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## Physicochemical properties of three food proteins treated with transglutaminase

### Propriedades físico-químicas de três proteínas alimentares tratadas com transglutaminase

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#### ABSTRACT

*Three sources of food proteins were treated with microbial transglutaminase (EC 2.3.2.13) in order to assess changes in the physicochemical properties of reactivity, solubility, emulsification, and free amino groups of the formed polymers. Samples of lactic casein (LC), isolated soy protein (ISP), and hydrolysed animal protein (HAP), were incubated with the enzyme for one or two hours. LC and ISP showed a reduced solubility of 15% and 24% respectively, with HAP showing no alteration on solubility. Amino nitrogen content was 7%, 3% and 2% reduced for HAP, LC and ISP respectively. LC and ISP demonstrated lower emulsifying activity when they were enzymatically treated but the formed emulsions were stable, contrasting with HAP, which exhibited no changes in emulsifying properties.*

**Key words:** *transglutaminase, food proteins, functional properties of proteins.*

#### RESUMO

*Três fontes protéicas alimentares foram tratadas com transglutaminase microbiana (EC 2.3.2.13) e as características físico-químicas como reatividade, solubilidade, emulsificação e grupos amino livres dos polímeros formados foram avaliadas. Amostras de caseína láctea (CL), proteína isolada de soja (PIS) e de proteína animal hidrolisada (PAH), foram incubadas com a enzima por uma ou duas horas. CL e PIS mostraram uma redução na solubilidade de 15% e 24% respectivamente, enquanto PAH não sofreu alteração de solubilidade. A quantidade de nitrogênio livre na forma amina apresentou uma redução de 7%, 3% e 2% para PAH, CL e PIS respectivamente. CL e PIS demonstraram baixa atividade emulsificante quando tratadas enzimaticamente, porém as emulsões formadas se mostraram estáveis, em contraste com PAH que não alterou suas propriedades emulsificantes.*

**Palavras-chave:** *transglutaminase, proteínas alimentares, propriedades funcionais.*

#### INTRODUCTION

Food industry is on constant search for improved, enzymatically modified forms of proteins, which are the most important functional food ingredients, capable of either introducing or modifying texture, appearance, and flavour (SGARBIERI, 1996).

Food proteins can have their functionality altered by physical methods such as temperature, or by chemical and enzymatic treatments, the latter being the only process with controlled specificity. Solubility and emulsifying properties of proteins are determined by a set of characteristics such as molecular weight, structural conformation and flexibility, net charge and hydrophobicity, besides their interactions with other food components (ZHU et al., 1995; BABIKER et al., 1996).

Transglutaminase (EC 2.3.2.13) is the enzyme that catalyses an acyl transfer reaction of  $\gamma$ -carboxyl groups of glutamine residues to several acceptors like  $\epsilon$ -amino groups of lysine in proteic substrates. Transglutaminases are broadly found in nature, in animal tissues, plants and micro-organisms. They are studied due to their biological importance, especially in hemostasis. In food processing, this enzyme has been used to promote polymerisation, texture improvement, meat restructuring, gel formation, and increased nutritional value of proteins by means of the incorporation of limiting amino acids on the original material (NIELSEN, 1995; MOTOKI & SEGURO, 1998). To date, Activa TG<sup>TM</sup> is the only available commercial transglutaminase (MOTOKI & SEGURO, 1998), produced from an actinomycete strain

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characterized by ANDO et al. (1989). It has widespread and growing applications in the food processing industry (ZHU et al., 1995; KOLLE & SAVELL, 2003). Recent efforts have been done to improve enzyme production (ZHENG et al. 2001; MEIYING et al., 2002), and to identify novel and alternative microbial sources for transglutaminase production (SOARES et al. 2003).

Many authors quote that the common functional attributes of food proteins depend on their degree of solubility (KINSELLA, 1977; MOTOKI et al., 1984; ZHU et al., 1995). What was studied in this work was some physicochemical and functional aspects of isolated soy protein, milk casein and hydrolysed mechanically deboned poultry meat protein before and after treatment with microbial transglutaminase.

## MATERIALS AND METHODS

### Protein sources

Casein was obtained from acid precipitation at the isoelectric point of commercial UHT, 3% fat content milk. HCl 0.5M was added until pH 4.8 was reached. After centrifugation and filtration, fat, minerals, lactose and moisture contents were removed through successive precipitation with absolute ethanol and diethyl ether, followed by filtration. Isolated soy protein (ISP) was Samprosoy 90 NB produced by Ceval Alimentos S. A., Esteio, RS, Brazil. Hydrolysed animal protein (HAP) was obtained in our laboratory as mechanically deboned poultry meat treated with Flavourzyme protease (SOARES et al., 2000) from Novo Nordisk Bioindustrial do Brasil Ltda, Araucária, PR, Brazil.

### Enzyme

Activa TG-B was a commercial formulation of microbial origin produced by Ajinomoto Co. (Japan) and kindly provided by Ajinomoto Interamericana Ind. Com. Ltda, São Paulo, SP, Brazil. Prior to use, suspensions of 20g L<sup>-1</sup> in water were always made fresh, centrifuged at 10,000g during 5 min to remove insoluble materials.

The activity of enzyme preparations were established as 0.5U mL<sup>-1</sup>, determined using the hydroxamate formation procedure and L-glutamic acid  $\gamma$ -monohydroxamate as standard (FOLK & COLE, 1966). One unit of transglutaminase activity is defined as the formation of 1 $\mu$ mol of L-glutamic acid  $\gamma$ -monohydroxamate per minute at 37°C.

### Polymerisation reaction

Solutions containing 10g L<sup>-1</sup> of soluble proteins to be tested were prepared in 0.1M sodium

phosphate buffer, pH 7.0. To these solutions, enzyme was added at concentration of 0.1g L<sup>-1</sup> of the commercial product. Reaction temperature was maintained at 37°C for 60 or 120min. Enzyme reaction was stopped at 95°C, 2 minutes.

### Soluble protein determination

Samples were centrifuged for 5min at 10,000g and soluble protein was determined according to the Lowry method (SCOPE, 1994). Calibration curves were prepared with bovine serum albumin (BSA) as standard.

### Emulsifying properties

The emulsifying properties of samples (incubated for 120min with transglutaminase) were determined by the PEARCE & KINSELLA (1978) method with the following modifications. Emulsions were prepared by mixing 3.0mL of sample with 1.0mL of maize oil and swirling for 1 minute. 0.1 of mL of this reaction was taken from the bottom of the tubes at different times as shown in Figure 4 and diluted with 4.9mL 1g L<sup>-1</sup> solution of sodium dodecyl sulfate (SDS). Absorbance was then measured at 500nm. According to the method, emulsifying activity is determined immediately after emulsion formation (time = 0min), and the emulsion stability is estimated by the life span of the formed emulsion over time up to 10 minutes.

### SDS-Polyacrylamide gels electrophoresis

SDS-PAGE was carried out in a Hoefer miniVE system (Amersham Pharmacia Biotech, San Francisco, USA) (LAEMMLI, 1970). Stacking and resolving gels were 4.0% and 10.0% respectively. Samples were denatured in a boiling water bath during 4 min in a tris-glycerol buffer containing 20g L<sup>-1</sup> of SDS and 50g L<sup>-1</sup> of 2-mercaptoethanol. Samples containing 20mg of protein were applied to gels and separation was carried out in a tris-glycine buffer with 1.0g L<sup>-1</sup> SDS. Gels were stained with an alcoholic solution of Coomassie Brilliant Blue R250 and revealed in a solution of 5.0% methanol and 7.5% acetic acid.

### Amino groups determination

The amount of free amino groups (N-terminals or alpha-amines and e-amino of lysine residues) were determined by the trinitrobenzenesulphonic acid (TNBS) assay (ADLER-NISSEN, 1979; IKURA et al., 1980). Protein samples of 0.25 mL were mixed with 1.75mL 0.2M phosphate buffer, pH 8.0, and 2.0mL of a 0.5g

L<sup>-1</sup> solution of TNBS (Sigma-Aldrich Corp., USA). After 60min of incubation at 50°C in darkness, 4.0mL 0.1M HCl were added to stop the reaction. After cooling, absorbance was measured at 340nm. A calibration curve was prepared using L-leucine as standard. The values were expressed in amino equivalents of L-leucine per gram of protein (mEq Leu-NH<sub>2</sub>g<sup>-1</sup>).

### Statistical analysis

One-way Analysis of Variance (ANOVA) and Tukey's test were used in order to compare the means of samples along time (0, 60 and 120min). Due to the impossibility to compare proteins with different degrees of hydrolysis, significance of results were only carried out for samples inside each treatment.

## RESULTS AND DISCUSSION

Results of the cross-linking and polymerisation reactions of each proteic substrate are shown in the SDS-PAGE gel (Figure 1). For casein (lanes 2, 3 and 4), an accumulation of polymerised protein on the stacking gel can be seen, indicating protein polymerization. Soy protein (lanes 5, 6 and 7) showed a more complex pattern, with several bands appearing in the gel at different molecular weights. A

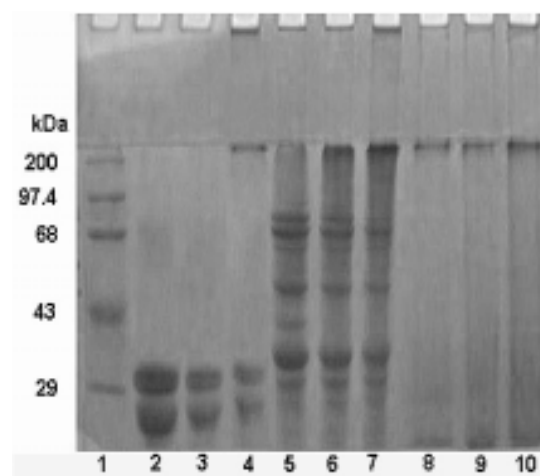


Figure 1 - SDS-PAGE patterns of proteic substrates treated with transglutaminase. Lane 1: molecular weight markers. Lanes 2, 3 and 4 show non-treated, 60 min and 120 min transglutaminase-treated casein respectively. Lanes 5, 6 and 7 show non-treated, 60 min and 120 min treated-ISP respectively. Lanes 8, 9 and 10 show non-treated, 60 min and 120 minutes treated HAP respectively

considerable modification of protein profile can be seen, with several bands reacting strongly after transglutaminase treatment, producing fractions heavier than 200 kDa. The molecular pattern of hydrolysed meat protein is highly diffuse and apparently produced no accumulation of heavier peptides, as shown in lanes 8, 9 and 10. Except for HAP, results are in agreement with the expected increase on molecular weight of the formed polymers, as reported by other authors (MOTOKI et al., 1984; BABIKER, 2000). The fact that HAP showed no apparent reaction might be due to the higher initial amount of free amino groups in this protein source when compared with casein or soy protein. This can be clearer seen in Figure 2, where HAP source showed an initial amino nitrogen content more than 3 times higher than for LC or ISP. The hydrolysis procedures to prepare HAP produced a low weight peptide solution. According to SGARBIERI (1996), non-hydrolysed protein maintains structural integrity, preventing the interaction of amino acids residues under transglutaminase action. This could explain the different content of free amino groups among treatments: while there was a 7 % reduction of its content for the enzymatic reaction of HAP, treatments of LC and ISP produced reductions of only 3 % and 2 % on free amino groups, respectively, although our results showed no statistical significance. Casein components such as  $\alpha$ -casein and  $\beta$ -casein are highly reactive, and  $\kappa$ -casein<sup>1</sup> (8 to 15 % of total milk casein) is less reactive (BABIKER et al., 1996).

Soluble protein content of samples are shown in figure 3. The solubility of HAP was not altered with transglutaminase treatment. LC and ISP presented reductions of 15 % (not statistically significant) and 24 %

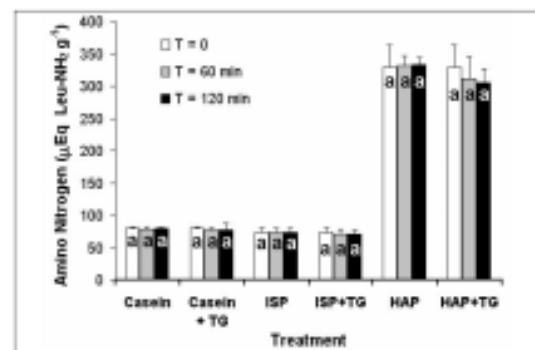


Figure 2 - Effect of treatment time on amino nitrogen content of different proteic substrates. Casein, ISP and HAP are the non-treated substrates. Cas+TG, ISP+TG, HAP+TG are the substrates treated with transglutaminase. Means followed by the same letter were not significantly different (P<0.05).

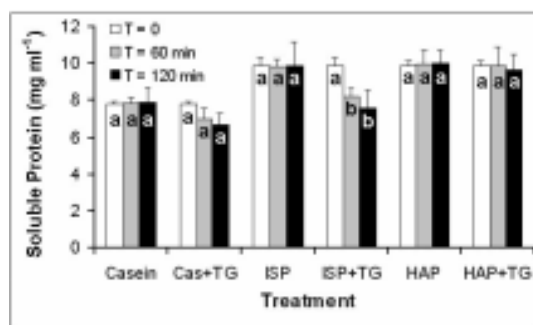


Figure 3 - Effect of treatment time on soluble protein content of different proteic substrates. Casein, ISP and HAP are the non-treated substrates. Cas+TG, ISP+TG, HAP+TG are the substrates treated with transglutaminase. Means followed by the same letter were not significantly different ( $P<0.05$ ).

respectively, fact that can be associated to the increase in molecular weight of these proteins. Our results contradict findings of BABIKER et al. (1996) who reported that hydrolysed gluten peptides exhibited an increased solubility when treated with transglutaminase, due to decreased molecular hydrophobicity.

The emulsifying properties of LC, ISP and HAP before and after 120min incubation with transglutaminase are presented in Figure 4. Results showed lowering emulsifying activities with time in the range of the experiment, both for LC and ISP. But the formed emulsions of enzymatically treated LC and ISP were more stable and presented higher turbidity values as compared to non treated material. FÆRGEMAND et al. (1998), studying droplet size formation, stated that extensive cross-linking of sodium caseinate and  $\beta$ -lactoglobulin reduced stability of the emulsions to the point of coalescence or strong flocculation. The ability of a protein of forming an emulsion is related to its capacity of efficiently adsorbing and stabilizing the oil-water interface.

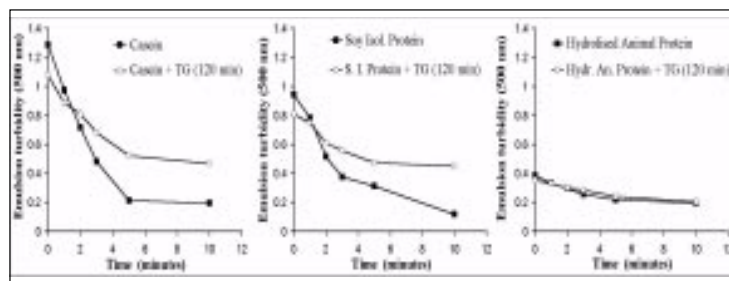


Figure 4 - Emulsifying properties of the three food proteins without treatment and after 120 minutes of treatment with transglutaminase. Results represent the mean of four experiments.

According to SHARMA et al. (2002) the emulsion stability mediated by proteins could only be improved if cross-linking is carried out after emulsification.

HAP, either treated or not with transglutaminase, presented no considerable changes in emulsification values.

The results presented in this work demonstrated that enzymatic treatment with transglutaminase of some proteins like isolated soy protein and casein can improve emulsifying properties, with increased stabilities. Nevertheless, the present commercial prices of this enzyme makes its use prohibitive on cheap protein sources such as ISP. Thus, cheaper sources of transglutaminase are essential if industry is to benefit from the use of this protein-enhancer in general process.

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