Effects of Cooking with Ginger Juice or Kiwifruit Juice on Collagen and Lipid Contents of “Kakuni Pork”

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This study was performed to clarify structural changes in collagen, changes in ASC, PSC and ISC contents, caused by moist heating for a prolonged period, and accompanying changes in lipid content, using kakuni pork as a sample. In addition, the influence of plant protease on these contents was also examined.

Regarding collagen content, no ISC was present, ASC content was significantly increased, and PSC content was significantly decreased after steaming. In the subsequent simmering process, the decrease in PSC content was not significant. When ginger juice or kiwifruit juice was added, no difference was noted in collagen content after steaming compared to that of the control. In the subsequent simmering process, FSC content decreased; a significant reduction was observed compared to the control when kiwifruit juice was added. This FSC content reduction in the simmering process may have resulted from protease action on the resultant collagen without globular domains during pre-treatment and heat-denatured regions of collagen in the early steaming phase. Fat and cholesterol decreased after cooking in all conditions. The contents were significantly reduced when kiwifruit juice was added compared to those of the control. These findings suggest that although heating weakens collagen fibers in pork chunks during cooking, a large amount of lipid is retained. The addition of kiwifruit juice further weakens the collagen fibers, increasing the dissolution of fat and cholesterol.

Keyword: Pork, collagen, fat, cholesterol

Introduction

Meat consists of muscular, connective and adipose tissues. Collagen, a major constituent of intramuscular connective tissue, has a triple helical domain and globular domains, which are referred to as telopeptides (Cross et al., 1973, Miller, 1984). Collagen molecules assemble in fibrils and are immobilized by cross-links that are preferentially formed between the triple helix and globular domains. Fat contained in meat is accumulated as adipose tissue between collagen fibers (Yosioka and Andou, 1999). Some cross-links are labile to heat and acid treatments, and thus some collagen can be solubilized by heat and acid treatments (Paul et al., 1973, Penfield and Meyer, 1975). Heating treatment also converts the triple helix structure to a globular structure. The heat-induced globular form is referred to as gelatin. Nagatsuka et al. (2005, 2006) have shown the rheological properties of nikogori, which contains gelatin, prepared by boiling fish and meat with seasonings.

Previously, we reported that fat and cholesterol were decreased during the actual cooking process (Sugiyama and Ishinaga, 1999, Sugiyama et al., 2000), and described specific actions of ginger and kiwifruit proteases on globular domains, telopeptides and gelatin (Sugiyama et al., 2005 a). Ohyama et al. (1993) have also reported the site of action of kiwifruit protease. We also clarified that the addition of ginger juice or kiwifruit juice to meat slightly increased the reduction of fat and cholesterol contents while grilling (Sugiyama et al., 2005 b).

However, no reports exist on the structural changes in collagen during actual cooking and accompanying lipid dissolution. The objective of this study was to clarify the structural changes in collagen induced by prolonged moist heating and the accompanying reductions in fat and cholesterol contents, using kakuni pork (ton-pou-roum) as a sample, as well as the influence of the addition of plant protease. Kakuni pork is prepared by steaming or boiling pork chunks for a prolonged period, followed by cutting the meat into cubes and braising with seasonings. The result is a smooth, rich taste from the gelatinized collagen and fat contained in the collagen fibers (Shiota and Matsuoka, 1986, Yamasaki et al., 2003).

Materials and Methods

1. Preparation of kakuni pork

Pork blocks (belly; 5 cm in width ×5 cm in thickness ×20 cm in length; about 400 g) were purchased in Hiroshima City. Experiments were repeated several times using pork purchased on different days, but samples for a
single experiment were purchased simultaneously. As shown in Fig.1, whole sections of pork belly were purchased and areas with similar characteristics were selected. Three pork chunks were cooked under each condition. Ginger juice or kiwifruit juice was added to several samples before steaming, and the pork was wrapped to spread the juice over the entire surface. The amount added was set at 20% (w/w) of the pork weight, and the pork was stored at 0℃ overnight. Ginger and kiwifruit juices were prepared as previously reported (Sugiyama et al., 2005b). The pH at the center of the ginger or kiwifruit juice–immersed pork was pH 5.2 or pH 4.3, respectively, while the pH of raw pork was 5.2. Steaming with constantly boiling water was continued for 3 hours using a steamer. Steaming for 0, 10, 20 and 30 min resulted in pork chunk temperatures of about 55, 65, 70 and 90℃, respectively. Thereafter, a temperature of about 93℃ was maintained.

The weight of the pork was reduced to about 280 g by steaming, and subsequently the pork was cut into 2-cm pieces (about 25 g/piece). Pork was simmered with 30% (w/w) water relative to the pork weight after steaming and seasoning (15% soy sauce, 15% sake, 15% mirin, and 4% sugar) over medium heat until no cooking liquid remained (30 min). Several pieces, with the exception of either end of the original chunk, were used for the subsequent analysis.

In a preliminary sensory test involving members of the Cookery Science Laboratory, the ginger or kiwifruit juice–immersed pork had a slight ginger or kiwifruit flavor and the texture was easily distinguished from the control. However, no differences in overall palatability were observed for all conditions.

2. Measurement of collagen and collagen–related peptides

Six pieces of *kakuni* pork prepared under each condition were homogenized (2 pieces per homogenate), and collagen was extracted using the method of Sato et al. (1988). Collagen was fractionated into acid–soluble collagen (ASC), pepsin–solubilized collagen (PSC), and insoluble collagen (ISC). The amount of collagen and collagen–related peptides liberated from pork was assessed by the hydroxyproline (HYP) content after acid–hydrolysis with 6 N HCl at 130℃ for 3.5 hours. HYP was determined by the method of Woessner (1961).

3. Measurement of fat and cholesterol contents

Six pieces of *kakuni* pork prepared under each condition were individually homogenized, and lipids were extracted by the method of Bligh & Dyer (1959). Fat and cholesterol contents of each sample were measured using gas chromatography, as previously reported (Sugiyama et al., 2005b).

4. Statistical analysis

Differences among samples in collagen, fat and cholesterol contents or in weights before and after cooking were evaluated by the Student’s *t*-test or Tukey’s HSD test using PASW Statistic 17.0 (SPSS Japan Inc.).

Results and Discussion

1. Effect of cooking process on collagen and collagen–related peptides from pork

Figure 2 shows cooking–induced changes in collagen content. The ASC, PSC, and ISC contents were 0.12, 1.52, and 0.31 g in raw pork, respectively. After steaming, the total collagen content was significantly decreased. ASC and PSC contents were significantly increased and decreased, respectively. The increase in the ASC content may have been due to changes of ISC to PSC and PSC to ASC in the steaming process. ISC was not observed. In the subsequent simmering process, the decrease in PSC content was not significant. We previously reported reduced ASC and PSC contents and increased ISC content in grilled chicken thigh (Sugiyama et al., 2010). ISC, which remained in an insoluble form after pepsin digestion, is likely due to tightly constricted collagen cross–linkages between helical domains. It is necessary to investigate whether these differences were due to the
region or the cooking method.

2) With ginger juice or kiwifruit juice

The addition of ginger juice or kiwifruit juice before steaming slightly reduced the PSC content compared to that without addition. The reduction was marked when kiwifruit juice was added, although the difference was not significant. We previously reported that plant protease could not act on non-denatured triple helical regions of collagen and acted only on globular domains under an acidic condition, and this action was marked when kiwifruit juice (pH 3.) was added as compared to ginger juice (pH 6.0) (Sugiyama et al., 1997). Thus, this reduction of collagen could be interpreted as follows: the degradation of globular domains by plant protease proceeded during the pre-steaming process.

After steaming, no ISC was present, similar to the situation in the control, and ASC and PSC contents were significantly increased and decreased, respectively. Ginger and kiwifruit proteases are inactivated by heat. However, these protease activities were observed at 80°C with denatured collagen as substrate, because the temperature needed to initiate collagen denaturation is lower than the protease-inactivating temperature (Sugiyama et al., 2005 a). Therefore, proteases rapidly acted on collagen molecules denatured in the early phase of steaming.

In the subsequent simmering process, PSC content decreased, and the reduction was marked when kiwifruit juice was added, showing a significant difference compared to the control. The expedited reduction of PSC content in the simmering process may have resulted from the protease action on the resultant collagen without globular domains during the pre-treatment and heat-denatured regions of collagen in the early phase of steaming. Therefore, the degradation of connective tissue during the heating process with these proteases depends on physicochemical structural changes in collagen molecules and inactivation/activation of protease by heating.

2. Effect of cooking process on fat and cholesterol from pork

1) Without ginger juice or kiwifruit juice (control)

Table 1 shows cooking-induced changes in the fat and cholesterol contents. The fat retention rates after cooking were 85.7% (Lot. A), 81.7% (Lot. B) and 83.6% (Lot. C). The retention rates of cholesterol were 94.7% (Lot. A), 94.6% (Lot. B) and 92.8% (Lot. C). Some samples in Lot. C were boiled instead of steamed, but no significant dif-
ferences were observed in the retention rate of fat (83.1%) or cholesterol (95.5%) between the steamed and boiled meats (data not shown). These results are consistent with a previous report showing that the cholesterol content was not readily decreased by cooking, due to its presence as a cell membrane component (Sugiyama et al., 2000).

As shown in Fig.2, the retention rate of collagen was low, but the retention rates of fat and cholesterol were high. The adipose tissue of meat is present between collagen fibers, and fat is accumulated as fat droplets in the adipose tissue. It is suggested that heating weakens the collagen fibers of cut pork chunks, whereas the fat is mostly retained. The retention of fat without destruction of the adipocyte cell membrane in smoked bacon has been reported (Yosioka and Andou, 1999). Additionally, structural changes in squid connective tissue after heating were observed using transmission and scanning electron microscopes (Ando et al., 1998). To elucidate the structural changes in collagen during the cooking process, microscopic observation of connective tissues is thought to be essential.

2) With ginger juice or kiwifruit juice

When ginger juice or kiwifruit juice was added, the fat and cholesterol contents were significantly reduced, and the decreases were marked when kiwifruit juice was added. As described above, the physicochemical structural changes in collagen molecules before the simming process may have affected protease action during simmering. This may have been the major factor associated with the significant reduction of fat and cholesterol contents by the addition of kiwifruit juice.

3. Changes in pork weight

Figure 3 shows cooking–induced weight changes in pork. The weight reduction in steamed pork chunks was greater than that in the control when ginger juice or kiwifruit juice was added. No significant weight reduction was noted in the subsequent simmering process, but a significant reduction was noted in the control. Subsequently, the overall weight changes from the raw meat weight were 63.0, 61.3, and 57.6%, respectively, showing no significant differences among the conditions.

The binding of tissue collagen with water in beef shank, chicken wing, and flounder has been shown using NMR (Nagatsuka et al., 2005), and gelatin produced by the cleavage of collagen fibers to low–molecular–weight molecules has a water–holding capacity. It was suggested that the absence of weight reduction in the simmering process in pork with added ginger juice or kiwifruit juice was due to differences in the structure of collagen molecules in the steamed pork. It was unclear whether gelatin, which is formed from low–molecular–weight collagen, was entirely dissolved from pork or retained in the meat because gelatin was removed in the myofibrillar protein elimination process before the extraction of collagen. PSC content was lower than that in the control when ginger juice or kiwifruit juice was added (Fig.2), suggesting that the involvement of the water–holding capacity of the collagen type was unlikely. Gelatin may have been partially retained in pork and its water–holding capacity may have retained cooking liquid in the pork during the simmering process.

Conclusion

Experiments were performed using kakuni pork (tonpou–ron) as a sample to clarify prolonged moist heat–induced structural changes in collagen and accompanying changes in the lipid content, as well as the influence of plant protease.

Regarding the collagen content, no ISC was present af-
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After steaming, and ASC and PSC contents were significantly increased and decreased, respectively. In the subsequent simmering process, the decrease in PSC content was not significant. When ginger juice or kiwifruit juice was added, PSC content was slightly decreased before steaming, although the decrease was not significant. The collagen content after steaming was not significantly different from that of the control, but a significant difference was noted in the subsequent simmering process, and the PSC content reduction was marked when kiwifruit juice was added as compared to ginger juice. This PSC content reduction in the simmering process may have resulted from the protease action on the collagen telopeptide and heat-denatured regions before and in the early steaming phase. The fat and cholesterol retention rates after cooking were high, but when kiwifruit juice was added, the contents were significantly reduced.

Based on these findings, although heating weakened collagen fibers during the cooking of pork chunks, a large amount of fat was retained. When kiwifruit juice was added, total collagen and PSC contents were markedly decreased, showing that collagen fibers were weakened, thereby promoting the dissolution of fat and cholesterol.

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豚角煮の加熱過程における生姜搾汁、キウイフルーツ果汁の添加がコラーゲン量と脂質量に及ぼす影響

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和文抄録
本研究は、長時間の湿式加熱におけるコラーゲンの構造変化（酸可溶性コラーゲン（ASC）、ペプシン可溶化コラーゲン（PSC）、不溶性コラーゲン（ISC）量の変化）とそれに伴う脂質量の変動、さらに植物プロテアーゼの影響について明らかとすることを目的とし、豚角煮を試料として実験を行った。

蒸し加熱後には、ISCは認められず、ASCは有意に増加、PSCは有意に減少した。その後の煮る過程でのPSCの減少は有意ではなかったが、続く煮る過程ではPSCは減少し、キウイフルーツ果汁を添加した場合にコントロールよりも有意な減少を示した。この煮る過程におけるPSCの減少は、蒸し加熱前および蒸し加熱初期に植物プロテアーゼがコラーゲンのテロペプタイド部位および熱変性部位に作用した結果であると推察された。いずれの条件でも調理後の脂肪およびコレステロールは減少した。キウイフルーツ果汁を添加した場合は、コントロールよりも有意な脂肪量およびコレステロール量の減少が認められた。

これらのことから、豚塊肉の調理では、加熱によりコラーゲン繊維は脆弱化するものの、脂肪の多くは保持されること、キウイフルーツ果汁を添加した場合にコラーゲン繊維が脆弱化し、脂肪およびコレステロールの溶出が増加することが示された。

キーワード：豚肉、コラーゲン、脂肪、コレステロール