Electronic Journal of Polish Agricultural Universities is the very first Polish scientific journal published exclusively on the Internet, founded on January 1, 1998 by the following agricultural universities and higher schools of agriculture: University of Technology and Agriculture of Bydgoszcz, Agricultural University of Cracow, Agricultural University of Lublin, Agricultural University of Poznan, Higher School of Agriculture and Teacher Training Siedlce, Agricultural University of Szczecin, and Agricultural University of Wroclaw.

ELECTRONIC JOURNAL OF POLISH AGRICULTURAL UNIVERSITIES

2003
Volume 6
Issue 1
Series FOOD SCIENCE AND TECHNOLOGY

LEŚNIEROWSKI G., KIJOWSKI J., STANGIERSKI J. 2003. DSC, SDS-PAGE AND SPECTROPHOTOMETRY FOR CHARACTERIZATION OF MODIFIED LYSOZYME **Electronic Journal of Polish Agricultural Universities**, Food Science and Technology, Volume 6, Issue 1.
Available Online http://www.ejpau.media.pl

DSC, SDS-PAGE AND SPECTROPHOTOMETRY FOR CHARACTERIZATION OF MODIFIED LYSOZYME

Grzegorz Leśnierowski, Jacek Kijowski, Jerzy Stangierski
Department of Food Quality Management, August Cieszkowski Agricultural University of Poznań, Poland

ABSTRACT

The aim of the study was an attempt to apply selected analytical methods for the evaluation of preparations of modified lysozyme. Lysozyme isolated from hen egg white was modified using thermal, thermal-chemical, chemical and membrane methods and subsequently the obtained preparations were evaluated with the use of the spectrophotometric method, as well as electrophoresis and differential scanning calorimetry (DSC).

It was found that the spectrophotometric method may be used in the evaluation of changes in the hydrolytic activity in lysozyme after its modification. The application of electrophoresis makes it possible to evaluate the quantitative composition of the preparations. The amount of the polymerized enzyme in the obtained preparations ranged from 27 to 55%. The calorimetric analysis (DSC), makes it possible to determine the parameters of thermal processes (temperature, enthalpy) and to assess the method of lysozyme modification. For the thermally modified enzyme a characteristic descending curve is obtained with no definite maximum. For lysozyme modified without the temperature factor two peaks are observed in the graph. On the other hand, lysozyme monomer during the calorimetric analysis shows only one thermal process.

Key words: differential scanning calorimetry (DSC), spectrophotometry, electrophoresis, lysozyme modification, lysozyme dimer, hydrolytic activity.

INTRODUCTION

Lysozyme is a globular protein of basic character, approx. 14.4 kDa. It is found as a single polypeptide chain consisting of 129 amino acids, in which lysine is the N-end amino acid and leucine is the C-end one. In a lysozyme molecule there are four disulfide bridges (S-S), which cause high thermal stability of the enzyme, together with six helix regions. The enzyme molecule is a compact complex in the shape similar to an ellipsoid with the dimensions of 4.5 x 3.0 x 3.0 nm. A characteristic property of the enzyme is its ability to form complex compounds with other proteins and to form biopolymers. One of the forms of protein-protein interactions is a natural association of lysozyme with ovomucin, ovoalbumin, ovomucoid and conalbumin [8, 20]. Another important property of this enzyme is its association ability. Under precisely defined conditions, at the low ionic strength and pH 5-9, dimerization is possible, whereas above pH of 9.0 higher oligomers may be formed [21].

Lysozyme monomer exhibits strong antibacterial activity against Gram-positive organisms. This phenomenon has found a practical application in the food processing industry, in medicine and pharmaceutical industry. The use of lysozyme in the food processing industry is connected primarily with its application as a natural preservative. Enzyme is widely used as a preservative for meat, fish and their products, for milk and dairy products, as well as fruit and vegetables [1, 3]. The pharmaceutical industry uses this enzyme in the manufacture of adjuvant drugs for antibiotics and analgesics in viral and bacterial infections, in the treatment of leukemia and neoplastic diseases [20]. Lysozyme is also used as a diagnostic agent, being an indicator of the occurrence and the progression of pathological changes in humans and animals [10].

The range of the practical applications of lysozyme may be considerably extended as a result of its modification. Numerous authors showed that a new specific activity of the enzyme against Gram-negative bacteria results from dimerization, without any loss of bactericidal properties against Gram-positive bacteria exhibited by the monomer [5, 13, 18, 19]. The dimeric form of lysozyme has been used in the treatment of bacterial and viral animal diseases. A drug produced on the basis of lysozyme dimer, known on the market under the name of Lydium – KLP, shows immunostimulating and immunocorrective activity, stimulates phagocytosis, and also increases the production of interferon alpha by lymphocytes. The drug strengthens antiviral defense and enhances the activity of antibiotics, such as penicillin or oxytetracycline. It was shown that it does not exhibit toxic or mutagenic action, it is well tolerated and safe even when administered in a bigger dose than the therapeutic dose [2, 4, 9].

The basic source of lysozyme monomer is hen egg white. At present the fundamental research problem is to develop effective methods of its modification in order to increase the range of the enzyme activity. Such studies have been conducted in several research centers around the world, primarily in Japan [5, 6, 7, 18, 19] and at our Department of Food Quality Management, the Agricultural University of Poznań [11, 12, 13, 16]. Another very important research problem is the quality assessment of the modified lysozyme. It is difficult to conduct because of a change - as a result of the modification – in the properties of the enzyme connected with e.g. the new specific activity against Gram-negative bacteria or a change in its hydrolytic activity. So far no method to assess these changes has been developed. In this study such an attempt has been undertaken using spectrophotometry, electrophoresis and scanning calorimetry (DSC).

MATERIALS AND METHODS[1]

The lysozyme preparations used in the investigations, i.e. L1 (11,000 U/mg) and L2 (16 U/mg), were produced by the authors and modified using chemical, thermal, chemical-thermal and membrane methods. The following denotations were adopted:

- Ch11 – lysozyme modified chemically for 11 days,
- Ch20 – lysozyme modified chemically for 20 days,
- T – lysozyme modified thermally,
- Tch1 – lysozyme modified using the chemical-thermal method (chemical modification for 1 day),
- Tch14 – lysozyme modified using the chemical-thermal method (chemical modification for 14 days),
- M – lysozyme modified using the membrane method.

Chemical modification. Lysozyme preparation (2.5 g) was dissolved in 100 cm^3 of the acetate buffer with pH of 4.4. The modification was conducted with the 10% share of an oxidant at the temperature of approx. 4°C for 11 and 20 days.

Thermal modification. Lysozyme preparation (2.5 g) was dissolved in 100 cm^3 of the acetate buffer with pH of 4.4 was heated for 20 min in a water bath at the temperature of 60°C. Next the solution was quickly cooled to 10-12°C.

Chemical-thermal modification. Lysozyme preparation (2.5 g) was dissolved in 100 cm^3 of the acetate buffer with pH of 4.4. The chemical modification was performed with a 10% share of an oxidant at the temperature of approx. 4°C for 1 and 14 days, and subsequently the solutions were heated for 20 min in a water bath at 75°C. After the modification was completed the solutions were intensively cooled to 10-12°C.

Membrane modification was conducted in a DDS 20-0.36 LAB ultrafiltration module with the use of membranes with the threshold permeability of 6 kDa. The lysozyme solution with pH of 7.0 was subjected to the process of diafiltration at the temperature of 50°C and the pressure of 20 Ba. The modification lasted for 150 min.

Drying of preparations. After each modification was completed lysozyme was dried in a Mini spray drier B-191 by Buchi under the following conditions:

- Input temperature of 150°C,
- Output temperature of 70°C,
- The flow of compressed air of 600 l/h,
- The efficiency of the aspirator of 80.

Hydrolytic activity. The hydrolytic activity was measured using the spectrophotometric method with *Micrococcus lysodeikticus* bacteria [17]. The method is based on the application of the phenomenon of the decomposition of *Micrococcus lysodeikticus* cell walls by lysozyme. The lysis effect, manifested in the decreasing turbidity of the bacterial suspension prepared in a 0.066 mole phosphate buffer with pH of 6.24 under the influence of the added enzyme, was measured as a change in the solution absorbency. In the initial stage of the reaction (the first several minutes) the dependence of the decrease in absorbance ΔA on the concentration of the added lysozyme is linear. In order to conduct the measurement 2.5 cm^3 of the bacterial suspension was placed in an absorption cell with the optical length of 1 cm and absorbency was measured at the wavelength of 450 nm. Next the amount of 100 µl of lysozyme solution was added and precisely after 60 s since that moment absorbency was measured again and the change in ΔA was calculated. To prepare the standard curve the values of ΔA were determined in solutions containing 1, 2, 3, 4 and 5 µg lysozyme in 100 µl. Curve was plotted of the dependence of absorbance changes on the concentration of standard samples. From the standard curve the concentration (activity) of lysozyme was determined in the investigated samples. The hydrolytic activity of lysozyme was expressed in units of activity (U), the numerical value of which defines the decrease in absorbency by 0.001 precisely 1 minute after the moment 0.1 cm^3 lysozyme solution was added to 2.5 cm^3 of bacterial suspension prepared in 0.066 M of the phosphate buffer the following conditions: pH of medium 6.24, temperature 25.0°C, wavelength γ 450 nm, optical length (the width of the absorption cell) 10.0 mm.

Electrophoresis. The analysis was conducted with the use of the SE-600 apparatus by Hoefer Scientific Instruments using the SDS-PAGE method [14,15]. Electrophoresis was performed on polyacryloamide gel. A 6% consolidating gel and 12.5% separating gel were prepared for separation. The examined samples were prepared in the Tris-HCl buffer with pH of 6.8, containing glycerol and bromophenol blue. Samples were heated for 5 minutes at 100°C and next transferred to the gel in the amount of 10 µl. The separation was performed at the current intensity of 60 mA and 90 mA. After electrophoresis the gel was stained with 0.25% w/v Coomassie brilliant blue R-250 in ethanol/ acetic acid/water (5:1:4 by volume), and destained in ethanol/acetic acid/water (5:1:4 by volume), followed by 10% acetic acid. Ready gels were scanned and kept in the form of computer files. The percentages of individual forms of the protein were assessed densimetrically using the QuantiScan 2.0 software by Biosoft.

Scanning calorimetry. Samples were examined using the DSC-7 differential scanning calorimeter by Perkin-Elmer. An empty aluminum capsule was used as reference. In order to ensure identical conditions for analysis, samples were prepared for the examinations in a precisely defined way. In every case 100 µl of distilled water was added to 100 mg of powder preparation and mixed thoroughly in order to obtain a homogenous matter. Next 13-15 mg of the sample were placed in a calorimetric cell, closed tight and subjected to thermal analysis conducted at the heating rates of 1, 5 and 10°C/min. Cadmium and gallium (Merck) were used as standards for the calibration of temperatures and enthalpy.

Statistical analysis. The results were subjected to statistical analysis using the STATISTICA PL version 6.0 software. The average value, minimum, maximum, standard deviation, standard error and confidence interval were calculated for each variable. The significance of results was verified with the use of the Schaffe test. The one-factor analysis of variance was conducted (ANOVA). Curves of regression and the Pearson coefficient of correlation were determined.

RESULTS AND DISCUSSION

Spectrophotometry. The spectrophotometric measurement was used to investigation the hydrolytic activity of lysozyme monomer and modified lysozyme.

It was shown that each modification of lysozyme led to a lowering of hydrolytic activity. Its absolute value for individual preparations ranged from approx. 6,000 to 11,000 U/mg (Table 1). The value of hydrolytic activity was affected not only by the modification itself, but also the initial level of its value in the preparations of lysozyme monomer. For this reason the degree of its decrease in comparison to the initial activity of the monomer was adopted as an objective indicator in the assessment of the hydrolytic activity of modified lysozyme preparations (Table 1). It results from the presented data that a decrease in hydrolytic activity was big and depending on the adopted method of modification it ranged from 31 to 60%. The highest lowering of the activity amounting to almost 60% was observed in preparations modified using the membrane (pressure) method. Most probably it was caused by a simultaneous action of three factors denaturing the enzyme, i.e. pressure, temperature and friction forces present while the solution was penetrating the membrane and was passing between the plates of the ultrafiltration module.

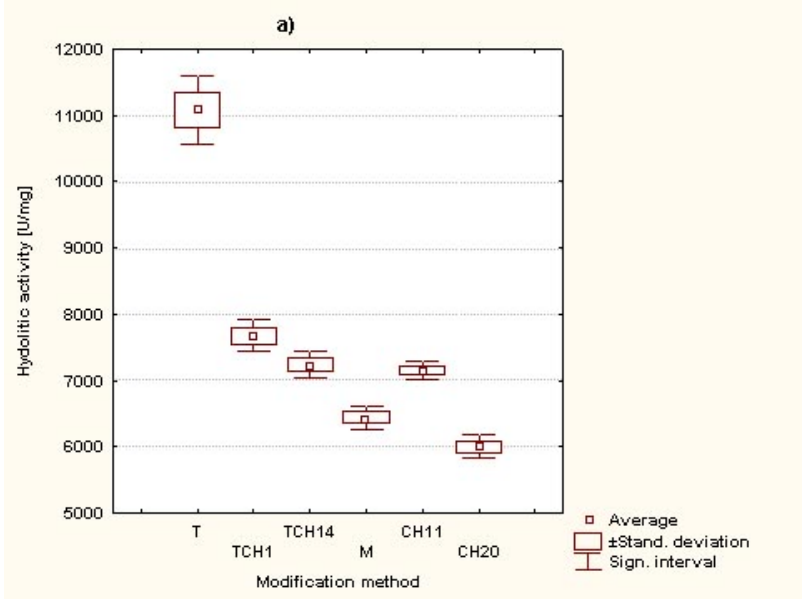
Table 1. The analysis of hydrolytic activity of lysozyme preparations

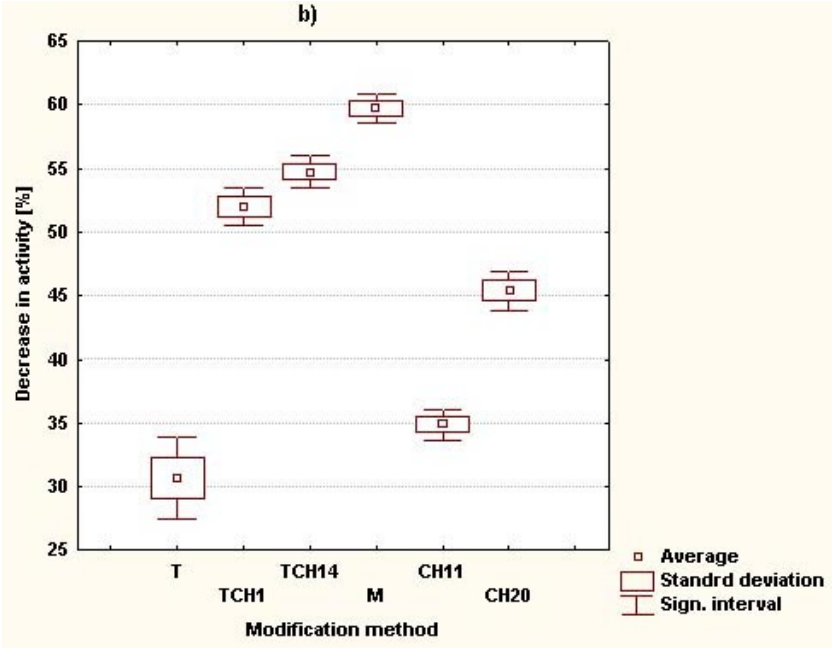
Sample before modification	Activity (U/mg)	Sample after modification	Activity (U/mg)	Decrease in activity (%)
L2	16000	**T**	11088^{A}	30.7^{A}
L2	16000	**Tch1**	7678^{B}	52.0^{B}
L2	16000	**Tch14**	7240^{C}	54.7^{C}
L2	16000	**M**	6443^{D}	59.7^{D}
L1	11000	**Ch11**	7161^{C}	34.9^{E}
L1	11000	**Ch20**	6007^{E}	45.4^{F}

A-F – means in the same column with the different superscripts are significantly different ($p < 0.05$)

In case of thermal-chemical modification the activity was lowered by 52-54%. Here the denaturing factor consisted in the joint action of the oxidant and temperature at which the enzyme was modified. However, the effect of only temperature itself during thermal modification caused a lowering of activity by merely 31%, whereas only the oxidant, depending on the time of interaction changed the activity during chemical modification by 35-45% (Fig. 1).

Figure 1. The application of spectrophotometry to evaluate preparations of modified lysozyme: a) hydrolytic activity b) decrease in hydrolytic activity

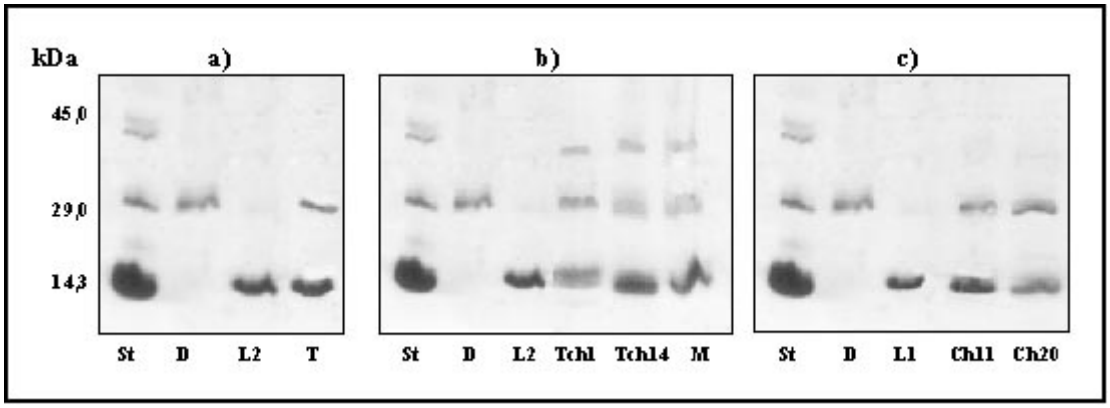


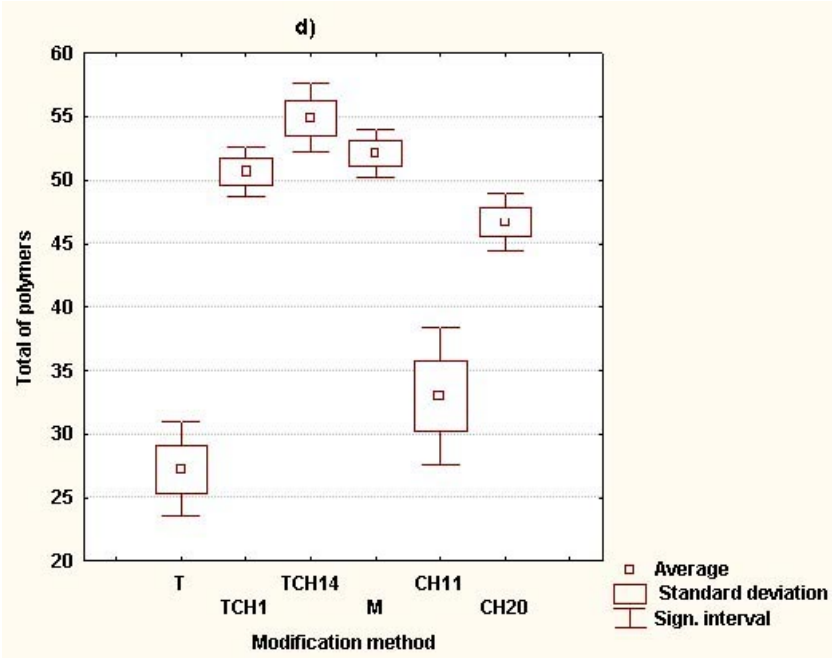


Electrophoresis. Electrophoretic analysis was used to assess the purity of the obtained enzymatic preparations and to determine their content of polymeric forms of lysozyme. Apart from modified lysozyme, protein standards (molecular weight of 14, 29 and 45 kDa), lysozyme dimer (Lydium KLP) and lysozyme monomer subjected to modification (L1 or L2) were placed on each gel.

Electrophoretic analysis indicated a high chemical purity of the enzymatic preparations. The presence of polymeric forms of lysozyme was observed in all the modified samples of this enzyme (Fig. 2A). The enzyme modified thermally (T) and chemically (Ch) showed in its composition the presence of the dimer, whereas lysozyme modified using the thermal-chemical (Tch) and membrane (M) methods contained also very small amounts of the trimer. Using computer software (Quanti Scan 2.0) the percentages of polymeric forms were determined in individual samples (Table 2).

Figure 2. The application of electrophoresis to evaluate preparations of modified lysozyme: A - electrophoresis of lysozyme modified using the thermal (a), thermal-chemical (b) and membrane method (c) B - average percentage of the total of polymers (d) and dimer (e)





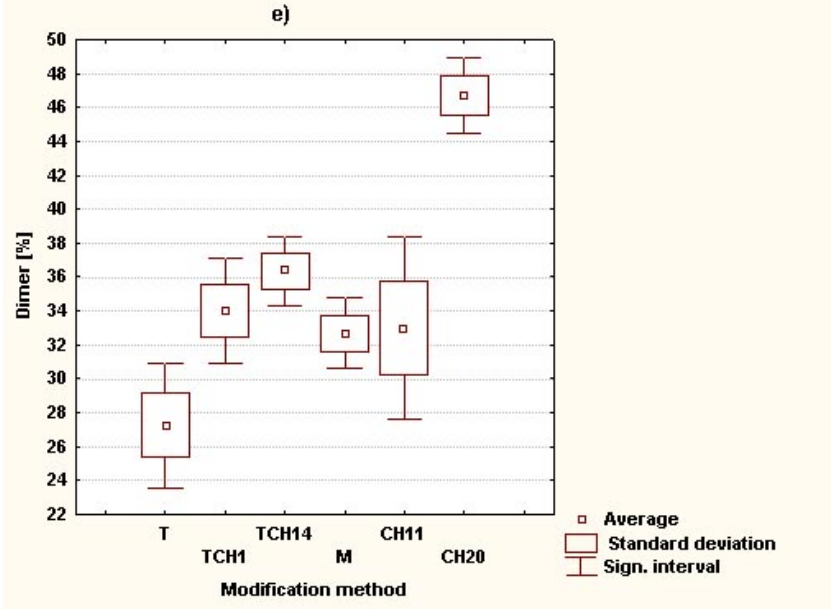


Table 2. The analysis of the percentage of lysozyme polymeric forms in modified enzymatic preparations

Sample after modification	Total of polymers (%)	Monomer (%)	Dimer (%)	Trimer (%)
T	27.2^{A}	72.7^{A}	27.2^{A}	0
Tch1	50.6^{B}	49.3^{B}	34.0^{B}	16.6^{A}
Tch14	54.9^{C}	45.1^{BD}	36.3^{B}	18.6^{B}
M	52.1^{BC}	47.9^{BD}	32.7^{B}	19.4^{B}
Ch11	33.0^{D}	67.0^{C}	33.0^{B}	0
Ch20	46.7^{E}	44.9^{D}	46.7^{C}	0

A-E – see Table 1

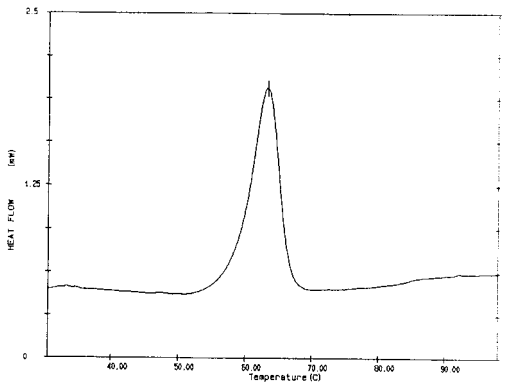
The total content of polymers in the samples ranged from 27 to 55% depending on the applied method of modification. A high percentage of polymers was observed in the samples modified using the thermal-chemical and membrane methods (approx. 50%). In that case, apart from the dimer (32-36%), also the trimer was observed (below 20%). In the samples modified chemically only the dimer was found and its percentage depended on the time of action of the oxidant (Ch 11 - 33%, Ch20 - 46.7%). The polymerization of the enzyme occurred as a result of thermal modification (the share of the dimer of 27%) (Fig. 2B).

The investigations showed that the application of electrophoresis along with the scanning program made it possible to conducted a precise analysis of the preparations of modified lysozyme. The percentages of polymeric forms of the enzyme were precisely determined in the preparations and it was shown that these percentages depended on the method of modification.

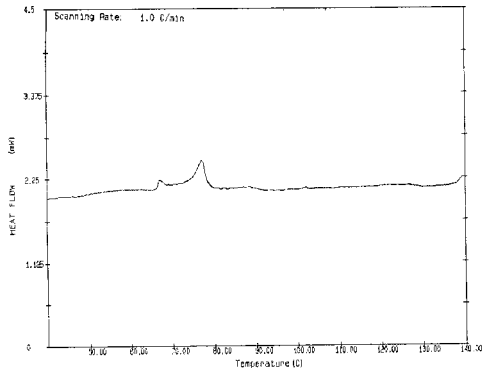
Calorimetry (DSC). Graphs resulted from this analysis, recorded characteristic changes taking place while the samples were heated (Fig. 3). Lysozyme monomer (L1) always showed only one thermal transition, which – depending on the heating rate of the sample – occurred at 59.1 to 64.4°C. The enthalpy of this transition was 16.9 J/g. Lysozyme modified chemically (Ch11 and Ch 20) two thermal transitions were observed, represented in the graph by two peaks. For the Ch11 sample the temperature of the first transition was similar to the temperature found for lysozyme monomer. However, the height of this peak was definitely lower than the height of the consecutive peak, for which the process occurred at a higher temperature (Fig. 3a). On the other hand, for the Ch20 sample the peak of first transition was more pronounced (a higher peak) than the second. The temperatures of both transitions were higher than those obtained for the Ch11 sample and for lysozyme monomer (Fig. 3b). A characteristic phenomenon was the lowering of the enthalpy of the processes along with the increase in the dimer content in the preparations. The highest enthalpy was observed for the monomer (16.9 J/g), whereas the lowest for the Ch20 preparation (5.2 J/g), which contained 46.7% of the dimer (Tables 2 and 3). However, no dependency was found between the height of the peak and the percentages of the monomer and dimer in the sample.

Figure 3. The aplication of calorimetry (DSC) to evaluate preparation of modified lysozyme: a) chemical modification (Ch11) b) chemical modification (Ch20) c) thermal (T), thermal-chemical (Tch) and membrane (M) modification

a)

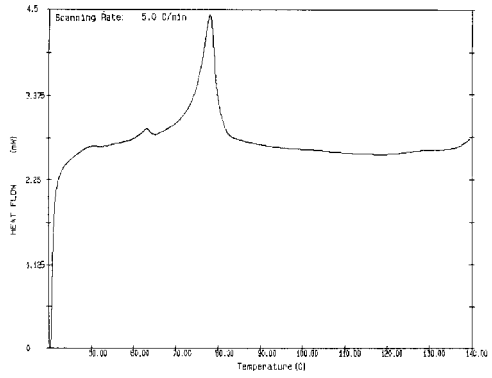


L1-Lysozyme before modification

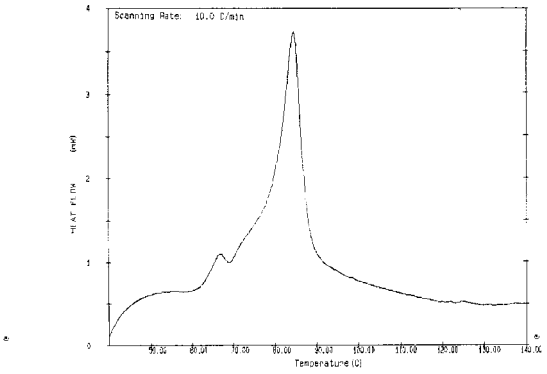


Ch 11-1°C/min

Fig. 3a cont.

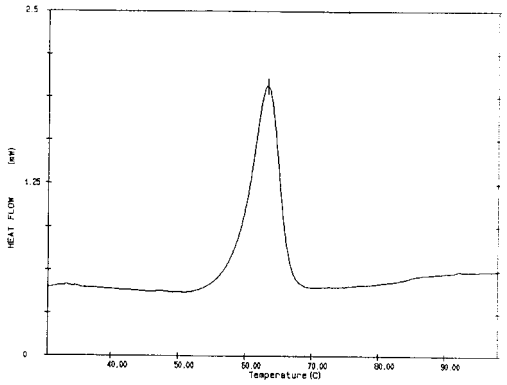


Ch11 -5°C/min

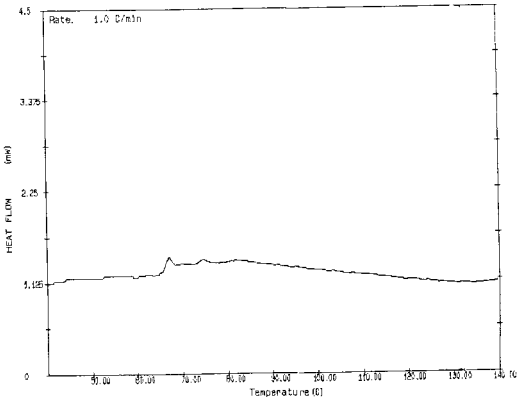


Ch11-10°C/min

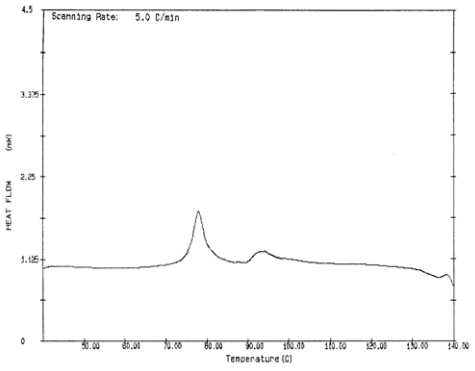
b)



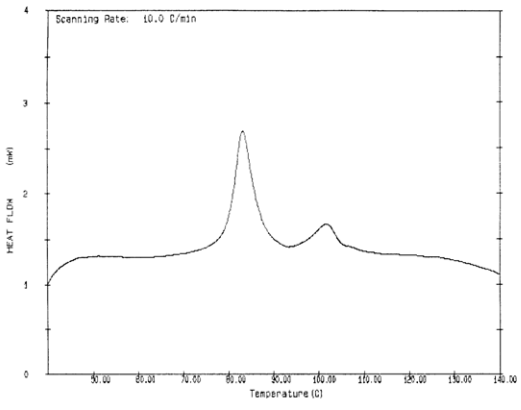
L1-Lysozyme before modification



Ch20-1°C/min

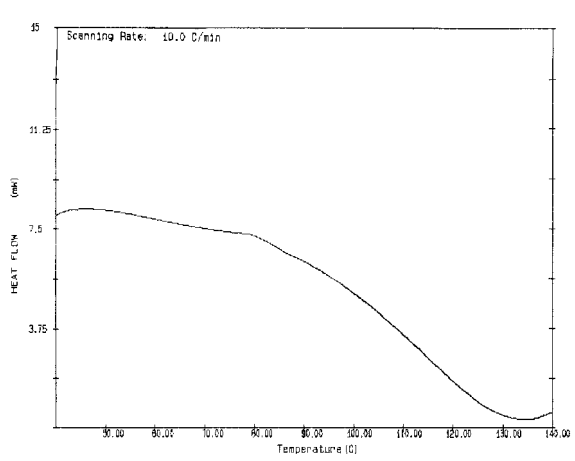


Ch20-5°C/min

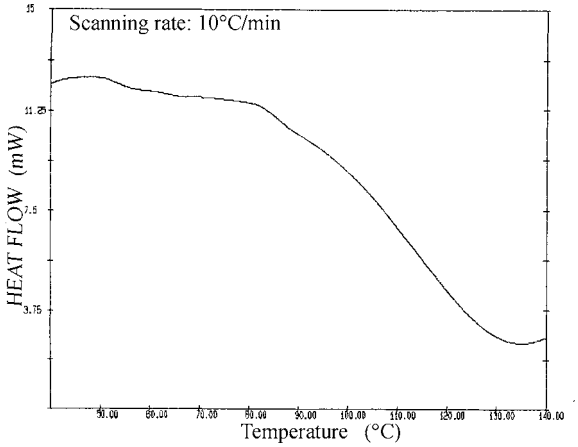


Ch20-10°C/min

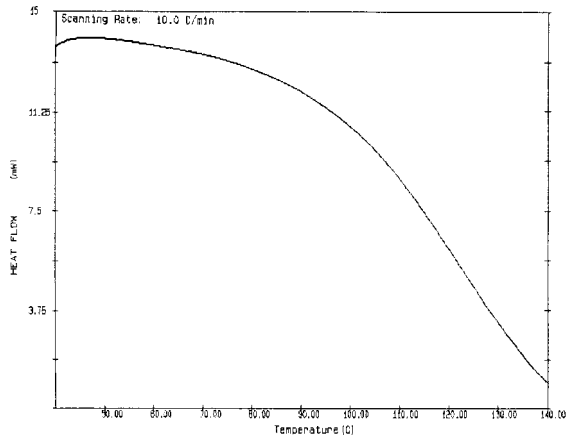
c)



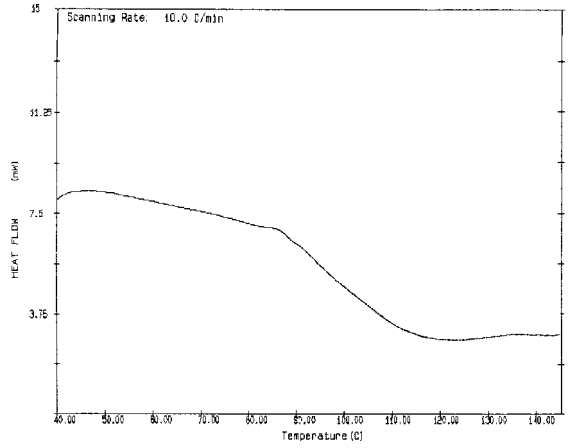
T



Tch 1



Tch 14



M

Table 3. The calorimetric analysis of modified enzymatic preparations

Sample	First thermal transition (°C)			Second thermal transition (°C)			Enthalpy (J/g)
	Heating rate			Heating rate			
	1°C/min	5°C/min	10°C/min	1°C/min	5°C/min	10°C/min	
L1	59.1^{Aa}	61.5^{Ab}	64.4^{Ac}	-	-	-	16.9^{A}
CH11	61.8^{Ba}	64.1^{Bb}	67.7^{Bc}	73.5^{Ad}	78.1^{Ae}	84.2^{Af}	11.9^{B}
CH20	68.7^{Ca}	78.0^{Cb}	83.0^{Cc}	75.0^{Bd}	94.1^{Be}	101.1^{Bf}	5.2^{C}

A-C – See Table 1
a-f – means on the same row with the different superscripts are significantly different ($p<0.05$).

In the course of investigations a very interesting phenomenon, connected with the heating rate of the samples, was observed. The most pronounced peaks were registered when samples were heated quickly. Lowering the heating rate decreased in the peak height (Figs. 3a, b). At the same time increasing the heating rate raised the temperature of the thermal transition (Table 3).

A different shape of the curves was observed for samples modified thermally (T), those modified using the thermal-chemical (Tch) and membrane (M) methods. In this case no peaks were observed, but only curves descending in a characteristic way (Fig. 3c). The cause of this phenomenon was the adoption of the temperature factor during the modification of the enzyme. It turned out that changes, which occurred in the preparations under the influence of temperature during modification, caused a different course of the curves registered during the calorimetric analysis. In this case it was impossible to determine the temperature of the transitions or their enthalpy.

The results of the investigations presented above indicate that the DSC technique may be successfully used also to assess the method of lysozyme modification. If it was conducted with the application of temperature, then as a result of calorimetric analysis a characteristically descending curve not exhibiting a maximum value was produced. If lysozyme was modified using techniques with no application of temperature, e.g. chemically, then two peaks of thermal transitions were obtained. On the other hand, if lysozyme was not modified at all, then calorimetric analysis showed only one thermal transition in the form of one peak.

CONCLUSIONS

The paper presents three methods to examine modified lysozyme. Each of them made it possible to assess different characteristic properties of lysozyme. Thus, # spectrophotometry is necessary to assess the changes in hydrolytic activity taking place in lysozyme as a result of its modification. The application of # electrophoresis made it possible to conduct a detailed qualitative and quantitative analysis of the modified enzyme. The percentages of polymeric forms of lysozyme were precisely determined in the obtained preparations. Using the # DSC technique it was detected whether the investigated lysozyme preparation was subjected to modification and the modification method was determined. In case of the monomer one peak of thermal transition was obtained. In case of the preparation containing polymeric forms obtained chemically two peaks were produced, whereas in case of thermal modification a characteristic curve with no peaks was generated. # The DSC technique made it possible to determine the temperature of the transitions and their enthalpy.

Thus, a simultaneous application of the three-presented methods makes it possible to conduct a precise qualitative and quantitative assessment of lysozyme. The suggested methods may be used to investigate the enzyme both under laboratory and industrial conditions.

The project was financed by the Committee for Research Projects, Grant no. PO6G 03818.

REFERENCES

1. Akashi A., 1972. The effect of egg white lysozyme on the growth of cheese starter organisms. J. Fac. Agric., 17, 67-69.
2. Cąkała S., 1994. Nowy preparat Lydium – KLP w leczeniu chorób zakaźnych zwierząt. [A new preparation Lydium-KLP in the treatment of contagious diseases of animals]. Życie Wet., 4A, 138-142 [in Polish].
3. Eisai Co. Ltd. 1972. Japanese patent, no. 5710/72.
4. Garbuliński T., 1994. Lydium – KLP w farmakoterapii weterynaryjnej. [Lydium-KLP in veterinary pharmacotherapy]. Życie Wet., 4A, 137 [in Polish].
5. Ibrahim H.R., Kato A., Kobayashi K., 1991. Antimicrobial effects of lysozyme against Gram-negative bacteria due to covalent binding of palmitic acid. J. Agric. Food Chem. 39, 2077-2082.
6. Ibrahim H.R., Hatta H., Fujiki M., Yamamoto T., 1994. Enhanced antimicrobial action of lysozyme against Gram-negative and Gram-positive bacteria due to modification with perillaldehyde. J. Agric. Food Chem., 42, 1813.
7. Ibrahim H.R., Higashiguchi S., Juneja L.R., Kim M., Yamamoto T., 1996. A structural phase of heat-denatured lysozyme with novel antimicrobial action, J. Agric. Food Chem., 44, 1416.
8. Jolles P., Jolles J., 1984. What is new in lysozyme research? Mol. Cell. Biochem., 63, 165.
9. Kiczka W., 1994. Od monomeru do dimeru lizozymu. [From lysozyme monomer to dimer]. Życie Wet., 4A, 131-136 [in Polish].
10. Kijowski J., Leśnierowski G., 1995. Wykorzystanie lizozymu do utrwalania żywności, w diagnostyce medyccnej I farmakologii. [The application of lysozyme to preserve food in medical diagnostic and pharmacology]. Biotechnologia, 2(29), 130 [in Polish].
11. Kijowski J., Leśnierowski G., Fabisz-Kijowska A., 1998. Methods of lysozyme separation, enzyme molecular form and functional quality of the residual egg white. Proc. 2nd Int. Symp. on Egg Nutrition and Newly Emerging Ovo-Technologies, Canadian Inovatech Inc., Banff, Alberta, Canada, 5-8 April 1998, p. 54.
12. Kijowski J., Leśnierowski G., 1999. Separation, polymer formation and antibacterial activity of lysozyme. Pol. J. Food Nut. Sci. 8/49(3), 3-16.
13. Kijowski J., Leśnierowski G., Fabisz-Kijowska A., 2000. Lysozyme polymer formation and functionality of residuals after lysozyme extraction. In: Egg Nutrition and Biotechnology. J. S. Sim, S. Nakai, W. Guenter (eds). International CAB, Wallingford, UK.
14. Laemmli U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227, 680.
15. Leśnierowski G., 1997. Otrzymywanie lizozymu z białka jaja kurzego metodami krystalizacji, ultrafiltracji oraz z użyciem wymieniacza jonowego. [Isolation of lysozyme from hen egg white using the crystallization, ultrafiltration and ion exchange methods. Diss., Agr. Univ. Poznań. [in Polish].
16. Leśnierowski G., Cegielska-Radziejewska R., Kijowski J., 2001. Antibacterial activity of thermally modified lysozyme. EJPAU, 4(2), 1-9.

17. Leśnierowski G., Kijowski J., 1995. Metody badania aktywności enzymatycznej oraz oznaczanie ilościowe lizozymu z białka jaja kurzego. [Methods of investigating enzymatic activity and quantitative determination of lysozyme from hen egg white]. Przem. Spoż., 12, 476 [in Polish].
18. Nakamura S., Kato A., Kobayashi K., 1991. New antimicrobial characteristic of lysozyme-dextran conjugate. J. Agric. Food Chem., 39, 647.
19. Nakamura S., Kato A., Kobayashi K., 1992. Bifunctional lysozyme-galaktomannan conjugate having excellent emulsifying properties and bactericidal effect. J. Agric. Food Chem., 40, 735.
20. Proctor V.A., Cunningham F.E., 1988. The chemistry of lysozyme and its use as a food preservative and a pharmaceutical, CRC Crit. Rev. Food Sci. Nutr., 26(4), 359.
21. Sophianopoulos A.J., Van Holde K.E., 1964. Physical study of muramidase (lysozyme). J. Biol. Chem., 239, 2516.

[1] Due to the confidentiality of the investigations no details concerning the methodology have been disclosed.

Grzegorz Leśnierowski, Jacek Kijowski, Jerzy Stangierski
Department of Food Quality Management,
August Cieszkowski Agricultural University of Poznań, Poland
Wojska Polskiego 31, 60-624 Poznań, Poland
e-mail: lesnier@au.poznan.pl
