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Tenderization of meat by salt-fermented sauce from shrimp processing by-products

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Abstract

The suitability of using salt-fermented shrimp sauce prepared from processing by-product (head, shell, and tail) of southern rough shrimp (*Trachypena curvirostris*) as a meat tenderizer was investigated. Pork neck portions were soaked in 10% saline (saline-treated meat), and in 10% salinity shrimp sauce (sauce-treated meat) for 3 min. The soaked samples were drained, left to stand at 20 ± 2 °C for 3 h and then stored at 4 °C for 5 days. During storage, sauce-treated samples were significantly different ($p < 0.05$) in decrease of moisture contents (75.2–74.0%, after 3 days) and pH (5.9–5.8, after 4 days), but showed an increase ($p < 0.05$) in their volatile base nitrogen (VBN, 16.1–18.9 mg/100 g, after 2 days). The colour of sauce-treated pork was scarlet for up to 3 days of storage, but after that it was similar to that of untreated and saline-treated pork (L value of approximately 50). The hardness and water-holding capacity (WHC) of sauce-treated pork decreased more than those of saline treatment. SDS–polyacrylamide gel electrophoresis (SDS–PAGE) patterns of sauce-treated pork indicated depolarization of myosin heavy chain (MHC) and complete disappearance of Z line and muscle fiber using electron microscopy. Sensory score (4.5, 1 day of storage) for tenderness was significantly higher ($p < 0.05$) than that right after the treatment. Thus, salt-fermented sauce from shrimp processing by-products can be used as a meat tenderizer.

Keywords: Shrimp sauce; Shrimp by-products; Sensory properties; Meat tenderization; Water holding capacity

1. Introduction

Shrimp is a rich source of protein, calcium, vitamins and various extractable compounds and has been used as one of the most popular and important raw materials for many Korean and international dishes, especially in the production of salt-fermented shrimp (Jeot-gal) (Han, 1997). Recently, consumption of shrimp has increased with the rapid growth of the fast food industry despite a decrease in its catch because of environmental pollution in the coastal areas (The Fisheries Association of Korea, 1999). Generally, the head, shell and tail portions of shrimp are removed during processing and these account for approximately 50% of the catch (Heu, Kim, Shahidi, Jeong, & Jeon, 2003). Shrimp processing by-products contain large amounts of nutritive components, extractives and enzymes (Heu et al., 2003), and serve as an excellent source of raw material for production of salt-fermented products such as sauce (Kim, Shahidi, & Heu, 2003). A small portion of shrimp processing by-products is used as fertilizer while the rest is dumped in landfills or hauled into the ocean, thus causing environmental pollution. Therefore, attention must be paid to fully utilize shrimp processing by-products in order to address such concerns. Studies on shrimp

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include those on salt-fermented products (Lee, Ahn, Oh, & Lee, 1986; Mok & Song, 2000; Mok et al., 2000), removal of the shells (Kolbe, Lee, & Babbitt, 1980), change of freshness during cold storage (Jeong, Jo, Lim, & Kang, 1991; Lannelongue, Finne, Hanna, Nickelson, & Vanderzant, 1982; Lee & Um, 1995; Moon, Beuchat, Kinkaid, & Hays, 1982), lipid components (Johnston, Ghanari, Wheeler, & Kirk, 1983; Kazynowek & Panunzio, 1989), enzyme characteristics (Doke & Ninjoor, 1987) and isolation of novel antioxidative substances (Pasqual & Babbitt, 1991). Efforts have also been underway to utilize shrimp shell by-products for the extraction of the carotenoprotein (Camo-Lopez, Simpson, & Haard, 1987; Simpson & Haard, 1985), chitin and chitosan (Ahn & Lee, 1992; Benjakul & Sophanodora, 1993; Chung, Kim, Hur, & No, 1996), pigments (Johnson, 1992) and their application in food processing (Heu et al., 2003). However, studies on the preparation of sauce from shrimp processing by-products, including the head, shell and tail, and their use as a meat tenderizer have not been reported. In this paper, we report on improvement of meat tenderness using salt-fermented sauce from shrimp processing by-products.

2. Materials and methods

2.1. Materials

Southern rough shrimps, *Trachypena curvirostris* (9.0 ± 0.2 cm in length, 8.7 ± 1.4 g in weight), caught near Tongyeong, Korea in October of 1999 were purchased after 2 days from the time of harvest from a commercial fish market, where they were kept on ice, hand peeled and their by-products transported to the laboratory in ice. Pork (neck portion; mainly sternohyoid muscle, 1 year old) was purchased from a local supermarket and transported to the laboratory in ice.

2.2. Preparation of salt-fermented sauce from shrimp processing by-products

Before preparation of salt-fermented sauce, by-products were presoaked in a 0.2% solution of sodium erythorbate for 30 min, then drained, frozen at −20 °C and pulverized. The sauce was prepared by fermentation at 20 ± 2 °C for 3 months. Salt-fermented sauce from shrimp processing by-products was then centrifuged at 12,000*g* for 15 min and the salinity of the supernatant was subsequently adjusted to $10 \pm 2\%$. The sauce so prepared was then used for meat tenderization.

2.3. Preparation of sauce- and saline-treated pork

Pork (neck portion; mainly sternohyoid muscle) from 1-year-old animals was cut into 8 cm × 8 cm × 1 cm pieces and two portions of it were fully soaked in 100 ml of 10% saline (saline-treated pork meat), or in salt-fermented sauce from shrimp by-products (sauce-treated meat) for 3 min. The soaked samples were drained, left to stand at 20 ± 2 °C for 3 h and then wrapped into aluminum foil and stored at 4 °C for 5 days. The untreated meat was used as a control in this study.

2.4. Proximate composition of sauce- and saline-treated pork

The proximate composition of sauce- and saline-treated pork was determined according to AOAC (1990); moisture content by oven drying, crude lipid by Soxhlet, crude protein by Kjeldahl and crude ash by direct ashing method. The salinity was determined by a salt meter (Model 460CP, Istek Co., Seoul, Korea).

2.5. pH and volatile basic nitrogen

The pH was determined, following grinding and homogenization of 5 g of sample with 50 ml of distilled water for 10 min and then using a Metrohm pH meter (Metrohm, 744, Switzerland). The content of volatile basic nitrogen (VBN) was determined by the micro-diffusion method of Conway (Pharmaceutical Society of Japan, 1980).

2.6. Hunter colour values

The Hunter colour values of meat were determined using total colour difference (Nippon Denshoku Kogyo Co., Ltd., ZE-2000, Japan). A white tile with *L*, *a*, *b* values of +91.60, +0.28 and +1.97 was used for calibration, respectively.

2.7. Hardness and water holding capacity

The hardness was measured by a cutting test using a rheometer (CR-100D, Sun Rheometer, Japan). The meat was heated at 80 °C for 1 h, cooled at ambient temperatures for 1 h and then cut into 3.8 cm × 1.6 cm × 1.0 cm pieces with a cutting knife. The water holding capacity was determined by the method of Yun, Lee, and Park (1998). The chopped meat (about 25 g) was weighed into centrifuge tubes, heated at 70 °C for 30 min, cooled at 25 °C for 10 min, centrifuged (1700*g*, 10 min, ambient temperature), and then the free liquid measured. Water holding capacity was expressed as percentage (w/w) of moisture content of the sample.

2.8. SDS–polyacrylamide gel electrophoresis

SDS–polyacrylamide gel electrophoresis (SDS–PAGE) was carried out by the method of Laemmli (1970) using a 5% stacking gel and a 10% separating

gel. For sample preparation for SDS–PAGE, 0.2 g of meat were added to 2.5 ml of an 8 M solution of urea containing 2% (v/v) mercaptoethanol and 2% (w/v) sodium dodecylsulphate (SDS), and then boiled at 100 °C for 3 min.

2.9. Observation by transmission electron microscope

Meat was cut into 1 mm × 1 mm × 1 mm pieces, placed in a prefixing solution (2.5% glutaraldehyde solution) at 4 °C for 24 h, and then washed with a 0.1 M sodium phosphate buffer, pH 7.2, for 20 min at 4 °C. Post-fixing with 1% osmium tetroxide was done at ambient temperature for 1–2 h, and samples washed again with a 0.1 M sodium phosphate buffer (pH 7.2). The washed sample was dehydrated by immersing in ascending concentrations of ethanol (50%, 70%, 80%, 90% and 100%) for 20 min, and the same procedure was repeated with propylene oxide. Epon-812 was added to the hydrated samples, incubated for 4 h at ambient temperature, and then placed in an oven (65 °C) for 48 h to polymerize. The prepared specimen was sliced with an ultramicrotome (Nova Co., LKB, Sweden), and stained with uranyl acetate at ambient temperature for 2 h. After washing in distilled water it was stained with lead citrate for 2 min and washed again with boiled distilled water. The specimen was then examined at 80 kV using a transmission electron microscope (Jem 1200EX-II, Jeol, Japan).

2.10. Sensory evaluation

The tenderness of saline- and sauce-treated meat during cold storage was assessed by seven trained panelists. A 5-point hedonic scale (4 and 5, superior quality; 3, good quality; 1 and 2, inferior quality) was used to evaluate the tenderness of raw pork treated with saline solution or shrimp by-product sauce (14 g; 22 °C).

2.11. Statistical analysis of data

All data were expressed as means ± standard deviation. Analysis of variance was performed using Duncan's multiple range test to determine the difference in mean values ($p < 0.05$).

3. Results and discussion

3.1. Proximate composition and salinity

The proximate composition and salinities of untreated, saline- and sauce-treated meats are shown in Table 1. The moisture and crude ash contents of untreated meats were 72.7% and 1.2%, respectively, while those of saline- and sauce-treated samples were within the 75.4–75.2% and 3.2–3.3% range, respectively. These differences ($p < 0.05$) were considered to be due to the transfer of moisture and salt from the solution. The contents of crude protein were decreased from 23.9% in untreated to 19.4% in a saline-treated and to 19.6% in a sauce-treated pork. These differences ($p < 0.05$) were considered to be due to the transfer of soluble protein to the soaking solution. There was no difference in crude lipid content among the untreated, saline- and sauce-treated pork samples. Also, there was no difference ($p > 0.05$) in proximate composition between saline- and sauce-treated samples. The salinity was about 2% for both saline- and sauce-treated meats.

Table 1
Proximate composition and salinity of untreated, saline- and sauce-treated pork meats (g/100 g)

Components	Untreated	Saline-treated	Sauce-treated
Moisture	72.7 ± 0.2^{a}	75.4 ± 0.3^{b}	75.2 ± 0.2^{b}
Crude protein	23.9 ± 0.0^{c}	19.4 ± 0.1^{a}	19.6 ± 0.0^{b}
Crude lipid	1.4 ± 0.1^{a}	1.2 ± 0.3^{a}	1.2 ± 0.2^{a}
Crude ash	1.2 ± 0.0^{a}	3.2 ± 0.1^{b}	3.3 ± 0.0^{b}
Salinity	0.2 ± 0.0^{a}	2.2 ± 0.1^{b}	2.2 ± 0.0^{b}

Untreated, raw pork meat; saline-treated, treated with soaking 100 ml of 10% saline solution for 3 min at 20 °C; sauce-treated, treated with soaking 100 ml of 10% salinity of shrimp byproducts sauce for 3 min at 20 °C. Values are means of three determinations ± standard deviation. Means with different letters within the same row are significantly different ($p < 0.05$).

3.2. Changes in moisture content

The changes in moisture contents of untreated, saline- and sauce-treated meats during storage are shown in Table 2. The moisture content of untreated pork was 72.0% and those of saline- and sauce-treated pork were 74.2% and 74.0%, respectively, after 3 days of cold storage. This reflects a decrease of only 1%, but these differences were significant ($p < 0.05$) compared with values prior to the storage. In general, muscles are known to lose moisture during cold storage (Ahn, Kim, Lee, Lee, & Lee, 1991; Lee, Joo, Kim, Cho, & Lee, 1994). Impermeability of the wrap used during cold

Table 2
Changes in moisture content of untreated, saline- and sauce-treated pork meats during cold storage (g/100 g)

Storage period (days)	Untreated	Saline-treated	Sauce-treated
0	72.7 ± 0.2^{c}	75.4 ± 0.3^{g}	75.2 ± 0.2^{fg}
1	72.4 ± 0.3^{bc}	75.0 ± 0.4^{fg}	75.0 ± 0.1^{fg}
2	72.8 ± 0.5^{c}	74.9 ± 0.3^{fg}	74.7 ± 0.2^{fg}
3	72.0 ± 0.4^{ab}	74.2 ± 0.2^{de}	74.0 ± 0.4^{d}
4	71.8 ± 0.4^{a}	74.2 ± 0.3^{de}	73.9 ± 0.2^{d}
5	71.6 ± 0.3^{a}	74.3 ± 0.2^{de}	74.0 ± 0.1^{d}

Treatments are the same as in Table 1. Values are means of three determinations ± standard deviation. Means with different letters are significantly different ($p < 0.05$).

Table 3
Changes in pH and volatile basic nitrogen (VBN) content of untreated, saline- and sauce-treated pork meats during cold storage

Storage (days)	pH			VBN (mg/100 g)		
	Untreated	Saline-treated	Sauce-treated	Untreated	Saline-treated	Sauce-treated
0	5.60 ± 0.04^{ef}	5.62 ± 0.02^{f}	5.89 ± 0.00^{h}	7.0 ± 2.0^{a}	7.5 ± 1.0^{a}	16.1 ± 1.0^{f}
1	5.58 ± 0.03^{def}	5.58 ± 0.00^{def}	5.88 ± 0.02^{h}	9.1 ± 1.0^{b}	9.8 ± 0.0^{bc}	17.5 ± 1.0^{fg}
2	5.54 ± 0.02^{cd}	5.57 ± 0.04^{de}	5.87 ± 0.02^{h}	12.6 ± 0.0^{e}	11.2 ± 0.0^{cde}	18.9 ± 1.0^{g}
3	5.52 ± 0.00^{bc}	5.55 ± 0.02^{cd}	5.85 ± 0.03^{h}	11.9 ± 1.0^{de}	$11.\ 2 \pm 0.0^{cde}$	18.9 ± 1.0^{g}
4	5.49 ± 0.03^{ab}	5.48 ± 0.03^{ab}	5.80 ± 0.04^{g}	12.6 ± 0.0^{e}	11.2 ± 0.0^{cde}	18.5 ± 1.0^{g}
5	5.47 ± 0.02^{a}	5.48 ± 0.02^{ab}	5.80 ± 0.03^{g}	11.2 ± 0.0^{cde}	10.5 ± 1.0^{bcd}	18.9 ± 1.0^{g}

Treatments are the same as in Table 1. Values are means of three determinations ± standard deviation. Means with different letters are significantly different ($p < 0.05$).

storage in our study rendered good waterproofing and hence is considered responsible for our observations (Fennema, 1985).

3.3. Change in pH and VBN

The changes in pH and contents of VBN of untreated, saline- and sauce-treated meats during storage are shown in Table 3. The pH values of untreated and saline-treated meats, immediately after treatment, were similar, 5.60 and 5.62, respectively, while that of the sauce-treated sample was 5.89, perhaps due to the influence of pH of salt-fermented sauce which is around 8.0. The pH of sauce-treated pork after 4 days, and those of untreated and saline-treated samples after 2 days showed a significant ($p < 0.05$) decrease. Although the levels of VBN remained relatively low during cold storage, increases of 16–60% in VBN contents in 5 days stored samples are considered insignificant, as explained by Ahn, Kim, Choi, Kim, and Park (1998).

3.4. Change in colour values

The changes in colour values of untreated, saline- and sauce-treated pork meats during cold storage are shown in Table 4. The Hunter *a* values of the meats immediately after treatment were similar, 12.34 in untreated meat and 12.30 in saline-treated samples. For sauce-treated meat, the Hunter *a* value was 12.94 which is higher than the untreated and saline-treated meats. Compared with untreated and saline-treated meats, sauce-treated meat maintained a scarlet colour during the 3 days of storage. Transferring of carotenoids from salt-fermented sauce might be responsible for this observation, but a colour similar to those of untreated and saline-treated meats were eventually attained. Oxidation of carotenoids during the later stages in the preparation of salt-fermented sauce which was added to meat may explain this observation (Kang & Kim, 1999). The Hunter *L* values of untreated and saline-treated meats immediately after the treatments were 47.82 and 47.90, respectively, and there was no difference in Hunter *L* values between these two meat samples. The sauce-treated meat had *L* value of 45.56, which is lower than those of the other samples. Regardless of treatments, all meat samples showed an increase in their Hunter *L* value, but their *a* values decreased during cold storage. The degree of increase in Hunter *L* value and the decrease in Hunter *a* values reflected no difference between untreated and saline-treated meats, but sauce-treated meat showed a larger change as compared to the other two meats. It is suggested that myoglobin in pork was oxidized during cold storage.

3.5. Changes in hardness

The changes in hardness of saline- and sauce-treated meats during cold storage are shown in Fig. 1. The hardness of saline-treated pork was decreased considerably immediately after saline treatment at ambient temperature, but decreased slowly during cold storage. The hardness of sauce-treated meat decreased rapidly up to

Table 4
Changes in Hunter *L* and *a* colour values of untreated, saline- and sauce-treated pork meats during cold storage

Storage (days)	*L* value			*a* value		
	Untreated	Saline-treated	Sauce-treated	Untreated	Saline-treated	Sauce-treated
0	47.82 ± 0.24^{bc}	47.90 ± 0.34^{c}	45.56 ± 0.31^{a}	12.34 ± 0.23^{f}	12.30 ± 0.37^{f}	12.94 ± 0.25^{g}
1	47.68 ± 0.27^{bc}	47.88 ± 0.22^{bc}	45.88 ± 0.18^{a}	12.32 ± 0.28^{f}	12.30 ± 0.43^{f}	12.60 ± 0.51^{fg}
2	48.02 ± 0.21^{c}	48.05 ± 0.25^{c}	47.45 ± 0.30^{b}	11.20 ± 0.18^{e}	11.22 ± 0.37^{e}	11.68 ± 0.42^{e}
3	48.63 ± 0.19^{d}	48.72 ± 0.20^{d}	48.59 ± 0.30^{d}	10.10 ± 0.21^{d}	10.01 ± 0.48^{cd}	10.09 ± 0.32^{d}
4	48.89 ± 0.24^{de}	48.95 ± 0.17^{de}	49.32 ± 0.12^{ef}	9.90 ± 0.17^{cd}	9.86 ± 0.15^{c}	9.49 ± 0.07^{cd}
5	49.43 ± 0.32^{f}	49.52 ± 0.23^{f}	50.45 ± 0.28^{g}	8.80 ± 0.27^{b}	8.86 ± 0.15^{b}	7.97 ± 0.33^{a}

Treatments are the same as in Table 1. Values are means of three determinations ± standard deviation. Means with different letters are significantly different ($p < 0.05$).

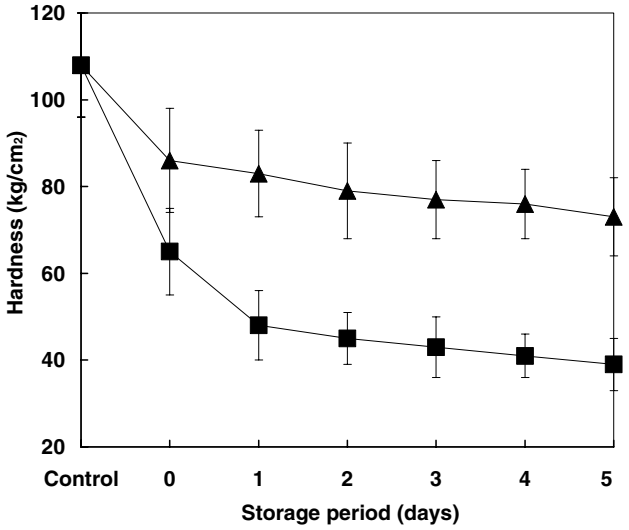

Fig. 1. Changes in hardness of saline (▲)- and sauce (■)-treated pork meats during cold storage. Treatments are the same as in the footnote of Table 1. Error bars represent the standard deviation of three determination on samples.

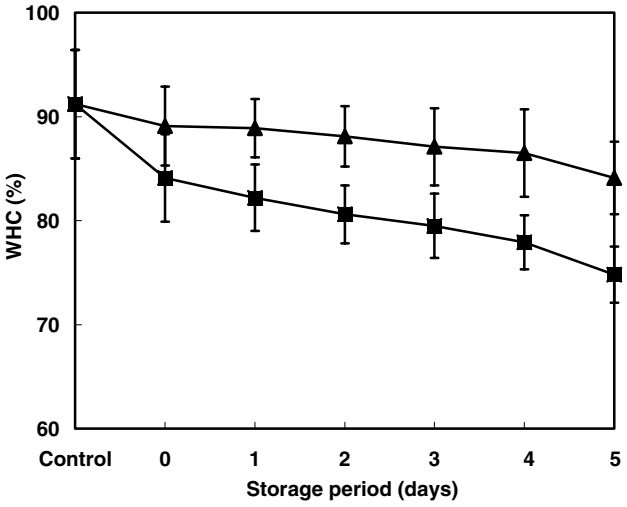

Fig. 2. Changes in water holding capacity (WHC) of saline (▲)- and sauce (■)-treated pork meats during cold storage. Treatments are the same as in the footnote of Table 1. Error bars represent the standard deviation of three determination on samples.

1 day of cold storage and this proceeded slowly afterwards. The optimum temperature for proteolytic and autolytic enzymes derived from shrimp processing by-products is generally above the ambient temperature (Park & Joo, 1986), and these cannot attain a high activity at low temperatures. The hardness of sauce-treated meat was considerably less than that of saline-treated pork, thus salt-fermented sauce from shrimp processing by-products exerted a tenderizing effect.

3.6. Changes in water holding capacity

The changes in water holding capacity of saline- and sauce-treated meats during cold storage are shown in Fig. 2. The water holding capacity of raw pork meat (control) was 91.4%. Regardless of the treatment, the water holding capacity of saline- and sauce-treated meats decreased during cold storage, but the degree of the decrease (84.1–74.8%) in sauce-treated pork meat was more than that (89.1–84.1%) of the saline-treated sample. In general, the closer the pH of meat is to the isoelectric point of its proteins (pH 5.0–5.1), the net charge and hence the number of water molecules to combine with them decreases (Song, 1989). Judging from the above results and this fact, the difference of water holding capacity in saline- and sauce-treated meats might be due to the difference in pH between saline- (pH 5.48–5.62) and sauce-treated pork samples (pH 5.80–5.89). Therefore, it suggested pork treated with salt-fermented sauce from shrimp processing by-products could improve the water

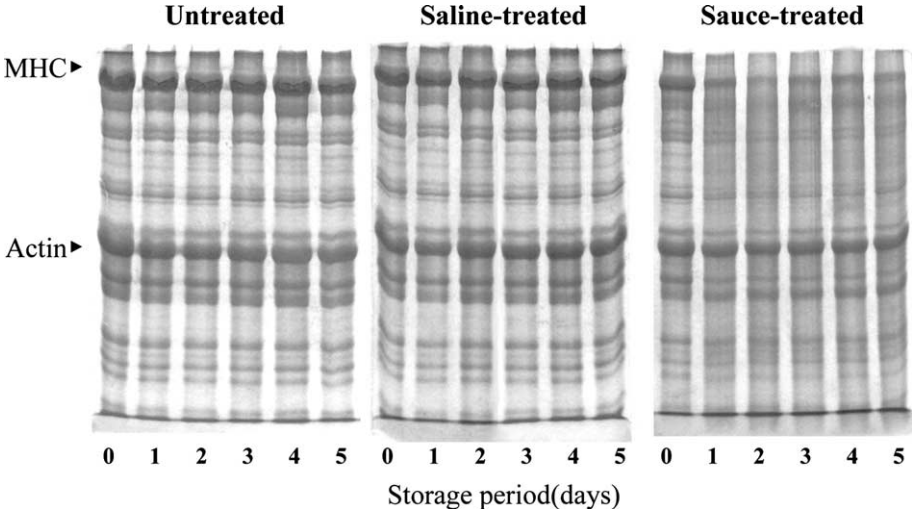

Fig. 3. SDS–PAGE patterns of myofibrillar proteins from untreated, saline- and sauce-treated pork meats during cold storage. Treatments are the same as in te footnote of Table 1. MHC, myosin heavy chain.

holding capacity of meat products, decrease their weight loss during processing and storage, and enhance their texture quality and juiciness.

3.7. Changes in myofibrillar proteins of pork

The changes in myofibrillar proteins of untreated, saline- and sauce-treated meats during cold storage, reflecting the degree of tenderization of meat, are shown in Fig. 3. Untreated and saline-treated pork samples showed almost no change in their myosin heavy chain (MHC) and other proteins during cold storage. Thus, untreated and saline-treated meats were not tenderized. Sauce-treated meat, however, showed a distinctive change in its MHC and other proteins after 1 day of cold storage. From these results, salt-fermented sauce from shrimp processing by-products can tenderize meat thereby improving its quality.

3.8. Transmission electron microscopy

The results of transmission electron microscopy on saline- and sauce-treated pork during cold storage are shown in Fig. 4. Saline-treated pork immediately after fermentation at ambient temperatures, had a dark muscle fibre, a bright extracellular matrix structure and a Z line which were clearly distinguishable; the Z line and the muscle fibre band were slightly blurred after 5 days of cold storage. In sauce-treated pork, the Z line and the muscle fibre band blurred immediately after fermentation at ambient temperature to a much greater extent than that of saline-treated pork at the earliest stage; after 5 days of cold storage, bundles of fibrous protein began to hydrolyze and the typical form of muscular cell

Table 5
Sensory scores on tenderness of saline- and sauce-treated pork meats during cold storage

Storage period (days)	Saline-treated	Sauce-treated
Control	3.0 ± 0.0^{c}	3.0 ± 0.0^{c}
0	3.4 ± 0.3^{b}	3.9 ± 0.3^{b}
1	3.5 ± 0.3^{b}	4.5 ± 0.2^{a}
2	3.5 ± 0.3^{b}	4.5 ± 0.2^{a}
3	3.4 ± 0.2^{b}	4.6 ± 0.2^{a}
4	3.6 ± 0.2^{b}	4.5 ± 0.2^{a}
5	3.6 ± 0.4^{b}	4.5 ± 0.2^{a}

Treatments are the same as in Table 1. Values are means of 10 determinations ± standard deviation. Means with different letters within the same row are significantly different ($p < 0.05$).

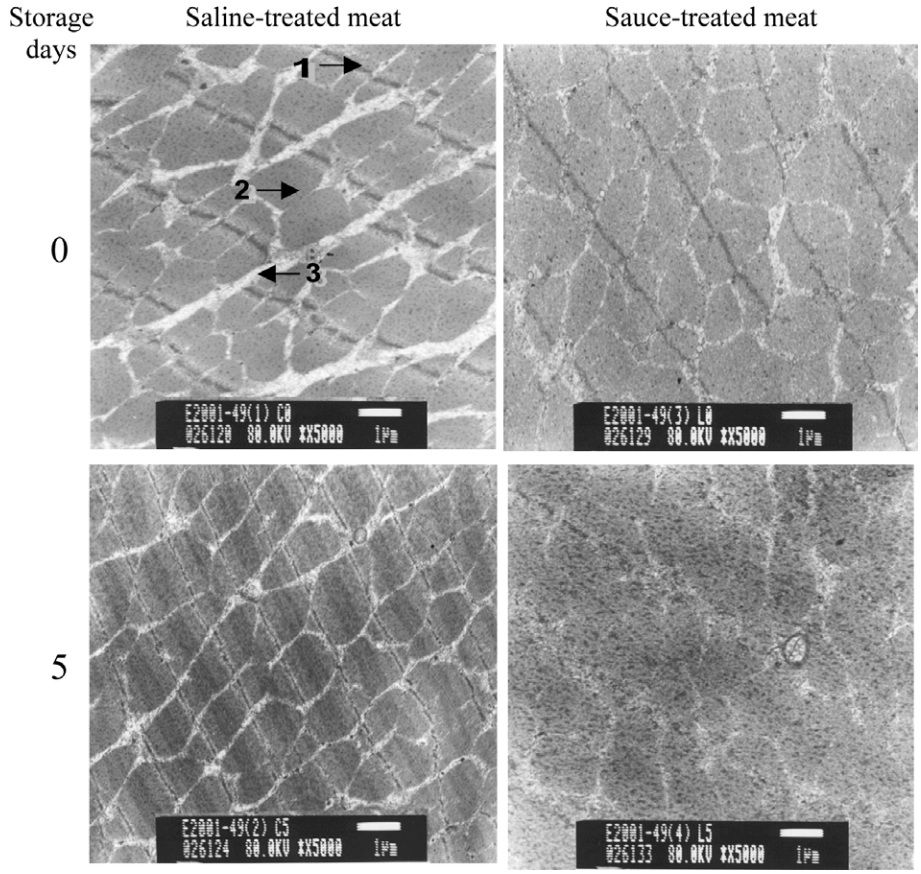

Fig. 4. Transmission electron microscopy of saline- and sauce-treated pork meats during cold storage. Treatments are the same as in the footnote of Table 1. Arrow 1, Z-line; arrow 2, muscle fibre; and arrow 3, extracellular matrix structure.

completely disappeared and bundles of fibers spread widely.

3.9. Sensory evaluation

The results of tenderness evaluation by sensory means for saline- and sauce-treated meats during cold storage are shown in Table 5. Saline-treated meat was tenderized slightly right after soaking in saline, but there was no change afterwards. During cold storage, sauce-treated meat was superior to that of saline-treated samples in terms of its tenderness.

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