

International Journal of Food Properties

ISSN: 1094-2912 (Print) 1532-2386 (Online) Journal homepage: https://www.tandfonline.com/loi/ljfp20

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To cite this article: Hanife Aydan Yatmaz & Nalan Gokoglu (2016) Effects of Plant Extract-Sulphide Combinations on Melanosis Inhibition and Quality in Shrimp (*Aristeus Antennatus*), International Journal of Food Properties, 19:2, 359-370, DOI: 10.1080/10942912.2015.1031247

To link to this article: https://doi.org/10.1080/10942912.2015.1031247

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Published online: 18 Nov 2015.

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International Journal of Food Properties, 19:359–370, 2016
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ISSN: 1094-2912 print/1532-2386 online
DOI: 10.1080/10942912.2015.1031247

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Effects of Plant Extract-Sulphide Combinations on Melanosis Inhibition and Quality in Shrimp (*Aristeus Antennatus*)

Hanife Aydan Yatmaz[1] and Nalan Gokoglu[2]

[1]*Akdeniz University, Food Safety and Agricultural Research—Development Center Antalya, Turkey*
[2]*Akdeniz University, Fisheries Faculty, Antalya, Turkey*

In this study, shrimp (*Aristeus antennatus*) immediately after trawling was treated with plant extracts (rosemary and green tea), sodium metabisulphite solution, and their combinations. After dipping into the solutions, melanosis and selected quality changes of shrimp were investigated during the storage at 4°C. Plant extracts were found effective in delaying melanosis in shrimp. Combinations of sodium metabisulphite and plant extracts were found more effective than those groups separately treated with plant extracts. Plant extracts, sodium metabisulphite, and combinations showed lower trimethylamine and total volatile nitrogen values as compared to the control group. It is concluded that use of plant extracts decreased the need for sodium metabisulphite.

Keywords: Shrimp, Melanosis, Plant extract antimelanosis activity, Onboard dipping.

INTRODUCTION

Shrimp contains very low fat with rich in essential amino acids.[1] In addition, it is easily digestible due to weak connective tissue. Shrimp are perishable with limited shelf life due to microbial spoilage and melanosis formation.[2,3] Melanosis is a biochemical process that triggered by the polyphenoloxidase (PPO) enzyme. Melanosis is an enzymatic browning process that occurs after harvest and proceeds during the storage. It is a natural mechanism caused by enzymatic reactions the presence of oxygen of the atmosphere from atmosphere after harvest. After harvest and death, PPO systems are still active and can promote the development of black pigments. This process begins with oxidation of phenols to quinones and polymerized to colored compounds.[3,4]

The presence of black spot is not dangerous to human health but it reduces the shrimp's market value and consumer acceptability. Reducing compounds are very effective to control of browning. Sulphiting agents are the most widely applied reagents for the control of browning in the food industry. Melanosis in crustaceans is normally controlled by means of certain sulphite derivatives.[5] Sulphides (especially asthmatics) cause harmful effects, thus alternative agents became necessary.[6,7] These compounds are known to produce allergic reactions and serious disturbances in asthmatic subjects.[8] Increased regulatory attention and heightened consumer awareness of the risks associated with sulphited foods

Received 15 December 2014; accepted 16 March 2015.
Address correspondence to Nalan Gokoglu, Akdeniz University, Faculty of Fisheries, Department of Seafood Processing Technology, Dumlupinar boulevard, 07058, Antalya, Turkey. E-mail: ngokoglu@akdeniz.edu.tr

have created a need for a safe effective sulphite alternative for use in foods.[9] Antioxidants are used to preserve food, such as delaying rancidity or discoloration which is result of oxidation. Both synthetic and natural phenolic antioxidants are used in foods.[10] Plant phenolic compounds such as flavonoid compounds, tocopherols, coumarins, and cinnamic acid derivates have an antioxidative effect.[11] Gokoglu and Yerlikaya[10] reported that grape seed extracts delayed melanosis formation in shrimp (*Parapenaeus longirostris*).

Rosemary (*Rosemary offiicinalis L.*) contains some antioxidative compounds such as rosmanol, rosmariquinone, rosmaridiphenol, and carnosol. Due to its phenolic compounds content, rosemary is four times effective antioxidant compared to butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT).[12] Tea contains some antioxidant components.[13] Antioxidant activity and total phenolic content of green tea are higher than those of other teas.[14] Green tea is rich source of catechins, epicatechins, epicatechin gallate, epigallocatechin, and epigallocatechin gallate. Antioxidant activities of polyphenols of green tea are higher than those of BHA, BHT, and DL-α-tocopherol.[15]

Natural antioxidants especially plant compounds became very important in recent years. Different plants have been investigated for their antioxidant activity.[16,17] In addition, several studies related to use of plant extracts for retarding of melanosis have been reported.[10, 18–21] In this study, different extracts which already are not used, were tested. Moreover, comparison of effectiveness of combinations of plant extracts and sodium metabisulphite was studied for the first time. The aim of this study was to use natural antimelanotic agents and their combinations with sodium metabisulphite immediately after harvesting and compare their effects on melanosis formation and quality in shrimp.

MATERIALS AND METHODS

Material and Reagents

Shrimp, *Aristeus antennatus*, were caught by trawling net in the Gulf of Antalya, Turkey (fishing area between 36º.40′.721″/31º.02′.870″ and 36º.43′.425″/31º.05′.040′). Average weight, length, and carapaces length were 16.71 ± 7.47 g, 14.02 ± 1.75 cm, 5.78 ± 0.95 cm, respectively. Dried green tea (*Camelia sinensis*) was purchased from the commercial market. Rosemary (*Rosemary officinalis*) was collected from the nature and dried until became the constant weight at 25–30°C.

Folin–Ciocalteau reagent, sodium carbonate, potassium persulphate, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), magnesium oxide, hydrochloric acid, sodium hydroxide, trichloroacetic acid, formaldehyde (FA), anhydrous toluene, potassium hydroxide, picric acid were obtained from Merck (Darmstadt, Germany). Slicone antifoam solution was obtained from Fluka (Steinheim, Germany). Tashiro indicator was obtained from Riedel (Seelze, Germany) for analyses.

Extraction

The plant extracts were prepared according to Yerlikaya and Gokoglu[22] extraction method. Dried and powdered samples were extracted with ethanol (1:20 [g/mL]) in a water bath with shaker at 40° C for 4 h. The extracts were filtered and concentrated in a rotary evaporator, then stored under nitrogen at –20°C until use. Concentrations of sodium metabisulphite and plant extracts were determined after preliminary trials which applied to shrimp of their different concentrations. For preliminary trials, shrimp were dipped into plant extracts of 1.0, 2.0, 3.0, and 5.0 g/100 mL and 0.5, 1.0, 1.25, and 2.0 g/100 mL sodium metabisulphite. At last, the best concentrations in terms of sensory properties and sulphite residue were used.

Treatments

For the experiment, eight different solutions with plant extracts and sodium metabisulphite were prepared in distilled water. These groups as follows:

R: Rosemary extract (2 g/100 mL);
G: Green tea extracts (2 g/100 mL);
S: Sodium metabisulphite (1.25 g/100 mL);
R+G: Rosemary extract (2 g/100 mL) + green tea extract (2 g/100 mL) combination;
R+S: Rosemary extract (2 g/100 mL) + Sodium metabisulphite (1.25 g/100 mL) combination;
G+S: Green tea extracts (2 g/100 mL) + Sodium metabisulphite (1.25 g/100 mL) combination;
R+G+S: Rosemary extract (2 g/100 mL) + green tea extract (2 g/100 mL) + Sodium metabisulphite (1.25 g/100 mL) combination;
C: control (distilled water).

Treatments with solutions were conducted on board. Immediately after catching, shrimp were dipped into the solutions of 15°C ± 1 (1:1 solution/shrimp [L/kg]) for one minute. After dipping, shrimp were placed on polystyrene plates and packed by overwrapping with polyvinylidene chloride film. Packed plates were stored at 4°C for 5 days. During the storage, melanosis formation was investigated and quality control analyses were conducted at daily intervals.

Analyses

Determination of phenolic content and antioxidant activity in the extracts: Total phenolic contents of the extracts were determined according to the Folin-Ciocalteau colorimetric method[23] using the Folin Ciocalteau reagent. Extract solution (100 mL for each) obtained by diluting 900 mL distilled water, 5 mL of 0,2 N Folin-Ciocalteau reagent and 4 mL saturated sodiumcarbonate solution (75 g/L) were added. The samples were kept at room temperature for 2 h and the results were evaluated with standard gallic acid curve, and expressed as gallic acid/100 g.

Antioxidant activities of the plant extracts were determined using the ABTS method.[24] Solution of potassium persulphate of 2.45 mM was added into the ABTS radical solution of 7 mM and kept in the dark for 12–16 h until it became radical. The ABTS radical solution was diluted with ethanol to show absorbance at 734 nm. Different concentrations of the extracts and Trolox solutions were prepared in ethanol and inhibition percentage was calculated. Graphs of inhibition percentage were plotted and calculated the slope. The ratio of the slopes of Trolox solution and plant extract was determined. The results were expressed as Trolox equivalent antioxidant capacity.

Dry matter content and pH of green tea and rosemary extracts

For dry matter content, extracts were dried at 105°C to a constant weight.[25] For pH analysis, samples were homogenized and diluted with distilled water in 1:1 ratio. Measurement was carried out by immersion pH-meter (WTW Inolab, Weilheim, Germany) probe.[26]

Total volatile basic nitrogene (TVB-N)

Total volatile basic nitrogen content was determined according to distillation method of Shormüller.[27] A 10-g sample was washed into the distillation flask and 1 mg magnesium oxide was added with a drop or two of silicone antifoam solution. Samples were boiled and distilled into 10 mL of 0.1 N HCl solutions in a 500 mL conical flask with added Tashiro-indicator. After distillation, the contents of conical flask were titrated with 0.1 N NaOH.

TABLE 1
Scale for melanosis evaluation (Otwell & Marshall 1986)

Melanosis scores	*Description*
0	Absent
2	Slight, noticeable on some shrimp
4	Slight, noticeable on most shrimp
6	Moderate, noticeable on most shrimp
8	Heavy, noticeable on most shrimp
10	Heavy, totally unacceptable

Trimethylamine (TMA)

A 10 g sample was blended with 90 mL of 5% trichloracetic acid (TCA) using an ultraturrax homogenizer (IKA Labortechnic, Staufen, Germany) and filtered. A 4 mL aliquot was transferred into test tubes and 1 mL FA (20%), 10 mL anhydrous toluene, 3 mL KOH (50%) solutions were added. The tubes were shaken and a 5-mL toluene layer was pipeted, to which a 5 mL picric acid (0.02%) had been added. The supernatant was then transferred to a spectrophotometric cell. Absorbance at 410 nm was measured with a UV-Vis spectrophotometer (Schimadzu UV-160A). At the same time, a series of standards were prepared and measured.[27]

Color measurement

Color measurements were carried out using CR-400 chromometer (Minolta, Osaka, Japan). L^* (lightness/darkness), a^* (redness/greenness), and b^* (yellowness/blueness) values of the samples were measured. Color measurements were made in head and body regions of five different shrimp. The average results were expressed as average values of all data.

Evaluation of melanosis

Melanosis formation was evaluated using Otwell and Marshall's[28] melanosis scale (Table 1). Melanosis for each experiment was evaluated daily by 10 experienced panellists from the Department of Food Engineering, University of Akdeniz. Random letters in each sample were coded before the beginning of the panel. Daylight was used to illuminate.

Statistical Analysis

Two replications of the experiment were conducted at separate times and all analyses were performed in duplicates. Data were analysed by a two factor factorial arrangement in a completely randomized design. The two factors were the eight extract treatments (C; S; G; R; R+G; R+S; G+S; and R+G+S) and the six storage times (0, 1, 2, 3, 4, and 5 days). Data were subjected to analysis of variance followed by Duncan's multiple range tests as appropriate Statistical Analytical Systems (SAS). Correlations between sensory melanosis scores and L^*, a^* values were calculated. Significance was defined either at $p < 0.05$ or at $p < 0.01$.

RESULT AND DISCUSSION

Dry Matter Contents of Extracts

Dry matter content of green tea and rosemary extracts were determined as 37.95 ± 0.27 g/100 g and 26.03 ± 0.54 g/100 g, respectively. Values of pH of the plant extract solutions were found as 6.04 ±

0.07 (C), 4.22 ± 0.01 (S), 4.03 ± 0.11 (G), 5.06 ± 0.01 (R), 3.95 ± 0.01 (R+G), 4.42 ± 0.01 (R+S), 4.62 ± 0.01 (G+S), 4.56 ± 0.01 (R+G+S).

Antioxidant and Phenolic Contents of Extracts

Total phenolic content of green tea extract (1.476 g GAE/100 g) was found to be higher than those of rosemary extract (0.234 g GAE/100 g). Total phenolic contents of methanol and water extracts of teas were found as 20,556 mg GAE/100 g and 19,126 mg GAE/100 g, respectively by Chan et al.[13] Ivanova et al.[29] reported that total phenolic content of green tea in water was 317.62 μM (quercetin equivalent). Yerlikaya and Gokoglu[22] reported that total phenolic content of green tea extract was 2.278 g GAE/100 g. Total phenolic content of rosemary extract in methanol was found as 162 mg GAE/g.[30] Gomez-Estaca et al.[31] reported that total phenolic content of rosemary extract in water was 665 μg (caffeic acid/mL extract). In the other research, total phenolic content of rosemary extract was reported as 185 mg GAE/g.[32] The reasons of differences in the values may be different solvents, time, and temperature and method of extraction and the pre-treatments applied to the products.

Total antioxidant activity of green tea extract was higher than those of rosemary extract. Antioxidant activities of green tea and rosemary extracts were determined as 814.32 μM Trolox and 51.0559 μM Trolox, respectively. In previous studies, many differences are observed for antioxidant activities. The reasons of these differences are estimated due to the differences in methods for antioxidant activity and extraction. In addition, phenolic content of the extract, solvent concentration, and type[33] affect antioxidant activity. Almajano et al.[34] reported that total antioxidant activity of green tea in hot water was 6344 mmol Trolox. Chan et al.[13] reported that ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) values of green tea extract in methanol were 126 mg GAE/g and 3000 mg (ascorbic acid/100 g), respectively. FRAP and DPPH values of green tea in water were found as 123 mg GAE/g and 26,210 mg ascorbic acid (AA)/100 g, respectively. Total antioxidant activity of rosemary extract in methanol was found as 45.7g Trolox/100 g by Oxygen Radical Absorbance Capacity (ORAC) method.[35] Antioxidant activity of rosemary extract in water was reported as 0.141 mg (ascorbic acid/mL) by Gomez-Estaca et al.[31] Erkan et al.[1] reported that antioxidant activity of rosemary extract in methanol was found as 15.7 mM (Trolox equivalent) by ABTS method. Antioxidant activity of green tea extract in water was found as 5.91 mM (Trolox equivalent).[29] Antioxidant activity of green tea extract in methanol was found as 1.772 mM Trolox by Yerlikaya and Gokoglu.[22] According to preliminary trials, the best concentrations in terms of sensory properties and sulphite residue were determined as 2 g/100 mL for plant extracts and as 1.25 g/100 mL for sulphite. These concentrations were used for the experiment.

Quality Parameters

During the storage, significant ($p < 0.01$) changes were found in pH values of treatment groups. The group with green tea extract has reached the highest pH value at the end of the storage. While there were significant differences ($p < 0.05$) in pH values among the treatments at 1st, 3rd, and 5th days of storage, insignificant differences were found at the 2nd and 4th days (Table 2). The real reason for increase in pH value is basic compounds produced by microbial activity.[36] A relationship between the consumer acceptability and pH value shows that pH value is a good indicator for freshness. Critical point for freshness is 7.8. In addition, 7.7 and lower pH values indicate the best quality; 7.70–7.95 values indicate acceptable quality; 7.95 and more values show unacceptable quality.[37] According to these criteria, all treatments have maintained the quality of shrimp during the storage. Nirmal and Benjakul[11] reported higher pH values for pacific white shrimp (*Litopenaeus vannamei*) treated with sodium metabisulphite than those the group treated with

TABLE 2
Changes in TVB-N, TMA-N, and pH values of shrimp treated with different solutions[a]

	Storage days	Treatments							
		C	*S*	*G*	*R*	*R+G*	*R+S*	*G+S*	*R+G+S*
TVB-N	0	22.20 ± 0.27 F	22.20 ± 0.27 F	22.20 ± 0.27 F	22.20 ± 0.27 F	22.20 ± 0.27 F	22.20 ± 0.27 F	22.20 ± 0.27 F	22.20 ± 0.27 F
	1**	35.90 ± 1.09 de E	40.81 ± 0.29 b E	43.02 ± 0.88 a E	36.52 ± 0.51 d E	34.60 ± 1.17 e E	38.95 ± 0.01 bc E	40.01 ± 0.65 b E	37.21 ± 0.96 cd E
	2**	77.38 ± 0.67 a D	59.56 ± 2.73 c D	57.20 ± 0.60 cd D	40.61 ± 0.02 f D	59.25 ± 1.26 c D	63.18 ± 0.52 b D	55.20 ± 0.11 de D	52.65 ± 0.82 e D
	3**	92.35 ± 3.86 b C	72.21 ± 0.57 d C	80.76 ± 2.42 c C	61.16 ± 0.49 f C	91.89 ± 2.33 b C	108.10 ± 1.54 a C	68.06 ± 0.98 de C	63.63 ± 0.49 ef C
	4**	131.57 ± 2.76 a B	120.43 ± 3.68 b B	88.85 ± 0.56 e B	91.96 ± 0.0007 e B	103.25 ± 0.42 d B	110.77 ± 0.27 c B	80.30 ± 3.61 f B	103.65 ± 0.16 d B
	5**	136.67 ± 0.30 a A	125.39 ± 0.41 c A	130.22 ± 0.07 b A	118.24 ± 2.13 d A	136.96 ± 0.51 a A	115.46 ± 0.48 de A	115.10 ± 0.66 e A	134.49 ± 2.42 a A
TMA-N	0	0.50 ± 0.02 F	0.50 ± 0.02 D	0.5 ± 0.02 E	0.5 ± 0.02 F	0.5 ± 0.02 E	0.5 ± 0.02 D	0.5 ± 0.02 F	0.5 ± 0.02 D
	1**	13.08 ± 0.60 a E	4.05 ± 0.66 d C	6.30 ± 0.41 bc D	4.56 ± 0.01 d E	4.64 ± 0.01 dD	4.76 ± 0.79 cd C	7.80 ± 0.26 b E	1.35 ± 1.38 e D
	2**	16.09 ± 0.16 a D	15.45 ± 0.04 a B	12.73 ± 1.36 b C	9.14 ± 0.03 c D	13.37 ± 0.59 b C	13.54 ± 0.73 b B	11.93 ± 0.36 b D	13.66 ± 1.08 b C
	3**	17.90 ± 0.23 a C	17.15 ± 0.46 a A	14.54 ± 0.82 b BC	11.74 ± 0.46 c C	18.28 ± 0.10 a B	14.47 ± 0.36 b B	15.23 ± 1.16 b C	17.61 ± 0.80 a B
	4**	19.87 ± 0.80 aB	17.46 ± 1.17 bcA	16.34 ± 0.74 cdB	14.20 ± 0.79 dB	19.24 ± 1.06 abB	16.76 ± 1.56 cA	17.34 ± 0.37 bcB	19.70 ± 0.20 aA
	5**	21.66 ± 0.20 aA	18.15 ± 0.18 deA	19.89 ± 1.12 bcdA	19.48 ± 0.87 dA	21.58 ± 0.39 abA	17.38 ± 0.66 eA	19.73 ± 1.17 cdA	21.40 ± 0.05 abcA
pH	0	6.79 ± 0.02 D	6.79 ± 0.02 E	6.79 ± 0.02 E	6.79 ± 0.02 D	6.79 ± 0.02 D	6.79 ± 0.02 E	6.79 ± 0.02 C	6.79 ± 0.02 E
	1*	7.06 ± 0.09 a C	7.01 ± 0.01 ab D	6.92 ± 0.04 bc D	6.99 ± 0.04 abc C	6.95 ± 0.06 bc C	6.98 ± 0.01 abc D	6.94 ± 0.03 bc B	6.9 ± 0.03 c D
	2	7.09 ± 0.03 ab C	7.02 ± 0.01 ab D	7.08 ± 0.07 ab C	7.01 ± 0.07 b C	7.10 ± 0.05 ab B	7.08 ± 0.05 ab C	7.12 ± 0.05 a A	7.06 ± 0.01 ab C
	3**	7.28 ± 0.01 a B	7.16 ± 0.02 cd C	7.12 ± 0.03 d C	7.23 ± 0.05 b B	7.13 ± 0.02 cd B	7.18 ± 0.05 bc B	7.11 ± 0.005 d A	7.13 ± 0.01 cd BC
	4	7.30 ± 0.12 abc B	7.34 ± 0.04 ab B	7.38 ± 0.08 a B	7.29 ± 0.04 abc AB	7.28 ± 0.01 abc A	7.19 ± 0.06 bc B	7.16 ± 0.12 c A	7.22 ± 0.10 bc B
	5**	7.50 ± 0.15 abc A	7.58 ± 0.05 ab A	7.60 ± 0.05 a A	7.36 ± 0.07 cd A	7.33 ± 0.14 cd A	7.41 ± 0.08 bc A	7.21 ± 0.05 d A	7.33 ± 0.07 cd A

[a]All values reflect mean ± standard error;

Uppercase letters in the same column indicate differences between treatments, lowercase letters in the same row indicate differences ($p < 0.05$) of the storage days;

**Significantly different ($p < 0.01$);

*Significantly different ($p < 0.05$).

ferulic acid at iced storage. In the present study, the highest pH value was found for the shrimp treated with green tea extract. The lowest value was found in the group treated with combination of green tea and sodium metabisuphite. Differences in initial pH value of shrimp can be attributed to differences in extract solution, shrimp species, and catching season.

TVB-N values of shrimp are shown in Table 2. TVB-N values of all samples increased during the storage. The increases in TVB-N value are statistically significant ($p < 0.01$). Control, R+G, and R+G+S groups reached the highest TVB-N values during the storage compared to other groups. Treatment groups of R, R+S, and G+S had the lowest values at the end of the storage. Rosemary extract indicated a good protective effect compared to green tea extract. Separate combinations of plant extracts with sodium metabisulphite (R+S and G+S) had a clear protective effect. However, in case of combination of all treatments (G+R+S) there was no significant effect. Sensory, chemical, physical, and microbiological analysis methods are used to control of the quality of fishery products. TVB-N is one of the most widely used analysis among the chemical methods. It is reported that the increase in TVB-N value of fishery products during the storage was parallel with increase of storage time. A level of 35 mg/100 g has been considered the upper limit for human consumption.[37] In the present study, TVB-N values exceeded the limit value at the first day of the storage. Initial TVB-N value of shrimp was found as 22.20 mg/100 g. Similar TVB-N values were reported for *Parapenaeus longirostris, Palaemon adspersus, Marsypenaeus japonicas* in the previous studies.[2,38,39]

Trimethylamine nitrogen (TMA-N) values in all treatment groups increased during the storage (Table 2). There were significant differences ($p < 0.01$) in TMA-N values among the plant extracts, sodium metabisulphite, and their combinations. At the first day of the storage, while the highest TMA-N content was found in control group, the lowest content was found in R+G+S group. During the storage R group had the lowest TMA-N values. At the end of the storage R+S was the most successful group. Rosemary extract was more effective to maintain the quality of shrimp. When plant extracts were used alone they were more effective. R and G groups were more effective than S group when compared each other. TMA occurs during degradation of fishery products. Dimethylamine (DMA), FA, and TMA in fish products originate by breakdown trimethylamine oxide (TMAO). The amount of TMA-N is important in terms of indicating the level of microbial growth for fresh seafood. The amount of 10 mg/100 g has been considered the upper limit for human consumption.[40] In the present study; while in the control group limit value was exceeded at the first day of storage, it was exceeded after two days in the other groups. Varlik et al.[38] reported that TMA-N value was found as 19.7 mg/100 g on the 4th day of the storage. The effect of organic acids on melanosis formation and quality in the shrimp (*Penaeus japonicus*) has been investigated.[2] While control group reached the highest TMA-N value, the lowest values was observed in the groups treated with citric and lactic acids at the end of the storage. The amount of TMA-N may vary with species, fishing area, fishing methods, and storage conditions.[40] There are some differences in TMA-N values due to differences in shrimp species, fishing season, extract type, and the initial TMA-N content.

Color Measurements

The results of color measurement are shown in Table 3. L^* value indicates whiteness, lightness or darkness. L^* value increases, also lightness increases and darkness decreases. There were significant differences among the groups at the 1st and 3rd days of the storage. The highest values were found for S and R+S groups. Sodium metabisulphite and its combinations were more effective than those of individual plant extracts to maintain lightness. This may arise from some properties of sulphides. They are effective reducing agent which has bleaching properties.[6]

During the storage, a^* values of shrimp decreased. At the end of the storage, C, G, R and R+G +S groups had the lowest a^* values. However, the groups treated with combination of plant

TABLE 3
Changes in color (L^*, a^*, b^*) values of shrimp treated different solutions[a]

	Storage days	Treatments							
		C	*S*	*G*	*R*	*R+G*	*R+S*	*G+S*	*R+G+S*
L*	0	36.81 ± 0.24 A	36.81 ± 0.24 B	36.81 ± 0.24 A	36.81 ± 0.24 A	36.81 ± 0.24 A	36.81 ± 0.24 A	36.81 ± 0.24 A	36.81 ± 0.24 AB
	1**	36.19 ± 2.02 c A	39.74 ± 0.57 a AB	36.53 ± 0.30 c A	36.27 ± 1.21 c A	35.40 ± 0.12 c A	39.32 ± 0.05 ab A	37.33 ± 0.17 bc A	36.73 ± 0.34 c AB
	2	36.22 ± 1.08 a A	38.53 ± 3.44 a AB	35.74 ± 1.39 a A	36.83 ± 2.46 a A	34.72 ± 2.26 a A	39.07 ± 0.83 a A	37.81 ± 0.16 a A	38.21 ± 0.74 a A
	3**	33.21 ± 0.87 e B	40.95 ± 0.22 a A	35.98 ± 0.74 cd A	34.43 ± 1.36 de A	34.24 ± 1.02 de A	39.31 ± 0.52 ab A	37.72 ± 0.60 bc A	36.88 ± 2.16 bcd AB
	4*	32.11 ± 0.71 d B	39.99 ± 0.19 a AB	35.37 ± 2.85 bcd A	33.69 ± 0.84 cd A	34.23 ± 2.63 bcd A	39.95 ± 2.53 a A	38.28 ± 0.68 ab A	37.71 ± 1.62 abc AB
	5	32.74 ± 1.03 c B	36.62 ± 0.63 ab B	34.53 ± 0.25 abc A	34.77 ± 1.67 abc A	33.45 ± 2.32 bc A	37.73 ± 2.37 a A	36.70 ± 1.42 ab A	34.96 ± 0.75 abc B
a*	0	10.56 ± 2.63 A	10.56 ± 2.63 A	10.56 ± 2.63 A	10.56 ± 2.63 A	10.56 ± 2.63 A	10.56 ± 2.63 A	10.56 ± 2.63 A	10.56 ± 2.63 A
	1	8.85 ± 0.01 a AB	10.32 ± 0.79 a AB	8.72 ± 1.51 a AB	10.23 ± 0.85 a A	8.56 ± 1.03 a AB	9.95 ± 0.55 a A	10.22 ± 0.65 a A	9.81 ± 0.32 a AB
	2**	7.44 ± 0.14 bc B	8.78 ± 0.46 ab AB	7.30 ± 0.81 bc AB	6.51 ± 0.68 c B	7.52 ± 0.17 bc B	9.56 ± 0.38 a A	10.10 ± 0.49 a A	9.68 ± 1.44 a AB
	3**	6.16 ± 0.58 b B	8.37 ± 0.86 a AB	7.18 ± 0.47 b B	6.37 ± 0.21 b B	7.26 ± 0.15 b B	8.57 ± 0.40 a A	9.30 ± 0.51 a A	9.50 ± 0.02 a AB
	4**	6.10 ± 0.30 c B	8.31 ± 0.15 a AB	6.51 ± 0.15 bc B	6.50 ± 0.07 bc B	7.17 ± 0.30 b B	8.40 ± 0.16 a A	9.20 ± 0.49 a A	9.10 ± 0.97 a AB
	5**	5.99 ± 0.15 cd B	7.15 ± 0.83 b B	5.77 ± 0.22 d B	5.97 ± 0.23 cd B	7.12 ± 0.30 bc B	7.76 ± 0.91 b A	9.15 ± 0.03 a A	6.94 ± 0.20 bc B
b*	0	8.16 ± 1.89 A	8.16 ± 1.89 A	8.16 ± 1.89 A	8.16 ± 1.89 A	8.16 ± 1.89 A	8.16 ± 1.89 A	8.16 ± 1.89 B	8.16 ± 1.89 B
	1**	6.55 ± 0.30 d A	7.93 ± 0.22 c A	8.68 ± 0.13 bc A	9.25 ± 0.97 abc A	9.35 ± 0.12 ab A	9.22 ± 1.07 abc A	9.15 ± 0.20 bc AB	10.53 ± 0.06 A a
	2**	6.48 ± 0.47 c A	7.18 ± 0.37 bc A	7.67 ± 0.29 b A	6.89 ± 0.62 bc A	9.25 ± 0.31 a A	9.44 ± 0.08 a A	10.10 ± 0.32 a AB	9.93 ± 0.42 a AB
	3**	6.03 ± 0.45 c A	7.47 ± 0.06 b A	8.35 ± 0.58 b A	7.97 ± 1.05 b A	8.45 ± 0.07 b A	8.68 ± 0.47 b A	10.38 ± 0.02 A a	10.43 ± 0.07 a A
	4**	6.40 ± 0.47 c A	8.03 ± 0.36 b A	8.56 ± 0.13 b A	8.01 ± 0.66 b A	8.72 ± 0.003 b A	8.29 ± 0.02 b A	10.96 ± 0.14 a A	10.35 ± 0.01 a A
	5**	6.88 ± 0.65 cd A	6.45 ± 0.39 d A	9.8 ± 0.71 a A	7.48 ± 0.21 c A	8.75 ± 0.32 b A	7.58 ± 0.19 c A	9.71 ± 0.002 ab AB	9.33 ± 0.27 ab AB

[a]All values reflect mean ± standard error;

Uppercase letters in the same column indicate differences between treatments, lowercase letters in the same row indicate differences of the storage days;

**Significantly different ($p < 0.01$);

*Significantly different ($p < 0.05$).

TABLE 4
Changes in melanosis values of shrimp treated different solutions[a]

Storage time (days)	*Control***	*S***	*G***	*R***	*R+G***	*R+S***	*G+S***	*R+G+S***
0	0 D	0 F	0 E	0 F	0 E	0 F	0 D	0 D
1**	3.55 ± 0.62 a C	0.72 ± 0.23 c E	3.05 ± 0.07 ab D	3.38 ± 0.39 a E	3.33 ± 0.78 a D	0.94 ± 0.07 c E	1.5 ± 0.39 c C	1.91 ± 0.82 bc C
2**	6.11 ± 1.1 a B	2.5 ± 0.39 c D	5.05 ± 0.54 ab C	6.11 ± 0.47 a D	4.72 ± 0.07 b C	2.61 ± 0.39 c D	2.55 c C	2.71 ± 0.69 c C
3**	7.66 ± 1.25 a AB	4.88 cd C	5.72 ± 0.39 bc C	7.05 ± 0.39 ab C	6.16 ± 0.54 bc B	3.55 ± 0.31 d C	4.27 ± 0.54 d B	3.57 ± 0.03 d BC
4**	8.38 ± 0.70 a A	5.83 ± 0.39 b B	8 ± 0.62 a B	8.27 ± 0.07 a B	7.61 ± 0.39 a A	4.5 ± 0.70 b B	5 b B	4.75 ± 1.06 b B
5**	9.5 ± 0.70 a A	7.88 ± 0.31 bcd A	9 ab A	9.22 ± 0.31 a A	8.27 ± 0.23 abc A	6.61 ± 0.23 de A	6.5 ± 0.86 e A	7.38 ± 0.86 cde A

[a]All values reflect mean ± standard error;
Uppercase letters in the same column indicate differences between treatments, lowercase letters in the same row indicate differences of the storage days;
**Significantly different ($p < 0.01$);
*Significantly different ($p < 0.05$).

extracts and sodium metabisulphite showed higher a^* values. The highest a^* value was found in G +S group. Treatment groups showed no significant changes in b^* values during the storage. However, there were significant differences among the treatment groups. Higher b^* values were found in sulphide combination groups. During storage, b^* values did not change significantly; however, there were significant differences among the treatment groups. Higher b^* values were found in the groups treated with sulphide combination. Group with green tea (G) showed also a high b^* value. Insignificant changes in b^* values of shrimp were reported during the storage.[6,10]

Melanosis

Melanosis scores of all groups significantly ($p < 0.01$) increased during the storage. The lowest melanosis scores were found in sulphide treated and sulphide combined groups. During the storage, control group had the highest melanosis scores (Table 4). At the end of the storage, the lowest melanosis score was found for G+S group. On the 4th day of the storage, melanosis score in C, G, and R groups was found as 8 (means heavy and noticeable on most shrimp). At the same time, appearance of these groups was unacceptable at the end of the storage. Treatments of plant extracts alone and as combination were no effective in delaying melanosis. More effective results were obtained when they combined with sodium metabisulphite. Even S, R+S, G+S, and R+G+S groups were found within the acceptable limits on 4th day of the storage. Melanosis formation was delayed by the treatment with grape seed extract for 2 days during the storage at 4°C.[10] Gokoglu[2] reported that while control group was unacceptable on the 4th day, sodium metabisulphite and organic acids combinations were unacceptable at the 6th day of the storage. Melanosis formation was delayed 12–14 days when shrimp treated with 4-hexyresorcinol combined with L-ascorbic acid and acetic acid.[3] These differences in different studies may be due to differences between species of shrimp, storage conditions, types of dipping solution, and variety of melanosis inhibitors. In this study, plant extracts combined with sodium metabisulphite were effective to delay melanosis. There was a good correlation between melanosis scores and L^* values except combination of sodium metabisulphite groups. Also there was a good correlation between melanosis scores and a^* values (Table 5).

CONCLUSIONS

According to quality parameters, rosemary extract was more effective than green tea extract. Plant extracts and their combinations with sodium metabisulphite were very effective on keeping quality of shrimp. Plant extracts, sodium metabisulphite group and combinations showed lower TMA-N and TVB-N values when compared with control group. Plant extracts were effective in delaying

TABLE 5
Correlations between sensory and instrumental color scores for all treatments

	L*	a*
C	–0.8635	–0.99008
S	0.0304	–0.96911
G	–0.9408	–0.98714
R	–0.7544	–0.93034
R+G	–0.9817	–0.94618
R+S	0.2091	–0.97901
G+S	0.1827	–0.96553
R+G+S	–0.49622	–0.95709

melanosis in shrimp. Combinations of sodium metabisulphite and plant extracts were more effective than other groups. It is concluded that use of plant extracts decreases the need for sodium metabisulphite. Rosemary and green tea extracts can be used as alternative antimelanotic agent against melanosis in shrimp. In addition, these plants are commonly found in nature and can be utilized in extract production. At this point economic importance of these plants can be increased. This research will constitute a base for further studies. Different plants and different extraction methods, different concentrations, and different storage conditions should be investigated.

ACKNOWLEDGMENT

The authors wish to thank Dr. Mehmet Gokoglu for his help in providing the samples.

FUNDING

The authors would like to thank the Scientific Research Project Administration Unit of Akdeniz University under Project No. 2010.02.0121.005 supported this research.

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