EXTRACTION AND UTILIZATION OF EGG WHITE LYSOZYME

***Mija Nina, Silvia Rubţov, Leon Bugan**

Technical University of Moldova – Chişinău, Moldova

***Mija Nina, ninamija@yahoo.com**

Abstract: In the era of advanced molecular technologies a great importance have the utilization of functional qualities of food ingredients. Lysozyme is one of the protein fractions of egg white that possess enzymatic and antibacterial qualities well pronounced. Lysozyme preparation, obtained experimentally was tested by turbidimetric method for estimating the antimicrobial qualities. Also, were studied physico-chemical and spectral characteristics of lysozyme preparation, emphasized the possibilities for their use in food preparation.
Keywords: egg white lysozyme, lysozyme preparation, antibacterial properties, turbidimetric methods, spectral characteristics

Introduction

Egg white proteins make up the several fractions: ovoalbumin (69.7 %), conalbumin (9.5 %), ovoglobulin (6.7 %), ovomucoid (12.7 %), ovomucin (1.9 %), lysozyme (3 %) and avidin (0.05 %) [4.6].

Egg ovoalbumin and conalbumin are compounds with high biological value, easily assimilated (up to 95%) and represents a valuable source of amino acids. Protein ovoglobulin determines the ability of the egg white to formed foam, ovomucin stabilized foam. Ovotransferin and lysozyme has antibacterial qualities. Lysozyme from egg white is only enzyme with litic activities used as a commercial available food additive [2.4].

The first investigations on lysozyme were performed by Russian researcher P.N. Laşcencov in 1909. Lysozyme as ingredients of natural materials with pronounced antimicrobial qualities was investigated by English microbiologist A. Fleming since 1921.

Today it is known that the food that contains significant amounts of lysozyme is the egg white - 1.5 g/100g and in whey - 4 mg/100g. Other natural sources of lysozyme: human blood serum - in the amount 0.002 to 0.02 mg/ml, tears - in trace amounts [6.7].

Lysozyme damage cellular wall of bacteria. It shows high activity against mesophilic and thermophilic spore-forming Gram-pozitive bacteria, and can realize good returns. The cellular wall of G-pozitive bacteria is composed of several layers (up to 25 layers of Peptidoglucanes). Cellular wall of Gram-negative bacteria can not be degraded by lysozyme (cellular wall contains ≈ 10% Peptidoglucane), and the outer layer consists of lipopolysaccharides. Due to the fact that the cellular wall of Gram-negative bacteria is more complicated in structure and the composition, lysozyme is less effective against them.

In practice, antibacterial role of lysozyme is so significant that if in the milk is absent lysozyme M (M-Milk) this fact is considered an insalubrious index of this product [5,8].

Fig. 1. Primary structure of lysozyme (blue-amino acids, red disulfide links)

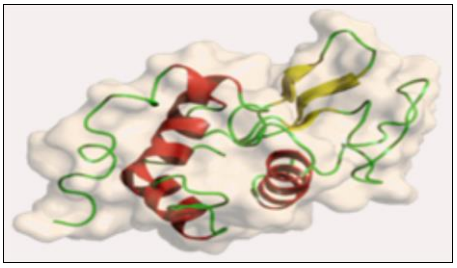

Fig. 2. Ssecondary structure of lysozyme

Being a protein, lysozyme has three levels of organization of its structure. Primary structure of lysozyme is a polipeptide in which 129 amino acids are arranged in a linear sequence to form a single polypeptide chain. In the composition of amino acids predominate histidine, lysine, arginine. The eight cysteine residues are involved in the formation of four disulfide links of the chain placed at different distances. Those amino acids that are part of the active site binding of substrate apparently are masked (Fig. 1). Spatial arrangement and secondary structure of the protein is in the form of five helical regions (α-helix standard) and 5 β-sheet folding regions (Fig. 2). Tertiary structure of native lysozyme in various physiological conditions, is a compact globular formation. Outside, on the surface of the lysozyme molecule is a long cleft, where is located the active site involved in binding to bacterial peptidoglucanes.

Methods of extracting of pure lysozyme is too costly, therefore are used several variants of obtaining of lysozyme preparations [2,3,7].

Lysozyme from egg white is used as a food additive (E 1105) in cheese manufacturing industry because it can affect sporulated bacteria of the genus *Clostridium*, in particular *Clostridium tirobuturicum* It is used in cheese to prevent contamination because it does not inhibit on starter and secondary cultures required for the fermentation and ripening of the cheeses. Sunrise *Clostridium tirobuturicum* spores are resistant to pasteurization, thay remaining active and metabolize lactic acid to butyric acid and CO_2. Undesirable effects are bloating of cheeses, forming large irregular holes, breaking of cheese [8].

Addition of lysozyme in milk is 2.5 g/100 l and about 90% of this amount is present in manufactured cheeses. The preparations of lysozyme can substitute nitrates used as preservatives in sausage. Addition of lysozyme in raw meat increases the validity time of sausages. At concentrations of cooking salt greater than 1.2% lysozyme loses its activity.

The aim of this article was to investigate conditions for obtaining a preparation of eggs white lysozime and estimate its properties.

Materials and methods

The research was conducted for egg white obtained from hen eggs produced by hens species *Leghorn* on poultry factory Valea Perjei, Taraclia. For each sample was used to determine the average mix obtained from six egg whites from a homogeneous batch of eggs. The white alkalinity was determined by titration with HCl 0.1N, used bromothymol blue as indicator. The amount of proteins in filtrates was determined by treatment with sol. $CuSO_4$, 3.1 % and spectrometric estimation at λ = 440 nm.

Separation process of lysozyme protein fraction was performed in a manner proposed by the authors [2], the design of the experiment is shown in Fig. 3. Spectral characteristics of the lysozyme preparations were recorded in the range 190-1000 nm, using for this the UV-VIS spectrometer, model Hach Lange DR 5000.

Antibacterial activity of obtained lysozyme preparation was tested by turbidimetric method [8]. Bacterial suspension was prepared with addition of *M.lyzodeicticus*. Statistical processing of the experimental data was performed with program Excel 06.

Results and discussions

Extraction of the lysozyme preparation The treatment of egg white at t = 60 ° C produced a slight foaming of the sample, the liquid becomes opaque. Foaming of the sample is due to the initial denaturation of ovoalbumin and ovoglobulin, protein that are unstable to thermal processing.

When sample was treated at 80 °C there was a massive denaturation of most protein fractions, accompanied by distortion of the structure of polypeptide chain and massive hydration of protein molecule. Coagulum is massive as presence and looks as a strongly hydrated colloid, is filtered with difficulty. Sample treated at 90 °C of egg white has a dense, compact consistency, free liquid is easily filterable.

Acidic environment favors the thermal separation of protein fractions. In all three filtrates is contained lysozyme fraction. In the filtrate of albumen treated at 90 °C is present practically only lysozyme as the most stable egg white protein while filtrates obtained at 60 and 80 ° C are less pure, containing other protein fractions.

Treatment conditions and physico-chemical parameters of obtained lysozyme preparations are given in Tab. 1

Estimation of antibacterial activity of lysozyme preparation Assessment of antibacterial activity of lysozyme preparation is based on estimation the degree of lyses of bacterial cell suspension and recording the effect of clearing of dense suspensions of a susceptible bacteria. In *t*he thermostat (at t = 37 ± 2 ° C) was maintained the subjected samples, prepared by mixing lysozyme preparation and bacterial suspension in a ratio of 1:1 (v / v). Bacterial suspension was prepared by adding in a pure saline water the culture of bacteria *M.lyzodeicticus* up to 40% increased turbidity, measured at λ = 670 nm.

Tab. 1. Treatment conditions and physico-chemical parameters of lysozyme preparations

Tempe-rature of treat-ment	Acetic acid (pH=3.5), ml	Egg white, g	Amount of filtrate, ml	Characteristics of lysozyme preparations				
				Output, %	Dry substan-ces,%	Protein, %	Acidity, °T	Clarity (Transmi-ttance T at λ=440 nm)
60	100	50	121	81	7.2	6.8	40	1
80	100	50	60	40	3.5	3.2	35	0.13
90	100	50	88	59	4.8	4.5	35	0.02

Evolutions of bacteria culture (development or inhibition) were studied for various concentrations of lysozyme after 6 hours maintenance at temperature (Table 2).

Tab. 2. Optical parameters of samples after 6 hours of maintenance at temperature

Optical parameters of samples at λ = 670 nm.	Lysozyme concentration in the test medium, mg/ml				
	0	0,4	1,0	2,5	4,0
Transmittance T,%	42	47	60	38	30
Absorbance D	0,16	0,25	0,22	0,43	0,52

Clarifying effect is more pronounced as the concentration of lysozyme above 2.0 to 2.5 mg/ml substrate (Fig.4.). Additional studies are needed to verify optimum conditions for expression that lysozyme have antibacterial qualities (addition of salts, pH of the environment, treatment with ray etc.).

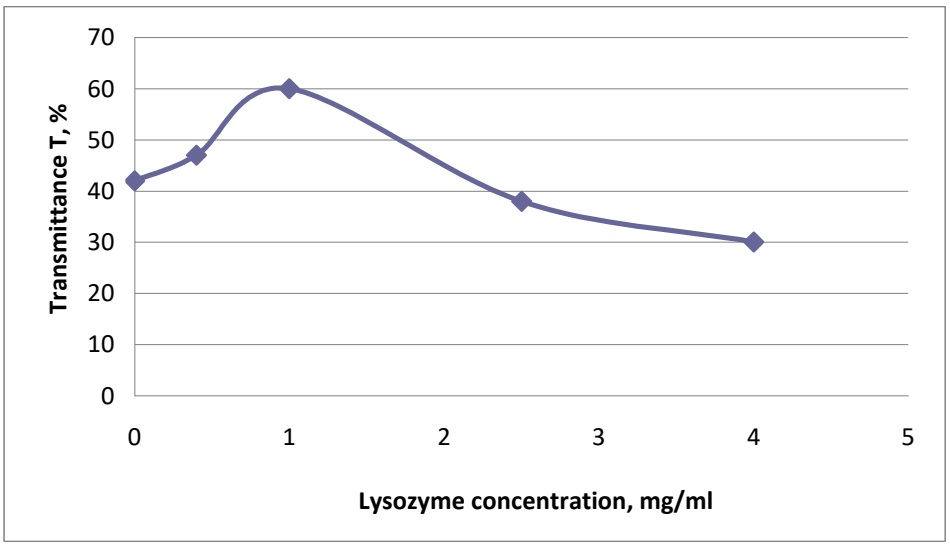

Fig. 3. Effect of reducing turbidity by addition to bacteria the lysozyme preparation

Spectral characteristics of the lysozyme preparation To determine the status of spatial configuration and composition complexity of lysozyme preparation were recorded the absorption spectrum. According to this, the observed spectrum have a maximum of absorbance 2.63 (a.u.) at 240 nm for all three samples, processed respectively at 60, 80, 90 °C, typical for lysozyme, which is present in all protein fractions combinations.

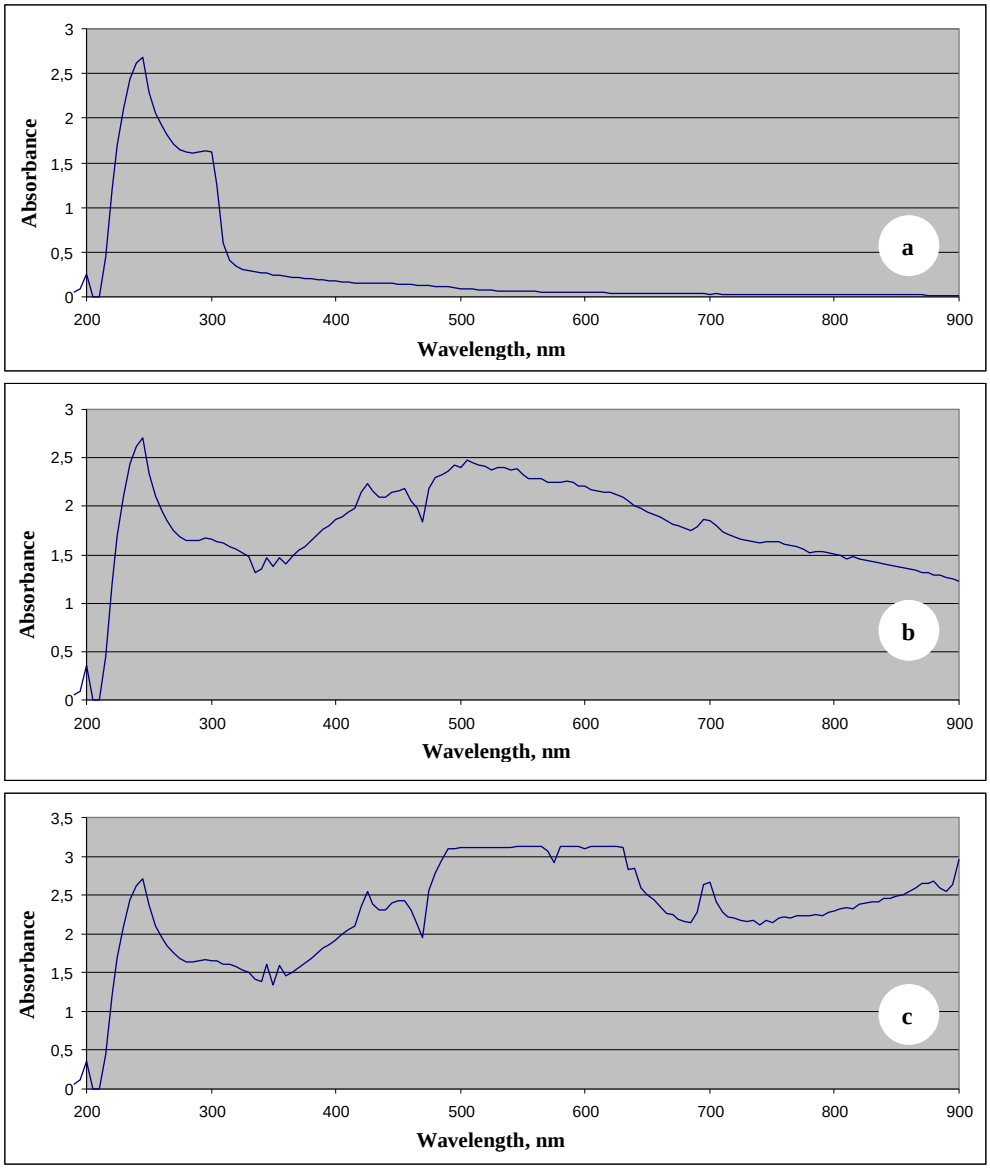

***Fig.* 4** Absorbance profile of lysozyme preparation: a - treated at 60 °C; b – at 90 °C; c – at 90°C.

The Figure 4.a shows a reduced optical activity of protein fractions of the filtrates, because functional groups of protein molecules are located inside the molecule quite compact. Absorbance profile of spectrum In Figure 4.b indicates that the filtrate contains a mixture of protein fractions, strongly hydrated, what can induce different absorption peaks. Molecular and ion oscillations are maximum in this case. In Figure 4.c the amount of mixed protein substances is relatively low, the preparation of lysozyme is pure.

Thus, it is well to know more methods of obtaining and purification of lysozime preparation.

Conclusions

1. Being a protein resistance to temperature lysozime can be separated from other fractions of egg white proteins by heat treatment in the range 80-95 ° C.
2. Optimal concentration of lysozyme, which inhibits bacterial growth, in the medium typical for milk products must exceed 2.0 - 2.5 mg / ml.
3. The absorbance spectrum of the lysozyme preparation have a maximum of absorbance at 240 nm, and minimum of absorbance - at 205 nm.

References

1. ISO/TS27105 | IDF/RM 216:2009 (ISO TC 34/SC) Milk and milk products. Determination of hens egg white lysozyme content by HPLC. Geneva, 2009.
2. Yu-Kaung Chang. Method development for direct recovery of lyzozime. In: Biochemistry Eng. J, 2006, V.30, p .63-75.
3. B.Kerkaert, F.Mestdagh. Detection of hen's egg white lysozime in food: Comparison between a sensitive HPLC and commercial ELISA method. In: Food Chemistry, 2010, V. 120, p. 580-584.
4. Y.Mine. Recent advanced in the understanding of egg white protein functionality. In: Trends in Food Science &Technology, 1995, July, V. 6, p. 225-232.
5. M.L.Vidal, J. Gautron. Development of an ELISA for quantifying lysozime in hen egg white. In: J.Agric. Food Chem., 2005. V.53.p. 2379-2385.
6. E.Şindilar. Expertiza sanitar-veterinară a alimentelor de origine animală Ch. Tipigrafia Centrală, 1996.- 325 p.
7. Баранов А.А., Дорофейчук П.А. Лизоцим – теория и практика. – М. – Н.Новгород, 1999.- с. 114.
8. Шидловская В.П. Ферменты молока.М.:Агропромиздат, 1985.-с. 152.