ADJUSTING THE pH, ADDING POTASSIUM NITRATE, AND CALCIUM PROPIONATE

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ABSTRACT

The performance of tuna se'i can be enhanced by adding natural red yeast rice dye (angkak); however, its short shelf life of only 3 days limits its distribution. Spoilage primarily results from bacterial and mold growth. This study aimed to extend the shelf life of tuna se'i using antimicrobial agents—potassium nitrate and calcium propionate—within permitted BPOM-2019 limits (2 g/kg and 50 mg/kg, respectively). A factorial experiment was conducted by applying these preservatives in curing solutions at pH levels of 4, 5, and 6, with storage at room temperature for 0, 4, 8, 12, and 16 days. Parameters evaluated included hedonic sensory attributes, Total Plate Count (TPC), yeast and mold counts, water content, peroxide value, thiobarbituric acid (TBA), color, and texture. Results showed that microbial growth remained within safe limits until day 12, while physicochemical indicators—water content, peroxide value, and TBA—remained below the SNI thresholds even at day 16. The untreated control was safe for consumption up to day 8. Declines in red color intensity, texture, and sensory acceptance were observed during storage. The best formulation was cured at pH 4, extending shelf life to 15 days with a TPC of 5.4×10^4 cfu/g, acceptable sensory ratings (mean score of 5 out of 7), and compliance with quality standards. Angkak also contributed nutraceutical compounds with antioxidant, antimicrobial, anti-inflammatory, and anti-gout potential, highlighting its dual functionality as a colorant and preservative.

Keywords: Organoleptic, Se'I, Total Plate Count, TBA

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INTRODUCTION

Tuna se'i, a traditional smoked delicacy from Kupang, East Nusa Tenggara, is uniquely flavored with kosambi leaves and typically prepared using land animal meats. As the dish gains popularity, innovations have emerged using boneless fish like tuna to improve convenience and market appeal. Unlike conventional smoked fish, which retains bones and skin, tuna se'i offers a cleaner presentation and easier packaging. However, the traditional use of potassium nitrate for its signature reddish color poses health risks due to nitrosamine residues. To address this, natural alternatives such as red yeast rice have successfully enhanced the color, taste, and safety of tuna se'i through a curing process before smoking, offering a healthier twist on a cherished regional specialty.[1] Red yeast rice is a natural product from traditional fermentation originating from China. Red yeast rice gets its red color from the *Monascus purpureus* mold, which is grown on a rice substrate. Angkak dye is considered to have advantages, namely stable and consistent, soluble in water, the resulting color can be mixed with other pigments, and is safe for consumption. The addition of saltpeter or the addition of natural dyes such as angkak can improve the performance of meat se'i or fish se'i, but there are still problems or obstacles in the form of a short shelf life of se'i or other smoked fish products. Beef se'i is only edible for 2-3 days, while the addition of liquid smoke can inhibit bacterial growth so that it is still suitable for consumption until the seventh day of storage.[3] Fish meatballs only last for 1 day, while the addition of 3.33% liquid smoke lasts until the 4th day. The addition of liquid smoke to smoked skipjack tuna is also only able to extend the shelf life to 3 days of room temperature storage.[5] The use of Kosambi bark extract in tuna can improve the organoleptic performance of color and taste up to 2 days. The use of chitosan can extend the shelf life of smoked tuna up to four days with the best concentration of 2%[7] %. Vacuum packaging increases the shelf life of smoked fish in Bangkalan - Madura

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from 2-3 days to 4-7 days.[8] Smoked freshwater fish, such as tawes fish and tilapia, with direct smoking have a shelf life of 4.45 days, while with liquid smoke, it has a shelf life of up to 6.09 days. The maximum storage time of liquid smoked tuna is 6 days with a total plate count (TPC) or total bacteria value of 1.8 x 10^5[9]. The addition of 2% extract to se'i can extend the shelf life up to 5 days. Shelf life constraints are related to the growth of bacteria and fungi in se'i products.[10] Conventional smoking, the addition of liquid smoke, and the addition of natural ingredients such as gelatin, pectin, red yeast rice, and chitosan have not been able to significantly increase the shelf life of se'i, where the maximum shelf life of se'i is only 7 days. Therefore, other preservatives are needed that are antibacterial and antifungal. The most widely used antibacterial and antifungal preservatives are organic preservatives such as propionate, sorbate, and citrate. Calcium propionate is effective as a preservative for mold and yeast at pH 4.9[11], with a maximum usage limit of 2g/Kg of material.[12] In addition, inorganic preservatives such as nitrate salts are also often used. Nitrate salts are generally used in the curing process, namely sodium or potassium nitrate, which is useful for preventing bacterial growth. Potassium nitrate effectively inhibits bacteria in the pH range of 5.0-5.5[12]. The use of nitrate as a meat preservative has a limit of 50 mg/kg.[13] The use of nitrate as a preservative can maintain the color of fish to be brighter, accelerate the curing process, and act as an antimicrobial. The activity of food preservatives is not only limited by the concentration of use but is also influenced by the optimum pH and soaking time. Found that the best soaking time or curing process for se'i tuna is 6 hours.[1] Bacteria that generally grow on se'i products can be pathogenic bacteria such as Staphylococcus aureus, Bacillus cereus, *Salmonella*, and *E. coli,* and can be inhibited at low pH.[10] The types of mold identified in smoked tuna are Aspergillus sp. and Penicillium sp. Microorganisms will grow very well at a pH of around 7, with a range between 6.6 - 7.5.[10] To extend the shelf life of se'i, it is essential to investigate the optimal soaking time of fish meat in a pH-regulated curing solution and its effect on storage at room temperature. This study aims to identify the best pH and soaking duration of a curing solution combining potassium nitrate and calcium propionate to prolong the shelf life of tuna se'i. The research evaluates the physical, microbiological, and sensory qualities of the product during storage. This focus addresses a critical research gap, as prior studies have examined individual additives like red yeast rice, liquid smoke, or chitosan, but none have explored the combined antimicrobial effects of potassium nitrate and calcium propionate in a pH-controlled curing process. The antimicrobial effectiveness of these preservatives depends not only on their concentrations but also on the pH and contact time during curing. Beyond overcoming technical hurdles in traditional food preservation, this study supports the Sustainable Development Goals (SDGs), especially SDG 3: Good Health and Well-being, by promoting safer preservation techniques that minimize the risk of carcinogenic nitrosamine formation associated with excessive synthetic nitrate use. These findings highlight the vital role of innovation in advancing traditional food technologies to meet contemporary health and sustainability standards.

EXPERIMENTAL

Materials and Methods

This study utilized fresh tuna (*Thunnus sp.*) sourced from the Sendang Biru Market in East Java, chosen for its bright color, minimal mucus, and firm texture that quickly returns to its original shape when pressed. To prepare the curing solution, red yeast rice powder, potassium nitrate, and calcium propionate were combined with a blend of spices including salt, shallots, garlic, sugar, pepper, and coriander. The pH of this curing solution was carefully adjusted by adding vinegar or sodium hydroxide to achieve different pH levels for experimentation. For the smoking process, natural fuels such as coconut fiber, dried coconut shells, and charcoal were employed to impart the desired flavor and preservation qualities. Chemical analyses of the smoked tuna involved various reagents, including glacial acetic acid, chloroform, sodium thiosulfate, potassium iodide, tapioca flour, 1-butanol, and TBA reagent. Microbiological assessments were carried out using PDA and PCA media, along with other laboratory supplies such as sodium chloride, distilled water, alcohol, cotton, and polypropylene plastics. Additionally, sensory evaluations were conducted with coffee powder and mineral water provided as palate cleansers. The tools used throughout the study were organized based on their function. Equipment for preparing the curing mixture included pans, stoves, spoons, and digital scales. Tuna processing was carried out using knives, cutting boards, containers, blenders, tongs, brushes, smokers, spoons, and scales. The smoking was performed in a zinc smoke cabinet equipped with

a cover and shelves specifically designed to hold the tuna during the smoking process. The tools used in testing the physicochemical characteristics consisted of a CR-400 chromameter, digital scales, 250 mL and 500 mL Erlenmeyer flasks (Pyrex, Duran), burettes, volumetric pipettes (Pyrex, HBG), suction bulbs, desiccators, ovens (Memmert), test tubes, water baths, spectrophotometers, cuvettes, separating funnels (Herma), measuring cups (Pyrex, Herma), spatulas, 100 mL and 500 mL beaker glasses (Pyrex, Iwaki). Tools for testing microbiological parameters include autoclave (Hirayama), large pan, test tubes (Pyrex, Herma), test tube rack, 250 mL Erlenmeyer flask (Iwaki), 500 mL Erlenmeyer flask (Iwaki), colony counter (Interscience), incubator (Binder), petri dish (Iwaki, Citotest), micropipette (Socorex), digital scale, volume pipette, manual pipette controller, vortex (Therma Scientific), 100 mL measuring cup (Iwaki), oven (Memmert), and spatula. As well as tools for organoleptic testing, between sample plates, drinking water glasses, pens, and questionnaires.

General Procedure

The method used in this study is experimental. The experimental method is carried out by treating tuna fish meat with variations in the pH of the curing solution from a mixture of red yeast rice, potassium nitrate, calcium propionate, and spices (A) and variations in the storage time of se'i (B). The treatment of the variation factor of the pH of the curing solution consists of control pH, pH 4.0, 5.0, and 6.0, while the treatment of the storage time factor of se'i consists of 0, 4, 8, 12, and 16 days. The treatment of the pH of the curing solution is based on the optimal pH for potassium nitrate, which ranges from pH 5.0-5.5, and calcium propionate is optimal at low pH, to pH 4.0-5.0[13], while the control is not regulated by the pH of the curing solution. Based on the treatment applied, the experimental design was designed with a factorial Completely Randomized Design with 3 replications.

Preparation of Fish and Curing Solution

The fish used was tuna (*Thunnus* sp.), which was cleaned and washed. After that, the fish is filleted, the bones are removed, and the skinning process is carried out to obtain tuna fish meat. Furthermore, the skinless fish meat is cut lengthwise with a width of approximately 3 cm. The preparation of the curing solution begins with making a red yeast rice solution and then mixing it with potassium nitrate and calcium propionate according to BPOM regulations. The concentration of potassium nitrate used is 2 g/Kg of material, and the concentration of calcium propionate is 50 mg/Kg of material.[13] The red yeast rice solution is made by weighing 25 grams of red yeast rice powder and dissolving it in 1 liter of water. The red yeast rice solution is boiled until boiling, cooled, precipitated, and then filtered with a filter cloth to obtain the red yeast rice solution filtrate. One liter of red yeast rice filtrate is added with potassium nitrate and calcium propionate according to the concentration, then homogenized to obtain a curing solution. Furthermore, the mixture solution per liter is added with a fine spice formulation (60 g salt, 200 g brown sugar, 140 g garlic, 100 g shallots, 28 g coriander, and 16 g pepper)[14], and the pH is adjusted according to the pH of the treatment using acetic acid and/or NaOH solution so that a curing solution is obtained that is ready to soak tuna meat.

Making Se'i Tuna

After preparing the fish, making a solution of a mixture of red yeast rice, potassium nitrate, and calcium propionate, and adding fine spices, I continued with the process of making se'i tuna. The tuna meat that has been prepared is soaked in a curing solution (a mixture of red yeast rice, potassium nitrate, calcium propionate, and spices). Longitudinal pieces of tuna meat are soaked for 6 hours at room temperature, then drained for about 15 minutes, and the hot smoking process is carried out for 6 hours.[15] Smoking is done at UKM Anugerah Mina Lestari, Jl. Gilimanuk IV, No.3, Gang 4, Lowokwaru, Malang City. The smoke used in the smoking process comes from burning dry coconut shells, coconut fiber, and charcoal. After being cooked, the meat is removed and immediately put into polypropylene (PP) plastic packaging, and sealed after the se'i tuna has cooled. The packaged se'i meat is stored at room temperature for 0, 4, 8, 12, and 16 days for testing. Parameter Analysis. The parameters analyzed include hedonic organoleptic, and scoring of taste, aroma, color and texture (Meilgaard *et al*., 1999)[16], peroxide value (AOAC, 2005)[17], water content (BSN, 2015)[18], TBA (AOCS, 2003)[19], Color (Hutching, 1999), Texture (Khaledi *et al.*, 2015)[20], Total Yeast Mold and TPC (BSN, 2015).[18]

Analytical Discussion

The research data were analyzed using the Minitab version 20.0 application. Parameters Total Plate Count (TPC), yeast mold, texture, color, water content, peroxide number, and thiobarbituric acid (TBA) were analyzed using ANOVA (Analysis of Variance) and Tukey's further test.

RESULTS AND DISCUSSION

A. Organoleptic Characteristics of Se'i Tuna During Storage

The organoleptic characteristics related to preservative factors and storage time of se'i tuna observed were hedonic from the attributes of color, texture, aroma, taste, and overall. Hedonic organoleptics are used to measure the level of panelists' preference for se'i tuna products based on their attributes.

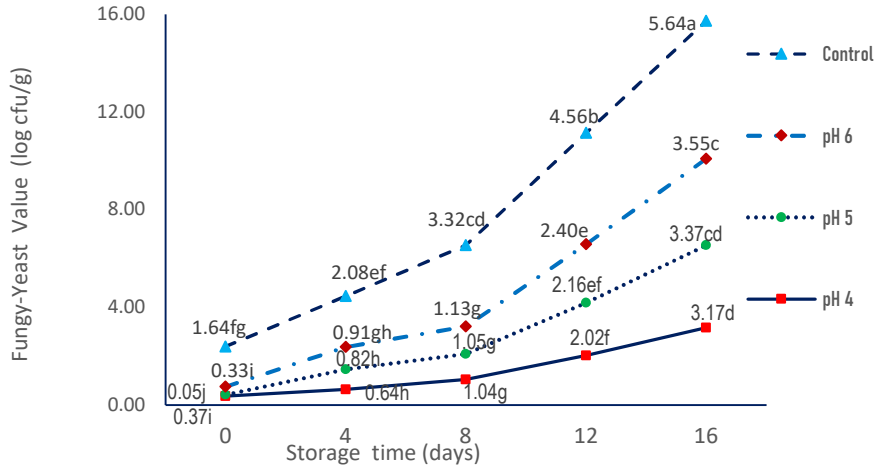
Table-1: Organoleptic Hedonic of Se'i

Parameter	Treatment	Storage (Day)				
		0	4	8	12	16
Color	Control	5.86^{a}	5.75^{a}	5.39^{a}	5.86^{a}	5.14^{a}
	pH 4,0	5.79^{a}	6.00^{a}	5.82^{a}	5.89^{a}	5.64^{a}
	pH 5,0	5.79^{a}	5.54^{a}	5.82^{a}	5.93^{a}	5.89^{a}
	pH 6,0	6.07^{a}	6.14^{a}	6.07^{a}	5.90^{a}	5.71^{a}
Texture	Control	5.00^{ab}	5.00^{ab}	3.85^{d}	3.45^{f}	3.15^{g}
	pH 4,0	5.10^{ab}	5.10^{ab}	4.90^{c}	4.70^{de}	3.75^{e}
	pH 5,0	5.65^{a}	5.05^{ab}	4.95^{c}	4.75^{de}	3.7^{ef}
	pH 6,0	5.00^{ab}	5.00^{ab}	4.90^{c}	4.70^{d}	3.15^{g}
Aroma	Control	6.35^{ab}	5.90^{abc}	4.10^{e}	3.97^{f}	3.55^{g}
	pH 4,0	6.52^{a}	5.35^{bc}	4.92^{c}	4.83^{d}	4.03^{ef}
	pH 5,0	5.66^{b}	5.14^{bc}	4.72^{cd}	4.70^{cd}	4.21^{de}
	pH 6,0	5.21^{bc}	4.97^{c}	4.93^{c}	4.48^{d}	4.45^{d}
Taste	Control	6.08^{ab}	5.96^{ab}	4.85^{de}	3.81^{fg}	3.04^{g}
	pH 4,0	5.84^{b}	5.25^{cd}	5.19^{cd}	4.19^{f}	3.92^{fg}
	pH 5,0	6.73^{a}	5.72^{bc}	5.04^{d}	4.50^{ef}	4.12^{f}
	pH 6,0	5.85^{b}	5.35^{c}	5.39^{c}	4.62^{ef}	4.42^{ef}
Overall	Control	6.11^{a}	5.32^{bc}	4.86^{c}	3.96^{e}	3.39^{f}
	pH 4.0	6.07^{ab}	5.71^{ab}	5.61^{b}	4.57^{d}	3.96^{e}
	pH 5.0	6.11^{a}	6.18^{a}	5.54^{b}	4.71^{cd}	3.76^{ef}
	pH 6.0	6.18^{ab}	5.68^{ab}	4.79^{c}	4.36^{d}	3.89^{ef}

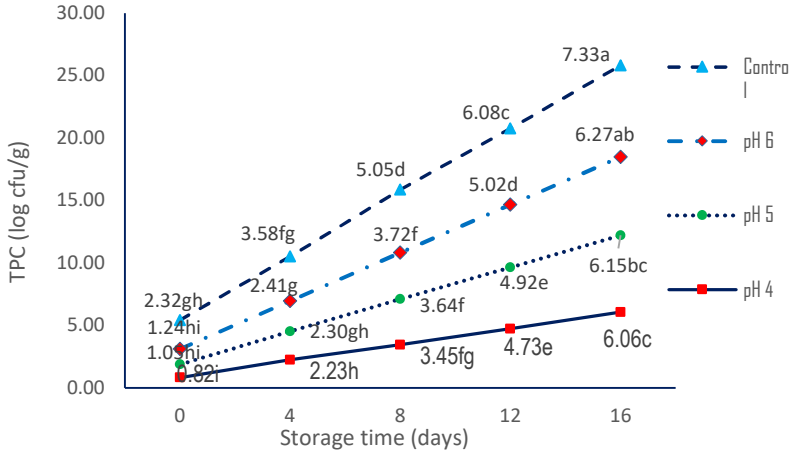
Table-1 shows that the level of preference for the attributes of se'i tuna is influenced by the pH of the curing solution and the storage period ($p < 0.05$), except for the level of preference for the color of se'i tuna. Color is one of the organoleptic attributes of a product that is seen based on visual properties observed using the sense of sight. Color attributes are often used to assess the organoleptic quality of a product based on the level of preference (hedonic) or the intensity of the visible color. The phenomenon of the level of preference for the red color indicates that the se'i product is preferred (average value of 5.64) and does not change with the pH of the curing solution and during storage. The red color of angkak is caused by the compounds rubropunctamine ($C_{21}H_{23}NO_4$) and monascorubramin ($C_{23}H_{27}NO_4$) produced by yeast of the genus Monascus.[21] The level of preference for the color of tuna se'i is reinforced by the color of beef se'i, with a score of 4 (quite like)[22], and smoked skipjack tuna with a score of 5 (quite like).[23] In addition, the color of se'i can be caused by the reaction between carbonyl and dicarbonyl in smoke with free amino acids in food products, which will give a brownish color that interacts with the red color of red yeast rice.

Microbiological Characteristics of Tuna Se'i

The microbiological quality characteristics of tuna se'i can be seen using the total plate count (TPC) and the number of yeast and mold. The limit of a product is still considered suitable for consumption based on its TPC, which is a maximum of $5x10^5$ cfu/g or 5.69 log cfu/g, while its AKK is $5x10^2$ cfu/g or 2.69 log cfu/g (BSN, 2009). The results of observations of the TPC and AKK values of tuna se'i during storage can be seen in Fig.-1.



(B)



(A)

Fig.-1: Total Number of Microbes (A) and (B) Amount of Yeast Molds in Tuna se, i During Storage at Room Temperature

The total number of microbes during storage was seen to continue to increase significantly ($p<0.05$), only the increase in TPC of control tuna se'i was faster than that of preservative tuna se'i. The safe limit for consumption of control tuna se'i is 8 days of storage, while tuna se'i added with preservatives is up to day 12. This shows that the addition of potassium nitrate and potassium propionate preservatives through the curing process can extend the shelf life of tuna se'i. Based on the difference in curing pH, it can be seen that the lower the curing pH, the slower the increase in the number of microbes, whereas the pH 4 curing solution has a lower number of microbes than pH 5 and pH 6. Microbial growth is a factor that influences changes in the organoleptic properties of tuna se'i, although there are other factors, such as water content, that will support microbial growth. The phenomenon of the number of molds and yeasts during storage is similar to the phenomenon of the total number of microbes, namely an increase in the number of molds and yeasts during storage, where the control tuna se'i experienced a higher increase, while in the lower curing pH treatment the increase in the number of molds and yeasts was lower (Fig.-1B). To obtain maximum shelf life, a log data plot of the number of microbes during storage was carried out, as shown in Table-2.
Based on the growth of microbes in se'i tuna given potassium nitrate and potassium propionate preservatives, the maximum shelf life reached 15.58 days. Potassium nitrate is antibacterial with a usage limit of 50 mg/kg, while calcium propionate is antifungal with a maximum usage limit of 2g/Kg of material.[13] This shelf life is much higher than beef se'i, which only reaches 2-3 days. Beef se'i plus liquid

smoke can be stored for up to 7 days (Syarifudin, 2023), and smoked tuna given 2% chitosan has a shelf life of 4 days[7] and vacuum-packed smoked tilapia has a maximum shelf life of 7 days (Hermawan *et al*., 2021).

Table-2: Plot of the Relationship between pH and Storage Time of TPC and the Amount of Yeast Molds of Se'i Tuna During Storage

No	Treatment	Formula	Maximum Shelf-Life
		TPC	
1	Control	$Y_c = 0.3134x + 2.3653$; $R^2 = 0.9975$	8.63 days
2	pH 6	$Y_{pH6} = 0.3168x + 1.198$; $R^2 = 0.9997$	14.22 days
3	pH 5	$Y_{pH5} = 0.3207x + 1.046$; $R^2 = 0.9998$	14.53 days
4	pH 4	$Y_{pH4} = 0.3243x + 0.864$; $R^2 = 0.9996$	15.09 days
		AKK	
5	Control	$Y_C = 0.262x + 1.3533$; $R^2 = 0.9806$	5.18 days
6	pH 6	$Y_{pH6} = 0.1981x + 0.08$; $R^2 = 0.9343$	13.7 days
7	pH 5	$Y_{pH5} = 0.1995x - 0.1027$; $R^2 = 0.9505$	13.67 days
8	pH 4	$Y_{pH4} = 0.1745x + 0.05$; $R^2 = 0.9241$	15.58 days

Natural Red Yeast Dye Compound Composition

The results of compound analysis in red yeast rice using LC-MS are presented in the form of an LC chromatogram (Fig.-7), which shows the presence of 17 peaks or 17 compounds identified based on molecular mass and their role as natural dyes and nutraceuticals (Table-3).

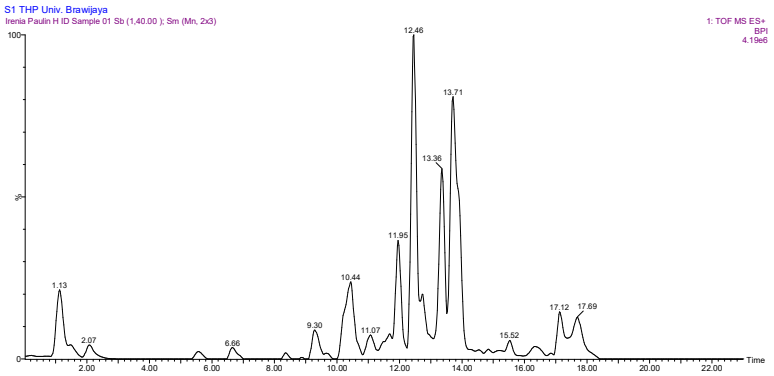


Fig.-2: LC chromatogram of Red Yeast Rice used in the Manufacture of Tuna-Red Yeast Rice se'i

Table-3: Results of LC-MS Analysis of Red Yeast Rice Extract, Identification of Compounds, and their Role or Function as Nutraceuticals

RT	Compound	Molecule Formula
1.13	Pentanamide (Fatty amides/ Valeramide)	$C_5H_{11}NO_2$
2.07	Ethyl-4-hydroxy-8-methoxy-3-quinoline carboxylate (Derivative ethyl ester alkaloid)	$C_{13}H_{13}NO_4$
5.56	Redoxcitrinin	$C_{13}H_{16}O_4$
6.66	2-(4-Methoxyphenyl)ethylamine (Alkaloid derivatives)	$C_9H_{13}NO$
8.35	4'-Hydroxy-5,7-dimethoxyflavanone	$C_{17}H_{16}O_5$
8.88	2-Phenoxyethyl 4-[4-(benzyloxy)-3-methoxyphenyl]-7-(4-methoxyphenyl)-2-methyl-5-oxo-1,4,5,6,7,8-hexahydro-3-quinolinecarboxylate	$C_{40}H_{39}NO_7$
9.30	Octaverine	$C_{23}H_{27}NO_5$
9.41	Hexahydrocurcumin	$C_{21}H_{26}O_6$
10.44	Piperine	$C_{17}H_{19}NO_3$
11.07	Gomisin A	$C_{23}H_{28}O_7$
11.10	Citreoviridin	$C_{23}H_{30}O_6$
11.95	Myricanone	$C_{21}H_{24}O_5$
12.46	Monascin	$C_{21}H_{26}O_5$

13.36	Armillarivin (terpenoid)	$C_{23}H_{28}O_5$
13.71	Ankaflavin	$C_{23}H_{30}O_5$
15.52	Dodemorph	$C_{18}H_{35}NO$
17.12	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$
17.69	Pheophytin b or (Methyl (3S,4S,21R)-14-ethyl-13-formyl-4,8,18-trimethyl-20-oxo-3-(3-oxo-3-{[(2E,7R,11R)-3,7,11, 15-tetramethyl-2-hexadecen-1-yl]oxy} propyl)-9-vinyl-21-phorbinecarboxylate)	$C_{55}H_{72}N_4O_6$

This study directly contributes to SDG 3: Good Health and Well-being by developing safer preservation methods for traditional foods like tuna se'i. The use of a controlled pH curing solution combining potassium nitrate and calcium propionate reduces the risk of harmful nitrosamine formation, which can occur with excessive synthetic nitrate use and is linked to cancer. By extending the shelf life of tuna se'i through natural and regulated preservatives, the research helps ensure food safety, reduces foodborne illnesses, and promotes healthier consumption. This aligns with global health goals to improve food quality, protect consumer health, and support well-being through innovative and safe food technologies.

CONCLUSION

The optimal formulation to enhance the durability and functionality of naturally colored se'i tuna was the curing process using red yeast rice (angkak), potassium nitrate (50 mg/kg), and calcium propionate (2 g/kg) at pH 4. This treatment extended the shelf life of se'i tuna up to 15 days at room temperature while maintaining acceptable sensory attributes (overall hedonic score ≥ 5) and complying with SNI microbial and chemical safety standards. The use of angkak not only contributed to improved visual appeal and antioxidant capacity but also introduced nutraceutical compounds with potential health benefits. The incorporation of safe, natural additives aligns with SDG 3 (Good Health and Well-being) by promoting the development of functional and safe food products that reduce reliance on synthetic preservatives. Further research is recommended to explore vacuum packaging, cold storage, or modified atmosphere packaging in combination with this curing formulation to further extend shelf life while preserving quality. Studies on consumer acceptance and potential nutraceutical bioavailability in angkak-treated products are also suggested.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

This article is related to the SDG-3: *Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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