FISHERIES SCIENCE 2005; **71**: 889–895

Changes in purine content of tilapia surimi products during processing

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ABSTRACT: Changes in purine-related compounds of tilapia surimi product during processing were investigated. The washing step could result in about 60% decrease of total purine content in tilapia mince during processing. The main released purine substance was inosine monophosphate. The major reducing effect was conducted in the first 10 min during washing. No significant changes were observed after washing for 20 and 30 min. The lowest total purine content of tilapia surimi product was obtained with repeating the washing step twice. Thus, this procedure could reduce the purine content of tilapia mince from a high purine content level to a middle level. The gel strength of tilapia surimi product increased with increasing washing duration within 30 min. However, tilapia surimi product with a middle purine content and acceptable gel strength might be produced by washing twice in 10 min during processing.

KEY WORDS: **gel strength, purine content, surimi, tilapia, washing.**

INTRODUCTION

Tilapia *Oreochromis niloticus* is widely consumed in Taiwan and classified as a high purine content food, since they contain a total purine level in the range of 139 mg/100 g to 180 mg/100 g.[1,2] Low dietary purine intake is suggested for sufferers of gout to reduce the serum uric acid concentration.[1,3–8] Therefore, the utilities of tilapia might be limited. However, the purine content of surimi products on the local market was 53.1 ± 27.8 mg/100 g in Taiwan[2] and 21.4–67.6 mg/100 g in Japan.[9] However, the raw material of the surimi products has not been studied. These data indicated that the purine content of fish raw material might be reduced during the processing of surimi products. The effects of cooking, drying and storage on purine content in foods have been widely studied.[7–15] In previous studies, it was certified that the moist heat methods could reduce purine contents of food and the reducing effects were in the following order: boiling > steaming > microwave cooking.[8] The released purine compounds were indeed transferred into the cooking liquid.[16] However, no information is available on the changes of purine content during the processing of surimi products. Thus, it is valuable to evaluate the processing steps resulting in loss of purine-related compounds in surimi products.

The objectives of this study were to investigate the changes in purine compounds of tilapia mince during processing of surimi products and to find out the major processing step which might reduce the purine content in the products. The major processing steps included mince (after chopping), after washing (washing), after grinding (surimi paste), and after cooking (surimi product). A proper procedure will be also suggested to obtain lower purine content in surimi products. The gel strength of tilapia surimi products was also evaluated during the processing.

MATERIALS AND METHODS

Materials

Freshly caught, cultivated tilapia *Oreochromis niloticus* were purchased in a local market. The average weight was 600 ± 67 g and average length was 32 ± 4 cm. The fish were packed in ice and brought to the laboratory within 1 h. After removal of heads, viscera, tails, bone and skin, the dorsal muscle was collected and chopped to cubes of <3 mm.

Standards of purine-related compounds, adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine

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Received 27 October 2004. Accepted 14 February 2005.

monophosphate (IMP), inosine (Ino), adenine (Ade), guanine (Gua), hypoxanthine (Hyp), and xanthine (Xan) 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 and perchloroacetic acid were supplied by Merck (Darmstadt, Germany).

Preparation of surimi paste and surimi product

Preparation of surimi paste

About 150 g of mince were weighed into a 2.0-L beaker and washed with cold water (kept below 5°C) in a water/mince ratio of 5:1. The mince was washed for 10 min under 100 r.p.m. rotation (Stirrer-PC320, Corning Co. Ltd, NY). After washing, the mince was centrifuged at 450 ×*g* for 2 min. The moisture of the dewatered mince was determined with an infrared drying moisture meter (YST-YL-1, Kao Shing Enterprise Co. Ltd, Chang-wha, China) and adjusted to a moisture content of about 80%. After the mince was ground for 5 min in an ice bath, 2.5% NaCl was added and ground for 2 min continously.[17]

Preparation of surimi product

The 2.5% NaCl added and chopped surimi paste was stuffed into a 10 cm circumference, 15 cm length polyvinylidene chloride casing and heated at 90°C for 30 min. Then, the surimi product was quickly cooled with running water immediately and stored at 4°C.

Washing conditions

To consider the washing conditions, the washing duration and the washing times were studied individually. During the preparation of surimi product, the mince samples were subjected to washing for 10, 20 and 30 min individually. In the study of washing times, the mince samples were washed once, twice and three times for 10 min individually and washing water was replaced after each washing.

Preparation of analytical samples

The samples were collected separately at the following processing steps: tilapia mince (after chopping), after washing (washing), after grinding (surimi paste), and after cooking (surimi product). All these samples were chopped, freeze-dried and ground in a mixer (Itecator 400 W, Sweden). The obtained powder was stored at –20°C until subsequent analyses.

Determination of purine contents

A modified method according to Lou and others was used to estimate the purine content in tilapia.[8] The powdered samples (about 100 mg) were digested in a scrubbed glass tube with a 5-mL mixture of $CF_3COOH/HCOOH/H_2O$ (5/5/1 : v/v) at 100°C for 35 min. The resultant hydrolysates were transferred into a 250-mL flask and dried by a rotary vacuum evaporator at 50°C. The evaporated step was repeated twice, adding *c*. 5-mL distilled water into the flask each time, until no acid existed in the dried residue. To dissolve the purine bases, 10 mL of 0.02 M KH_2PO_4 buffer solution (pH 3.2) was added to the flask and placed in an ultrasonic water bath for 3 min. The solution was then filtered through a 0.2-μgm membrane filter. The purine bases were separated by high-pressure liquid chromatography (Gold System Chromatography, Beckman, USA) using a reversed phase column (Lichrospher $5C_{18}$, 250 × 4.6 mm, i.d., Merck, Germany) with 0.02 M KH_2PO_4 buffer containing 2.5 mM dimethyloctylamine, pH 3.2. The flow rate was 1 mL/min. The elute was passed through an ultraviolet detector at 254 nm and the concentrations of purine bases, namely Ade, Gua, Hyp 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).

Determination of adenosine triphosphate-related compounds

The ATP-related compounds including ATP, ADP, AMP, IMP, inosine and hypoxanthine were determined by the method described previously by Tsuchimoto and others.[18] Cold (5°C) perchloroacetic acid (15 mL) was added to 1.0 g of freeze-dried powders. Sample homogenization was carried out with CAT X520D homogenizer (M. Zipperer, Germany) at 9000 r.p.m. for 1 min in an ice bath. The homogenate was then centrifuged at 20 000 ×*g* for 5 min at 5°C. The supernatant was transferred to a beaker. The precipitate was extracted with 10 mL cold perchloroacetic acid again. After homogenization and centrifugation, the obtained supernatant

was collected and added to the previous supernatant beaker. A 10-mL aliquot was delivered to a beaker. The pH of the aliquot was then adjusted to 6.5–6.8 with 1 or 10 M of KOH solution. The aliquot was placed in an ice bath for 30 min followed by filtration with Whatman no. 1 filter paper. The filtrate was collected into a 25-mL volumetric flask. The residue was washed with neutralized perchloroacetic acid (5%). Then, the volumetric flask was made up to 25 mL with neutralized perchloroacetic acid (5%). This solution was then filtered through a 0.2-μm membrane filter and subjected to ATP-related compounds measurement by hihg-pressure liquid chromatography method (Gold System Chromatography). A reversed phase column (Lichrospher $5C_{18}$, 250×4.6 mm, i.d., Merck) with 0.05 M KH_2PO_4/0.05 M K_2HPO_4 (1/1, v/v) mixed buffer solution (pH = 6.9) was used. The flow rate was 1 mL/min. The elute was passed through an ultraviolet detector at 254 nm and the concentrations were computed on the basis of peak area. Peak identification was based on retention time and spectrum scanning with a photodiode array detector (SPD-M6A, Shimazu).

Evaluation of gel quality in surimi product

After the tilapia surimi product was cut into pieces with a thickness of 3 cm, samples were subjected to the test of breaking force (g) and deformation (cm). They were determined with a rheometer (Sun Rheometer, CR-150, Tokyo, Japan) using a ball plunger (5 mm in diameter) at a compression speed of 60 mm/min. The gel strength was calculated as breaking force (g) × deformation (cm). For each treatment, four determinations were performed and the mean values were calculated.[17]

Statistical analysis

The data were analyzed by an ANOVA using the SAS software package.[19] The significance of difference between the means was determined by Duncan's multiple range test ($P < 0.05$).

RESULTS AND DISCUSSION

Change in purine contents of tilapia during processing

The purine contents of tilapia were determined by the acid hydrolysis method. This method could release the purine bases from nucleosides, nucleotides and nucleic acid. Therefore, the obtained purine bases represented a sum of its related compounds. The changes in purine content of tilapia were followed during the processing of surimi product (Table 1). The major purine base was Hyp with a value of 28.08 ± 1.59 μmole/g dry basis. The level of Hyp decreased significantly after washing from 28.08 ± gmole/g dry basis to 8.32 μmole/g dry basis. It was meaningful since Hyp and Ade were more uricogenic than Gua and Xan.[3] Many studies showed that free Hyp-related compounds were very soluble and released quickly from foods during cooking.[10–12,15,20] The authors' previous study[16] certified that the released free purine-related compounds in grass shrimp during cooking were indeed transferred to the cooking juice. In the steps after grinding and after cooking, the content of Hyp decreased slightly, presumably because of the amount of Hyp released and the surimi product being filled in a sealed polyvinylidene chloride case during cooking. Ade also decreased after washing from 5.66 to 3.60 μmole/g dry basis, then decreased steadily after grinding and cooking. There were no differences in Gua during processing. This phenomenon might be because of the low magnitudes and the low solubility of Gua.[6,21] The total purine content of tilapia mince was 36.22 ± 1.92 μmole/g dry basis, equal to 111.6 ± 1.0 mg/100 g, which was classified to the high purine content food.[1] Total purine content decreased during washing at a much faster rate than grinding and cooking. The released amount

Table 1 Changes in purine content of tilapia during processing of surimi product* (μmole/g dry basis)

Processing steps	Adenine	Guanine	Hypoxanthine	Total purine†	Total purine (mg/100 g)
Mince	5.66 ± 0.24^{a}	2.48 ± 0.09^{a}	28.08 ± 1.59^{a}	36.22 ± 1.92^{a}	111.6 ± 1.0^{a}
After washing	3.60 ± 0.49^{bc}	2.40 ± 0.31^{ab}	8.32 ± 0.81^{bc}	14.32 ± 1.61^{b}	35.6 ± 0.8^{c}
After grinding	3.60 ± 0.19^{b}	2.12 ± 0.02^{b}	8.04 ± 0.18^{b}	13.76 ± 0.39^{b}	48.1 ± 0.1^{b}
After cooking	3.00 ± 0.15^{c}	2.41 ± 0.14^{a}	7.28 ± 0.30^{c}	11.69 ± 0.17^{c}	33.2 ± 0.3^{c}

*Values (mean ± standard deviation of three determinations) in the same column with the same letter are not significantly different ($P > 0.05$).

†Total purine = Adenine + Guanine + Hypoxanthine.

Table 2 Changes of adenosine triphosphate-related compounds in tilapia during processing of surimi product* (μmole/g dry basis)

Processing steps	ATP	ADP	AMP	IMP	Inosine	Hypoxanthine
Mince	0.16 ± 0.01^{b}	4.53 ± 0.20^{a}	0.93 ± 0.14^{bc}	23.73 ± 2.92^{a}	ND	3.23 ± 0.13^{c}
After washing	0.27 ± 0.05^{a}	1.70 ± 0.08^{b}	0.78 ± 0.05^{c}	6.77 ± 0.28^{b}	ND	1.77 ± 0.10^{d}
After grinding	0.27 ± 0.06^{a}	1.60 ± 0.14^{b}	1.23 ± 0.06^{a}	4.50 ± 0.12^{c}	0.55 ± 0.13^{a}	5.24 ± 0.13^{a}
After cooking	0.19 ± 0.09^{ab}	1.63 ± 0.09^{b}	0.47 ± 0.02^{d}	4.83 ± 0.25^{c}	0.21 ± 0.02^{b}	4.23 ± 0.25^{b}

*Values (mean ± standard deviation of three determination)s in the same column with the same letter are not significantly different ($P > 0.05$).

ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; IMP, inosine monophosphate; ND, not detected.

was about 60% from 36.22 to 14.32 μmole/g dry basis after washing. Grinding and cooking could only lead to a slight decrease of total purine content. After washing, the total purine content in fresh basis decreased from 111.6 to 35.6 mg/100 g, which was classified to be a middle purine content food.[1]

Collectively, these data suggested that washing was the major step to reduce the purine content of tilapia mince during the processing of surimi product. The main released purine substance was Hyp-related compounds. Thus, the obtained product (surimi product) could be classified to be a middle purine content food, which was quite different from the high purine content food in tilapia mince.

In order to find out what kind of purine-related compounds were released, the ATP-related compounds and their changes during processing were also investigated (Table 2). IMP was the major purine-related compounds in the tilapia and decreased after washing from 23.73 μmole/g dry basis to 6.77 μmole/g dry basis. Then, the level of IMP decreased gradually by a small magnitude during grinding and cooking. The contents of ADP and free Hyp were 4.53 and 3.23 μmole/g dry basis, respectively. Both decreased significantly after washing. There were no changes of ADP during grinding and cooking, while the level of free Hyp increased after grinding. Then, it decreased slightly after cooking. The increasing free Hyp level might be due to the degradation of IMP to Hyp proceeded by enzymatic reaction during grinding, since the most IMP and free Hyp were released into solution during washing. The changes of ATP and AMP were small during processing, although the level of AMP increased slightly during grinding, presumably from some other Ade nucleotide compounds.

These indicated that the reduction of purine content during the processing of surimi product was conducted mainly in the washing step. The major released purine substance was IMP. The content of ADP and free Hyp were also reduced by small magnitudes during washing. Thus, the high purine content of tilapia mince could be used to produce a middle purine content food.

Effect of washing duration on purine contents of tilapia surimi product

Changes in purine contents of tipalia as a result of washing duration were evaluated (Fig. 1). The contents of Hyp decreased significantly after 10 min washing, after which it decreased slightly at 20 min and then increased slightly up to 30 min. Similar changes were observed in the levels of total purine content for washing. The content of Ade decreased after 10 min washing and then was constant. No changes of Gua were observed during washing.

The reducing effect during washing on ATP-related compounds in tilapia mince was also investigated (Fig. 2). The content of IMP decreased dramatically for the first 10 min, then fluctuated by small magnitudes up to 30 min. ADP, AMP and Ino decreased slightly during the first 10 min of washing, then were steady during washing. However, no changes of free Hyp and ATP were found.

These changes in purine content during washing indicated that the primary effective time of the washing step to reduce purine substances was during the first 10 min. The major released purine compound was IMP, which was more uricogenic than other purine substances and might lose some taste intensity. Increasing washing time up to 30 min could only make small changes of purine content during washing.

Effect of washing times on purine contents of tilapia surimi product

In certain cases, the washing step might be repeated to two or three times to obtain a better quality of gel. Therefore, the reducing effects of various washing times in 10 min on purine content of tilapia surimi product were also investigated. The

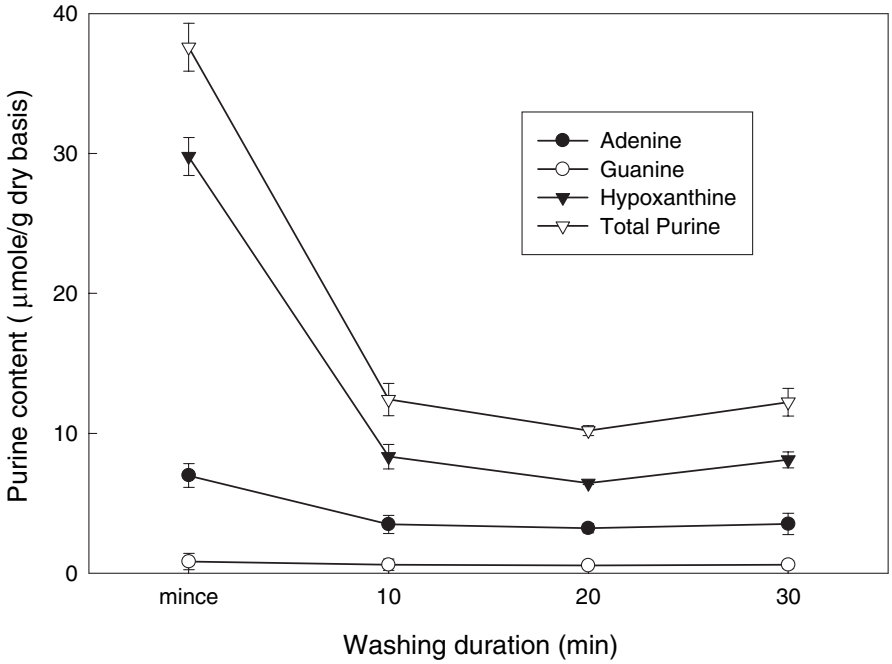

Fig. 1 Changes in purine content of tilapia mince after different washing durations.

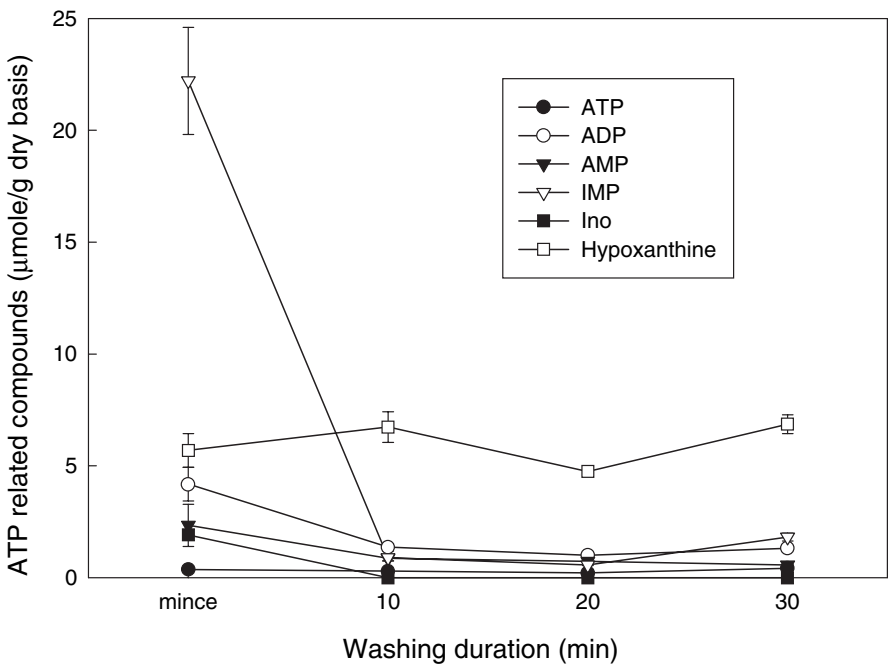

Fig. 2 Changes in adenosine triphosphate-related compounds of tilapia mince after different washing durations.

results indicated that the level of Hyp decreased significantly with increased washings (Fig. 3). Consequently, similar decreasing phenomenon of the total purine content was also observed with different washing times. The ratios of reducing effect were 67, 80 and 88% with one, two and three times washing, respectively. The Ade decreased, however, during the first washing and then no change was found during subsequent washings. The level of Gua was constant during different numbers of washings.

The changes in ATP-related compounds of the tilapia surimi product with various washing times were also evaluated (Fig. 4). The level of IMP decreased dramatically after the first washing, then fluctuated by small magnitudes for washing twice

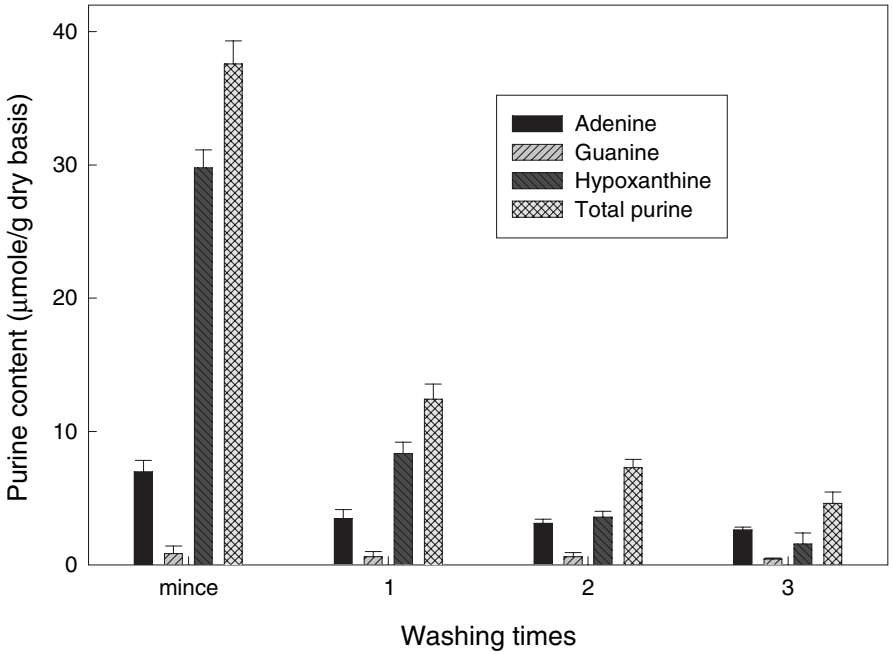

Fig. 3 Changes in purine content of tilapia mince after different numbers of washings.

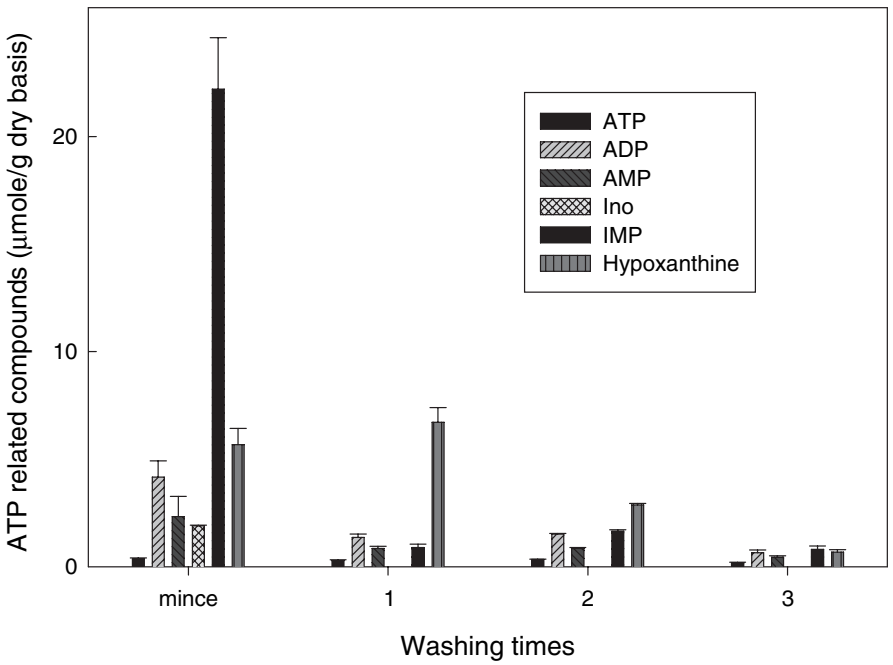

Fig. 4 Changes in adenosine triphosphate-related compounds of tilapia mince after different numbers of washings.

and three times. Free Hyp increased slightly during the first washing and then decreased gradually. As mentioned above, the increasing free Hyp might be because of the degradation of IMP during washing. Thus, the extraction effect and enzymatic degradation effect were performed at the same time during the washing step, which might result in the reduction of purine substances, especially for IMP. The contents of ADP, AMP and Ino decreased gradually with increased washings.

It is concluded that the more washings up to three times, the more purine content was released from tilapia mince. The major released purine substance was IMP. The reducing effect might be due to the effect of extraction and enzymatic degradation. Thus, the tilapia surimi products with middle

Table 3 The gel strength of tilapia surimi product with different washing conditions

Washing conditions	Gel strength (g. cm)
Washing duration	
10 min	160.9 ± 13.1[c]
20 min	503.2 ± 30.5[b]
30 min	703.2 ± 34.4[a]
Washing times (for 10 min)	
once	160.9 ± 13.1[c]
twice	328.6 ± 25.3[a]
three times	281.0 ± 12.1[b]

*Values (mean ± standard deviation of four determinations) in the same column with different letters are significantly different ($P < 0.05$).

purine content could be produced after proper washing treatment.

Changes in gel strength of the tilapia surimi product after different washing conditions

The gel formation quality of the tilapia surimi product prepared by different washing durations and numbers of washings was also compared (Table 3). The gel strength of tilapia surimi product increased with increased washing duration. The gel strength of product was 160.9 g × cm for washing 10 min, while it increased to 703.2 g × cm for 30 min of washing. For different numbers of washings, the largest gel strength was obtained at washing twice, while it decreased for washing three times. Collectively, longer washing time could result in better gel strength of the tilapia surimi product. Washing twice might be a proper number to get a better gel strength of the tilapia surimi product.

CONCLUSION

The washing step could reduce the total purine content of tilapia surimi product during processing. The main released purine compound was IMP. Consequently, the high purine content tilapia mince could be converted to a middle purine content surimi product after processing.

ACKNOWLEDGMENTS

This research was supported by the National Science Council, Taiwan, under grant No. NSC -88-2313-B-197-005.

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