Effects of Cold Storage, Freezing and Thawing on Browning of Cooked Scallop Adductor Muscle

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The effects of cold storage, freezing and thawing on the browning of scallop adductor muscle, which occurs during the cooking process, were studied in relation to the content of glycogen, glucose and sugar phosphates (glucose-6-phosphate, fructose-6-phosphate, and fructose-1,6-diphosphate) in the muscle. The changes in content of glycogen, glucose and sugar phosphates were not observed in frozen muscle. Frozen muscle did not show browning after cooking at 110°C for 90 min as unfrozen muscle immediately after death. During cold storage (5°C) of unfrozen muscle, glycogen slowly decreased, glucose and sugar phosphates gradually increased, and browning of the muscle gradually increased. When frozen muscle was thawed either slowly (in still air at 0°C) or rapidly (in running water at 15°C), decrease in glycogen and substantial increases in glucose and sugar phosphates occurred, and strong browning of the muscle during the cooking process was observed. During cold storage (5°C) of thawed muscle, glycogen and sugar phosphates gradually decreased, whereas glucose considerably increased, and browning of the muscle gradually weakened. The content of glucose-6-phosphate and the degree of browning were closely related (r = 0.85).

Key words: scallop adductor muscle, cold storage, thawing, browning, glycogen, glucose-6-phosphate, Maillard reaction

Scallop adductor muscle is widely utilized not only as raw material for various dishes, but also for dried, smoked, and canned products. Browning of muscle is frequently observed in these products, which is thought to occur mainly during the cooking process. Because extended browning of cooked muscle impairs the taste and flavor as well as the appearance of the products, it is important to clarify the cause of browning of cooked muscle and to establish methods of prevention.

Tarr1) found that glucose-6-phosphate (G6P) induced marked browning in fish flesh under experimental conditions. Yamanaka et al.2-4) clarified that the main compounds responsible for orange discoloration of canned skipjack meat were G6P and fructose-6-phosphate (F6P). Nagayama and Kimura5) studied the boil-shucking of scallop and reported the relation between G6P formation during boil-shucking of scallop and browning of canned scallop muscle. Hiltz and Dyer6) reported that the hexose monophosphates in cold stored Canadian sea scallop (Placopecten maghellanicus) adductor muscle were present in sufficient amounts for Maillard reactions upon heating.

In this report, the effects of cold storage, freezing and thawing on browning of scallop were studied in relation to the contents of glycogen, glucose and sugar phosphates in the adductor muscle.

Materials and Methods

Materials

Live scallop Patinopecten yessoensis was obtained from Tokyo Central Wholesale Market in September 1993 and the striated part of the adductor muscle immediately after exuviation was used. Each of 5 samples was divided into 3 portions, then frozen in a deep freezer (−70°C). It took three and a half hours for the central part of the scallop adductor muscle to reach −70°C. One portion was stored at −70°C for 1 month and used for frozen samples and the other two portions were used either thawed in still air at 0°C (slow thawing) or thawed in running water at 15°C (rapid thawing). Slow thawing took four and a half hours for the central part of the scallop adductor muscle to reach 0°C, while rapid thawing took 45 min. In order to examine the effects of cold storage on unfrozen muscle and thawed muscle, 5 samples were stored at 5°C, and another 5 samples were frozen quickly, thawed rapidly and then stored at 5°C.

Preparation of Extract

About 2.5 g of muscle was homogenized in 10 ml of ice-cold 6% perchloric acid and the suspension was centrifuged at 10,000 rpm for 10 min. The procedure was repeated and the supernatants were combined. After neutralization with KOH solution, the supernatant was made...
up to 25 ml with distilled water. These solutions were measured for glucose, glycogen and sugar phosphates.

**Determination of Glucose and Glycogen**

Glucose was determined using hexokinase (E.C.2.7.1.1, Boehringer Mannheim Co.) and G6P dehydrogenase (E.C.1.1.1.49, Boehringer Mannheim Co.) on the basis of the increment in optical density of NADPH at 340 nm according to the method of Keppler and Decker. Glycogen was hydrolyzed with amylglucosidase (E.C.3.2.1.3, Boehringer Mannheim Co.) and the glucose formed was determined in the same manner.

**Determination of Sugar Phosphates**

G6P, F6P and fructose-1,6-diphosphate (FDP) were determined by the method of Michal and Beutler. G6P was determined using G6P dehydrogenase (E.C.1.1.1.49, Boehringer Mannheim Co.) on the basis of the increment in optical density of NADPH at 340 nm. F6P was converted to G6P with phosphoglucose isomerase (E.C.5.3.1.9, Boehringer Mannheim Co.) and then determined as stated above. FDP was converted to F6P with fructose-1,6-diphosphatase (E.C.3.1.3.11, Sigma Chemical Co.) and F6P formed was determined in the same manner. The contents of glucose, glycogen and sugar phosphates were described using the average and standard deviation (SD) of 5 samples.

**Measurement of Degree of Browning**

Three grams of muscle and an equal weight of distilled water were sealed in a high-density polyethylene bag and cooked in an autoclave at 110°C for 90 min. This cooking condition was the same as that of canning. The cooking liquid thus obtained was deproteinized by adding perchloric acid, then its optical density at 450 nm was measured as the degree of browning of the cooked muscle.

**Results**

**Changes in Content of Glycogen, Glucose and Sugar Phosphates**

Figure 1 shows the content of glycogen, glucose and sugar phosphates in unfrozen, frozen and thawed scallop adductor muscle. Average content of glycogen, glucose, G6P, F6P and FDP immediately after death was 1.34 g/100 g (SD 0.16), 14.6 mg/100 g (SD 5.4), 64.5 mg/100 g (SD 5.7), 12.1 mg/100 g (SD 1.3) and 1.3 mg/100 g (SD 0.9) respectively. Frozen muscle did not show changes in the content of these sugars. Glucose and sugar phosphates increased and glycogen decreased in thawed muscle and the changes were almost the same in both rapid thawed and slow thawed muscle. G6P and F6P increased to the level of more than twice that of the initial amount, FDP in creased to 20 times that of the initial amount, and glucose increased 1.2 times, whereas glycogen decreased by approximately 0.1 g/100 g.

The changes in content of glycogen, glucose and sugar phosphates in unfrozen scallop muscle stored at 5°C are shown in Fig. 2. The initial content of glycogen, glucose, G6P, F6P and FDP in the cold stored muscle was 1.22 g/100 g (SD 0.11), 20.7 mg/100 g (SD 4.6), 64.0 mg/100 g (SD 7.0), 14.0 mg/100 g (SD 3.3), and 4.4 mg/100 g (SD 1.5), respectively. Glucose, G6P and F6P gradually increased and FDP gradually decreased during storage at 5°C. The amount of glucose reached the level of three times that of the initial amount and those of G6P and F6P reached twice that of the initial amount after 2 days storage. Glycogen showed a slow decrease during storage and reached 1.17 g/100 g (SD 0.10) after 3 days storage.

Figure 3 shows the changes in content of glycogen, glucose and sugar phosphates in thawed muscle during cold storage at 5°C. The content of glycogen, glucose, G6P, F6P and FDP in the samples of this group immediately after death was 1.36 g/100 g (SD 0.16), 13.6 mg/100 g (SD 2.5), 55.8 mg/100 g (SD 5.9), 10.5 mg/100 g (SD 2.7), and 4.1 mg/100 g (SD 0.6), respectively. Glycogen decreased by thawing and during subsequent cold storage and reached 0.94 g/100 g (SD 0.29) on the third day of storage, whereas glucose increased markedly during storage after thawing and reached 104.5 mg/100 g (SD 14.8) on the same day. G6P, F6P and FDP significantly increased by thawing, but they gradually decreased during storage at 5°C after thawing. These changes were especially large in G6P.
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Fig. 3. Changes in content of glycogen, glucose and sugar phosphates in thawed scallop adductor muscle during storage at 5°C. 0*: Immediately after thawing.

Fig. 4. Changes in content of glucose-6-phosphate and degree of browning* in unfrozen scallop adductor muscle during storage at 5°C. *expressed as optical density at 450 nm of muscle liquid obtained after cooking at 110°C for 90 min.

Fig. 5. Changes in content of glucose-6-phosphate and degree of browning* in thawed scallop adductor muscle during storage at 5°C. *same as Fig. 4. 0*: Immediately after thawing.

Fig. 6. Relation between content of glucose-6-phosphate and degree of browning*.

Relations between Degree of Browning and Content of Sugar Phosphates

The suitability of reflectance of the cooked muscle and optical density of cooking liquid as indices of the degree of browning was examined. The reflectance showed a large variation according to the parts of the muscle measured. The optical density of the cooking liquid showed not only good agreement to the degree of browning but also consistent results. Consequently, the optical density of the cooking liquid at 450 nm was used as an index of the degree of browning in this study. When yellowish browning was observed in the cooked muscle, the optical density of its cooking liquid was more than 0.35. Muscle with deep browning had an optical density of more than 0.45, and also had a slight burned odor.

Changes in the content of G6P and the degree of browning in unfrozen scallop adductor muscle during storage at 5°C are shown in Fig. 4. Muscle immediately after death did not show browning by cooking at 110°C for 90 min. During cold storage of unfrozen muscle, optical density gradually increased with increasing G6P content. Browning of the cooked muscle was observed after 2 days storage, and G6P content was nearly double at that point.

Figure 5 shows changes in the content of G6P and the degree of browning in thawed scallop adductor muscle during storage at 5°C. Though not shown in Fig. 5, frozen muscle did not cause browning by cooking. Strong browning was observed when thawed muscle was cooked, then browning gradually weakened with the decrease in G6P content during cold storage.

The relation between the content of G6P in the muscle and the degree of browning expressed as the optical density of the cooking liquid is summarized in Fig. 6. The content of G6P and the degree of browning were closely related, and their correlation coefficient was 0.85. Total amounts of G6P, F6P and FDP also showed the same result, with a correlation coefficient of 0.84.

Discussion

Freezing and frozen storage at -70°C up to 1 month did not produce any changes in the content of the sugars, nor the degree of browning of scallop adductor muscle. Frozen muscles can be utilized as unfrozen fresh muscles if care is taken to avoid thawing before the start of cooking. The results showed that the degree of browning of cooked muscle had a high correlation with the content of
sugar phosphates in the muscle. Similar to the results of Hiltz and Dyer\textsuperscript{6} on Canadian sea scallop, G6P accounted consistently for 78–85\% of the total amounts of G6P, F6P and FDP, and thus G6P seemed to be most responsible for the browning of the cooked muscle among these sugar phosphates. The rate of decrease in glycogen during cold storage differed markedly between unfrozen muscle and thawed muscle. Because the amount of change was markedly higher in thawed muscle than that in unfrozen muscle, thawing seemed to accelerate glycogen degradation considerably. The decrease in the content of glycogen in unfrozen muscle was nearly proportional to the increase in the content of glucose and sugar phosphates. However, the rate of decrease in glycogen in thawed muscle far exceeded the rate of increase in glucose. This discrepancy may be explained by the results\textsuperscript{10} we previously obtained that octopine and lactic acid accumulation in thawed muscle was considerably higher in thawed muscle than that in unfrozen muscle. Hiltz and Dyer\textsuperscript{6} found that the decrease in glycogen in thawed Canadian sea scallop muscle was greater than the formation of hexose monophosphates and octopine, and suggested the existence of another pathway of glycogen degradation or other intermediates accumulated in thawed muscle. We\textsuperscript{10} previously clarified that D-lactic acid is one of the end-products in glycolysis in scallop adductor muscle. Since Hiltz and Dyer did not determine D-lactic acid, it is probable that the compound they suggested is D-lactic acid.

Hiltz and Dyer\textsuperscript{6} also found that G6P content was highest in the thawed Canadian sea scallop muscle during 1–2 days of storage at 5°C, and browning occurred at that point. On the contrary, our results showed that the maximum accumulation of G6P and strongest browning occurred in the muscle immediately after thawing. Furthermore, Hiltz and Dyer\textsuperscript{6} reported that octopine formation was exceptionally large upon thawing, and subsequent accumulation was relatively slow, particularly in the rapidly thawed muscle, where it was much slower than that of the unfrozen control, and G6P accumulation appeared to be delayed until octopine production is at or near its maximum. We found in this study and in a previous study\textsuperscript{10} that in scallop adductor muscle, G6P accumulation was largest upon thawing when octopine formation was very low, and that G6P gradually decreased during storage after thawing but octopine formation was maximum on the first day of storage. These differences may be caused by differences in species of the samples used in the experiments.

Nagayama\textsuperscript{11} found no linear correlation between browning of heated fish flesh and ribose or glucose content. Nagasawa\textsuperscript{12} reported that browning of canned crab meat was caused by Maillard reaction with glucose and ribose as sugars. Our results showed that the increase in glucose (0.55 μmol/g) far exceeded the decrease in G6P (0.18 μmol/g) during cold storage of thawed muscle, but at the same time a decrease in the degree of browning occurred. Therefore, glucose is considered to contribute less to the browning of scallop adductor muscle unlike in the case of fish flesh and crab meat.

References