Biological evaluation of mechanically deboned chicken meat protein hydrolysate[1]

Avaliação biológica do hidrolisado de proteína da carne de galinha desossada mecanicamente

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ABSTRACT

Objective

The objective of this study was to evaluate the biological properties of a protein hydrolysate obtained by enzymatic hydrolysis of mechanically deboned chicken meat.

Methods

Mechanically deboned chicken meat was hydrolysed using Alcalase 2.4 L FG and then dried in a spray-drier. Three groups (n=6) of male *Wistar* rats received diets containing casein, mechanically deboned chicken meat protein hydrolysate and a protein-free diet. The rats were randomly assigned to individual cages with controlled temperature (22°C) for 12 days.

Results

The mechanically deboned chicken meat diet resulted in a good net protein utilization (3.74) and high true digestibility (96%). The amino acid composition of the hydrolysate was relatively well balanced, but the concentrations of methionine and cystine were low, making them the limiting amino acids. The proximate chemical composition of the hydrolysate showed protein content to be as high as 62%.

Conclusion

The results obtained in this work suggest that mechanically deboned chicken meat hydrolysate can be used as a protein enhancer in food preparations such as enteral formulations, and as an edible protein enhancer in general applications.

Indexing terms: Biological factors. Hydrolyzed vegetable protein. Protein. Epidemilogy, experimental.

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RESUMO

Objetivo

O objetivo do estudo foi avaliar a qualidade biológica da proteína hidrolisada obtida a partir da carne mecanicamente separada de frango.

Métodos

A carne mecanicamente separada de frango foi hidrolisada com a enzima Alcalase 2,4 L FG e o hidrolisado obtido foi submetido a secagem em atomizador. Foram utilizados três grupos (n=6) de ratos machos Wistar os quais receberam dietas contendo caseína, proteína hidrolisada de carne mecanicamente separada de frango ou uma dieta com proteína livre. Os animais foram distribuídos aleatoriamente em gaiolas individuais, com temperatura controlada (22°C), por um período de 12 dias.

Resultados

A dieta utilizando carne mecanicamente separada de frango resultou em elevada utilização líquida de proteína (3,74) e elevada digestibilidade verdadeira (96%). A composição de aminoácidos da proteína hidrolisada apresentou bons resultados, embora metionina e cistina tenham apresentado baixos valores, sendo considerados aminoácidos limitantes. A composição química mostrou altos valores de proteína no hidrolisado obtido (62%).

Conclusão

Os resultados obtidos neste trabalho sugerem que a proteína hidrolisada de carne mecanicamente separada de frango poderá ser utilizada como um suplemento em formulações alimentares, tais como formulações enterais, ou como fonte de complementação protéica na indústria de alimentos em geral.

Termos de indexação: *Fatores biológicos. Proteína hidrolisada vegetal. Proteína*. Estudos experimentais.

INTRODUCTION

Brazil is the world's third largest chicken meat producer and the leading exporter of chicken products, with a total production of 9.7 millions tons in 2007[1]. It is reckoned that at least 20% of chicken fresh-cut carcasses are transformed into Mechanically Deboned Chicken Meat (MDCM), which could be used in processed meats, such as meat emulsion, paste meat and chicken nuggets[2]. MDCM is a by-product of the poultry industry increasingly used in processed meat products. It is also a source of high biological quality proteins that could be used to produce protein preparations with specific characteristics. MDCM is mostly obtained from necks and back parts of chicken and turkey carcasses[3].

Protein hydrolysates constitute a promising alternative to intact proteins and elemental formulas in the development of special formulations designed to provide nutritional support for patients with different needs[4]. The number of amino acid residues remaining in the peptide chains after hydrolysis is an essential characteristic in the production of protein hydrolysates with desired functional properties[5]. Protein hydrolysates find applications mainly in the nutritional management of individuals who cannot digest whole/intact protein. The most prevalent application of protein hydrolysates has been in formulations for infants with food hypersensitivity, in order to improve nutritional and functional properties[6]. Other possible applications of hydrolysates have been suggested and include the preparation of geriatric products, high energy supplements and weight control and therapeutic or enteric diets[7].

The protein hydrolysates most commonly used in nutritional products are casein, whey protein and soy protein. Other sources of protein hydrolysates, such as fish, chickpea, shark muscle, and shrimp, were studied and can also be used in supplementation diets, high protein sports drinks, and therapeutic or enteric diets [8-10]. Considerable attention has been given to the use of MDCM as an ingredient in the manufacture of sausages and other meat products[11], especially considering its nutritional quality[2,12,13]. However, few studies have

been published on the production and quality of MDCM protein hydrolysate. The aims of this work were to study the biological quality of MDCM protein hydrolysate and its amino acid composition in order to use it as a nutritional enhancer in food products and diets.

METHODS

Mechanically deboned chicken meat was supplied by Avipal S.A, which is a large, local poultry processing plant. The enzyme used was Alcalase 2.4L FG, an endopeptidase from *Bacillus licheniformis,* provided by Novozymes Latin America, Brazil.

Batches of 8L of MDCM were hydrolyzed in optimum conditions using Alcalase (50ºC, pH 8.0 and enzyme concentration of 2.0%). The hydrolysate obtained was centrifuged at 8g for 10min (Sorvall RC-5B, Du Pont Instruments, USA) and concentrated to 20% of solids in a rotavap (Labortora, Heidolph 400, Germany). The hydrolysate was spray-dried in a laboratory spray-dryer (Model Mini Spray Dryer Buchi B 191, Sweden) with an inlet temperature of 160ºC, outlet temperature of 96ºC and feed flow rate of 160 $mL.h^{-1}$, using 5% of maltodextrin as a carrier and 1% of calcium carbonate as anti-humectant agent.

The samples of MDCM and protein hydrolysate were collected and analyzed for moisture, ash, total fat[14] and crude protein content (N x 6. 25) by the Kjeldahl method[14].

Young, 21-23 days old, white male Wistar rats, weighing 40.0-55.0g, were obtained from the nursery of the *Centro de Ciências Biológicas, Universidade Federal do Rio Grande do Sul*, Brazil. Animals were randomly divided into three groups, each group consisting of 6 rats. Protein-free diets were offered to the first group. The second group was fed a casein diet as control. The third group received a protein hydrolysate diet. All animals received diets formulated with casein supplementation during an acclimation period of 2 days. The diets were formulated according to Reeves *et al.*[15], adapted to contain 10% of protein for casein (control) and for MDCM protein hydrolysate. The diet compositions are listed in Table 1. Water and feed were offered *ad libitum.* The animals were individually caged and kept in a room at 22ºC and a 12h light and dark cycle. Feces were collected five times for each rat for a period of ten days. The feces were dehydrated, weighed and their nitrogen content was analyzed by the Kjeldahl method[14].

Amino acid composition was determined after acid hydrolysis with 6 N HCl for 24 hours at 110ºC, following methodology described by Spackman *et al.*[16], using a Beckman HPLC-amino

Table 1. Calculated composition of the experimental diets as 10% protein of the total. *Porto Alegre* (RS), Brazil, 2007.

Ingredients	Casein	Protein-free diet	Protein hydrolysate
	g		
Casein	11.86	-	-
MDCM protein hydrolysate	-	-	17.10
Sucrose	10	10	10
Corn oil	7	7	7
Cellulose	5	5	5
Mineral mix	3.5	3.5	3.5
Vitamin mix	1.0	1.0	1.0
L-cystine	0.3	-	-
Choline bitartrate	0.25	-	-
Tert-Butylhydroquinone	0.0014	0.0014	0.0014
Cornstarch	61.08	73.50	56.40

MDCM: mechanically deboned chicken meat.

acid analyzer. Tryptophan was analyzed according to Spies[17].

The data obtained from this experiment were used to calculate True Digestibility (TD) (Eq.1) and Net Protein Retention (NPR) (Eq.2) by employing the following formulas, according to Sgarbieri[18]: TD = (($Ni - NF_1 - NF_2$) /Ni) x 100 (1) Where: Ni = Nitrogen intake of animals fed the tested diet; N_{F1}= Nitrogen excreted in feces of animals fed the tested diet; N_{F2} = Nitrogen excreted in feces of animals fed the protein-free diet.

NPR = weight gain of test group + weight loss of protein-free group / weight of test protein consumed. (2)

Feed intake, protein intake and body weight were used to compute the following nutritional indices according to Sogi *et al.*[19] showed in equations 3 to 5:

Feed efficiency (FE) = Gain in body wt (g)/Feed intake (g) (3)

Feed utilization (FU) = Feed intake (g)/Gain in body wt (g) (4)

Protein utilization (PU) = Protein intake (g)/Gain in body wt (g) (5)

The data were subjected to Analysis of Variance (ANOVA) in a completely randomized design to determine the significant differences among the various groups.

RESULTS AND DISCUSSION

Proximate chemical composition

The chemical composition of mechanically deboned chicken meat and its spray-dried hydrolysate is presented in Table 2. MDCM presented much higher fat and lower protein contents. The high amounts of fats in the MDCM might indicate a process where more skin off-products are being used, while the unusually low ash content is indicating that less bones and cartilaginous tissues were used. The fat content in the hydrolysate was greatly reduced during the exclusion of the insoluble protein fraction by centrifugal separation[20]. Spray-dried hydrolysate showed very high protein content (61.9%). Comparatively, Hamid *et al.*[21], used 2.0% Alcalase, pH 8.0 and 55 ºC to produce fish protein hydrolysate powder with 49.6% of total protein and 2.8% of fat, while Soares *et al.*[22], using Alcalase at 1.2%, pH 7.0 and 50ºC to hydrolyze mechanically deboned chicken meat, obtained around 40.5% of total protein after the spray-drying process.

Table 2. Proximate composition of mechanically deboned chicken meat and the spray-dried hydrolysate. *Porto Alegre* (RS), Brazil, 2007.

Composition (%)	MDCM[a]		Spray-dried hydrolysate[b]	
	M	SD	M	SD
Moisture	63.65	3.57	6.92	0.17
Protein[c]	32.56	1.07	61.93	1.10
Fat[c]	55.58	1.08	1.08	0.30
Ash[c]	2.15	0.05	30.07	0.06

[a]Values are means and standard deviations of quintuplicate measurements; [b]Values obtained using 5% of maltodextrin as a carrier for spray drying. Values are means of duplicate measurements; [c]Dry weight basis.
MDCM: mechanically deboned chicken meat; M: mean; SD: standard deviation.

Nutritional indices

The protein hydrolysate showed good nutritional quality when compared with casein. Table 3 shows the nutritional indices obtained in the biological assay. Rats fed casein, with a total consumption of 107.08g of feed, gained less weight than those fed protein hydrolysate (total consumption of 100.96g of feed, although these results were not significantly different (p<0.05). Protein intake, feed efficiency, feed utilization and protein utilization were not significantly different (p<0.05) when compared with casein.

Digestibility is the measure of the percentage of proteins that are hydrolyzed by digestive enzymes and absorbed as amino acids by the organism, and it determines the protein quality of the diet. The compared digestibility of

Table 3. Nutritional indices* of casein and protein hydrolysate diets. *Porto Alegre* (RS), Brazil, 2007.

Parameter*	Casein		Protein hydrolysate	
	M	SD	M	SD
Feed intake (g)	107.08	19.38	100.96	21.48
Protein intake(g)	12.48	2.26	10.97	2.33
Initial weight (g)	46.25	2.36	52.32	2.72
Weight gain (g)	34.47	9.45	35.46	15.82
Feed efficiency	0.32	0.48	0.35	0.67
Feed utilization	3.11	2.05	2.85	1.49
Protein utilization	0.36	0.24	0.31	0.14
NPR	3.21	4.18	3.74	6.77
TD (%)	97.64[a]	0.74	96.43[b]	0.68

*Values are means and standard deviations of 6 rats per group over an experimental period of 10 days; [a,b]Different superscripts are significantly different ($p<0.05$) according to the Student's *t* test.
M: mean; SD: standard deviation; NPR: net protein retention; TD: true digestibility.

protein hydrolysate (96.43%) and casein (97.74%) were proximately the same, indicating that protein hydrolysate is exceptionally good as a source of amino acids, rendering highly digestible protein meals. The Net Protein Ratio method estimates the protein used for growth and maintenance. The NPR value of protein hydrolysate (3.74) was greater than that of casein (3.21), although not significantly different ($p<0.05$). Our results showed that MDCM protein hydrolysate, as prepared in this work, was a much better source of protein when compared with other preparations listed in the literature. Rebeca *et al.*[23], who used eviscerated mullet to produce fish protein hydrolysate, found NPR values of 3.07. Negrão *et al.*[2], using mechanically deboned chicken flour, reported NPR values of 3.19 and true digestibility of 92.90%, which was lower than that of MDCM protein hydrolysate, suggesting that hydrolysis can contribute to better digestibility because its absorption by the organism might be more effective.

Amino acid composition

The nutritional value of dietary proteins is determined by the pattern and quantity of essential amino acids present. The presence of one or more of the essential amino acids in adequate amounts increases the nutritive value of the protein[24]. The quality of MDCM protein hydrolysate as source of amino acids can be assessed by comparing the essential amino acids with the World Health Organization's (WHO)[25] recommended standard, and is shown in Table 4. The amino acid profile of the mechanically deboned chicken meat protein hydrolysate contained higher amounts of all essential amino acids, except for methionine and cystine when compared with the WHO's reference for adults. Considering the demands for children (2-5-year-olds), the results indicated that MDCM protein hydrolysate contained adequate amounts of lysine, treonine, isoleucine and histidine[25]. The low concentrations of methionine and cystine are reported not only in animal proteins, which are generally considered reference proteins, but also in vegetable sources, normally lacking sulfur amino acids[18]. This low content of methionine and cystine can be increased by adding other food ingredients containing high or moderate amounts of sulfur amino acids or by supplementing L-methionine to the formulation, especially when used for infants. However, a good protein needs more than just containing amino acids in adequate proportions to meet all the requirements of an organism. The amino acids also need to have high bioavailability, i.e., they can be absorbed in a metabolic active form, in order to achieve specific functions in the body. Despite the relatively low amounts of methionine and cystine in MDCM protein hydrolysate, animals in our study had good

Table 4. Amino acid composition of mechanically deboned chicken meat (MDCM) protein hydrolysate. *Porto Alegre* (RS), Brazil, 2007.

Amino acid	Protein hydrolysate (mg/g protein)	FAO (2-5 years)	FAO (adults)
Essential			
Histidine	24	19	16
Isoleucine	34	28	13
Leucine	62	66	19
Lysine	70	58	16
Metionine+Cystine*	15	25	17
Phenylalanine+Tyrosine	65	63	19
Treonine	38	34	5
Tryptophan	9	11	9
Valine	36	35	13
Non essential			
Aspartic acid	86	-	-
Glutamic acid	128	-	-
Serine	36	-	-
Proline	37	-	-
Glycine	48	-	-
Alanine	50	-	-
Arginine	54	-	-

*limiting amino acid. MDCM: mechanically deboned chicken meat; FAO: Food and Agriculture Organization.

weight gain and the NPR and the true digestibility of MDCM protein hydrolysate values were excellent, suggesting that the amino acids were promptly available for digestion, rendering this source as adequate.

CONCLUSION

Proximate analyses revealed that the spray-dried mechanically deboned chicken meat protein hydrolysate consisted of proximately 62% protein and very low lipid content, characteristics that are usually desired for food preparations with special ends such as diets for weight control, enteral nutrition, among others. Biological parameters indicated that protein hydrolysate, a low-cost product, presented very good nutritional properties, despite its low content of methionine and cystine, the limiting amino acids. Protein hydrolysate could be used in industrial preparations and specialized adult nutritional products and supplements and, when supplemented with the limiting amino acids, its use could be tested in infant formulas.

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COLLABORATORS

D.M. ROSSI participated in all the stages of the study. J.G. VENZKE helped with the animal experiments and enzymatic hydrolysis of the mechanically deboned chicken meat. S.H. FLÔRES co-supervised the study, following all the stages. M.A.Z. AYUB supervised and coordinated the project and wrote the manuscript.

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