CHANGES IN PURINE RELATED COMPOUNDS OF MILKFISH SURIMI BASED PRODUCT DURING PROCESSING

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ABSTRACT:

Changes in purine related compounds of milkfish surimi based product during processing were investigated. The objective samples were collected at the processing steps as mince (after chopping), after washing, surimi and surimi based product, separately, and subjected to purine content determination. The results indicated that washing step could result in about 60% decrease of purine substances of milkfish during processing. The main released purine substances were inosine monophosphate (IMP) and inosine. The major reducing effect was observed in the first 10 min during 30 min washing. No further change was found after washing for 20 or 30 min. However, the gel strength of milkfish surimi based product increased with increasing washing time. The total purine content in milkfish surimi based product decreased as the washing times increased. The best gel strength was obtained for washing twice. These data suggested that the total purine content of milkfish could be reduced from high purine content level (>100 mg/100 g) to middle purine content level (25-100 mg/100 g) during processing of milkfish surimi based product by washing twice for 10 min in each time. The gel strength of this milkfish surimi based product was still good acceptable.

Keywords: purine related compounds, washing, milkfish, surimi, gel strength

Introduction

Dietary purine content is closely associated with the disease gout. Therefore, the sufferers of gout is recommended to reduce their daily intake of high purine content food (Clifford and Story, 1976; Ho, 1986; Herbel and Montag, 1987; Wolfram and Colling, 1987; Montag and others, 1989; Lou, 1998; Lou and others, 2001). According to Ho's study (1986), it was suggested that food was divided into three groups, namely low, middle and high purine content ranging from 0- 25 mg/100 g, 25-100 mg/100 g and above 100 mg/100 g, respectively. Milkfish (*Chanos chanos* Forskal) is widely consumed in Taiwan and was grouped to high purine content food, since they contained about 139 mg /100 g total purine content (Lou and others, 1996) and 180 mg/100 g (Ho, 1986). However, the purine content of fish surimi based products is lower than that in raw material. The purine content of surimi based products on local market was 53.1 ± 27.8 mg/100 g in Taiwan (Lou and others, 1996) and 21.4 – 67.6 mg/100 g in Japan (Shinoda and others, 1981), which were classified as middle purine content foods. These data indicated that purine content could be released from raw material during the processing of surimi based products. Because the quality and quantity of purine compounds in food might be changed during storage and processing, the effects of cooking, drying and storage on purine content in foods have been widely studied (Young, 1982,1983; Shinoda and others, 1982; Brule and others, 1989; Colling and Wolfram, 1989; Lou and Montag, 1994; Lou and others, 1997,2001; Lou, 1998). In our previous studies, it was certified that the moist heat methods could reduce purine contents of food and the reducing effect were in following order: boiling ＞ steaming > microwave cooking (Lou and others, 2001). The released purine compounds indeed transferred into the cooking liquid (Lou and others, 1998).

Fish surimi based products are very popular in Asia market for consumption. The major processing steps included chopping, washing, grinding and cooking. The washing step could probably play an important role to release the purine compounds from food matrix during the processing. However, still no information is available on the changes of purine content during surimi based products processing. Thus, it is valuable to evaluate the processing step resulted in loss of purine related compounds of surimi based product. These might be modified and extended to reduce the purine content of another food products, which have similar processing steps.

Our objectives were to investigate changes in purine compounds of milkfish during processing of surimi based product and to find out the major processing step, which might reduce the purine content of products. A processing method will be also suggested to obtain lower purine content surimi based products. The gel strength of milkfish surimi based product was also evaluated during the processing.

Materials and Methods

Materials

Freshly caught, aquaculture grown milkfish (*Chanos chanos* Forskal) were purchased in local market. The average weight was 500 ± 78 g and average length was 38 ± 3 cm. The fish were packed in ice and brought to the laboratory within 1 hr. After removal of heads, viscera, tails, bone and skin, the dorsal muscle was collected and chopped to cubes of less than 3 mm.

Standards of purine related compounds, adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine 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 and surimi based product

Surimi. About 150 g mince were weighted into a 2.0 L beaker and washed with cold water (keep below 5ºC) in a water/mince ratio of 5/1. The mince was washed for 10 min under 100 rpm rotation (Stirrer-PC320, Corning Co. Ltd., NY). After washing, the mince was centrifuged at 1800 rpm 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, R.O.C.) and readjusted the moisture content to about 80%. The mince was then ground for 5 min in an ice bath, while 2.5% NaCl was added after 2 min grinding.

Surimi based product. Surimi was injected into 10 cm circumference, 15 cm length polyvinylidene chloride casing and heating at 90ºC for 30 min. Then, the surimi based product was quickly cooled with running water immediately and stored at 4ºC.

Washing condition

During the preparation of surimi, the mince was subjected to individual washing

conditions for 10, 20 and 30 min. The effect on purine content of different washing times for 10 min was also investigated. The mince was 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: mince (after chopping), washing (after washing), surimi (after grinding) and surimi based product (after cooking). All these samples were chopped, dried by freeze-dryer (Labconco, Germany) at -50ºC for 48 hrs and ground in a mixer (Itecator 400 W, Sweden). The obtained dry powder was stored at -20ºC until subjected to subsequent analyses.

Determination of purine contents

A modified method according to Benkmann (1995) and Lou (1998) was used to estimate the purine content in milkfish. 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 100ºC for 35 min. The resultant hydrolysates were transferred into a 250 mL flask and dried by a rotary vacuum evaporator at 50ºC. The evaporated step was repeated for three times, added ca. 5 mL distilled water into flask each time, until no acid existed in 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 μm membrane filter. The purine bases were separated by HPLC (Gold System Chromatography, Beckman, USA) using a reversed phase column (Lichrospher $5C_{18}$, 250×4.6 mm, i.d., Merck,

Germany) with 0.02 M KH_2PO_4 buffer included 2.5 mM Dimethyloctylamine, pH 3.2. The flow rate was 1 mL/min. 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).

Determination of ATP related compounds

The ATP related compounds included ATP, ADP, AMP, IMP, inosine and hypoxanthine were determined by the method described previously by Tsuchimoto and others (1985). Cold (5ºC) 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 rpm for 1 min in an ice bath. The homogenate was then centrifuged at 12000 rpm for 5 min at 5ºC. 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 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 HPLC method(Gold System Chromatography, Beckman, USA). A reversed phase column (Lichrospher $5C_{18}$, 250×4.6 mm, i.d., Merck, Germany) 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 a UV detector at 254 nm and the concentration 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, Japan).

Assessment of gel quality

Surimi based 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, Sun Scientific Co., LTD. 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) times deformation (cm). For each treatment, 6 determinations were performed and the mean values were calculated.

Results and Discussion

Change in purine related compounds of milkfish surimi based product during processing

The purine contents of milkfish were determined by 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 data indicated that adenine, guanine and hypoxanthine were found in milkfish (Fig.1). Hypoxanthine was the main purine base in milkfish and much more than the other two purine bases. The level of hypoxanthine decreased obviously after washing step from 45.7 μmole/g dry basis to 13.8 μmole/g dry basis. The ratio of hypoxanthine loss was about 69%. The total purine content, consequently, decreased dramatically about 61% from 54.2 to 21.1 μmole/ g dry basis. This might be due to the extraction effect of washing solution on free hypoxanthine related compounds, since they are very soluble and are released quickly from foods during washing (Young 1982, 1983; Brule and others 1989; Lou 1997; Lou and others 1997). In our previous study (Lou and others 1998) certified that the released ATP related compounds in grass shrimp during cooking were indeed transferred to the cooking juice. The changes in adenine and guanine after washing were very small. This phenomenon might be because of the low magnitudes of this two purines and the low solubility of guanine (Clifford and others 1976; Montag and others 1989). No obviously change in adenine, guanine and hypoxanthine was found during surimi and surimi based product processing steps. However, the amounts of total purine decreased slightly during surimi based product processing step, presumably from slightly extraction effect during cooking process, since the surimi based product were filled in a sealed polyvinylidene chloride case.

An analysis of ATP related compounds was carried out to elucidate the distribution of purine related compounds in milkfish. The results indicted that IMP was the major component in the milkfish (Fig. 2). The level of IMP and inosine were 21.0 and 3.1 μmole/g dry basis, respectively, while the content of free hypoxanthine was only 0.6 μmole/g dry basis. However, the contents of ATP, ADP and AMP were lower than 0.7

μmole/g dry basis. These data suggested that IMP and inosine might be the major accumulations resulting from nucleotide degradation in milkfish. The accumulation of IMP and inosine in milkfish during postmortem rigor-mortis, seasonal variation and various muscle parts have been reported (Shiau and others 1996; Chiou and others 1995; Lou and Chen 1998). The IMP level decreased dramatically from 21.0 to 5.3 μmole/g dry basis during washing step, then decreased gradually with a small magnitude during surimi and surimi based product processing steps. A decrease of inosine from 3.1 to 0.9 μmole/g dry basis during washing process was also observed, after which no further change ($p>0.05$) occurred during surimi and surimi based product processing steps. During these three processing steps, the change of ATP, ADP and AMP levels were only slightly, since the mince of milkfish contained less ATP, ADP and AMP initially. However, the content of ADP increased in a small amount during washing process. The exact reason for this phenomenon is still unclear.

Collectively, these data suggested that the washing step will reduce purine content of milkfish about 60%, especially the level of IMP. The reducing effects of washing on purine content in milkfish were primarily on IMP, since it was good soluble and the milkfish contained low level ATP , ADP and AMP.

Effect of washing duration on purine related compounds and gel quality of milkfish surimi based product

As mention previously, washing step might play an important role to release the purine substance to the washing solution during processing of surimi based product. The changes in purine content of milkfish as a result of washing duration should be evaluated (Fig. 3). The contents of hypoxanthine decreased from 45.7 μmole/g dry

basis to 14.5 μmole/g dry basis after 10 min washing, after which it decreased slightly up to 30 min. Similar changes were observed in the levels of total purine content for washing during 10 to 30 min. The total purine content decreased about 60% from 54.2 μmole/g dry basis (i.e. 184.8 mg/ 100 g) to 21.6 μmole/g dry basis (i.e. 55.3 mg /100 g) after 10 min washing. However, the content of adenine and guanine were constant during washing.

The reducing effect during washing on ATP related compounds in milkfish was also investigated (Fig. 4). The content of IMP decreased obviously for the first 10 min, then decreased from 5.7 to 2.7 μmole/g dry basis after 30 min. However, no change of ATP, ADP, AMP, inosine and free hypoxanthine were observed during washing.

These data indicated that the purine content in milkfish could be reduced above 60% in 10 min washing during surimi based product processing. The high purine content milkfish (184.8 mg/100 g) could be, therefore, produced to middle purine content milkfish surimi based product (55.3 mg /100 g). No change was observed after washing more than 10 min up to 30 min. The major released purine substance was IMP, which might cause some loss of taste intensity.

The gel formation quality during different washing time was also compared (Fig. 5). The gel strength of milkfish surimi based product increased with long time washing. The gel strength of product was 523 g×cm for washing 10 min, while it increased to 815 g×cm for 30 min washing. The deformation maintained steady up to 20 min washing, then increased at 30 min washing. The highest breaking force was observed at 20 min washing.

Collectively, washing step of milkfish surimi based product processing could reduce the purine content of products. The primary effective time of washing step was at the first 10 min. No obviously change was observed after washing more than 10

min. The major released compound was IMP. However, the gel strength of the milkfish surimi based product increased with long time washing up to 30 min.

Effect of washing times on purine related compounds and gel quality of milkfish surimi based product

In certain case, the washing step might be repeated to 2 or 3 times washing to obtain better quality of gel formation capability. Therefore, the reducing effects of various washing times for 10 min washing duration on purine content of milkfish surimi based product were also investigated. The results indicated that the level of hypoxanthine decreased from 45.7 μmole/g dry basis to 14.5, 10.3 and 5.4 μmole/g dry basis with increasing washing times for 1, 2 and 3 times, respectively (Fig. 6). The percentage of reducing effect on hypoxanthine were 68% for 1 times, 77% for 2 times and 88% for 3 times washing. The total purine content also decreased, consequently, with increasing washing times. The percentage of reducing effect was 60%, 66% and 76% with 1, 2 and 3 times washing, respectively. The change in ATP related compounds of milkfish surimi based product with various washing times were also evaluated (Fig. 7). The changes in levels of IMP with different washing times were similar to the changes of the hypoxanthine and the total purine content. The other purine related compounds were constant for the various washing times. It was concluded that the more washing times up to 3 times, the more purine content was released from milkfish surimi based product. Thus, the less purine content in milkfish surimi based product was found.

The changes in breaking force, deformation and gel strength of milkfish surimi based product with various washing times were also investigated. The gel strength

increased for washing twice, but it decreased gradually for washing three times. However, a steady increase of breaking force and deformation were observed with increasing washing times (Fig. 8).

Collectively, the more washing times during processing of milkfish surimi based product, the lower purine content in surimi based product products were found. A better gel strength was obtained if washing step was performed only twice.

Conclusion

The purine content of milkfish surimi based product was much lower than the milkfish mince cause of the reducing effect of washing step during the processing of surimi based product. The high purine content milkfish raw material could be consequently produced to surimi based products classified as middle purine content food. The major released compound was IMP. The lowest purine content in milkfish surimi based product could be produced by 30 min washing for 3 times, but it was time consumption and loss gel strength. A procedure of washing for 10 min, repeating twice might be a good condition to reduce the purine content in milkfish surimi based product. In the meanwhile, the gel strength was still good acceptable.

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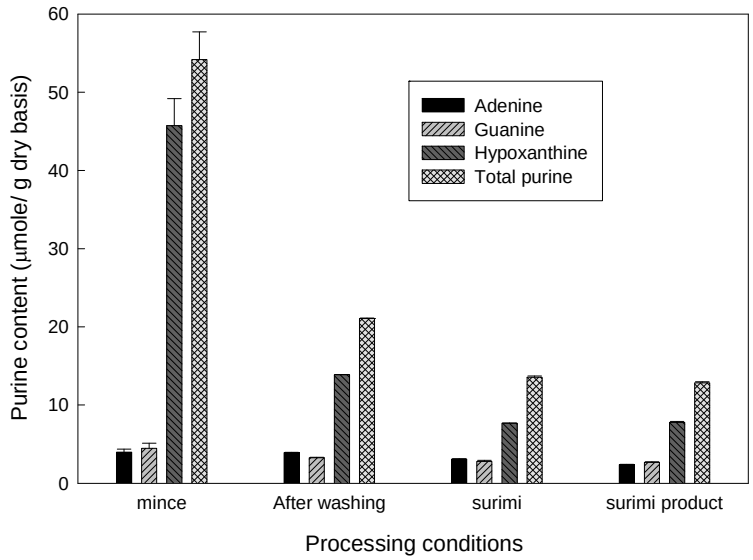

Fig. 1 Changes in purine content of milkfish during surimi based product processing

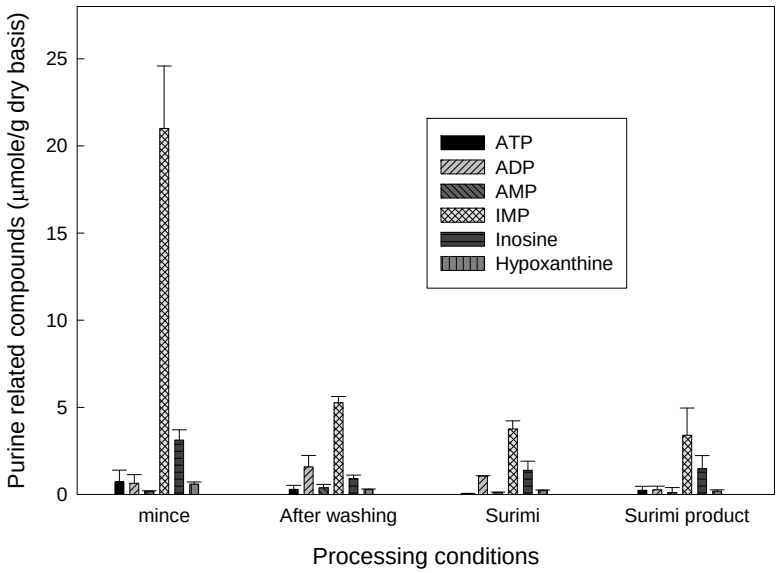

Fig. 2. Changes in purine related compounds of milkfish during surimi based product processing

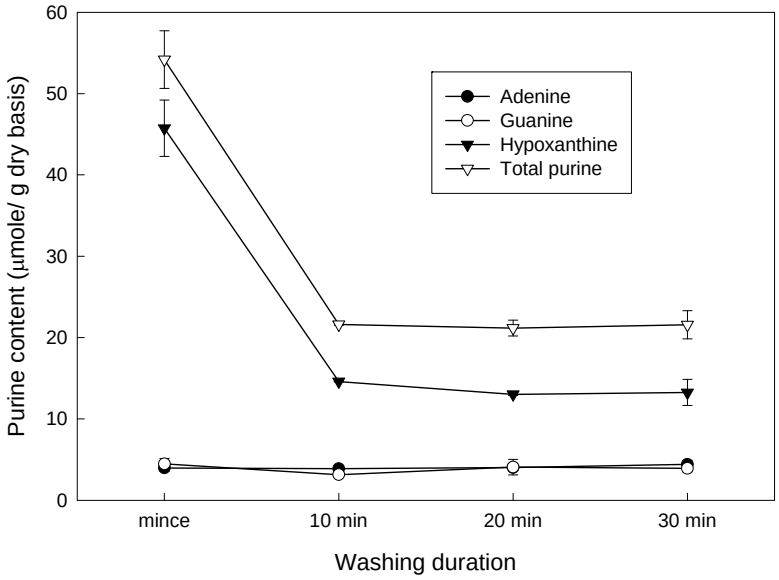

Fig. 3. Change in purine content of milkfish surimi based product after different washing duration

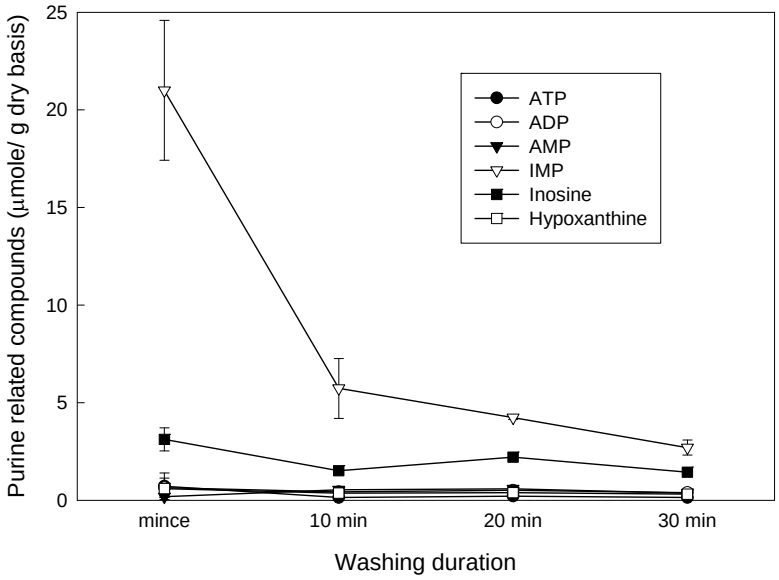

Fig. 4. Change in purine related compounds of milkfish surimi based product after different washing duration

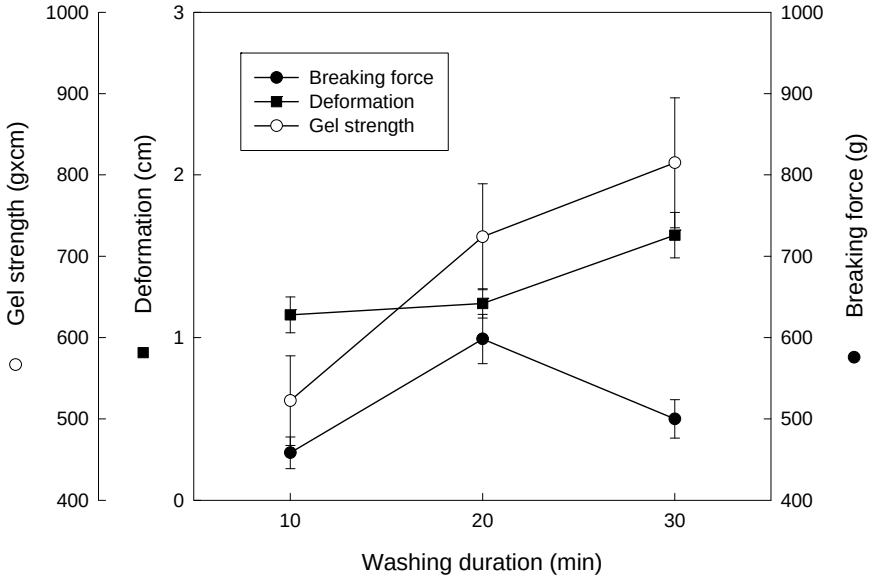

Fig. 5. Changes in breaking force, deformation and gel strength of milkfish surimi based product with different washing duration during processing

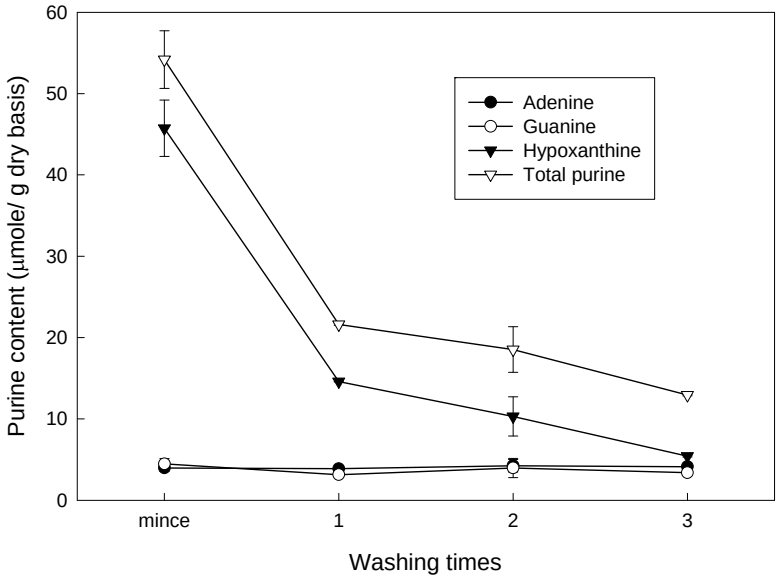

Fig. 6. Change in purine content of milkfish surimi based product with different washing times during processing

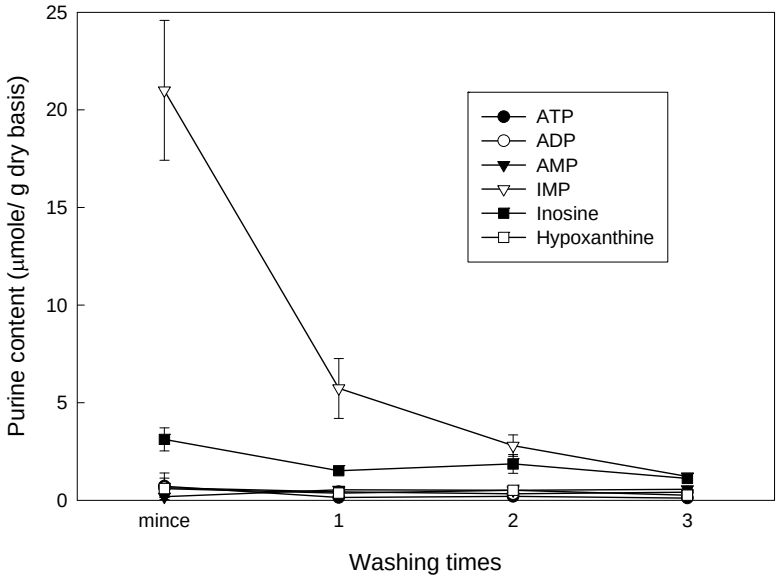

Fig. 7. Change in purine related compounds of milkfish surimi based product with different washing times during processing

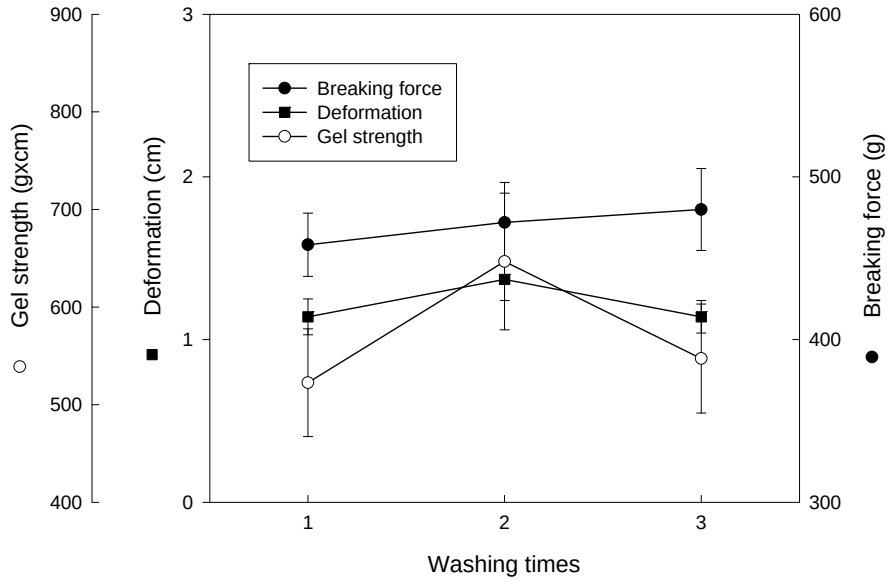

Fig. 8. Changes in breaking force, deformation and gel strength of milkfish surimi based product with different washing times during processing