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The Recovery and Nutritional Evaluation of Alkali Extracted Protein Coagulates from Crushed Bone Residues

Krystyna Palka,* Zdzisław E. Sikorski† & Maria Rakowska‡

* Agricultural Academy of Kraków, Faculty of Agriculture Kraków; † Technical University Politechnika Gdańska, Faculty of Chemistry, Gdańsk; ‡ Institute of Breeding and Reclimatization of Plants, Radzików, Poland

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ABSTRACT

Non-collagenous proteins were recovered with 80% yield from crushed beef and pig bone residues leaving a commercial deboner, by single step extraction at room temperature with NaOH solution at pH 10·5–10·7 followed by precipitation at pH 4·5–5·0. The indices of nutritional value of the proteins, calculated on the basis of amino acid composition, were similar to those of meat. No lysinoalanine was detected by thin-layer and ion-exchange chromatography above the detection limit of 100 μg/g and 80 μg/g protein, respectively. The results of rat growth and nitrogen balance experiments were similar to those obtained for control diets.

INTRODUCTION

The crushed bone leaving the industrial mechanical separators in abattoirs still contains residual soluble proteins. These crushed bones are utilized mainly for the production of fodder meals, but after removal of the non-collagenous proteins they can be converted to gelatin. In gelatin factories the non-collagenous proteins removed in the first stages of the process, if recovered, are at best used for fodder purposes.

Soluble proteins have been extracted with NaOH solutions from mechanically separated poultry residues and from meat industry

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byproducts by several investigators (Hamilton, 1978; Jelem *et al.*, 1979, 1982). After acid precipitation, the coagulate has been used as a component in sausages and luncheon meats. However, alkali treatment of proteins may lead to nutritionally objectionable changes in amino acid residues, mainly due to the formation of lysinoalanine (LAL) and lanthionine (LAN) with a corresponding loss of cystine and lysine (Whitaker, 1980) and racemization of some residues to the D-forms. According to numerous published results, especially by Friedman and co-workers (Masters & Friedman, 1980), the rate of these undesirable changes in proteins is significantly high only at pH about 13 and temperature above 30 °C.

Thus it seemed reasonable to discover whether soluble proteins could be extracted from crushed bone residues with sufficient yield at room temperature with alkaline solutions at pH below 11, whether the acid-precipitated coagulate contained detectable LAL and whether the product had a nutritional value comparable to that of the original meat.

MATERIALS AND METHODS

Beef and pig bone residues from a commercial Seffelaar and Loyen deboner were extracted (Fig. 1) in a vertical cylindrical extractor of 18 litres.

The fat content in the bones and in the products was determined in a Soxhlet apparatus using ligroin as the solvent.

The crude protein content in the raw material and in the products was calculated from the Kjeldahl nitrogen using a conversion factor of 6·25. Collagen was determined colorimetrically after Stegman & Stadler (1967); the conversion factor was 7·46. The PAG electrophoresis of proteins was carried out after Weber & Osborn (1969). The samples for electrophoresis were dissolved in 0·01 M sodium phosphate buffer at pH 7·0, 1 % in SDS and 1 % β-mercaptoethanol. The protein concentration in the samples was 0·2–0·6 mg/cm^3 and 7·5 % acrylamide gels were used. The amino acid composition was determined in the Beckman 119 CL analyser after 24 h hydrolysis in 6 M HCl at 110 °C. The sulphur amino acids were oxidized by performic acid prepared by mixing 88 % formic acid and 30 % hydrogen peroxide in the ratio 9:1. The tryptophan content was assayed using the spectrophotometric method (Guiragossian *et al.*, 1977) with

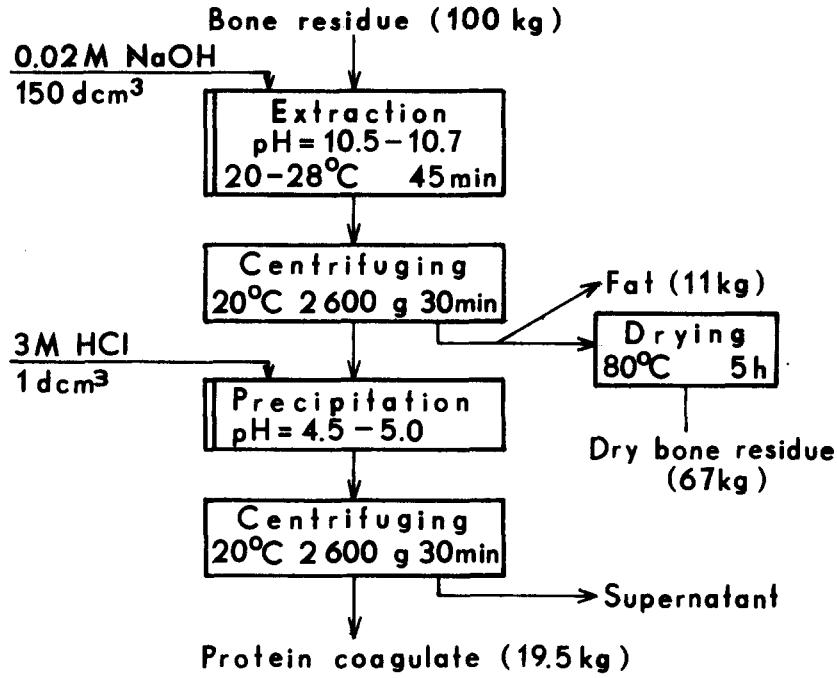

Fig. 1. A flow sheet of the process of protein recovery from bone residues.

glyoxylic acid after hydrolysis of 100 mg samples of the proteins with papain.

On the basis of the amino acid composition of the protein coagulates, % EAA, CS and EAA_{index} were calculated using whole egg protein as a standard. PER was calculated using the equations (Alsmeyer *et al.*, 1974):

(1) PER = $-0{\cdot}684 + 0{\cdot}456$ (Leu) $- 0{\cdot}047$ (Pro)
(2) PER = $-0{\cdot}468 + 0{\cdot}454$ (Leu) $- 0{\cdot}105$ (Tyr)
(3) PER = $-1{\cdot}816 + 0{\cdot}435$ (Met) $+ 0{\cdot}780$ (Leu) $+ 0{\cdot}211$ (His) $- 0{\cdot}944$ (Tyr)

The LAL content was determined after Fritsch and Klostermeyer. The detection limit was 80 μg LAL/g protein. Also thin-layer chromatography (TLC) was used to detect LAL and LAN. About 500 mg of the coagulate were hydrolysed for 24 h with 20 ml 6 M HCl at 110 °C. After cooling the hydrolyzates were filtered, evaporated at 40 °C, twice dissolved in 20 ml of deionized water and evaporated, and dissolved in 10 ml of water. Five microlitres of the final solution was applied on 0·25 mm layers of cellulose MN 300. The chromatograms were developed in *n*-butanol: 88 % formic

acid:water = 23:4:5 to about 17 cm from the start, dried at room temperature, sprayed with ninhydrin reagent, dried at 100 °C and sprayed with a solution containing 2 mg $CuSO_4 \cdot 5H_20$, 10 ml of distilled water, and 30 ml of 95 % ethanol. The detection limit of LAL was 100 μg/g protein.

In the 14 days growth experiments two groups of eight Wistar rats of 45–50 g body weight, were fed with isoprotein diets. The coagulate mixture from bovine and pig bones (1:1) was tested against casein supplemented with 1 % methionine. The diet fed *ad libitum* contained about 70 % wheat starch, 10 % crude protein, 8 % fat, 5 % salts, 5 % water, 1 % fibre, and 1 % vitamins. The net protein ratio (NPR) was calculated as

$$\mathrm{NPR} = \frac{\Delta W + \Delta W_0}{P_i}$$

where: ΔW = body mass gain of rats after 2 weeks of experiment, ΔW_0 = loss of mass of rats on proteinless diet, P_i = protein intake.

In the 4 day nitrogen balance study after the growth experiments, rats of 85–113 g in body weight were used and also growing rats (43–47 g each), firstly acclimatized during 4 days to the tested diet. The results were used for calculating the metabolic faecal nitrogen: $F' = (0{\cdot}081x + 3{\cdot}01) \times 5$, where x = mean mass of each animal in the experiment. Endogenous urinary nitrogen: $U' = (0{\cdot}147x + 19{\cdot}43) \times 5$. True digestibility: $D_t = (N_i - [F - F']/N_i) \times 100(\%)$, where: N_i = nitrogen intake, F = faecal nitrogen. Biological value: $\mathrm{BV} = (N_i - [F - F'] - [U - U']/N_i - [F - F']) \times 100(\%)$, where U = urinary nitrogen, and net protein utilization: $\mathrm{NPU} = (D_t \times \mathrm{BV})/100(\%)$.

RESULTS AND DISCUSSION

Single step (45 min) alkali extraction at pH 10·5–10·7 at room temperature (Fig. 1) of crushed bone residue, containing about 2 % of non-collagenous proteins (Table 1), removed from the raw material about 80 % of these proteins. By acidifying the extract with 3 M HCl to pH 4·5–5·0, about 95 % of the dissolved proteins could be precipitated. The overall yield of protein in the coagulate was 77 % in respect to the content of soluble proteins in the raw material.

The electrophoretic pattern of the coagulate proteins (Fig. 2) is typical for myofibrillar proteins in a large range of pH used for precipitation.

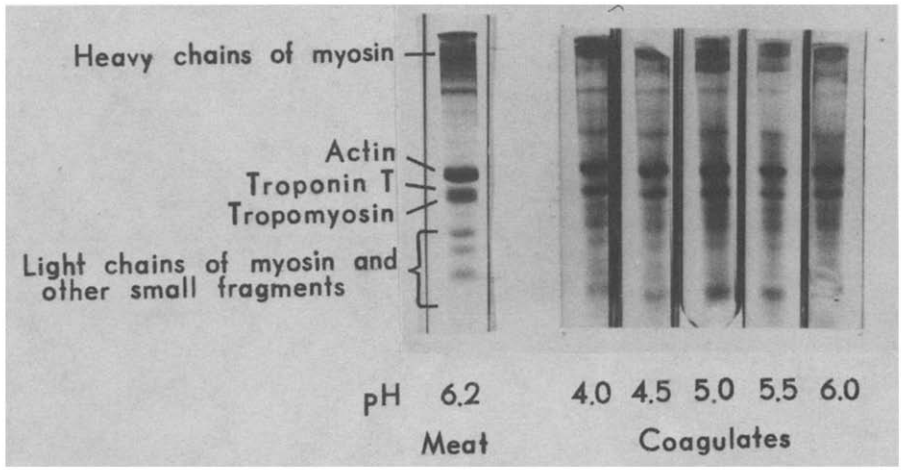

Fig. 2. A typical electrophoretic pattern of proteins of the coagulate.

Also the amino acid composition of the product is similar to that of whole meat proteins (Table 2). Thus the indices of nutritional value of the proteins are similar to those of meat (Table 3) and the limiting amino acids in the coagulate, as in meats, are the sulphur-containing residues.

The presence of LAL in the coagulates was not detected above the detection limits of 100 μg/g protein and 80 μg/g protein, respectively, of the TLC and ion-exchange chromatography (Tables 2 and 4). Similar results regarding the contents of LAL in alkali-extracted chicken protein

TABLE 1
The Chemical Composition of Raw Materials and Coagulates ($n = 20$)

Sample		*Total crude protein Mean ± s.d* (%)	*Collagen Mean ± s.d.* (%)	*Fat Mean ± s.d.* (%)	*Water Mean ± s.d.* (%)	*Ash Mean ± s.d.* (%)
Bone residue from a commercial deboner	Pork	20·8 ±1·26	18·6 ±0·27	16·3 ±1·50	33·8 ±1·55	29·2 ±1·85
	Beef	20·6 ±0·81	18·7 ±0·41	18·8 ±1·02	26·4 ±1·38	34·2 ±1·29
Coagulate	Pork	9·01 ±0·80	0·01 —	2·88 ±0·50	87·8 ±0·90	0·30 ±0·03
	Beef	9·11 ±0·70	0·01 —	2·80 ±0·30	88·2 ±0·70	0·30 ±0·03

TABLE 2
Amino Acids in Hydrolysates of Protein Coagulates and Pork Loin (g/100 g protein)

Amino acid	*Protein coagulate*		*Pork Loin*
	Pork	*Beef*	
Cys	1·08	1·08	0·98
Asp	9·55	9·27	8·90
Met	2·73	2·85	2·92
Thr	4·43	4·54	4·94
Ser	4·38	4·24	3·96
Glu	15·00	15·2	15·8
Pro	4·28	4·05	3·77
Gly	4·24	3·99	4·01
Ala	7·27	7·34	7·29
Val	5·14	5·23	4·71
Ile	4·74	4·99	4·79
Leu	8·94	9·10	7·90
Tyr	3·52	3·26	3·58
Phe	4·30	4·45	4·11
His	2·81	2·84	4·36
Lys	8·66	8·78	9·64
Arg	7·78	7·43	6·88
Trp	1·17	1·38	1·44
LAL	0	0	—

Hydroxyproline was not determined in the amino acid analyser.

TABLE 3
Nutritional Value of Protein Coagulates and Pork Loin

Specification	*Pork protein coagulate*	*Beef protein coagulate*	*Pork loin*
Eqn 1	3·2	3·3	2·7
Eqn 2	3·2	3·3	2·7
Eqn 3	3·6	4·0	3·2
% EAA	40·1	41·3	40·4
% EAA index[a]	84·1	87·0	85·2
CS	66·8 Met + Cys	69·0 Met + Cys	68·4 Met + Cys

[a] With Cys and Tyr.

TABLE 4
R_f Values of the First Three Spots of Amino Acids on TLC Plates

Kind of sample	R_f			
Coagulate pH = 4·5[a]	—	0·23	0·27	0·32
Coagulate pH = 5·0	—	0·23	0·27	0·31
Coagulate pH = 5·3	—	0·22	0·26	0·31
Standard LAL	0·10	—	—	—
Standard LAN	0·14	—	—	—

[a] pH of precipitation.

TABLE 5
Biological Value of Protein Coagulate

Specification	*Diet*		*n*
	With coagulate	*With casein*	
NPR	4·6 ± 0·2	4·6 ± 0·2	8
ΔW (g)	52·0 ± 7·6	52·4 ± 4·1	8
D_t (%)	96·2 ± 1·4	96·1 ± 1·0	13
BV (%)	77·3 ± 2·2	91·5 ± 2·7	13
NPU (%)	74·3 ± 2·0	87·9 ± 2·5	13

at 20 °C have been published by Lawrence & Jelen (1982). Thus, at 10 % coagulate protein in the diet, the maximum content of LAL in the feed was below 8 μg/g and should not be expected to cause any harmful effects in the biological experiments. The quantities of LAL which caused nephrocytomegaly in the kidneys of rats has been shown by Feron *et al.* (1978) to be 30 (LD-isomer) to 1000 (DD-isomer) μg/g of the diet.

The results of the growth and nitrogen balance experiments have shown indeed (Table 5) that only the BV and NPU of the protein coagulate were about 13 % lower than those of the methionine-fortified casein. However, NPU for beef, pork loin, and milk powder are 79, 72 and 83 %, respectively (Rakowska *et al.*, 1978).

The protein content in the coagulate, being only 9 %, is significantly lower than in meat. Although in laboratory conditions the microbiological contamination of the coagulate was not very significant (Table 6), a product of such high water activity must be treated as most perishable

TABLE 6
The Microbiological Contamination of Raw Materials and Protein Coagulates

Sample	*n*	*Total count of aerobic micro-organisms in 1 g*
Beef bone residues	12	$1{\cdot}2 \times 10^5$–$2{\cdot}1 \times 10^6$
Pork bone residues	21	$3{\cdot}0 \times 10^4$–$9{\cdot}6 \times 10^5$
Coagulate from beef bone residue	3	$3{\cdot}0 \times 10^5$
Coagulate from pork bone residue	3	$4{\cdot}0 \times 10^5$

and should be immediately frozen. Our preliminary experiments have shown that the frozen coagulate could have been used as a meat extender in sausages in quantities not higher than about 10% in respect to meat, without causing undesirable changes in texture.

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