RELATIONSHIP BETWEEN ADDED PYROPHOSPHATE CONTENT AND SAUSAGE QUALITY

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Introduction

In the previous paper[1], we reported that inorganic pyrophosphate (PP) added to experimental sausage emulsions was readily hydrolyzed by endogeneous enzymes on incubation under similar conditions to that previal during cooking of commercial products. It is therefore important from practical view point to know whether or not the desirable effects of added PP in meat products persist even after the PP had been hydrolyzed. Both water-holding capacity (WHC) and binding ability are the two important factors that determine the quality of meat products. These properties are closely interrelated. Both properties are improved by the addition of PP. In this study, we measured the WHC as an index of quality of the experimental sausages, made with the addition of various concentrations of PP.

Another purpose of this work was to determine the minimum level of PP necessary to produce sausages with high quality. In commercial practice 0.3% and over polyphosphates (on weight basis) are added to meat emulsion. The high content of phosphates in diet not only interfere with the absorption of certain minerals such as Ca^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, and Fe^{2+} from the intestine but also causes some physiolgical ill-effects in the body[2,3].

Materials and Methods

Reagents: Reagents used in this study were of the highest purity grade.

Preparation of porcine meat: Porcine minced meat was prepared as described in previous paper[1].

Water-holding capacity: Two per cent NaCl, 0 to 0.5% sodium pyrophosphate (Na-PP), and 30% water (on the weight basis of meat) were added to procine minced meat. Sausage emulsions were prepared from this mixture by homogenization for 1 min at 10,000 rpm with a Nihon Seiki homogenizer equipped with an ice-jacket. The emulsions were put into Pyrex-glass centrifuge tubes of 2 cm diameter and the tubes were covered with Parafilm and aluminum film. After incubation for 30 min at 12°C to improve the reproducibility of the results according to HONIKEL *et al.*[4], the emulsions were centrifuged for 40 min at 5,200×g and 4°C to remove air bubbles. They were cooked at 70°±2°C for 50 min and chilled in a refrigerater.

The experimental sausages were taken out of the centrifuge tubes, wiped away the juice on the surface with filter paper, and weighed for determining cooking loss. A piece of the experimental sausage, which was cut to 1 cm thickness, was put between filter paper. The weight of exuded juice from the sample was measured by weighing the sample before and after pressing at pressure of 10 kg/m^2 for 20 sec.

WHC was calculated by the following formula.

$$\mathrm{WHC}(\%)=\left(1-(W_c+W_p)/W_d\right)\times 100 \qquad (1)$$

where W_d is the total weight (g) of water in the emulsion, W_c is the weight (g) of juice released from the sample during cooking (cooking loss), and W_p is weight (g) of juice exuded by pressing.

Determination of the amounts of proteins and myosin ectracted from meat: Homogenate was prepared by a similar procedure as described above except that a larger amounts of water was used in this case (10 vol instead of 0.3 vol). After centrifugation at 5,200×g for 30 min, the volume of supernatant was measured. The supernatant was centrifuged again at 27,000 ×g for 30 min and the protein concentration of this second supernatant was determined by the biuret procedure[5] with bovine serum albumin as a reference standard. A part of the supernatant was subjected to slab gel electrophoresis in the presence of sodium dodecyl sulfate (SDS). Densities of bands in destained slab gels were measured with a recording densitometer, Toyo DVC-33C. Contents of myosin in the sample are expressed herein (Fig. 2) as the ratio of myosin heavy chain to total proteins as determined by a densitometer.

Gel electrophoresis: Slab gel electrophoresis with SDS was performed in a gel containing 10% acrylamide and 0.27% Bis (pH 8.7) by the method of PORZIO and PEARSON[6].

Results

Relationship between water-holding capacity and pyrophosphate concentration: Addition of Na-PP to sausage emulsions improved WHC of resulting products as shown in Fig. 1. WHC increased linearly with increase in Na-PP content 0 to 0.2% and reached the maximum level at 0.2% Na-PP in the presence of 2% NaCl. Thereafter WHC did not increase further by raising the Na-PP content beyond 0.2%.

Relationship between the extractability of proteins and pyrophosphate concentration: Figure 2 shows the extractability of total proteins and myosin from the 1-day aged or 8-days aged meat in the presence of various concentration of Na-PP and 2% NaCl. It can be seen that with increasing Na-PP concentration up to 0.3% the total protein extracted from both 1- and 8-days aged meat increased to about 80 mg/g meat, thereafter the amount did not increase with further increasing the Na-PP content to 0.5%. On the other hand myosin showed a maximum extractability from 1- and 8-days aged meat at 0.2% concentration of Na-PP. The myosin extractability from both the meats remained almost constant up to 0.4%

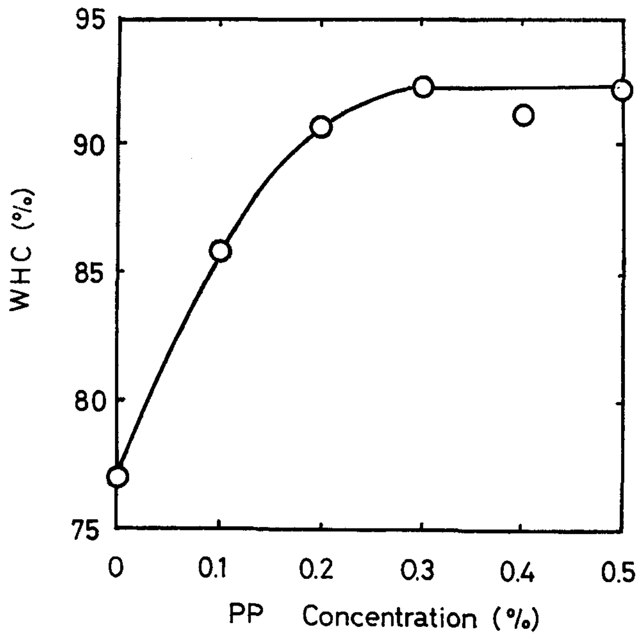

Fig. 1. Relationship between water-holding capacity and pyrophosphate concentration. Minced meat was aged for one day at 4°C before experimentation. WHC was calculated by equation (1).

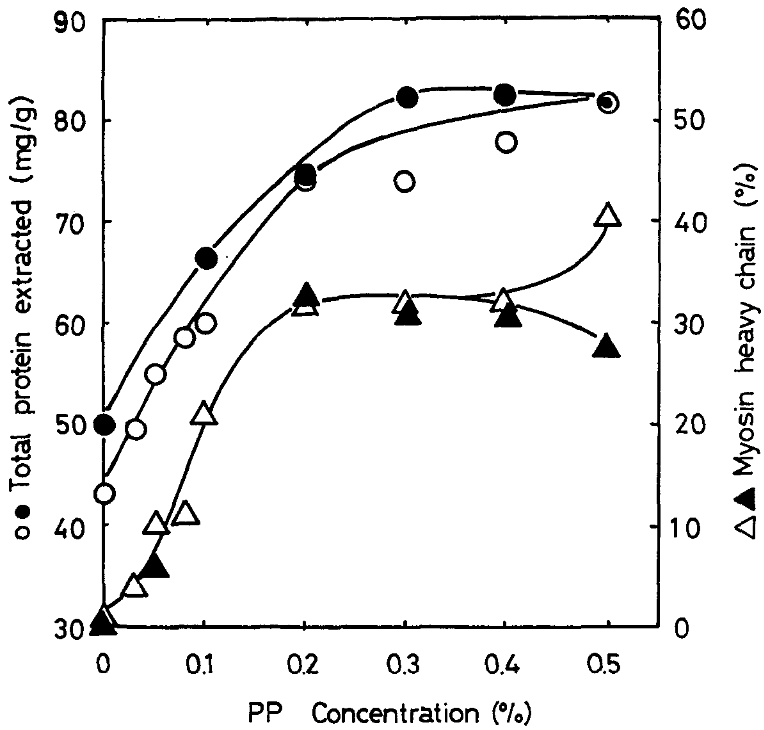

Fig. 2. Relationship between the extractability of proteins and pyrophosphate concentration. Porcine minced meat was aged one day (○, △) or eight days (●, ▲) at 4°C. Circles show the total protein concentration in the supernatant after centrifugation (see "Materials and Methods"). Triangles show the per cent of myosin heavy chain in the total protein calculated by using densitograms after gel electrophoresis.

Na-PP content, however it tended to increase in 1-day aged meat and decrease in 8-days aged meat at 0.5% Na-PP concentration.

Discussion

In the production of sausages, phosphates are always used along with sodium chloride. The combined effects of phosphates and NaCl on the quality of products were stressed by Hamm and Grau[7]. Hellendoorn[8] reported that the effect of PP or tripolyphosphate (TP) on meat hydration increases with increasing NaCl content. However, the concentration of NaCl used in sausages is limitted to about 2% in the final product today. Thus we thought it is important to study the effect of various concentration PP on the quality of sausage keeping the concentration of NaCl constant at 2%. The ionic strength of 2% NaCl is about 0.3, which is high enough to cause dissolution of myosin molecules from myofibrils.

Figure 1 shows that WHC increased with increasing PP content and reached the maximum level at 0.2% Na-PP under our experimental conditions. This suggests that the minimum concentration of Na-PP required to produce sausages with high WHC is about 0.2%. Based on the results described in the previous paper[1], it can be expected that almost all the PP added is hydrolyzed in the experimental sausages after cooking. This is consistent with the view presented by BENDALL[9] and YASUI *et al.*[10], *i. e.*, the effect of PP is due to an increased solubility of muscle proteins caused by the dissociation of actomyosin. That is, PP exerts the effect on sausages only during making of emulsions. NERAAL and HAMM[11] also reported that the increase in WHC of beef emulsion caused by the addition of PP is not reduced after complete hydrolysis of PP. Although there is a close relationship between WHC and binding ability, study on the effect of PP on the binding ability by some methods such as viscoelasticity will be required.

Addition of 0.2% or more Na-PP to emulsion increased the amounts of total proteins and myosin molecules extracted from meat (Fig. 2). By using densitograms of gel electrophoresis, we could distinctly measure the myosin contents in the extracted (water-soluble plus salt-soluble) proteins. These results also support that the minimum concentration of Na-PP required is about 0.2%. Above 0.4% Na-PP, slightly more myosin was extracted from 1-day aged meat and less was extracted from 8-days aged one. Probably, some biochemical properties of myosin such as affinity for substrates changed during long aging process. But this difference in extractability of myosin did not affect the WHC (Fig. 1). Several studies[12~15] have shown that actin and tropomyosin have an enhancing effect on the heat-induced gel formability and on maintaining the stability of the gel. At 0.2% PP, sufficient amounts of myosin is extracted which can form gel of desirable strength in the sausage. Water is immobilized in the gel networks. Since slightly more amount of myosin was ectracted from the 1-day aged meat at 0.4% Na-PP concentration, possibly a high amount of actin or tropomyosin will be required in this case to form a gel of appropriate rigidity with high WHC[13,16]. We could not measure the amounts of actin and tropomyosin on the densitograms of gel electrophoresis. Further studies on this aspect is needed.

Summary

The improvement of water-holding capacity of sausages caused by the addition of inorganic pyrophosphate is not reduced after complete hydrolysis of the pyrophosphate.

The minimum concentration of sodium pyrophosphate required to produce sausages with high water-holding capacity is about 0.2 per cent in the presence of 2 per cent sodium chloride. A close relationship between water-holding capacity and the amounts of myosin extracted from meat was confirmed by using densitograms of gel electrophoresis in the presence of sodium dodecyl sulfate. These results support the idea that the gel formation of myosin molecules is the main factor determining the water-holding capacity and binding ability of sausages.

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