ACTA SCIENTIARUM POLONORUM *Acta Sci. Pol., Technol. Aliment. 4(2) 2005, 111-118*

SOME SELECTED PROPERTIES OF PROTEIN PREPARATIONS MADE BY ENZYMATIC TREATMENT OF ANIMAL BLOOD RED CELL FRACTION

Piotr Konieczny, Waldemar Uchman, Krystyna Krysztofiak, Jarosław Przyborski

Agricultural University of Poznan

Abstract. Products prepared from animal blood intended for human consumption and collected in accordance with GHP rules are considered as of high nutritional value for their use for food purposes. The properties of a globin protein prepared from red cell fraction by an enzymatic method were studied. The efficiency of process as well as functional and nutritional characteristics of hydrolysates were evaluated. Liquid preparation obtained commercially from a culture of *Bacillus subtilis* contained a complex of proteolytic and some amylolitic enzymes was used in the experiment. 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. The extinction of some hydrolysates at 540 nm was lower than 0.05. When compared with spray dried blood plasma or 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 freeze dried showed good solubility. Having very good emulsifying and foaming properties, the examined preparations indicate the potential as a valuable agent for incorporation into food formulations.

Key words: animal blood, red cells, enzymatic hydrolysis

INTRODUCTION

Efficient utilization of meat by-products is important for the profitability of the meat industry. In the past, by-products were largely used in several countries for food purposes but traditional markets for edible meat by-products have gradually been disappearing, because of low prices and health concerns. For these reasons, meat processors have directed their interest towards non-food uses, such as pet food, pharmaceuticals, cosmetics, animal feed or organic fertilizers and soil improvers [Uchman and Konieczny 1984, Pezacki 1991, Konieczny and Uchman 1997].

Corresponding author – Adres do korespondencji: dr hab. Piotr Konieczny, Department of Food Qauality Management of Agricultural University of Poznan, Wojska Polskiego 31, 60-624 Poznań, email: pikofood@au.poznan.pl

By-products originating from slaughterhouses, including organs, fat or lard, skin, feet, abdominal and intestinal contents, bone and blood of cattle, pigs and lambs represent 66.0, 52.0 and 68.0% of the live weight, respectively. More than half the animal byproducts are not suitable for normal consumption, because of their unusual physical and chemical characteristics. As a result, a valuable source of potential revenue is lost, and the cost of disposing of these products is increasing [Liu 2004]. If blood is discharged into the abattoir effluent instead of being utilized, it increases extremely the biological oxygen demand (BOD) of the effluent. Environmental impact of animal blood represents very important issue of new animal by-products regulations and associated EU proposals [Regulation EC No 1774/2002...].

In the last decades the recognition of animal blood and its fractions as a potential source of protein for human with a fairly high nutritive value and interesting technological properties led to a wide range of products. To ensure that the safety of blood originating products is preserved, blood for human consumption is collected with close system only and according to Good Manufacturing Practices (GMPs) [Mujisers 1994, Rozporządzenie... 2003]. The safety aspect has been under intense scrutiny due to first reports about bovine spongiform encephalopathy (BSE). Despite very intensive research and detailed epidemiological evidence, no BSE infectivity has been detected in animal (bovine) blood in either natural or experimental cases. The Office International des Epizooties (OIE), European Commission Scientific Steering Committee (SSC) and World Health Organization (WHO) all include blood and plasma products in Category IV, tissues with no detected infectivity [Russell 2001].

In Poland, a significant and still growing part of the animal blood is used for food purposes. Of blood fractions, fresh, chilled or frozen plasma has found the largest application being used in the production of various meat products such as sausages and canned meat [Stiebing and Wirth 1986, Pyrcz et al. 2005]. Although the hemoglobin of animal blood contributes to an increase in the nutritional value of food and has functional properties of great utility in industrialized products, its use in diets, mostly in form of red cell fraction, is restricted because of its dark color and flavor [Schaper Bizzotto et al. 2005]. Nevertheless, this problem can be solved by removing the haem group, producing an isolated globin with great potential use in food. Among the several methods suggested to obtain decolorized 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 and Drepper 1981].

Following growing interest of meat industry searching an effective method to resolve the problem of red cell utilization, this research was undertaken in our lab both to optimize conditions of enzymatic hydrolysis of red cell fraction and to determine selected functional properties of obtained globin preparation.

MATERIAL AND METHODS

Preparation of globin

Red cell fraction was obtained from a slaughterhouse, where the blood (pork) was collected under hygienic conditions and separated in the 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 a liquid food-grade enzymatic preparation commercially made in Poland from *Bacillus subtilis* culture. It was recommended by producer that the optimal hydrolysis temperature should be 45°C, while optimal pH value should be equal to 4.0. The other 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 min using JANETZKI Model: K 24, Germany, centrifuge.

Chemical analysis

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

To evaluate the decoloration degree, liquid hydrolysates were subjected to extinction measurement by 540 nm using spectrophotometer SPECOL, Germany.

The molecular weight distribution of protein mixture was determined with lab apparatus and disc membranes 1, 5, 20 kD purchased from SPECTRA / POR, Netherlands.

A part of obtained liquid hydrolysates were also dried using laboratory freeze dryer (model LGA 05, Germany). Dry products were subjected to following analyses:

– determination of amino acid composition by MIKROTECHNA analyser, model 339. Tryptophan content was determined colorimetrically using modified procedure of Lombard and 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]. Detailed results of this part are presented in the separate study [Uchman and Konieczny 1990],

– 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], foam capacity (FC) and foam stability (FS) (after 30 min) by the method described by Shahidi et al. [1984]. Water and fat absorbtion of dried protein preparations were also evaluated due to methods described by Uchman and Konieczny [1990]. Both, commercially made spray dried plasma and as sodium caseinate were used as reference materials in this experiment.

All analytical determinations were performed at least in triplicate. Values of different parameters were expressed as the mean values.

RESULTS AND DISCUSSION

Preliminary studies indicated that the enzymatic preparation is suitable for decoloration 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 decoloration, these parameters were suggested for potential application.

Table 1. Effect of hydrolysis conditions on selected properties of red cell hydrolysates
Tabela 1. Wpływ warunków hydrolizy na wybrane właściwości hydrolizatów krwinek

Time of hydrolysis Czas hydrolizy h	Amount of enzyme, ml per 100 g of red cells Ilość enzymu, ml na 100 g krwinek	Efficiency of hydrolysis Efektywność hydrolizy %	Extinction by λ = 540 nm Ekstynkcja przy λ = 540 nm	Protein fractionation of hydrolysate, % Skład frakcyjny hydrolizatu, %		
				< 5 kD	5-20 kD	> 20 kD
6	5.0	23.3	0.29	7.7	14.7	77.6
	10.0	44.4	0.25	11.5	19.8	68.7
	20.0	57.0	0.13	12.8	18.4	68.8
18	5.0	44.3	0.08	14.3	21.3	64.4
	10.0	59.0	0.05	16.7	20.2	63.1
	20.0	64.4	0.06	17.4	21.2	61.4
48	5.0	49.5	0.04	16.9	20.3	62.8
	10.0	54.9	0.03	22.0	19.7	58.3
	20.0	64.9	0.02	24.8	19.2	56.0

The results of fractionation of examined hydrolysates by the use of molecularporous ultrafiltration technique clearly confirm increasing of protein decomposition. 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, whereas a fraction below 5 kD is about 17.0%.

The obtained hydrolysates, however, do not differ significantly from amino acid composition (not presented here). 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 of peptides with Q < 1300 are not bitter [Ney 1971, Drepper and Drepper 1981]. Q-values of red cell hydrolysates (including previous selected 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 [Uchman and Konieczny 1990].

Functional properties of protein preparations are the most important from the technological point of view. Three groups of parameters are the most interesting: sorption properties, emulsifying properties and gel forming ability. Among the functional properties of proteins, emulsifying properties are the most important for their utilization in comminuted meat products [Zayas 1996].

The emulsification behavior of proteins is primarily affected by two factors: the diffusion rate of proteins to the interface and the film formation behavior of proteins at the interface. Both of these factors depend on the structure and size of protein molecules [Tornberg and Hermansson 1977].

The second one is gel forming ability. A gel from globular proteins can be described as an intermediate state between a protein precipitate, formed above a certain critical level of concentration with just the right balance of protein-protein and protein-solvent interaction [Hermansson 1979]. It means that too small molecules of proteins or polypeptides do not create a gel form. But the structure of protein has a large importance for both of these parameters.

All of the above mentioned factors are changed during processing used in our investigations. For that the properties of all obtained preparations differentiate very much from properties of red cell fraction. The most important parameters are shown in Table 2.

Most of functional properties of protein preparations modified by any enzymatic method are connected with the obtained degree of hydrolysis and mean value of molecular weight of protein [Thomas 1994]. Therefore, attempts were also made to determine correlations between results of determining emulsifying properties and results of determining molecular weights of protein fractions present in these hydrolizates.

The linear correlations between these results (dependent variable-y) and the amount of proteins of molecular weight higher than 20 kD (independent variable-x) were found.

They are for:

– content of oil phase – r = + 0.93

– emulsifying capacity – r = + 0.94

– emulsion stability – r = + 0.72

In each case high and positive values of correlation coefficients were obtained. This indicates that the emulsifying properties of the system depend, to a large extent, on high molecular protein fractions. However, it must be emphasized again that the most of these preparations have better emulsifying properties than the raw material itself, which means that the relationship between emulsifying properties and molecular weights of proteins has a parabolic character, at optimum corresponding to mild conditions of hydrolysis. It is connected with the balance of hydrophilic and hydrophobic properties of detached peptides. Nevertheless, this optimum does not coincides with the optimum of the red cell decoloration.

As the result of mild hydrolysis, there is a significant improvement in emulsifying properties in comparison with the raw material, it means non modified red cells. Majority of the obtained preparations have better properties than even such an excellent emulsifier as blood plasma. This indicates that there is a real possibility of using these preparations to modify quality of meat products.

Some functional characteristics determined for previous selected red cell hydrolysate (as freeze dried preparation) confirmed its useability as a valuable agent for potential

Table 2. Some functional characteristics of examined protein preparations
Tabela 2 Wybrane właściwości funkcjonalne badanych preparatów białkowych

Preparation Preparat	Protein Białko %	Solubility Rozpuszczalność %	Emusifying properties Właściwości emulgujące		Foaming properties Właściwości pianotwórcze		Water absorption Absorpcja wody	Fat absorption Absorpcja tłuszczu
			EC ml oil	ES %	FC ml	FS ml	WA %	FA %
Nonmodified freeze dried red cells Niemodyfikowane liofilizowane krwinki	70.6	65.6	32.5	57.0	20.0	4.0	lack	lack
Freeze dried enzyme treated red cells Liofilizowany hydrolizat enzymatyczny krwinek	70.0	91.0	96.5	60.6	169.0	36.0	lack	lack
Spray dried blood plasma Suszone rozpyłowo osocze krwi	72.0	98.4	62.9	61.4	130.0	15.0	570	85
Spray dried sodium caseinate Suszony rozpyłowo kazeinian sodu	88.7 (N x 6.38)	89.2	47.5	62.0	148.0	24.0	300	75

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-treated 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, it 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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WYBRANE WŁAŚCIWOŚCI PREPARATÓW BIAŁKOWYCH OTRZYMANYCH METODĄ HYDROLIZY ENZYMATYCZNEJ GĄSZCZU KRWINEK

Streszczenie. Mimo rosnącego zainteresowania przemysłu mięsnego niespożywczymi kierunkami zagospodarowania krwi zwierzęcej zarówno ona, jak i jej frakcje (osocze, krwinki) są postrzegane ciągle jako źródło białek o korzystnym składzie aminokwasowym i strawności. Z tego powodu produkty z krwi, zebranej zgodnie z wytycznymi Dobrej Praktyki Higienicznej (GHP), charakteryzują się dużą wartością odżywczą i są wykorzystywane do celów spożywczych. W pracy badano wybrane właściwości globiny otrzymanej z krwinek poddanych hydrolizie enzymatycznej. Badania skoncentrowano na doborze optymalnych warunków hydrolizy i ich wpływie na stopien odbarwienia tej frakcji krwi. Oceniano skuteczność procesu, a także wybrane właściwości funkcjonalne i żywieniowe otrzymanych płynnych hydrolizatów. Do hydrolizy krwinek używano płynnego, otrzymywanego przemysłowo, preparatu enzymatycznego pochodzącego z kultury bakteryjnej *Bacillus subtilis.* Zarówno czas hydrolizy (6-48 godzin), jak i stężenie enzymu istotnie oddziaływało na stopień odbarwienia frakcji krwinek i wszystkie badane właściwości otrzymanych hydrolizatów. Charakteryzowały się one zawartością białek o różnej masie cząsteczkowej i różnym składem aminokwasowym. Obliczony indeks Q otrzymanych hydrolizatów nie przekraczał 1280, zatem nie wykazywały one smaku gorzkiego. Ekstynkcja niektórych z badanych hydrolizatów mierzona przy $\lambda = 540$ nm nie przekraczała 0,05. W porównaniu z innymi preparatami białkowymi (suszone rozpyłowo osocze krwi, kazeinian sodu), preparat globinowy otrzymany w wytypowanych warunkach omawianą metodą hydrolizy enzymatycznej (czas hydrolizy: 18 h, 10 ml enzymu na 100 g krwinek) i wysuszony metodą liofilizacji, charakteryzował się dobrą rozpuszczalnością. Ze względu na bardzo dobre właściwości emulgujące i pianotwórcze, badany preparat przedstawia wartościowy produkt przeznaczony do wykorzystania w recepturach wybranych produktów spożywczych.

Słowa kluczowe: krew zwierzęca, krwinki, hydroliza enzymatyczna

Accepted for print – Zaakceptowano do druku: 3.10.2005 r.

***For citation – Do cytowania**: Konieczny P., Uchman W., Krysztofiak K., Przyborski J., 2005. Some selected properties of protein preparations made by enzymatic treatment of animal blood red cell fraction. Acta Sci. Pol., Technol. Aliment. 4(2), 111-118.*