Purine Content in Grass Shrimp during Storage as Related to Freshness

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ABSTRACT

In grass shrimp (*Penaeus monodon*), the combined total content of the uricogenic bases adenine (Ade) and hypoxanthine (Hyp) decreased gradually during storage. Whether stored at 5°C or at room temperature (22°C), a negative regression of log Ade and a positive regression of the Kp value (Hyp/Ade) were observed. Volatile basic nitrogen (VBN) increased during storage at 5°C and 22°C. Correlations between the content of log Ade and VBN at 5°C and 22°C were −0.9248 ($p<0.01$) and −0.8139 ($p<0.05$, while those between the Kp value and VBN were 0.9557 ($p<0.001$) and 0.8197 ($p<0.05$), respectively. At the point where the shrimp would remain acceptable, the upper limits of Kp were 1.42 and 1.29 for storage at 5°C and 22°C, respectively; the corresponding lower limits of Ade were 18.72 and 20.42 μmole/g dry wt.

Key Words: grass shrimp, purine content, storage, uricogenic bases, freshness

INTRODUCTION

URIC ACID IS THE END PRODUCT OF PURINE metabolism in humans. Dietary purine intake influences serum uric acid levels, and this has been reported to be associated with hyperuricemia and gout (Clifford and Story, 1976; Ho, 1986; Herbel and Montag, 1987; Wolfram and Colling, 1987; Montag et al., 1989, Lou et al., 1996). Grass shrimp (*Penaeus monodon*) are popular in Taiwan and contain the highest total purine content of any commercial seafood (Ho, 1986; Lou et al., 1996). Because the quality and quantity of purine compounds in food might change during storage and processing, the effects of cooking on purine content in some selected foods have been studied (Young, 1982, 1983; Shinoda et al., 1982; Brule et al., 1989; Lou and Montag, 1994; Lou et al., 1997). Raw grass shrimp have a higher purine content than cooked shrimp (Lou, 1997a). Little information is available, however, on the quantity and composition of purine in uncooked grass shrimp during storage.

The purine compounds are closely associated with nucleotide catabolites in many foods. However, the degree to which changes in the quantity and composition of purine compounds affect the freshness of grass shrimp during storage has not been reported. The K value, which is a ratio of inosine (Ino) and hypoxanthine (Hyp) to the sum of ATP, ADP, AMP, IMP, Ino and Hyp, has been proposed as a freshness index of seafood (Saito et al., 1959). Instead of K value, a modified Kp value, equal to hypoxanthine/adenine, and the logarithm of adenine content (log Ade) are hypothesized as a freshness index in this study. The common chemical quality indicator, volatile basic nitrogen (VBN), has been used or suggested as a quality index for shrimp (Suwetja et al., 1989; Shamshad et al., 1990; Matsumoto and Yamanaka, 1990; Yamagata and Low, 1995). Therefore, our objective was to investigate changes in purine content of grass shrimp stored at different temperatures and to evaluate freshness of the shrimp based on Kp value, log Ade and VBN.

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MATERIALS & METHODS

Materials

Freshly caught, aquaculture grown grass shrimp (*Penaeus monodon*) were purchased locally. The average weight was 22.4±5.3g and average length was 14.9±1.2 cm. Shrimp were immediately packed in ice and transported to the laboratory within 1h. Upon arrival, shrimp were washed and packed in polyethylene bags with three shrimp in each bag. The shrimp were divided into three groups for storage at room temperature (22±2°C), 5°C or −20°C. Three bags from each group were sampled at regular intervals and subjected to the following analyses.

Determination of purine contents

The purine content was estimated according to Benkmann and Montag (1993) as modified by Lou and Chen (1997). After removal of heads and shells, shrimp were chopped, freeze-dried and ground with pestle and mortar. The resulting powder (200 mg) was digested in a glass tube with a mixture of CF_3COOH/HCOOH (1/1, v/v) at 90°C for 14 min. The resultant hydrolysates were transferred into a 250 mL flask and dried by rotary vacuum evaporator at 50°C. To dissolve the purine bases, 10 mL 0.02M KH_2PO_4 buffer solution (pH 3.2) was added to the flask. This solution was then filtered through a 0.2 μm membrane filter. The purine bases were separated by HPLC using a reversed phase column (Lichrospher $5C_{18}$, 250 × 4 mm, i.d., Merck, Germany) with 0.02M KH_2PO_4 buffer, pH 3.2. The eluate was passed through a UV detector at 254 nm and the concentrations of the purine bases, Ade, Hyp, guanine (Gua) and xanthine (Xan) were computed automatically on the basis of peak areas. The purine standards were obtained from Sigma Chemical Co., (St. Louis, MO). Our new proposed Kp value was calculated from the ratio of hypoxanthine/adenine. All values were computed on the basis of μmole/g dry weight.

Determination of volatile basic nitrogen (VBN) and pH

The VBN was estimated according to the microdiffusion method of Conway (1950). To determine pH, 5g shrimp muscle was homogenized in 45 mL distilled water. The pH of the filtrate was measured using a pH meter (Corning, NY).

Sensory evaluation

Sensory evaluation was done by a panel of 7 trained judges consisting of laboratory staff and students, who were regular shrimp consumers. The judges participated in five training sessions to recognize the characteristic odor of raw fresh shrimp and were additionally trained in the detection of putrid odors of ice-stored shrimp until they recognized the characteristic odors of decomposition. Sensory evaluation was conducted on each sample at the time it was removed from storage. One to three raw deheaded and peeled shrimp from each test sample were randomly evaluated by panelists. All samples were placed on an open plate and identified by a 3-digit code. Sensory testing was done in a clean, well-lighted and ventilated room. The panelists classified the shrimp for odors into one of three stages individually: 1. acceptable (no putrid odor); 2. initial decomposition (faintly putrid odor); 3. advanced decomposition (putrid odor). A structured scale was used. No classification was recorded unless at least 4 judges evaluated the sample at the same stage (Matsumoto and Yamanaka, 1990).

Statistical analysis

The data were subjected to an analysis of variance followed by Duncan's multiple range test ($p<0.05$) (SAS Institute, Inc., 1985). Pearson correlation coefficients be-

tween purine and storage time and VBN were determined.

RESULTS & DISCUSSION

Changes of purine content during storage

The changes in purine content of grass shrimp were followed during storage at different temperatures (Table 1). Adenine (Ade) decreased ($p<0.05$) from 4.95 to 1.82 mg/g dry wt in the first 12h at 22°C and then decreased to 1.39 mg/g dry wt over the next 12h. However, hypoxanthine (Hyp) levels increased from 1.68 to 2.50 to 3.59 mg/g dry wt after 0, 4 and 8h, respectively, after which no further changes occurred. The fact that xanthine (San) was not initially detected indicates that the shrimp samples were very fresh. Xan content increased gradually during storage. The changes in guanine (Gua) content were also small. The only significant change in total purine content occurred at 24h.

Collectively, these data suggested that adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP) and adenosine degraded at the early stage of storage at room temperature. This might be due to the increase of inosine monophosphate (IMP), inosine (Ino) and Hyp, which would lead eventually to xanthine formation. The decreasing Ade levels suggested an enzymatic effect of AMP deaminase or adenosine deaminase; the degradation of ATP to IMP proceeded by endogenous enzymes in the kuruma prawn muscle (Matsumoto and Yamanaka, 1991). AMP deaminase and adenosine deaminase have been reported in several species of marine shrimp (Cheuk et al., 1979).

The Ade content decreased steadily from 4.95 to 0.54 mg/g during 144h storage at 5°C, whereas Hyp increased from 1.68 to 5.46 mg/g in the first 120h and then decreased slightly. Xan increased during storage, Gua increased slightly, reached a peak at 72h, and then decreased slightly. The changes in total purine fluctuated during storage, although a significantly lower value was observed at 144h. Thus similar changes were observed in levels of purine bases during storage at these two temperatures, but changes at 22°C occurred at a faster rate than at 5°C. The nucleotide degradation pathway appeared to be the same as that for fish. We also concluded that IMP, Ino or Hyp accumulated in grass shrimp during storage.

There were no differences ($p>0.05$) in Ade, Gua and Xan in shrimp stored up to 10 wk at −20°C. This may indicate that the temperature was sufficiently low to retard enzymic activities. However, although Hyp was constant for the first 4 wk, it then decreased slightly. The total purine content also decreased. The moisture content decreased from 76.50% to 67.42%, presumably from drip loss during thawing. This could have caused the slight decrease of Hyp., since Hyp is very soluble and is released quickly from foods during cooking (Young, 1982, 1983; Brule et al., 1989; Lou, 1997a).

Table 1—Changes in purine contents of grass shrimp during storage (mg/g dry wt)

Storage time	Moisture (%)	Adenine	Guanine	Hypoxanthine	Xanthine	Total purines[h]
Storage at 22°C (h)						
0	76.5±0.9[a]	4.95±0.03[a]	0.56±0.00[b]	1.68±0.14[c]	N.D.[g]	7.19±0.14[a]
4	76.3±1.3[a]	3.63±0.15[b]	0.58±0.11[b]	2.50±0.79[bc]	0.07±0.02[d]	6.78±0.81[a]
8	77.3±1.1[a]	2.76±0.19[c]	0.75±0.06[a]	3.59±0.32[a]	0.10±0.00[cd]	7.20±0.38[a]
12	77.4±0.7[a]	1.82±0.15[de]	0.74±0.04[a]	3.67±0.43[a]	0.12±0.01[bc]	6.35±0.46[ab]
16	78.7±0.5[a]	1.75±0.29[de]	0.70±0.08[ab]	3.65±0.69[a]	0.15±0.02[b]	6.25±0.75[ab]
20	77.6±1.2[a]	1.95±0.33[d]	0.81±0.00[a]	3.63±0.07[a]	0.13±0.00[b]	6.52±0.34[a]
24	77.1±0.8[a]	1.39±0.19[e]	0.59±0.05[b]	3.51±0.12[ab]	0.29±0.02[a]	5.78±0.23[b]
Storage at 5°C (h)						
0	76.5±0.09[a]	4.95±0.03[a]	0.56±0.00[bc]	1.68±0.14[c]	N.D.	7.19±0.14[ab]
24	75.7±0.8[a]	2.53±0.13[b]	0.55±0.03[c]	3.63±0.23[d]	0.05±0.00[d]	6.75±0.27[aabc]
48	76.4±1.2[a]	1.67±0.06[c]	0.65±0.01[a]	4.11±0.24[cd]	0.11±0.00[cd]	6.54±0.25[bc]
72	76.0±0.5[a]	1.29±0.04[d]	0.72±0.02[a]	4.86±0.30[ab]	0.24±0.04[bc]	7.11±0.31[ab]
96	76.1±0.3[a]	0.96±0.10[e]	0.57±0.03[bc]	4.70±0.15[bc]	0.29±0.10[b]	6.52±0.31[bc]
120	77.9±1.1[a]	0.88±0.05[c]	0.64±0.05[ad]	5.46±0.34[a]	0.58±0.03[a]	7.56±0.35[a]
144	77.6±1.2[a]	0.54±0.03[f]	0.43±0.04[d]	4.53±0.47[bc]	0.59±0.08[a]	6.10±0.48[c]
Storage at −20°C (wk)						
0	76.5±0.9[a]	4.95±0.03[a]	0.56±0.00[ab]	1.68±0.14[a]	N.D.	7.19±0.14[a]
2	68.9±0.9[cd]	4.79±0.93[a]	0.55±0.05[ab]	1.62±0.06[a]	N.D.	6.96±0.93[ab]
4	71.6±0.8[b]	4.23±0.06[a]	0.63±0.00[ab]	1.66±0.08[a]	0.02±0.00	6.54±0.10[ab]
6	69.9±0.9[bc]	4.62±0.00[a]	0.54±0.02[b]	1.42±0.00[b]	0.05±0.00	6.63±0.02[ab]
8	69.5±1.0[bcd]	4.66±0.04[a]	0.57±0.01[ab]	1.31±0.02[b]	N.D.	6.54±0.05[ab]
10	67.4±1.1[d]	4.38±0.02[a]	0.66±0.09[a]	1.23±0.09[b]	N.D.	6.27±0.13[b]

[a-f]Means (n=3) with the same superscripts in a column at separate storage temperature are not significantly different ($p>0.05$).
[g]N.D.=not detected.
[h]Total purine = adenine + guanine + hypoxanthine + xanthine.

The changes in purine content of shrimp during different storage conditions indicate that AMP deaminase, adenosine deaminase or xanthine oxidase may be important in the breakdown pathway of nucleotides in grass shrimp. Lowering the temperature reduces enzyme activities and consequently has a major impact on the patterns of purine compounds in stored grass shrimp.

Change in uricogenic bases during storage

Generally, the "low purine diet" has been mainly based on total purine content. Clifford and Story (1976), however, reported that Ade and Hyp were more uricogenic than Gua and Xan. The quantity and composition of purine content in grass shrimp changed after storage and were different from those in fresh shrimp. In our study, during the period in which the shrimp were still acceptable (i.e., 24h at 5°C and 8h at 22°C), the sum total of the uricogenic purine bases Ade and Hyp decreased by <0.5 mg/g during storage at 5°C and 22°C (Table 1). However, after 10 wk storage at −20°C, total uricogenic purine bases decreased from 6.63 to 5.61 mg/g. Thus, long term frozen storage may reduce the uricogenic purine content in shrimp, while the quality of the shrimp at this long term frozen storage remains acceptable (Table 2).

Change in VBN, pH and sensory quality

Changes in VBN and pH of shrimp stored at different temperatures together with sensory ratings were compared (Table 2). The initial VBN of 8.63 mg% decreased to 4.24 mg% after 4h at 22°C, then increased to 30.26 mg% after 12h storage, at which time the sensory rating reached the advanced decomposition stage. The VBN for 24h storage reached 77.34 mg%. The pH decreased slightly at 4h storage, then slowly increased again. At 5°C the VBN content increased continuously with time, reaching the advanced decomposition stage at 72h, and the pH increased. During storage at −20°C for 10 wk, pH fluctuated somewhat, but VBN did not significantly change and sensory ratings remained acceptable.

The VBN value for spoiled Gulf shrimp has usually been in excess of 30 mg% (Cobb et al., 1973), while a shrimp muscle VBN level of 28.5 mg% has been considered the upper limit for acceptable quality (Shamshad et al., 1990). In our results, however, VBN levels of 25.56 and 30.26 mg% were associated with advanced decomposition stage for 5°C and 22°C storage, respectively. These differences might be due to species and storage temperature; VBN may not be an appropriate freshness index for grass shrimp during storage at 5°C and 22°C.

Correlations between purine compounds, storage time and VBN

Pearson correlation coefficients (r) between purine compounds and storage time were developed (Table 3). Correlations between storage time were significant for Ade ($p<0.01$), Hyp ($p<0.05$) and Xan ($p<0.01$) at 22°C storage. Log Ade and a proposed Kp value (Hyp/Ade) also correlated well with storage time. Similar conditions were found for 5°C storage, although at −20°C storage time only correlated significantly with Hyp levels.

Changes of purine compounds during storage depend on the activities of enzymes (Matsumoto and Yamanaka, 1991). Cheuk et al. (1979) suggested that the activity of adenosine deaminase and AMP deaminase may

Table 2—Changes in VBN, pH and sensory ratings in grass shrimp during storage

Storage time	VBN (mg%)	pH	Sensory ratings[g]
Storage at 22°C (h)			
0	8.63±0.88[e]	6.86±0.02[d]	1
4	4.24±0.28[f]	6.68±0.03[e]	1
8	12.22±2.04[d]	7.02±0.01[c]	1
12	30.26±0.78[c]	7.12±0.00[b]	3
16	45.34±1.04[b]	7.16±0.02[b]	3
20	75.98±2.01[a]	7.27±0.01[a]	3
24	77.34±1.83[a]	7.28±0.01[a]	3
Storage at 5°C (h)			
0	8.63±0.88[e]	6.86±0.02[e]	1
24	12.75±2.98[d]	7.22±0.02[c]	1
48	14.72±1.77[d]	7.28±0.01[b]	2
72	25.56±2.52[b]	7.26±0.01[b]	3
96	20.12±1.12[c]	7.30±0.01[b]	3
120	27.92±1.41[b]	7.56±0.03[a]	3
144	34.54±1.18[a]	7.55±0.01[a]	3
Storage at −20°C (wk)			
0	8.63±0.88[a]	6.86±0.02[c]	1
2	8.62±1.07[a]	7.12±0.01[a]	1
4	9.56±0.64[a]	6.84±0.02[c]	1
6	8.74±0.51[a]	6.82±0.01[c]	1
8	9.47±0.59[a]	6.95±0.01[b]	1
10	8.46±0.61[a]	7.18±0.00[a]	1

[a-f]Means (n=3) with the same superscripts in a column at separate storage temperatures are not significantly different (p>0.05).
[g]1=Acceptable; 2=initial decomposition; 3=advanced decomposition.

Table 3—Pearson correlation coefficients (r) between purine contents, storage time and VBN

Purine	Storage time	VBN
Storage at 22°C		
Ade	−0.9073**	−0.7586*
Gua	0.3918	0.3348
Hyp	0.7723*	0.5942
Xan	0.9052**	0.7926*
log Ade	−0.9380**	−0.8139*
Kp	0.9365**	0.8197*
Storage at 5°C		
Ade	−0.8750**	−0.8199*
Gua	−0.2415	−0.1732
Hyp	0.8046*	0.7646*
Xan	0.9663***	0.9360**
log Ade	−0.9770***	−0.9248**
Kp	0.9927***	0.9557***
Storage at -20°C		
Ade	−0.5777	−0.4220
Gua	0.5182	0.1033
Hyp	−0.9460**	0.1040
Xan	0.0786	0.0879
log Ade	−0.5616	−0.4245
Kp	−0.6882	0.3348

*p<0.05; **p<0.01; ***p<0.001.

potentially serve as quality indices for shrimp held on ice. The negative regression between storage time and log Ade, the linear increase of Kp value and Xan during storage, and high correlation coefficients lead to the conclusion that the Kp value, Xan and log Ade all have potential as freshness indices for grass shrimp.

In Japanese prawn (*Pandalus hypsinotus*), Arai (1966) proposed two pathways for the degradation of ATP. One involved the deamination of AMP to IMP, and the second involved dephosphorylation of AMP to adenosine, followed by deamination to Ino. Stone (1970) and Fatima et al. (1981) considered the major pathway for adenine nucleotide degradation to be deamination of AMP to IMP in several shrimp species. Matsumoto and Yamanaka (1990, 1991) have shown that IMP accumulated in kuruma prawn during storage. However, the accumulation of Ino during ice storage in some other shrimp species supports the adenosine deamination pathway (Tarr and Commer, 1965; Flick and Lovell, 1972; Cheuk et al., 1979). Thus, it is difficult to determine which nucleotide catabolites may be involved in the K value to effectively estimate the freshness of a given species of shrimp. Basing the K value on total purine bases and their ratio rather than single nucleotide catabolites might help circumvent any effects of different breakdown pathways.

Because log Ade content, Xan and Kp values had a strong linear relationship to storage time at 5°C and 22°C, these measures were compared with the common chemical quality indicator VBN (Shamshad et al., 1990; Matsumoto and Yamanaka, 1990; Yamagata and Low, 1995). Several strong correlations were found (Table 3). These correlations further demonstrated the suitability of log Ade and Kp as potential freshness indices for grass shrimp during storage at 5°C and 22°C. The change in purine content of kuruma shrimp (*Penaeus japonicus*) during iced storage has been studied in our laboratory. Very good correlation between Kp value, VBN and storage time were reported in kuruma shrimp during storage under icing. The Pearson correlation between the Kp (Hyp/Ade) value and storage time and those between the Kp value and VBN were 0.9859 and 0.9643, respectively (Lou, 1997b). Xan cannot be recommended as a freshness indicator, however, because of the small magnitude of changes. Ade levels above 18.72 μmole/g dry wt (from Table 1: the concentration of Ade after 24h at 5°C, i.e., 2.53 mg/g ÷ the molecular weight of Ade) at 5°C and 13.47 μmole/g dry wt (from Table 1: the concentration of Ade after 8h at 22°C, i.e., 2.76 mg/g ÷ the molecular weight of Ade) at 22°C would be acceptable, and the upper limits for Kp value should be 1.42 and 1.29 at 5°C and 22°C, respectively.

CONCLUSION

THE URICOGENIC PURINE CONTENT OF grass shrimp decreased during storage at −20°C. Correlations between log Ade, storage time and volatile basic nitrogen (VBN) and those between the Kp (Hyp/Ade) value, storage time and VBN suggested that both log Ade and Kp value could be used as freshness indicators for grass shrimp during 5°C and 22°C storage.

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