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Physical, sensory, antioxidant and nutritional properties on Riceberry rice porridge fortified with bio-calcium and protein hydrolysate from salmon (*Salmo salar*) frame for elderly

Pakanun Charoensri [a], Kongkarn Kijroongrojana [a,*], Sineenath Sukkwai [a], Krisana Nilsuwan [b], Soottawat Benjakul [b]

[a] *Program of Food Science and Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla, 90110, Thailand*
[b] *International Center of Excellence in Seafood Science and Innovation, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla, 90110, Thailand*

A R T I C L E I N F O

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A B S T R A C T

This study aimed to enhance the nutrition of Riceberry rice porridge (RB), the color rice which is rich in bioactive compounds, by fortifying with bio-calcium (BC, 300, 444, and 589 mg/100g) and protein hydrolysate (PH, 1.0, 1.25, and 1.5 %) from salmon frames. Increasing in BC or PH levels resulted in increasing the total color difference (ΔE^*) and the difference in Chroma or color intensity (ΔC^*) compares to the control (without BC or PH) ($P < 0.05$). Both fortifications had no adverse on rheology ranging from 790 to 1010 cP ($P > 0.05$). The antioxidant activities including 2,2-diphenyl-1-picrylhydrazyl radical scavenging, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging, Ferric reducing antioxidant power and metal chelating activities of 1.5 % PH sample were two to almost eight times the capability of the control. Nevertheless, BC had no impact on the activities ($P > 0.05$) with values of 350–540 μmol TE/g RB), 2760–3400 μmol TE/g RB, 545–630 μmol TE/g RB, and 17–40 μmol EDTA/g RB, respectively. The RB with 589 mg BC or 1.5 % PH received the highest acceptance scores (6–7) from the 50 elderlies. The fortified RB with both 589 mg BC and 1.5 % PH contained 6 g protein/serving of which glutamic and aspartic acids were most abundant amino acids. Moreover, It provided 294 mg Ca/serving accounting for 36.75 % of Thai Recommended Daily Intake. Therefore, PH and BC are effective ingredients that can supplement a product for managing nutritional deficiencies in the elderly.

1. Introduction

As people age, their ability to chew, swallow, and digest food decreases, which can lead to sarcopenia and malnourishment [1]. Moreover, the reduction of calcium intake in the elderly from several causes i.e. absorption of calcium in the intestinal tract, swallowing disorder, lactose intolerance, medication interactions post menopause, and the chronic disease osteoporosis changes bone formation and strength. Therefore, developing food products for the elderly is crucial. These products should provide adequate energy and nutrients to meet their maintenance needs and be presented in forms that are easy to consume.

Rice porridge is a traditional food in Asia countries, particularly Thailand, China, Japan, Singapore, and the Philippines which is frequently served to persons who have problems with their digestive system, patients who have lost their appetite and the elderly whose ability to swallow has declined [2]. According to Future Market Insights research (2024) [3], the porridge market is anticipated to grow at a compound annual growth rate (CAGR) of 5.5 %. The market value is expected to rise from USD 591.6 million in 2022 to USD 1010.5 million by 2032. The Asia Pacific region, particularly China, holds a significant share of the global porridge market due to its large population and increasing income levels. The market growth in this region is further supported by changing lifestyle patterns and rising demand for convenient and healthy food options. While rice porridge is the most commonly consumed, it is typically made from starchy plants, especially grains like rice, oats, and wheat, which often lack the necessary nutritional value or daily nutrient intake needed by the elderly or patients.

Riceberry rice (*Oryza sativa* L.) is non-glutinous pigmented rice that contains health-promoting compounds such as vitamin E, anthocyanin, γ-aminobutyric acid (GABA), phenolic acids, flavonoids, phytosterol, and antioxidant compounds [4]. Moreover, the biological activities of Riceberry rice including anti-cancer, antioxidant, anti-hyperlipidemic,

* Corresponding author.
E-mail address: kongkarn.k@psu.ac.th (K. Kijroongrojana).

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anti-hyperglycemic, and anti-inflammatory could be due to the abundance of compounds like peonidin-3-glucoside (P3G) and cyaniding-3-glucoside (C3G) [5].

Salmon (*Salmo salar*) meat from salmon frames can be proteolyzed (a peptide bonds hydrolysis reaction) by enzymes. The PH obtained had a higher solubility, dispersibility, and emulsification, and is easily absorbed [6]. In addition, the hydrolysates provide antioxidant activities and are good nutritional composition in which essential amino acids are abundant [7].

Calcium supplements are mostly used in the form of different calcium salts including calcium carbonate and calcium citrate due to the high amount of elemental calcium [8]. Bio-calcium (BC) is peptides chelated calcium which could prevent calcium precipitation in phosphate salt forms [9]. Therefore, the solubility and bioavailability of calcium in BC from salmon frames were higher in comparison with $CaCO_3$ [10].

Riceberry rice, protein hydrolysate, and bio-calcium can provide several desirable compounds. The combination of ingredients may have a synergistic advantage and strengthen one's health among the elderly or patients. Thus, this study aimed to investigate the impact of PH and BC supplements on the physical, sensory, antioxidant, and nutritional properties of the RB for maintaining seniors' nutrition.

2. Materials and methods

2.1. Preparation of protein hydrolysate (PH) from salmon frames (Salmo salar)

PH was prepared according to Sinthusamran et al. (2020) [11] and Idowu et al. (2018) [12] with slightly modification. The salmon chuck was homogenized, and hydrolyzed with Protease P1000 until obtained approximately 30 % DH, then inactivated the enzymatic at 90 °C for 15 min. The protein hydrolysate obtained was debittered with 2-butanol, then centrifuged. The supernatant collection (lower fraction) was dehydrated using freeze-dryer.

2.2. Preparation of bio-calcium powders (BC) from salmon frames (Salmo salar)

The preparation of BC has followed the method of Idowu et al. (2020) [13]. Dried salmon bones from part 2.1 were ground with the high-speed blender (Panasonic, Model MX-898N, Berkshire, UK) and further ground using a ball mill machine (Model PM 100, Retsch GmbH, Haan, Germany) The ground bones were sieved through 200 mesh to obtain fine powders with particle sizes less than 75 μm [14].

2.3. Preparation of Riceberry rice porridge (RB)

Riceberry rice (*Oryza sativa* L.) (Mah Boonkrong Rice Brand, Patum Rice Mill, and Granary Public Company Limited, Thailand) was washed, soaked, drained, ground, and sieved through a mesh to obtain particles smaller than 2.0 mm. The ground rice was cooked with water at a ratio of 1:10, boiled for approximately 45 min until excess water evaporated. The cooked rice was combined with chicken soup (6 °Brix) prepared according to Charoensri et al. (2024) [15]. The obtained Riceberry rice porridge was supplemented with 0 (control), 1, 1.25, or 1.5 % PH per 100 g of RB or with 0 (control), 300, 444, or 589 mg BC per 100 g of RB. The RB was then simmered for 5 min, cooled, and subjected to analyses.

2.4. Analyses

2.4.1. Color

Color measurement of the samples was conducted using a colorimeter (C04-1005-631 ColorFlex, Hunter Lab Reston, VA, USA), and the results were reported in the CIE system. The parameters L* (lightness), a^* (redness-greenness), b^* (yellowness-blueness), ΔE^* (total color difference), and ΔC^* (chroma difference) were documented. The calculation of ΔE^* and ΔC^* followed the method by Idowu et al. (2020) [11].

2.4.2. Rheology

The steady rotational shear measurement of the viscosity and flow behaviors of the porridges using a Rheometer (HAAKE RS75, Germany) with the parallel-plate measuring system following the method of Kim et al. (2017) [16] with slight modification. The flow curves were obtained by registering shear stress (τ) at various shear rates (γ) ranging from 0.1 to 100/s in 300 s. The power-law model was used to describe the data on the shear-induced behavior of the porridge.

2.4.3. Antioxidant activities

Prior to analysis, the RB fortified with PH or BC was dissolved in distilled water with RB to water ratio of 1:1. The diluted RB was determined for their antioxidative activities according to Idowu et al. (2018) [12].

2.4.3.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. 1.5 mL of RB was vigorously mixed with the DPPH reagent. After incubation in dark for 30 min, the absorbance of the mixture was measured at 517 nm using a spectrophotometer (Shimadzy, Kyoto, Japan).

2.4.3.2. 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)(ATBS) radical scavenging activity. 7.4 mM ABTS solution was mixed with a 2.6 mM $K_2S_2O_8$ solution and stored in the dark for 12 h. The solution was diluted by mixing 1 mL of ABTS solution with 50 mL of methanol to achieve an absorbance of 1.1 ± 0.02 at 734 nm. The absorbance of sample (1 mg/mL; 150 μL) mixed with 2850 μL of the solution, was measured at 734 nm using spectrophotometer.

2.4.3.3. Metal chelating activity assay. The sample (10 mg/mL; 4.7 mL) was added with 0.1 mL of 2 M $FeCl_2$ and 0.2 mL of 5 M ferrozine. The absorbance of reaction mixture was then read at 562 nm using a spectrophotometer.

2.4.3.4. Ferric reducing antioxidant power (FRAP) assays. FRAB reagent was prepared by mixing acetate buffer (30 mM, pH 3.6), 10 mM TPTZ solution in 40 mM HCl and 20 mM $FeCl_3$ solution in proportion of 10:1:1 (v/v). The diluted RB (100 μL) was mixed with 3 mL of working FRAP reagent and measure for absorbance at 593 nm using a spectrophotometer.

Blank of all activities was prepared with distilled water instead of the sample. All activities were expressed as μmol Trolox equivalent (TE)/g RB; except for metal chelating activity, which was reported as μmol EDTA equivalent/g RB.

2.4.4. Acceptance test

An acceptance test was performed by 50 consumer-type panelists aged 60 and over. The panelists were selected by their familiarity to rice porridge, dietary habits and health consideration. The panelists assessed the RB both with and without PH or BC, in term of liking for appearance, color, odor, taste/flavor, mouth-feel, viscosity, aftertaste, and overall using a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely). The samples labeled with random three-digit codes were served at 50–60 °C in random of presentation. After tasting each sample, panelists rinsed their mouths with bottled water [17].

2.5. Nutritive values of RB fortified with 1.5 % PH and 589 mg BC

The RB was added with 1.5 % PH and 589 mg BC which levels obtained the highest acceptance scores from 2.4.4 and subjected to determination of amino acid contents, nutritional and energy values.

2.5.1. Amino acid composition

The amino acid composition of Riceberry rice porridge fortified with 589 mg of BC and 1.5 % PH was determined via analytical method No. of 994.12 [18].

2.5.2. Nutritional and energy values

The nutrition labeling of PH and BC fortified RB was determined according to Thai nutrition labeling. The analyses consisted of serving size, calories, total fat content, cholesterol content, sodium content, total carbohydrate content, protein content, vitamin (A, B1, B2) and mineral (calcium and iron) using analytical method No. 996.06, 996.06, 981.10, 985.29, 984.27, 984.27, 984.27, 945.38C, and 945.38B, respectively [18].

2.6. Statistical analysis

Experiments were carried out in triplicate, with results presented as mean ± standard deviation. A complete randomized design (CRD) was utilized for physical and chemical analyses. For the acceptance test, a randomized complete block design (RCBD) was implemented. Blocking by the panelists was necessary to account for the variation that may arise from using different ranges of scales of each participant [17]. Data were analyzed using analysis of variance (ANOVA), and mean comparisons were performed using Duncan's Multiple Range Test using SPSS software (SPSS version 10.0 for Windows, SPSS Inc., Chicago, IL, USA). All tests were considered a 5 % confidence level.

3. Results and discussion

3.1. Physical properties of RB fortified with different levels of BC or PH

3.1.1. Color

Table 1 showed that the color of the porridges without BC (control) exhibited a darker color as indicated by lower L^* values (Lightness), compared to those added with BC ($P < 0.05$). Higher L^* values and lower a^* value (Redness) of porridge were observed with increasing levels of BC used. However, no difference in b^* value (yellowness) was noticed among the samples ($P \geq 0.05$). The natural color of Riceberry rice possessed a dark reddish color as influenced by anthocyanin pigment. BC from salmon bones was removed from meat residue as well as blood during alkaline treatment and became a creamy whitish color leading to the increases in L^* value of porridges with lower redness and yellowness. Furthermore, increases in both the total color difference (ΔE^*) and the chroma difference (ΔC^*) values of the porridge were also found when the amount of BC was increased. The total or collective color difference of the porridges was described as the magnitude of a color difference, however, does not indicate in any way in what direction those differences may be. Moreover, the porridges showed positive Chroma or saturation differences which were more Chroma or more saturated than the control (more color intensity). Increasing BC levels demonstrated the high magnitude of a difference between the porridges and more saturated or color intensity than the control. Overall, fortification of BC affected directly the color especially lightness and redness of the porridges, depending on the amount of BC addition. The result was in agreement with Idowu et al. (2019) [19] who observed that the addition of bio-calcium to the cracker led to higher lightness and lower redness when compared the control. Moreover, fish spread fortified with sea bass bio-calcium obtained the higher lightness, redness, and chroma due to heat-stable white milky color of bio-calcium [20].

Color values of RB fortified with PH are depicted in Table 1. Higher b^* (yellowness) of the porridges, were obtained as the level of PH increased. This was in accordance with the higher ΔE^* and ΔC^* in the former. The control (without PH) showed a lighter color as indicated by higher L^* values, compared to those added with PH ($P < 0.05$) except that with 1.0 % hydrolysate. However, L^* and a^* values decreased as the level of PH increased ($P < 0.05$). Overall, the porridge became greyish purple, when PH level increased ($P < 0.05$). The increases in both ΔE^* and ΔC^* values of the porridge were also found when the amount of PH increased. Salmon PH used in the present study was yellowish-pale in color. It was in accordance with the salmon frame PH prepared by Sinthusamran et al. (2020) [11] and Idowu et al. (2018) [12]. The salmon PH had carotenoid pigments due to disruption of the protein-carotenoid complex during protease hydrolysis, and liberating carotenoid [21]. Increasing PH fortification resulted in a rising magnitude of a difference and saturation or color intensity of the porridges ($P < 0.05$).

3.1.2. Rheology

A viscosity curve of all porridges showed a downward trend, representing shear-thinning flow behavior ($n < 1$). A lower apparent viscosity value was obtained in the porridge when the level of BC increased ($P < 0.05$) as shown in Table 1. However, there was no significant decrease in

Table 1
Color and rheological properties of various Riceberry rice porridges (RB) fortified with protein hydrolysate (PH) and bio-calcium (BC) from salmon frame.

Samples	Color properties					Rheological properties		
	L^{a}	a^{a}	b^{a}	ΔE^{a}	ΔC^{a}	Viscosity (mPa•s) at shear rate of 50 s^{-1}	Consistency coefficient (K)	Flow behavior index (n)
Control[a]	30.1 ± 0.1^{dA}	8.36 ± 0.05^{aA}	5.59 ± 0.03^{aD}			970 ± 40^{aAB}	15 ± 2^{aB}	0.32 ± 0.05^{aA}
300 mg BC	32.6 ± 0.2^{c}	8.05 ± 0.06^{b}	5.57 ± 0.03^{a}	3.2 ± 0.5^{d}	0.05 ± 0.02^{c}	950 ± 60^{a}	12 ± 2^{ab}	0.35 ± 0.03^{a}
444 mg BC	34.2 ± 0.2^{b}	7.57 ± 0.08^{c}	5.55 ± 0.06^{a}	8.7 ± 0.7^{b}	0.32 ± 0.06^{b}	840 ± 50^{b}	10.9 ± 0.7^{bc}	0.35 ± 0.02^{a}
589 mg BC	35.5 ± 0.1^{a}	7.19 ± 0.07^{d}	5.53 ± 0.02^{a}	15.4 ± 0.6^{a}	0.69 ± 0.09^{a}	820 ± 30^{b}	9.4 ± 0.5^{c}	0.39 ± 0.02^{a}
1.00 % PH	29.95 ± 0.06^{B}	6.82 ± 0.03^{B}	6.4 ± 0.3^{C}	2.05 ± 0.00^{C}	1.53 ± 0.06^{C}	1100 ± 100^{A}	15 ± 2.5^{AB}	0.33 ± 0.01^{A}
1.25 % PH	29.08 ± 0.06^{C}	6.60 ± 0.07^{C}	7.04 ± 0.03^{B}	2.7 ± 0.1^{B}	2.7 ± 0.1^{B}	910 ± 60^{B}	11.7 ± 0.04^{B}	0.35 ± 0.02^{A}
1.50 % PH	28.92 ± 0.05^{D}	6.53 ± 0.06^{C}	7.65 ± 0.06^{A}	4.36 ± 0.05^{A}	3.67 ± 0.09^{A}	870 ± 80^{B}	11.4 ± 0.9^{B}	0.34 ± 0.03^{A}

All values are means ± standard deviation (n = 9).
[a-f, A–C] Different lower case (a, b, c) and upper cases (A, B, C) superscripts in the same column indicate significant difference within the same group including control ($P < 0.05$).
[a] Control: RB without addition of BC or PH.

apparent viscosity between the control and the porridge added with BC up to 300 mg ($P \geq 0.05$). Through ion-dipole interaction, calcium ions have the potential to form complexes with starch molecules. These interactions may disrupt the hydrogen bonding network within the starch granules, thereby reducing their capacity to absorb water and swell. Moreover, calcium ions could compete with water molecules for binding sites on starch molecules and hinder the formation of a cohesive gel network during cooking or heating, leading to reduced swelling and viscosity in the resulting starch porridge. Furthermore, calcium ions may participate in crosslinking reactions with starch molecules. Excessive crosslinking could result in retrogradation, where the starch gel became more rigid and less prone to swelling. Nevertheless, the Wijayanti et al. (2022) [22] revealed that higher viscosity was observed in mayonnaise added with sea bass bone bio-calcium (0–10 %) when compared that of control due to dense and small droplet size of bio-calcium powder. Moreover, they found that the sea bass bio-calcium at level of 10 % which led to increase firmness, viscosity and consistency of fish spread [20].

The power law model best describes the flow behavior index of the porridges with R^2 values higher than 0.9. The porridge fortified with BC showed a lower consistency coefficient (K) value than that without BC ($P < 0.05$) except for adding 300 mg. However, no significant difference in the flow behavior index (n) was observed among the porridges ($P \geq 0.05$), indicating similar shear-thinning behavior in all samples.

All porridges fortified with PH also shear-thinning behavior as indicated by lower flow behavior index values ($n < 1$) ($P \geq 0.05$). The control (without PH) showed a higher viscosity, compared to those added with PH ($P < 0.05$) except 1.0 % PH. The apparent viscosity at a shear rate of 50 s^{-1} was reduced as the level of PH increased ($P < 0.05$). The samples with 1.25 and 1.5 % PH had similar viscosity values and consistency coefficients (K) ($P \geq 0.05$).

PH contained peptides and amino acids which could interact with starch molecules. These interactions could disrupt the starch-water interactions and affect the swelling capacity of the starch granules. The exposure of charges or polar residues of low molecular weight PH showed a high affinity for retaining water, which might compete with starch for water molecules, thereby reducing the availability of water for starch swelling. Additionally, the proteins might interfere with the formation of a cohesive gel network during heating, finally leading to decreased viscosity of the starch system.

RB with BC or PH at all levels were classified as a honey-like consistency by the National Dysphagia Diet Task Force; NDD 3 (US standard) or The International Dysphagia Diet Standardization Initiative (IDDSI) level 2.

3.1.3. Antioxidant activities

3.1.3.1. DPPH radical scavenging activity. DPPH radical scavenging activity as shown in Fig. 1 has been employed to evaluate the antioxidative properties of compounds as hydrogen donors or free radical scavengers. Furthermore, the DPPH assay is effective for lipophilic compounds [23]. There was no significant difference in DPPH activities of the porridge without and with different BC additions ($P \geq 0.05$). Although salmon BC contained some amino acids that functioned as proton donors such as aspartic and glutamic acids, as well as hydrophilic and lipophilic amino acids, the content was low. Additionally, the BC was fortified with a small amount (300–589 mg/100 g porridge), which might not significantly enhance the antioxidant activities.

Settapramote et al. (2018) [24] reported that Riceberry rice flour provided 14.51–16.13 % DPPH scavenging. Generally, Riceberry rice contained high levels of anthocyanin contents which were responsible for its dark color. Anthocyanins are potent antioxidants that could neutralize free radicals and reduce oxidative stress in the body [25]. In addition to anthocyanins, Riceberry rice is rich in various phenolic compounds including flavonoids and phenolic acids as well as vitamins particularly vitamin E, and minerals e.g. zinc, and selenium which have antioxidant properties [26]. These nutrients work synergistically with anthocyanins and phenolic compounds to enhance the overall activity capacity of the rice. However, the porridge exhibited higher DPPH activity as the PH concentration increased ($P < 0.05$), indicating an enhanced hydrogen donating ability, which led to more effective scavenging of free radicals. The results showed that RB fortified with 1.25 % and 1.5 % PH could donate hydrogen atoms to radicals, forming stable diamagnetic molecules and terminating the radical chain reaction. Idowu (2018) [12] reported that the DPPH radical scavenging activity of salmon PH ranged from 240.15 to 298.81 μmol TE/g sample. Various factors, such as amino acid composition, side chains, and chain length, are known to influence antioxidant activity [23,27].

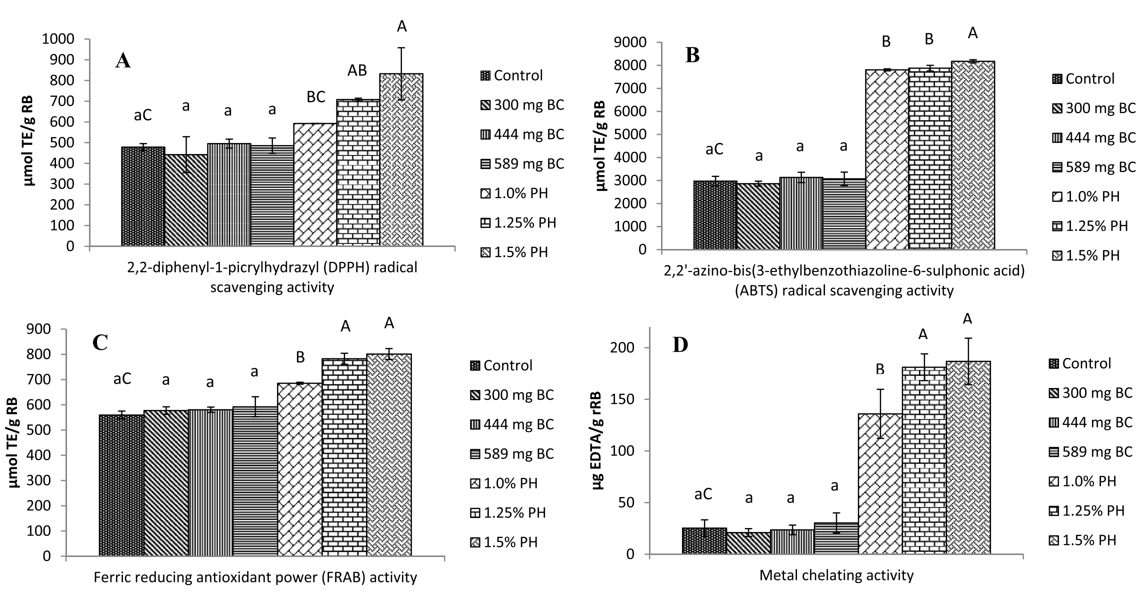

Fig. 1. Antioxidant activities (2,2-diphenyl-1-picrylhydrazyl radical scavenging (A), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging (B), Ferric reducing antioxidant power (C) and metal chelating activities (D)) of Riceberry rice porridge (RB) fortified with Bio-calcium (BC) or protein hydrolysate (PH) from salmon frame. Bars indicate stand derivation (n = 3). [a-f, A−C] Different lower case (a, b, c) and upper cases (A, B, C) superscripts indicate significant difference within the same group including control ($P < 0.05$). *Control: RB without addition of BC or PH.

3.1.3.2. ABTS radical scavenging activity. The ABTS radical scavenging activity assay as shown in Fig. 1 evaluates the ability of antioxidants to donate a hydrogen atom or an electron to free radicals, resulting in the formation of nonradical species [28]. This assay is widely used for assessing both lipophilic and hydrophilic compounds [29]. No difference in ABTS radical scavenging activities of RB added with various BC contents was observed ($P \geq 0.05$). Idowu et al. (2020) [11] reported that BC from salmon frame contained low content of both hydrophilic and lipophilic or hydrophobicity amino acids. Nevertheless, the activities notably increased when fortified with PH, reaching 2.6 to 2.8 times higher compared to the control. Idowu (2018) [12] reported that the PH provided ABTS radical scavenging activity in the range of 418.34–470.12 μmol TE/g sample. The higher antioxidative capacity of PH demonstrated the peptides' ability to scavenge and stabilize free radicals, thereby slowing down the chain reaction.

3.1.3.3. Ferrous reducing antioxidant power (FRAP). FRAP as shown in Fig. 1 measures a compound's ability to donate an electron to free radicals [23]. It is typically used to assess a compound's capacity to reduce the TPTZ-Fe (III) complex to the TPTZ-Fe(II) complex, or ferric iron (III) to ferrous iron (II). The FRAP values of porridges with added BC did not significantly differ from the control ($P \geq 0.05$), while the addition of PH notably increased FRAP ($P < 0.05$). The reducing properties are dependent on the electron-donating capacities of peptides and amino acids, particularly hydrophobic amino acids like alanine, isoleucine, leucine, methionine, phenylalanine, proline, and valine [28,30]. High amounts of low-molecular-weight peptides enhance electron donation to free radicals, thus terminating the chain reaction [31]. The tested compounds demonstrated greater radical scavenging and reducing power at higher concentrations. This result aligns with Idowu et al. (2018) [12], who reported FRAP values of PH from salmon frame ranging from 722.64 to 753.72 μmol TE/g. Additionally, FRAP values for tuna PH and peptide fractions of 1–5 kDa were 2254.50 ± 30.35 and 2251.85 ± 28.03 μmol TE/mg protein, respectively [9]. The greater reducing power indicates that RB fortified with PH can donate electrons to free radicals, thereby preventing or slowing the propagation phase [32].

3.1.3.4. Metal chelating activity. BC additions had similar effects on the metal chelating activity of the porridges ($P \geq 0.05$). Nevertheless, the continuous increase in metal chelating activity of the porridges with PH was noticeably enhanced by 5.4–7.4 times when compared to the control. All PH fortifications had peptides capable of chelating the prooxidative metal. Idowu (2018) [12] found that the metal chelating activity of salmon PH was 503–562.84 μmol EDTA/g sample. The variation in metal ion chelating activity at different PH levels (1.0 and 1.25–1.50 %) may be attributed to differences in the number of peptides. Peptides containing histidine have the capacity to trap radicals and sequester metals due to the imidazole ring [33]. Hydrolysate derived from salmon frames likely acts as a secondary antioxidant, capable of chelating prooxidative metal ions [34]. Metal ions are effective prooxidants that can accelerate the initiation process; therefore the ability of hydrolysate to chelate these ions can help prevent lipid oxidation.

The results of the antioxidant activities revealed the potential of PH to scavenge or neutralize free radicals and bind to metal ions, thereby reduce oxidative stress, which is a major contributor to inflammation, aging and aging-related diseases. This occurs through key mechanisms such as hydrogen and electron-donating ability, metal ion chelation, inhibition of lipid peroxidation. These properties alleviate cellular damage linked to chronic conditions, including cardiovascular and metabolic diseases [35].

3.1.4. Acceptance test

The effect of BC at various levels on the acceptability of RB is shown in Table 2. There are no differences in acceptance scores of appearance, taste, and mouth-feel between the control and the samples fortified with all BC levels ($P \geq 0.05$) which ranged from 7.1 to 7.3 (like moderately). BC was a fine powder (particle size less than 75 μM) and tasteless, therefore it did not impact on appearance, taste, and mouth-feel of the porridge sample. However, a significant decrease in thickness, color, odor, and viscosity liking score was observed when increased BC levels up to 589 mg ($P < 0.05$). Decreasing in color likeness was related to lighter color measurement when addition of BC as shown in Table 1. With an increase in BC levels, the porridge turned to a lighter color. However, the sample with 300 mg BC obtained the highest color likeness. Thickness and viscosity likeness also decreased as BC levels increased due to a decrease in apparent viscosity at a shear rate of 50 s^{-1} as depicted in Table 1. Furthermore, the aftertaste likeness of the porridge slightly decreased when increasing in BC levels. This may be affected by the residue of BC particles after swallowing. Although the particle size of BC was reduced to lower than 75 μm, the consumers could perceive the residue when BC was added at higher concentrations. In addition, the odor likeness of the porridge decreased as BC concentration increased ($P < 0.05$). The BC used in the present study was treated with hexane to remove the formation of offensive odor or rancidity. However, at the high amount of BC up to 444 mg, the consumers could detect the offensive odor, which led to a lower odor acceptance score. The most prevalent volatiles observed in Bio-calcium powders were aldehydes which contributed to off-flavor and off-odor and were used as indicators of lipid oxidation [36]. Butanal, a benchmark for flavor degradation in fish products, and hexanal were also observed in BC [12,37]. In addition, BC from pink salmon also contained ketones (2-octanone, 2-propanone, 2-hexanone, and 2-butanone) and alcohols from the decomposition of hydroperoxides [38]. The overall liking scores of RB declined when adding BC at 444 mg BC and higher. However, no differences in acceptance scores of samples with 589 mg

Table 2
Mean acceptance scores of Riceberry rice porridge (RB) with different levels of bio-calcium (BC) and protein hydrolysate (PH).

Samples	Appearance^{ns}	Thickness	Color	Odor	Taste^{ns}	Mouth-feel^{ns}	Viscosity	Aftertaste	Overall
Control[a]	7 ± 1	6.8 ± 0.9^{ab}	7 ± 1^{ab}	7.2 ± 0.8^{a}	7 ± 1	7.0 ± 0.9	7 ± 1^{ab}	7.0 ± 0.9^{ab}	7.3 ± 0.8^{a}
300 mg BC	7.3 ± 0.9	7 ± 1^{a}	7.3 ± 0.9^{a}	7.1 ± 0.9^{a}	7.2 ± 0.8	7.2 ± 0.9	7.1 ± 0.9^{a}	7.2 ± 0.8^{a}	7.4 ± 0.8^{a}
444 mg BC	7.1 ± 0.9	6.9 ± 0.9^{ab}	7 ± 1^{ab}	7 ± 1^{ab}	7 ± 1	7.1 ± 0.8	7 ± 1^{ab}	7.0 ± 0.9^{b}	7 ± 1^{b}
589 mg BC	7.0 ± 0.9	7 ± 1^{b}	7 ± 1^{b}	7 ± 1^{b}	7.1 ± 0.9	7.0 ± 0.8	7 ± 1^{b}	7 ± 1^{b}	7 ± 1^{b}
Samples	Appearance^{ns}	Thickness	Color^{ns}	Odor	Taste	Mouth-feel	Viscosity	Aftertaste	Overall
Control[a]	7.1 ± 0.8	7 ± 1^{x}	7.0 ± 0.9	7 ± 1^{xy}	7.3 ± 0.9^{x}	7 ± 1^{x}	6.9 ± 0.8^{x}	7.1 ± 0.9^{x}	7.3 ± 0.9^{x}
1.00 % PH	7.0 ± 0.8	6.8 ± 0.9^{x}	7 ± 1	7 ± 1^{y}	7 ± 1^{y}	6.7 ± 0.9^{y}	6.7 ± 0.9^{xy}	6.7 ± 0.9^{y}	7 ± 1^{y}
1.25 % PH	7.1 ± 0.8	7 ± 1^{x}	6.9 ± 0.9	7 ± 1^{xy}	6.9 ± 0.9^{y}	7 ± 1^{y}	7 ± 1^{y}	7 ± 1^{y}	7 ± 1^{y}
1.50 % PH	7 ± 1	6 ± 1^{y}	7.0 ± 0.9	7 ± 1^{x}	7 ± 1^{xy}	6.8 ± 0.9^{y}	7 ± 1^{y}	7 ± 1^{y}	7 ± 1^{y}

Values are expressed as mean ± S.D., n = 50.
Different superscript letters (a,b,c) and (x,y,z) in the same column indicate significant differences within the same group including control ($P < 0.05$).
ns: non-significance, Acceptance score: 1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely.
[a] Control: RB without addition of BC or PH.

and 444 mg BC were noted (P ≥ 0.05).

Similar result was observed by Wijayanti et al. (2022) [20], who found that the addition of BC from sea bass bone (0–10 %) had no impact on odor, flavor, taste, and sandy, mouth-feel likeness scores of fish spread samples (P > 0.05). However, the spread with 10 % bio-calcium gained the highest overall acceptability.

The effect of PH at various levels on the acceptability of RB demonstrated that no differences in appearance and color acceptance scores between the control and samples fortified with all concentrations of PH were observed (P ≥ 0.05). The thickness likeness was declined when adding PH up to 1.5 % (P < 0.05). The PH addition at all levels led to a decrease in mouth-feel, viscosity, aftertaste, and overall liking scores. In addition, decreases in odor and taste liking scores were observed in the sample supplemented with PH at low concentrations. Nevertheless, the scores of 1.5 % PH porridge were not different from the control.

Appearance and color likeness of the samples were mostly affected by the color of Riceberry rice which possessed a dark purple color contributed by anthocyanin [39]. Although PH containing astaxanthin which is a red-orange carotenoid pigment impacted L^*, a^*, and b^* values as demonstrated in Table 1, the changes did not influence color likeness. Moreover, PH which is an amorphous powder and hygroscopic can be soluble completely due to the low molecular weight of peptides and become homogenous in the porridge. Thereby, it did not influence the appearance of the sample. PH fortification led to a decrease in taste-liking scores when compared to the control. Salmon PH contained metallic, fish, trimethylamine (TMA), and rancid flavor [40]. It also provided a bitter taste. Although the PH used in this study was debittered by the removal of hydrophobic amino acids with butanol, the bitterness remained at a low extent. However, there was no difference in the scores among samples with various PH concentrations (P > 0.05). This effect was attributed to umami compounds like L-glutamic acid, L-aspartic acid, and short peptides present in the PH [41]. Umami compounds not only contribute to the umami taste but also can enhance or suppress other tastes. They exhibit a unique synergy among the five basic tastes [42]. The porridges with 1.5 % PH obtained the highest odor and taste likeness which were not different from the control. Moreover, bitter taste detection and recognition thresholds of the elderly panelists increased due to a decline in bitter taste sensitivity. The suppression of bitterness in foods when interacting with the substances e.g. the bitter-umami interaction that umami substances have been proposed to suppress the bitterness of various chemicals in human sensory evaluation was reported by Kim et al. (2015) [43]. Additionally, thickness, mouth-feel, and viscosity likeness decreased when added with high PH levels which attributed the decreased apparent viscosity and consistency coefficients as previously mentioned in Table 1. Yarnpakdee et al. (2012) [44] found that fortifying milk with 0.3 % protein hydrolysate from Indian mackerel led to a decrease in fishy flavor, taste and overall liking scores most likely due to fishy odor caused by lipid oxidation of the protein hydrolysate. Additional, Singh et al. (2022) [45] studied on biscuit supplemented with salmon frame hydrolysate from 0 to 25 g/100 g of the total weight and found that adding the hydrolysate higher than 15 g/100 g led to decreased likeness score for taste and flavor. This also related to lower overall-likeness score due to the presence of fishy odor. Moreover, Idowu et al. (2019) [19] showed that fortification of BC (2.5–10 %) and PH (2.5–10 %) into whole wheat crackers likeness resulted in decrease in all sensory attributes.

Although the liking scores of each attribute for the sample with the highest PH (1.5 %) or BC (589 mg/100 g RB) content tended to decrease, the overall liking score were still within the moderately like range, suggesting the potential of the elderly market. Therefore, considering both health benefits and acceptability, these levels were chosen for further study.

3.2. Amino acids and nutritional value of Riceberry rice porridge fortified with bio-calcium (BC) and protein hydrolysate (PH) powders from salmon frames

3.2.1. Amino acid content

The amino acid composition of the porridge fortified with 589 mg BC and 1.5 % PH, expressed as mg/100 g, is shown in Table 3. The RB contained all essential amino acids, except tryptophan. The glutamic acid/glutamine (272 mg/100 g porridge), aspartic acid/asparagine (223 mg/100 g porridge), L-alanine (188 mg/100 g porridge), and glycine (183 mg/100 g porridge) were abundant amino acid present in the RB. The result was similar to several previous studies showing that, among all amino acids, glutamic acids and aspartic acids were found to be higher in most of the Atlantic salmon (*Salmo salar*) PH [33,46], while tryptophan was not detected [47]. Hydrophobic amino acids including leucine (179 mg/100 g porridge), proline (149 mg/100 g), valine (105 mg/100 g), phenylalanine (83.9 mg/100 g), isoleucine (79.2 mg/100 g), and methionine (20.4 mg/100 g) and branched-chain amino acids such as valine, proline and leucine, provide bitter taste [48]. It was noted that the porridge contained hydroxyproline (57.5 mg/100 g), indicating that collagen derivatives were present. During hydrolysis of fish frame (fish collagen), especially localized in bone or muscle, the collagen could be solubilized and hydrolyzed by protease used. The PH used had a high content of amino acids and could serve as a supplement in food to compensate for imbalanced dietary protein. Furthermore, amino acids exerted a significant influence on sensory attributes, including two umami amino acids (glutamic acid and aspartic acid), six bitter amino acids (tyrosine, isoleucine, leucine, valine, phenylalanine, and lysine), five sweet amino acids (threonine, serine, proline, glycine, and alanine), and two sulfur-containing amino acids (cystine and methionine). Free amino acids are not only crucial for flavor but also act as flavor precursors involved in the Maillard reaction. This reaction generates volatile compounds and contributes significantly to the robust meaty aroma of the samples [49].

3.2.2. Nutritional and energy values

Nutritional values of RB fortified with 589 mg BC and 1.5 % PH are presented in Table 4. The sample with a serving size of 200 g had a total energy of 100 kcal (30 kcal energy from fat). The major component was carbohydrate (11 g), followed by protein (6 g) and total fat (3.5 g). The

Table 3
Amino acids composition of Riceberry rice porridge (RB) fortified with 589 mg Bio-calcium (BC) and 1.5 % protein hydrolysate powders (PH).

Amino acids	Quantity (mg/100g)
Essential amino acid	
Histidine	152
Isoleucine	79.2
Leucine	179
Lysine	171
Methionine	20.4
Phenylalanine	83.9
Tyrosine	54.9
Threonine	89.3
Tryptophan	Not detect
L-Arginine	149
Valine	105
Non-essential amino acid	
L-Alanine	188
Aspartic Acid	223
Cystine	<10.0
Glycine	183
Glutamic acid	272
Proline	149
Serine	99.9
Hydroxylysine	<10.0
Hydroxyproline	57.5

Table 4
Nutritional values of Riceberry rice porridge (RB) fortified with 589 mg Bio-calcium (BC) and 1.5 % protein hydrolysate powders (PH).

Nutrient	Quality (per 200g porridge)	% Daily value
Total Fat (g)	3.5	5
Saturated fat (g)	1	5
Cholesterol (mg)	less than 5	1
Protein (Nx6.25)(g)	6	
Total Carbohydrate, Include DF (g)	11	4
Dietary Fiber (g)	1	4
Sugars (g)	1	
Sodium (mg)	330	17
Vitamin B1 (mg)	0.04	2
Vitamin B2 (mg)	0.02	0
Calcium (mg)	294	35
Iron (mg)	0.40	2

protein content of RB might be contributed by PH, BC, and chicken soup which contained 82.01, 12.07, and 11.64 % protein, respectively.

The micronutrients of the RB included 330 mg sodium and 294 mg calcium Salmon BC fortified in the porridge contained 294 mg Ca, and 136 mg P, presenting as hydroxyapatite along with collagenous proteins [19]. Recommended Nutrient Intakes (RNIs) of calcium and protein for adults as well as children aged 19 years and older are 1000 mg/day and 50 g/day, respectively [50]. Thus, the RB provides Ca and protein at levels of 36.75 % and 12 % of the Thai RDI Recommendation (800 mg per day), respectively, which could be classified as an excellent source of Ca and a good source of protein. Moreover, the fortification with PH was previously shown to have potential antioxidant activities which are crucial for elderly health.

Therefore, the Riceberry rice porridges fortified with PH and BC had gained significant potential benefits for elderly health. The PH from salmon frames offered high-quality protein that was easily digestible, supporting muscle maintenance and immune function of the elderly. Moreover, Bio-calcium derived from salmon frames was a bioavailable form of calcium, which was easily absorbed and provided other beneficial minerals such as phosphorus and magnesium which worked synergistically with calcium to support bone health [51]. The porridge provided 294 mg of calcium per serving; it could contribute a significant portion of the elderly's daily calcium needs. The Riceberry rice porridge fortified with BC and PH from salmon frames provided a balanced nutritional profile for of the elderly.

4. Conclusions

Fortification of BC and PH on Riceberry rice porridge improved the calcium levels, amino acid contents, and antioxidant activities, as would be expected, but it also affected other qualities that depended on the concentration used. All concentrations tested had benefits on the nutritional values of rice porridge but the optimal concentrations of BC and PH were 589 mg and 1.5 %, respectively, and were the most acceptable by elderly consumers.

However, the bioavailability testing and *in vivo* studies of the porridge should be further conducted to determine the amount of the fortified nutrients that are accessible and effective for the elderly individuals in real-life situations.

CRediT authorship contribution statement

Pakanun Charoensri: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Kongkarn Kijroongrojana:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Conceptualization. **Sineenath Sukkwai:** Methodology, Investigation, Formal analysis. **Krisana Nilsuwan:** Methodology. **Soottawat Benjakul:** Resources, Funding acquisition.

Ethical approval

The evaluation received ethical approval from the Health Science Human Research Ethics Committee of Prince of Songkla University, Thailand (HSc-HREC: 63-037-1-1).

Declaration of competing interest

The authors declare no conflict of interest.

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Data availability

Data will be made available on request.

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