RECOVERY OF PROTEIN FROM MECHANICALLY SEPARATED CHICKEN RESIDUE USING DIFFERENT METHODS OF EXTRACTION

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Summary: Mechanically separated chicken residue from skinless chicken backs was subjected to: a) alkali extraction at pH 10,5 followed by acid precipitation; b) salt extraction using 6% NaCl solution and c) enzimatic hydrolysis using the enzime alcalase at enzime: substrate ratio of 2g:100g protein. Five trials were conducted for each treatment.
The bone residue used in the trials had a protein content in the range of 15,9 - 19,7% and fat content in the range of 8,9 - 11,7%. The alkali extract had moisture and protein contents in the range of 88,1 - 92,9% and 5,1 - 11,1% respectively. Fat content was around 1,5%. The extract produced using saline solution contained protein and fat contents in the range of 4,1 - 6,3% and 1,9 - 4,8% respectively. As in the alkali extract the moisture content showed great variability between the trials and was in the range of 88,1 - 91,9%. The enzimatic extraction produced a slurry with a moisture and protein contents in the range of 92,3 - 93,8% and 4,8 - 6,4% respectively. The fat content was around 0,8%. The results showed no differences between the techniques of extraction on the protein content, and fat content was reduced to low levels in all trials.

Introduction: The production and utilization of protein extracts from by-products are not new. In the 1960's a lot of work was done to produce a good fish protein concentrate (MEINKE et al. 1972).
Many investigators have been studying the viability of protein extraction from by-products using different methology as the enzimatic (Webster et al. 1974) and alkali extraction (YOUNG, 1975). The main objective in recovering the protein from by-products is the production of protein concentrates of quality for human consumption.
With the advances in mechanical deboning, the meat that remains in the bone can be recovered in an economical way. Detailed information about different equipments, processes, raw-materials and yields can be obtained in the works of NEWMAN (1981), FIELD (1988) and BERAQUET (1989). Mechanical separation produces considerable amounts of bone residues with meat attached that are destined to animal food.
Considering that the mechanical separation yield in industrial conditions is in the range of 65 - 76% (SCHULER, 1985) the percentage of bone residue is in the range of 25 - 35%. As Brazil has a production potential of more than 200.000 ton of mechanically separated chicken meat, the residue volume is in the range of 50.000 to 70.000 ton per year.
The bone residue contains considerable amounts of protein, around 18 - 20% (LAWRENCE et al. 1982; YOUNG et al. 1986). Its protein content is higher than the protein content of the mechanically separated chicken meat that is around 13% (BERAQUET, 1990).
Several investigators have been studying the possibility of recovering the protein from the bone residue using different methods of extraction. KIJOWSKI & NIEWIAROWICZ (1985) evaluated the saline method to extract the protein. Sales et al. (1991) investigated the effect of using different enzymes to recover the protein from the residue. LAWRENCE et al. (1982) reported that although most of its protein is collagen, more than 18% are sacoplasmatic and myofibrillar proteins and can be alkali extractable.
This work aimed to determine, on a laboratory scale, the influence of alkali and salt extraction on the recovery of protein from mechanically separated chicken residue.

Material and Methods: Five trials were conducted using bone residues resulting from mechanical deboning of chicken skinless backs using a POSS deboning machine. The bone residue was subjected to: a) alkali extraction at pH 10,5 and room temperature (20°C) for 30 minutes followed by centrifugation at 3.000 rpm for 15 minutes. The precipitate was separated and

acid (HCl 4N) was added to bring the sobrenadant to pH 5,0 that was centrifuged at 3.000 rpm for 15 minutes; b) salt extraction using cold 6% NaCl solution at room temperature for 30 minutes followed by centrifugation at 3.000 rpm for 15 minutes and c) enzimatic extraction using the enzyme alcalase, temperature of 50°C and pH 8,0. The enzime:substrate ratio was 2,0g:100,0g protein and the digestion time was 3 hours. The degree of hydrolysis was controlled using the pH-stat technique as decribed by ADLER NISSEN (1977). For all extractions the bone residue was mixed with tap water or solution using 1:1,25 ratio.

Proximate Analysis: Proximate analysis (moisture, protein, fat and ash) was performed on the protein slurries collected after the extraction and on the bone residues to assess the degree of the sample variability. Moisture content was determined by during 10g sample at 105°C for 24 hours as decribed by HORWITZ (1980). Fat content was assayed by the Soxhlet technique using as a solvent petroleum ether as described by HORWITZ (1980). The total nitrogen was determined using the macrokjedahl method as decribed by the Torry Research Station (1973) for protein determination. The ash content was determined by drying 2 - 3g sample at 550°C until constant weight was achieved. All determinations were conducted in triplicate. The mean values were analysed using the Tukey test.

Results and Discussion: The proximate composition of the bone residue used in all trials is presented in Table 1. The moisture content was in the range of 58,1 - 63,0%. Higher contents, in the range of 60,4 - 63,0% were observed in trials 1, 2 and 5 but differences were not statistically significant. The fat content, in the range of 8,9 - 11,7%, was statistically different only between trials 1 and 3. Protein content was around 15,8 - 17,1%, except in trial 5, which was around 19,7%, statistically higher from the others. Ash content in trial 1, of 12,9% was statistically different from the others, except from trial 4. Higher contents of 11,6 and 12,9% were obtained in trials 1 and 4 respectively. In the other trials, the ash content was around 9,8 - 10,6%. The values found in this study were similar to those reported by McCURDY et al. (1986) who found moisture, fat and protein contents of 58,0; 11,7 and 18,2% respectively. LAWRENCE & JELEN (1983) obtained fat content in the range of 16 - 20%. These differences between authors are related with differences between type of cut, meat:bone ratio in the raw material and pressure used in the deboning machine.

The proximate composition of the alkaline extract is presented in Table 2.

Moisture content and fat content were in the range of 88,1 - 92,9% and 1,0 - 2,0% respectively. Protein content showed the greatest variability. Contents around 5,1% were obtained in trials 1 and 5. In trials 2 and 4, the protein content was in the range of 6,3 - 7,0%, but in trial 3 it increased to 11,1%. LAWRENCE et al. (1982) obtained extracts with a moisture and protein contents in the range of 80 - 88% and 1 - 10% respectively.

Table 3 shows the proximate composition of the saline extract. As observed with the alkaline extract, the moisture content was in a range of 88,1 - 91,9%. Higher contents, of around 90,7 and 91,9%, were observed in trials 4 and 1 respectively. Protein content was high in trials 2 and 4, with a content of 6,3 and 5,8% respectively. In the other trials it was situated in the range of 4,1 - 4,8%. KIJOWSKI & NIEWIAROWITZ (1984) using the same method of extraction obtained protein extracts with higher moisture content of 92,7%, but lower protein and fat content of 2,5% and 0,4% respectively.

Table 4 shows the degree of hydrolysis (DH) as a function of time in the enzimatic extraction. The degree of hydrolysis in trials 3 and 4 was higher than in the other trials, around 13,5% in trial 4 and 14,0%, in trial 3. In the other trials the DH was in the range of 10,8 - 11,3%. Sales et al. (1991), using the same enzyme, obtained, after 3 hours of extraction, DH around 8%. In all trials, the reaction came to a halt after around 120 minutes. The proximate analysis of the enzimatic extract is presented in Table 5. The moisture content was in the range of 92,3 - 93,8%. Fat content, obtained in trials and 2, was statistically higher than those obtained in the other trials. In trials 1 and 2, the fat content was around 1,6 and 0,8%

Table 1. Proximate composition of the bone residue from skinless chicken backs in the different trials.

Trial	Moisture (%)	Fat (%)	Protein (%)	Ash (%)
1	$60,6^{ab}$	$8,9^{b}$	$15,8^{b}$	$12,9^{a}$
2	$63,0^{a}$	$10,2^{ab}$	$15,9^{b}$	$9,9^{b}$
3	$59,1^{c}$	$11,7^{a}$	$16,1^{b}$	$10,3^{b}$
4	$58,1^{bc}$	$11,7^{ab}$	$17,1^{b}$	$11,6^{ab}$
5	$60,4^{ab}$	$11,2^{ab}$	$19,7^{a}$	$10,6^{b}$
$\bar{M}$	60,2	10,7	16,9	11,1

Means withine columns followed by the some letter don't differ significantly ($P<0,05$)

Table 2. Proximate composition of the alkaline extract in the different trials.

Trial	Moisture (%)	Fat (%)	Protein (%)	Ash (%)
1	$92,9^{a}$	$1,0^{a}$	$5,1^{b}$	$0,6^{a}$
2	$91,0^{b}$	$1,1^{a}$	$7,0^{c}$	$0,6^{a}$
3	$88,1^{c}$	$1,6^{b}$	$11,1^{a}$	$0,4^{a}$
4	$89,6^{d}$	$2,2^{c}$	$6,3^{bc}$	$0,6^{a}$
5	$92,1^{a}$	$1,8^{bc}$	$5,1^{b}$	$0,9^{a}$
$\bar{M}$	90,7	1,5	6,9	0,6

Table 3. Proximate composition of the saline extract in the different trials

Trial	Moisture (%)	Fat (%)	Protein (%)	Ash (%)
1	$91,9^{a}$	$2,2^{c}$	$4,8^{b}$	$0,4^{c}$
2	$89,9^{c}$	$2,1^{c}$	$6,3^{a}$	$1,3^{b}$
3	$88,1^{c}$	$4,8^{a}$	$4,1^{b}$	$4,2^{a}$
4	$90,7^{b}$	$1,9^{c}$	$5,8^{a}$	$4,3^{a}$
5	$89,0^{d}$	$3,6^{b}$	$4,1^{b}$	$4,3^{a}$
$\bar{M}$	89,9	2,9	5,0	2,9

Table 4. Degree of hydrolysis (DH) as a function of time.

Time (min)	Degree of hydrolysis (%)				
	Trial				
	1	2	3	4	5
0	0	0	0	0	0
30	5,3	6,1	7,8	5,5	6,2
60	7,4	8,7	10,4	9,7	7,9
90	8,6	9,5	12,0	11,3	9,6
120	10,0	10,4	13,1	12,1	10,2
150	10,2	10,8	14,0	13,5	10,8
180	11,2	10,8	14,0	13,5	10,8
200	11,3	10,8	14,0	13,5	10,8

Table 5. Proximate composition of the enzimatic extract in the different trials.

Trial	Moisture (%)	Fat (%)	Protein (%)	Ash (%)
1	$92,7^{ab}$	$1,6^{a}$	$4,8^{c}$	$0,8^{a}$
2	$93,8^{a}$	$0,8^{b}$	$5,5^{b}$	$0,8^{a}$
3	$92,3^{b}$	$0,3^{c}$	$6,4^{a}$	$0,9^{a}$
4	$92,5^{b}$	$0,3^{c}$	$5,4^{b}$	$0,8^{a}$
5	$93,7^{a}$	$0,2^{c}$	$5,2^{bc}$	$0,8^{a}$
$\bar{M}$	93,0	0,6	5,5	0,8

respectively. In trials 3, 4 and 5 the fat content was much lower in the range 0f 0,2 - 0,3%. The high contents observed in trials 1 and 2 can be related with the efficiency of the centrifugation process. The protein content obtained in trial 3, of 6,4% was higher than the contents observed in the other trials. In trials 2, 4 and 5 there were not differences between the protein contents, that were in the range of 5,2 - 5,5%. Ash content did not differ between the trials and was around 0,8 - 0,9%. SALES et al. (1991) obtained extracts with 7,9% of fat. In this study, the highest content was 1,6%.

Conclusions: The results showed no differences between the techniques of extraction on protein recovery. Fat content was reduced to low levels in all extracts. Higher levels of fat and ash contents were obtained using saline extraction.

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