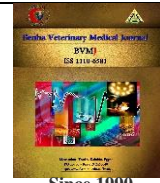




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Soybean 11S globulin spray boosts chilled shrimp shelf-life, physicochemical, and sensory attributes beyond twelve days

Wesam Dawam¹, Mai Elsheikh¹, Ali Osman², Shimaa Edris¹, Mahmoud Sitohy², Islam Sabeq^{1*}

¹Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Benha University, Tukk, Qalyubia 13736, Egypt.;

²Department of Biochemistry, Faculty of Agriculture, Zagazig University (ZU) Zagazig 44519EGYPT;

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ABSTRACT

Strict temperature control, to limit bacterial development, preserve freshness, and give fresh shrimp a longer shelf life, could not be entirely reliable along the production chain, demanding a combined antimicrobial strategy with insignificant implications for delicate shrimp quality. The current study assessed the antioxidant and antibacterial impacts of soybean (*Glycine max* L.) 11S globulin (SBEG) spray on tiger shrimp (*Penaeus monodon*) shelf-life, physicochemical, and sensory traits. Fresh peeled tiger shrimp were sprayed with 100 µg/ml (SESG1) or 200 µg/ml (SESG2) of SESG at a rate of 2 ml/100 g. Hygienic, oxidative, and sensory qualities were monitored every four days throughout twelve days in a refrigerator at 2 ± 0.5 °C. Only sterile distilled water was supplied to the control shrimp. The shrimp's physicochemical parameters were not affected by SESG spray, chilling length, or contact. The pH curve of shrimp sprayed with 200 µg/ml was the lowest and maintained unchanged during the chilling time, contrary to the V-shaped pH curves of the control and SESG1-sprayed shrimp. SBEG's antioxidant and antibacterial properties significantly reduced all shrimp's oxidative and hygienic markers in a dose-dependent manner. Compared to ruined control shrimp after 8 chilling days at 2.5 °C, SBEG levels were effective in suppressing native aerobic spoilage bacteria below six logs cfu/gm and maintaining satisfactory fresh shrimp organoleptic indices beyond 12 days. Shrimp are more perishable than beef and have a shorter chilling shelf life. However, a combination of SESG spray with chilling at 2.5 °C provides a natural and field-applicable preservation strategy.

1. INTRODUCTION

Shrimp is a widely spread and globally favored fishery product, with increasing market demand (Peng et al., 2022). Shrimp are highly perishable and quickly deteriorate after harvesting, resulting in an obvious off-taste and mushy texture due to their high quantity of non-protein nitrogen molecules, autolytic enzymes, and susceptibility to common bacteria (Mastromatteo et al., 2010).

Several mechanisms contribute to the deterioration of shrimp quality during storage (Lin et al., 2022). Endogenous autolytic proteolytic enzymes are the primary internal spoiling mechanism in shrimp immediately after harvest, beginning with chilling before the other two mechanisms, microbial and oxidative alterations. This can be attributed to the higher pH of seafood, which is typically between 6.2 and 6.5 as opposed to 5.5–5.8 in animal muscle (Kontominas et al., 2021).

Externally, *Pseudomonas*, *Alcaligenes*, *Aeromonas*, *Enterobacter*, *Bacillus*, *Enterococcus*, *Psychrobacter*, *Escherichia coli*, *Listeria*, *Brochothrix*, and *Shewanella* species were among the significant genera of spoilage bacteria that have been identified in fresh and processed fish and fisheries products (Tahiluddin et al., 2022). Moreover, lipid oxidation is among the primary non-microbial factors contributing to shrimp quality degradation, much like it is in meat and meat products (Lorenzo and Gómez, 2012). However, compared to other meat products, seafood is more prone to lipid autoxidation due to its significant amounts of unsaturated fatty acids, which initiate the generation of fatty

acid radicals. Lipoxygenase, which is found in seafood tissues or originates from microbial presence in the food, can also catalyze the generation of fatty acid radicals, such as hydroperoxides (Damodaran et al., 2007). Furthermore, polyphenol oxidase (PPO) is an indigenous shrimp oxidative enzyme that degrades quality by hastening the natural post-mortem biochemical process of melanosis (Miraglia et al., 2021).

Conventional techniques for preserving shrimp, like freezing, chilling, and cold storage, are ineffective at preventing spoiling and preserving shrimp quality over extended periods of time (Arancibia et al., 2015). Additionally, some people experience allergic-type reactions when exposed to chemical preservatives such as sulfiting chemicals (Gunnison et al., 1987). Moreover, consumers become more conscious of the health risks associated with chemicals and more resistant to their use in food. Nowadays, consumers are more concerned about the freshness and safety of aquatic products (Peng et al., 2022). Therefore, regulatory bodies are being forced to strictly restrict the use of chemicals in food.

The use of active functional substances (e.g., protein hydrolysates, essential oils, and plant extracts) has demonstrated anti-melanosis, antibacterial, and antioxidant properties, making it an efficient strategy for maintaining shrimp quality (Lin et al., 2022). Certain kinds of legumes, like common and soybean beans, are widely used in the food industry because of their diverse functional qualities (viscosity, emulsion and gelling abilities, foaming) that contribute to texture and sensory attributes. Additionally,

* Correspondence to: islam.sabek@fvvm.bu.edu.eg.

when integrated into meat and other foods, globulins extracted from legumes showed potential preservation capabilities (Abdel-Shafi et al., 2019; Mahgoub et al., 2011; Osman et al., 2018, 2014, 2013; Sitohy et al., 2011, 2012). Considering the quick deterioration mechanism of shrimp that occurs even at cooling temperatures and the adverse impacts on overall quality, the effect of these legume-derived globulins on delaying shrimp deterioration and overall quality, including melanosis, has never been investigated. Therefore, the purpose of this study was to determine the effect of soybean-11S globulin (SEG) on the shelf life and quality of tiger shrimp.

2. MATERIAL AND METHODS

2.1. Experiment management and approval.

The methods employed in this work were approved by the Institutional Animal Care and Use Committee Research Ethics number (BUFVTM) of the Faculty of Veterinary Medicine at Benha University, with the number BUFVM 13-05-2023.

2.2. Soybean 11S globulin (SBEG) preparation

Soybean 11S globulin is one of four fractionated products of soybean protein isolate (SPI), the others being 7S globulin, higher soybean residue, and lower soybean residue (Wei et al., 2021). Soybean 11S globulin (SBEG) preparation extracted from defatted soybean powder according our previous published papers (Sitohy et al., 2012).

2.3. Shrimp preparation and groups distribution.

Fresh peeled tiger shrimp, *Penaeus monodon*, weighing 15 ± 5 g each, were procured from a nearby fish vendor on the day of the experiment and promptly transported to the laboratory for examination. Subsequently, they were randomized and sprayed with either 100 μ g/ml (SESG1) and 200 μ g/ml (SESG2) of SESG, or with sterile distilled water (control). The individual treatment consisted of twenty-four shrimp divided into two replicates of twelve pieces each. For the entire period of each replication, three shrimps were assigned to each of the four storage locations. At a rate of two milliliters per 100 grams of shrimp, the SESG1 and SESG2 shrimp groups were sprayed separately and respectively with a freshly prepared solution containing either 100 μ g/gm or 200 μ g/gm of SESG in sterile distilled water (DW). With the goal for uniform SESG dispersion throughout all pieces, shrimp were stirred by gentle rotation, and then left in the zippered packaging for 30 minutes. The control group was handled using the same methods with Sterile DW. The shrimp were then packed in a bag and stored at $2 \pm 0.5^\circ\text{C}$ in a cooling incubator (Binder KB, BINDER GmbH, Tuttlingen, Germany). On days 1, 4, 8, and 12, the shrimp's physicochemical quality, antioxidant stability, and shelf-life were tested.

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. At room temperature, the pH meter was calibrated using three pH values (10, 4, and 7) and a temperature metal probe.

2.4.2. Water-holding capacity estimation

The water-holding capacity (WHC) of shrimp was assessed using the FPPM 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 assessment of chilled shrimp

2.5.1. Determination of 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).

2.5.2. Determination of coliform count

Coliform enumeration was measured using the same ten-fold 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). Colonies were enumerated and reported in logarithmic colony-forming units per gram of sample.

2.6. Malondialdehyde estimation

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

2.7. Sensory analysis of Shrimps during Storage

To assess the color, texture, and odor of shrimp, five highly skilled panelists from the department were selected based on ISO 8586-2023 (International Organization of Standardization, 2023) sensory evaluation standards. Every component was assigned a score on a continuous demerit point scale from 1 to 10 when changes occurred during storage (Figure 1).

(Figure 1a)

Demerit point scale	Traits	Period (Days)	SBEG			SEM	P values		
			SBEG1	SBEG2	SEM		SBEG	Time (T)	SBEG*
1	Color (0-3:black; 4-6, slightly red or black; 7-10, Shiny / normal)	1 st	10	10	10	0.654	<0.001	<0.001	<0.001
		4 th	6.4	8.2	9.2				
		8 th	4.8	8.4	9.4				
		12 th	2.2	4.8	7.2				
3	Odor (0-3, ammonia; 4-6, Neutral; 7-10, Inherent)	1 st	10	10	10	0.837	<0.001	<0.001	<0.001
		4 th	5.2	7.8	9.6				
		8 th	1.4	7.8	9				
		12 th	0.6	6	7.4				
7	Texture (1-3, Bad/soft; 4-6, Fat/denser; 7-10, Good/dense).	1 st	10	10	10	0.805	<0.001	<0.001	<0.001
		4 th	5.2	8.6	9.6				
		8 th	2.2	8.2	8.4				
		12 th	0.6	6.4	7.2				

(Figure 1b)



Figure 1 illustrates the effects of soybean 11s globulin (SBEG) spray levels on tiger shrimp sensory attributes (a) and visual properties (b). The sensory elements (color, texture, and odor) were graded using a continuous demerit point system with a range of 1 to 10.

Figure 1b compares the visual properties of tiger shrimp sprayed with soybean 11s globulin (SBEG) to the control after 12-day aerobic chilling at $2 \pm 0.5^\circ\text{C}$. Control: no SBEG; SBEG1: 100 μ g/ml; SBEG2: 200 μ g/ml.

2.8. Statistical Analyses

The data was analyzed using SPSS Version 22 (SPSS Inc, Chicago, IL, USA). The effects of treatments (control,

SESG1, and SESG2), chilled-storage intervals (1, 4, 8, and 12 days), and their interactions on shrimp physicochemical, microbiological and antioxidant parameters were investigated using general linear mixed models (LMM). The results are shown with their means and standard errors. The statistical model used Tukey's b multiple comparison test to quantify the effects of SESG and their levels in comparison to the control, and to determine the significance of differences between storage point averages of the same treatment. Significant differences were identified using a *P*-value of less than 0.05.

3. RESULTS

Apart from pH, based on a general linear statistical comparison, the assessed physicochemical parameters were not affected by the SESG treatment (Table 1). Drip loss was not influenced by either fixed or random variable, nor by

their interaction, however chilling time had a substantial impact on pH and WHC findings. Additionally, no noticeable effect was observed on any of the physicochemical shrimp characteristics because of the interaction between treatment and chilling length. Except for the first and last chilling day, when treated shrimp's pH level was lower than control, especially when the SESG level was high—200 µg/gm—the SESG treatment and levels had no consequence. The pH curves of the control and SESG-treated shrimp 100 µg/gm showed an upward trend, however the pH of the high SESG level, 200 µg/gm, remained constant for the entire chilling period. The chilling period did not impact the water holding capacity of control or high SESG-treated shrimp (200 µg/g), while low pretreatment shrimp displayed an ascending curve (*P* < 0.05).

All hygienic indices were strongly impacted by the SBEG, chilling length, and their interaction, according to a general linear statistical comparison (Table 2).

Table 1 displays the effects of soybean 11s globulin inclusion levels on various tiger shrimp physicochemical characteristics.

Groups	Storage (Days)	Control	SBEG		SEM	<i>P</i> value		
			SBEG1	SBEG2		SESG	Time (T)	SESG *T
pH	1 st	7.54 ^{aB}	7.24 ^{aB}	6.92 ^b	0.023	0.000	0.002	0.085
	4 th	7.31 ^C	7.22 ^B	7.20				
	8 th	7.36 ^C	7.21 ^B	7.15				
	12 th	7.78 ^{Aa}	7.55 ^{abA}	7.25 ^b				
WHC	1 st	94.65	90.58 ^{AB}	88.20	0.722	0.497	0.013	0.747
	4 th	85.27	88.24 ^B	85.24				
	8 th	93.08	92.79 ^{AB}	91.35				
	12 th	93.22	95.08 ^A	94.22				
Purge loss	1 st	6.49	5.80	7.92	0.669	0.150	0.491	0.657
	4 th	9.58 ^a	6.63 ^{ab}	3.97 ^b				
	8 th	5.71	3.60	3.68				
	12 th	10.68	6.51	3.58				

¹SESG, soybean 11s globulin; SBEG1, 100 µg/ml of soybean 11s globulin; SBEG2, 200 µg/ml of soybean 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.

Both high and low SBEG levels were able to inhibit native aerobic spoilage microorganisms below six logs cfu/gm beyond 12 days (*P* < 0.05) in comparison to deteriorated control shrimp after 8 chilling days at 2.5 °C. There was a dose-dependent (*P* < 0.05) antibacterial suppressing effect of SBEG. After twelve cooling days, coliform levels were 1-1.7 logs cfu/gm lower in the treated shrimp (*P* < 0.05), indicating a similar suppression effect. SBEG spray

significantly reduced MDA production, with dose-dependent effects from the fourth chilling day to storage end (*P* < 0.05). The SBEG, chilling time, and their combination had a significant influence on all organoleptic indices (Figure 1a and 1b). Compared to spoiled control shrimp after 8 chilling days at 2.5 °C, both high and low SBEG levels were able to retain acceptable fresh organoleptic indices beyond twelve days (*P* < 0.05).

Table 2 displays the antimicrobial effects of varying doses of soybean 11s globulin incorporation on tiger shrimp bacteriological, oxidative indices and shelf-life.

Groups	Storage time (Days)	Control	SBEG		SEM	<i>P</i> value		
			SBEG1	SBEG2		SBEG	Time (T)	SBEG *T
APC	1 st	4.43 ^C	4.32 ^C	4.36 ^B	0.026	0.000	0.000	0.000
	4 th	5.56 ^{aB}	4.32 ^{bc}	3.45 ^{cC}				
	8 th	7.48 ^{aA}	5.06 ^{bb}	4.30 ^{dB}				
	12 th	7.79 ^{aA}	6.11 ^{ba}	4.95 ^{cA}				
Coliform	1 st	3.03 ^{dD}	<2.0 ^{bd}	<2.0 ^{bb}	0.02	0.000	0.000	0.000
	4 th	3.99 ^{aC}	2.93 ^{bc}	2.15 ^{dB}				
	8 th	5.82 ^{aB}	4.47 ^{bb}	4.23 ^{ba}				
	12 th	6.07 ^{aA}	5.00 ^{ba}	4.32 ^{aA}				
TBA	1 st	29.15 ^D	28.63 ^A	27.91 ^A	0.239	<0.001	<0.001	<0.001
	4 th	32.85 ^{aC}	21.7 ^{bb}	17.92 ^{bb}				
	8 th	34.15 ^{aB}	29.47 ^{ba}	29.09 ^{aA}				
	12 th	37.48 ^{aA}	32.04 ^{ba}	28.08 ^{aA}				

¹SESG, soybean 11s globulin; SBEG1, 100 µg/ml of soybean 11s globulin; SBEG2, 200 µg/ml of soybean 11s globulin; APC, aerobic plate count; TBA, thiobarbituric acid; 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.

4. DISCUSSION

While soy protein is widely used in the meat industry to improve the quality of meat products (Schmidt and Oliveira, 2023; Yanni et al., 2024), there hasn't been much field research done to take advantage of its antimicrobial properties to extend shelf-life and reduce associated spoilage losses of highly perishable seafood such as shrimp.

The control v-shaped pH curve can be linked to the production of acid compounds including lactic acid via glycolysis, which lowers pH. Enzymes and microorganisms degrade down proteins, amino acids, and nitrogen-containing components in shrimp to generate alkaline chemicals such ammonia, trimethylamine, indole, and histamine, resulting in a rise in muscle pH curve prolonged storage (Huss et al., 1998; Xu et al., 2019). Conversely, shrimp sprayed with SEG, especially those treated with greater concentrations of SEG2, showed a very slow rising trend over prolonged storage times. This could be explained by the antibacterial and antioxidant properties of SEG, which postponed the postmortem microbial and endogenous autolytic glycolytic and proteolytic activities. This assumption may be supported by the observation that shrimp sprayed with SEG2 had lower pH levels at the first and last storage points than shrimp sprayed with SEG1, a lower dose, and control. Additionally, the initial pH of shrimp sprayed with SEG2 is closer to the optimal score obtained before in white shrimp (Xu et al., 2019).

The SBEG spray displayed no discernible effect on WHC or purge loss; nevertheless, over an extended storage, it was plausible to detect a relationship between SEG and persistent or higher WHC trends and decreased purge loss trends in comparison to the contrast trends observed in the control group. This improvement could be attributed to the legumes' protein technological activities, which are extensively used to improve Techno-Functional elements in meat products (Neji et al., 2022). Protein structure and function alterations caused by lipid oxidation, enzymatic activity, and microbial spoilage (Zhang et al., 2015) may be responsible for the control shrimp's increased drip loss and reduced water holding capacity.

When compared to decomposed control shrimp after eight cooling days at 2.5 °C, SBEG, particularly SBEG2, were able to suppress native aerobic spoilage count below six logs cfu/gm beyond 12 days. SBEG had an antibacterial suppressive effect that was dose-dependent. Native and modified soybean fractions, including SBEG, revealed antibacterial activity comparable to or higher than penicillin and nisin against the spoilage and pathogenic bacteria and fungi in milk and meat (Abdel-Shafi et al., 2019; Mahgoub et al., 2011; Osman et al., 2016, 2013; Sitohy et al., 2012). Not all microorganisms growing in raw fish contribute in the spoilage process; however, certain Spoilage Organisms (SSOs), which are commonly present in small quantities and vary depending on the kind of seafood, are primarily involved in the spoiling process in raw fish (Gram and Dalgaard, 2002).

Previous in vitro antioxidant studies found that soy products had significant amounts of antioxidants comparable to gallic acid and vitamin C standards (Robbani et al., 2022). This could explain SBEG's significant reduction in shrimp thiobarbituric acid, which also contributed to enhanced sensory qualities as compared to control shrimp that were severely affected by greater oxidative rancidity MDA levels (Takeungwongtrakul and Benjakul, 2013).

After eight days of cooling at 2.5 °C, the control shrimp exhibited unpleasant smells in comparison to the fresh odors of the SBEG-treated shrimp. Shrimp off-flavor during

storage is caused by volatile organic compounds (VOCs), which accumulate with postmortem spoilage bacteria break down of shrimp soluble and low molecular weight components (Broekaert et al., 2013). Melanosis development, protein denaturation, and fatty acid oxidation by autolytic enzymes and spoilage bacteria, as well as increased water loss due to longer shrimp storage, all reduce light reflection and result in a darker color (Kustyawati et al., 2021; Yan et al., 2010), as noticed in control after 12 days (Figure 1b).

5. CONCLUSIONS

All shrimp oxidative and hygienic markers were markedly decreased by SBEG's antioxidant and antibacterial capabilities in a dose-dependent way. SBEG levels were found to be efficient in reducing native aerobic spoilage bacteria below six logs cfu/gm and preserving acceptable fresh shrimp organoleptic indices beyond 12 days, when compared to ruined control shrimp after eight chilling days at 2.5 °C. Compared to beef, shrimp have a shorter cooling shelf life and are more perishable. Nonetheless, a field-applicable and natural preservation method is offered by combining SESG spray with chilling at 2.5 °C.

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