Effects of genistein on melanosis and microbial quality of *Litopenaeus vannamei* during ice storage

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Abstract

Utilization of genistein as a natural inhibitor was studied alone and in combination with conventional treatments on melanosis and microbial quality of fresh *L. vannamei* during 10 days ice storage. Treatments were as followed: A (dipping samples in distilled water), B (dipping in 1.25 % sodium metabisulphite solution), C (dipping in 0.01 % genistein + 2% glycerol solution), D (dipping in 0.1 % genistein + 2% glycerol solution), E (dipping in 1% NaCl + 0.05 % EDTA + 0.5 % ascorbic acid + 0.5 % lactic acid solution), F (dipping in 0.01% genistein + 2 % glycerol + 1 % NaCl + 0.05 % EDTA + 0.5 % ascorbic acid + 0.5 % lactic acid solution) and G (dipping in 0.1 % genistein + 2 % glycerol + 1 % NaCl + 0.05 % EDTA + 0.5 % ascorbic acid + 0.5 % lactic acid solution). Results showed that G treatment was more affective on inhibiting of melanosis in *L. vannamei*. Mesophilic and psycrophilic bacterial counts in G treatment were lower than other treatments during ice storage.

Keywords: Genistein, Melanosis, *Litopenaeus vannamei*, Microbial quality

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Introduction

Deterioration of shrimp is associated with microbiological, chemical and physical changes during postmortem storage (Nirmal and Benjakul, 2010). Melanosis or black spot, which appears in a few hours after capture, causes some deleterious changes in the organoleptic properties of shrimp and other crustacean and result in shorter shelf-life and poor quality of them (Montero *et al*., 2006). Although, melanosis does not affect the eating quality and are not dangerous to human health but it strongly reduces the marketability of the products (Omar, 1998; Kim *et al*., 2000). Melanosis is caused by the action of an enzyme called polyphenoloxidase (PPO), which oxidizes phenols to quinones. Polymerization of the colorless quinones gives rise to the black high-molecular-weight pigments (Montero *et al*., 2006).

It has proved that effectiveness of the melanosis inhibitors is dependent on several factors such as the type of inhibitor, inhibitor concentration, method of application, interspecies variations, seasonal variationsetc. The most common melanosis inhibitors are sulfite-based compounds, mainly metabisulfite which is currently used to prevent or at least delay melanosis (Montero *et al*., 2004). Due to its adverse reaction effect in some groups of people such as causative agent of asthma attacks (Collins-Williams, 1983), many studies have been carried out in order to find better alternatives for melanosis inhibition without negative effect. Among these agents we can mention different kinds of acids such as citric acid, ascorbic acid, acetic acid, ferulic acid and erythrobic acid (Marshall *et al*., 2000; Gökoğlu, 2004; Montero *et al*., 2006; Nirmal and Benjakul, 2010), sodium chloride, phenolic antioxidant (Marshall *et al*., 2000), chealator compounds such as EDTA, phosphates, kojic acid (Chen *et al*., 1991; Marshall *et al*., 2000; Montero *et al*., 2006), polysaccharides (Marshall *et al*., 2000), 4-hexylresorcinol (Guandalini *et al*., 1998; Montero *et al*., 2006), everfresh, composed of 4-hexylresorcinol and NaCl (Slattery *et al*., 1995), and also removing oxygen from shrimps and ice boxes (Ogawa *et al*., 1983).

However, increasing regulatory attention and awareness of consumers against synthetic additives in food processing have led to the interest in natural additives to prevent melanosis in shrimp (Nirmal and Benjakul, 2010). In this regards, plant phenolic compounds (Jayaprakash *et al*., 2001), grape seed extract (Gokoglu and Yerlikaya, 2008) and catechin with ferulic acid (Nirmal and Benjakul, 2010) are used to prevent the melanosis in shrimp. These compounds also showed antioxidant and antibacterial effects. Genistein is a simple type of isoflavonoid in leguminosae with different effects such as phytoestrogen, anticancer, and reducing the risks of heart diseases (Dixon and Ferreira, 2002).

There is no information about the effects of soybean extracted genistein

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on the melanosis prevention as well as shelf-life extension of Pacific white shrimp (*L. vannamei*). The aim of this study was to investigate the inhibitory effect of genistein on melanosis as well as microbial changes of *L. vannamei* during iced storage.

Materials and methods

Chemical

Metabisulfite, NaCl, Ethylenediaminetetracetic acid (EDTA), ascorbic acid, lactic acid, sulfuric acid, boric acid, thiobarbituric acid (TBA) and malonaldehyde were supplied by Merck (Germany) and pure genistein was purchased from LC laboratories (US).

Sample collection and preparation

Freshly cultured Pacific white shrimps (*L. vannamei*) with the mean weight of 17 g were purchased from Choebde (Abadan, Iran) and transported in ice to the Department of Food Hygiene, Shahid Chamran University of Ahvaz (Ahvaz, Iran) within 2 hours. Upon arrival, shrimps were washed in cold water and randomly divided into seven treatments lots. Completely random design was used for this research. Treatments were as: A (control, dipping samples in distilled water), B (dipping in 1.25 % sodium metabisulphite solution), C (dipping in 0.01 % genistein), D (dipping in 0.1 % genistein), E (dipping in 1 % NaCl + 0.05 % EDTA + 0.5 % ascorbic acid + 0.5 % lactic acid solution), F (0.01 % genistein added to treatment E) and G (0.1 % genistein added to treatment E). 2 % glycerol solution was used in treatments C, D, F and G. In all treatments, samples were dipped for 5 min. Then treated samples were kept in separate insulated boxes containing ice with a shrimp: ice ratio of 1: 2 (w: w) for ten days. Sampling was carried out on days 0 (immediately after treatment), 2, 4, 6, 8 and 10 of storage.

Microbiological analysis

Microbiological counts were determined by placing a sample (25 g) in 225 mL of 0.85 % NaCl solution and homogenizing it with a stomacher for 60s. From this dilution, other decimal dilutions were prepared and plated in the nutrient agar (PCA) (Dowens *et al*., 1992).

Determination of mesophilic bacterial count

For determination of mesophilic bacteria, plates were incubated at 35ºC for 48 h. The microbiological data were transformed into the logarithms of the number of colony-forming units (log CFU/g) (Nirmal and Benjakul, 2009).

Determination of psychrotrophic bacterial count (PBC)

The inoculated plates were incubated at 4ºC for 7 days for psychrotrophic (PTC) counts. The microbiological data were transformed into the logarithms of the number of colony-forming units (log CFU/g) (Nirmal and Benjakul, 2009).

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Melanosis assessment

Melanosis or blackening of Pacific white shrimp was evaluated through visual inspection by 8 trained panelists using 4-point scoring test (Montero *et al*., 2004). Panelists considered appearance, attachment of the head (carapace) to the abdomen, and presence of yellow-green coloring of the viscera. Melanosis (manifested as black spots on the shell, especially on the head) was assessed according to a visual scale from 1 to 4 of 9 samples, where 1= complete absence of black spots, 2= a few small spots on the carapace, 3= considerable spotting on the carapace and 4= substantial spotting over the entire shrimp.

Statistical analysis

The data were analyzed using one and two way analysis of variance (ANOVA) followed by LSD test using SPSS version 16. The significant level of results was based on 5%. All data are expressed as mean ± S.D. Triplicate measurements were made for each analysis.

Results

Determination of mesophilic bacterial count

Changes in mesophilic bacterial count of Pacific white shrimp during iced storage as the functions of treatments and storage time are shown in Table 1. The initial mesophilic bacterial count for control samples (treatment A) was 3.51 log CFU/g. This bacterial count was lower than the results of Nirmal and Benjakul (2009) for control samples of Pacific white shrimp at day 0. There were no significant differences in mesophilic bacterial count between control and treated samples at day 0 (Table 1). In general, mesophilic bacterial count increased continuously in all treatments throughout the storage for 10 days in ice (p<0.05). The effect of treatments on mesophilic bacteria was found from day 2 in treatments of D and G. The lowest content of mesophilic bacteria during storage period was detected in treatment G (dipping in 0.1% genistein + 2% glycerol + 1% NaCl + 0.05 % EDTA + 0.5 % ascorbic acid + 0.5 % lactic acid solution), followed by treatment D (dipping in 0.1 % genistein + 2% glycerol solution) (Table 1).

Determination of psychrotrophic bacterial count (PBC)

Changes in psychrotrophic bacterial count of Pacific white shrimp during iced storage as the functions of treatments and storage time are shown in Table 2. The initial psychrotrophic bacterial count for control samples (treatment A) was 2.93 log CFU/g. This bacterial count was higher than the results of Nirmal and Benjakul (2009, 2010) for control samples of Pacific white shrimp at day 0. Treating with different solution insignificantly reduced psychrotrophic bacterial count at day 0 immediately after treatment. Psychrotrophic bacterial count increased continuously in all treatments throughout the storage for 10 days in ice (p<0.05).

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Table 1: Changes in mesophilic bacterial count (log CFU/g) content in treated *Litopenaeus vannamei* during ice storage.

Days of storage	Treatments						
	A	B	C	D	E	F	G
0	3.51±0.09 ^{Ad}	3.54±0.08 ^{Ad}	3.58±0.27 ^{Ad}	3.51±0.17 ^{Ad}	3.41±0.22 ^{Ad}	3.40± 0.15 ^{Ac}	3.41 ± 0.24 ^{Ae}
2	3.79±0.17 ^{Ad}	3.78±0.14 ^{Ad}	3.79±0.16 ^{Ad}	3.33±0.10 ^{Bd}	3.78± 0.14 ^{Ac}	3.80± 0.16 ^{Ac}	3.50 ± 0.07 ^{Bde}
4	4.37±0.28 ^{ABc}	4.35±0.27 ^{ABc}	4.47±0.19 ^{Ac}	3.96±0.18 ^{BCc}	4.37±0.28 ^{ABb}	4.51± 0.32 ^{Ab}	3.81 ± 0.07 ^{Cd}
6	5.29±0.35 ^{Ab}	5.34±0.25 ^{Ab}	5.32±0.22 ^{Ab}	4.70±0.36 ^{Bb}	5.20± 0.16 ^{Aa}	5.37± 0.30 ^{Aa}	4.37 ± 0.20 ^{Bc}
8	5.54±0.15 ^{Ab}	5.62±0.23 ^{Aab}	5.53±0.23 ^{Aab}	5.27±0.15 ^{Aa}	5.35± 0.08 ^{Aa}	5.49± 0.22 ^{Aa}	4.76 ± 0.28 ^{Bb}
10	5.99±0.12 ^{Aa}	5.87±0.19 ^{ABa}	5.87±0.16 ^{ABa}	5.52±0.19 ^{BCa}	5.55±0.26 ^{BCa}	5.66±0.25 ^{ABa}	5.21 ± 0.08 ^{Ca}

Values are means and S.D. of triplicate; Means with the same uppercase superscripts in a row were not significantly different at $p<0.05$ level in different treatment. Means with the same lowercase superscripts in a column were not significantly different at $p<0.05$.

Table 2: Changes in psychrotrophic bacterial count (log CFU/g) content in treated *Litopenaeus vannamei* during ice storage.

Days of storage	Treatments						
	A	B	C	D	E	F	G
0	2.93± 0.13 ^{Ae}	2.91± 0.20 ^{Ae}	2.90± 0.24 ^{Ae}	2.79 ± 0.34 ^{Ae}	2.96± 0.19 ^{Ae}	2.87± 0.27 ^{Ae}	2.86± 0.15 ^{Af}
2	3.25±0.20 ^{Ade}	3.29±0.19 ^{Ade}	3.31± 0.10 ^{Ad}	2.97 ±0.30 ^{Ade}	3.22±0.10 ^{Ade}	3.28±0.14 ^{Ade}	3.06± 0.09 ^{Ae}
4	3.59±0.18 ^{Acd}	3.51±0.08 ^{Acd}	3.55±0.20 ^{Acd}	3.37±0.17 ^{ABcd}	3.38±0.03 ^{ABc}	3.56±0.02 ^{Acd}	3.27± 0.04 ^{Bd}
6	3.78± 0.14 ^{Ac}	3.79± 0.17 ^{Ac}	3.79± 0.16 ^{Ac}	3.78 ± 0.14 ^{Ac}	3.43± 0.09 ^{Bc}	3.80± 0.15 ^{Ac}	3.49±0.04 ^{Bc}
8	4.35±0.27 ^{ABb}	4.37±0.28 ^{ABb}	4.47± 0.19 ^{Ab}	4.37± 0.28 ^{ABb}	3.96±0.18 ^{BCb}	4.51± 0.32 ^{Ab}	3.82±0.08 ^{Cb}
10	5.34 ± 0.25 ^{Aa}	5.29± 0.35 ^{Aa}	5.32± 0.22 ^{Aa}	5.34 ± 0.27 ^{Aa}	4.70± 0.36 ^{Ba}	5.37± 0.30 ^{Aa}	4.53±0.12 ^{Ba}

Values are means and S.D. of triplicate; Means with the same uppercase superscripts in a row were not significantly different at $p<0.05$ level in different treatment. Means with the same lowercase superscripts in a column were not significantly different at $p<0.05$.

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The lowest psychrotrophic bacterial count during storage period was found in treatment G, followed by treatment E.

Melanosis assessment

Melanosis score of control and treated samples of *L. vannamei* with different solutions and those changes during ice storage are given in Table 3. As it is shown in Table 3, there was no melanosis (score=1) in control and treated shrimps at day 0. Similar results were reported by Montero *et al*. (2006) and Nirmal and Benjakul (2010). Melanosis score was increased with elongation of ice storage.

However, the increasing rate of melanosis varied with treatments. Melanosis was detected at day 2 in treatments A (control) and C (dipping in 0.01 % genistein + 2% glycerol solution), while it was detected in day 6 for treatments B, D, E and F and even in day 8 for treatment G. Melanosis was retarded with higher concentration of genistein alone and with combination of conventional treatments. Similar results have been reported by Montero *et al*. (2006) on the pink shrimp treated with different concentrations of 4-hexylresorcinol based formulations. Genistein in 0.1% concentration in treatments of G and D showed the best retarding results on melanosis in Pacific white shrimp during 10 days ice storage. The two way ANOVA analysis shows that the effects of treatments, storage period and interaction between these two factors were significant for mesophilic and psychrotrophic bacterial count. For melanosis score, although the effect of treatments and storage period were significant (p<0.05), but the interaction between them was not significant (Table 4).

Table 3: Changes in malanosis in different treatments during ice storage

Days of storage	Treatments						
	A	**B**	**C**	**D**	**E**	**F**	**G**
0	1.00± 0.00 ^{Ad}	1.00± 0.00 ^{Ad}	1.00± 0.00 ^{Ad}	1.00 ± 0.00 ^{Ac}	1.00± 0.00 ^{Ad}	1.00 ± 0.00 ^{Ad}	1.00± 0.00 ^{Ac}
2	1.22± 0.44 ^{Ad}	1.00± 0.00 ^{Ad}	1.11± 0.33 ^{Ad}	1.00 ± 0.00 ^{Ac}	1.00± 0.00 ^{Ad}	1.00 ± 0.00 ^{Ad}	1.00± 0.00 ^{Ac}
4	1.66± 0.50 ^{Ac}	1.00± 0.00 ^{Cc}	1.33±0.50 ^{Bcd}	1.00 ± 0.00 ^{Cc}	1.00 ± 0.00 ^{Cd}	1.00± 0.00 ^{BCd}	1.00± 0.00 ^{Cc}
6	2.00± 0.00 ^{Ac}	1.67±0.50 ^{ABc}	1.67±0.50 ^{ABc}	1.33±0.50 ^{BCc}	1.33±0.50 ^{BCc}	1.33± 0.50 ^{BCc}	1.00± 0.00 ^{Cc}
8	2.66± 0.50 ^{Ab}	2.66± 0.50 ^{Ab}	2.67± 0.50 ^{Ab}	2.22±0.44 ^{ABb}	2.11 ± 0.33 ^{Bb}	2.22± 0.44 ^{ABb}	1.67± 0.50 ^{Cb}
10	3.66± 0.50 ^{Aa}	3.33± 0.52 ^{Aa}	3.66± 0.50 ^{Aa}	2.66 ± 0.60 ^{Ba}	3.00 ± 0.00 ^{Ba}	3.00 ± 0.00 ^{Ba}	2.33± 0.50 ^{Ca}

Values are means and S.D. of triplicate; Means with the same uppercase superscripts in a row were not significantly different at p<0.05 level in different treatment. Means with the same lowercase superscripts in a column were not significantly different at p<0.05.

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Table 4: Two way ANOVA results

Factor	Treatments	Storage	Interaction	Error
Mesophilic bacterial count	0.00	0.00	0.05	0.0437
psychrotrophic bacterial count	0.00	0.00	0.02	0.0457
Melanosis score	0.00	0.00	0.31	0.1174

Discussion

In present study, the effect of genistein alone (treatments C and D) and in combination with acids and EDTA (a chelating agent) was studied on melanosis formation and microbial changes in *L. vannamei* during 10 days ice storage and also compared with the effects of sodium metabisulphite solution. Genistein (4', 5, 7-trihydroxyisoflavone) is the simplest isoflavonoid compound of the Leguminosae. It is a central intermediate in the biosynthesis of more complex isoflavonoids and common precursor in the biosynthesis of antimicrobial phytoalexins and phytoanticipins in legumes (Dixon and Ferreira, 2002). The mesophilic bacterial count was increased in all treated and control samples during 10 days ice storage. The lowest bacterial count was found in treatment G. Higher reduction effect on mesophilic count were also found in treatments D and E in comparison to other treatments. Nirmal and Benjakul (2009) reported a continuous increase in mesophilic count up to day 4, followed by a slight increase until the end of 10 days storage . They mentioned that this increase could be due to the tolerance of those microorganisms to cold conditions up to a certain limit. In addition to low temperature storage effect on the bacterial count, the results of this study indicated the higher antimicrobial activity of 0.1 % genistein and combined solution of ascorbic acid, lactic acid and EDTA and those synergic effects towards mesophilic bacteria in white shrimps during iced storage. For psychrotrophic bacteria, the best bacterial retarding results was found in treatment G (dipping in 0.1% genistein + 2% glycerol + 1% NaCl + 0.05 % EDTA+0.5 % ascorbic acid+0.5 % lactic acid solution), while the treatment with different concentration of genistein showed similar effects compared with the control and samples treated with sodium metabisulphite. These results were in accordance with the study of ferulic acid on the mesophilic and psychrotrophic bacteria (Nirmal and Benjakul, 2009), the effects of catechin and ferulic acid on the psychrotrophic bacteria (Nirmal and Benjakul, 2010), and the effect of green tea extract treatment and modified atmosphere packaging on psychrotrophic bacteria (Nirmal and Benjakul, 2011) in Pacific white shrimp. It has been reported that ferolic acid might disrupt the cell wall of microorganism. Phenolic compound

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might form complexes with proteins in cell wall and cause the lyses of cell wall (Chanthachum and Beuchat, 1997). Furthermore, phenolics, especially catechin and its derivatives in the extract, might chelate some metal ions required for microbial growth (Nirmal and Benjakul, 2011).

The mesophilic and psychrotrophic bacterial count did not reach the value of 7.9 log CFU/g which is the microbiological upper limit for the fresh fish used by Salam (2004) during 10 days ice storage.

As it is evident from Table 3, treatment of *L. vannamei* samples with 0.1% genistein had better retarding effects on the melanosis up to end of ice storage in comparison to sodium metabisulphite, , while the results of treating with 0.01% genistein was not different with control samples. The melanosis inhibition of genistein was increased with combination of ascorbic and lactic acids and chelating factor. Similar results have been reported by Montero *et al*. (2006) who investigated the melanosis inhibition in deep water pink shrimp with various concentrations of 4-hexyresorcinol alone and in combination with ascorbic acid, acetic acid etc. The combination of acids and EDTA both inhibited the melanosis formation (treatment E) and enhanced the effect of genistein (treatment G) (Table 3). It has been reported that presence of acids in the inhibitor solution reduces the pH, and melanosis is more easily avoided in shrimp (Montero *et al*., 2001; Montero *et al*., 2006).

EDTA is a chelating agent which in addition to direct action on the active site of the polyphenol-oxidase (PPO), acts as the metalloprotease inhibitor and consequently at least partially may inhibit the formation of free tyrosine and phenylalanine, substrates for the action of PPO (Slattery *et al*., 1995; Montero *et al*., 2006).

Our results showed that the G treatment had more inhibiting affect on the formation of melanosis in *L. vannamei*. The Mesophilic and psycrophilic bacterial counts in the G treatment were also lower than the other treatments during ice storage.

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