EFFECT OF CURING AGENTS ON PROTEASES INVOLVED IN THE INCREASE IN FREE AMINO ACIDS AND PEPTIDES DURING THE CURING-PROCESS OF PORK

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INTRODUCTION

It is well-known that flavor of meat products such as hams and sausages is improved in their curing-process. This improvement is thought to be caused by the increase in free amino acids and peptides during the curing-process of meat products. Free amino acids were shown to increase in the curing-process of sausages and the ripening of Iberian cured ham (1). The increase in free amino acids is caused by the actions of neutral aminopeptidases in the curing-process of meat products as well as during postmortem aging of meats. However, there is few information about the effects of curing agents on these aminopeptidases. Furthermore, there is no information about the changes in peptides during the curing-process of meat products, although peptides as well as free amino acids are important taste compounds in meat (2, 3). The increase in peptides during curing-process is thought to be caused by the actions of proteinases, such as cathepsins and calpains. Cathepsins B, D, H and L were shown to be still active after 8 months of dry curing (4). Cathepsins D and H were also clarified to be strongly affected by NaCl. However, the effect of curing agent on the activity of calpain is not known yet. Thus, since there are few research on the increases in free amino acids and peptides during the curing-process of meat products, the mechanism of their increase has not clarified completely.

This work was performed to elucidate the changes in free amino acids and peptides during the curing-process of pork loin and the effect of curing agents on proteases involved in their increases.

METHODS

Curing method-One-tenth volume of 20% NaCl/1% KNO_3 solution or distilled water was homogeneously injected into pork loin aged for 2 days, and these loins were stored at 4 ℃ for 5 days. These loins were called a cured or uncured pork.

Analyses of free amino acids and peptides- A cured or uncured pork loin was homogenized with distilled water before and after its storage. The homogenate was mixed with one-tenth volume of 50% trichloroacetic acid solution in order to remove proteins. The supernatant after centrifugation was used for the analyses of free amino acids and peptides. Free amino acids were analyzed with an amino acid analyzer. The amount of peptides was also determined with the same analyzer, after the sample solution was hydrolyzed in 6 N HCl at 110 ℃ for 24 hr.

Preparation and assay of calpain and cathepsins-Calpain was partially purified from porcine muscle (*M. longissumus dorsi*) according to the method reported by Ishiura *et al.* (5). Activity of calpain was measured at pH 7.5 by using casein as substrate.

Cathepsins B and L were prepared from the same porcine muscle according to the method reported by Okitani *et al.* (6). Activities of cathepsins B and L were measured at pH 6.2 by using benzoyl-Arg-β-naphthylamide (BANA) and benzyloxycarbonyl-Phe-Arg-7-(4-methyl)coumarylamide (Z-Phe-Arg-MCA), respectively.

Chromatography of aminopeptidases in porcine muscle-Muscle (*M. longissimus dorsi*, 10 g) from the cured and uncured pork loin was minced and homogenized with 30 ml of 40 mM Tris-HCl (pH 7.2) in a Waring blender for 1 min. The supernatant after centrifugation was dialyzed against 10 mM Tris-HCl buffer (pH 7.2) containing 0.1% 2-mercaptoethanol (buffer A). This muscle extract (20 ml) was applied on a DEAE-cellulose column equilibrated with buffer A. Adsorbed proteins were eluted with linear gradient of NaCl concentration from 0 to 0.35 M. An aminopeptidase activity of each fraction was measured with β-naphthylamide derivatives of amino acids (AA-NA) according to the method reported previously (7).

RESULTS AND DISCUSSION

Changes in peptides during curing-process-Peptides increased during the storage of pork in the presence of curing agents (2% NaCl and 0.1% KNO_3) as well as in the absence of them (Fig. 1). Its increment was smaller in pork stored with curing agents than without them. Its increments in porks with and without curing agents were 5.0 and 15.0 μmol amino acids/g meat, respectively.

Calpain and cathepsins B and L are shown to be involved in the increase in peptides during the storage of meats. Calpain was not inhibited by both curing agents at all. On the other hand, a curing agent, 2% NaCl, inhibited about 17 % of the activities of cathepsins B and L. The inhibition of the activities of cathepsins in the presence of NaCl seems to result in the depression of the peptides increase during the storage of pork with curing agents.

Changes in free amino acids during curing-process-Free amino acids increased during the storage of pork in the presence of curing agents as well as in the absence of them (Fig. 2). Their increases were smaller in pork stored with curing agents than without them. Their increments in pork with and without curing agents were 6.3 and 9.1 μmol/g meat, respectively. In both meats, the increases in Ala, Leu, Glu and Ser were large. This result was almost consistent with that in the report on the ripening of Iberian cured ham (1). There were scarcely differences in the pattern of the increase in free amino acids between porks stored with and without curing agents. These results indicate that the increase in free amino acids is caused by aminopeptidases C and H in the curing-process of meat products

as well as during postmortem aging of meat.

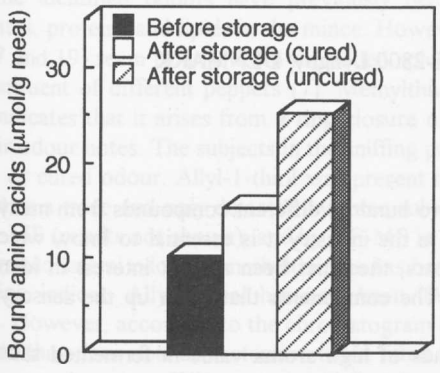

Fig. 1 Changes in the amounts of total bound amino acids during the storage of pork with and without curing agents for 5 days.

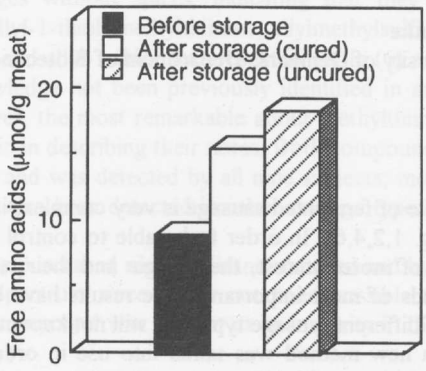

Fig. 2 Changes in the amounts of total free amino acids during the storage of pork with and without curing agents for 5 days.

Effect of curing agents on aminopeptidase activities-Five aminopeptidases containing aminopeptidases C and H in an uncured or cured pork were separated and eluted at 0, 0.11, 0.13 and 0.20 M NaCl concentration on ion-exchange chromatography. Although the activities of these aminopeptidases in both porks after storage were smaller than those in pork before storage, there were scarcely differences in the intensities of their activities between porks stored with and without curing agents. Furthermore, aminopeptidases C and H showed higher activities than other aminopeptidases in uncured and cured porks, suggesting that both enzymes are major ones contributing to increase in free amino acids in the curing-process of meat as well as during postmortem aging of meat.

The effects of curing agents on aminopeptidases in pork were examined (Table 1). A curing agent, 0.1% KNO_3 did not affect activities of all amiopeptidases. However, 2% NaCl inhibited the activities of almost all aminopeptidases. Aminopeptidase C activity for Lys-β-naphthylamide (Lys-NA) was about 45 % inhibited in the presence of 2% NaCl. Aminopeptidase H activity for Leu-NA was also about 32 % inhibited. These results suggested that inhibition of both aminopeptidases by NaCl caused the depression of the increase in free amino acids during the storage of pork in the presence of curing agents.

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Table 1 Effect of curing agents on aminopeptidase (APase) activities

APase	Substrate	None	0.1% KNO_3	2% NaCl	2% NaCl +0.1% KNO_3
0 M	(Leu-NA)	100	129.0	245.0	270.4
0.11 M	(Leu-NA)	100	90.0	85.1	85.1
0.13 M	(Lys-NA)	100	95.4	66.7	65.5
0.20 M #	(Leu-NA)	100	124.4	68.4	65.8
(APase H)	(Glu-NA)	100	99.5	108.6	101.0
0.20 M	(Leu-NA)	100	101.3	87.4	83.8
(APase C)	(Lys-NA)	100	98.1	55.3	49.6

#; The activity of aminopeptidase C or H in the 0.20 M NaCl fraction was measured in the presence of iodoacetic acid or EDTA, respectively.