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THE EFFECT OF ORGANIC ACID TREATMENTS ON THE MELANOSIS INHIBITION AND SHELF-LIFE IN SHRIMP (*PENAEUS JAPONICUS*)

N. GÖKOĞLU*

Department of Food Engineering, Faculty of Agriculture, University of Akdeniz, Antalya. Turkey

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The effect of organic acids on the melanosis inhibition and shelf-life on shrimp was investigated. Shrimps were treated with 1% solutions of lactic, citric, acetic acids, sodium metabisulphite (0.3%) and their various combinations. After treatments the shrimps were stored at 4 °C and evaluated for melanosis every day and quality changes on every other day. Combinations with sodium metabisulphite were the most effective in delaying melanosis. Citric and lactic acids extended shelf life to a lesser extent but acetic acid had no effect.

Keywords: shrimp, melanosis, quality, organic acid treatments

Shrimps are one of the most important and also expensive marine products in Turkey. Total amount of shrimp caught in Turkey was 890 metric tons in 1999 (ANON, 2001). Shrimp is a highly perishable product with limited shelf life due to melanosis and microbial spoilage. Discolouration due to melanosis is a serious problem in shrimp processing. Melanosis in shrimp, commonly referred to as "black spot" is a defect caused by activity of polyphenoloxidase (BENNER et al., 1994). The endogenous shrimp enzyme, polyphenoloxidase, catalyzes the initial step in black spot formation and remains active throughout post-harvest processing unless the shrimps are frozen or cooked (MCEVILY et al., 1991). This defect initially occurs in appearance but increasingly spoils the flesh and therefore quality is lost. This is a very important factor limiting the storage life of fresh shrimp. Proliferated enzymes present in the head region are responsible for discoloration. The breakdown of tyrosine, an amino acid, brings about melanin formation causing black spot. Tyrosine liberated during proteolysis in shrimp muscle undergoes oxidation to dihydroxyphenyl alanine and leads to the formation of melanin pigments. These compounds are formed from phenols by oxidation as well as enzymatically initiated and metal-catalyzed polymerization (SIKORSKI et al., 1989). pH of shrimps and prawns has been suggested as a good index of freshness (CHUNG & LAIN, 1979). Increases in shrimp pH during the storage at low temperatures may be due to postmortem enzymatic ammonia production. Ammonia producing adenosine deaminase and adenosine monophosphate deaminase enzymes can remain active at low temperatures (MARSHALL & WIESE-LEIGH, 1997).

* Fax: 90 2422274564; e-mail: ngokoglu@akdeniz.edu.tr

Sulfating agents were introduced in the 1950s to inhibit black spot formation. Adverse reactions to sulfites, with particular concern for sensitive asthmatics, as well as other risks associated with their use are well documented (MCEVILY et al., 1991).

Increased regulatory attention and heightened consumer awareness of the risks associated with sulfated foods have created a need for a safe, effective sulfite alternative for use in foods (TAYLOR et al., 1986; MCEVILY et al., 1991). It has been shown that substation of 4-hexylresorcinol for sulfating agents had several advantages for both the fisherman and consumer.

Quality changes in shrimp during storage occur due to microbial activity and tissue enzymes (COBB & VANDERZANT, 1971; FLICK & LOVELL, 1972; COBB et al., 1976). Microbial activity is one of the main causes of quality deterioration of shrimp, but endogenous enzymatic activity also causes quality deterioration in shrimp (COBB et al., 1976). Changes in pH, microbial counts, trimethylamine, total volatile nitrogen, non-protein nitrogen, free amino acids, volatile acids and indole have been used as quality indices of shrimp (CHEUK et al., 1979). Total volatile nitrogen (TVN) production is the combined result of tissue enzymes and microbial activities (CHEUK et al., 1979). Total volatile nitrogen is produced at a low but constant rate in shrimp until bacterial levels exceed 10^6 per gram (COBB et al., 1976). Crustaceans contain considerable trimethylamine oxide (TMAO), and TMAO is broken down to trimethylamine (TMA) by psychotropic bacterial enzymes during cold storage (YAMAGATA & LOW, 1995).

The purpose of this study was to investigate the effect of organic acids (lactic, citric, acetic acids) and their combinations with sodium metabisulphite on melanosis inhibition and to determine the shelf life of shrimp treated with organic acids during refrigerated storage.

1. Materials and methods

Fresh shrimps (*Penaeus japonicus*) were obtained directly from fishing boats in the gulf of Antalya, Turkey and immediately packed in ice and transferred to the laboratory within 3 h.

Shrimps were divided into eight groups and dipped into treatment solutions for 1 min. Dip solutions consisted of the following (1) control, (2) 1% lactic acid, (3) 1% citric acid, (4) 1% acetic acid, (5) 0.3% sodium metabisulphite, (6) 1% lactic acid with 0.3% sodium metabisulphite, (7) 1% citric acid with 0.3% sodium metabisulphite, (8) 1% acetic acid with 0.3% sodium metabisulphite. All dip solutions were prepared using seawater. After dipping, shrimps were drained at ambient temperature (20 °C) for 5 min. Ten shrimps from each treatment were placed on a polystyrene container, packed by wrapping with polyvinylidene chloride film and stored at 4 °C. Melanosis formation was evaluated according to the melanosis scale (Table 1) published by OTWELL and MARSHALL (1986).

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

1.1. Melanosis inhibition

Shrimps from each treatment were evaluated daily for degree of melanosis by an experienced 5-member panel. A score of 4 or less indicated high quality product with minimal melanosis. A score between 4 and 10 was considered indicative of shrimp with measurable quality defects. A score of 8 or greater represented severe defects, approaching unacceptable (OTWELL & MARSHALL, 1986; MCEVILY et al., 1991; BENNER et al., 1994).

1.2. Quality parameters

1.2.1. Sample preparation. The samples were homogenized using a blender and all analyses were performed with samples of the homogenates. Total volatile nitrogen (TVN), trimethylamine nitrogen (TMA-N), pH and sensory analyses were conducted at intervals of 2, 4 and 6 days. All analyses were performed in duplicates.

1.2.2. TVB-N analysis. Magnesium oxide was added to homogenized samples and all volatile nitrogenous compounds from the samples were distilled and these were collected in hydrochloric acid. The unreacted acid was titrated with NaOH solution and TVB was calculated (SCHORMULLER, 1968).

1.2.3. TMA-N analysis. Volatile bases were extracted with trichloracetic acid. Bases other than TMA were fixed with formaldehyde. Toluene was used to extract the TMA from a basic medium and then reacted with picric acid to yield a coloured picrate salt and analyzed spectrophotometrically (SCHORMULLER, 1968).

1.2.4. pH measurement. The pH of meat was measured in a mixture of homogenized sample and distilled water (1:1).

1.2.5. Sensory analysis. Sensory evaluation was carried out by a trained panel of 10 panellists. The panelists evaluated the shrimp for odour, colour and general appearance on a 9 point hedonic scale (9: extremely good, 5: neither good nor poor, 1: extremely poor). The average score for each parameter was calculated. An overall quality score was calculated as the mean score of odour, colour and appearance. In order to determine if the shrimps contain any acid taste, the taste of shrimp treated with organic acids was tested after treatments. For this purpose the shrimps were cooked in a closed jar over boiling water and presented to the panellists.

1.2.6. Statistical analysis. For statistical analysis, data were analyzed using analysis of variance. Significant differences between means were determined by Least Significant Difference.

2. Results and discussion

Mean melanosis scores of shrimp increased during the storage at 4 °C (Table 2). Black spot developed rapidly in control shrimps and exceeded the limit score after 2 days. The acid treatments showed delayed melanosis. However, the combinations of organic acid-sodium metabisulphite were more effective ($P<0.05$) than organic acid treatments alone. Previous studies have shown that bisulfite treatments had considerable effect on preventing melanosis (CAMBER et al., 1957; TAYLOR et al., 1986; SLATTERY et al., 1991; ARMENTIA-ALVAREZ et al., 1994). The effect of sodium metabisulphite on melanosis was confirmed in the present study. Limit score, which indicates poor quality, was exceeded after 5 days. Lactic acid-metabisulphite and citric acid-metabisulphite combinations were more effective ($P<0.05$) than acetic acid-metabisulphite combination.

Control shrimps were judged unacceptable after 4 days compared to lactic and acetic acids treatments, which were unacceptable after 5 days (Table 2). Citric acid treatment and acid-sulphite combinations were unacceptable after 6 days.

Table 2. Melanosis scores of shrimp during the storage

Storage days	C	LA	CA	AA	LMS	CMS	AMS	MS
1	4.4±0.13	2.8±0.04	2.0±0.08	2.8±0.08	0	0	0	0
2	6.0±0.17	2.8±0.44	2.8±0.06	3.2±0.14	0	0	2.8±0.2	0
3	6.4±0.12	5.6±0.16	4.8±0.04	6.0±0.19	3.2±0.14	3.2±0.04	5.2±0.32	3.2±0.12
4	8.8±0.16	6.0±0.27	5.6±0.41	6.8±0.34	5.8±0.27	4.8±0.26	5.2±0.21	4.0±0.47
5	9.6±0.22	8.4±0.28	7.2±0.17	8.4±0.17	8.0±0.2	8.0±0.12	8.0±0.12	7.2±0.13
6	10±0	9.2±0.12	9.6±0.18	9.6±0.13	9.6±0.08	9.2±0.1	10±0	8.0±0.1

C: Control; LA: lactic acid; CA: citric acid; AA: acetic acid; LMS: sodium metabisulphite with lactic acid; CMS: sodium metabisulphite with citric acid; AAS: sodium metabisulphite with acetic acid; MS: sodium metabisulphite.
* All values reflect the means ± standard errors (n=5).

2.1. Sensory analysis

Sensory shelf life of shrimp judged by sensory evaluation is shown in Table 4. There was no significant difference ($P>0.05$) among treatments and control till to the fourth day of storage. Acceptability decreased with increasing storage time. In the taste studies no acidic taste was detected up to the fourth day in any of the treatments.

Table 3. The results of variance analysis related to TVB-N, TMA-N and pH values

Source		DF	Sum squares	F
TVB-N	Time	3	731.17785	76.85**
	Treatment	3	106.961	11.24**
	Time × treatment	9	56.879106	5.98**
	Error	16	9.514	
TMA-N	Time	3	125.3854667	72.01**
	Treatment	3	2.297900	1.32*
	Time × treatment	9	0.411900	0.24
	Error	16	1.7412750	
pH	Time	3	0.35663646	3.99*
	Treatment	3	0.18978646	2.12
	Time × treatment	9	0.03489757	0.39
	Error	16	0.08936563	

* significant ($P<0.05$)
** significant ($P<0.01$).

Table 4. Sensory analysis scores of shrimp treated with citric, acetic and lactic acids[c]

Storage (h)	Control (n=10)	Citric acid (n=10)	Acetic acid (n=10)	Lactic acid (n=10)
0	9±0.21	8.9±0.16	8.7±0.24	8.8±0.25
2	5.4±0.24	7.7±0.14	7.2±0.14	7.6±0.23
4	2.1±0.21	5.6±0.035	2.3±0.09	6.3±0.14
6	1.1±0.09	2.1±0.035	1.2±0.09	3.3±0.1

[a] Data are means ± standard errors.

2.2. TVB-N

Initial TVB-N content of shrimp was 18.29 mg/100 g and this value gradually increased during storage at 4 °C for all treatments (Fig. 1). It was reported that TVB-N increased with time and temperature (SHAMSHAD et al., 1990; YAMAGATA & LOW, 1995). TVB-N contents showed significant differences ($P<0.01$) according to storage time and organic acid treatments (Table 3). A level of 30 mg/100 g has been considered the upper limit above which fishery products are considered unfit for human consumption (MONTGOMERY et al., 1970). The shrimps treated with acetic acid and control shrimps reached the limit value after 4 days, whereas shrimps treated with citric acid and lactic acid exceeded the limit after 6 days.

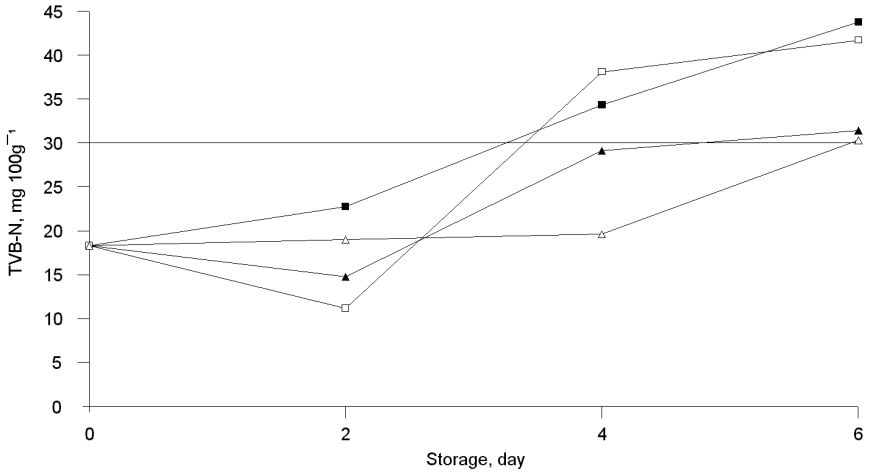

Fig. 1. Changes in the total volatile nitrogen (TVN) content of shrimp treated with citric, acetic and lactic acids during storage. ■: Control; ▲: citric acid; □: acetic acid; Δ: lactic acid

2.3. TMA-N

TMA-N content increased throughout the storage at 4 °C for all treatments. TMA-N level of untreated shrimps was 0.05 mg/100 g. TMA-N reached 9.1 mg/100 g in the control treatment. TMA-N contents were significantly different ($P<0.01$) between storage times (Table 3). The effect of organic acid treatments on TMA-N contents was significant ($P<0.05$) (Table 3). A TMA-N value of 5 mg/100 g is the limit of acceptability for shrimp in Australia and Japan (MONTGOMERY et al., 1970). Our samples (all treatments and control) exceeded the limit value after 4 days (Fig. 2).

2.4. pH

pH values of shrimp tissue increased throughout the storage for all treatments. Although the effect of storage time on pH value was significant ($P<0.05$), organic acid treatments did not have a significant effect (Table 3). The pH of untreated shrimps was 7.10 and it reached 7.31, 7.50, 7.55 in shrimps treated with acetic, citric and lactic acids, respectively (Fig. 3). However, the pH of control group was higher than that of others throughout the storage ($P<0.05$).

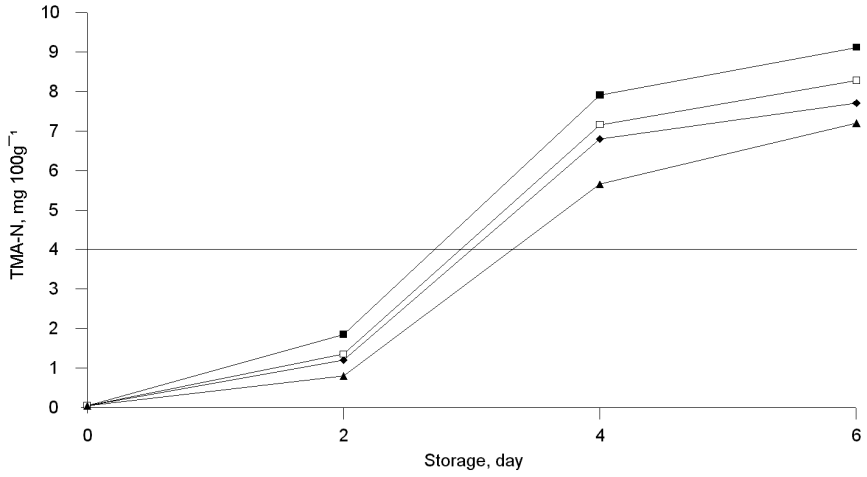

Fig. 2. Changes in the tyrimethylamine nitrogen (TMA-N) content of shrimp treated with citric, acetic and lactic acids during storage. ■: Control; ▲: citric acid; □: acetic acid; Δ: lactic acid

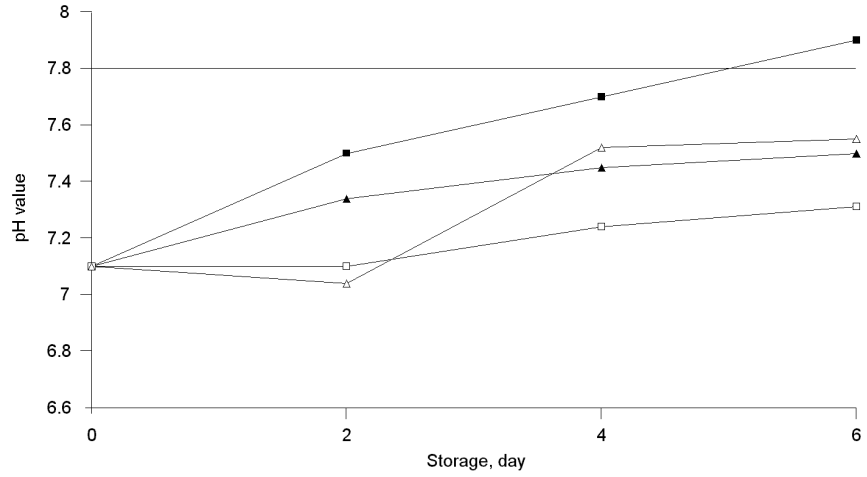

Fig. 3. pH changes of shrimp treated with citric, acetic and lactic acids during storage. ■: Control; ▲: citric acid; □: acetic acid; Δ: lactic acid

SHAMSHAD and co-workers (1990) reported that the pH increased with time and temperature and they noted a relationship between pH and acceptability. The pH of shrimp has been suggested as a good index of freshness and pH 7.8 was a critical

margin for acceptability (CHUNG & LAIN, 1979). It was also reported that shrimp pH of 7.7 or less indicates prime quality, 7.70–7.95 shows poor but acceptable quality and 7.95 or greater represents unacceptable quality (MARSHALL & WIESE-LEIGH, 1997). According to these criteria, our samples treated with organic acids were of good quality throughout the storage, whereas control samples were of acceptable quality up to 2 days only. ZHUANG and co-workers (1996) reported that the pH of shrimp immediately after treatment with sodium acetate was lower than that of control. They also stated that sodium lactate or propyl gallate had no effect on pH throughout the 13-day test.

3. Conclusion

Organic acids and their combinations with metabisulphite had an inhibitory effect on melanosis in fresh shrimp. Citric acid was the most effective followed by lactic and acetic acids. The combinations of organic acids with metabisulphite were more effective than organic acids alone in preventing melanosis. Moreover, citric and lactic acids extended the shelf life. Acetic acid had no effect on the shelf-life.

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