BONE PARTICLE DETERMINATI0N IN MECHANICALLY SEPARATED RABBIT MEAT– PRELIMINARY RESULTS

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ABSTRACT

The standard laboratory technique used in the U.S. for bone particle determination takes 13 or more hours and relies on enzymatic digestion using papain followed by separation in carbon tetrachloride, acetone and ether. This study was conducted to modify the existing laboratory method for isolation of bone fragments in mechanically separated meat to increase the accuracy, to shorten the time required, and to avoid use of noxious reagents. Preliminary studies with mechanically separated rabbit meat indicated that bromelain and papain in equal concentrations (0.25%) and ficin at 0.002% provided better separation than papain alone. Furthermore, separation was improved when the samples were autoclaved after incubation with the enzymes rather than before. Two incubations of one hour each were as effective as a five hour followed by an 8 hour incubation. Washing with water was as effective at separating the bone fragments as the use of acetone, carbon tetrachloride, and ether. It was concluded that this shortened, more environmentally friendly method would prove useful once validation studies are completed.

Key words: Rabbit meat, deboning, bone particle determination.

INTRODUCTION

Structured markets that offer rabbit meat at prices competitive with similar products must be established to maintain stability and viability of the rabbit industry in the United States (GHEBREMEDHIN, 1990). Although rabbit meat is generally found as whole or cut-up carcasses in the market, the likely consumer base for rabbit meat prefers further processed, microwave ready products (MCLEAN-MEYINSSE *et al*.,1994). Deboning whole carcasses or the lower value portions and restructuring them into nuggets, patties, rolls or other forms would allow marketing these cuts in forms that would have greater consumer appeal (SECRIST, 1987).

Rabbit carcasses can be rapidly and efficiently deboned with a belt-and-drum deboner (Baader 603, Baader North America Corp., Fort Meyers, Fla.) (MCNITT *et al*., 2002; 2003). The United States Department of Agriculture has established regulations for mechanically separated meats for the maximum calcium and minimum protein and fat contents as well as limitations on the sizes of bone particles that may be in the mince. No more than 2% of the bone particles can be larger than 0.5 mm in their greatest dimension and no particles can exceed 0.85 mm (US Code of Federal Regulations, 2003). Previous studies have shown that mechanically deboned rabbit mince easily satisfies the requirements for calcium, protein and fat but there were too many large bone fragments (MCNITT *et al*., 2002; 2003). However, closer examination revealed that many of the large fragments were dried connective tissue adhered to smaller bone fragments.

The standard laboratory technique for bone particle determination is based on the method of HILL and HITES (1968) which takes 13 or more hours and relies on enzymatic digestion using papain followed by separation in acetone, carbon tetrachloride, and ethyl ether. Papain does not completely digest all types of connective tissue so some connective tissue gets included as bone. Ficin and bromelain also actively degrade connective tissue (MIYADA and TAPPEL, 1956; WHITAKER, 1957). ROMANS *et al*. (1994) pointed out that papain has little degradative action on collagen while bromelain is very effective on collagen but does not strongly affect muscle fibers or elastin. Ficin is effective in degrading both collagen and elastin.

This work was carried out to modify the existing laboratory method for isolation of bone fragments in meat to increase the accuracy, shorten the time required, and avoid the use of carbon tetrachloride and other noxious reagents.

MATERIAL AND METHODS

Collection of mince

Whole carcasses (not deboned) of 12 New Zealand White fryers 8-12 weeks of age that had been vacuum-packed and stored for two to three months at -20 °C were thawed and ground in one pass through a Butcher Boy Model A52HF grinder (Lasar Mfg. Co. Inc., Los Angeles, CA) using 9.5 mm plate apertures. Immediately after collection, 5 g samples of the ground rabbit were stored at -20 °C for future studies of bone particle separation methods.

Separation of meat from bone

The major steps in the HILL and HITES (1968) method for bone particle determination are shown in Table 1.

Table 1. Major steps in the Hill and Hites (1968) method for bone determination and the changes that were made to improve the procedure

HILL and HITES (1968)	- Multiple enzymes	- Multiple enzymes - Autoclave after incubation	- Modified (final) procedure - Multiple enzymes - Autoclave after incubation - Wash with water
Autoclave 10 min.	Autoclave 10 min.		
Add papain	Add papain, ficin, bromelain	Add papain, ficin, bromelain	Add papain, ficin, bromelain
Incubate 5 h	Incubate 5 h	Incubate 5 h	Incubate 1 h
Wash	Wash	Wash	Wash
Add papain	Add papain, ficin, bromelain	Add papain, ficin, bromelain	Add papain, ficin, bromelain
Incubate 8 h or overnight	Incubate 8 h or overnight	Incubate 8 h or overnight	Incubate 1 h
		Autoclave	Autoclave
Wash and rinse with acetone and water	Wash and rinse with acetone and water	Wash and rinse with acetone and water	Wash and rinse with water
Wash with CCl_4 and acetone (2 times)	Wash with CCl_4 and acetone (2 times)	Wash with CCl_4 and acetone (2 times)	
Wash with ethyl ether	Wash with ethyl ether	Wash with ethyl ether	
Dry contents	Dry contents	Dry contents	Dry contents
Weigh residue	Weigh residue	Weigh residue	Weigh residue

According to the HILL and HITES (1968) procedure, 5 g of mince is placed in a 250 ml Erlenmeyer flask containing 100 ml of water, dispersed by stirring and autoclaved for 10 min. The contents are cooled and tissue floating on the surface is skimmed off. Five ml of buffer (0.8M phosphate buffer, pH 7) and 10 ml of papain solution (0.5 g papain) are added, and the volume adjusted to 200 ml with water. The content is incubated for 5 h at 35 °C stirring occasionally. At the end of incubation, the supernatant that contains suspended soft tissue is discarded. Fresh medium is added and incubated for another 8 h or overnight. Following incubation, the supernatant is discarded and the residue rinsed with water. The residue is washed with two 10 ml changes of acetone. About 50

ml of CCl_4:acetone mixture (2.5:1) is added to the residue, mixed well and allowed to settle for a few seconds. The supernatant is discarded, and the residue is washed twice with 10 ml ethyl ether and dried at 80 °C for 12 h. The dried residue is weighed and the bone content reported as 'percent bone per g of sample'.

Effects of multiple enzymes and incubation times

Preliminary studies indicated that bromelain and papain in equal concentrations and ficin at 0.002% provided better separation than papain alone (NEGATU, unpublished data). A completely randomized design was used with 2 factors (incubation time and enzyme concentration) in a 2 x 5 factorial arrangement (2 incubation times of 2 and 4 h; 5 concentrations of papain and bromelain: 0.0025, 0.025, 0.25, 0.50, 1.00 per cent, w/v) with 8 replicates for each treatment combination. The experimental units were 5 g samples of mince. Ficin was used at a fixed concentration of 0.002%. The samples were incubated with the enzymes prior to autoclaving. The medium was changed once during incubation. After incubation, the samples were washed with water, acetone, a CCl_4:acetone mixture and ethyl ether according to the method of HILL and HITES (1968). Seven replicates of mince incubated according to the unmodified HILL and HITES (1968) method were used as controls.

Effects of timing of autoclaving

Autoclaving samples before digestion caused meat hardening so elimination of this step to improve digestion efficiency was studied in a completely randomized design with two treatments (control and treatment). Equal numbers (n=12) of the 5 g experimental units were randomly assigned to each treatment. The controls were incubated after autoclaving the sample according to the HILL and HITES method. The treatments were first incubated with papain (0.25%), bromelain (0.25%) and ficin (0.002%) and then autoclaved.

Use of water for separation

To avoid the use of noxious chemicals, water was assessed as a medium for washing the supernatant. A completely randomized design with two treatments was used to compare separation with water or with acetone, CCl_4:acetone mixture and ethyl ether. Equal numbers of the 5 g experimental units (n=30) were incubated in 0.25% papain, 0.25% bromelain, and 0.002% ficin, then washed with acetone and CCl_4:acetone mixture (HILL and HITES, 1968) or water.

Statistical comparisons were made using the GLM procedure of SAS (SAS, 1999).

RESULTS AND DISCUSSION

Effects of multiple enzymes and incubation times

As shown in Table 2, there were no differences ($P<0.05$) as a result of the enzyme concentrations or the time of incubation. This meant that a fairly low enzyme concentration (0.25%) and shorter incubation times could be used.

Table 2. Effect of incubation time and concentration on bone separation efficiency

Concentrations of papain and bromelain %	2 h incubation %bone (mean ± s.e.)	4 h incubation %bone (mean ± s.e.)	HILL and HITES (1968) method (papain only) %bone (mean ± s.e.)
0.0025	7.55 ± 0.75	7.87 ± 0.63	
0.025	7.35 ± 0.94	7.60± 0.71	
0.25	8.17 ± 0.52	9.07 ± 1.11	
0.5	8.92 ± 0.31	8.18 ± 0.83	6.78 ± 0.47 (n=7)
1.0	8.08 ± 0.65	8.36 ± 0.41	

Effects of timing of autoclaving

Samples incubated in 0.25% papain, 0.25% bromelain, and 0.002% ficin were autoclaved before or after incubation. Bone recovery was 5.39 ± 0.55% for the samples autoclaved before incubation and 6.20 ± 0.35% for the samples autoclaved after incubation. While these differences were not statistically significant, bone recovery was increased on a numerical basis by autoclaving after incubation.

Effects of separation with water

Samples incubated with 0.25% papain, 0.25% bromelain and 0.002% ficin were separated with an acetone and CCl_4:acetone mixture (HILL and HITES, 1968) or with water. The bone fragment recovery was 6.00 ± 0.34% for the 15 samples using acetone wash and 6.65 ± 0.38% for the samples washed with water. These results showed that acetone and carbon tetrachloride could be replaced with water in the bone separation procedure.

CONCLUSIONS

These preliminary results show that bone fragment separation can be greatly simplified from the method published by HILL and HITES (1968). This simplification will provide equal or improved separation of bone and soft tissues in a shorter period of time without

the use of environmentally hazardous chemicals. Further work is under way to validate the method.

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