Reprinted from Journal of Food Science
TECHNICAL LIBRARY
U.S. ARMY NATICK R & D COMMAND
NATICK, MA 01760
R79-118

ACTION OF SPLEEN EXTRACTS ON STORED PRE-COOKED FREEZE-DRIED BEEF

S. H. COHEN, J. A. KOSTICK, F. M. ROBBINS, R. A. SEGARS and J. E. WALKER

ABSTRACT

Weight changes in stored freeze-dried meat samples following rehydration indicated a greater H_2O uptake by enzyme-treated samples. Although sarcomere length measurements and Instron testing demonstrated that enzyme-treated samples (at all sampling periods) were significantly more tender and more uniform in texture than the control samples, protein extractability was not changed significantly.

INTRODUCTION

TREATMENT of beef muscle with the catheptic enzymes from bovine spleen causes loss of myofibrillar Z band structure and degradation of intramuscular connective tissue (Robbins and Cohen 1976). These changes are similar to those observed during the aging of muscle, and suggest that spleen extract may have a potential as an exogenous muscle tenderizer. Treatment of meat with spleen extract produces textural changes similar to those occurring during aging, and results in a product with more uniform textural properties (unpublished observations). This action is due to the selective degradation of myofibrillar proteins by the catheptic enzymes (Chatterjee et al., 1977), and is in contrast to the effect of commercial tenderizers containing papain which indiscriminately degrades myofibrillar proteins causing an undesirable mushy texture.

This study describes the effect of spleen extract on the properties after rehydration of precooked, freeze-dried beef, which had been in storage up to 1 yr.

MATERIALS & METHODS

Preparation of spleen extract

Spleen extract was prepared from bovine spleen as previously described (Robbins and Cohen, 1976). The extract was assayed for cathepsin D using hemoglobin at pH 3.5 (Keilova and Tomasek, 1976), and for cathepsin B1 using N-benzoyl arginine p-nitroanilide (Barrett, 1972). The activities of cathepsin D and cathepsin B1 were 0.11 units per milligram, 0.001 units per milligram, respectively. Prior to its use the enzyme solution was passed through a Millipore filter (pore size 0.2 μm) so that it would be free of bacteria.

Sample preparation

Each of 10 commercial grade eyes of the round were cooked to an internal temperature of 71°C, cooled and cut perpendicular to the muscle fiber axis into 24 0.6 cm slices. After labeling, slices from each of the 10 roasts were placed into cans which were then vacuum sealed. Care was taken to insure at least two slices from each roast were included within all cans.

Immediately after canning, two cans were opened and 12 slices from each can were removed for testing. The remaining cans were stored at room temperature to be opened after 3-, 6-, 9- or 12-months storage.

Authors Cohen, Robbins and Walker are with the Biochemistry & Nutrition Group, Food Sciences Laboratory, U.S. Army Natick Research & Development Command, Natick, MA 01760. Author Kostick is with the Food Technology Group, Food Engineering Laboratory, and Author Segars with the Food Chemistry Group, Food Sciences Laboratory, U.S. Army Natick Research & Development Command, Natick, MA 01760.

Weight determination

At each of the storage periods, two of the stored cans were opened and the 24 freeze-dried slices were divided into either a control or enzyme group, consisting of 12 slices each. The samples were then weighed and rehydrated.

The controls were rehydrated for 30 min in 1% NaCl solution adjusted to pH 5.5 with HCl and blotted to remove excess solution and weighed again. The enzyme group was rehydrated in the NaCl solution containing 0.32 mg/ml spleen extract for 30 min, blotted to remove excess solution and weighed again. All slices were then placed in plastic bags and immersed in a 65°C H_2O bath for 10 min, then cooled to room temperature for testing.

Sarcomere length measurements

So that we could follow sarcomere length changes within the same muscle over the duration of the storage period, four slices from the same roasts, which were stored in different cans, were examined at each sampling period.

Small 1-cm^3 pieces were dissected from each sample and fixed for 1 hr in 2.5% glutaraldehyde in KCl-borate buffer pH 7.1 (Robbins and Cohen, 1976) then washed twice in the buffer solution.

Next, groups of muscle fibers were teased from the larger pieces, placed in a drop of buffer between a glass slide and coverslip and examined with laser diffraction as described by Cohen (1977).

Five measurements were made on each of four random samples from each slice.

Rheology

An Instron Universal Testing Instrument Floor Model TTDM fitted with a punch and die test cell (Segars et al., 1975) was used to evaluate the texture of the rehydrated freeze-dried beef slices.

A 1-cm diameter punch was used in all tests. It was driven through the 0.6 cm thick meat slices at a velocity of 5 cm/min. The resulting force-deformation curve provided several parameters to characterize the meat; however, only one, maximum shear stress, (γ_m), which is derived from the maximum force required to shear the slice, is reported in this paper since no significantly different information was provided by the other parameters. Three measurements per slice were taken, giving a total of 36 data points for each treatment.

Evaluation of control and enzyme-treated samples was made after 0 (initial samples), 3, 6, 9 and 12 months of storage.

Chemistry

Extraction of protein. Each of the rehydrated beef slices was homogenized in 10% volume/wet weight of Weber-Edsal Buffer solution (Perry, 1953) (0.6M KCl, 0.04M $NaHCO_3$, 0.01 $NaCO_3$). The extraction medium was centrifuged at 20,000 × G for 1 hr. The supernatant was considered the "salt soluble proteins." The residue was re-extracted with the same volume of Weber-Edsal Buffer which also had been made 8M in urea. The slurry was centrifuged at 20,000 × G and the supernatant was considered the "urea soluble proteins." Protein determinations were made by the Biuret method (Gornall et al., 1949) using bovine serum albumin as the standard.

Electrophoresis. The electrophoresis of the salt soluble and urea soluble fractions were performed after the method of Weber and Osborn (1969). The following proteins served as reference molecular weight markers: Bovine serum albumin, ovalbumin, chymotrypsinogen and myoglobin. The electrophoresis of all the samples was performed in 5% acrylamide gels.

RESULTS & DISCUSSION

Weight determinations

The weight relationships between dry and rehydrated control and enzyme-treated samples are presented in Figure 1. There are essentially no significant differences among the dry samples; however, following rehydration the enzyme-

treated samples absorbed significantly (Table 1) more H_2O than the controls, with the percentage differences between the two groups gradually increasing from about 6% at 0 months to 16% at 12 months.

Although there is a trend towards greater weight differences between the two groups as the storage period progresses, the 6-month values are inexplicably inconsistent with the other periods. Nevertheless, the greater amount of H_2O absorbed by the enzyme groups at all other sampling periods is indicative of a higher H_2O absorbing capability due to enzyme treatment.

Sarcomere length measurements

The gradual sarcomere length shortening of all samples during the 12-months storage indicates a gradual toughening of the freeze-dried meat (Fig. 2). After enzyme treatment there was a significant (Table 1) increase in sarcomere lengths which suggest that the enzyme is able to reverse some of the toughening-related changes. Samples rehydrated with the enzyme had sarcomere lengths as long as non freeze-dried raw meat (unpublished data).

Although the mean sarcomere lengths of both groups decreased at each sampling period, those of the enzyme-treated samples ranged between 7.7% (0 months) and 12.9% longer than the controls.

That the enzyme treated samples exhibited more uniform texture was shown by a sarcomere length shortening of 14.9% during the 12 months, whereas the controls shortened by an average of 19.8%. Since all the samples toughened upon storage, the difference between these measurements are an indication of the greater amount of tenderization produced by the enzyme on tougher samples.

Rheology

Slices rehydrated in the enzyme solution gave maximum shear stress (γ_m) values parallel to, but averaging approximately 80% lower than the controls (Fig. 3). Previous experience with the punch and die test cell on beef slices (Segars et al., 1975) demonstrated a high correlation with taste panel evaluation. The observed decrease in parameter values represents a significant (Table 1) enzyme-produced tenderization.

The maximum shear stress value of 26.9 N/cm^2 for the pre-cooked freeze-dried, rehydrated (at 0 months storage)

Fig. 1—Weight change following rehydration of control and enzyme-treated samples during the 12-month storage period. Dry samples to be rehydrated in ▨ saline or ■ enzyme. Samples rehydrated in saline ▥ or enzyme □ . N = 12 for each test.

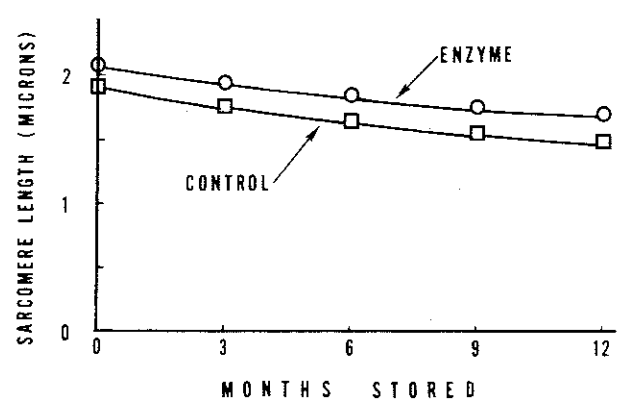

Fig. 2—Comparison of means of 20 sarcomere length measurements of microns made on four control □ and four enzyme treated samples ○ at each sampling period during the 12 month storage period.

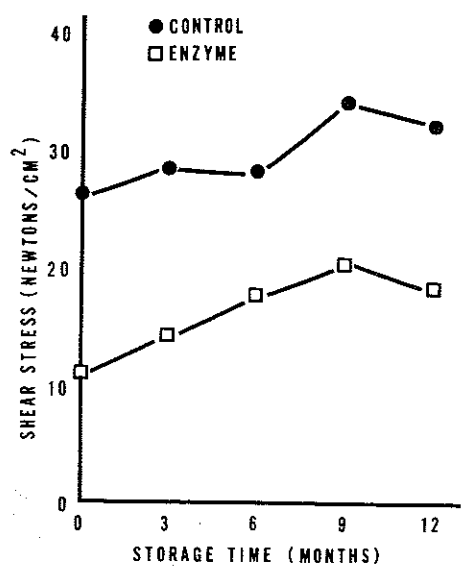

Fig. 3—Mean shear stress (γ_m) values in Newtons/cm² for control slices of meat rehydrated in saline solution compared with slices rehydrated in enzyme solution at five sampling periods during the 12-month storage period. Three measurements were made on each of 12 slices per treatment.

Table 1—Statistical evaluation[a] of enzyme treated vs control samples

	During storage period					
	Weight (kg)		Sarcomere length (μ)		Shear stress (N/cm^2)	
	E	C	E	C	E	C
Mean	41.69	38.93	1.88	1.69	16.53	30.21
Standard deviation	6.080	6.325	0.2001	0.3016	5.74	6.88
Variance	36.966	40.01	0.4004	0.9097	32.949	47.334
Degrees of freedom	60	60	400	400	180	180
Significance	$P < 0.05$		$P < 0.01$		$P < 0.01$	

[a] The student's t test was used to calculate the significance

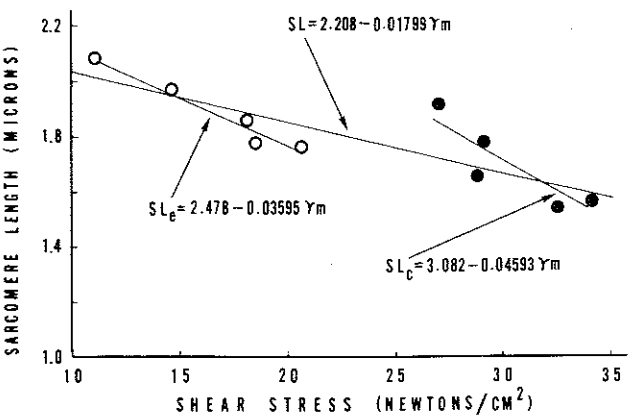

Fig. 4—Regression line representing correlations between sarcomere length and shear stress values of control and enzyme treated samples. SL_e ○ represents sarcomere length of enzyme-treated samples plotted vs shear stress. SL_c ● represents control lengths plotted vs shear stress.

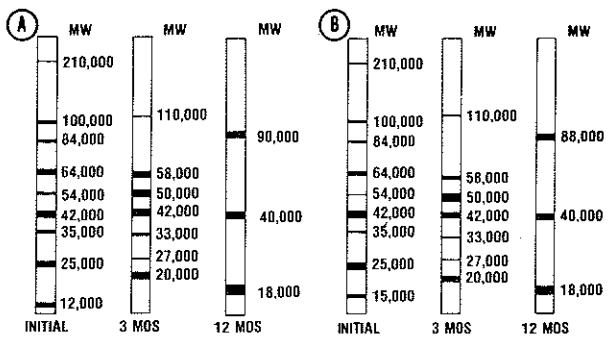

Fig. 5—Representative disc gel electrophoresis patterns of precooked freeze-dried beef slices (N-120) stored at 22°C and extracted with Weber-Edsal Buffer with urea: (A) Controls; (B) Enzyme treated slices. The trends towards the loss of specific protein bands of the treated and control 0, 3 and 12 month gels are compared.

muscle used in this study is similar to the value obtained by Segars et al. (1975) for U.S. Choice Grade semitendenosus (23.7 N/cm^2) but lower than the γ_m = 39.7 N/cm^2 number they found for U.S. Commercial Grade semitendenosus.

The lower value given here results in part from the inherent variation within each grade (the semitendinosus is probably among the most tender of the bovine muscles) and in part from the tenderization produced by freeze-drying which causes a partial breakdown of the muscle microstructure. On the other hand toughening associated with freeze-dried meats occurs upon storage and is shown by both the decreased sarcomere length values (Fig. 2) and the larger γ_m values in Figure 3.

The data in Figure 3 show that the mechanism which causes the meat to toughen during storage was never completely reversed by enzyme treatment after storage; nevertheless, the enzyme-treated samples at all sampling periods are more tender than the controls, and after 12 months, remain more tender than the initial controls.

Reduction in textural variation between slices is also shown in Figure 3 by the less erratic point scatter exhibited in the γ_m plot by the enzyme-treated samples as compared to the controls. This observation agrees with other data which show a greater enzymatic action on tough meat than on more tender slices.

It is informative to correlate the sarcomere length (SL) with the maximum shear stress (γ_m) even though the limited number of data points (n=10) requires a correlation of r = 0.765 for a 1% level of significance.

A linear regression of SL vs γ_m in Figure 4 using both control and enzyme-treated samples yields the equation SL = 2.208 − 0.01799 γ_m with a correlation of r^2 = 0.69 (r = 0.83). Thus 69% of the variation in sarcomere length is predicted by the Instron measurement of maximum shear stress and the requirement for a 1% level of significance is satisfied.

Based on this correlation one can conclude that the structural changes primarily responsible for tenderization are those which also increase sarcomere length. The effects resulting from action of the enzyme present in the rehydration fluid provide a reasonable explanation for the observed enzymatic tenderization.

Chemistry

Over the 12-month storage period, both control and enzyme-treated samples showed a decrease in the total number of proteins (based on molecular weight) (Fig. 5). The observation is probably a reflection of the limited and selective degradation of the muscle proteins by catheptic enzymes which significantly alter their influence on tissue texture and average sarcomere length but does not greatly effect their solubility characteristics relative to the untreated controls.

CONCLUSION

REHYDRATION with spleen extract containing catheptic enzymes increased the water absorbing capability of stored freeze-dried meat.

Enzyme treatment of meat samples at several intervals during the storage period resulted in longer average sarcomere lengths and more textural uniformity among the treated samples than the untreated controls.

Measurement of shear stress (γ_m) showed that after enzyme treatment at all sampling periods, meat samples were more tender and had a more uniform texture than the controls. This reduced textural variation implies a greater degree of enzymatic action on tougher meat.

Regression analysis showed a high correlation between sarcomere length and shear stress.

A decrease in protein extractability during the 12-month storage period was not significantly changed by enzymatic treatment.

REFERENCES

Barrett, A.J. 1972. Lysosomal enzymes. In "Lysosomes, a Laboratory Handbook," Ed. Dingle, J.T., p. 119. North-Holland Publishing Co., Amsterdam, Holland.

Chatterjee, S., Robbins, F.M., Cohen, S.H. and Walker, J.E. 1977. Role of exogenous proteases in tenderization of meat. Nucleus 55: 12.

Cohen, S.H. 1977. The determination of tenderness. Nucleus 55: 14.

Gornall, A.G., Bardawill, C.J. and David, M.M. 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 177: 751.

Keilova, H. and Tomasek, V. 1976. Further characteristics of Cathepsin D inhibitor from potatoes. Collection Czech. Chem. Commun. 41: 2440.

Perry, S.V. 1953. The protein components of the isolated myofibril. Biochem J. 55: 114.

Robbins, F.M. and Cohen, S.H. 1976. Effects of catheptic enzymes from spleen on the microstructure of bovine semimembranosus muscle. J. Texture Studies 7: 137.

Segars, R.A., Hamel, R.G., Kapsalis, J.G. and Kluter, R.A. 1975. A punch and die test cell for determining the textural qualities of meat. J. Texture Studies 6: 211.

Weber, K. and Osborn, M. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244: 4406.

Ms received 12/6/78; revised 3/1/79; accepted 3/10/79.

The authors thank Dr. Edward W. Ross, Jr., Staff Mathematician, for his helpful suggestions.

This paper reports research undertaken at the U.S. Army Natick Research & Development Command and has been assigned No. TP 2005 in the series of papers approved for publication. The findings in this paper are not to be construed as an official Department of the Army position.