Effect of Carotenoid Extract from Dried Chili Pepper (*Capsicum annuum*) on Growth Performance and Pigmentation in Cooked Giant River Prawn (*Macrobrachium rosenbergii*)

Siripong Wongphonprateep* and Abdulkareem Kasirak

ABSTRACT

Buyers often evaluate cooked shrimp based on the size and color of the animals. The objective of this study was to enhance the color of giant river prawn (*Macrobrachium rosenbergii*) by using a crude extract from chili pepper (*Capsicum* spp.). Three types of chili pepper, namely Korean chili (*C. annuum*), red chili pepper (*C. annuum*), and Guinea pepper (*C. frutescens*), were processed with absolute ethyl alcohol to extract carotenoids. Korean chili peppers and red chili peppers were identified as suitable options for enhancing prawn color due to their affordability and high carotenoid content. Subsequently, the chili pepper extracts were encapsulated in alginate microcapsules at ratios of 0, 25, and 50 $mg \cdot kg^{-1}$ and fed to the prawns for a duration of 60 days. The study indicated no significant differences in terms of survival, growth, or feed utilization among the three treatment groups ($p>0.05$). However, prawns that received 50 $mg \cdot kg^{-1}$ of chili pepper extract demonstrated an improved ability to store pigment in their muscles and showed potential for converting ß-carotene into other derivatives. The crude extracts obtained from prawn meat revealed the presence of a xanthophyll sub-derivative with an absorption value ranging from 470–500 nm that accumulates in the muscles.

Keywords: *Capsicum annuum*, Enhancing color, Natural carotenoid extracts, Prawn, Vivid color

INTRODUCTION

It has been well accepted that incorporating vital elements like carotenoids into feed can enhance color of cooked shrimp (Amaya and Nickell, 2015). In addition, carotenoids function as antioxidants and can boost immunity (Delgado-Vargas and Paredes-Lopez, 2003), which has positive implications for both the well-being of the prawn and the satisfaction of consumers.

The color of crustaceans plays a crucial role in determining their quality and market price. These creatures cannot produce pigments internally, so they rely on their diet to accumulate pigments, whereby astaxanthin and β-carotene are the primary pigments that determine their body color. Carotenoids, which can be obtained from natural sources or artificially synthesized compounds, are commonly used as colorants in animal feeds (Ashokkumar *et al*., 2023). However, the production of synthesized carotenoids is a more complex and costly process (Szucs *et al*., 2011; Ruangdej and Laohavisuti, 2014).

Peppers (*Capsicum annuum*) are extensively utilized in diverse culinary traditions worldwide due to their distinctive flavors, which can vary from spicy and hot to sweet or occasionally sour (Mohd Hassan *et al*., 2019). Furthermore, the distinct color and taste of chili pepper extracts make them highly desirable for inclusion in various medical products (Palevitch and Craker, 1996). They can be used to add color to food or to decorate products

Department of Agricultural Science, Faculty of Agriculture, Rajamangala University of Technology Srivijaya, Nakhon Si Thammarat, Thailand
*Corresponding author. E-mail address: siripong.w@rmutsv.ac.th
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in order to attract consumer's attention (Parisenti *et al*., 2011; Amin, 2016; Dave *et al*., 2020). Chili pepper are also renowned for their substantial nutritional value, as they are abundant in vitamin A, vitamin C, and a variety of carotenoids. The carotenoid content typically ranges from 1,500 to 2,500 $mg \cdot kg^{-1}$ in chili pepper (Yanar *et al*., 2016). Lutein, violaxanthin, neoxanthin, and β-carotene are among the primary compounds identified in *C. annuum* (Villa-Rivera and Ochoa-Alejo, 2020); in red chili peppers, Mohd Hassan *et al*. (2019) reported 28.47±19.34 mg of β-carotene per 100 g of fresh weight. Aquatic animals, specifically shrimp and crab, possess the ability to convert ß-carotene into astaxanthin (Benjakul, 2011). This conversion occurs when these animals consume carotenoids as part of their diet and absorb them into their bodies. The process involves the formation of compounds such as crustacyanin and carotenoproteins, which bind with the carotenoids and ultimately lead to the production of astaxanthin, chemically known as 3,3'-dihydroxy-ß, ß-carotene-4,4'-dione (Parisenti *et al*., 2011). When changes occur in the proteins present in shrimp muscles, they release the stored carotenoids (Pongsetkul *et al*., 2014).

Farming of giant river prawn (*Macrobrachium rosenbergii* [De Man, 1879]) contributes around 5% of global shrimp and prawn aquaculture production (FAO, 2022). Although most of the production is sold as live prawn (Yang *et al*., 2012), the quality, especially color after cooking, is also important. The color of cooked prawn could be enhanced using 200 $mg \cdot kg^{-1}$ feed of synthetic astaxanthin (Kumar *et al*., 2009). However, astaxanthin is a high-value coloring substance that needs to be imported from abroad (Wade *et al*., 2015). Therefore, the exploration of local products that contain abundant carotenoids, such as chili peppers, is crucial. This exploration is based on the capacity of crustacean animals, like shrimp and prawn, to modify and utilize a variety of carotenoid types. The main objective of this study was to investigate the effect of carotenoids from chili pepper on growth and color of the giant river prawn after cooking. The insights gained from this research will be valuable in commercial applications using giant river prawns and other shrimp species.

MATERIALS AND METHODS

A preliminary study was conducted to determine the amount of carotenoid extract that could be produced from three types of chili pepper: Korean chili powder, red chili powder (prepared from whole dried chili pepper), and Guinea chili powder. Korean chili and Guinea chili were purchased pre-ground (Chung Jung One and Raitip brands), while the whole dried chili pepper was obtained from a farmer in Pak Phanang District, Nakhon Si Thammarat Province, Thailand, and was ground before use.

The peppers were finely ground and subjected to extraction by fermentation using 20 g pepper powder and 1,200 mL absolute ethanol. The extraction process continued until the solution became clear and colorless (approximately 96 h). The solvent was then evaporated using a vacuum evaporator (Rotavapor R-3; Buchi).

Carotenoid analysis

The maximum absorption range (400–700 nm) was measured using UV-visible spectrophotometer in scan mode (Orion Aquamate 81000; Thermo Scientific), following the method described by Rodriguez-Amaya and Kimura (2004). The carotenoid content was determined by calculating the UV-visible absorption value at approximately 450 nm. The extracted substances from the three varieties of peppers belonged to the ß-carotene group, consistent with the description provided by Rodriguez-Amaya and Kimura (2004). Then the extraction of carotenoids was encapsulated in micrometer particles using the ionotropic gelation method described by Pongjanyakul (2012). This involved spraying a mixture of $CaCl_2$ and Alginate (in a 1:1 ratio) into the solvent solution of carotenoid with alginate, while continuously stirring with a magnetic rod. After allowing the mixture to settle for a day, the clear part was removed. The quantity of carotenoids trapped in the particles was calculated using the equation provided by Rodriguez-Amaya and Kimura (2004).

Feed preparation

The basal diet selected for the experiment was a commercial feed (9043; CP., Thailand), including a minimum crude protein content of 32%, crude lipid content of 4%, a maximum crude fiber content of 6%, and a maximum moisture content of 12%. To incorporate the pepper extracts into the feed, the commercial feed was sprayed with the encapsulated pepper extracts in a colloidal suspension state at proportions of 0, 25 and 50 $mg \cdot kg^{-1}$. Following this, the treated feeds were left to air-dry for 24 h at room temperature. All the prepared feeds were then stored at 4 ºC until they were ready for feeding.

Feeding trial

A total of one hundred male giant river prawns (average weight = 11.70±1.42 g) were obtained from a private farm in Suphanburi Province, Thailand. The all-male *Macrobrachium rosenbergii* was used to avoid potential growth inhibition of males caused by peptide hormones from females, which has been reported in crustaceans (Guo *et al*., 2018; Liu *et al*., 2021). The prawns were acclimated for four weeksin a fiberglass tank measuring 1 m wide, 2 m long, and 1 m high, filled with 2 t of water. During the acclimation period, the prawns were fed a control diet (without chili extract) four times a day at specific intervals (09.00 a.m., 12.00 a.m., 4.00 p.m. and 8.00 p.m.).

After the acclimation period, the prawns with similar size (11.80±0.20 g initial body weight) were assigned to one of three treatments (different concentrations of the encapsulated carotenoid: 0, 25, and 50 $mg \cdot kg^{-1}$ feed), with eighteen prawns assigned to each treatment. To prevent cannibalism, the prawns were individually housed in plastic boxes measuring 20 cm in width, 28 cm in length, and 11.8 cm in height, which were placed on a set of shelves. The structure, with three shelves, was made of steel and measured 200 cm in width, 48 cm in length, and 120 cm in height. Eighteen boxes were positioned on each shelf. Each box was securely attached to a drainage channel using a 3/4-inch PVC joint to facilitate the downward flow of water from one box to another, leading into a 300-liter plastic container designed to collect the water. A 25-watt pump continuously pumped the water up to the top shelf through PVC pipes. Each prawn served as an experimental unit (Figure 1).

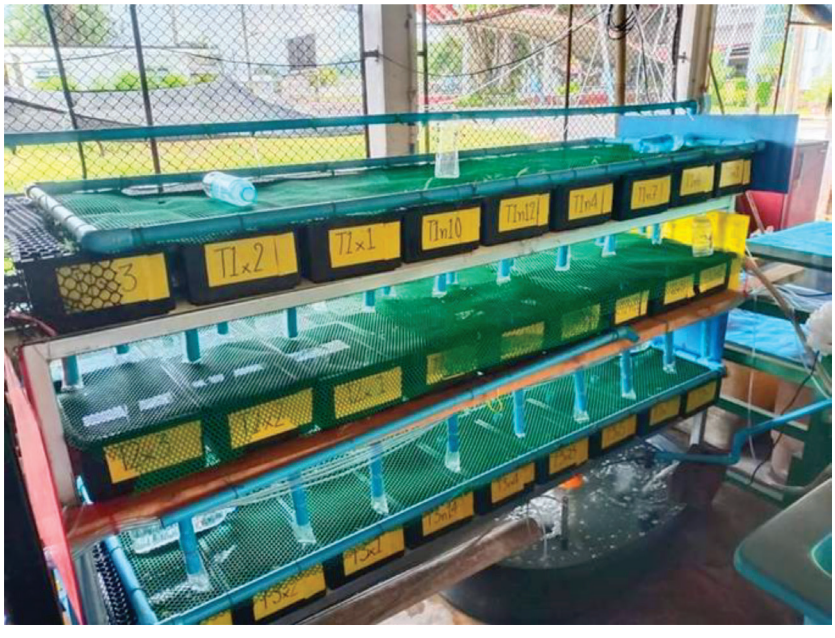

Figure 1. Water recirculating system for prawn feeding experiment.

The feeding protocol for the experiment was the same as during the acclimation period. A recirculating water system with continuous aeration was employed to maintain dissolved oxygen levels above 5 $mg \cdot L^{-1}$. Water quality was adjusted using lime materials ($CaCl_2$, $MgCl_2$, $Ca[HCO_3]_2$) to achieve an alkalinity value of 120 $mg \cdot L^{-1}$ as $CaCO_3$ and a hardness value of 200 $mg \cdot L^{-1}$ as $CaCO_3$ (Tapparangsee *et al*., 2013). During the trial, the pH and temperature were measured four times a day using a portable pH and temperature meter (Lutron pH-223). The water samples were collected and analyzed using a Lovibond MD 600 spectrophotometer and tablet for ammonia (M60-Ammonia T) and nitrite (M270-Nitrite T) levels. Calcium, magnesium, alkalinity, and hardness were measured every three days using Para-Test (Aquacare). Water changes were performed every seven days at 50%.

Experimental design and monitoring of prawn growth rate

The treatments were assigned following a completely randomized design (CRD) with three treatments (each experimental set used 18 prawns). The time it took for prawns to reach a satisfied state during each feeding was estimated to be 15 min. Individual body weight was determined for random samples of 10 prawns per treatment every 30 days. Average daily gain (ADG), specific growth rate (SGR), survival rate (SR) and feed conversion ratio (FCR) were determined using equations 1–5:

Weight gain (WG; g) = final weight-initial weight (1)

Average daily gain (ADG; $g \cdot day^{-1}$)
= weight gain/rearing period (days) (2)

Feed conversion ratio (FCR)
= feed consumed/weight gain (3)

Specific growth rate (SGR; $\% \cdot day^{-1}$)
= [Ln (final weight)-Ln (initial weight)]
×100/days (4)

Survival rate (%) = [(final count of prawns)/(initial count of prawns)]×100 (5)

Color measurement

At the end of the experiment (day 60), ten prawns per treatment were euthanized using the hypothermia method by placing them in ice. Subsequently, the deceased prawns were boiled at 100 ºC for 1 min. Shell color parameters were then measured using the Konica Minolta CR-400 instrument. Measurements were taken on both sides of the shell (left and right) and on the 2nd carapace from the cephalothorax. The measured CIE parameters included Lab* (L* for lightness, a* for red-green, and b* for yellow-blue) and CIE LCH* (L* for lightness, C* for chroma, and h for hue angle). The color measurements can convert qualitative values into quantitative ones, potentially identifying statistical differences.

Quantification of carotenoid in prawn shell

After boiling the prawns, the shell was peeled away from the body and the skin was carefully removed to access the dermis layer, which was about 1 cm thick. We conducted a hexane extraction protocol similar to the one used for extracting carotenoids from peppers (1 g:100 mL). The resulting solutions were then examined to determine their maximum absorption value.

Carotenoid analysis

The thin-layer chromatography technique was utilized to ascertain the relative rates of flow of the beta-carotene standard solution, crude extract derived from chili pepper, and the crude extract sourced from giant river prawns fed at 50 $mg \cdot kg^{-1}$. The retention factor (Rf) is used to measure the movement of compounds along the TLC plate. Rf is defined as the distance travelled by an individual component divided by the total distance travelled by the solvent. The obtained value is always between zero and one.

Statistical analysis

Data were statistically analyzed using one-way ANOVA. The differences between means were tested using Levene's test and the Scheffe method. The tests were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Carotenoid analysis

The results revealed that the extracted substances from the three varieties of peppers belonged to the ß-carotene group, consistent with the description provided by Rodriguez-Amaya and Kimura (2004). The carotenoid content of the three pepper varieties was as follows: 55.216± 0.795 μg·g^{-1} for Korean Chili pepper, 42.562±0.397 μg·g^{-1} for red chili pepper and 28.562±0.292 μg·g^{-1} for Guinea pepper. Based on these results, red chili pepper was selected for use in the experiments due to its acceptable carotenoid content and lower cost compared to Korean chili.

Effect of the chili extract on prawn growth rate

The growth trial results showed no statistically significant differences among means of each growth parameter measured at day 30 and day 60 (Table 1 and 2). However, it was noted that prawns fed a carotenoid mixture from red chili pepper at a dose of either 25 or 50 mg·kg^{-1} tended to have a lower survival rate at the end of the trial.

The growth rate of prawns remained unaffected by the utilization of carotenoid extract derived from chili peppers. Nevertheless, prawns that were provided with carotenoids at concentrations of 25 and 50 mg·kg^{-1} displayed a greater appetite in comparison to the control group, and thus FCR tended to increase although without statistical support. This might be a result of capsaicin present in the extract. Capsaicin has been found to notably reduce the accumulation of body fat in mice, while their energy metabolism was enhanced (Ohnuki *et al*., 2001). This mechanism might have been triggered in the prawns fed chili pepper extract in the present study and resulted in no growth difference relative to that of the control although they consumed a larger quantity of feed, as seen from their higher FCR. This finding is consistent with a study conducted by Göçer *et al*. (2006) on tiger shrimp (*Penaeus semisulcatus*) and in another study on trout (Yanar *et al*., 2016). Furthermore, the presence of capsaicin, the compound responsible for the

Table 1. Growth parameters of giant river prawns fed with diets supplemented with chili pepper extract at 30 days of rearing.

Treatment	WG (g)	ADG (g·day^{-1})	FCR	SGR (%·day^{-1})	Survival (%)
0 mg·kg^{-1}	2.41±1.15	0.08±0.04	2.61±1.01	0.59±0.30	100
25 mg·kg^{-1}	2.32±1.36	0.08±0.05	2.99±1.80	0.65±0.42	94.44
50 mg·kg^{-1}	3.36±2.37	0.11±0.08	2.45±2.22	0.94±0.74	83.33
p-value	0.481	0.312	0.462	0.314	-

Note: WG = weight gain; ADG = average daily growth; FCR = feed conversion ratio; SGR = specific growth rate

Table 2. Growth parameters of giant river prawns fed with diets supplemented with chili pepper extract at 60 days of rearing.

Treatment	WG (g)	ADG (g·day^{-1})	FCR	SGR (%·day^{-1})	Survival (%)
0 mg·kg^{-1}	5.36±2.95	0.18±0.10	2.44±1.68	0.60±0.32	100
25 mg·kg^{-1}	4.64±2.70	0.15±0.09	2.59±1.78	0.58±0.31	88.89
50 mg·kg^{-1}	5.59±2.55	0.19±0.09	1.89±1.26	0.77±0.40	83.33
p-value	0.560	0.591	0.213	0.350	-

Note: WG = weight gain; ADG = average daily growth; FCR = feed conversion ratio; SGR = specific growth rate

spiciness of chili peppers, can have a long-term impact on the prawn's digestive tract and might account for the trend in survival reduction. Notably, examination of dead shrimp carcasses during the experiment revealed unsuccessful molting, which is vital for crustacean growth. This contrasts with a study by Amin (2016), which reported that prawns receiving lutein-mesozeaxanthin extract from marigolds exhibited greater weight gain, specific growth rate, and growth coefficient compared to prawns not receiving marigold extract. This suggests that carotenoids can enhance nutrient utilization and energy expenditure in aquatic animals.

Throughout the experiment, water quality fell within the recommended range for freshwater aquaculture (National Bureau of Agricultural Commodity and Food Standards, 2009): temperature was 24–26 ºC; average ammonia content 0.12±0.01 $mg \cdot L^{-1}$; average nitrite level<0.05 $mg \cdot L^{-1}$; average calcium concentration 42.22±9.2 $mg \cdot L^{-1}$ as $CaCO_3$; average magnesium concentration 42.22±9.2 $mg \cdot L^{-1}$ as $CaCO_3$; average hardness 622.22±62.9 $mg \cdot L^{-1}$ as $CaCO_3$; and average alkalinity 98.78±14.7 $mg \cdot L^{-1}$ as $CaCO_3$.

Color of cooked giant river prawn

The cooked giant river prawns were assessed for their shell color after being administered different amounts of carotenoids from chili peppers (0, 25, and 50 $mg \cdot kg^{-1}$). The brightness (L*) of the prawns did not show statistically significant difference ($p>0.05$) among treatment groups. However, the measurement of redness (a*) was higher for the prawns receiving 50 $mg \cdot kg^{-1}$ compared to the other treatments ($p<0.05$).

Moreover, both the yellow value (b*), chroma (C*) and the change in color angle (H*) displayed similar trends. This implies that when the shrimp received carotenoids extracted from chili peppers at a level of 50 $mg \cdot kg^{-1}$, it resulted in an enhanced skin tone (Table 3 and Figure 2).

Carotenoid in prawn shell

The crude extract of prawns fed with 25 $mg \cdot kg^{-1}$ of carotenoids displayed a maximum absorption range of 460–473 nm. Similarly, when fed 50 $mg \cdot kg^{-1}$ of carotenoids, the prawns exhibited a maximum absorption range of 448–474 nm. In comparison, the control group exhibited a maximum absorption value of beta-carotene dissolved in hexane at 450–477 nm. This indicates that the substances accumulated in the prawns mainly consisted of beta-carotene, with minor quantities of other carotenoid derivatives. The beta-carotene from the consumed feed accumulates in the prawn muscles which contain green, blue, and purple color pigments. When the prawn is cooked, the protein compounds release carotenoids, causing a color shift from green or blue to yellow, orange, and red. The absorption of these carotenoids takes place within a wavelength range of 470–472 nm (Parisenti *et al*., 2011), with astaxanthin being detectable at 476 nm (Dave *et al*., 2020). The results of the present study indicate that prawns fed with carotenoids from chili peppers exhibited different carotenoid derivatives compared to the control group. However, the maximum absorption value for these derivatives still fell within the range of 470–472 nm. On the other hand, the retention factor (Rf) values in thin layer chromatography were 0.96, 0.96, and 0.98 for beta-carotene standard solution, crude extract

Table 3. Color of cooked giant river prawn fed different carotenoid concentrations in diet for 60 days.

Treatment	L*	a*	b*	C*	H*
0 $mg \cdot kg^{-1}$	70.23±2.52^{a}	4.39±1.79^{a}	13.09±2.32^{a}	13.84±2.68^{a}	72.02±5.04^{ab}
25 $mg \cdot kg^{-1}$	71.04±1.32^{a}	5.18±0.86^{a}	13.45±1.15^{ab}	14.43±1.19^{a}	68.93±3.20^{b}
50 $mg \cdot kg^{-1}$	71.33±1.04^{a}	7.51±0.86^{b}	15.78±0.91^{b}	17.49±0.97^{b}	64.56±2.65^{a}
p-value	0.542	0.002	0.020	0.006	0.013

Note: Means ±SD in each row superscripted with different lowercase letters are significantly ($p<0.05$) different; L*= lightness; a* = red-green coordinate; b* = yellow-blue coordinate; C* = chroma; H* = hue angle

Figure 2. Color of cooked giant river prawn fed 0, 25 and 50 mg·kg^{-1} carotenoid from chili pepper extract for a rearing period of 60 days.

derived from chili pepper, and crude extract sourced from giant river prawns fed at 50 mg·kg^{-1}, respectively. These findings suggest that prawns have the ability to convert β-carotene from chili peppers into various other carotenoid derivatives. The crude extracts obtained from prawn meat revealed the presence of a xanthophyll sub-derivative with an absorption value ranging from 470–500 nm that accumulates in the muscles. In a study conducted by Goodwin (1984), it was described that crustaceans possess a mechanism to convert β-carotene into canthaxanthin, a process similarly noted by Harpaz *et al*. (1998). However, identification of the specific compound involved in the present study was not possible due to the requirement for advanced investigative tools (Maoka, 2020). To make the most of the subtle observable changes in shell coloration among prawns, it is suggested that studies be conducted on ornamental shrimps that exhibit vibrant red shell coloration.

CONCLUSION

Preliminary screening of three varieties of chili (red chili pepper, Korean pepper, and Guinea chili) indicated that red chili pepper had a high content of carotenoids in absolute ethanol extract. The primary carotenoid identified was β-carotene. The supplementation of encapsulated β-carotene in the feed of giant river prawn at levels of 0, 25, and 50 mg·kg^{-1} of food for durations of 30 and 60 days did not have a significant impact on prawn growth. However, survival rates tended to decrease

in groups fed with chili extract doses. Nevertheless, the prawns that received carotenoids extracted from red chili peppers at a dosage of 50 $mg \cdot kg^{-1}$ showed an enhanced skin color.

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