INCORPORATION OF BONE PROTEIN EXTRACTS INTO COOKED SAUSAGES

H. W. Ockerman and H. A. Caldironi

The Ohio State University, Columbus, and The Ohio Agricultural Research and Development Center, Wooster, Ohio, U.S.A. and Instituto de Investigaciones Bioquimicas, UNS-CONICET, Bahia Blanca, ARGENTINA.

INTRODUCTION

Bones are a good source of proteins which have proven to be useful as an animal feed supplement (Nash and Mathews, 1971). Most of the bone proteins also could be incorporated into human food since they have good functional properties and high nutritional values (Golan and Jelen, 1979).

Collagenic proteins can be solubilized from crushed bones using hot water (Duerr and Earle, 1974). However, high temperature extraction denatures the proteins and yields a gritty, non cohesive precipitate (Jelen et al., 1979). A procedure using low temperatures and alkaline solutions appears to be the most satisfactory method to extract a suitable protein concentrate to incorporate into human foods (Toledo, 1973; Golan and Jelen, 1979).

This research evaluated the emulsifying capacity (EC) and water holding capacity (WHC) of bone proteins extract, and the acceptability of these concentrates as a partial substitute for meat in sausages.

EXPERIMENTAL

Bone protein extracts were prepared from beef bones (in natural proportions) which were ground so that the largest particle size was 1.2 cm (maximum diameter).

Three experiments were performed, using 2 batches of material each time. A batch consisted of 3,000 g of ground bones, 3,000 ml distilled water and 50 ml of 0.3N NaOH (pH of mixture approximately 10.0). This mixture in a plastic bag was tumbled for 1 hour (12 rpm) at 4°C and maintained at this temperature for an additional period of 24 hours. The slurry was separated by filtration through cheese-cloth and the proteins were precipitated from this red solution by adjusting (0.5N HCl) the pH to 5.6. After centrifugation (10 minutes at 3,000 x g) the supernatant containing most of the fat was separated and a pink, meaty paste was collected.

Plasma proteins were obtained from beef blood collected (1.5 liters) during slaughter into a jar containing 1.5 liters of 0.9% NaCl solution and 0.5% Na-citrate to prevent coagulation. The blood solution was cooled 24 hr at 4°C before the separation of plasma by centrifugation at 3,000 x g for 10 minutes at 4°C. The proteins were isolated according to the procedure of Caldironi and Ockerman (1981).

Moisture, total protein, fat and ash contents were determined according to Ockerman (1980). Water holding capacity (WHC) was measured by the press technique described by Tsai and Ockerman (1981). Emulsifying capacity (EC) was determined as previously reported (Caldironi and Ockerman, 1981). Salt-soluble proteins were quantified by the Saffle and Galbreath (1964) procedure.

Bone protein extract and combinations with plasma proteins and sodium pyrophosphate concentrations were introduced into a sausage formula to evaluate modifications in flavor, texture, odor and color. The sausage formula was similar to that previously reported by Caldironi and Ockerman, (1981). Briefly it consisted of 36% (weight) of lean, beef trimming (7% fat), 36% regular pork trimming (28% fat), 24% of crushed ice and seasoning, cure, sugar and ascorbate.

Measured amounts of meat proteins were replaced in the sausage formula by bone protein extract (BPE) and/or plasma proteins as specified in Tables 2 and 3, maintaining the same protein content as the controls.

A panel of six experienced (averaged 10 years of meat evaluation) members evaluated the product for flavor, texture, color and odor using a scale of nine levels (Flavor: 9 - intensive bologna flavor, 5 - moderate, 1 - off flavor; Texture: 9 - hard, 5 - moderate, 1 - soft; Odor: 9 - intensive bologna odor, 5 - moderate, 1 - off odor; Color: 9 - dark, 5 - moderate, 1 - light).

The statistical analysis was conducted with the general linear model of the Statistical Analysis System (SAS Institute, 1979) and means were compared with the control by a t-test (Snedecor and Cochran, 1979).

RESULTS AND DISCUSSION

Protein extracts from several batches of ground beef bones were obtained in this experiment. Total protein, soluble protein, fat, moisture and ash from these extracts, ground bones, lean beef meat (muscle) and blood plasma proteins are given in Table 1. Protein and ash levels were in agreement with those reported by Jelen et al. (1979) who used a similar extraction procedure. The bone protein extract resulted in a very acceptable pink meaty paste with meat odor.

Emulsifying capacities, plotted as a function of total protein obtained from BPE, plasma proteins and beef muscle proteins, are compared in Figure 1a. Meat proteins showed significantly ($P<0.01$) higher EC values than plasma proteins and bone extracts. Lower values were obtained with bone protein extract.

Among sausage ingredients, soluble protein has been shown to play an important role in the ability to emulsify fat (Hason, 1960). According to Satterlee et al. (1973), the quality of salt (1N NaCl) soluble proteins can be determined by comparing EC values on a per 100 mg of soluble protein basis. In the current research EC values (ml oil/100 mg soluble protein) were approximately the same for beef muscle protein and bone protein extract but those from plasma proteins were approximately 2 to 3 times higher (Figure 1b).

Table 1 - Composition of bones and protein sources, and WHC values of the protein extracts.

	Ground bones[a]	BPE[b]	PP[c]	Beef muscle[d] Sample A	Beef muscle[d] Sample B
Moisture %	14.4(0.5)	84.2(3.0)	79.9(1.8)	78.0(0.1)	77.9(0.2)
Total protein %	22.4(0.8)	9.9(1.7)	18.4(2.0)	19.4(0.3)	19.7(0.7)
Soluble protein (as % total prot.)[e]	--	35.7(0.4)	16.0(0.1)	62.3(0.3)	--
Fat %	15.5(2.9)	1.9(0.3)	0.9(0.2)	1.5(0.3)	2.5(0.4)
Ash %	42.7(2.4)	1.2(0.4)	--	1.0(0.2)	--
WHC[f]	--	81.6(5.0)	42.3(2.1)	50.6(1.4)	64.8(2.4)
Fat/protein ratio	--	0.21	0.05	0.08	0.13

BPE: bone protein extract; PP: blood plasma protein; WHC: water holding capacity

[a]Values are expressed as mean and (standard deviation); 3 experiments, each duplicated.

[b]Bone protein extract mean and (SD) of 6 different extractions.

[c]Plasma protein mean and (SD) of 6 determinations from two samples.

[d]Mean and (SD) of 3 determinations.

[e]At pH 5.6

[f]Water holding capacity values from 8 determinations, mean and (SD).

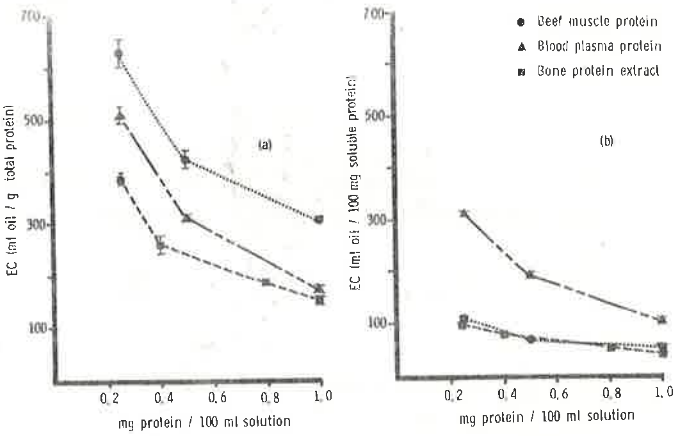

Figure 1 - Emulsifying capacities of bone protein extracts, plasma proteins and beef muscle protein. a. As a function of total protein content. b. As a function of salt soluble protein content.

Replacement of inefficient emulsifier proteins by more efficient ones was proposed by Morrison et al. (1971) as a solution to emulsion stability. To explore this, mixtures of BPE and meat proteins were prepared and the EC determined (Figure 2a). The EC values were between those of the components; other curves were dependent on the proportions of each constituent in the sample. Mixtures including up to 10% bone proteins showed no significant differences in emulsifying capacity when compared with meat proteins and mixtures with 30% bone protein extract showed even better EC values than plasma proteins which have proven to be a good fat emulsifier (Satterlee et al., 1973; Caldironi and Ockerman, 1981).

Alkaline phosphates are known to improve the EC and water holding capacity in meat and meat products (Young, 1976; Tsai and Ockerman, 1981). Particularly pyrophosphates have a synergistic effect with salt. The effect of sodium pyrophosphate on EC is shown in Figure 2b. Concentrations of 0.25% (w/w) and 0.50% (w/w) sodium pyrophosphate improve the EC values between 10 and 15% at low protein concentrations (0.25, 0.4 g/100 ml solution). At high protein concentrations (0.8 and 1.0 g/100 ml solution), 0.25% and 0.50% of sodium pyrophosphate also showed a significant improvement (4 and 8%, respectively) in these values. Emulsifying capacity of bone protein extracts plus pyrophosphate at both concentrations (0.25 and 0.50%) were not significantly different from those of plasma proteins.

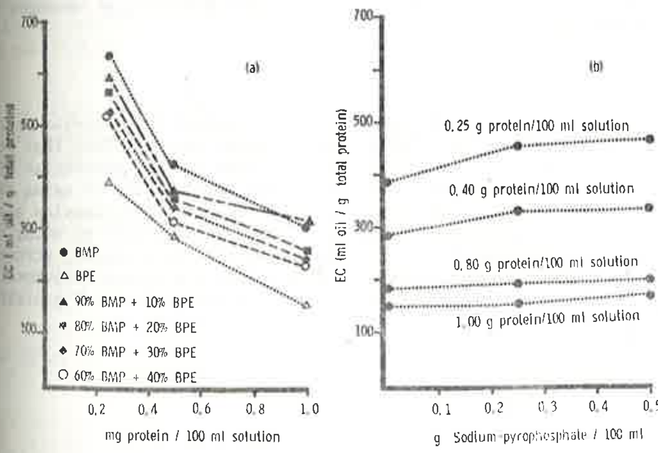

Figure 2 - Modifications in bone protein extracts (BPE) emulsifying capacity (EC) values by mixing a. with beef muscle proteins (BMP), b. with 0.25 and 0.50 gr Na-pyrophosphate.

Water holding capacity can be related to properties such as juiciness, drip loss, cooling shrink and yield on processing (Ockerman, 1980). Table 1 indicates the WHC values of bone protein extract, plasma protein and two different samples of lean beef meat (muscle). WHC values do not seem to be correlated with fat concentrations or protein levels. However, moisture contents were very similar for all the samples (79.9±2.9), and a close correlation (r=0.99) was found between WHC and fat/protein ratio. This could be related to the narrow limits between which the three components: protein, fat and water (moisture) were varied to obtain satisfactory physical properties (Morrison et al., 1971). Other proportions of moisture with different source of proteins, probably would result in different correlation (in sign or value).

Combinations of bone protein extract and plasma protein were included in sausages, since the latter can lighten the color (Caldironi and Ockerman, 1981) and convey elasticity and binding strength (Terrel et al., 1979; Suter et al., 1976) when incorporated into meat products.

Table 2 shows the sensory evaluation obtained from five types of sausages including combinations of 10% and 20% of bone protein extract, both plus 0.2% (w/w) sodium pyrophosphate, 10% bone protein extract + 10% plasma protein and a control.

Table 2 - Sensory evaluation panel values[a/] for different combinations of meat with bone protein extract (BPE), plasma protein (PP) and phosphate (0.2% (W/W) sodium-pyrophosphate).

	Flavor	Texture	Odor	Color
Control	4.8(1.9)	5.0(0.8)	4.6(0.6)	5.6(1.1)
10% BPE	4.8(1.3)	5.0(1.4)	4.8(1.6)	5.6(1.1)
10% BPE + Phosphate	5.6(1.3)	4.8(1.5)	5.0(1.6)	6.2(0.8)
20% BPE	3.8(1.8)	3.6(1.3)	4.4(0.9)	5.4(1.3)
20% BPE + Phosphate	5.4(1.9)	2.8(1.1)[b]	5.2(1.3)	6.0(0.7)
10% BPE + 10% PP	4.2(1.8)	5.0(1.0)	4.0(1.7)	6.4(0.9)

[a/] Values are for 5 determinations and are given as the mean and (standard deviation).

[b/] Significantly different from the control ($P < 0.01$). The rest of the values were not significantly different from the respective controls ($P < 0.1$).

Flavor was a limiting factor for acceptability when more than 20% bone protein extract or 10% bone protein extract + 10% plasma protein were included in sausages. Samples containing phosphate displayed more intensive flavor than the respective ones without phosphate. Some panel members objected to the flavor which was reflected in a larger standard deviation; however, differences were not statistically significant ($P<0.1$) from the control.

Preparations containing 20% bone protein extracts plus phosphate showed a significantly ($P<0.01$) lower value in texture than the controls. Although a similar tendency was observed in samples containing 20% BPE and no phosphate, these values did not differ significantly ($P<0.1$) from the controls.

No differences were found for odor except in the samples containing blood plasma but again these differences were not large enough to be statistically different from controls.

Color was judged as very acceptable in all the samples with no large differences observed. Samples with 10 and 20% bone protein extract were slightly darker but the values improved when phosphate or plasma protein were added.

Sausages (bolognas) containing 10% bone protein extract, 10% bone protein extract plus 5% plasma protein and these combinations plus 0.4% (w/w) sodium pyrophosphate were compared with control samples (Table 3) by a sensory panel. Non-significant differences from controls were obtained for all samples. Some objection to texture was expressed with samples containing phosphate which resulted in a large standard deviation. In addition, yield of samples during cooking with phosphate increased approximately 10% when compared with appropriate samples without phosphate (values not shown).

Bone protein extracted by alkaline solutions at low temperature have good functional properties and are acceptable in flavor, texture, odor and color when incorporated into sausages up to 20% of the protein content. Some objection to flavor, texture and odor was noted when this proportion was exceeded. Bone protein extract at the proportions tested were capable of holding sufficient water, and therefore the addition of phosphates is not recommended since the altered WHC causes the texture to become softer. Substitution of 10% bone protein extract or combinations of bone protein extract and plasma protein up to 15% for beef proteins in sausages gave both good functional properties and a very acceptable product.

Table 3 - Sensory evaluation panel values[a/] for cooked sausages containing different proportions of bone protein extract (BPE), plasma protein (PP) and phosphate (0.4% (W/W) sodium pyrophosphate).

	Flavor	Texture	Odor	Color
Control	6.6(0.5)	6.2(0.8)	5.8(1.5)	6.0(0.9)
10% BPE	6.6(0.5)	5.8(0.8)	6.0(1.4)	6.3(0.8)
10% BPE + Phosphate	6.4(1.3)	6.0(1.5)	5.8(1.5)	6.3(1.0)
10% BPE + 5% PP	6.4(0.9)	6.0(1.3)	6.6(1.1)	6.0(1.1)
10% BPE + 5% PP + Phosphate	6.8(0.4)	6.0(1.7)	6.6(1.1)	6.0(0.9)

[a/] All determinations are for 6 panelists scores and are given as the mean and (standard deviation). Values shown were not significantly different from the control ($P < 0.1$).

Considering the future demand for proteins in the world, the rising cost of meat, and the fact that bones and other by-products represent a high percentage of the total weight of animals, the possibility of using these in the production of foods for human consumption should be seriously considered. The possibility of using animal by-products in the meat industry in a simple, economical way for the production of foods for human consumption could be a response to the future demand for protein.

REFERENCES

Caldironi, H. A. and Ockerman, H. W. 1981. Incorporation of blood proteins into sausage. Journal Article No. 119-81 Ohio Agricultural Research and Development Center. Accepted for publication J. Food Sci..

Duerr, P. E. and Earle, M. D. 1974. The extraction of beef bones with water, dilute sodium hydroxide and dilute potassium chloride. J. Sci. Fd. Agric. 25:121.

Golan (Goldhirsh) A. and Jelen, P. 1979. Nutritional evaluation of low temperature alkaline extracts from beef bones. J. Food Sci. 44:332.

Hason, L. J. 1960. Emulsion formation in finely comminuted sausage. Food Technol. 14:565.

Jelen, P., Earle, M. and Edwardson, W. 1979. Recovery of meat protein from alkaline extracts of beef bones. J. Food Sci. 44:327.

Morrison, G. S., Webb, N. B., Blumer, T. N., Ivey, F. J. and Haq, A. 1971. Relationship between composition and stability of sausage-type emulsions. J. Food Sci. 36:426.

Nash, M. A. and Mathews, R. J. 1971. Food protein from meat and bone meal. J. Food Sci. 36:930.

Ockerman, H. W. 1980. Quality Control of Post-Mortem Muscle Tissue. Vol. 1, 11th Ed., Dept. Animal Science, The Ohio State University, Columbus, OH.

Saffle, R. L. and Galbreath, J. W. 1964. Quantitative determination of salt-soluble protein in various types of meat. Food Technol. 18:1943.

SAS Institute. 1979. SAS User's Guide. 1979 Edition SAS Institute Inc. Gary, NC.

Satterlee, L. D., Free, B. and Levin, E. 1973. Utilization of high protein tissue powders as a binder/extender in meat emulsion. J. Food Sci. 38:306.

Snedecor, G. W. and Cochran, W. G. 1979. Statistical Methods. 5th Ed. The Iowa College Press, Ames, Iowa.

Suter, D. A., Sustek, E., Dill, C. W., Marshall, W. H. and Carpenter, Z. L. 1976. A method for measurement of the effect of blood protein concentrates on the binding forces in cooked ground beef patties. J. Food Sci. 41:1428.

Terrell, R. N., Weinblatt, P. J., Smith, G. C., Carpenter, Z. L., Dill, C. W. and Morgan, R. G. 1979. Plasma protein isolate effects on physical characteristics of all-meat and extended frankfurters. J. Food Sci. 44:1041.

Toledo, R. T. 1973. Preparation and properties of low temperature extracted animal protein concentrates. J. Food Sci. 38:141.

Tsai, T. C. and Ockerman, H. W. 1981. Water binding measurement of meat. J. Food Sci. 46:697.

Young, L. L. 1976. Composition and properties of an animal protein isolate prepared from bone residue. J. Food Sci. 41:606.