FUNCTIONAL PROPERTIES CHANGE OF PIGSKIN COLLAGEN BY CHEMICAL MODIFICATION

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Summary

The relationship between the possible structural change due to chemical modifications and functionality changes was studied in pigskin collagen. Amino groups in collagen were modified by succinylation and reductive alkylation. Carboxyl groups were modified using carbodiimide. Thermal denaturation temperature of collagen increased remarkably by carboxyl groups modification whereas decreased by succinylation and reductive alkylation. Emulsifying capacity was improved by reductive alkylation and carboxyl groups modification while emulsion stability was improved by succinylation. Chemical modifications increased solubility whereas decreased the foaming capacity of collagen. Viscosity of collagen at various pH varied with methods of modification.
(Key Words: Collagen, Chemical Modification, Functional Properties)

Introduction

Collagen is the most abundant single protein in the mammalian body, comprising about 25% of the total protein in the body (Pearson and Young, 1989). Since collagen fibers are widely distributed in various tissues such as skin, bone, tendon, cartilage, blood vessels and organs, it has various functions. As functionalities can be modified by chemical and enzymatic methods, enormous progress in the technology of manufacturing collagen products has been made and new uses for collagen have been discovered in many fields (Chvapil, 1979).

However, collagen has not been utilized greatly as a food ingredient except gelatin due to the poor functionalities such as solubility, emulsifying capacity and foaming capacity. Therefore, in order to enhance its food use, the relationship between its functionality as a food ingredient and structural change of collagen by various chemical modification methods was studied. Chemically modified collagen, however, should be subjected to rigorous safety tests before being considered for food uses.

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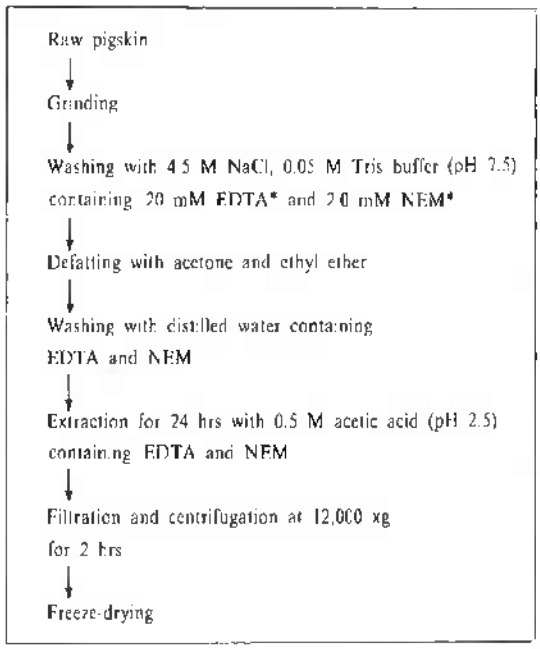
Materials and Methods

Sample preparation

Collagen was extracted from raw pigskin as described by Miller and Rhodes (1982) and freeze-dried (figure 1). Amino groups in collagen were



* EDTA ethylene diamine tetraacetic acid
NEM N-ethylmaleimide

Figure 1. Collagen preparation.

modified by succinylation according to the method of Eisele and Brekke (1981) and by reductive alkylation as in Wong et al. (1984). Carboxyl groups were modified using carbodiimide and glycinamide (Glazer et al., 1975). After modification, they were dialyzed against distilled water for 2 days and freeze-dried.

Analysis

Degree of modification was determined by the methods described in Fields (1972) for succinylation and reductive alkylation, and by Glazer et al. (1975) for carboxyl groups modification. Thermal transition temperature of collagens was measured using Perkin-Elmer DSC-4(U.S.A.) and viscosity using Brookfield Synchro-Lectric Viscometer (model LVF) at 20℃ and 60 r.p.m. with spindle #2. Solubility measurement was made using 1% solution at pH 7.0 according to the method of Morr et al. (1985). Emulsifying capacity and emulsion stability were measured using 0.7% suspensoids and Virtis homogenizer (The Virtis Co., U.S.A.) as described by Sung and Lee (1985). Foaming capacity determination was carried out by stirring 0.1% collagen suspensoids at high speed and then measuring the foam volume in a graduated cylinder.

Results and Discussion

Analysis on the degree of modification (table 1) showed that succinylation resulted in 79.5% of modification, reductive alkylation 68.5% and carboxyl groups modification 65.6%. The thermal transition temperature (Tm) measurements (table 1) showed that carboxyl groups modification increased Tm remarkably (94.4℃) whereas succinylation (Tm = 51.6℃) and reductive alkylation (Tm = 45.9℃) decreased the temperature compared with native collagen (Tm = 63℃).

As shown in table 2, functional properties of chemically modified collagens varied with methods of modification. Carboxyl groups modification and dimethylation (reductive alkylation) improved emulsifying capacity whereas succinylation made no difference. However, succinylation improved emulsion stability while other modifications did not. Solubility of collagen was increased by all the chemical modifications, among them succinylation increased most. Chemical modifications decreased the foaming capacity of collagen.

Figure 2 illustrates that viscosity of collagens varied with pH. At pH 3.0 only the carboxyl groups modification resulted in the decrease of

TABLE 1. DEGREE OF MODIFICATION AND THERMAL TRANSITION TEMPERATURE (Tm) OF COLLAGEN BY CHEMICAL MODIFICATIONS

	Succinylation	Reductive alkylation	Carboxyl groups modification
Degree of modification (%)	79.5	68.5	65.6
Tm (℃)	51.6	45.9	94.4

TABLE 2. FUNCTIONAL PROPERTIES OF VARIOUS COLLAGENS

Collagen	Solubility* (%)	Emulsifying capacity** (ml/g)	Emulsion stability*** (%)	Foaming capacity**** (ml)
Native	26.9	494	14.3	9
Reductive alkylated	47.2	591	15.8	6
Succinylated	75.5	494	0	7
Carboxyl groups modified	35.9	706	13.3	2

* with 1% suspensoids at pH 7.0.
** in 0.7% suspensoids; ml of soybean oil emulsified by g of the sample.
*** % separated volume.
**** with 0.1% suspensoids.

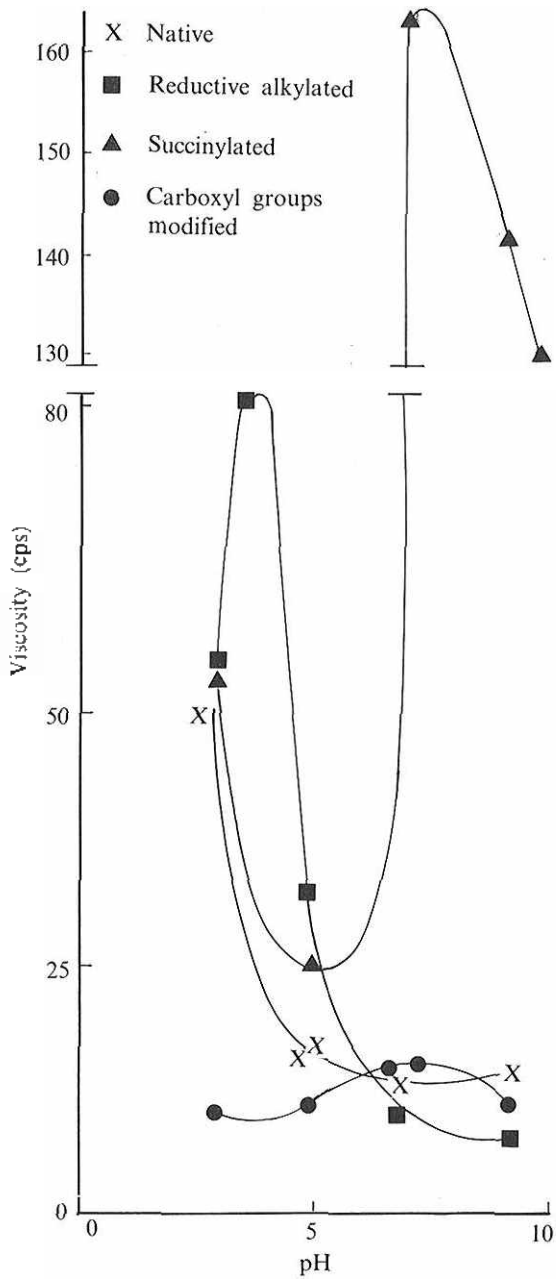


Figure 2. Viscosity of collagens (in 0.7% suspensoids) at various pH values.

viscosity. With the increasing pH, while the continuous fall of viscosity was observed in native collagen, the decrease first and then sharp increase in succinylated, the increase and then continuous decrease in reductive alkylated, and no significant change in carboxyl groups modified collagen were observed.

Chemical modifications that increase surface charge on proteins tend to improve their functional properties and heat stability by increasing charge repulsion (Ball and Winn, 1982). Heat stability of proteins could be brought about by providing for internal cross-linking (Hegg and Lofqvist, 1974). In this study, the modifications that increase negative charge (succinylation) or abolish the charge (reductive alkylation) decreased the heat stability while that preserving positive charge (carboxyl groups modification) increased the stability. It was reported that different proteins that were modified by the same chemical procedure responded to heat treatment differently (Ball, 1987).

Changes in charge can also affect the conformation of the protein and thus its overall functional characteristics. The attachment of hydrophilic groups to protein could increase solubility of the protein by increasing its affinity for water (Feeney, 1977). The net negative charge of the protein *increases upon succinylation causing* changes in the electrostatic environment and conformation of the protein molecule. The end products tend to dissociate, are more soluble and posses high intrinsic viscosity (Feeney, 1977).

Improvement of emulsifying capacity is attributed to enhanced solubility and expanded molecular structure which enhanced emulsion formation. Decrease of emulsifying capacity in a chemically modified protein could be due to the excessive net charge and subsequent impairment of formation of a cohesive film. Electrostatic repulsion between emulsified droplets enhanced stability (Kinsella, 1982). The modified proteins have been found to exhibit different hydration and surfactancy properties (Shukla, 1982).

The intermolecular protein-protein interactions are primarily responsible for the observed viscosity behavior. Since many factors such as conformation, hydration, exposure of hydrophobic groups, and charge distribution contribute to the intermolecular interactions that affect viscosity, the viscosity is not necessarily correlated to the solubility. However, in a protein the solubility change by altering the pH would change the viscosity because soluble molecules would experience greater protein-protein interactions (Shen, 1981).

Acknowledgements

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Literature Cited

Ball, H. R., Jr. 1987. Functional properties of chemically modified egg white proteins. JAOCS 64(12): 1718.

Ball, H. R., Jr and S. E. Winn. 1982. Acylation of egg white proteins with acetic anhydride and succinic anhydride. Poultry Sci. 61:1041.

Chvapil, M. 1979 Industrial uses of collagen. In "Fibrous Proteins: Scientific, industrial and medical aspects (D.A.D. Parry and L. K. Creamer, eds.)", Vol. 1, pp. 247, Academic Press, Inc., N. Y., N. Y.

Eisele, T. A. and C. J. Brekke. 1981. Chemical modification and functional properties of acylated beef heart myofibrillar proteins. J. Food Sci. 46:1095.

Feeney, R. E. 1977. Chemical modification of food proteins. In "Food Proteins. Improvement through chemical and enzymatic modification (R. E. Feeney and J. R. Whitaker, eds.)", ACS. Washington, D. C. pp. 3.

Fields, R. 1972. The rapid determination of amino groups with TNBS. Methods in Enzymology. Vol. 25A, pp. 264. *Academic Press, Inc.*

Glazer, A. N., R. J. DeLange and D. S. Sigman. 1975. Chemical modification of proteins. North-Holland Pub. Co., Amsterdam.

Hegg, P. and B. Lofqvist. 1974. The protective effect of small amounts of anionic detergents on the thermal aggregation of crude ovalbumin. J. Food Sci. 39:1231.

Kinsella, J. E. 1982. Protein structure and functional properties: emulsification and flavor binding effects. In "Food Protein Deterioration. Mechanisms and Functionality (J. P. Cherry, ed.)" ACS, Washington, D.C. pp. 275.

Miller, E. J. and R. K. Rhodes. 1982. Preparation and characterization of the different types of collagen. Methods in Enzymology. Vol. 82, pp. 33. Academic Press, Inc.

Morr, C. V., B. German, J. E. Kinsella, J. M. Regenstein, J. P. Van Buren, A. Kilara, B. A. Lewis and M. E. Mangino. 1985. A collaborative study to develop a standardized food protein solubility procedure. J. Food Sci. 50:1715.

Pearson, A. M. and R. B. Young. 1989. Muscle and Meat Biochemistry. Academic Press, Inc., San Diago, CA.

Shen, J. L. 1981. Solubility and viscosity. In "Proteins Functionality in Foods (J. P. Cherry, ed.)", ACS, Washington, D.C. pp. 89.

Shukla, T. P. 1982. Chemical modification of food proteins. In "Food Protein Deterioration. Mechanisms and Functionality (J. P. Cherry, ed.)", ACS, Washington, D.C. pp. 275.

Sung, S. K. and M. Lee. 1985. A study on the utilization of pork preblends in emulsion-type sausage production. K. J. Animal Sci. 27(1):34.

Wong, W. S. D., D. T. Osuga and R. E. Feeney. 1984. Pyridine borane as a reducing agent for proteins. Analyt. Biochem. 139:58.