RESEARCH ARTICLE

Nutritive value and safety aspects of acidified mantis shrimp during ambient storage

Asitlendirilmiş mantis karidesinin besin değeri ve ortam sıcaklığında depolanması süresince güvenlik değerlendirilmesi

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Abstract: In this study effects of acidification with an organic acid (3 %, formic acid - FA) and an organic-inorganic acid mixture (1.5 % FA + 1.5 % sulphuric acid - FASA) were evaluated on a non-target species (mantis shrimp - *Erugosquilla massavensis*). Nutritional composition (proximate analysis and fatty acid composition), chemical (biogenic amine concentrations, non-protein nitrogen -NPN and pH) and microbiological assessments (total viable counts -TVC and lactic acid bacteria counts -LAB) were conducted under 27-28 °C. The analysis conducted for a 60 day period at 1st, 7th, 14th, 30th, 42nd and 60th day intervals. Moisture contents of acidified shrimp were significantly lower than the raw material. No changes in protein contents and an increase in lipid content only in FASA group were observed. Palmitic acid, stearic acid, palmitoleic acid, oleic acid and docosahexaenoic acid (DHA) were observed to be the dominant fatty acids in raw and acidified mantis shrimp. The initial pH value of mantis shrimp was 7.71 and became stable (4.14-3.97) throughout the storage period. An increase was observed in NPN contents and FA and FASA were 0.56 and 0.51 g 100 g⁻¹, respectively at the end of the storage. Putrescine (3.00 mg 100 g⁻¹), tyramine (2.94 mg 100 g⁻¹) and serotonin (2.71 mg 100 g⁻¹) were found to be the dominant biogenic amines in raw mantis shrimp. No significant changes in biogenic amine concentrations were observed in general during the storage period. TVC was found as 4.16 log cfu g⁻¹ at the beginning of the storage period. Bacterial load was decreased after the addition of acids and stayed low throughout the storage period. The results showed acid treated mantis shrimp could be considered potential feed component due to its high nutritional value and safe in regards of biogenic amines.

Keywords: Mantis shrimp, fatty acid composition, biogenic amines, acidification, total viable count, lactic acid bacteria

Öz: Bu çalışmada organik asit (% 3, formik asit - FA) ve bir organik-inorganik asit karışımı (% 1.5 FA + % 1.5 sülfirik asit - FASA) ile asitleşmenin etkileri, hedef dışı bir tür (mantis karidesi - *Erugosquilla massavensis*) üzerinde değerlendirildi. Besin kompozisyonu (besin içeriği ve yağ asidi kompozisyonu), kimyasal (biyojenik amin konsantrasyonları, protein olmayan azot -NPN ve pH) ve mikrobiyolojik değerlendirmeler (toplam bakteri sayını - TVC ve laktik asit bakteri sayısı - LAB) 27-28 °C'de yapılmıştır. Analizler 1., 7., 14., 30., 42. ve 60. günlerde 60 günlük bir süre boyunca yapıldı. Asitlenmiş karideslerin nem içeriği hammaddeden önemli derecede düşük bulunmuştur. Protein içeriğinde herhangi bir değişiklik gözlenmezken sadece FASA grubunda lipi içeriğinde bir artış gözlendi. Çiğ ve asitlendirilmiş mantis karidesinde palmitik asit, stearik asit, palmitoleik asit, oleik asit ve dokosaheksaenoik asit (DHA) dominant yağ asitleri olarak gözlenmiştir. Mantis karidesinin başlangıç pH değeri 7.71 olurken, depolama süresi boyunca kararlı hale gelmiştir (4.14 - 3.97). NPN içeriklerinde bir artış gözlenmiş ve depolamanın sonunda FA ve FASA sırasıyla 0.56 ve 0.51 g 100 g⁻¹ olmuştur. Çiğ mantis karidesinde baskın biyojen aminler olarak, putresin (3.00 mg 100 g⁻¹), tiramin (2.94 mg 100 g⁻¹) ve serotonin (2.71 mg 100 g⁻¹) bulunmuştur. Depolama süresi boyunca genel olarak biyojenik amin konsantrasyonlarında belirgin bir değişiklik gözlenmemiştir. TVC, depolama periyodunun başlangıcında 4.16 log kob g⁻¹ olarak bulunmuştur. Asit ilavesi yapıldıktan sonra bakteri yükü azalmış ve depolama süresince düşük kalmıştır. LAB'da artışlar gözlenmiş ve bu değerler depolama periyodunun sonunda sırasıyla FA ve FASA grubu için 4.50 ve 5.68 log kob g⁻¹ olmuştur. Elde edilen sonuçlar, asitle işleme tabi tutulan mantis karidesinin yüksek besin değeri nedeniyle potansiyel besleme bileşeni olarak kabul edilebileceğini ve biyojenik aminler açısından güvenli olduğunu göstermiştir.

Anahtar kelimeler: Mantis karidesi, yağ asidi kompozisyonu, biyojenik aminler, asidifikasyon, toplam bakteri sayımı, laktik asit bakterileri

INTRODUCTION

Incorporation of non-targeted species into the economy of the fishing industry has a great potential to become an important source of income for fishermen. The utilization of seafood processing waste or discard species enhances the efficiency of animal protein usage, minimizes the environmental problems, and adds a nutritional benefit to diets prepared from such materials. Mantis shrimp (*Erugosquilla massavensis*) which migrated by the Suez Channel is in great numbers in the Mediterranean Sea. However, its consumption as a food source is refused because of its small size. Therefore it may be use for feed source for animal nutrition.

It is stated that the composition of the acidified seafood products is very similar to the raw materials in general (Mach and Nortvedt, 2009; Arruda et al., 2007). Acidification of seafoods for use as animal feed has many advantages like easy production, low investment, energy and labour. Acidification can be done either by organic and inorganic acid. The selection of acid type interconnected with the cost and availability of the acids. For fatty and bony fish, 3-6% of concentrated sulphuric acid is recommended, while for organic acids such as formic acid and propionic acid recommended value is 2-3%. Although sulphuric acid is cheaper than organic acid, it needs to be used in larger quantities and must be neutralized prior to use on the feed (Rurangwa et al., 2014). For this reason, organic and inorganic acids can be mixed and should be considered to be used for researches.

Biogenic amines may be a potential risk in acidified fish feed because of its low pH and chemical properties, and they may reduce the biological value of the feed because of toxicity. The amount of biogenic amines formed are influenced by factors such as fish species, the presence of decarboxylase enzymes, microbial growth, availability of free amino acids, water activity, pH and elevated temperature conditions. In spite of the broadly acknowledged association between histamine and scombroid fish poisoning, histamine seems to be inadequate to cause toxicity single-handedly. Putrescine and cadaverine have also been known to strengthen histamin's toxic activity by limiting the intestinal histamine-metabolizing enzymes, diamine oxidase (Hungerford and Arefyev, 1992) and histamine N-methyltranferase (Stratton et al., 1991). For this reason, it is important to investigate the safety of acidified feed stuffs in regards of biogenic amines. In order to research the possibilities of using as an animal feed, mantis shrimps were stabilized through ensilage by direct acidification with 3% formic acid (FA) and 1.5 % formic acid + 1.5 % sulphuric acid (FASA) in this study. Chemical, microbiological and nutritional evaluations and also biogenic amines concentrations were performed for determining the safety and nutritive values of acidified mantis shrimp.

MATERIALS AND METHODS

Acidification of mantis shrimp

Average weight and length of mantis shrimp (*Erugosquilla massavensis*) were 11.93 ± 1.37 g and 19.87 ± 5.68 cm, respectively (n=40). All samples that were provided by local fisherman were minced by a grinder and divided into two equal groups. The first group (FA) was mixed with 3 % formic acid (v/w) and the second group (FASA) was mixed with 1.5% formic acid and 1.5 % sulphuric acid (v/w). After that, 250 mg kg⁻¹ butylated hydroxytoluene (BHT) as an antioxidant and 2.2 g kg⁻¹ potassium sorbate as fungicide were added. Both groups (FA and FASA) were stored at 27-28 °C in plastic jars with caps and stirred daily until ripening. Each group was managed as triplicates, stored 60 days and sampled for analyses at 0th, 7th, 30th and 60th day.

Nutritional composition analyses of acidified mantis shrimp

The moisture content and crude ash of acidified mantis shrimps were detected in an oven at 103 and 550 °C, respectively until the weight of samples became stable. The crude protein and lipid were analysed according to AOAC (1999, 981.10) and Bligh and Dyer (1959) procedure, respectively. Lipid samples were turned into their constituent fatty acid methyl esters (FAMEs) by using the method of Ichihara et al., (1996). Gas chromatography (Clarus 500, Perkin Elmer, Shelton, CT, USA) was used for the separation and quantification of the fatty acid methyl esters.

Chemical analyses of acidified mantis shrimp

pH was measured using a digital pH metre (WTW 315i, Germany). Non-protein nitrogen (NPN) analysis was used to estimate the protein autolysis. Samples (40 g) were stirred with TCA (60 mL, 20 % thrichloracetic acid) and filtered. After that, Kjeldahl's procedure was used for determining the nitrogen content and the results were expressed as NPN (AOAC 2002; method 991.21). Trimethylamine (TMA) and biogenic amine (BA) analysis were completed using a rapid HPLC (high-performance liquid chromatography) method (Özogul et al., 2002). A Shimadzu Prominence HPLC apparatus (Shimadzu, Kyoto, Japan) equipped with a SPD-M20A diode array detector, two binary gradient pumps (Shimadzu LC-10AT), auto sampler (SIL 20AC), column oven (CTO-20AC) and valve unit FCV-11AL with a communication bus module (CBM-20A) was used. The column was a reverse-phase, ODS Hypersil, 5µ,

250x4.6mm (Phenomenex, Macclesfield, Cheshire, UK). Oven temperature was 30 °C and mobile phase was acetonitrile and HPLC grade water.

Microbiological analyses of acidified mantis shrimp

10 g of acidified mantis shrimp from three different jars for each group were randomly sampled for total aerobic bacteria counts (TVC) and lactic acid bacteria analysis. TVC were incubated on PCA (Fluka 70152, Switzerland) plates for 2 days at 30°C. LAB counts were carried out by the pour plate method and they were grown on MRS (Fluka 69964 Steinheim, Spain) agar at 30°C for 5 days.

Statistical analysis

Statistical analyses were performed using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). All analyses were performed at least triplicate and differences between means were analysed by one-way analysis of variance (ANOVA) and mean comparisons were carried out using Duncan's (Duncan, 1955) multiple range tests and t-test. Data were expressed as mean \pm standard deviation (SD) and significant differences were defined as P < 0.05.

RESULTS AND DISCUSSION

Nutritional composition of acidified mantis shrimp

The moisture, ash, protein and lipid content of raw material were found 74.19 %, 7.30 %, 15.99 % and 1.11 %, respectively (Table 1). Moisture contents of acidified shrimps were significantly less than the raw material (P<0.05). Ash contents also increased in both groups significantly (P<0.05). However, other than the lipid content of FASA group, no significant changes were observed in lipid and protein contents of acidified shrimps. It can be concluded that the acidification process for preserving mantis shrimp caused minor variations in protein and lipid contents for the raw mantis shrimp. Among fatty acids, palmitic acid, stearic acid, palmitoleic acid, oleic acid and docosahexaenoic acid (DHA) were the dominant fatty acids in raw and acidified mantis shrimp (Table 1). Generally, there were no significant changes in fatty acid contents after acidification treatment. DHA was determined as the dominating fatty acid within PUFA in both groups, even after the acidification process.

Chemical assessment

The initial pH value of mantis shrimp was 7.71 and the pH value in both groups showed significant decrease (4.14 - 3.97) during the storage period (Figure 1). During storage period, pH of the acidified

shrimps remained stable under 4.5 which was the recommended pH value for acidified fish (Espe and Lied, 1999). The non-protein nitrogen (NPN) contents which were known as protein solubilisation values for the acidified shrimps are presented in Figure 1. The increase of NPN was likely occurred because of the release of NPN components either by the breakdown of compounds like trimethylamine oxide (TMAO) or protein hydrolysis by enzymes. Non-protein nitrogen (NPN) contents were found as 0.56 and 0.51 g 100g⁻¹ for FA and FASA, respectively at the end of the storage periods.





Figure 1. The changes of pH, Non-protein nitrogen values, in acidified mantis shrimps during storage period. Means values of three (n=3) independent determinations. Standard deviations are indicated by bars

		Raw	54	54.64	
	E. massavensis		FA	FA+SA	
Proximate composition (%)	Moisture	74.19±0.14 ^c	72.63±0.12 ^b	71.45±0.46ª	
	Ash	7.30±0.33ª	8.57±0.26 ^b	9.07±0.23 ^b	
	Protein	15.99±0.23ª	15.98±0.20ª	15.61±0.51ª	
	Lipid	1.11±0.03ª	1.20±0.05ª	1.89±0.19 ^b	
	C12:0	3.77±0.27 ^b	2.02±0.22ª	3.93±0.01 ^b	
	C14:0	4.16±0.06 ^b	3.82±0.13ª	4.18±0.08 ^b	
	C15:0	0.32±0.03ª	0.25±0.01ª	0.32±0.04ª	
	C16:0	18.14±0.72 ^a 21.75±2.08 ^b		18.32±0.91ª	
	C17:0	0.49±0.01 ^b 0.31±0.04 ^a		0.49±0.01 ^b	
	C18:0	8.82±0.17° 10.86±2.84°		8.73±0.06ª	
	C20:0	0.27±0.01ª 0.29±0.06ª		0.27±0.01ª	
	C22:0	0.04±0.01° 0.14±0.06 ^b		0.04±0.01ª	
	C14:1	0.45±0.05 ^b	0.26±0.00ª	0.46±0.06 ^b	
	C15:1	0.04±0.01ª	-	0.04±0.01ª	
	C16:1	5.83±0.28ª	6.95±0.19 ^b	5.93±0.32ª	
Fatty acid composition (% of total fatty acids)	C17:1	0.12±0.00 0.10±0.00		0.12±0.00	
	C18:1 n9	7.39±0.39 ^a 8.23±0.66 ^a		7.52±0.47ª	
	C18:1n7	4.26±0.02 ^a 4.71±0.95 ^a		4.25±0.01ª	
	C20:1	0.84±0.03 ^b 0.61±0.07 ^a		0.82 ± 0.00^{b}	
	C22:1n9	0.15±0.00 0.63±0.00		0.15±0.00	
	C18:2n6	1.37±0.23ª 1.41±0.13ª		1.27±0.21ª	
	C18:3n3	0.25±0.04ª	0.28±0.02ª	0.27±0.01ª	
	C20:2 cis	5.87±0.56ª	4.55±0.70ª	5.71±0.69ª	
	C20:4 n6	0.34±0.06ª	0.23±0.08ª	0.31±0.01ª	
	C20:5n3	1.20±0.08 ^b	0.47±0.06ª	1.24±0.07 ^b	
	C22:2 cis	1.34±0.06ª	1.25±0.13ª	1.32±0.04ª	
	C22:6 n3	8.14±0.31ª	8.79±0.64ª	8.17±0.43ª	

Table 1. Proximate and fatty acid compositions of acidified mantis shrimps

*The values are expressed as mean \pm standard deviation, n=3

^{a-c}Values in a same line followed by different letters indicate significant differences (P<0.05)

As a result of the activities of assorted endogenous and bacterial decarboxylase enzymes, it was observed that acidified mantis shrimp had significant levels of free amino acids which were the precursors for biogenic amines. The initial TMA level in raw mantis shrimp was 30.39 mg 100g⁻¹(Table 2). After formic and sulfuric acid addition, the TMA value considerable decreased during 30 days (16.88-24.27 mg 100g⁻¹) and then increased in 60th day (44.42 mg 100g⁻¹). However there were no significant differences between FA group and FASA group at 60 day of storage in regards of TMA value.

Similarly, high TMA value in acidified fish products had been also observed in other studies (Jiang et al 2007; Achinewhu and Oboh 2002).

The production of 10 biogenic amines was found in raw and acidifed mantis shrimps (Table 2). Putrescine (3.00 mg 100g⁻¹), tyramine (2.94 mg 100g⁻¹) and seratonine (2.71 mg 100g⁻¹) were the major biogenic amines in the raw mantis shrimp. The presence of histamine, spermidine, tryptamine, cadaverine, agmatine, 2-phenylethylamine and spermine was found to be in lower concentrations (0.08-1.27 mg100g⁻¹)

Days	0	7		30		60	
		FA	FASA	FA	FASA	FA	FASA
TMA	30.39±17.73	52.18±0.57ª	16.88±0.03 ^b	46.79±1.67ª	24.27±1.88 ^b	44.30±13.31ª	44.42±1.58°
HIS	0.22±0.00	0.94±0.48ª	0.22±0.01 ª	0.32±0.08ª	3.71±2.96ª	0.2±0.00 ^a	1.24±1.29ª
PUT	3.00±0.70	2.29±0.23ª	1.02±0.68ª	3.09±0.62ª	2.61±0.22ª	2.19±0.19ª	3.00±0.41 ª
CAD	1.27±0.19	1.04±0.14ª	0.45±0.21ª	1.33±0.34ª	1.36±0.19ª	1.03±0.05ª	1.32±0.18ª
TYR	2.94±0.09	6.44±0.16ª	9.72±6.34ª	5.82±1.82ª	7.16±1.56ª	4.53±1.28ª	8.69±3.11ª
SPD	0.11±0.02	0.07±0.01ª	0.17±0.12ª	0.09±0.02ª	0.31±0.22ª	0.07±0.00ª	0.30±0.22ª
SPN	0.11±0.00	0.10±0.00	-	0.34±0.03ª	0.47±0.05ª	0.11±0.00ª	0.59±0.57ª
TRP	0.08±0.05	0.15±0.13ª	0.06±0.00ª	0.07±0.04ª	0.15±0.17ª	0.43±0.37ª	0.15±0.16ª
PHEN	0.21±0.00	0.19±0.08ª	0.24±0.06ª	0.29±0.15ª	0.45±0.4ª	0.14±0.04ª	0.33±0.23ª
AGM	0.99±0.55	4.82±0.03ª	7.29±0.01 ^b	7.71±4.46ª	13.64±0.05ª	8.64±3.75ª	11.35±2.61ª
SER	2.71±0.92	4.99±1.58°	8.45±0.42ª	4.33±0.34ª	7.52±2.39ª	4.37±0.38ª	9.25±1.31ª

Table 2. TMA and biogenic amine concentrations of acidified mantis shrimp groups (FA and FASA, mg 100g⁻¹)

TMA= Trimethylamine, PUT=Putrescine, CAD=Cadaverine, SPD=Spermidine, TRP=Tryptamine, PHEN= 2-Phenylethylamine, SPN=Spermine, HIS=Histamine, SER= Seratonine, TYR=Tyramine, AGM= Agmatine

*The values are expressed as mean ± standard deviation, n=3

^{a-b}Values in a same column followed by different letters indicate significant differences of the parameter with respect to the kind of groups at same storage days

than putrescine, spermidine and tryptamine. High concentrations of histamine resulted in a reduction in weight gain and feed consumption, therefore present a eventual long-term health hazard for poultry and other animals. No regulation for histamine content in animal food can be found European Union (EU Directive 32/2002; Macan et al., 2006). High contents of histamine (50- 510 mg 100 g⁻¹) were reported in fish meals by some researchers (Pike 1991; Macan et al 2006). The initial histamine content in this study was 0.22 mg 100g⁻¹. The histamine concentrations were found considerably low level in both groups. During 60 days storage, maximum histamine content was 3.71 mg 100g⁻¹ and there were no significant differences in FA and FASA groups (P>0.05). On the other hand, the legal limits for histamine content for human consumption regarded by the EU (EEC 1991) as less than 10 mg 100 g⁻¹ have not been reached by both groups during storage period. Cadaverine and putrescine contents of acidifed mantis shrimps (FA, FASA) were not considerably changed during storage. However, they were no significant difrences were observed between groups at sampling days. At the 60th day of storage, cadaverine and putrescine concentrations were 1.03 and 2.19 mg 100g⁻¹ for FA group and 1.32 and 3.00 mg 100 g⁻¹ for FASA group, respectively. Considerably high putrescine (9.20 - 24.1 mg 100g⁻¹) and cadaverine (48.0 - 120.5 mg 100g⁻¹) concentaration were reported for some fish products (Mah et al., 2002; Mohamed et

al 2009). In the present study, maximum putrescine and cadaverine contents was found as 3 mg 100g⁻¹. The initial tyramine content was 2.94 mg 100 g⁻¹ in raw mantis shrimp and then considerably increased in both groups. However there were no significant difrences between FA ans FASA group at the same storage days. Kuley et al., (2011) reported that quantitatively the most common biogenic amine in fermented meat products was tyramine.

In this study, it was also observed that tyramine was found to be abundant in acidified mantis shrimp. On the other hand, the tyramine concentrations in this study were fairly lower than reported for limit of human consumption (80 mg 100g⁻¹) by Ten Brink et al., (1990).

Microbiological assessment

Figure 2 shows total viable counts (TVC) in acidified shrimps. Initial bacterial load was 4.16 log cfu g⁻¹. Bacterial growths sharply decreased after addition of acids. Initial lactic acid bacteria count was as low as 2.77 log cfu g⁻¹. The increase in lactic acid bacteria counts were observed in groups FA and FASA during storage. Jini et al., (2011) reported that lactic acid bacteria counts in visceral wastes of different freshwater fishes were found in the range of 4.22-5.88 log cfu g⁻¹. Similarly, lactic acid bacteria counts were found in range of 4.50-5.68 log cfu g⁻¹ in this study (Figure 2).



Figure 2. The changes of Total viable and Lactic acid bacteria counts in acidified mantis shrimps during storage period. Means values of three (n=3) independent determinations. Standard deviations are indicated by bars

CONCLUSIONS

According to chemical, microbiological and nutritional evaluation, acidified mantis shrimp has great potential as a feed component because of its nutritious components and storage stability. In general, the range of the biogenic amine concentrations detected in this study can be called as safe as were reported by many studies for fish products. It can be concluded that acid type used for preparation of acidification did

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not differently effected on biogenic amine formation. Therefore, formic acid or mixture of formic and sulfiric acid can be used for acidification of mantis shrimp without no adverse effect to animal health in regards of biogenic amines.

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