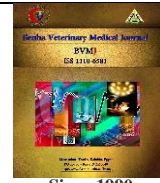




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Cowpea 11S globulin spray quadruples shrimp shelf-life and maintains freshness

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ABSTRACT

Shrimp preservation is of interest to food scientists and processors because of their restricted shelf life caused by microbial spoilage, black spot formation, and the associated financial losses and food safety risks. Current study was conducted to determine the preservative impacts of Cowpea (*Vigna unguiculata*) 11S globulin (CPEG) spray on tiger shrimp (*Penaeus monodon*) shelf-life and physicochemical characteristics, as well as optimum field preservative levels. Fresh peeled tiger shrimp were sprayed with either 100µg/ml (CPEG1) or 200µg/ml (CPEG2) of CPEG at a rate of 2 ml/100 gm and refrigerated at 2±0.5°C. The control shrimp received only sterile distilled water. After 12 storage days, CPEG2 sprayed shrimp displayed a steadily raising pH trend, reaching 7.3, whereas control and CPEG1 sprayed shrimp demonstrated a V-pH curve. Though was not entirely significant, but 200 µg/ml of CPEG spray improved shrimp WHC and drip loss attributes. Compared to the apparent increasing curves of control shrimp's native microbial curves, which culminated in clear spoiling on the eighth day, CPEG-treated shrimp at both doses did not exceed 6 log cfu/gm for the entire twelve-day chilling period. Also, both CPEG levels exhibited a significant antioxidant impact compared to the control. The retarding effect of CPEG on treated shrimp native microbial curves and malondialdehyde was dose dependent. Conclusively, both CPEG levels kept the fresh tiger shrimp's organoleptic aspects and postponed the spoilage beyond twelve-days. Therefore, the shrimp's shelf-life can potentially be extended while preserving its safety and quality by using the CPEG spray, which works best at 200 µg/ml.

1. INTRODUCTION

Aquaculture is anticipated to grow and increase substantially by 2050, nearly doubling current production (FAO, 2022). Aquatic food products are extremely perishable due to their biological content (Tahiluddin et al., 2022). In particular, shrimp's popularity as a food stems from its high content of high-quality fatty acids and protein while being low in carbohydrates, as well as a great nutritional supply of specific vitamins and minerals such as vitamin B3, folate, calcium, magnesium, phosphorus, and potassium (Wright et al., 2018). Fish spoils quickly due to both internal and external influences (Ghaly et al., 2010). Intrinsically, shrimp's high water, free amino acid, and unsaturated fatty acid content makes it more susceptible to deterioration than other muscle meals, resulting in the production of unpleasant aromas and odors (García-Soto et al., 2015). Shrimp are decomposed by three main processes: microbial spoilage, lipid oxidation, and enzymatic autolysis (García-Soto et al., 2015). Enzymes involved in protein hydrolysis, melanin synthesis, and ATP breakdown in shrimp include trypsin-like protease, cathepsin B, polyphenoloxidase, prophenoloxidase, and ATPase. Muscle degradation, melanosis, and umami loss are the results of these processes (Peng et al., 2022). Lipid oxidation is a widespread deteriorative chemical reaction that mostly affects fish species. Fish fat contains more polyunsaturated fatty acids (PUFA) with five or six double bonds compared to meat fat

from mammals, which has mostly two double bonds (Secchi and Parisi, 2016). Psychrotrophic gram-negative bacteria are the main cause of spoiling in chilled seafood. These microorganisms are highly environment-dependent and include *Pseudomonas* species, *Shewanella putrefaciens*, *Aeromonas* species, and *Photobacterium phosphoreum* (Fan et al., 2022). Icing or refrigeration have typically been used to retard different shrimp spoilage including melanosis. However, during iced or refrigerated storage, Psychrotrophic bacteria and endogenous shrimp proteolytic and oxidative enzyme including melanosis still occurs because PPO remains active under these settings (Li et al., 2022). Artificial preservatives are one of the various strategies employed by the fishing industry to decrease or avoid enzymatic browning during shrimp marketing. Sulfite-containing food additives, such as sodium metabisulfite (E 223) and 4-hexylresorcinol, are frequently used to prevent crustaceans from developing melanosis (Sae-Leaw et al., 2017). Customers are dissatisfied of the usage of preservatives since certain substances can have detrimental effects on sensitive individuals. Shrimp storage always results in a change in quality; however for the fishing and food businesses, shrimp preservation is crucial. Investigating preservation techniques to preserve shrimp quality has received a lot of attention (Lin et al., 2022). Thus, there has been a lot of interest towards developing feasible and effective preservation technologies to significantly

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preserve shrimp quality (Peng et al., 2022). Legumes are not only a good source of protein (20.4%), but they are also less expensive than animal protein, linked to several health benefits, including a lower risk of cardiovascular diseases, type 2 diabetes mellitus, and certain types of cancer (Yanni et al., 2024), with well-established functional activity in food technology (Schmidt and Oliveira, 2023) and intriguing antibacterial capabilities (Abdel-Shafi et al., 2019; Ebrahim et al., 2022; Mahgoub et al., 2011; Osman et al., 2014; Sitohy et al., 2011, 2012). So, the current study was designed to estimate the best field preservative dosages and the preservative benefits of Cowpea (*Vigna unguiculata*) 11S globulin (CPEG) spray on tiger shrimp shelf-life and physical-chemical properties.

2. MATERIAL AND METHODS

2.1. Experiment management and approval

Under the number BUFVM 10-06-2023, the Institutional Animal Care and Use Committee Research Ethics number (BUFVTM) of Benha University's Faculty of Veterinary Medicine approved all of the protocols used in this investigation

2.2. Cowpea 11S globulin (CPEG) protein extraction

The two main types of cowpea seed proteins are 7S and 11S globulins, which make up more than 51% of the total seed protein content, while albumins make up about 45%. The 11S fraction has a molecular mass of approximately 350 kDa and is composed of acidic (37–42 kDa) and basic polypeptide (20 kDa) subunits joined by a disulphide bond (Freitas et al., 2004). Cowpea (*Vigna unguiculata* (L.) Walp.) 11S globulin (CPEG) was prepared from defatted powder according to our previous published papers (Abdel-Shafi et al., 2019).

2.3. Sample preparation and distribution

Fresh peeled tiger shrimp (*Penaeus monodon*) weighing 15 ± 5 g each were obtained from a local fish supplier and immediately delivered to the laboratory for analysis. Shrimps were randomly assigned to one of three treatments: no CPEG spray (control), CPEG1, and CPEG2. The individual treatment involved twenty-four shrimp divided into two replicates of twelve pieces each. Each replicate received three shrimps every single day for four days of assessment. Shrimps in the CPEG1 and CPEG2 groups were sprayed with 100 µg/ml and 200 µg/ml of CPEG in sterile distilled water (DW) at a rate of 2 ml/100 gm of shrimp, respectively. After spraying, shrimp were mixed with moderate rotation and left in the zippered bag for 30 minutes to ensure equal dispersion of CPEG throughout all pieces. The same protocols were followed for the control group, which only received Sterile DW. The shrimps were bagged and refrigerated in a Binder KB chilling incubator (BINDER GmbH, Tuttlingen, Germany) at $2 \pm 0.5^\circ\text{C}$. The physicochemical quality, antioxidant stability, and shelf life of the shrimp were assessed on days 1, 4, 8, and 12.

2.4. Physicochemical evaluation of shrimp attributes

2.4.1. pH analysis

The pH was determined by directly introducing pH-meter electrodes (Jenway 3510 pH-meter, Cole-Parmer, Staffordshire, United Kingdom) into the shrimp's first two abdominal segments.

2.4.2. Water-holding capacity estimation

The water-holding capacity (WHC) of shrimp was assessed using the filter paper pressing method, which required pressing 0.2–0.4 g of shrimp on Whatman No. 1 filter paper with a weight of 5 kg for 30 seconds. After removing the top plate, the meat WHC can be computed as the ratio of loose water to the sample's initial weight (Honikel and Hamm, 1994).

2.4.3. Purge estimation

The purge loss at each checking point is calculated as the percentage of shrimp weight loss from the initial weight recorded on the first day of chilling (1, 4, 8, and 12) (Honikel, 1998)

2.5. Microbiological evaluation of shrimp

2.5.1. Aerobic plate count

The aerobic plate count (APC) of shrimp samples was determined on standard plate count agar in the same way that beef and chicken gibbet samples were earlier measured using the surface spread technique (Gamil et al., 2024; Sabike et al., 2015). A homogenous suspension (10%) of each sample was prepared by aseptically weighing 10g of the sample and mixing it with sterile 90 mL of distilled water using the Stomacher 400R (Seward, UK). The resulting sample homogenates were serially diluted tenfold in sterile distilled water. Then, one milliliter of each dilution was surface-plated into sterile Petri dishes, with two separate plates for each dilution. The solidified inoculated plates were then incubated for 24 hours at 37°C (ISO, 2013). Colonies were determined and reported as log colony-forming units per gram of food (cfu/g).

2.5.2. Coliform count

Coliform enumeration was measured using the same tenfold serial dilutions of sample homogenates previously produced for APC, except Violet red bile agar (Himedia Laboratories, India) was used and incubated for 24 hours at 37°C (International Organization of Standardization, 2006).

2.6. Malondialdehyde evaluation of shrimp

Malondialdehyde (MDA) levels in homogenized shrimp samples were evaluated using HPLC (Agilent HP 1200 series equipment, USA) according to the previous established procedure (Ahmed-Farid et al., 2017).

2.7. Organoleptic evaluation of shrimp

Based on ISO 8586-2012 sensory evaluation guidelines (International Organization of Standardization, 2023), five highly qualified panelists from the department were chosen to evaluate the color, texture, and odor of shrimp. When alterations happened during storage, each component was given a score on a continuous demerit point scale ranging from 1 to 10. (Figure 1).

2.8. Statistical analysis

SPSS Version 22 was used to analyze the data (SPSS Inc. Chicago, IL, USA). General linear mixed models (LMM) were implemented to examine the effects of treatments (control, CPEG1, and CPEG2), chilled-storage intervals (1, 4, 8, and 12 days), and their interactions on shrimp physicochemical, microbiological, and antioxidant parameters. The means and standard errors of the results are displayed. The effects of CPEG and their levels in relation to the control were measured by the statistical model using Tukey's b multiple comparison test, which was also utilized

to assess the significance of differences between storage point averages of the same treatment. A P-value of less than 0.05 was used to identify significant differences.

3. RESULTS

Statistically, CPEG, chilling time, and their interaction had a significant impact on all physicochemical parameter indices, except for water holding capacity (WHC), which was exclusively affected by chilling period (P < 0.05) (Table 2, figure 1). Higher CPEG-pretreated shrimp had lower pH levels on the first and eighth days, while shrimp with low CPEG concentration pretreated shrimp had the highest pH, with the control group in the middle of the range (P < 0.05). When comparing control shrimp to shrimp treated with CPEG, drip loss was significantly higher at the fourth and twelve chilling periods. The pH rose with an extended chilling period in all shrimp groups. While the WHC of the CPEG-treated shrimp tended to increase over time, the

control shrimp's WHC showed a slight decline. Longer chilling periods tended to increase drip loss in control shrimp, but not in CPEG-treated shrimp, especially in shrimp with low CPEG contents (100µg/g).

CPEG addition, chilling duration, and their interaction all had a statistically significant effect on bacterial hygiene indicators (Table 2). The native aerobic count of control shrimp showed a clear increasing pattern with prolonged chilling times, reaching evident spoiling on the eighth day. CPEG-treated shrimp at both doses remained below 6 log cfu/gm for the whole twelve-day chilling period. CPEG-treated shrimp at 200µg/gm drastically decreased coliform growth curve, which clearly commenced at the 8th day of chilling and continued thereafter. The growth retardation effect of CPEG in treated shrimp was dose dependent on all indices, with the exception of the initial count of APC, which was lower in shrimp treated with low CPEG contents than with higher ones

Table (1): the consequences of Cowpea 11S globulin (CPEG) inclusion levels on tiger shrimp physico-chemical traits

Traits	Period (Days)	Control	CPEG		SEM	P values		
			CPEG1	CPEG2		CPEG	Time (T)	CPEG *T
pH	Zero	7.19 ^{hAB}	7.31 ^{ab}	6.92 ^{cc}	0.012	0.001	<0.001	0.002
	4 th	7.09 ^{AB}	7.09 ^C	7.13 ^B				
	8 th	7.14 ^{hB}	7.31 ^{ab}	7.20 ^{hB}				
	12 th	7.50 ^A	7.45 ^A	7.30 ^A				
WHC	1 st	93.11	91.05 ^A	91.62 ^{AB}	0.826	0.900	<0.001	0.668
	4 th	82.62	80.48 ^B	78.36 ^C				
	8 th	92.12 ^b	94.64 ^{aa}	90.01 ^{cAB}				
	12 th	90.90	93.43 ^A	96.03 ^A				
Drip loss	1 st	4.47 ^B	6.27	4.91 ^{AB}	0.352	0.019	0.011	0.026
	4 th	6.69 ^{hB}	4.32 ^b	2.05 ^{cc}				
	8 th	3.43 ^B	5.82	2.80 ^{AB}				
	12 th	12.36 ^{hA}	5.28 ^b	5.64 ^{hA}				

¹ CPEG, Cowpea 11S globulin; CPEG1, 100 µg/ml of Cowpea 11S globulin; CPEG2, 200 µg/ml of Cowpea 11S globulin; WHC, water holding capacity; standard error mean. ² Different small letters within the row show significant effect of treatments (P < 0.05), while different capital letters within the column indicate significant differences across chilling times.

Table (2): the antimicrobial effects of different Cowpea 11S globulin (CPEG) inclusion levels on native shrimp bacteriological, oxidative indices as well as shelf life

Traits	Period (Days)	Control	CPEG		SEM	P values		
			CPEG1	CPEG2		CPEG	Time (T)	CPEG*T
APC	1 st	4.43 ^{cc}	4.26 ^{bc}	4.42 ^{hB}	0.029	<0.001	<0.001	<0.001
	4 th	5.53 ^{hB}	4.02 ^{bd}	3.62 ^{cd}				
	8 th	7.48 ^{hA}	4.76 ^{hB}	4.00 ^{cc}				
	12 th	7.66 ^{hA}	5.98 ^{hA}	5.15 ^{ca}				
Coliform	1 st	2.72 ^C	2.15 ^D	2.00 ^C	0.027	<0.001	<0.001	<0.001
	4 th	4.04 ^{hB}	2.95 ^{bc}	2.00 ^{cc}				
	8 th	5.92 ^{hA}	4.84 ^{hB}	4.08 ^{hB}				
	12 th	6.15 ^{hA}	5.90 ^{hA}	4.92 ^{ca}				
MDA	1 st	28.66 ^D	27.72 ^A	29.83 ^A	0.501	<0.001	<0.001	0.016
	4 th	33.6 ^C	19.1 ^B	19.28 ^B				
	8 th	34.89 ^{hB}	29.64 ^{hA}	28.84 ^{hA}				
	12 th	37.8 ^{hA}	28.14 ^{ca}	28.71 ^{ba}				

¹ CPEG, Cowpea 11S globulin; CPEG1, 100 µg/ml of Cowpea 11S globulin; CPEG2, 200 µg/ml of Cowpea 11S globulin; APC, aerobic plate count; MDA, Malondialdehyde; standard error mean. ² Different small letters within the row show significant effect of treatments (P < 0.05), while different capital letters within the column indicate significant differences across chilling times.

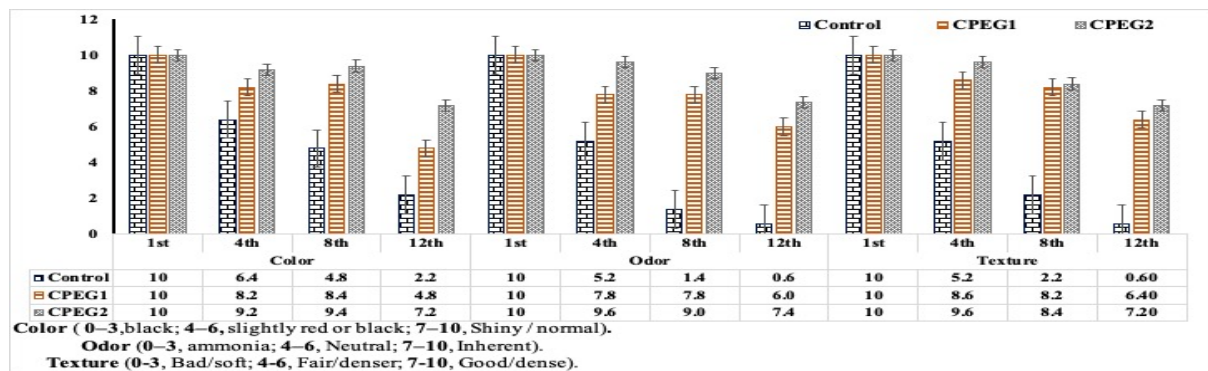


Figure 1. the consequences of Cowpea 11S globulin (CPEG) spray levels on tiger shrimp sensory traits (color, texture, and odor). A continuous demerit point scale with a range of 1 to 10 was used to grade the sensory components. Control, no CPEG; CPEG1 = 100 µg/ml and CPEG2 = 200 µg/ml.

Figure 1 reveals that the SBEG, chilling duration, and their combination had a substantial impact on all organoleptic criteria. Both high and low SBEG levels were able to retain acceptable fresh organoleptic indices for more than twelve days ($P < 0.05$) when compared to spoiled control shrimp after eight chilling days at 2.5°C. With a demerit estimation score range of 7.2 to 7.4, CPEG2 maintained its original smell, shiny/normal color, and nice dense texture numerically. A very low range of 6 to 2.2 was noted in the control sample, but shrimp sprayed CPEG1 showed a demerit organoleptic evaluation score range of 4.8 to 6.4. The CBEG spray maintained the shrimp's color lighter than the control one even after a 12-day period, according to visual inspection in Figure 2.

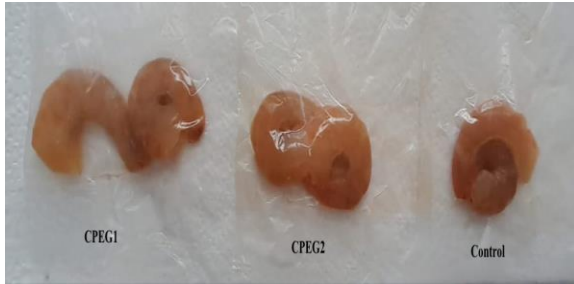


Figure 2. The visual properties of tiger shrimp treated with cowpea 11S globulin (CPEG) compared to the control after 12 day aerobic chilling at $2\pm 0.5^\circ\text{C}$. CPEG1 = 100 µg/ml and CPEG2 = 200 µg/ml

4. DISCUSSION

Cowpea protein is not as widely used in the production of processed foods as soy (*Glycine max* L.) (Robbani et al., 2022), although having a similar nutritional makeup. This is because less is known about the biotechnological characteristics of cowpea protein. Surprisingly little research, aside from a few noteworthy studies that concentrate on microbiological qualities, has been published on the physicochemical and preservation influences of cowpea protein on shrimp (Abdel-Shafi et al., 2019; Osman et al., 2021). One of the reference standards for determining the freshness of shrimp muscle is to measure variations in its pH value (Kural et al., 2008). While published studies found that shrimp pH levels between 6.5 and 7.5 provide ideal conditions for bacterial growth (Xu et al., 2019), others proposed that pH 7.7 or lower is adequate for shrimp freshness (Mu et al., 2012), which is consistent with current pH ranges. The accumulation of alkaline substances, such as ammonia and amines, produced by the disintegration of amino acids, peptides, and protein is associated with an increase in pH, but the drop in initial pH in control and CPEG1 sprayed shrimp could be attributed to the shrimp's glycogen glycolysis occurring an hour after death (Dai et al., 2016). In contrast, CPEG2 sprayed shrimp showed no decrease but rather a steadily rising trend till reaching 7.3 after 12 storage days. This could imply that higher doses of CPEG have a considerable inhibitory effect on these processes, glycogen glycolysis, or alkaline substances, which are primarily boosted by autolytic endogenous enzymes as well as microorganism enzymes. During 12 days of storage, shrimp treated with CPEG2 had the lowest aerobic plate, coliform, and malondialdehyde levels, as well as pH, compared to all other treatments, confirming a CPEG inhibitory effect. The lower trend in WHC and larger drip loss with longer storage time is due to degrading changes in the structure of the muscle cytoskeleton protein, including collagen molecules, myofibrils, and extracellular matrix structure caused by enzymes and microbes. As a result, the structure between the myofibrils loosens, reducing WHC

and causing muscle texture softening, decreased flexibility, and quality degeneration (Xu et al., 2019). The control shrimp's native aerobic count showed a discernible rising curve with longer chilling intervals, culminating in clear spoilage on the eighth day. Psychrotrophic gram-negative bacteria are very resistant and adaptable to low temperatures, and they could restore their reproducing capacity following temperature fluctuations with prolonged storage time (Zhang et al., 2015). For the full twelve-day period of chilling, the CPEG-treated shrimp at both doses did not exceed 6 log cfu/gm. Shrimp sprayed with 200 µg/gm of CPEG significantly reduced the coliform growth curve, which was evidently initiated on the eighth day of chilling and persisted subsequently. All indices showed that the growth retardation impact of CPEG on treated shrimp was dosage dependent. According to previously published findings, cowpea seed protein hydrolysates (CPH) and their peptides shown high antibacterial efficacy with MIC ranges of 25 to 150 µg/mL against nearly all evaluated spoilage and pathogenic microorganisms (Osman et al., 2021). Cowpea 11S globulins cause entire membrane degeneration, cell swelling, and vacuole development, which eventually leads to full cell lysis (Abdel-Shafi et al., 2019). CPEG levels exhibited a substantial antioxidant effect compared to the control, resulting in a V-shaped trend in malondialdehyde over the twelve storage days. Previously published study demonstrated antioxidant potential of Cowpea Seed derived protein that reduced myoglobin oxidation and undesirable coloring caused by metmyoglobin (Abdel-Shafi et al., 2019). Obvious control shrimp off-odor at the 8th chilling day is mainly attributable to spoiling bacteria reached more than 7 log CFU/gm. H_2S -producing bacterial species can manufacture hydrogen sulfide, ammonia, and pyruvic acid through the action of cysteine desulfhydrase (Lapin & Koburger, 1974). Moreover, NH_3 is produced during storage by the metabolism of proteins and amino acids in aerobic bacteria. The offensive odor of decomposed shrimp may also be caused by other volatile organic compounds (VOCs), which depend entirely on bacterial species. These VOCs include butanone, acetone, methyl mercaptan, dimethyl disulphide, ethyl acetate, acetic acid, and 1,2-butanediol, 2-propanol, 2-pentanone, and butanone. Specific spoilage organisms (SSOs) in shrimp during postmortem storage may vary based on the shrimp category, growth region, and storage conditions (Lin et al., 2022).

Furthermore, oxidative degradation of polyunsaturated fatty acids in shrimp contribute the generation of off-flavors and off-odors (Zhang et al., 2015). The color of seafood is critical in terms of consumer perception of quality and has a significant role in consumer purchasing decisions (Zhang et al., 2015). The considerable drop in shrimp lightness values during storage was previously attributed to changes in light absorption and scattering induced by the denaturation of shrimp proteins (Lopkulkiaert et al., 2009). The drop in redness might mostly be attributable to the breakdown of astaxanthin (carotenoid pigment) and lipid oxidation (Sundararajan et al., 2011), as well as melanosis progression (Li et al., 2022). Previously, it was reported that Pacific white shrimp had an eight-day shelf life when kept under ice (Okpala et al., 2014). According to recent organoleptic and microbiological estimates, control tiger shrimp have a shorter shelf life than eight days at 2.5 c. Here, the fresh tiger shrimp's organoleptic characteristics were preserved by both 100 and 200 µg/ml of CPEG, and the aerobic plate count was kept below the spoiling threshold of six log CFU/gm for a period exceeding twelve days.

5. CONCLUSIONS

CPEG demonstrated a dose-dependent significant preservative activity that could extend shrimp shelf-life and acceptable sensory characteristics beyond 12 cooling days. Here, 100 and 200 µg/ml of CPEG maintained the organoleptic properties of the fresh tiger shrimp, and the aerobic plate count was maintained below the spoiling threshold of six log CFU/gm for more than twelve days.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data

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