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Study The effect of Brown Alga (*Colpomeni sinousa*) on Melanosis, Quality and Lipid Oxidation of Western Pacific Shrimp (*Litopenaeus vannamei*) During The storage on Ice

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THE present survey was performed to evaluate the effects of *Colpomeni sinousa* (*C. sinousa*) on melanosis, quality and lipid oxidation of Western Pacific shrimp (*Litopenaeus vannamei*) during the storage on ice. Shrimp samples were classified into four groups [Control, *C. sinousa* treatments (1 and 10%) and 3% sodium metabisulfite], packed and stored on ice for 20 days. Quality properties of shrimp samples were assessed daily. Sensory properties of fried shrimp samples were assessed at the end of the experiment. Peroxide, anisidine and TBA contents of shrimp samples were measured at days 0, 10 and 20 of the experiment using standard techniques. The highest qualitative scores were given to shrimp samples treated with sodium metabisulfite(3%), followed by *C. sinousa* extract (10%). Peroxide, anisidine and TBA contents of shrimp samples were significantly increase during the storage period ($P < 0.05$) with the highest content for the control group. C. sinousa extract (10%) caused significant decrease in the production of peroxide, anisidine and TBA contents of shrimp samples during the storage ($P < 0.05$). Sensory evaluators specified the highest sensory scores to hardness, color, taste and overall acceptance for the shrimp samples treated with sodium metabisulfite(3%), followed by *C. sinousa* extract (10%). Obtained findings were not statistically significant between shrimp samples treated with *C. sinousa* extract (10%) and sodium metabisulfite(3%). Therefore, considering the high cost of sodium metabisulphite, *C. sinousa* extract (10%) is an appropriate candidate to improve the shelf-life and oxidative stability of shrimp during the storage on ice.

Keywords: Colpomeni sinousa, Litopenaeus vannamei, Melanosis, Quality, Lipid oxidation.

Introduction

Shrimp is a good source of protein, fatty acids, minerals and vitamins. Its consumption guarantees the preparation of nutrients materials and further human health [1]. In keeping with this and according to the high contents of nutritional parameters, particularly protein, fatty acids and minerals, shrimp is severely prone to chemical corruption [2]. Occurrence of lipid oxidation and black spot are two important issues regarding the long term storage of shrimp even in low temperature [3, 4]. Lipid oxidation is responsible for the formation of off-flavour, rancidity and toxic materials associated with decrease in shelf-life of shrimp [5]. Several indicators of lipid oxidations including Anisidine (AV), peroxide (PV), and Thiobarbituric acid (TBA), were currently used to assess the rate and levels of lipid oxidation in foodstuffs [5]. PV is an indicators of lipid oxidation and increase in an initial stages of oxidation, while AV and TBA are increased in the late stages of lipid oxidation [5]. Black spot or melanosis is a routine discoloration or darkening occurred initially in the swimmerets, tail, chest, head, and shell [6]. It is an enzymatic issue which

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caused presence of brown to black pigments that facilitated with the presence of oxygen [6]. Occurrence of melanosis negatively affects quality perception and marketing of shrimp samples [7].

According to the high economic and nutritional values of shrimp and high possibility of the occurrence of lipid oxidation and melanosis, manufacturers have made great efforts to increase the shelf-life and chemical quality of shrimp samples [8]. Several techniques were used including, low temperature, synthetic packaging, synthetic antioxidant agent and chemical preservatives are known to increase shelf-life and decrease the chemical corruption of shrimp [9, 10]. Nevertheless, inefficiency and low effects, high production costs, disturbance on odor and taste and toxic effects (carcinogenicity, mutagenicity and fetal deformity) of these methods have been confirmed in various studies [11, 12].

Colpomenia sinuosa (*C. sinuosa*) is a brown algae of Mediterranean seas and oceans belongs to Scytosiphonales order [13]. High nutritional [14], antioxidant [15] and biological [16] effects of *C. sinuosa* have been reported in diverse investigations. This brown algae is lengthily employed as ingredients in human food and animal feed owing to its high nutritional values (vitamins A, B, C, D and E, niacin, pantothenic acid, riboflavin, and folic acid, and polyunsaturated fatty acids, and diverse antioxidant agents such as phenols, saponins, triterpenoids, flavonoids, anthraquinones, tannins, alkaloids, steroids, and glycosides) [17]. However, scarce surveys have been conducted to assess its biological effects, particularly on its application as food preservative. Rendering the high antioxidant effects of *C. sinuosa* and high sensitivity of shrimp to melanosis and lipid oxidation, the present survey was performed to assess the effects of *C. sinuosa* extract on melanosis, quality and lipid oxidation of western pacific shrimp (*Litopenaeus vannamei*) during the storage on ice.

Materials and Methods

Shrimp samples

The present survey was conducted on Persian Gulf and Oman Sea Ecological Research Institute, Bandar Abbas, Iran. White leg (*Litopenaeus vannamei*) shrimp were cultivated in Hormozgan province, Iran. Valve method was used to harvest shrimp samples from the pool. Species identification of shrimp samples was performed by an expert professor of the field of aquatic sciences (No: PG-SH-Vanami 14). Fresh live shrimps were transferred to the laboratory of the National Aquatic Processing Research Center in boxes containing ice within about 60 min. Samples were washed with tap water. Shrimp samples were packed in 10 different packages (1 Kg each) at the day 0 of the experiment.

C. sinousa extract

The test samples of *C. sinousa*, weighing approximately 20 Kg, were brought by diving on a depth of 8 to 10 meters of the north parts of the Hormoz Island, Persian Gulf, Iran and were then transferred to the laboratory using the sea water. Species identification was performed by a professor of the field of the marine botany of the Persian Gulf and Oman Sea Ecological Research Institute, Bandar Abbas, Iran (Herbarium No PG-GA-Co.sunusa 121). Algae samples were first cut and then placed in a freeze dryer device (Maham Azma, Tehran, Iran) for 48 hours. The dried *C. sinousa* samples were powdered using a mill (Azin Lab, Tehran, Iran) and kept at -24 °C until further processing. Extraction was achieved using the soaking method [18]. Three different stages of soaking, filtering the extract and separating the solvent were performed for this purpose. Briefly, acetone (Merck, Germany) was added to powdered samples and then, samples were placed in dark at room temperature for 72 hours. Achieved solution was filtered (Whatman paper No. 1). Achieved extracts were transferred to rotary device (Heidolph, laborota 4000) at 45 °C.

Treatments

Shrimp samples were divided into two main groups of control and treatment. Control group was subdivided into two separate subdivisions of shrimps: shrimp samples without any additives, and another shrimp processed with sodium metabisulfite. Sodium metabisulfite (3%) was used for about 10 min for shrimp's processing. Treatment group was also subdivided into two separate subdivisions of shrimps: shrimp samples immersed in *C. sinousa* extract (1%), and another shrimp immersed in *C. sinousa* extract (10%) for 30 min. Then, all processed samples were packed in a vacuum package. Each group contained 60 shrimp samples. All shrimp samples were stored at 4 °*C* for 20 days. Chemical analysis of samples was performed at days 0, 5, 10, 15 and 20 of the experiment.

Lipid oxidation measurement

Fifty grams of each sample (whole shrimp with head, legs and viscera) was put into a

500 mL beaker and 300 mL of n-hexane (1:6) added. Extractions were performed at 50 °C for 3 h. After extraction, the suspensions were filtered (Whatman paper No. 1). The hexane was evaporated under vacuum at ambient temperature. Anisidine (AV) and peroxide (PV) values of the extracted oil were determined by the colorimetric procedure (spectrophotometer model 2100 UV, Cole Parmer Instruments Company 625 East Bunker Court Vernon Hills, USA). Thiobarbituric acid (TBA) was also measured according to standard method [19]. For peroxide analysis, 2.35 mL of ethanol (75% w/v), 50 µl of ammonium thiocyanate (30% w/v) and 50 µl of ferrous chloride solution were added to 50 µl of shrimp oil sample and mixed well. The absorbance of the colored solution was read after 3 min at 500 nm using a spectrophotometer (Shimadzu, Japan). For TBA analysis, 2.5 mL of a mixture containing 0.375% TBA (w/v), 15% TBA (w/v) and 0.25 M HCl was added to 0.5 g lipid extracted from shrimp samples and heated at 95 °C for about 10 min. Mixture was centrifuged on 3600 rpm at 25 °C for about 20 min (Hettich, Germany). Finally, the absorbance of solution was measured at 532 nm by a spectrophotometer (Shimadzu, Japan). For AV, 100 mg of shrimp oil sample was dissolved in isooctane (25 mL). Then, 2.5 mL of achieved solution was added to 0.5 mL r-anisidine (0.5%) and remained in acetic acid for 10 min. Finally, the absorbance of achieved solution was measured at 350 nm by spectrophotometer (Shimadzu, Japan).

Quality properties

Quality evaluation of shrimp samples was performed using the data presented in table 1. For this purpose, 10 trained sensory evaluators (5 men and 5 women) were checked the physical quality of shrimp samples during the study period. Evaluation was performed from day 0 to day 20 in a daily pattern.

TABLE 1. Scoring the quality characteristics of shrimp samples of different treatments during storage [20].

Characters	Scoring methods			
	1	2-4	5-7	8-10
Color	Complete darkness of the head, chest, tail fins and shells	Darkness of head and chest, some tail fins and dark lines on the shell	Colorless, slightly transparent and the appearance of some black spots	Completely colorless, transparent and no dark lines
Head/chest and tail	Peeling off most of the heads and tails	Connection is low and easily removed, loosening and tearing of a few tails and heads are visible	Continuous but not completely rigid and onset of lethargy in some areas	Tight and perfectly continuous
Legs, shells and tentacles	Most of the legs, tentacles and shells are removed	Starting of legs and tentacles loosing	The legs and tentacles are less stiff	Complete and tight
Eyes	Most of eyes are removed	Color reduction and some eyes are peeled off	Decreased transparency and slightly darker	Transparent and tight
Odor	odor of ammonia, sulfide and nauseous	Slight odor of fish	Without odor	odor like seaweed, the odor of the sea, pleasant
Meat (texture, color and veins)	Blackening, yellow and green spots on the meat and rupture of blood vessels	The appearance of darkness of the meat and self-digestion of the vessels	Low stiffness, soft, matte white, less resistance of vessels without opacity	Hard, juicy, white and transparent, hard and resistant vessels

Sensory evaluation

In order to assess the sensory properties of shrimp samples of different groups of the experiment, all shrimp samples were fried using vegetable oil (Oila, Iran) at 60 °C for about 30 min (Philips, Netherlands). Then, organoleptic properties of shrimp samples were assessed by 10 trained sensory evaluators (5 men and 5 women). Five point hedonic tests were used for this purpose. The evaluation was performed in a quiet place, with natural light and without any specific odor. Drinking water was used between the evaluations of samples of different groups. Totally, hardness, color, taste, and overall acceptance sensory properties were evaluated. For this purpose, 5-point hedonic scale, where 1 represented "dislike extremely" and 5 represented "like extremely" were used. For hardness evaluation, 5 delineated "very high" and 1 delineated "very low" levels of hardness [21].

Statistical analysis

Statistical test was performed in a completely randomized design with factorial arrangement for 2 treatments. Two way Analysis of variance (ANOVA) was performed using SPSS 21.0 statistical software (ver. 21, SPSS Inc., Chicago, IL, USA) and the comparison of means was performed by LSD and Duncan test at 95% confidence level. Significance level ($P < 0.05$) was also determined.

Results

Table 2 shows the scores given to qualitative properties of shrimp samples of the control group during the storage period. Table 3 shows the scores given to qualitative properties of shrimp samples treated with *C. sinousa* extract (1%) during the storage period. Table 4 shows the scores given to qualitative properties of shrimp samples treated with *C. sinousa* extract (10%) during the storage period. Table 5 shows the scores given to qualitative properties of shrimp samples treated with sodium metabisulfite (3%) during the storage period. Scores were given as 1 (the lowest) to 10 (the highest). Findings revealed that the evaluators given the highest qualitative scores to shrimp samples treated with sodium metabisulfite (3%), followed by *C. sinousa* extract (10%). In fact, the lowest scores were given to shrimp samples of the control group. Additionally, scores were reduced during the storage period.

TABLE 2. Scores given to qualitative properties of shrimp samples of the control group during the storage period.

Storage period (day)	Qualitative scores					
	Color	Head/chest and tail	Legs, shells and tentacles	Eyes	Odor	meat
1	10 ± 2^{a}*	10 ± 2^{a}	10 ± 2^{a}	10 ± 1^{a}	10 ± 1^{a}	10 ± 1^{a}
10	1 ± 0^{b}	1 ± 0^{b}	1 ± 0^{b}	1 ± 0^{b}	1 ± 0^{b}	1 ± 0^{b}
20	1 ± 0^{b}	1 ± 0^{b}	1 ± 0^{b}	1 ± 0^{b}	1 ± 0^{b}	1 ± 0^{b}

*Dissimilar small letters in each column shows statistically significant differences about $P < 0.05$.

TABLE 3. Scores given to qualitative properties of shrimp samples treated with C. sinousa extract (1%) during the storage period.

Storage period (day)	Qualitative scores					
	Color	Head/chest and tail	Legs, shells and tentacles	Eyes	Odor	meat
1	10 ± 2^{a}*	10 ± 2^{a}	10 ± 2^{a}	10 ± 1^{a}	10 ± 1^{a}	10 ± 1^{a}
10	2 ± 0^{c}	2 ± 0^{c}	2 ± 0^{c}	3 ± 0^{c}	2 ± 0^{c}	2 ± 0^{b}
20	1 ± 0^{c}	1 ± 0^{c}	1 ± 0^{c}	1 ± 0^{c}	1 ± 0^{c}	1 ± 0^{b}

*Dissimilar small letters in each column shows statistically significant differences about $P < 0.05$.

TABLE 4. Scores given to qualitative properties of shrimp samples treated with C. sinousa extract (10%) during the storage period.

Storage period (day)	Qualitative scores					
	Color	Head/chest and tail	Legs, shells and tentacles	Eyes	Odor	meat
1	10±1^{a}*	10±2^{a}	10±1^{a}	10±1^{a}	10±2^{a}	10±1^{a}
10	5±0^{a}	6±0^{a}	5±0^{b}	6±0^{a}	4±0^{b}	6±0^{a}
20	1±0^{a}	1±0^{a}	1±0^{b}	1±0^{a}	1±0^{b}	1±0^{a}

*Dissimilar small letters in each column shows statistically significant differences about P <0.05.

TABLE 5. Scores given to qualitative properties of shrimp samples treated with sodium metabisulfite (3%) during the storage period.

Storage period (day)	Qualitative scores					
	Color	Head/chest and tail	Legs, shells and tentacles	Eyes	Odor	meat
1	10±1^{a}*	10±2^{a}	10±1^{a}	10±1^{a}	10±2^{a}	10±1^{a}
10	6±0^{a}	7±0^{a}	7±0^{a}	7±0^{a}	5±0^{b}	7±0^{a}
20	1±0^{a}	1±0^{a}	1±0^{a}	1±0^{a}	1±0^{b}	1±0^{a}

*Dissimilar small letters in each column shows statistically significant differences about P <0.05.

Table 6 signifies the changes of the Peroxide (PV) content of shrimp samples of different groups during the storage period. PV content of all shrimp samples were significantly increased during the storage period (P <0.05). Shrimp samples treated with sodium metabisulfite (3%) had the lowest (10.93±1.16 meq O2/Kg oil) PV content at the end of the storage period, while those of control group had the highest (26.73±1.69 meq O2/Kg oil) (P <0.05).

Table 7 signifies the changes of the anisidine content of shrimp samples of different groups during the storage period. Anisidine content of all shrimp samples were significantly increased during the storage period (P <0.05). Shrimp samples treated with sodium metabisulfite (3%) had the lowest (3.05±0.17) anisidine content at the end of the storage period, while those of control group had the highest (9.63±0.82) (P <0.05).

Table 8 signifies the changes of the Thiobarbituric acid (TBA) content of shrimp samples of different groups during the storage period. TBA content of all shrimp samples were significantly increased during the storage period (P <0.05). Shrimp samples treated with sodium metabisulfite(3%) had the lowest (2.01±0.18 mg MDA/Kg oil) TBA content at the end of the storage period, while those of control group had the highest (6.98±0.41 mg MDA/Kg oil) (P <0.05).

Table 9 represents the sensory properties of different groups of shrimp samples at the end of the storage period (day 20). Findings revealed that the sensory evaluators specified the highest sensory scores to hardness (4.21±0.19), color (4.38±0.27), taste (4.10±0.28) and overall acceptance (4.37±0.25) for the shrimp samples treated with sodium metabisulfite(3%). However, according to scores given to the control group, sensory evaluators found the shrimp samples of the control group unconsumable.

TABLE 6. Peroxide content (Mean ± Standard Deviation (SD)) of different groups of shrimp samples during the storage period.

Shrimp groups	PV (meq*** O2/Kg oil) content in the storage period (day)		
	0	10	20
Control	0.33±0.029 C*a**	18.24±1.37 Ba	26.73±1.69 Aa
C. sinousa extract (1%)	0.28±0.021 Ca	13.18±1.04 Bb	20.38±1.53 Ab
C. sinousa extract (10%)	0.25±0.015 Ca	8.31±0.55 Bc	13.17±1.08 Ac
Sodium metabisulfite (3%)	0.24±0.010 Ca	7.20±0.41 Bc	10.93±1.16 Bc

*Dissimilar capital letters in each row show a statistically significant difference P <0.05.
**Dissimilar small letters in each column show a statistically significant difference about P <0.05.
***milliequivalents

TABLE 7. Anisidine content (Mean ± Standard Deviation (SD)) of different groups of shrimp samples during the storage period.

Shrimp groups	Anisidine content in the storage period (day)		
	0	10	20
Control	0.22±0.018 C*a**	5.70±0.36 Ba	9.63±0.82 Aa
C. sinousa extract (10%)	0.24±0.013 Ca	3.33±0.27 Bb	6.01±0.45 Ab
C. sinousa extract (1%)	0.25±0.015 Ca	2.01±0.15 Bc	4.87±0.31 Ac
Sodium metabisulfite(3%)	0.25±0.014 Ca	1.19±0.10 Bd	3.05±0.17 Ad

*Dissimilar capital letters in each row show a statistically significant difference P <0.05.
**Dissimilar small letters in each column show a statistically significant difference about P <0.05.

TABLE 8. Thiobarbituric acid (TBA) content (Mean ± Standard Deviation (SD)) of different groups of shrimp samples during the storage period.

Shrimp groups	TBA (mg MDA***/Kg oil) content in the storage period (day)		
	0	10	20
Control	0.14±0.012 C*a**	3.44±0.28 Ba	6.98±0.41 Aa
C. sinousa extract (10%)	0.12±0.010 Ca	2.23±0.14 Bb	4.75±0.33 Ab
C. sinousa extract (1%)	0.11±0.010 Ca	1.08±0.09 Bc	2.24±0.17 Ac
Sodium metabisulfite(3%)	0.11±0.009 Ca	0.99±0.06 Bc	2.01±0.18 Ad

*Dissimilar capital letters in each row show a statistically significant difference P <0.05.
**Dissimilar small letters in each column show a statistically significant difference about P <0.05.
***Malondialdehyde

TABLE 9. Sensory properties (Mean ± Standard Deviation (SD)) of different groups of shrimp samples at the end of the storage period (day 20).

Shrimp groups	Sensory scores in day 20			
	Hardness	Color	Taste	Overall acceptance
Control	2.70±0.15 c	2.76±0.24 c	2.03±0.15 c	2.27±0.18 c
C. sinousa extract (10%)	3.81±0.24 b	3.55±0.31 b	3.50±0.28 b	3.63±0.29 b
C. sinousa extract (1%)	4.18±0.20 a	4.22±0.23 a	4.04±0.32 a	4.21±0.14 a
Sodium metabisulfite(3%)	4.21±0.19 a	4.38±0.27 a	4.10±0.28 a	4.37±0.25 a

**Dissimilar small letters in each column show a statistically significant difference about P <0.05.

Discussion

An existing survey was performed to assess the effects of *C. sinousa* extract on qualitative and sensory properties and lipid oxidation of shrimp samples during 20 days storage on ice. Findings indicated that treated of shrimp samples with *C. sinousa* extract (10%) caused significant increase in the qualitative and sensory scores given by the evaluators. Furthermore, treated of shrimp samples with *C. sinousa* extract (10%) caused significant decrease in the production of PV, anisidine and TBA oxidative parameters compared with the control group ($P < 0.05$). However, the best results of sensory, qualitative and oxidative inhibition were found for the shrimp samples treated with sodium metabisulfite(3%), but there were no statistically significant differences for the most findings between shrimp samples of this group and with those treated with *C. sinousa* extract (10%) ($P > 0.05$). Thus, it seems that *C. sinousa* extract (10%), similar to sodium metabisulfite(3%), can prevent from the oxidative rancidity of shrimp samples and retained their qualitative and sensory properties during the storage period. Another important advantage of *C. sinousa* extract in comparison with the sodium metabisulfite(3%) is the high cost of synthesis of sodium metabisulphite.

Melanosis signifies a thoughtful issue to the shrimp industry. It is mainly formed by the natural formation of dark pigments mostly in the head, thorax and joints region because of the enzymatic oxidation of phenolic complexes. Melanosis disturbs sensory features, reducing shrimp's shelf-life and also quality [22]. In the present survey, using *C. sinousa* extract, owing to its antioxidant effects, caused significant decrease in the levels of melanosis, particularly in head, thorax and legs of examined shrimp samples.

Antioxidant effects of diverse kinds of seaweeds were confirmed in previous investigations [23-25]. It seems that presence of certain antioxidant agents, particularly phenols and phenolic acids, flavonoids, vitamin E, and glucuronic acid, is the main factor for the antioxidant effects of *C. sinousa* extract which decreased the procedure of lipid oxidation of shrimp samples [26, 27]. Generally, at higher concentrations of *C. sinousa* extracts, due to the presence of more antioxidant complexes and, therefore, an upsurge in the number of hydroxyl groups present in the reaction, the probability of donating hydrogen to free radicals and afterward the oxidation inhibitory potency of the extract is increased. Sharifian et al. [28] reported that shrimp samples treated with phlorotannins (5%) extracted from seaweeds had the least pH, melanosis score, total volatile nitrogen and lipid oxidation with higher sensory scores given by evaluators. Similarly, Shiekh et al. [29], Nirmal and Benjakul (2011a) [7] and Nirmal and Benjakul (2011b) [30] reported that the shrimp samples treated with Chamuang extract (1%), lead seed extract (0.5%) and green tea extract (5 g/L) caused significant decrease in the melanosis production during the storage period, respectively. One of the probable reason for the lower increase in the PV, anisidine and TBA contents of shrimp samples treated with *C. sinousa* extract is presence of polyphenols which can inhibit the lipid oxidation and scavenge free radicals, and chelate metal ions [31].

Findings of the present research revealed that sensory evaluators given the highest scores for color, taste, texture and overall acceptance to the shrimp samples treated with sodium metabisulfite(3%) and *C. sinousa* extract (10%) after 20 days storage at ice. This finding is maybe due to the antioxidant effects of *C. sinousa* extract which decrease the procedure of oxidation and subsequent effect on taste, odor, texture and color of shrimp samples. Thus, using *C. sinousa* extract (10%), similar to sodium metabisulfite(3%), caused shrimp samples to reached to the end of their shelf-life (20 days). Our findings revealed that *C. sinousa* extract (10%) could improve the sensory score and extend the shelf-life of Pacific white shrimp up to 20 days. According to the high prevalence of food-borne pathogens in Iran [32-50], application of *C. sinousa* extract as a natural preservative has been suggested in future investigations.

Conclusions

In conclusion, *C. sinousa* extract (10%) can reduce the procedure of lipid oxidation and increase the shelf-life of shrimp samples during the storage on ice. Additionally, *C. sinousa* extract (10%) caused significant increase in the scores given to sensory and qualitative properties of shrimp samples. Put together, the ability of *C. sinousa* extract (10%) in oxidative stability and shelf-life improvement of shrimp samples was relatively similar to sodium metabisulfite (3%). Nevertheless, considering the high costs of synthesis and production of sodium metabisulphite, application of *C. sinousa* extract is recommended to increase the shelf-life of

shrimp samples. However, some supplementary experiment should perform to assess other effects of *C. sinousa* extract on shrimp samples.

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Conflict of interest

The authors declared that no conflict of interest.

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