FUNCTIONAL PROPERTIES OF PROTEIN ISOLATE EXTRACTED FROM BEEF BONES

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Abstract

Bone protein isolate (BPI) extracted from beef bones with 2% added salt *(i)* and without salt *(ii)* has been analysed for functional properties as these properties are of importance when the protein isolate is used in different industries.

Water absorption by the bone protein isolate *(i)* and *(ii)* was 0.35 and 0.46 H_2O/g protein, respectively.

The minimum solubility of protein isolate *(i)* was 65.85% at pH 3 while isolated protein *(ii)* had a minimum solubility of 52.30% at pH 5. An increase in solubility was observed below and above pH 3 for the protein isolate *(i)* (83.66% at pH 1 and 87.12% at pH 11) and below and above pH 5 for protein isolate *(ii)* (83.20% at pH 1 and 89.78% at pH 11).

The emulsifying capacity of the BPI is great, being much better than that of many classical proteins (sodium caseinate, soy protein isolate). The emulsifying capacity (ml oil/g protein) progressively decreased with increasing protein concentration. On the other hand, alkaline pH improved the mentioned property more than acidic pH. The maximum emulsifying capacity was 1152.60 and 986.78 ml oil/g protein at pH 9 for protein isolate *(i)* and *(ii)* respectively.

The best foaming capacity was observed at pH 4 for both protein isolates *(i)* and *(ii)* (96% and 89% volume increase, respectively). Foams had also high stability at pH 4, having 145 ml and 138 ml final volume for isolates *(i)* and *(ii)*, respectively. The stability of foams decreased with increase in pH.

Both protein isolates obtained had high solubility (NSI) and dispersibility (PDI); being 97.98%, 86.78% for NSI and 99.94%, 89.06% for PDI in case of isolates *(i)* and *(ii)*, respectively. Such isolates had a satisfactory protein solubility in the pH range existing in many food products.

Introduction

The search for new sources of protein became necessary to meet the increasing demand for proteins. One of the potentially important sources of protein is bone residue from meat deboning processes (YOUNG, 1975, 1976; JELEN et al. 1979).

To be useful and successful in food applications, proteins, in addition to providing essential amino acids should also possess several desirable char-

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acteristics referred to as functional properties (LÁSZTITY, 1988, RUTKOWSKI, 1986), namely emulsifying capacity, foaming capacity, solubility etc., which enable them to be successfully used in supplementing or substituting traditional proteins.

Emulsifying capacity of various purified beef muscle proteins was descendingly ranked from highest to lowest by HEGARTY et al. (1963).

ELDRIDGE et al. (1963) studied some factors (pH, salts, temperature etc.) affecting foam expansion, i.e. volume increase of soy protein foams.

Denatured proteins do not have good functional properties such as solubility, water binding ability, emulsifying and foaming capacities, as reported WARRIER et al. (1981).

YOUNG (1976) suggested that protein from bone residue might have some use as a food ingredient.

This work was designed to study the functional properties of the protein isolate extracted from beef bones as these properties are of the utmost importance when the bone protein isolate is used in different food industries.

Materials and methods

Materials

Cattle bones used in this study were obtained from 18–24 month old male animals from Mansoura slaughter house.

Methods

1. Protein extraction: Protein was extracted from bones according to the procedure described by PETER and MARY (1974) and HIROTOMO (1981) with appropriate modifications. 3000 g of finely ground bone material was mixed with 3000 g of tap water. The mixture was heated in an autoclave at a pressure of 2.2 kg/cm^2 for 2 hrs. The fat floating on the surface of the extract was skimmed. The extract was filtered to remove sedimented bone fragments in order to produce an extract with 10% solid concentration. The filtrate was mixed with 0.4 H_2O_2 at 70 °C for discoloration of the extract. The extract was divided into two batches, one batch only was mixed with 2% salt, then both batches were evaporated to dryness on a water bath, packed and stored at room temperature for subsequent analysis.

2. pH of protein isolate: 10 g of protein isolate was homogenized with 90 ml of distilled water for 2 minutes, then the pH of the homogenate was measured using a pH meter, Corning 3 model.

3. Water absorption was measured according to the method described by SMITH and CIRCLE (1939) for sunflower with appropriate modifications.

4. Solubility was determined according to the method of WARRIER and NINJOUR (1981).

5. Emulsifying capacity was determined according to the method described by CARPENTER and SAFFLE (1964). The emulsifying capacity of bone protein isolate was determined at concentrations of 0.5, 0.6, 0.7, 0.8, 0.9 and 1% protein isolate. The emulsifying capacity of 1% protein isolate at pH 4, 5, 6, 7, 8 and 9 was also determined.

6. The foaming capacity was determined according to the method described by RASEKH (1974). Foaming capacity of 1% protein isolate was determined for pH 4, 5, 6, 7, 8 and 9.

Foaming stability at different pH values was also determined after holding foams at 20 °C for 10, 20, 30, 40, 50 and 60 minutes.

% Nitrogen solubility index (NSI) and nitrogen dispersibility index (PDI) were determined according to the methods of A.O.C.S. (1973).

Results and discussion

It is worth to mention that bone protein isolate extracted from beef bones with 2% added salt contained 5.48% moisture, 71.40% protein, 2.36% fat and 21.02% ash, while bone protein isolate without salt consisted of 5.40% moisture, 83.65% protein, 8.36% fat and 3.62% ash on wet weight basis as found previously by ABDEL-GAWWAD and SHALABY (1986).

The bone protein isolate (BPI) we obtained has been analysed for functional properties, i.e. water absorption, solubility, emulsifying capacity, foaming capacity, nitrogen solubility index (NSI) and protein dispersibility index (PDI).

The pH value of salted protein isolate was 5.90, while the pH of unsalted protein isolate was 5.84. This difference in pH value might be due to the high concentration of sodium chloride.

Water absorption

Data presented in Table 1 showed that water absorption was 0.35 and 0.46 g/g protein for the salted and unsalted protein isolates, respectively. The unsalted protein isolate had a higher water absorption capacity than the salted one which was most probably due to its high protein content (83.65%) (the principal water binding substance) as compared with salted isolate (71.40%).

These values of water absorption of BPI were lower than those reported by SOSULSKI and FLEMING (1977) for soy protein isolate (3.6 g/g protein) and

Table 1

pH value and water absorption of beaf bone protein isolate

Trait	Salted protein isolate	Unsalted protein isolate
pH	5.90	5.84
Water absorption (%)	35	46

sunflower protein isolate (3.9 g/g protein) possibly due to the presence of fat and because of the high solubility of protein. However, the water absorption of proteins varied substantially depending on the method used in the determination (LIN et al. 1974).

Solubility of protein

From results in Table 2, salted bone protein isolate had a minimum solubility of 65.85% at pH 3; the solubility increased as the pH increased beyond 3 at which the protein was almost soluble (87.12%), i.e. at pH 11. An increase in solubility was also observed below pH 3; being 83.66% at pH 1. On the other hand, unsalted protein isolate had a minimum solubility of 52.30% at pH 5. The increase in solubility was moderately rapid as the pH increased beyond 7, and 89.78% of the protein was found to be soluble at pH 11. The solubility of unsalted protein isolate also increased below 5, 83.20% of the protein was soluble at pH 1.

It is suggested that the variation in solubility of the protein isolate may be explained on the basis of the ionogenic groups present in protein molecules in the lyophilic colloidal systems of protein solutions. Since the ionogenic

Table 2

Solubility of bone protein isolate as a function of pH

pH	Solubility of protein (%)	
	Salted protein isolate	Unsalted protein isolate
1	83.66	83.20
3	65.85	60.74
5	72.54	52.30
7	78.29	80.34
9	82.30	86.02
11	87.12	89.78

groups of protein are present largely at the isoelectric point of protein at given pH value, the protein molecule would precipitate and the protein become less soluble. Results of solubility of BPI are in general in agreement with those observed with beef haemoglobin and non-fat dry milk (CRENWELGE et al. 1974). YOUNG (1976) reported that the solubility of proteins extracted from chicken bones slightly decreased below pH 7.5 and increased above this pH value.

From data presented in Table 2 it could be also noticed that the unsalted bone protein isolate was less soluble than the salted one at pH values 1, 2, 3, 4, 5 while the solubility of unsalted protein isolate was higher at pH values 6, 7, 8, 9, 10, 11 although the solubility behaviour of salted and unsalted protein isolate was generally similar.

Emulsifying capacity

The emulsifying capacity of salted and unsalted bone protein isolate has been determined for concentrations of protein ranging from 0.5 to 1.0 g/100 ml water. From Table 3, the emulsifying capacity progressively decreased with increasing protein concentration. This finding concurred with that observed with meat proteins (ACTON and SAFFLE, 1972). IVEY et al. (1970) also concluded that as the protein concentration increased, the emulsifying capacity of meat proteins decreased. The decrease in emulsifying capacity (Table 3) could be attributed to the "overloading of the system", i.e. part of the emulsion remained on the side of the jar and there was incomplete mixing as the end point was approached (SAFFLE, 1968), or to the formation of thicker emulsifying agent (protein) layers caused by increasing protein concentration on the fat droplets, thus using more continuous phase per drop as suggested by IVEY et al. (1970).

So, from Table 3, it could be concluded that the emulsifying capacity as ml oil/g protein decreased with increasing protein concentration for both salted

Table 3

Emulsifying capacity of BPI (ml oil/g protein) as a function of protein concentration (PC) and pH and at a protein concentration of 1%

PC%	Emulsifying capacity ml oil/g protein		pH	Emulsifying capacity ml oil/g protein	
	Salted protein	Unsalted protein	PC + 1%	Salted protein	Unsalted protein
0.5	1563.48	1451.32	4	841.32	589.36
0.6	1433.19	1304.92	5	924.18	637.66
0.7	1260.48	1136.25	6	1002.92	790.05
0.8	1197.86	1053.44	7	1044.48	843.53
0.9	1089.79	963.54	8	1088.69	891.67
1.0	1022.12	891.83	9	1152.26	986.78

and unsalted protein isolates and the emulsifying capacity of unsalted BPI was lower than that of the salted one. This may be ascribed to the high protein content in the unsalted protein isolate (83.65%) when compared with salted protein isolate (71.40%). These results are in agreement with data given by SWIFT et al. (1961) who found that increasing the saline to meat ratio resulted in more complete protein solubilization which increased the ability of the meat to emulsify large quantities of fat.

As shown by results in Table 3, the emulsifying capacity increased with increasing pH for both salted and unsalted protein isolate. This dependence of the emulsifying capacity on pH was also proved by YOUNG (1976) who found that the emulsifying capacity was relatively low at low pH but improved as the pH increased.

It could also be observed from data presented in Table 3 that the emulsifying capacity for salted BPI was higher than for the unsalted one, being 841.32 and 589.36 ml oil/g protein at pH 4 and 1152.26 and 986.78 ml oil/g protein at pH 11 for salted and unsalted BPI, respectively.

Reviewing the data presented in Tables 2 and 3 it could be noticed that the emulsifying capacity of BPI was affected by the pH similarly to the solubility of protein isolate. This observation is in agreement with that made by SWIFT et al. (1961) and YOUNG (1976).

Foaming capacity

The capacity to form stiff and stable foams is an important characteristic required for proteins to be used in some food products. From data in Table 4, both salted and unsalted protein isolate had low foaming capacities (53 and 34% volume increase, respectively) as compared with sunflower (235%) (LIN et al. 1974).

From Table 4, the stability of the foam prepared from protein isolate was also low (complete collapse within 30 min at 20 °C). This low foaming ability

Table 4

Foaming capacity and foaming stability of bone protein isolate

Trait	Salted protein isolate	Unsalted protein isolate
Foaming capacity %	53	34
Foaming stability %:		
after 10 min.	28	9.0
after 20 min.	16	—
after 30 min.	—	—

Table 5

Foaming capacity and foaming stability of BPI as a function of pH

pH	Foaming capacity (% volume increase)		Stability of foaming capacity (% volume increase)											
			Salted protein isolate						Unsalted protein isolate					
	Salted protein isolate	Unsalted protein isolate	Time in (min.)						Time in (min.)					
			10	20	30	40	50	60	10	20	30	40	50	60
4	96	89	88	79	70	57	49	45	83	77	69	51	46	38
5	58	39	37	23	20	17	15	13	12	3	—	—	—	—
6	49	30	29	11	8	7	5	4	7	—	—	—	—	—
7	46	27	15	4	—	—	—	—	5	—	—	—	—	—
8	43	26	2	—	—	—	—	—	5	—	—	—	—	—
9	42	24	8	4	—	—	—	—	4	6	—	—	—	—

of BPI could be attributed to the low value of the surface tension of air/water interface caused by adsorption of protein molecules.

From data presented in Table 4, it could also be noticed that salted BPI had higher foaming capacity than the unsalted one. This improvement in foaming capacity as a result of added salt may be due to the increased protein solubility (Table 2).

From results given in Table 5, the foaming properties of salted and unsalted BPI were pH-dependent. Maximum increase in volume (96 and 89% for salted and unsalted BPI, respectively) was observed at pH 4 with a progressive lowering as the pH increased; being 42 and 24% volume increase at pH 9 for salted and unsalted BPI, respectively (Table 5).

To study the foam stability as a function of pH, the foams were held at 20 °C for 60 minutes. From Table 5, foaming stability decreased with increasing pH value for both salted and unsalted protein isolates. Such pH dependence of foaming stability has also been reported for soy protein (LIN et al. 1974) and could be attributed to the formation of stable molecular layers in the air-water interface of the foams.

Nitrogen solubility index (NSI) and protein dispersibility index (PDI):

The solubility or dispersibility of a protein is a physicochemical property that is related to the other functional properties and therefore, it is the first to be studied in systematic investigations of physical properties.

From Table (6), BPI had a high nitrogen solubility and protein dispersibility; the values being 97.98% and 86.78% for NSI and 98.94% and 89.06% for PDI for salted and unsalted protein isolate, respectively.

The high NSI and PDI values obtained for BPI are superior when compared with those of soy protein isolate as reported by JOHNSON (1970). It was found by YATSUMATSU et al. (1972) that the higher the NSI values of the isolate, the wider their application. JOHNSON (1970) also reported that high PDI values of soy protein improved the emulsifying action of the protein.

Table 6

Nitrogen solubility index (NSI) and protein dispersibility index (PDI) of salted and unsalted BPI

Samples	NSI	WSP*	PDI	WDP**
Salted protein isolate	97.98	70.86	98.94	71.16
Unsalted protein isolate	86.78	71.97	89.06	75.02

* Water soluble protein
** Water dispersible protein

It is wort mentioning that the isolate obtained in the present study had a satisfactory protein solubility in the pH range existing in many food products, which is very important in view of introducing the mentioned isolate in a product or another.

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