EFFECT OF PHYSICAL TREATMENTS ON DIGESTIBILITY AND ALLERGENICITY OF BEEF EXTRACT

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Background and Objectives.

In previous report (Han et al., 1999), we indicated that bovine serum albumin (BSA) and bovine gamma globulin (BGG) played an important role in allergenicity of beef. Cross-reactivity between beef- and cow's milk-allergens, and ineffectiveness of heat- and high-pressure treatment for reducing allergenicity were also suggested.

The uptake of undigested protein and/or polypeptides is low in adult organisms, but it is considerable in the immature gut of the newborn (Dannaeus et al., 1979). Fiocchi et al. (1995) suggested that digestibility must play an important role in the reduction of allergenicity of BSA. Cooking is a critical step to obtain a hygienically safe product, on the other hand, cooking can affect the proteolytic activity of gastrointestinal enzymes as a result of the protein structural changes due to heat denaturation (Prevalov 1990). On the basis of the hypothesis that allergenic proteins will lose their allergenicity, if the proteins are fully digested by gastric protease in the gut, we investigated the effect of heat- and high-pressure treatment on the digestibility of beef extract in vitro. Each digested samples was applied to immune assay with sera of beef allergic patients to investigate their allergenicity.

Material and Methods.

Sera of beef allergic patients. Sera of beef-allergic patients were obtained from Yoshida hospital.

Preparations of beef extract. The beef extract was prepared by centrifugation following homogenization with 10 volume of 20mM sodium phosphate buffer (pH 7.4).

Heat- and high-pressure treatments on beef extract. Beef extract was treated by heat (60℃,100℃) for 10 min and by high pressure (200,400,600 MPa) for 5min using NBIP (Nikkiso Isostatic Processor).

In vitro protein digestion. Beef extract was digested with pepsin in the ratio, 1:90 (enzyme to sample; w/w) at pH 2.5. After peptic attack, a solution containing of 0.5 mg/ml of trypsin was added to each sample in the ratio 1/36 (w/w) at pH 7.3. Proteolysis was carried on at 37 ℃ in water bath shaken at 100 beats/min. The reaction was stopped by TCA (final 10%) to determine total free peptide. The reaction was also stopped by pepstatin A and leupeptin to subject to SDS-PAGE and ELISA.

Total free peptide determination. Total free peptide was determined by measuring the absorbance at 280 nm.

SDS-PAGE. SDS-PAGE was carried out according to the method of Laemmli [28] with a slight modification using a 4% stacking gel and 10% separating gel.

ELISA and immunoblots. ELISA and immunoblots were performed according to the method of Engval & Perlmann (1972) and Towbin et al. (1979) with sera of beef allergic patients, respectively.

Results and discussion.

Fig.1 shows the digestibility of heat-treated beef extract. Control (non-treated) beef extract was rapidly hydrolyzed and total free peptide increased faster than those of heat-treatment at all stages of enzymatic attack, both pepsin and trypsin. Digestibility gradually decreased by increasing the temperature of heat-treatment. These results were also supported by their SDS-PAGE pattern (Fig.2). A similar result was reported by Restani et al. (1992). SDS-PAGE pattern of heat-treated sample showed that a trace of BSA remained even after 24 hours enzymatic attack (Fig.2), indicating that BSA is more stable to digestion than other proteins in the beef extract. Fig.3 shows the change of allergenicity of the control and heat-treated samples at each stage; sample treated at 100℃ could not be applied to ELISA due to its aggregation (refer to immunoblotting results). Even after enzymatic attack of 8 and 24 hours, heat-treated sample had residual allergenicity of 45% and 25%, respectively. On the contrary, control sample lost most of its allergenicity from 8 hours. In immunoblotting (Fig.4), the sample of 60℃ and 100℃ showed specific reaction even after 24 hours,

but no reaction was detected in control sample after 24 hours. However, Restani et al. (1997) suggested that heat-treatment reduced positive responses in skin prick test (SPT).

On the other hand, high-pressure treatment (200 MPa) may be effective to improve the digestibility as shown in Fig.5. The digestibility became to decrease from 400 MPa, but high-pressure treatment retained upper digestibility than those of heat treatment, corresponding to the results of SDS-PAGE patterns (Fig.6). Due to increase of digestibility, allergenicity of high-pressure (200 MPa) treated sample decreased more than those of heat-treated sample (Fig.7).

Conclusions.

The results are summarized as follows: 1) physical treatment (heat and high-pressure) alone was no effective to reduce allergenicity of beef, 2) heat treatment to beef extract decreased its digestibility in simulated gastric model, on the contrary high-pressure treatment improved the digestibility, 3) beef allergenicity decreased with the progress of digestion.

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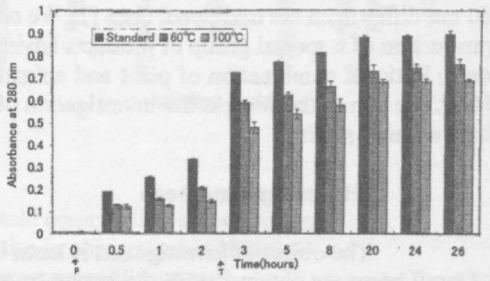

Fig. 1. Digestibility of heat-treated beef extracts by pepsin and trypsin.

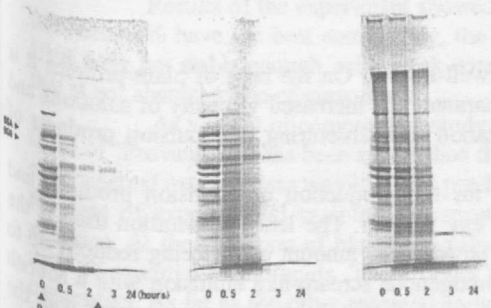

Fig. 2. SDS-PAGE (10%) of heat-treated beef extracts digested by pepsin and trypsin.

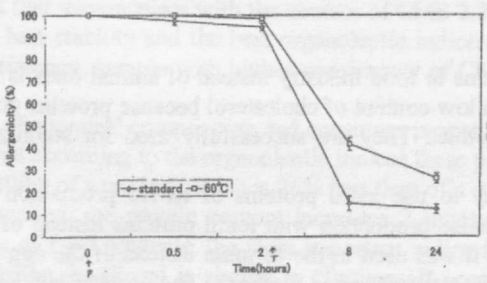

Fig.3. ELISA of heat-treated beef extracts digested by pepsin and trypsin.

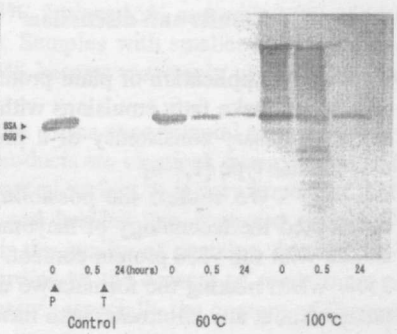

Fig.4 Immunoblot of heat-treated beef extracts digested by pepsin and trypsin.

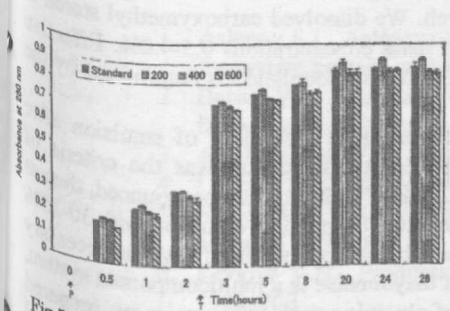

Fig.5. Digestibility of high-pressure-treated beef extracts by pepsin and trypsin.

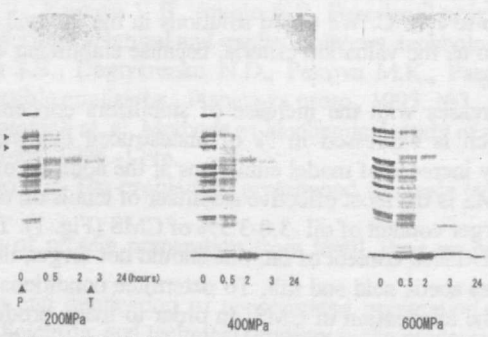

Fig.6. SDS-PAGE (10%) of high-pressure-treated beef extracts digested by pepsin and trypsin.

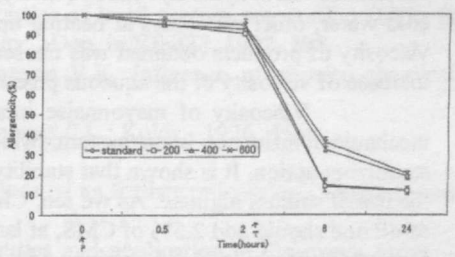

Fig.7. ELISA of high-pressure-treated beef extracts digested by pepsin and trypsin.