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Combined effect of sepia soaking and temperature on the shelf life of peeled shrimp *Penaeus kerathurus*

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Abstract

The effects of sepia ink (*Sepia officinalis*) extract solutions with concentrations of 0.0%, 0.01%, 0.2% and 2% on the microbial flora and the chemical composition of stored peeled shrimp (*Penaeus kerathurus*) were examined at two temperatures (−2 and 0 ° C). The quantification of aerobic and psychotrophic bacteria was performed by a plate count after each 3 days of storage. Results showed that partial freezing and coating treatments had synergistic effects in reducing the aerobic plate counts (APCs) with at least a 10-day extension of shelf life. The inhibition efficacy on the psychotrophic cells in shrimp meat increased with the increase in concentration of the added extract. However, the extract showed no effect after 16 days on the mesophilic bacteria. No detrimental effects of sepia coating on organoleptic parameters such as appearance, odour, and taste were observed. Partial freezing reduced significantly the formation of nitrogenous compounds in all samples, however total volatile bases (TVB) and trimethylamine (TMA) levels were lower in the sepia extract-treated samples. Initially, shrimp tissue ninhydrin positive substances (NPS) level was 9.74 ± 1.05 mM/100 g tissue. No significant change was observed in the treated-samples during the refrigerated storage, conversely, these concentrations increased in the raw shrimps.

Keywords: Sepia extract; Temperature; Chemical changes; APCs; *Penaeus kerathurus*

1. Introduction

The growing consumer demand for food without chemical preservatives has focused efforts towards research on natural antimicrobials and antioxidants. Thus, investigation in this field at academic and industrial laboratories has strengthened recently, and several investigations have been conducted in an attempt to extend the shelf life of seafood without adversely affecting its quality and safety [e.g. various spices and derivatives are frequently used as antimicrobial agents in food (Prasad & Seenayya, 2000; Sağdic & Özcan, 2003; Shelef, 1983), edible coating from polysaccharides, protein and lipids has also been applied on seafood products during storage (Gennadios, Hanna, & Kurth, 1997)]. Furthermore, the naturally occurring antimicrobials compounds in chitin were found to hinder both the microbial flora and the increase in the volatile basic nitrogen content in refrigerated raw shrimp (Simpson, Gagne, Ashie, & Noroozi, 1997). Numerous other studies have supported the antibacterial and antioxidative activities of chitin, chitosan and their derivatives in processed seafood products (Kamil, Jeon, & Shahidi, 2002; Shahidi, Arachchi, & Jeon, 1999; Tsai, Su, Chen, & Pan, 2002).

The ink (commonly called sepia) is produced by a side gland of the digestive tract in the mantle cavity of molluscs such as cuttlefish, squid or octopus. Because of its unusual black colour and delicate taste, cuttlefish ink is used to colour pasta in some Mediterranean countries and has become a classic worldwide dish. Moreover, the cuttlefish ink has been traditionally assumed to exhibit an antiseptic effect on cured cuttlefish meat produced in Japan (Mochizuki, 1979).

Accordingly, the unusual antimicrobial activity of cephalopod ink against different groups of microorganisms has received considerable attention in recent

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years (Palumbo & Jackson, 1995; Small & McFall-Ngai, 1999; Takai, Yamazaki, Kawai, Inoue, & Shinano, 1993a).

Takai et al. (1993a) ascertained that both *Staphylococcus epidermidis* and *Staphylococcus aureus* were inhibited by the extracts of squid ink. Furthermore, they suggested that there are heat stable inhibitory substances in squid ink. These authors (Takai, Yamazaki, Kawai, Inoue, & Shinano, 1993b) have also reported the inhibitory effect of ink on the formation of total volatile bases formation during the ripening process of squid meat. Moreover, elevated levels of peroxidase were found in the ink-producing tissues of the cuttlefish *Sepia officinalis*, which are known to have enzymatic pathways associated with antimicrobial activity (Small & McFall-Ngai, 1999).

Although the natural sepia ink has a significant preservative effect, even more benefit in terms of increased storage life and freshness characteristics can be achieved by choosing an appropriate storage temperature.

This study was conducted to determine whether different concentrations of sepia ink extract and low temperatures in combination could be used to extend shelf life of seafood such as shrimp by inhibiting bacterial growth and spoilage reactions.

2. Materials and methods

2.1. Sepia extraction

Fresh cuttlefishes (*Sepia officinalis*) were directly purchased from a fishermen (La Goulette fishing port, Tunisia). They were rapidly brought to the laboratory where they were dissected and the ink collected in centrifuging tubes (50 ml, Beckman). Ink samples were immediately centrifuged (27,000*g*) for 40 min. The transparent supernatant was collected in clean tubes and immediately stored at −20 °C until use. The ink solutions (0%, 0.01%, 0.2%, and 2%) were made by diluting appropriate volumes in autoclaved ultra-pure water.

2.2. Shrimp sample preparation

The royal shrimps (*Penaeus kerathurus*) used in the experiments were caught in the Golf of Gabès (Tunisia), during a fishing campaign on the research vessel Hannibal. They were kept alive in the laboratory aquarium before the experiments. Live shrimps were removed, headed, peeled and soaked in ink solutions (500 g of peeled shrimp in each solution) for 5 min with a gentle swirling using a sterile glass rod to ensure complete contact of the shrimp with the coating solution.

Drained shrimps were then packed in PE/PET bags (50 g per bag) and stored at −2 °C. Shrimp samples were taken before soaking (control), immediately after soaking and after 2, 5, 9, 16, and 23 days of storage at −2 °C.

The effect of storage temperature was studied by conducting a second trial in which a 2% – ink-treated shrimps were stored at 0 ° C. Samples were taken until organoleptic test indicated shrimp spoilage (0, 3, 6, 8, 10, 12 days).

2.3. Bacteriological analyses

Aerobic and psychrotrophic bacteria of packed shrimp were counted on 3 shrimp samples per treatment at each sampling time. The package exterior was wiped with ethanol before opening and all instruments were flamed with ethanol. From each sample, 10 g tissue were homogenised mechanically with 90 ml peptone solution (0.1%). The counts of mesophilic aerobic bacteria were assessed according to the standard method of AOAC (1995) using Nutrient Agar with incubation at 35 °C for 48 h and at 5 °C for 14 days for psychrotrophic bacteria.

2.4. Chemical analyses

1 g from each remaining samples was weighed (nearest 0.1 mg) and homogenised on ice with 1 ml ultrapure water (ELGA, UHQPS) for 1 min in a Polytron homogeniser (type PT 1200). One millilitre of 6% perchloric acid was added and the extract was homogenised for a further 1 min.

Homogenates were centrifuged at 14,000*g* for 20 min and the supernatants were used for trimethylamine (TMA) (Sadok, Uglow, & Haswell, 1996), total free amino acids measured as ninhydrin positive substances (NPS) (Sadok, Uglow, & Haswell, 1995) and total volatile bases (TVB) (Ruis-Capillas & Horner, 1999) analysis.

2.5. Sensory evaluation

An organoleptic test was applied in order to assess whether the ink treatments influenced the flavour of the shrimp flesh.. The panellists were also asked to comment on odour, colour, taste, and texture. The assessors (12) consisted of personnel and students from the INSTM department and of fishermen. On each sampling day, the sensory analysis was performed on raw shrimp. The effects of panellists and treatment were evaluated by analysis of variance (ANOVA). Treatments receiving scores >4 by 50% of the panellists were considered acceptable, those obtaining scores of 4 were at the borderline of acceptability and scores <4 were considered unacceptable and rejected. The maximum shelf life for a treatment was identified as the last sampling day where the treatment received a score of 4 or above.

2.6. Statistical analysis

Appropriate, normally-distributed data ($P > 0.05$, K–S Lillifors test) on TMA, TVB and NPS concentrations and TMA levels were subjected to Analysis of variance (one way ANOVA) using Sub-programme of the Statistical Package for the Social Sciences (SPSS) on a PC. The level of significance for F test, used in conjunction with ANOVA variance data, was at the 95% level of confidence.

3. Results and discussion

3.1. Sensory analysis

The sensory quality of shrimp is defined as a complex set of characteristics including appearance, aroma, taste and texture (Shahidi & Cadwallader, 1997). In this study, the results of sensory analyses for control and sepia-treated shrimp stored at different temperatures are given in Table 1. Temperature significantly affected all

Table 1
Penaeus kerathurus: sample treatment and sensory evaluation of control (L0-C and L0C-0) and sepia-treated shrimps (L1, L2, L3, and L3-0) stored at −2 °C and at 0 °C (L0C-0 and L3-0)

Sample	Ink level (%)	Maximum acceptable shelf life (days)[a]	Borderline sensory characteristics				Rejection sensory characteristics			
			Texture	Flavour	Colour	Odour	Texture	Flavour	Colour	Odour
L0-C	0	16–18	Soft	Slightly bitter (12)[b]	Pink	Neutral	Mushy	Strong off-flavour of sulphides (18)[c]	Brown	Slightly putrid
L1	0.01	20–21	Soft	Slightly sour (20)	Pink	Neutral	Soft	Slightly sour (23)	Pink	Slightly musty
L2	0.2	22–23	Slightly fibrous	Slightly sour (23)	Pink	Shellfishy	Soft	[d]	Pink	Neutral
L3	2	>23	Slightly fibrous	Neutral	White	Shellfishy	[d]	[d]	Slightly pink	Shellfishy
L0-C-0	0	6–8	Soft	Slightly sour (6)	Pink	Neutral	Mushy	Strong off-flavour of sulphides (10)[c]	Brown	Putrid
L3-0	2	10–12	Slightly fibrous	Neutral (9)	White	Shellfishy	Soft	Slightly sour (12)	Slightly pink	Neutral

[a] Based on last acceptable sampling day.
[b] Sampling day at which borderline is reached.
[c] Sampling day at which sample is unacceptable.
[d] Samples from treatment are still of borderline quality.

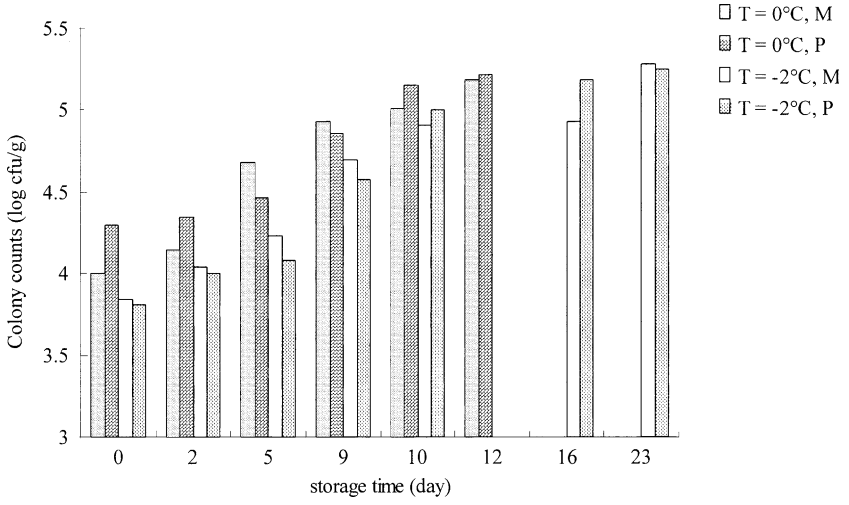

Fig. 1. *Penaeus kerathurus*: the effect of the storage temperatures (0 and −2 °C) on the mesophilic (M) and psychrotrophic (P) counts of peeled shrimp over the storage period.

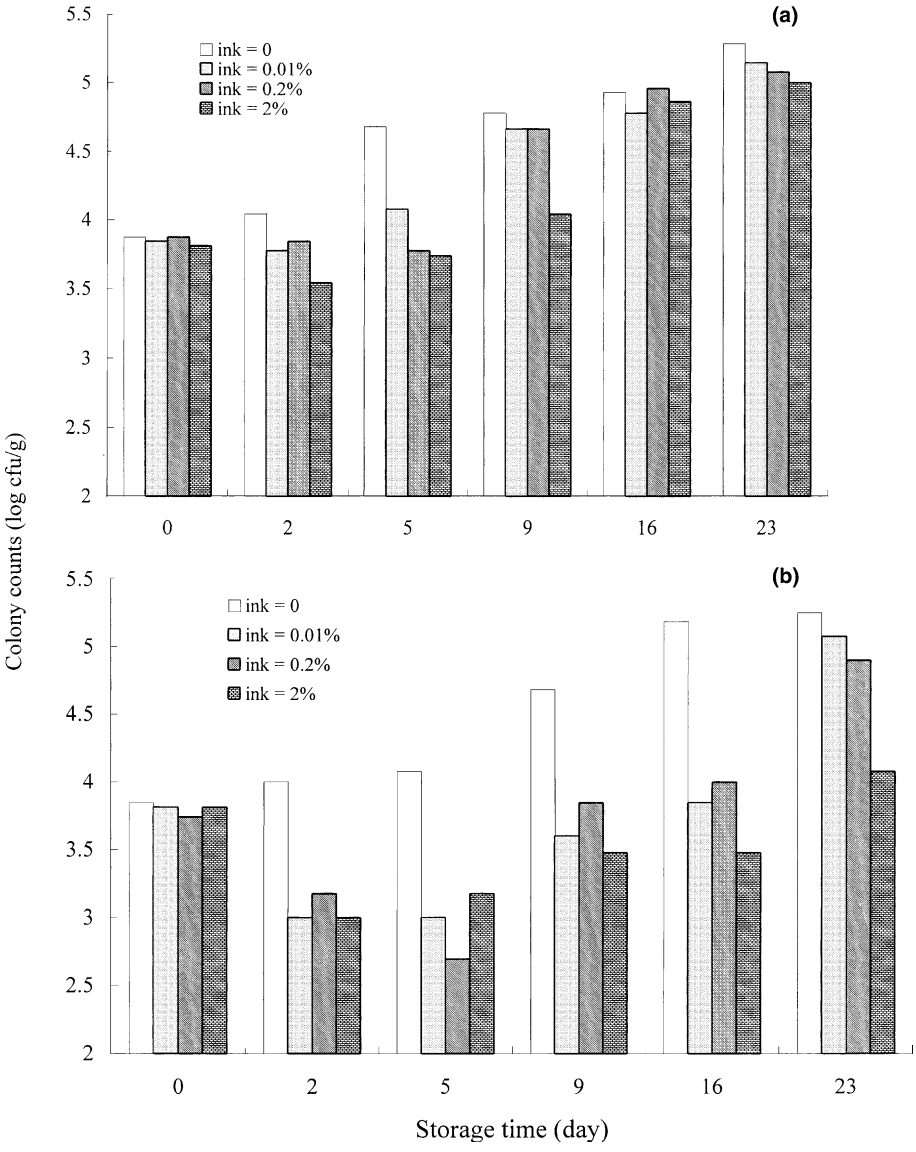

Fig. 2. *Penaeus kerathurus*: the effect of soaking with different sepia extract solution levels on the mesophilic (A) and psychrotrophic (B) counts of peeled shrimp over the storage period at −2 °C.

sensory attributes in raw and treated shrimp samples. Thus, the decrease of the storage temperature from 0 to −2 °C gave an increase in shelf life of 8 to 10 days for raw shrimps.

The spoilage patterns described by the panellists were similar in all treatments and analysis of variance (ANOVA) showed no significant differences ($P > 0.05$) among their evaluation on any sampling day (data not shown).

According to the test panel, the raw shrimp stored at −2 °C retained prime quality up to 11 days after which there was a loss of the characteristic sweet flavour associated with fresh shrimp. Such changes occurred after only 6 days in non-treated shrimp stored at 0 °C. From day 12 to approximately day 16, the −2 °C-raw shrimps were still of acceptable quality, showing a loss of characteristic flavour indicating borderline quality. After the 16th day on, pronounced off-flavour developed and the shrimp became unacceptable to the panellists. Higher test temperature induced a reduced shelf life. Similar results were reported for *Penaeus monodon* (Basavakumar, Bhaskar, Ramesh, & Reddy, 1998) and for *Penaeus merguensis* (Shamshad, Nisa, Riaz, Zuberi, & Qadri, 1990) stored on ice. The chilling temperature used in this study (−2 °C) is critical because it is at the upper threshold of freezing where 50% of the water is frozen out (Connell, 1995). Thus, the tissue is benefited by the lowest temperature possible without undergoing internal cell crystallisation inducing deleterious changes (Haard, 1992) and about a doubling of storage life can be obtained (Connell, 1995).

In overall acceptability, which is the combination of all sensory attributes, the panellists could not find a

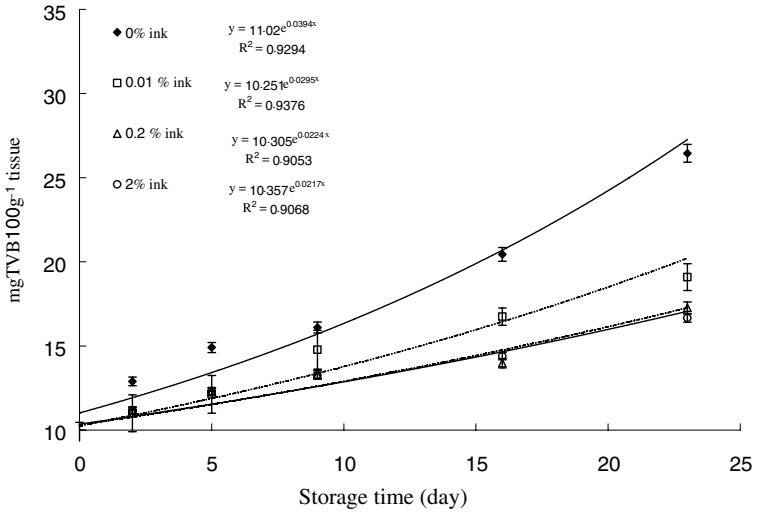

Fig. 3. *Penaeus kerathurus*: total volatile bases (TVB) levels in free and ink-treated peeled shrimp during 3 weeks of storage at −2 °C. Vertical bars = SE ($n = 6$, in each case).

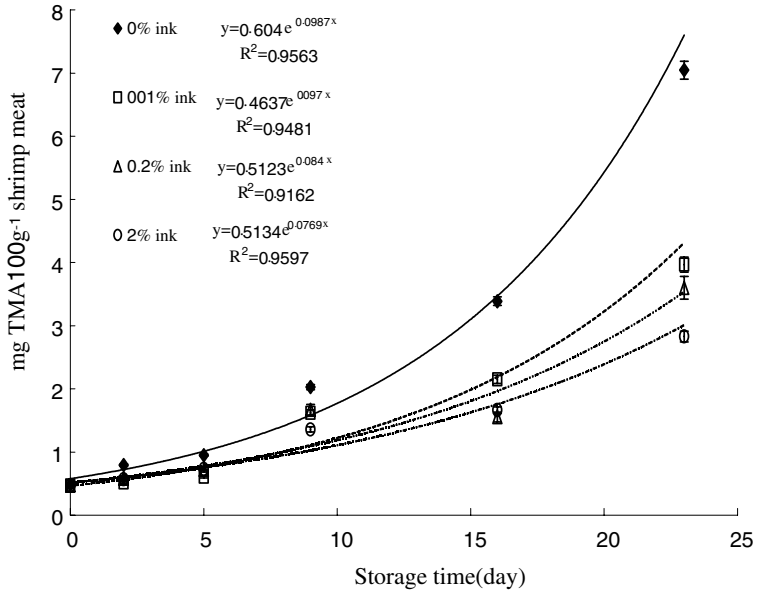

Fig. 4. *Penaeus kerathurus*: trimethylamine (TMA) levels in free and ink-treated peeled shrimp during 3 weeks of storage at −2 °C. Vertical bars = SE ($n = 6$, in each case).

significant difference ($P > 0.05$) between sepia-coated and untreated shrimp. Thus coating with solution up to 2% ink, did not affect the appearance, taste or odour of shrimp when compared to the control samples. However, increasing the coating the ink solution levels from 0.01% to 2% gave a gradual increase in shelf life of approximately 5 days for low ink samples and more than 2 weeks for the highest ink-treated shrimps stored at −2 °C.

3.2. Microbiological analysis

Counts of bacterial population in raw samples stored at different temperatures are shown in Fig. 1. The initial low total microbial charge found in all samples (<12 10^3 cfu/g) indicated a good initial quality of shrimp used in this study. Peeling reduced the bacterial charge present on the surface. Bacteria grew most quickly in packed shrimp kept at 0 °C whereas the lowest counts were found in the −2 °C stored shrimps. Partial freezing (−2 °C) has been shown by Haard (1992, 1995) to inhibit microbial growth.

The mesophilic counts of sepia-coated samples stored at −2 °C became statistically different from the control group after 5 days of storage (Fig. 2).

Coating with ink solutions of 0.0–2% provoked a decrease in psychrotrophic bacterial number to below 10^3 cfu. The lowest counts were with the 2% ink solution

where the log phase was apparently extended. It has been reported that cuttlefish ink exhibited an important effect on microbiological growth (Mochizuki, 1979; Takai et al., 1993a).

Our results showed a significant additive interaction effect of partial freezing and antimicrobial coating in reducing the growth of bacterial charge in peeled shrimp. This effect was also characterised by a significant shelf life extension in sepia-coated shrimp stored at −2 °C.

3.3. Chemical analysis

Fig. 3 illustrate the pattern of total volatile bases changes in the muscle of control and sepia-treated shrimp stored at −2 °C. The initial TVB concentration in the fresh muscle was relatively low (9.34 ± 0.15 mg TVB/100 g tissue). Control tissue TVB increased exponentially over the time of storage ($r^2 > 0.9$). A significant effect of sepia coating on TVB production was observed after 10 days of storage at −2 °C. Thus sepia-coated shrimps showed a significantly lower TVB levels than the control samples (Fig. 3). Concomitantly, trimethylamine showed a similar pattern of change in shrimps stored at −2 °C (Fig. 4), however at a much lower levels than TVB values.

Comparisons of the three type of sepia coating reveals the 2% solution as the most effective for inhibiting TMA production. The inhibitory effect of sepia on TMA and TVB production was reported by Takai, Kawai, Inoue, and Shinano (1992, 1993a). Here, TMA–N concentrations oscillated between 7.04 and 2.82 mg TMA/100 g shrimp meat in control and 2% – treated rejected samples. However, the high increase in shrimp tissue TMA cannot be correlated to the total aerobic plate count found in this study ($<10^{-6}$ cfu/g). A high cell concentration of $>10^8$ cfu/g of specific bacteria is required for the production of a TMA level normally found in spoiled fish (Dalgaard, 1995). This suggests that spoilage is due to autolytic changes, or more likely, that changes in the composition and metabolism of the microflora were not reflected by the microbiological methods used in this experiment.

Free amino acids (FAAs) are known as the most important taste compounds of molluscs and crustaceans but they reflect microbial spoilage, and are precursors of biogenic amines, factors of health concern and indicators of seafood decomposition (Antoine, Wei, Littell, Quinn, Hogle, & Marshall, 2001). The changes in the concentration of free amino acids measured as ninhydrin substances (NPS) during storage at −2 °C are shown in Fig. 5. Initially, the muscle contained 9.74 ± 1.05 mM/100 g tissue of NPS with no significant difference among the shrimps in three treatments. Such NPS levels were however lower than values observed in *Penaeus monodon* (Chen, 2000). Over the following 10 days of storage, the NPS showed an increase which escaped statistical significance (cf. control values) and, subsequently a significant NPS increase ($P < 0.05$) occurred only in the non-treated shrimp (Fig. 5). The ini-

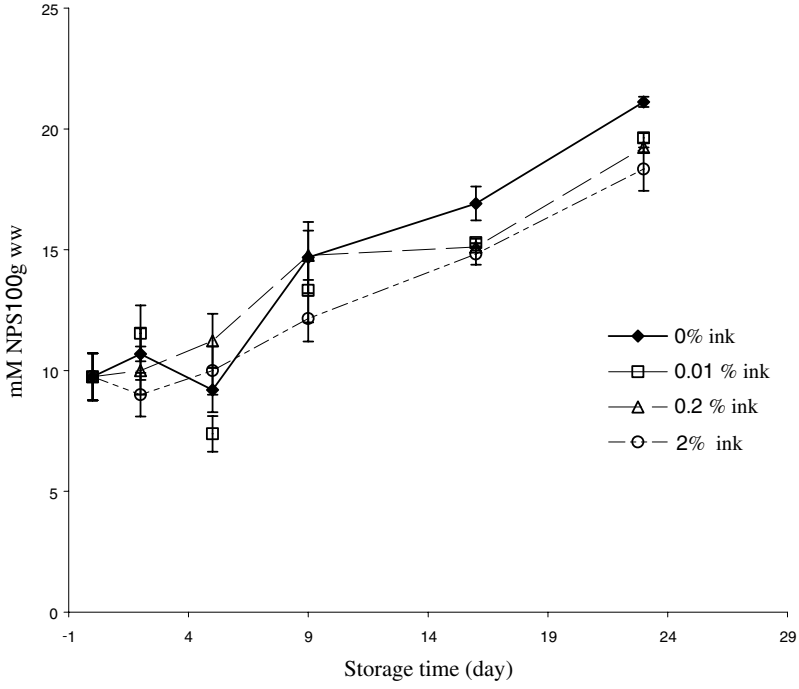

Fig. 5. *Penaeus kerathurus*: total free amino acid (as NPS) levels in free and ink-treated peeled shrimp during 3 weeks of storage at −2 °C. Vertical bars = SE ($n = 6$, in each case).

tial steps of raw fish and shellfish deterioration during refrigerated storage are related to hydrolytic reactions catalysed by endogenous enzymes, which produce nutrients that allow bacterial proliferation (Busconi, Folco, Martone, & Sanchez, 1989). Among hydrolytic reactions, proteolysis should be considered with special attention due to its likely influence on nutritional quality. Thus the NPS increase can be related to the action of endogenous collagenases inducing the degradation of collagen fibril in raw tissues after a relatively long period of refrigerated storage (Kolodziejska & Sikorski, 1997; Mizuta, Yoshinaka, Sato, & Sakaguchi, 1997). The treatment of samples with sepia showed an inhibitory effect on NPS formation, however further studies are needed to optimise the application of such natural antimicrobial ingredients.

4. Conclusion

The present study dealt with the control of bacterial growth and chemical changes in peeled shrimps using adequate temperatures combined with edible antimicrobial coating. The results showed significant potential for inhibiting aerobic bacteria, especially psychrotrophic flora, and as a result a shelf life extension was obtained. The appearance, flavour and taste were not affected by the treatment with sepia extract up to a concentration of 2%.

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