SOME PROPERTIES OF THE GLOBIN PREPARATION OBTAINED BY ENZYMATIC DECOLOURATION OF RED CELL FRACTION

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SUMMARY

The selected properties of a globin protein prepared from blood red cell fraction by an enzymatic method were studied. The experiments have been concerned with an optimisation of the hydrolysis parameters and their influence on the decolouration degree of this fraction. The efficiency of process as well as functional and nutritional characteristics of liquid hydrolysates were evaluated. For final investigations the enzymatic preparation PROTEOPOL BPS (commercially made in Poland) was chosed. It is a liquid preparation obtained from a culture of Bacillus subtilis contained a complex of proteolytic and some amylolytic enzymes.

Both, time of hydrolysis (6-48 h) and enzyme concentration significantly affected the degree of decolouration of red cell fraction and all properties of preparations obtained. The preparations demonstrated various molecular weight composition and showed various amino acid patterns. Calculated Q-index was not higher than 1280 and no bitter taste was observed in examined hydrolysates. The extinction of some hydrolysates at 540 nm was lower than 0.05.

When compared with other protein preparations (spray dried blood plasma, sodium caseinate), the globin preparation obtained under chosen conditions (18 h of hydrolysis, 10 ml of enzyme solution per 100 g of red cells) and finally freeze dried , showed good solubility. Having very good emulsifying and foaming properties , the examined preparation indicate the potential as a valuable agent for incorporation into food formulations.

Introduction

Among the several methods suggested to obtain decolourated globin protein from red cell fraction e.g. hem separation by organic solvent extraction (Tybor et al.,1973, Shahidi et al, 1984), treatment with strong oxidants (Wismer- Pedersen, 1980), hem adsorption by surface- active substances (Autio et al., 1982) or hem separation by hydrolysis method (Hellquist,1976, Fretheim et al., 1979), the enzymatic hydrolysis offers the best chance for its practical application in industrial scale (Drepper et al, 1981, Houlier,1986). The properties of globin preparation obtained is influenced by enzyme, enzyme to substrate ratio, and e.g. method of drying (Grigorov et al, 1989).

This work was undertaken both to optimize conditions of enzymatic hydrolysis of red cell fraction and as to determine selected properties of obtained globin preparation.

Material and methods

Preparation of globin

Bovine red cell fraction was obtained from a slaugtherhouse, where the blood was collected under hygienic conditions and separated in an Alfa-Laval centrifugal separator. One part of red cells were hemolysed by adding water (2 parts) and the pH and temperature of the suspension was adjusted to the desired values.The enzyme used was PROTEOPOL BPS, a liquid food-grade preparation of Bacillus subtilis. PROTEOPOL BPS is commercially available from polish company PEKTOWIN , Jaslo. For this enzyme it was found that the optimal hydrolysis temperature should be 45° C while optimal pH should be equal to 4.0. The other optimal parameters for the process (enzyme to substrate ratio, time of hydrolysis) were established as the result of investigations presented below. Before the analysis, the liquid hydrolysates were centrifuged at 14 000 rpm for 20 minutes.

Chemical analysis

Nitrogen content in hydrolysate was determined by Kjeldahl's method and the efficiency of hydrolysis process was shown as percent of total nitrogen.

To evaluate the decolouration degree liquid hydrolysates were subjected to extinction measurement by 540 nm using spectrophotometr SPECOL (Germany).

The molecular weight distribution of protein mixture was determined with disc membranes (1, 5, 20 kD from SPECTRA / POR , The Netherlands).

Next , a part of liquid hydrolysates were dried using laboratory freeze dryier LGA 05 (Germany) and final dry products were subjected to following analyses:

- determination of amino acid composition by MIKROTECHNA analyser type 339. Tryptophan content was determined colorymetrically using modified procedure of Lombard , de Lange (1965). The results were used to evaluate both biological value using the EAA-index (Walker, 1983) as and the bitterness of hydrolysates by calculation of Q-values (Ney,1971),
- assessment of protein functionality including solubility (NSI) (Lawhon and Cater, 1971), emulsifying capacity (EC), emulsion stability (ES) (Swift et al. 1961, Webb et al, 1970) and foam ability (FA), foam stability (FS) by the method described by Shahidi at al (1984). Both commercially made spray dried plasma and as Na caseinate were used as reference materials.

Results and Discussion

Preliminary studies indicated that the enzymatic preparation PROTEOPOL BPS is suitable for decolouration of red cell fraction. After initial experiments and selection of various possibilities the following parameters of hydrolysis have been suggested: temperature: 45° C, pH value:4.0, time of hydrolysis: up to 48 h, enzyme to substrate ratio: up to 30 ml of enzymatic preparation per 100 g of red cells (ca 35 g of total crude protein , N x 6.25).

The results reported in Table 1 indicate that the efficiency of hydrolysis process was significantly affected by time and amount of enzymatic preparation and varied in the range between 23.3 and 64.9 %. As expected , the best results were obtained for the boundary conditions (the longest time of hydrolysis and the highest concentration of enzyme). Since a time of 18 h and enzyme amount of 10 ml pro 100 g of red cells gives satisfactory results with respect to decolouration, these parameters were suggested for potential application.

The results of fractionation of examined hydrolysates by the use of molecularporous ultrafiltration technique clearly confirm increasing of protein decomposition (Table 1). As the time of hydrolysis and the amount of added enzyme increase, so goes the content of fractions with molecular weight below 5 kD up and, at the same time , the number of protein fractions above 20 kD decrease. For previous pointed variant (hydrolysis: 18 h , 10 ml of enzyme) was found , that it still contains about 63 % of protein with molecular weight above 20 kD, whreas a fraction below 5 kD is about 17.0 %.

The obtained hydrolysates , however, do not differ significantly to amino acid composition. Low isoleucine content is the main factor limiting their biological value (the EAA-index < 0.8). The amino acid contents are also suitable for predication of bitterness of protein hydrolysates and follow to calculate the Q-index ,due to method described by Ney , 1971. In general, protein hydrolysates or peptides with Q <1300 are not bitter (Ney,1971, Drepper and Drepper,1981). Q-values of red cell hydrolysates (including previous chosed variant: hydrolysis 18 h, 10 ml of enzyme) examined in this study, varied in the range between 1215 and 1287 and not bitter taste were observed indeed.

Some functional characteristics determined for previous selected red cell hydrolysate (as frezee dried preparation) confirmed its useability as a valuable agent for potential incorporation into food formulations. In agreement to results reported by Thomas,1994 the data in Table 2 reveal that both emulsifying properties and as foaming properties of enzyme-trated protein are better than the raw material itself (dried red cells). Having a good solubility (NSI > 90 %) , the final obtained globin preparation was comparable both to commercially made spray dried blood plasma and as sodium caseinate.

Conclusion

Protein hydrolysates with important functional properties can be manufactured by enzymatic hydrolysis of red cell fraction . The quality of protein obtained is affected by hydrolysis parameters and varied markedly due to preparation process. In general, is possible to obtain preparations with properties comparable to other commercially made protein preparations
(like dried blood plasma or sodium caseinate).

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Table 1 Selected properties of examined red cell hydrolysates
Table 2 Some functional characteristics of examined protein preparations