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Research Note

Specific Degradation of Myosin in Meat by Bromelain

ABSTRACT

The specificity of bromelain and papain for degradation of actin and myosin in meat was compared. Meat was treated with 0·1% enzyme for 0–60 min at 24°C and proteins, extracted with 6M urea containing 2% sodium dodecylsulfate (SDS) solution, were separated by SDS–polyacrylamide gel electrophoresis (PAGE). The profile of the extracted proteins clearly indicated that papain degrades myosin and actin at similar rates, whereas bromelain degrades myosin preferentially. This distinction could be useful in the development of freeze-dried meat products in which toughening of the meat due to the formation of high molecular weight aggregates from myosin cross-linking is minimized.

INTRODUCTION

Dehydration is a preservation technique widely used for long-term storage of foods. Freeze-drying is particularly useful for preservation of the structural integrity of the foods. Nevertheless, certain chemical changes take place during and subsequent to freeze-drying and lead to undesirable changes in sensory properties. For example, the dryness and toughness of freeze-dried meat are a well known problem (Harper & Tappel, 1957; Connell, 1962). Carbonyl-amine browning reaction involving glucose and proteins was suggested as a possible mechanism for the textural changes in freeze-dried meats (Regier & Tappel, 1956). More recently, the specific cross-linking of myosin by the Maillard-type reaction was identified as the

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primary cause for insolubilization of the proteins in freeze-dried meat (Kim *et al.*, 1984). It was also demonstrated that such cross-linking could be prevented by treating the meat with compounds or food ingredients such as N-α-acetyl-L-lysine or hydrolyzed vegetable protein. Apparently, the amino groups in the added amino acids compete with the ε-amino group of the lysine residues on the proteins for glucose in the meat and inhibit cross-linking of the proteins.

Alternatively, the hydrolytic properties of meat-tenderizing enzymes could be utilized to mask the effect of cross-linking. Papain, ficin and bromelain are plant-derived sulfhydryl proteases used in meat tenderizers (Liener, 1974). Their effectiveness for meat tenderization has been investigated (Miyada & Tappel, 1956; Fogle *et al.*, 1982). Papain and bromelain are more commonly used because ficin has such a high hydrolytic activity that it can render the meat too 'mushy' (Wells, 1966). Wells (1966) used these enzymes in rehydration solutions for freeze-dried chicken, and showed that the enzyme-induced tenderness is related to the destruction of muscle fiber. He also showed that papain is more effective than bromelain for tenderizing freeze-dried chicken. The molecular basis for such a distinction was not discussed.

These enzymes can also be used to hydrolyze the meat proteins prior to freeze-drying. If myosin, which is primarily involved in the cross-linking, could be preferentially degraded to smaller fragments without over-tenderizing the meat, the ability of myosin to cross-link might be substantially reduced since the unique arrangement of myosin rods in the myofibril seems to be responsible for the cross-linking (Kim *et al.*, 1984). Moreover, even if the myosin fragments were to become cross-linked, the molecular weight of the resulting adducts might not be high enough to cause toughening of the meat after freeze-drying or storage. In either case, it is desirable to be able to degrade myosin without affecting other myofibrillar proteins such as actin. It is the purpose of this paper to compare the specificity of papain and bromelain toward hydrolysis of myosin and actin, two major structural proteins in the meat.

MATERIALS AND METHODS

Sample preparation

A lean portion of raw beef round was chopped to obtain a homogeneous sample. Each of eight 0·8 g samples of the chopped meat was placed in a test tube. To each of four of these test tubes, 0·8 ml of 1 mg/ml papain (2 × crystallized, 80% protein; Sigma Chemical Co., St Louis, MO) solution in deionized water was added, and the contents were thoroughly mixed. The

test tubes were allowed to stand at room temperature (24°C) for 0, 10, 30 and 60 min, respectively, and heated in boiling water for 2 min to inactivate the enzyme. The chopped meat in the four test tubes was similarly treated with 1 mg/ml bromelain (50% protein; Sigma) solution. The pH of these digestion mixtures was approximately 5·8, which was between the pH optimum of bromelain (pH 5·0) and papain (pH 7·0). The digested samples were then freeze-dried for subsequent analysis.

SDS–PAGE

Proteins from 25 mg aliquots of the freeze-dried meat samples were extracted with 5 ml of 6 M urea solution containing 2% SDS by stirring gently for 2 h. The extracted proteins were then separated on a 7·5% polyacrylamide tube gel (Laemmli, 1970) as follows: the protein extract was mixed with an equal volume of sample buffer containing 0·0625 M Tris–HCl (pH 6·8), 3% SDS, 10% glycerol, and 5% 2-mercaptoethanol, and heated for 2 min in a boiling water bath. Electrophoresis was carried out for 3 h with a constant current of 2 mA/gel. The gels were stained with 0·05% Coomassie Brilliant Blue R250 solution and destained with 7·5% acetic acid solution. The amount of protein in the gel was measured by densitometric scanning with a Varian model 634 spectrophotometer-gel scanner system, and measuring the height of the protein peak of interest from the densitogram. These experiments were repeated and the amounts of myosin heavy chain or actin from two determinations were averaged.

Determination of extractable protein

In a separate experiment, a 5 g aliquot of the chopped meat was treated with 5 ml of 1 mg/ml bromelain solution in a 50 ml beaker for 2 h at 24°C and freeze-dried. A control sample of chopped meat was similarly treated with water and freeze-dried. Portions (0·5 g) of the freeze-dried meat samples, control and bromelain-treated, were heated at 100°C for 30 min in a sealed can. The heated and unheated samples were pulverized, and the amount of proteins extractable with 6 M guanidine–HCl and 20 mM dithiothreitol (DTT) solution was determined by the dye-binding method of Bradford (1976). A bovine serum albumin solution (1 mg/ml in guanidine–HCl, DTT) was used to construct a calibration curve.

RESULTS AND DISCUSSION

Since myosin is primarily responsible for the cross-linking and the accompanying changes in the texture, it is desirable to find an enzyme that

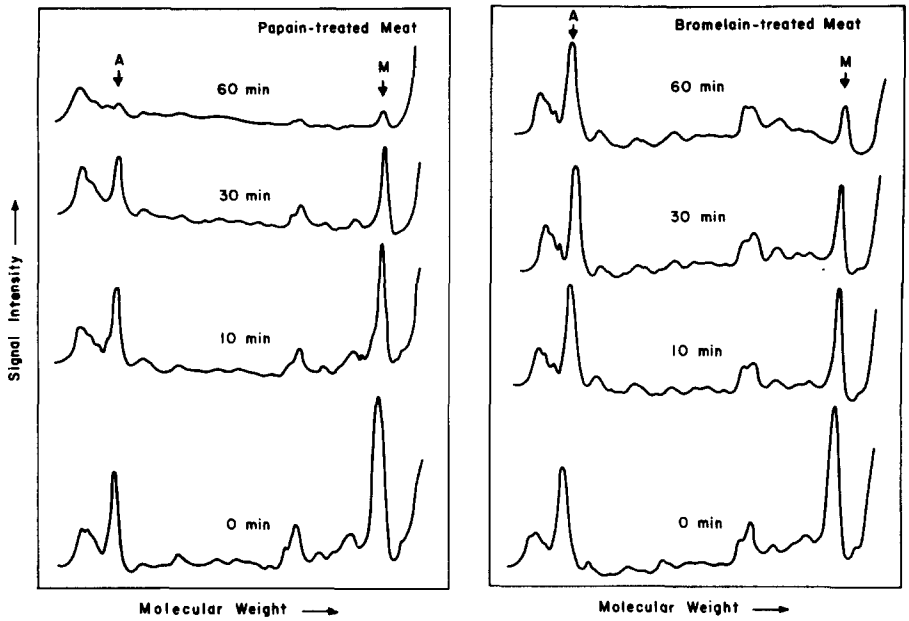

Fig. 1. Densitograms corresponding to SDS-PAGE profile of proteins extracted with 6M urea, 2% SDS solution from meat treated with papain and bromelain at 24°C for 0–60 min. Actin and myosin heavy chain are denoted by A and M, respectively.

will specifically degrade myosin. The purity of papain and bromelain used in the present work was different. As a result, a quantitative comparison of the degradative action of these enzymes on the myofibrillar proteins in the meat was not entirely meaningful. Nevertheless, a qualitative characterization of the specificity of these enzymes on the major myofibrillar proteins was possible by monitoring changes in the protein profile after digestion with the enzymes.

The SDS–PAGE profiles of proteins extracted from the enzyme-treated meats are shown in the densitogram in Fig. 1. It is clear that, when the meat is treated with papain, both myosin heavy chain and actin are degraded with appoximately the same efficiency. Bromelain appears to act primarily on myosin during the first 60 min. When the amounts of remaining myosin and actin were measured from the densitogram, the following results were obtained. In the papain-treated meat, 73% myosin heavy chain and 75% actin remained after 10 min digestion; 52% myosin and 50% actin after 30 min; and 9% myosin and 11% actin after 60 min. On the other hand, 76, 58 and 25% myosin heavy chain remained in the bromelain-treated meat after 10, 30 and 60 min, respectively, but only a 4% decrease in actin was observed after 60 min. When digestion by bromelain was prolonged, actin was also degraded. These results are consistent with the observation

TABLE 1
Effect of Bromelain Treatment on the Amount of Protein Extracted from Freeze-Dried Meat Before and After Heat Treatment

Sample	*Heat treatment*	*Extractable[a] protein (mg/mg sample)*
Control freeze-dried meat	None	0·483
	100°C 30 min	0·278
Bromelain-treated freeze-dried meat	None	0·468
	100°C 30 min	0·396

[a] Average of duplicate measurements.

(Kang & Rice, 1970) that papain shows stronger activity toward myofibrillar proteins than bromelain.

The observed specificity of bromelain toward myosin suggests that one could modify myosin prior to freeze-drying with minimal effect on other structural proteins in the meat. It was expected that protein insolubilization would be reduced in bromelain-treated meat compared with the control meat when they are freeze-dried and subjected to conditions where myosin cross-linking would occur. Results shown in Table 1 indicate that more proteins are extractable from the heated, bromelain-treated freeze-dried meat than from the heated control. The amount of extractable protein in the heated control sample decreased by 42%, presumably due to myosin cross-linking, compared with the unheated control sample. A similar decrease was reported previously (Kim *et al.*, 1984). The amount of extractable protein in the heated, bromelain-treated freeze-dried meat decreased by 15%. This observation is consistent with the idea that one might minimize the formation of high molecular weight myosin aggregates by pretreating the meat with bromelain.

Pretreating the meat with papain might also minimize the formation of high molecular weight protein aggregates, but it then again may lead to overtenderization due to the degradation of both myosin and actin. It has been pointed out by Kumar *et al.* (1977) that papain treatment leads to overtenderization and sogginess in freeze-dried mutton. Those authors also indicated that papain-treated freeze-dried meat is less juicy after reconstitution than bromelain- or ficin-treated meat. The lower water-

holding capacity seems to indicate complete breakdown of the myofibrillar structure.

The flavor of the bromelain-treated freeze-dried meat also appears better than the papain-treated meat. Bromelain-treated meat in a dental liquid product (freeze-dried meal for persons unable to chew solid food) showed less off-flavor than papain-treated meat upon storage (Shaw *et al.*, 1985). A similar result was obtained for freeze-dried mutton (Kumar *et al.*, 1977). Cathepsin D from muscle and spleen also degrades myosin heavy chain without affecting actin (Robbins *et al.*, 1979). Nevertheless, the hydrolysis of myosin by cathepsin D is much slower than by bromelain (typically 24 h at 37°C by cathepsin versus 1 h at 24°C by bromelain) and cathepsin D is more expensive than bromelain.

In summary, it appears that bromelain is an inexpensive enzyme commonly used in foods that can degrade myosin without affecting actin. It may be useful for making good quality, shelf-stable, freeze-dried meat products. Treatment conditions such as enzyme concentration, time and temperature of digestion, and the method of applying the enzyme need to be determined for optimal texture and flavor of the freeze-dried meat products.

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