New Procedures to Improve Productivity and Quality in the Manufacture of Boiled-dried Adductor Muscles of Scallop (shiraboshi)

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Shiraboshi is a boiled-dried adductor muscle of scallop, which is predominantly produced in North-Hokkaido, Japan. The manufacture of shiraboshi has certain limitations such as less productivity in opening and threshing of the shell for its processing and the appearance quality by excessive browning of the adductor muscle. In this study, a new improved procedure for the manufacture of shiraboshi was developed based on the consideration that the generation of glucose-6-phosphate was responsible for the browning. A new preheating treatment of the rare muscle introduced immediately after the shell opening-threshing process led to the inactivation, and the hot water extract obtained was a glycine and taurine enriched broth, a valuable by-product. Moreover, the shell opening-threshing process was improved by using saturated steam that allowed localized and quick heating of the shell surface. The new procedure increased the yield of the scallop adductor muscle and reduced the loss due to solving out during boiling/steaming process. Consequently, the new procedure introduced involves two modified processes: firstly, for improving the productivity of the shiraboshi preparation and secondly, for obtaining a novel value-added by-product.

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Key words: New procedures, productivity and quality, boiled-dried adductor muscles of scallop, by-products shiraboshi

The boiled-dried adductor muscle of scallop called “shiraboshi” is predominantly manufactured at North-Hokkaido area in Japan. Shiraboshi is processed from live scallop caught from the coast of Okhotsk Sea. To obtain the adductor muscle from the scallop, the scallop is left in the air without seawater overnight, the molluscan body in the shell is threshed from the scallop by boiling and/or steaming (100°C for 5 ~7 min), and then the muscle is separated manually from the molluscan body. The adductor muscle of the scallop is boiled in salt water for seasoning (9~14% NaCl for 11~16 min), and then is heated for 50 ~60 min at 80 ~90°C by a hot air heater. The drying process is performed daily using a hot air dryer (60°C for 60 min per day) and/or by sun drying, until the moisture content of the material decreases to 40% and re-iteration of procedure until it falls to 16%, thus requiring ~1 month for drying of the shiraboshi product.

Traditionally, the method employed by the scallop-processing industry involves boiling and/or steaming the whole scallop several minutes for opening the shell and threshing the molluscous body from the scallop. In our previous study, we reported an improved protocol for the opening and threshing technique in which the scallop was exposed to superheated-steam under the pressure, thus the heating the shell surface up to ca. 60°C for easier access to opening the shell and threshing the molluscous body while leaving the adductor muscle raw. However, certain modifications such as improving the efficiency of opening-threshing process, which requires leaving the scallop overnight for weakening, still need to be addressed. Additionally, exposing / subjecting the adductor muscles to high temperatures for extended time during the boiling and/or steaming reduces the yield owing to loss of the solid contents from the muscle by solving out.

The commodity/commercial value of shiraboshi is

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largely influenced by its appearance such as color and firmness. Thus, while moderately browning of the shiraboshi product is graded high, the excess browning of the product lowers the commodity value. The browning during cooking is thought to be the most likely cause of the Maillard reaction with D-glucose-6-phosphate (G6P) and D-fructose 6-phosphate (F6P). G6P and F6P are intermediates of the glycolytic pathway triggered during the postmortem changes in glycogen of the adductor muscle. Thus, the color figuration of the adductor muscle during the manufacturing process is affected by endogenous sugar phosphate, and therefore it is necessary to use flesh materials and to be heated to avoid the excess production of G6P and F6P. Hence, enhancing the productivity and prevention of solving out of the solid content are important for the scallop-processing industry, the understanding of which remains limited by the availability of only a few reports on the processing of the scallop adductor muscle.

The development of individual process involved manufacturing shiraboshi, including scallop heating with superheated steam, seasoning by salt soaking at low temperature, and introduction of programmed controlling of the temperature and humidity for the drying process to improve the product quality having better appearance, and to shorten the processing time are addressed in the current study. Particularly, the bottleneck in manufacturing shiraboshi, like, low productivity due to leaching out of adductor constituents and generation of G6P during the processing, have been described and the new method is developed to provide solutions.

**Materials and methods**

1. **Materials**

   Frozen adductor muscle of scallop (Patinopecten yessoensis) was purchased from ice making and freezing factory of Tokoro Fisheries Cooperative Association.

   Live scallop, Japanese scallop (P. yessoensis), occurring in Yubetsu sea area in Sea of Okhotsk was purchased from Yubetsu Fisheries Cooperative Association.

2. **Determination of glucose 6-phosphate**

   G6P was determined using the modified F-kit for the estimation of D-glucose, D-glucose-HK (Megazyme Itn. Ltd., Ireland). Glucose 6-phosphate dehydrogenase (Oriental Yeast Co. Ltd., Japan) solution was used instead of mixture solution of hexokinase and glucose 6-phosphate dehydrogenase in F-kit. First, 4 g of adductor muscle was soaked into 8 mL of straub solution (0.3 M KCl, 0.1 M KHPO4, 0.05 M KHPO2), transferred into a sterilized filter bag and then homogenized with a stomacher for 90 s. The homogenate was diluted with straub solution to an appropriate concentration, heated at 100°C for 10 min to inactivate endogenous enzymes present in the muscle, and then the G6P content of the sample was determined using the modified F-kit. Standard solution of G6P monosodium salt (Calzyme Lab. Inc., USA) was used for obtaining the standard curve.

3. **Preparation/Separation of the adductor muscle from the scallop**

   The traditional procedure was followed, in which, the scallop was boiled in water for 5 min at 90°C. The adductor muscle was manually extracted from the molluscs body obtained with boiling of scallop.

   The new procedure involved the use of the scallop sheller test model for laboratory (Nikko Co. Ltd.). This device allows spreading the saturated steam effectively and covers the entire shell surface, where it is adhere/attached to adductor muscle. To facilitate opening the shell, the scallop was heated for 10 s with saturated steam, after which the visceral organs were manually removed from the molluscs body. Further, the shell bound to the adductor muscle was heated for 10 s, thereby allowing the separation of the muscle from the shell.

4. **Determination of yield rate and denaturation by heating scallop in the new procedure**

   The solid content that solve out from the scallop materials during boiling and steaming treatment was calculated from the weight of the extract in each treatment and the Brix value from the sugar refractometer. The yield was calculated as a percentage of the substance to the total scallop material. The extent of denaturation of the adductor muscle during the heat treatment was estimated based on the whiteness of the muscle surface. L*, a* and b* values were obtained by measurement of the surface using a chroma meter with colorimeter (Konica Minolta CR-400). The whiteness was calculated by the following formula:

   \[ W = 100 - \left( \left( 100 - L^* \right)^2 + a^* + b^* \right)^{1/2} \]

5. **Production of shiraboshiby using the new procedure**

   The adductor muscles were isolated from 100 kg
of live scallops by using the traditional or new procedure and immediately boiled with tap water at 70°C for 25 min, following which the boiled adductor muscles were heated 3 times with superheated steam at 140°C for 5 min. The drying procedure with set program coordination of temperature and humidity was carried out for 10 days until the moisture content of the adductor muscles become ~16%.

6. Determination of free amino acids in extract as by-products

The scallop adductor muscles were boiled in water of the same weight at 70°C for 25 min. One gram of the broth was mixed with the same volume of 5% trichloroacetic acid (TCA) to precipitate the proteins contained in the broth. After centrifugation (10,000 rpm, 10 min) the supernatant was mixed with 2 ml of n-hexane and the mixture was allowed to stand for a few minutes after which, it was filtered through a cellulose acetate membrane (0.2 μm). The amino acid concentration in the filtrate was determined using an amino acid analysis system, LaChrom Elite (Hitachi High-Technologies Corp., Japan) with a post-column NIN method. The temperature for the ninhydrin reaction was set to 130°C (reagent flow rate: 0.25 ml/min). The reacted amino acids were detected by recording the absorbance at 570 nm.

Results and Discussion

1. Establishment of glucose-6-phosphate assay

Since an earlier report showed that the G6P and F6P were mainly responsible for involved in the browning of the cooked adductor muscle of the scallop, the author attempted to establish a new method specifically for assaying G6P by using a commercially available F-kit for d-glucose assay. The kit contains hexokinase, adenosine-5’-triphosphate (ATP), nicotinamide-adenine dinucleotide phosphate (NADP+) and glucose-6-phosphate dehydrogenase (G6P-DH). While hexokinase and ATP allow synthesis of G6P and adenosine-5’-diphosphate from glucose and ATP, G6P-DH catalyzes the oxidation of the G6P concomitantly reducing NADP+ to give nicotinamide - adenine dinucleotide phosphate (NADPH). Thus, it was expected that this assay could be applied for the detection of G6P instead of glucose by bypassing the reaction catalyzed by hexokinase. In the modified assay, G6P-DH instead of hexokinase and G6P-DH. To confirm the specificity of the modified method, glucose 1-phosphate, G6P, fructose-6-phosphate, fructose-1,6-phosphate and glucose were used as substrates. Remarkably, the assay (Fig. 1) exclusively detected G6P. Furthermore, it was confirmed that detection by the modified method demonstrated that an absorbance at 340 nm due to NADPH was proportional to concentration of G6P (Fig. 2). Consequently, it was concluded that the modified method is suitable for the quantitative assay / estimation of G6P.

2. Effect of thawing time on G6P production in frozen adductor muscles

Prior speculation that glycogen metabolites were responsible for the browning required the examination of the course of the G6P production in the adductor muscle as a function of time. For this purpose, frozen muscle was defrosted in the refrigerator (4°C), and the G6P present in the samples was extracted and assayed by the newly established methods for its quantification. The content of G6P in the adductor muscle increased...
between 3~12 h post thawing and then decreased (Fig. 3). The temperature at the center of the adductor muscle exceeded 0°C at 3 h post thawing and rose to 4°C before 6 h post thawing (data not shown). These findings suggest that the endogenous production of G6P commenced above 0°C in the adductor muscle. A previous report indicated that fast production of G6P occurred in the adductor muscle that was rapidly thawed under running water (15°C) for 45 min.

The effect of the preheating treatment of the adductor muscle for the prevention of G6P production was examined by preheating the muscle by boiling until their central temperature reaches to 40, 50, 60 and 70°C and then storing at 4°C for 0~48 h. The muscle G6P content collected at several time intervals is displayed in Fig. 4. It was observed that G6P content in the muscle without preheating treatment increased and decreased depending on the storage time (Fig. 4; Column A), whereas this change in the levels of G6P were not observed in the muscles that had been preheated above 50°C. The plausible reason could be that preheating could have inactivated the endogenous enzyme(s) of the involved in the glycolytic pathway. It was observed that inactivation of endogenous enzyme(s) by preheating within 6 h after death was critical for the retardation/prevention of G6P production in the adductor muscle during refrigeration.

3. New procedure for shiraboshi production and its effects on the productivity

Traditionally, the procedure for opening and threshing the shell reduced the yield of the adductor muscle owing to leaching of solid content in the broth during the extended boiling step. The new protocol employed saturated steam that allows rapid localized heating, thus aiding opening and threshing the shell. Thus, relatively lower yield rate was obtained according to the traditional procedure, because of the leaching of substantial solid content in the broth (Table 1) caused by extended exposure to high temperatures. Additionally, because the adhered substances on scallop, such as marine organisms and their metabolites are also eluted into

Fig. 3 Change in G6P content in the adductor muscles of the scallop during thawing

Frozen adductor muscles of the scallop (n = 5) were thawed and stored in the refrigerator (4°C) for 48 h. The G6P contents in the extracted muscle samples were estimated with the newly established assay.

Fig. 4 Effect of the preheating treatment of the adductor muscles of scallop on the generation of G6P in the muscle during cold storage

The adductor muscles of the scallop were preheated by boiling. Upon reaching a central temperature of 40~70°C, each sample was stored for 0~48 h in the refrigerator (4°C). Column A indicated the thawing of the frozen adductor muscle without preheating. The muscles were collected at several time intervals. The G6P content in the samples after the storage were determined by the new G6P assay. The G6P content in the adductor muscles without preheating treatment, which were stored at 4°C for 6 h, was set to 100%.
the broth, the broth is rendered unsuitable for secondary usage as by-products. On the other hand, saturated steam ensures the rapid heating of the shell surface to enable opening and threshing, thus reducing the amount of solid content in the broth and consequently increasing the yield rate of the scallop adductor muscle as compared to the traditional procedure.

The degree of whiteness/paleness of the adductor muscle surface represents the extent of denaturation caused by heating. No difference was observed between the degree of whiteness between raw muscle and that after saturated-steam treatment (data not shown). In the traditional opening-threshing procedure, whitening of the adductor muscle due to heat denaturation was observed along with less productivity ease in adductor muscle isolation process.

The adductor muscles obtained from the traditional and new procedures were further processed to furnish shiraboshi products. The yield rate of the final product was 3.00% on following the new procedure as against 2.25% (data not shown) by traditional procedures. The alteration in the isolation procedure of the adductor muscle clearly produced a difference in the productivity in the shiraboshi preparation. The new procedure, which uses saturated steam for a short time interval, improved productivity over 30% in comparison with the traditional procedure.

### 4. Utility value of the hot water extract obtained as the by-product in the new process

The adductor muscles of scallop obtained by the new procedure of opening and threshing the shells were boiled at 70°C for 25 min, to inactivate the endogenous enzyme (s) involved in G6P production. The amino acid content in the broth was analyzed (Table 2), and it was observed that glycine and taurine accounted for approximately 80% of the total free amino acids present in the extract. Glycine and taurine are constituents of bile acid and related to cholesterol metabolism in humans and animals. Whilst the former is sweet in taste, the latter shows valuable bioactivity**. Thus, the hot water extract, obtained on boiling the muscle to inactivate endogenous enzyme (s), would be a useful by-product.

The traditional procedure also gives off/supplies hot water extract of the adductor muscle at the seasoning step. However, since the extract contains high salt concentration and browning, it finds limited industrial use. Thus, although the extract contains taste components in abundance, it is either wasted or traded to condiment companies at a low price. Meanwhile, the hot water extract obtained from the new improved shiraboshi processing procedure is a novel by-product. The lyophilized powder of the extract showed low salinity (0.1%: data not shown) and no browning. The powder possesses acceptable flavor of the boiled adductor muscle of scallop. Additionally the extract can be applied to spray drying method without desalting.
A newly proposed method for manufacturing shiraboshi based on the results obtained from this study. Fresh scallops were steamed for 10s to enable opening and threshing the shells. The adductor muscles were manually isolated and boiled with tap water at 75~100°C for 15~25min to inactivate the endogenous enzyme (s). The materials were soaked into 6% NaCl water at 4°C for 20h for seasoning, heated 3 times at 140°C for 5min each, and then dried with programmed coordination of the temperature and humidity.

**Fig. 5** A new process for shiraboshi production

<table>
<thead>
<tr>
<th>Process</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh scallop</td>
<td>(material)</td>
</tr>
<tr>
<td>Isolated adductor muscle</td>
<td>Steaming for 10sec, each side of shell</td>
</tr>
<tr>
<td>Boiling with water</td>
<td>75<del>100°C, 15</del>25min</td>
</tr>
<tr>
<td>Soaking in low temp. salt water</td>
<td>6% NaCl (4°C, 20h)</td>
</tr>
<tr>
<td>Heating with super heated steam</td>
<td>140°C, 5min x 3 times</td>
</tr>
<tr>
<td>Drying with program coordination of temp. and humidity</td>
<td>[program parameter] Temp 40°C, humidity 85% ↓ linear gradient for 10 days Temp 20°C, humidity 45% moisture content (below 16%)</td>
</tr>
<tr>
<td>Shiraboshi</td>
<td>(product)</td>
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</tbody>
</table>

and addition of diluents.

In the new procedure, additional processing is required, in which the adductor muscle is boiled to prevent the generation of endogenous G6P immediately after the opening-threshing step (Fig. 5). G6P is known to be one of the causative agents for browning in cooked adductor muscle[10]. Thus, it is necessary to understand the role of G6P in the browning of shiraboshi products. The new procedure in conjunction with the use of saturated steam processing for opening-threshing the shell improved the productivity of the shiraboshi preparation. Further, the hot water extract obtained as a by-product in the inactivation step was identified as a valuable material.

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**References**


ホタテガイ白干し加工の生産性と
品質向上のための新しい製法

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ホタテガイの閉殻筋（貝柱）を、煮熟－乾燥させた白干しは北海道北部が主産地となっている。白干しの製造工程には様々な問題点があるが、主には貝の開殻、脱殻操作における生産低下や過度な褐変による製品の外観品質低下である。本研究では白干し製造の生産性を向上させる新しい操作と工程を開発した。第一に内在G6Pの産出が褐変に影響するという仮説をもとに、開殻脱殻工程後の生け近い貝柱を素早く加熱し失活させる新しい工程を導入した。この結果、失活工程で得られる熱水抽出液はグルシン、タウリンに富み高付加価値の副産物であった。第二に貝の外殻から限定的に短時間乾燥蒸気をあてた操作に開殻脱殻工程を改良した。この新しい操作によって従来の煮熟蒸煮中の固形分溶出に起因する貝柱歩留まりの低下やエキスの損失を改善した。2つの工程改良を含む新操作は白干しの生産性を改善すると共に新しい副産物を得るという結果を得た。

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