Changes in Purine Content of *Tilapia mossambica* During Storage, Heating and Drying

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Running Head: Purine content in stored and thermal processed tilapia

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ABSTRACT

The changes in the purine content of tilapia (*Tilapia mossambica*) during storage, heating and drying were investigated. In tilapia, the combined total content of the uricogenic purines adenine (Ade) and hypoxanthine (Hyp) decreased during storage for 10 wks at -20℃, while the fish were still acceptable. The content of these uricogenic purines in tilapia decreased 46.4% and 24.9% during 40 min boiling and steaming, respectively. However, 16.6% of uricogenic purines were lost in 7 min cooking by microwave, while the decreases were 20.0% and 12.6% for 3h drying at 70℃ and 80℃, respectively. These findings suggest that moist heat methods had a greater reducing effect on uricogenic purines in tilapia than dry heat methods. The major decreased purine substances were Hyp-related compounds regardless of whether the talapia was stored or processed.

Key words：tilapia, uricogenic purines, storage, heating, drying

INTRODUCTION

Tilapia (*Tilapia mossambica*) is a well-known species of fish in Taiwan and is widely consumed as part of the local diet. It contains a high total purine concentration in the range of 106 to 199 mg/ 100 g with or without skin (Ho, 1986; Lou et al., 1996). Dietary purine intake is known to influence human serum uric acid levels which are associated with hyperuricemia and gout. The purine content of various foods has been investigated in several studies (Fujii et al., 1971; Clifford et al., 1976; Clifford and Story, 1976; Matsumoto et al., 1977; Shinoda et al., 1982; Yokozawa et al., 1985; Ho,

1986; Herbel and Montag, 1987; Wolfram and Colling, 1987; Brule et al., 1988; Montag et al., 1989; Lou et al., 1996). The quality and quantity of purine compounds in food might be changed during storage and by different thermal processing methods. The purine content in grass shrimp decreased during storage at different temperatures, and a good correlation between the ratio of Ade/Hyp and freshness was observed (Lou, 1998). The effects of cooking procedures on the purine content in some selected foods have also been studied (Young, 1982, 1983; Shinoda et al., 1982; Brule et al., 1989; Colling and Wolfram, 1989; Lou and Montag, 1994; Lou et al., 1997). However, little information is available on changes in the quality and quantity of purine compounds in fishery products during storage and thermal processing (Shinoda et al., 1982; Brule et al., 1989; Lou et al., 1997). Thus, more information on the changes in the purine contents in fish during storage and heating is required in order for recommendations about dietary purine intake to be useful to consumers.

The objective of this study was to determine the changes in purine contents in tilapia during storage at different temperatures and the influence of different thermal procedures, namely boiling, steaming, microwave cooking and drying at 70℃ and 80 ℃, on the concentrations of purine in tilapia. The amounts of uricogenic purines in tilapia, namely the sum of Ade and Hyp, were also investigated.

MATERIALS & METHODS

Materials

Freshly caught, aquaculture grown tilapia (*Tilapia mossambica*) were purchased locally. The average weight was 176.7 ±42.7 g and average length was 20.0 ±3.3 cm. The fish were immediately packed in ice and transported to the laboratory within 2 h. Upon arrival, fish were washed and divided into three groups for storage, thermal processing and drying.

Purine standards, Ade , guanine (Gua), xanthine (Xan) and Hyp , were purchased from Sigma Chemical Co., (St. Louis, MO, USA), while trifluoroacetic acid and formic acid were obtained from RDH Chemical Co., (Seelze, Germany). Potassium dihydrogen phosphate was supplied by Merck (Darmstadt, Germany).

Storage

The fish were divided into three groups and stored at room temperature (20 ±2) ℃, 5℃ and -20℃ for 16 h, 96 h and 10 weeks, respectively. Three fish from each group were sampled at regular intervals. After removal of heads, viscera, tails, bone and skin, the edible portions, including the dorsal and ventral parts, were chopped, freeze-dried and ground. The resulting powder was then stored at -20℃ until subjected to purine determination.

Heating and drying

Three cooking treatments, boiling, steaming and microwave cooking, were carried out. Batches, each of three fish, were decapitated, gutted and chopped into 3-5 pieces each of about 5 cm in length. Each batch was cooked in boiled water (1/5; w/v) for 10, 20, 30 and 40 min. Fish samples in the steamed batch were then steamed over a water

bath for 10, 20, 30 and 40 min. Fish samples in the microwave cooking batch were placed on a dish and cooked by microwave oven (600W,Teco.Korea) for 4, 5, 6 and 7 min.

After filleting, fillets from the three batches were dried in a hot air blast oven (Channel, DCM45,Taiwan) at 70 and 80℃ for 1, 2 and 3 h, respectively. After processing, the edible portions of all these samples were chopped, freeze-dried and ground in a mixer (Itecator, 400W, Sweden). The obtained powder was stored at -20 ℃ until subjected to subsequent analyses.

Moisture content of tilapia

Moisture determinations of raw and processed tilapia were performed using standard AOAC methods (1995).

Determination of purine contents

A modified method according to Benkmann (1995) and Lou and Chen (1997) was used to estimate the purine content in tilapia. The powdered samples (about 100 mg) were digested in a glass tube with a 5 mL mixture of CF_3COOH/HCOOH (1/1:v/v) at 100℃ for 35 min. The resultant hydrolysates were transferred into a 250 mL flask and dried by a rotary vacuum evaporator at 50℃. To dissolve the purine bases, 10 mL of 0.02 M 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 (Gold System Chromatography, Beckman, USA) using a reversed phase column (Lichrospher $5C_{18}$, 250×4 mm, i.d., Merck, Germany) with 0.02 M KH_2PO_4 buffer, pH 3.2. The elute was passed through a UV detector at 254 nm and the

concentration of purine bases, namely Ade , Hyp, Gua and Xan , were computed on the basis of peak area. The concentration of a standard solution of purine bases was 0.1 mM. Peak identification was based on retention time and spectrum scanning with a photodiode array detector (SPD-M6A, Shimazu, Japan).

Statistical analysis

The data were analyzed by an analysis of variance (ANOVA) using the SAS software package (SAS Institute, Inc., 1985). The Significance of difference between the means was determined by Duncan's multiple range test ($p<0.05$)

RESULTS & DISCUSSION

Purine bases in tilapia

The total purine content in tilapia was 5.65 mg/g dry wt (equal to 126 mg/100g fresh tilapia). The Hyp level was 4.08 mg/g dry wt and constituted about 72% of the total purine (Table 1). The contents of Gua and Ade were lower than 0.95 mg/g dry wt, while no Xan was detected. This might have been due to the struggling during netting and transportation, which might have accelerated the degradation of Ade-related compounds, adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP). These data suggested that the Hyp- related compounds, namely inosine monophosphate (IMP), inosine (Ino) or Hyp, may be the major accumulations resulting from nucleotide degradation in tilapia. The accumulation of inosine in tilapia during iced storage has been reported (Azam,1996). In sea water acclimated tilapia, IMP accumulated during storage at 0, 10 and 20℃ (Yoon, 1996).

Changes in purine content during storage

The changes in purine content of tilapia were followed during storage at different temperatures (Fig. 1). No significant changes ($p>0.05$) in Hyp and total purine content were observed during storage up to 4 weeks at -20℃ after which the content of Hyp decreased steadily from 4.24 mg/g dry wt to 2.66 mg/g dry wt during 10 weeks storage. The total purine content also decreased from 5.63 mg/g dry wt to 3.87 mg/g dry wt at 10 weeks. The decreasing Hyp levels suggested the combined effects of endogenous degradative enzymes and drip loss. Since lowering the storage temperature was sufficient to retard the growth of bacteria, the effect of exogenous degradative enzymes was eliminated. Fish contain enzymes, including Xan oxidase, uricase, allantoinase and allantoicase, in which Hyp is degraded via uric acid to urea, ammonia and glyoxylate, the end product of purine metabolism in fish (Mertz, 1993, Eisenbrand, 1995). Sakabura et al (1996) reported that fish contained degradative enzymes, which are able to convert purines to urate, urea and glyoxylate, the end products of purine degradation. In the present study, the moisture content decreased during thawing from 78.7% to 74.4% (data not shown), presumably from drip loss. This could have caused the slight decrease of Hyp, since Hyp is soluble and is released quickly from foods during cooking (Young, 1982, 1983; Brule et al., 1989, Lou, 1997). The change of Hyp could have caused the decrease in total purine content, since there were no differences ($p>0.05$) in Ade, Gua and Xan in tilapia stored up to 10 weeks.

The contents of Hyp and total purine were constant for the first 12 h of storage at 20℃ and then decreased ($p<0.05$) slightly. No changes of Ade, Gua and Xan

occurred during storage. Similar changes were observed in the levels of purine bases during storage at 5℃, but the levels of Hyp and total purine were constant for the 96 h. The slight decrease of Hyp after 12 h storage was supposed to be due to the exogenous enzymes. Yokoyama et al. (1992, 1996) studied the changes of ATP-related compounds in the adductor muscle, mantle, gill and body trunk of oysters during ice storage. They found that Hyp increased slowly until the 14^{th} day, and then decreased in adductor muscle and body trunk of oysters, while Xan increased or was detected first at the 10^{th} day, and then increased. Their studies using the antibiotic chloramphenicol showed that the changes of Hyp and Xan during the decomposition stage were mainly caused by exogenous enzyme.

Collectively, these data suggest that IMP, Ino or Hyp accumulate in tilapia during storage at different temperatures. The degradative enzymes, endogenous and exogenous, may be important in the breakdown pathway of nucleotides during storage. Dietary regimens for the restriction of purines in patients with hyperuricemia have been mainly designed based on the amounts of total dietary purines. Ade and Hyp have been reported to be more uricogenic than Gua and Xan (Clifford and Story, 1976; Sarwar and Brule, 1991). In previous studies , tilapia were still acceptable (VBN<25 mg%) during storage at 4℃ for 96 h (Wang et al., 1994) or at 25℃ for 6 h (Hsue and Ko, 1998). In our study, during the period for which tilapia samples were still acceptable , the sum total of the uricogenic purine bases, Ade and Hyp, were constant during storage. However, after 10 weeks storage at -20℃, total uricogenic purine bases decreased from 4.70 to 3.21 mg/g. Thus, long term frozen storage may

reduce the uricogenic purine contents in tilapia even though the quality of tilapia remains acceptable for consumption.

Effect on purine bases during heating

Changes in purine bases during boiling were evaluated (Table 1). The Hyp content decreased ($p<0.05$) from 4.08 to 2.12 mg/g dry weight in tilapia boiled for 40 min. Ade levels also decreased ($p<0.05$) gradually with a small magnitude. However, Gua content increased, reaching a peak after 10 min boiling, and then decreased. Xan was not detected in either the fresh or boiled tilapia. Consequently, the total purine content decreased steadily from 5.65 to 3.53 mg/g dry wt during 40 min boiling. The moisture content also decreased from 78.72% to 72.68% in the first 10 min of boiling, then no further changes occurred.

Collectively, these data (Table 1) suggest that a long duration of boiling will reduce purine contents in tilapia, especially those of Hyp-related compounds. These changes could be due to the good solubility of Hyp, the extraction effect of hot cooking liquid and heat degradation. Similar changes were also observed in broiler chicken meat (Young, 1982, 1983), in haddock and beef (Brule et al., 1989; Sarwar and Brule, 1991), and in grass shrimp (Lou, 1997). Both the extraction effect and the heat degradation of nucleotides in grass shrimp was observed during cooking, since IMP, inosine and Hyp were detected in cooking juice, and no inosine or Hyp was found in fresh grass shrimp meat (Lou et al., 1998). The effects on nucleotide in tilapia were primarily on IMP related compounds, since the fresh tilapia contained

less Ade-related compounds. However, the content of Gua increased during boiling for the first 10 min, then decreased gradually. It was assumed that some of the soluble protein in tilapia was also extracted by hot water in the first 10 min of the boiling process (Young, 1983; Lou et al., 1997; Lou, 1997). Therefore, the dry matter of tilapia decreased which resulted in an increase in the Gua content. An increased Gua or total purine content was also observed in cooked grass shrimp (Lou, 1997) and some fish (Lou et al., 1997). The finding of increased purine content in chicken skin after cooking was previously reported as resulting from the release of soluble protein into cooking liquid (Young, 1983). Beyond 10 min, the insoluble Gua was slightly extracted during cooking. The amount of uricogenic purines, Ade and Hyp, decreased 46.4% from 4.70 to 2.52 mg/g dry wt. after 40 min boiling. Thus, a long duration of boiling may reduce the uricogenic purine contents in tilapia. However, most of the released purine substances from tilapia might be transferred into the cooking liquid.

The content of Hyp in tilapia decreased ($p<0.05$) from 4.08 to 3.01 mg/g after 40 min steaming, while Ade decreased in a smaller amount (Table 1). Gua content in tilapia increased during the first 20 min of steaming, after which it decreased gradually. Xan was initially not detected but was found after 30 and 40 min of steaming . This might have been due to the heating degradation of Hyp-related compounds and the drip loss of nucleotides during steaming. Total purine content was constant for the first 20 min of steaming, then decreased from 5.76 to 4.71 mg/g after 40 min. A steady decrease ($p<0.05$) in moisture was also observed during steaming decreasing from 78.7 to 70.1% during 40 min steaming. The amount of uricogenic purines decreased 24.9% from 4.70 to 3.53 mg/g during 40 min steaming. It was also

concluded that a long duration of steaming could reduce uricogenic purine contents in tilapia. Thus, similar changes were observed in levels of purine bases for these two processing methods, however, the changes due to boiling occurred at a faster rate and to a greater extent than the changes due to steaming.

Moisture content decreased from 78.7 to 71.7% during microwave cooking for 4 min (Table 1), then no further changes occurred during 7 min cooking. The Hyp content also decreased ($p<0.05$) from 4.08 to 3.35 mg/g only in the sample microwaved for 7 min . Ade levels decreased from 0.62 to 0.47 mg/g during the first 4 min of microwave cooking then increased to 0.57 mg/g at 7 min. However, Gua content increased slightly from 0.95 to 1.48 mg/g after 7 min cooking. Xan was detected after 4 min heating. This could have been the result of the heat degradation effect of Hyp-related compounds and the concentration effect described previously (Young, 1983; Lou et al., 1997; Lou, 1997). No significant changes in total purine content occurred during heating up to 7 min. The uricogenic purine contents decreased 16.6% from 4.70 to 3.92 mg/g after 7 min heating. These data suggest that microwave cooking could only slightly reduce the purine content in tilapia. The reducing effect of purine content in tilapia during different types of thermal processing followed in order of boiling > steaming > microwave cooking.

Changes in purine bases during drying

Changes in purine bases in tilapia as a result of drying at 70℃ and 80℃ for 3 h were also evaluated (Table 2). The moisture content decreased ($p<0.05$) from 78.7 to 54.1% and 34.7% during drying at 70℃ and 80℃, respectively. The content of Hyp decreased ($p<0.05$) from 4.08 to 3.53 mg/g for 3 h drying at 70℃ and from 4.08 to 3.66 mg/g at 80℃. The Xan levels increased only slightly during drying. These changes were considered to result from a combination of an enzymatic effect at the initial stage and a heat degradation effect during drying. The Ade content decreased slightly during drying at these two temperatures. However, the levels of Gua remained constant at 70℃ and 80℃ drying. The decrease of Hyp and Ade could have caused the decrease of the total purine content. The total purine content decreased from 5.65 to 4.75 and 5.10 mg/g for 3 h drying at 70℃ and 80℃, respectively. Similar content changes were also found for uricogenic purines. The levels of uricogenic purines decreased 20.0% from 4.70 to 3.76 mg/g at 70℃ drying, where it was reduced more than by drying at 80℃ (12.6%). Similar results have been reported in drying grass shrimp meat at 70℃ and 80℃ (Lou, 1997). This could be due to differences in lipid content , which might lead to a different dry weight basis. However, we did not determine lipid and protein levels in tilapia. The exact reason for this phenomenon is still unclear. Converting the dry weight basis to 100 g of fresh tilapia, the total purine content of fresh tilapia was 126 mg/100g. After drying at 70℃ and 80℃ for 3 h, the levels of total purine increased to 218 and 334 mg/100g, respectively.

Compared with thermal processing, the reduction in uricogenic purines contents by the drying method was similar to that of microwave cooking. Our results indicate that the moist heat methods reduced uricogenic purines contents more effectively

than dry heat methods. Presumably, this was due to the combined effects of hot water extraction and heating degradation during moist heat processing. However , no extraction effect occurred during dry heat processing.

CONCLUSION

The dominant purine bases in tilapia are Hyp-related compounds. These compounds accumulated during storage at different temperatures. Our results suggest that long term frozen storage may reduce the uricogenic purine contents in tilapia. Regardless of the type of processing, Hyp content decreased more than any of the other purine bases. The effects of processing on reducing uricogenic purines contents in tilapia were in following order: boiling > steaming > microwave cooking or drying. The combined effects of extraction and degradation of purine compounds by moist heat methods played an important role during processing. Most of the released purine compounds from tilapia during cooking may have been transferred into the cooking liquid.

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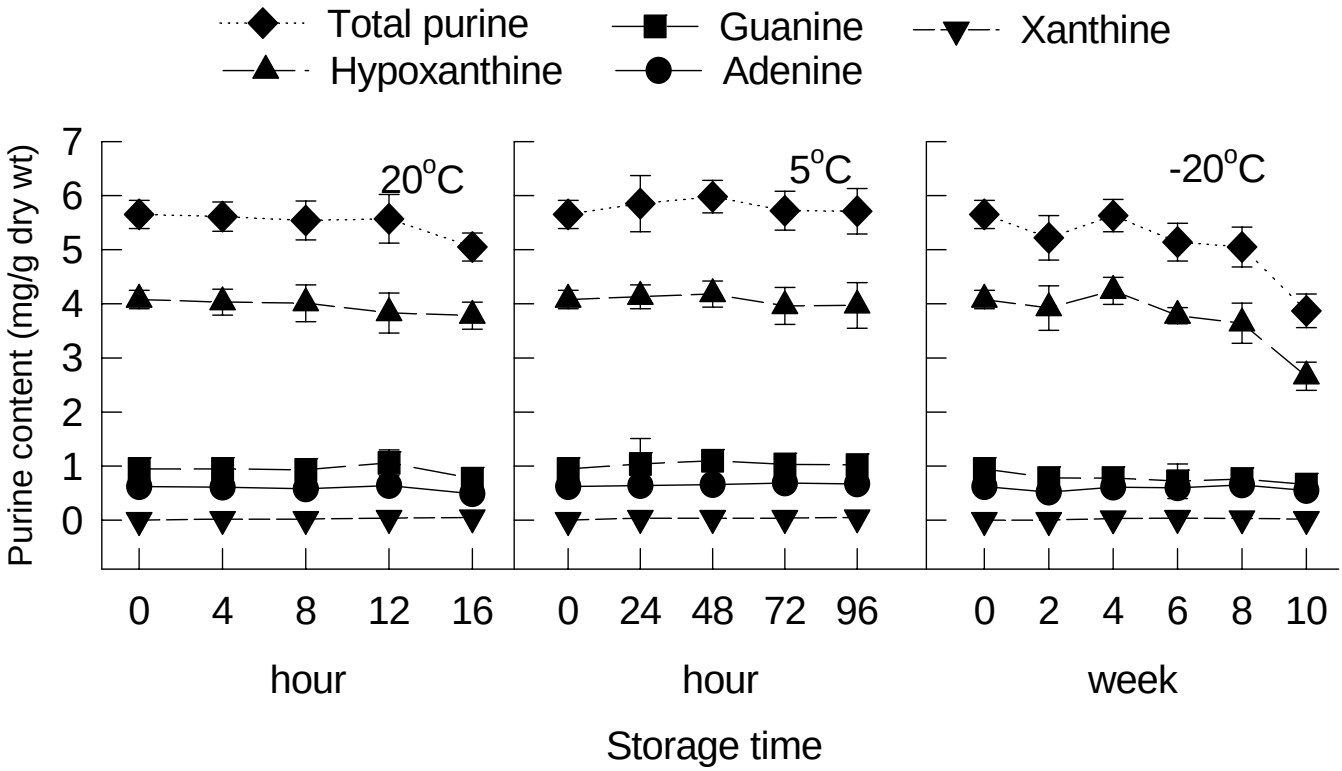

Fig. 1- Changes in purine contents of Tilapia during storage at different temperatures

Table 1- Changes in purine contents of tilapia during boiling, steaming and microwave cooking (mg/g dry wt.)[1]

Time (min)	Moisture(%)	Ade	Gua	Hyp	Xan	Total purine[2]
Boiling						
0	78.7±0.7^{a}	0.62±0.02^{a}	0.95±0.19^{c}	4.08±0.17^{a}	N.D.[3]	5.65±0.26^{a}
10	72.7±0.3^{b}	0.57±0.03^{b}	1.74±0.03^{a}	3.26±0.05^{b}	N.D.	5.57±0.07^{a}
20	73.0±0.3^{b}	0.51±0.07^{bc}	1.26±0.14^{b}	2.72±0.03^{c}	N.D.	4.49±0.16^{b}
30	73.6±0.6^{b}	0.50±0.01^{c}	1.10±0.36^{bc}	2.63±0.04^{c}	N.D.	4.23±0.37^{b}
40	72.7±0.8^{b}	0.40±0.01^{d}	1.01±0.16^{bc}	2.12±0.07^{d}	N.D.	3.53±0.17^{c}
Steaming						
0	78.7±0.7^{a}	0.62±0.02^{a}	0.95±0.19^{c}	4.08±0.17^{a}	N.D.	5.65±0.26^{a}
10	74.3±0.5^{b}	0.38±0.01^{e}	1.64±0.19^{a}	3.57±0.02^{b}	N.D.	5.59±0.19^{a}
20	73.3±0.1^{c}	0.56±0.06^{b}	1.74±0.01^{a}	3.46±0.02^{b}	N.D.	5.76±0.06^{a}
30	72.1±0.3^{d}	0.48±0.04^{d}	1.31±0.06^{b}	3.13±0.06^{c}	0.03±0.02	4.95±0.10^{b}
40	70.1±0.4^{e}	0.52±0.01^{c}	1.15±0.17^{bc}	3.01±0.04^{c}	0.03±0.01	4.71±0.17^{b}
Microwave cooking						
0	78.7±0.7^{a}	0.62±0.02^{a}	0.95±0.19^{c}	4.08±0.17^{a}	N.D.	5.65±0.26^{a}
4	73.4±1.0^{b}	0.47±0.01^{d}	1.28±0.15^{ab}	3.70±0.08^{b}	0.05±0.01^{a}	5.50±0.17^{a}
5	71.7±0.2^{c}	0.52±0.01^{c}	1.23±0.27^{ab}	3.79±0.15^{ab}	0.02±0.01^{b}	5.56±0.31^{a}
6	70.1±0.6^{d}	0.51±0.05^{cd}	1.16±0.02^{b}	3.65±0.16^{b}	0.02±0.01^{b}	5.34±0.18^{a}
7	71.2±0.4^{c}	0.57±0.02^{b}	1.48±0.16^{a}	3.35±0.10^{c}	0.03±0.01^{b}	5.43±0.19^{a}

[1]Means (n=3) with the same superscripts in a column were not significantly different ($p>0.05$)

[2]Total purine=Ade+Gua+Hyp+Xan

[3]N.D.= not detected

Table 2 - Changes in purine content of tilapia during drying at 70℃ and 80℃ (mg/g dry wt.)[1]

Drying time (hr)	Moisture(%)	Ade	Gua	Hyp	Xan	Total purine[2]
At 70℃						
0	78.7±0.7^{a}	0.62±0.02^{a}	0.95±0.19^{a}	4.08±0.17^{a}	N.D.[3]	5.65±0.26^{a}
1	68.9±0.6^{b}	0.58 ±0.08^{a}	1.05 ±0.27^{a}	4.06 ±0.27^{a}	0.05 ±0.01^{a}	5.74 ±0.39^{a}
2	65.8±0.4^{c}	0.36 ±0.04^{b}	1.01 ±0.13^{a}	3.83 ±0.86^{b}	0.09 ±0.08^{a}	5.29 ±0.87^{ab}
3	54.1±0.5^{d}	0.23 ±0.01^{c}	0.96 ±0.12^{a}	3.53 ±0.23^{b}	0.03 ±0.02^{a}	4.75 ±0.26^{b}
At 80℃						
0	78.7±0.7^{a}	0.62 ±0.02^{a}	0.95 ±0.19^{a}	4.08 ±0.17^{ab}	N.D.	5.65 ±0.26^{ab}
1	67.7±0.4^{b}	0.48 ±0.04^{b}	0.92 ±0.03^{a}	4.33 ±0.23^{a}	0.04 ±0.02^{a}	5.77 ±0.24^{a}
2	58.0±0.8^{c}	0.39 ±0.01^{c}	0.87 ±0.29^{a}	3.83 ±0.13^{bc}	0.03 ±0.01^{a}	5.12 ±0.16^{b}
3	34.7±0.8^{d}	0.45 ±0.01^{bc}	0.98 ±0.33^{a}	3.66 ±0.08^{c}	0.02 ±0.01^{a}	5.11 ±0.34^{b}

[1] Means (n=3) with the same superscripts in a column at separate drying temperatures are not significantly different ($p>0.05$)

[2] Total purine=Ade+Gua+Hyp+Xan

[3] N.D.= not detected