Iran Agricultural Research, Vol. 31, No. 1, 2012
Printed in the Islamic Republic of Iran
Shiraz University

Optimization of Lysozyme – Inulin Conjugation and Investigation on its Functional Properties

S. HAGHIGHI-MANESH[1], M. AMINLARI[1, 2*] and R. RAMEZANI[1*]**

[1] Department of Food Science and Technology, College of Agriculture, Shiraz University, I.R. Iran
[2]Department of Biochemistry, Veterinary School, Shiraz University, I.R. Iran

Received 21 September 2011, Accepted 2 October 2012, Available online November 7, 2012

ABSRRAC- In recent **y**ears, several oconjugation protocols have been developed to improve the functional properties of the enzyme lysozyme. In this study, the optimum conditions of lysozyme – oxidized inulin conjugation and functional properties of the modified lysozyme were investigated. Initially, inulin (MW about 25 kDa) was oxidized by periodate to provide the reactive carbonyl groups to attach to amino groups of lysozyme for Maillard reaction and was then conjugated to the enzyme at a molar ratio of oxidized inulin to enzyme of 5:1. A number of studies were conducted to investigate the optimization of lysozyme – Inulin conjugation consisting of different pH levels (3, 7, and 9), different temperatures (40, 60, and 80°C), and different reaction times. The degree of modification was determined by SDS-PAGE and sugar analysis of the product. The best condition for conjugation was determined to be 60°C at pH 7.0 for one week. The results showed that under these conditions, the inulin-lysozyme conjugate had 58% of the lytic activity of the native enzyme and had better emulsifying properties and heat stability than native lysozyme. Moreover, there was significantly higher protein solubility at pH 7.0 and 9.0 at different temperatures than heated lysozyme. Taken together, the results of this study indicated that lysozyme modification by oxidized inulin results in a new product with improved functional properties which may be employed for different industrial purposes.

Keywords: Conjugation, Functional properties, Inulin, Lysozyme, Optimization

INTRODUCTION

Inulin belongs to a class of carbohydrates known as fructans. Fructan, in general is a term used for any carbohydrate in which fructosyl-fructose links constitute the majority of the glycosidic bonds. Fructans are linear or branched fructose polymers, which are either β, 2 → 1 linked inulins or β, 2 → 6 linked levans. A starting glucose moiety may be present, but not necessary. Both GF_n and F_n compounds (where F is a fructosyl unit and G a glucosyl unit) are

---

* Former Graduate Student, Professor and Instructor, respectively
** Corresponding Author

thus included under the same nomenclature. In chicory inulin, n, the number of fructose units can vary from 2 to about 60 (13). The main sources of inulin used in the food industry are chicory and artichoke (10). During the last decade, a significant increase in the number of publications dealing with the technological and nutritional benefits of inulin has occurred (7), but it was Thomson who called this substance inulin (22). Inulin is classified as a dietary fiber in all European and most other countries. It also complies with the Codex Alimentarius definition of dietary fiber (Codex Guidelines on Nutrition Labelling CAC/GL 2-1985, Rev.1-1993). In several countries, the nutritional properties of inulin are used for formulating health claims on food products and food supplements (4).

Lysozyme is a single chain polypeptide of 129 amino acids cross-linked with four disulfide bridges. It hydrolyzes β (1→4) linkages between N-acetylmuraminic acid and N-acetyl-D-glucosamine residues in peptidoglycan and between N-acetyl-D-glucosamine residues in chitodextrin. The enzyme is often used for lysing bacterial cells by hydrolyzing the peptidoglycan present in the cell walls (5 and 8). Recently much attention has been directed towards the preparation of new functional proteins. A number of studies attempting to improve protein functionality have involved chemical modification, such as alkylation, esterification, amidination, deamidination, covalent attachment of carbohydrates and fatty acids, thiol-disulfide exchange, and enzymatic modification (18). Among the various modifications applied to food proteins, modification with carbohydrates through mild Maillard-type reaction usually makes marked changes in the functional properties of proteins (15 and 20). These properties make the modified proteins more functional in biotechnological processes, where increased solubility and heat stability of proteins is required to ensure efficient transformation processes (18).

The purpose of this investigation was to study the effect of pH, temperature, and time on the glycation of lysozyme with inulin, and to measure some functional properties of the modified lysozyme.

MATERIALS AND METHODS

Materials

Chicken egg-white lysozyme was obtained from Inovatech, Inc., Abbotsford, BC, Canada. Inulin from chicory (MW about 25kDa) and *Micrococcus lysodeicticus* cells were from Sigma Shlevi/ Mv/ (USA). Sodium dodecyl sulfate (SDS), concentrated hydrochloric acid, sodium hydroxide, Tris, copper acetate, boric acid, concentrated sulfuric acid, phenol, sodium-potassium tartarate, were all from Merck, Germany. All other chemicals were reagent grades and were commercially available.

Oxidation of Inulin

Initially, inulin was oxidized by periodate to provide the reactive carbonyl groups to attach to amino groups of lysozyme for Maillard reaction. Upon the inulin oxidation, selective scission of chemical bonds between two carbon atoms (C3 and C4) in the fructose ring occurs and two reactive aldehyde groups are formed. The glycoconjugates can be prepared by the direct reaction of the aldehyde groups of oxidized inulin with ε-amino groups of the lysine residues in the molecule of the enzyme (23).

Inulin with a molecular weight of 25 kDa (3 g) and potassium periodate (600 mg) were dissolved in 30 ml 0.02 M Tris buffer pH 7. The mixture was constantly stirred in the dark at room temperature for 24 h (21 and 23).

Separation of Oxidized From non-oxidized Inulin

The oxidized inulin was separated from unreacted inulin through a Sephephadex G-10 column (30 cm × 1.5 cm), and the samples were eluted with 0.02 M Tris buffer pH 7.0 (Fig. 1). The fructose content was estimated by the phenol sulfuric acid method and fructose was used as standard (6 and 21).

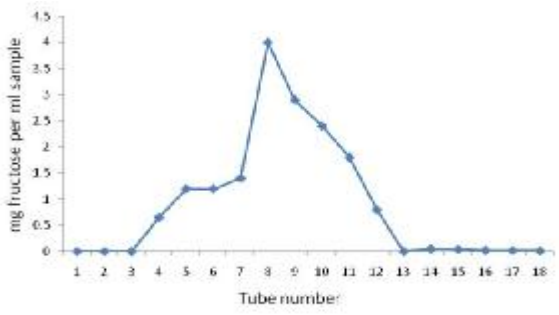

Fig. 1. G-10 Chromatography of oxidize inulin

Preparation of Lysozyme-Inulin Conjugate

One hundred mg lysozyme and 500 mg oxidized inulin were added to 2.0 ml 0.05 mol/L sodium phosphate buffer pH 9.0, 2.0 ml 0.05 mol/L sodium phosphate buffer pH 7.0, and 2.0 ml 0.05 mol/L sodium acetate buffer pH 3.5. The pH of the solutions was adjusted if necessary and after thorough mixing and incubating at room temperature for 1 h, the solutions were frozen at –35°C and then lyophilized. Lyophilized powder was incubated under relative humidity of 79%, provided by saturated KBr at 40, 60, and 80°C for 10, 7 and 2 days, respectively.

Separation of Conjugated Lysozyme From Non Conjugated Lysozyme

A Sephadex G-100 column chromatography which was equilibrated with 0.02 M Tris buffer pH 7.0, was used for separation of conjugated from non conjugated lysozyme. One hundred mg inulin-conjugated lysozyme was dissolved in 1 ml 0.02 M Trisbuffer, pH 7.0. The solution was gently mixed and centrifuged at 2500 ×g for 10 min to remove undissolved materials. The supernatant was applied to a 1.5 cm × 100 cm Sephadex G-100 column chromatography. Proteins were then eluted with the same buffer. The protein and the total sugar content of the collected tubes were measured by spectroscopic and phenol sulfuric acid methods, respectively (18).

Bulk Production of Lysozyme-Oxidized Inulin Conjugate

This stage was performed to provide the materials for tests of functional properties. Inulin (25 g) and potassium periodate (5 g) were dissolved in 250 ml 0.02 M Tris buffer pH 7.0. The mixture was stoppered tightly and maintained on stirrer in the dark at room temperature for 24 h. The mixture was then dialyzed against distilled water for 30 minutes in three steps. At the end of each step, the amounts of total sugars of the outer liquid of the dialysis tube were measured by the phenol sulfuric acid method; when it reached zero in the third step, dialysis was stopped and the amounts of total sugars of the inner liquid of the dialysis tube were measured in the same way. By using a vacuum oven, the mixture's volume was reduced to 20 ml. Regarding the amounts of total sugar in the mixture (20% w/w), proportionate amounts of lysozyme at a molar ratio of oxidized inulin to enzyme 5:1 were added and dissolved in 0.02

M Tris buffer pH 7 buffer. To perform the glycosylation process, the cited solution was incubated at 60°C near KBr (RH: 79%) for one week.

Electrophoresis

Slab SDS-PAGE was preformed according to the discontinuous buffer system of Laemmli (11). Protein samples were added to the loading buffer to give a final concentration of 1 mg ml^{-1} protein, 0.01 mol l^{-1} -HCl, pH 6.8, 0.4% SDS, 0.01 mol l^{-1} glycerol, and 0.04 g l^{-1} bromophenol blue. The running gel was made with 140 × 140 × 1mm dimensions and 150 g l^{-1} or 50–200 g l^{-1} polyacrylamide in 1.2 mol l^{-1} Tris Cl, pH 8.8 and 3 g l^{-1} SDS. The stacking gel contained 30 g l^{-1} acrylamide in 0.25 mol l^{-1} Tris-HCl, pH 6.8 and 2 g l^{-1} SDS. The electrode buffer comprised of 0.025 mol l^{-1} Tris-HCl, 0.192 mol l^{-1} glycine and 1.5 g l^{-1} SDS at pH 8.16. Electrophoresis was performed at a constant 25 mA current and gels were stained with silver in 500 g l^{-1} acetic acid/250 g l^{-1} methanol and de-stained with 100 g l^{-1} acetic acid/70 g l^{-1} methanol.

Color measurement and Analysis of the Samples

Color measurement and sample analysis were carried out by the Yam and Papadakis method. Three parameters, L* (lightness), a* (redness) and b* (yellowness), were used to study changes in the color. L* refers to the lightness of the samples and ranges from black = 0 to white = 100. A negative value for a* indicates green, while positive a* indicates a red- purple color. Positive b* indicates yellow while a negative b* indicates blue. Positive and negative b* indicate yellow and blue colors, respectively (24).

Total Sugar Content Measurement

. Determination of sugars using phenol-sulfuric acid is based on the absorbance of 490 nm of a colored aromatic complex formed between phenol and the carbohydrate. The amount of sugar present is determined by comparison with a calibration curve using a spectrophotometer as described by Dubios et al (6).

Enzyme Activity

The activity of lysozyme was determined using a microbiological assay consisting of lyophilized *Micrococcus lysodeikticus* cell wall. Nine milligrams of dried *M. lysodeikticus* cell wall was dissolved in 25 mL of 0.1 M potassium phosphate buffer (pH 7.0) and diluted to a final volume of 30 mL with the same buffer. Lyz or modified Lyz at a concentration of 1mg of protein/mL was dissolved in cold distilled water A cuvette was filled with 2.9 ml of cell suspension and incubated for 4–5 min in order to achieve temperature equilibration to establish blank rates. 0.1 ml of enzyme solution was then added to the cuvette. Changes in the absorbance at 450 nm were recorded per minute from the initial linear portion of the curve (20).

Protein Content

The total level of protein in solutions was determined by the Lowry protein assay. The total protein concentration is exhibited by a color change of the sample solution in proportion to protein concentration, which can then be measured using colorimetric techniques. The produced blue color is the result of the reaction of Cu^{2+} with peptide bonds of proteins and the reduction of phosphomolybdic/phosphotungestic acid by tyrosine and tryptophan groups of proteins. The bovine serum albumin (BSA) was selected as the standard protein (14).

Protein Solubility at Different pH and Temperatures

Samples of native, control (heated lysozyme in the absence of oxidized inulin) and conjugated lysozyme were dissolved (30 mg) in a proper buffer (sodium acetate 0.1M, pH 3.0 and 5.0, and sodium phosphate 0.1M, pH 7.0 and 9.0), homogenized and kept at 25 °C for one hour. The resulting solution was centrifuged at 2700 g for 15 min. The protein content of the supernatant was measured by Lowry method. Solubility percentage is defined as the ratio of the protein concentration in supernatant over total protein concentration of the sample, multiplied by 100 (12 and 16).

For determination of the effect of temperature on protein solubility of samples, the cited method for determination of protein solubility at different pH levels was used, except that the lysozyme samples were dissolved in sodium phosphate 0.1 M, pH 7.0 & 9.0 and heated in water baths maintained at 25, 40, or 60°C for 48 h (12 and 16).

Emulsifying Properties

The emulsion activity and stability was measured by the Pearce and Kinsella method (17). One ml corn oil was added to 3 ml of 1 mol/L protein in 0.1 mol/L sodium phosphate, pH 7.4 and the mixture was homogenized at fixed speed at 25°C for 1 min. At 1 min intervals (0 to 10 min), 0.1 ml of the emulsion was removed, added to 5 ml 0.1% SDS and absorbance at 500 nm was recorded immediately and absorbance against time plots were prepared. Emulsion activity was the absorbance at zero time. The time required to obtain a 50% reduction in absorbance was a measure of emulsion stability (17).

Heat Resistance

Heat resistance of inulin conjugated lysozyme was determined by measuring the turbidity (absorbance at 500 nm) of protein solutions (7.5 mg per 10 ml 0.1 mol/L sodium phosphate, pH 7.4) held at 50°C to 95°C. Starting at 50°C the temperature was increased 1°C per min and absorbance was recorded each 5 min (19).

Experimental Design and Data Analysis

The data were subjected to analysis of variance (ANOVA) using SPSS version 16.0. Significant differences between the means were determined using Duncan's multiple range test at $p<0.05$.

RESULTS AND DISCUSSION

Effect of Glycosylation on The Electrophoresis Behavior of Lysozyme SDS-PAGE

Figure 2 shows the SDS-PAGE pattern of glycosylated lysozyme compared to non glycosylated lysozyme on 10% polyacrylamide gel. The band intensity in column 1 is higher than that of column 2, which indicates the higher glycosylation process in tubes 21-29. Moreover, new bands were seen in conjugated samples (column 1) which were comparable to non conjugated samples (columns 2 and 3).

Optimization of Lysozyme – Inulin Conjugation

Using the SDS-PAGE technique, the best condition of glycosylation of lysozyme and oxidized inulin was detected to be 60°C at pH 7.0 for one week (Fig. 3). Although samples 9

and 6 represented better bands than sample 5, they were not selected as the best ones. That was firstly because of the deteriorative influence of high pH on food ingredients; additionally, the sample solubility reduced by increasing the temperature.

Separation of Conjugated from non Conjugated Lysozymes

The first and second peak in Figure 4, are related to conjugated and non conjugated enzymes, respectively. This is the consequence of the lower molecular weight of non conjugated enzyme as compared to that of conjugated lysozyme. There was an overlap between the total sugar content curve and the first peak of the chromatogram, which shows the existence of the conjugated lysozyme between tubes number 21 to 36.

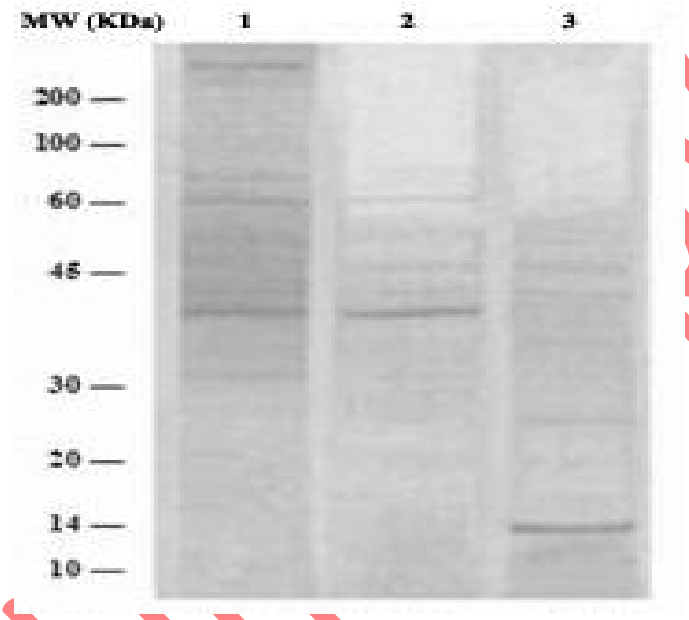

Fig. 2. The pattern of the electrophoresis of the glycosylated enzyme compared to non glycosylated lysozyme on the 10% polyacrylamide gel (columns number 1, 2, and 3 relate to tube numbers 21-29, 29-36, and 36-45 respectively)

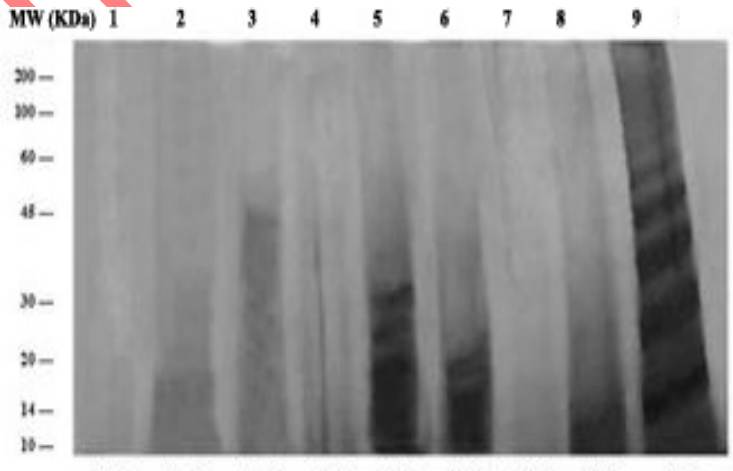

Fig. 3. The pattern of the electrophoresis of the glycosylated lysozyme (columns number 1, 2, 3, 4, 5, 6, 7, 8, and 9 relate to different conditions of glycosylation conditions of pH 3 at 40°C, pH 7 at 40°C, pH 9 at 40°C, pH 3 at 60°C, pH 7 at 60°C, pH 9 at 60°C, pH 3 at 80°C, pH 7 at 80°C and pH 9 at 80°C, respectively)

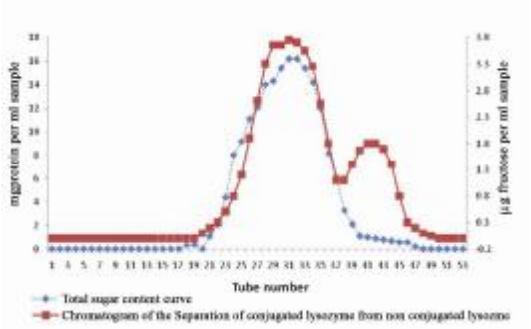

Fig. 4. Chromatogram of the separation of conjugated from non conjugated lysozyme by the sephephadex G-100 column chromatography (1 cm × 100 cm)

Color measurement and Analyzing of the Samples

Statistical analysis of the data presented in Table 1 with SPSS software indicated that the "L^* factor" was considerably affected by the glycosylation process. Moreover, glycosylation caused the decrease in the "a^* factor" which indicates the decreasing of redness. Comparison of the "b^* factor" of the samples with each other shows that, while heating had no significant (p<0.05) influence on the yellowness of lysozyme, glycosylation increased the yellowness of native and control lysozyme significantly (p<0.05).

Table 1. Color measurement and analyzing of the samples

Lysozyme samples	L^* factor	a^* factor	b^* factor
Native lysozyme	91.4 ^{a} (±0.3)*	-5.7 ^{a} (±0.4)	4.2 ^{a} (±0.2)
Control lysozyme	91.2 ^{a} (±0.7)**	-5.2 ^{a} (±0.5)	4 ^{a} (±0.4)
Conjugated lysozyme	81.2 ^{b} (±0.4)	-1.2 ^{b} (±0.7)	19.3 ^{b} (±0.6)

*** In each column different superscript letters indicate significant differences (p<0.05)**
**** Each point is the average of three replicates**

Enzyme activity

The enzyme activity of glycosylated, control and native lysozyme, were 85.3, 327.7 and 416.7 (unit/mg protein), respectively. Glycosylation of lysozyme with inulin resulted in the decrease of the enzyme activity by 80% and 75% as compared with native and control samples, respectively (Table 2).

The net charge of the lysozyme has an important role in enzyme activity against *Micrococcus lysodeikticus* cell wall; therefore, change in surface charge of lysozyme affects surface activities in lysozyme. Blockage of free amino groups in lysozyme with inulin decreases its positive charge and consequently decreases the tendency of lysozyme to *Micrococcus lysodeikticus* cell wall (having negative charge) (1 and 6).

Table 2. Enzymatic activity of glycosylated, control and native lysozyme

Lysozyme samples	Enzyme activity (unit/mg protein)	Enzyme activity %
Native lysozyme	416.7 ^{a} (±7.5)*	100 ^{a} (±0.01)
Control lysozyme	327.7 ^{b} (±26.3)**	78.5 ^{b} (±0.07)
Conjugated lysozyme	85.3 ^{c} (±16.5)	20.5 ^{c} (±0.37)

*** In each column different superscript letters indicate significant differences (p<0.05)**
**** Each point is the average of three replicates**

Protein Solubility at Different pH Levels and Temperatures

Table 3 shows the solubility of lysozyme samples at different pH's. Although native lysozyme exhibited the highest protein solubility (p<0.05) at all pH's, glycosylation resulted in remarkable decrease in solubility. One exception was seen at pH 9.0, where glycosylated enzyme was less different from native enzyme as compared to the other pH's.

One interesting characteristic of the covalent complex of protein and polysaccharides is the increase of protein solubility at the isoelectric point. The increase of hydration capacity of the enzyme during the conjugation of inulin to lysozyme improves solubility at pH 9.0. The sever decrease in solubility at pH 3.0 is the result of electric charge density change (9).

Table 3. Protein solubility (%) at different pH's at 25°C

Lysozyme samples	pH 3	pH 5	pH 7	pH 9
Native lysozyme	99.8 ^{a} (±0.94)*	99.7 ^{a} (±1.15)	99.6 ^{a} (±1.23)	98.3 ^{a} (±0.98)
Control lysozyme	75.4 ^{b} (±1.06)**	86.8 ^{b} (±1.31)	79.7 ^{b} (±1.01)	70.8 ^{b} (±1.76)
Conjugated lysozyme	44.4 ^{c} (±1.34)	55.5 ^{c} (±1.08)	70.8 ^{c} (±0.95)	88.6 ^{c} (±1.42)

*** In each column different superscript letters indicate significant differences (p<0.05)**
**** Each point is the average of three replicates**

Results from solubility studies at different temperatures at pH 7.0 and 9.0 are given in Table 4. Given the highest solubility of glycosylated lysozyme at pH 9.0 and the neutrality of most foodstuffs, it would be convincing to select pH 9.0 and 7.0 to perform this assay. Although compared to the control lysozyme, glycosylation at pH 7.0 improved lysozyme solubility at different temperatures, solubility of glycosylated lysozyme at pH 9.0 was significantly (p<0.05) lower than the control lysozyme. In brief, increasing the temperature at both pH 7.0 and 9.0, results in the decrease of the solubility of the native, control and glycosylated lysozyme. The improvement of the conjugated lysozyme solubility at pH 7.0, was the result of the great hydration capacity of the attached carbohydrate to the enzyme (3 and 18).

Table 4. Protein solubility (%) at different temperatures at pH 9.0 and 7.0

Lysozyme samples	25°C	40°C	60°C
Native lysozyme at pH 9.0	98.6 ^{a} (±1.13)*	95.1 ^{a} (±0.96)	78.1 ^{a} (±0.96)
Native lysozyme at pH 7.0	97.3 ^{a} (±1.22)**	95.6 ^{a} (±1.34)	88.2 ^{a} (±1.43)
Control lysozyme at pH 9.0	80.9 ^{b} (±1.36)	70.1 ^{b} (±1.22)	59.4 ^{b} (1.45)
Control lysozyme at pH 7.0	72.3 ^{c} (±1.32)	60.2 ^{c} (±0.94)	34.9 ^{c} (±1.52)
Conjugated lysozyme at pH 9.0	62.2 ^{c} (±1.44)	58.3 ^{c} (±1.02)	42.4 ^{c} (±1.51)
Conjugated lysozyme at pH 7.0	83.6 ^{b} (±1.25)	72.5 ^{b} (±1.16)	58.1 ^{b} (±1.24)

*** In each column different superscript letters indicate significant differences (p<0.05)**
**** Each point is the average of three replicates**

Emulsifying Properties

There was no significant difference ($p<0.05$) between the emulsifying properties of the native and control lysozyme; whereas the emulsifying activity and emulsifying stability of the glycosylated lysozyme were significantly ($p<0.05$) higher than the native and control lysozyme (Table 5).

Emulsifying properties of protein-polysaccharide compounds are ascribed to their amphiphilic properties. The residues of denatured hydrophobic protein are set in the oil-water interface on the oil droplets; the hydrophilic part of the carbohydrate aligns to the aqueous phase and prevents the aggregation of the oil droplets. Therefore, by the conjugation of a polysaccharide to a protein, a very stable emulsion is obtained (15 and 19).

Table 5. Emulsifying properties of the protein samples

Lysozyme samples	Emulsifying activity (A_{500} nm)	Emulsifying stability (min)
Native lysozyme	**0.06 [a] (±0.01)***	**2.27 [a] (±0.24)**
Control lysozyme	**0.05 [a] (±0.01)****	**2.70 [a] (±0.12)**
Conjugated lysozyme	**0.09 [b] (±0.01)**	**4.17 [b] (±0.14)**

*** In each column different superscript letters indicate significant differences ($p<0.05$)**
**** Each point is the average of three replicates**

Heat Resistance

According to the results summarized in Figure 6, increasing the temperature, would increase the turbidity of the protein samples (native, control and glycosylated lysozyme). At temperatures over 70°C, the increase in turbidity was more considerable in non glycosylated samples. The rate of turbidity increase by temperature increase (from 60°C to 90°C) in glycosylated lysozyme was less than its increase in the native and control lysozyme. For instance, the absorbance of 0.06 nm was achieved at 60, 55 and 70°C for native, control and conjugated lysozyme respectively.
Since the carbohydrate residues act as a protective agent for glycoprotein moiety and prevent aggregation of the denatured proteins, glycoproteins have less sensitivity to heat denaturation than natural proteins (9).

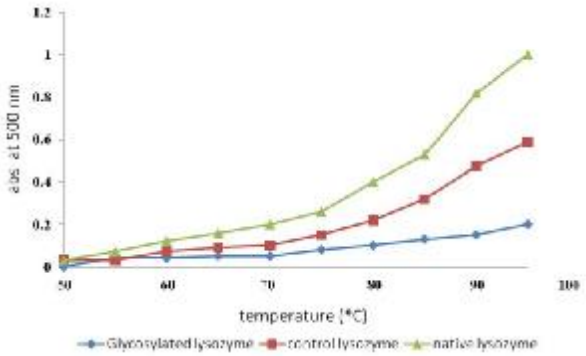

Fig. 5. Heat stability of the native.e, control and glycosylated lysozymes in the lyophilize state

CONCLUSION

To sum up, regarding the results, it is recommended that functional food products be made from lysozyme – inulin conjugates. The resulting conjugate would be resistant to the fluctuation of pH and temperature. Moreover, by considering the importance of the functionality of inulin, it can be put to widespread use in food and drug industries.

Specific Comments and Suggestions

This paper evaluated the optimization of the lysozyme – inulin conjugation and investigated its functional properties. Bioconjugation of lysozyme has been already studied by different authors. However, the possibility of lysozyme – inulin conjugation and functional properties of the modified lysozyme, as reported in this paper, deserves particular attention.

REFERENCES

1. Aminlari, M., R. Ramezani and F. Jadidi. 2005. Effect of Maillard-based conjugation with dextran on the functional properties of lysozyme and casein. Journal of Science of Food and Agricalture. 85: 2617-2624.

2. Arita, K., E. E. Babiker, H. Azakami and A. Kato. 2001. Effect of chemical and genetic attachment of polysaccharides to proteins on the production of IgG and IgE. Journal of Agriculture and Food Chemistry. 49: 2030-2036.

3. Babiker, E. E. Effect of chitosan conjugation on the functional properties and bactericidal activity of gluten peptides. 2002. Journal of Food Chemistry. 79: 367-372.

4. Coussement, P.A.A. Inulin and oligofructose: safe intakes and legal status. 1999. Journal of Nutrition. 129: 1412-1418.

5. Davis, A. C. A., A. Neuberger and B.M. Wilson. 1969. The dependence of lysozyme activity on pH and ionic strength. Journal of Biochemistry and Biophysics. 178: 294-301.

6. Dubois, M., K. A. Gilles, K. Hamilton, A. P. Rebers and F. Smith. 1956. Calorimetric method for determination of sugars and related substances. Journal of Analytical Chemistry. 28: 350-356.

7. Franck, A., L. De Leenheer. 2002. Inulin, Biopolymers. (1^{th} ed). Steinbuchel: VCH Weinheim, Chapter 6.

8. Imoto, T. and K. Yagishita. A simple activity measurement of lysozyme. 1971. Journal of Agriculture and Biological Chemistry. 35: 1154-1156.

9. Jimenez-Castano, L., M. Villamiel and R. Lopez-Fandino. 2007. Glycosylation of individual whey proteins by maillard reaction using dextran of different molecular mass. Journal of Food Hydrocolloid. 21: 433-443.

10. Kaur, N., and A. K. Gupta. 2002. Application of inulin and oligofroctose in health and nutrition. Journal of Biological Science. 27: 703-714.

11. Laemmli, UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Journal of Nature. 227: 680-685.

12. Lakkis, J. and R. Villota. 1992. Effect of acetylation on substructural properties of protein: A study using fluorescence and circular dichoism. Journal of Agriculture and Food Chemistry. 40: 553-560.

13. Leenheer, De. L and H. Hoebregs. 1994. Progress in the elucidation of the composition of chicory inulin. Journal of Starch. 46: 193-198.

14. Lowry, R. H, N. J. Rosebrough Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry. 193: 265-275.

15. Nakamura, S., K. Kobayashi and A. Kato. 1994. Role of positive charge of lysozyme in the excellent emulsifying proprieties of maillard-type lysozyme-polysoccharide conjugate. Journal of Food Chemistry. 42: 2688-2691.

16. Miller, G. L. 1959. Protein deterimination for large numbers of samples. J. Analy. Chem. 31: 429-442.

17. Pearce, K. M. and J. E. Kinsella. 1978. Emulsifying properties of proteins: Evaluation of a turbidimetric technique. Journal of Agriculture and Food Chemistry. 86: 716-723.

18. Scaman, C., S. Nakai, and M. Aminlari. 2006. Effect of pH, temperature and sodium bisulfite or cysteine on the level of maillard-based conjugation of lysozyme with dextran, galactomannan and mannan. J. Food Chem. 99: 368-380.

19. Shu, Y., S. Sahra, and A. Kato. 1996. Effect of the length of polysaccharide chains on the functional properties of the Maillard-type lysozyme-polysaccharide conjugation. Journal of Agriculture and Food Chemistry. 44: 2544-2548.

20. Shugar, D. 1952. The measurement of lysozyme activity and the ultra-violet inactivation of lysozyme. Journal of Biophysics. 8: 302–309.

21. Tabandeh, M. R. and M. 2009. Aminlari. Synthesis, physicochemical and immunological properties of oxidized inulin-L-asparaginase bioconjugate. Journal of Biotechnology. 141: 189-195.

22. Thomson, T. 2010. A System of Chemistry. (5th ed.). Philadelphia: Abraham Small, Chapter 4.

23. Vina, I., A. Karsakevich and M. Bekers. 2001 Stabilization of anti-leukemic enzyme L-asparaginase by immobilization on polysaccharide levan. Journal of Molecular Catalysis. 11: 551-558.

24. Yam, K. L., and S. E. Papadakis. 2004. A simple digital imaging methods for measuring and analyzing color of food surfaces. J. Food Eng. 61: 137-142.

بهینه سازی کانژوگه کردن لیزوزیم با اینولین و بررسی خواص عملکردی محصول تولید شده

سروش حقیقی منش[1]، **محمود امین لاری[2,1]** و **رقیه رمضانی[1]***

[1] بخش علوم و صنایع غذایی، دانشکده کشاورزی، دانشگاه شیراز، جمهوری اسلامی ایران
[2] بخش بیوشیمی، دانشکده دامپزشکی، دانشگاه شیراز، جمهوری اسلامی ایران

چکیده- در سال های اخیر در زمینه بیوکانژوگه کردن موافقیت هایی انجام شده است تا خصوصیات عملکردی آنزیم لیزوزیم بهبود یابد. در این تحقیق شرایط بهینه کانژوگه شدن اینولین اکسید شده –لیزوزیم و خواص عملکردی محصول تولید شده مورد ارزیابی قرار گرفت. ابتدا برای تأمین گروه های آلدهیدی فعال برای چسبیدن به گروه های آمینی شرکت کننده در واکنش های میلارد، اینولین (وزن ملکولی تقریبا 25kDa) توسط پرایودات، اکسیده و با آنزیم لیزوزیم با نسبت مولی اینولین اکسید شده به آنزیم 5 به 1، ترکیب گردید. مطالعات انجام شده برای بررسی شرایط بهینه کانژوگه کردن، شامل بررسی واکنش در شرایط مختلف pH (3،7و9) ، دماهای (80°C و ،60، 40) و شرایط زمانی مختلف می باشد. بهترین شرایط برای کانژوگه کردن pH 7، 60°C و مدت زمان یک هفته تعیین گردید. نتایج نشان داد که تحت چنین شرایطی کانژوگه اینولین – لیزویزیم دارای 58% از فعالیت تجزیه کنندگی، خصوصیات امولسیفایری بهتر و مقاومت حرارتی بالاتر در مقایسه با لیزوزیم خالص می باشد. همچنین این محصول دارای حلالیت پروتئینی بیشتری در pH 7 و pH 9 در دماهای مختلف نسبت به لیزوزیم کنترل بود. در مجموع نتایج این تحقیق نشان می دهد که اصلاح لیزوزیم توسط اینولین اکیسد شده باعث تشکیل محصول پایدار جدید با خواص عملکردی ارتقاء یافته می گردد، که ممکن است برای مصارف صنعتی مورد استفاده قرار گیرد.

واژه های کلیدی: اینولین، بهینه سازی، خواص عملکردی، کانژوگه کردن، لیزوزیم

---

*به ترتیب دانشجوی پیشین کارشناسی ارشد، استاد و مربی
** مکاتبه کننده