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Gelatin: A Valuable Protein for Food and Pharmaceutical Industries: Review

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ABSTRACT: Many works have appeared in various scientifically reputable journals and publications worldwide that seem to have made potential or satisfactory contribution to our knowledge on the functions and utilization of gelatin — an important source of animal protein. Irrespective of these worldwide publications, room still exists for more work to be done to fully understand the utilization, chemical, biological, physical and functional properties of gelatin. Chemical and enzymatic modifications as well as biological studies should be undertaken with accuracy to be able to extend the utilization of gelatin in food and pharmaceuticals.

KEY WORDS: manufacturing technology, chemical composition, model building, biological activity, physico-chemical properties, and protein modification.

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I. INTRODUCTION

Gelatin is a traditional water-soluble functional protein of high interest and value, having the ability of forming transparent gels under specific conditions. Generally obtained by heat dissolution at alkaline or acidic pH and partial hydrolysis of collagen in animal skins, bones and tendons, gelatin presents a structure with variable physical properties and chemical heterogeneity due to the differences in collagen sources and preparation techniques.[1,2,3,4,5] Although there are some differences in the manufacturing technology, gelatin is remarkably known for its unique gel-forming ability, which makes it a valuable material for investigating the fundamental functional properties in colloid studies.

Gelatin has long been used in the food industry as clarification agent, stabilizer, and protective coating material.[1, 6] Tonnages of gelatin have been reported to be used annually in the food industry, especially in desserts, candies, bakery products, jellied meat, ice cream, and dairy products. The amount of gelatin that finds its application in the pharmaceutical industry is not negligible as far as the manufacture of pharmaceutical capsules, ointments, cosmetics, tablet coating, and emulsions are concerned. Gelatin also finds application in photography and some specialized industries. Despite the wide utilization of this protein, very little information could be found in the patent literature. In the last few decades, during the twentieth century especially, advances in technology and social economic factors, including population growth, has dictated the need for higher levels of efficiency in the production industries and subjected the overall food industry to the requirement of finding new protein functionalities. Against this background, some investigations have been conducted and reported on gelatin for the following purposes:

1. The elucidation of the structure of gelatin, which involves model building of the possible make-up of gelatin molecule and the mechanisms of modification and denaturation as well as the fundamental building of the protein based on the amino acid composition. On this aspect, the works of Gordon et al.,[7] Bowes and Kenten,[8] Ward,[9] and Ames[10] are excellent illustrations.

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2. The pharmaceutical and medicinal applications, which deal essentially with the highly reactive properties of gelatin used in adequate quantities to achieve specific reactions in the treatment of bodily shortcomings or treatment of diseases as it is seen in the works of some scientists such as Yamagami et al.[11]
3. To elucidate the functional properties of gelatin in various food models and search for new functionalities through physical, chemical, and/or biological modifications, and enable the large utilization of gelatin as protein source. This part has gained much more attractive interest for the practical significance it promotes.

After succinctly tracing and documenting the progress of the last 2 decades, it can be concluded that basic studies of proteins have dramatically expanded our knowledge about proteins and their utilizations. Unfortunately, the literature is not evenly distributed and only relatively little attention has been focused on the study of gelatin. This paper aims to provide a succinct résumé of information regarding gelatin to serve as a reference for anyone embarking on the study of this potential protein. It deals essentially with the preparation techniques, the structure, chemical and physical properties, including interactions with other biopolymers, biological functions, current uses, as well as the chemical and enzymatic modifications of its functional properties.

II. THE MANUFACTURING TECHNOLOGY AND REFINING TECHNIQUES OF GELATIN

As stated previously, gelatin is the derived protein that can be obtained as a breakdown product of collagen by extracting collagen-source materials with hot water above 40°C. Collagen constitutes the main organic component of animal skins, bones, tendon, and loose connective tissues.[12] The nature and amount of collagen vary significantly from one tissue to another and also vary with the kind of animal. Due to this heterogeneity and the kind of gelatin of interest, the preparation techniques differ with different authors in terms of the raw material and chemical reagents used, the extent of treatment and some sequential procedures in the manufacturing technology. However, the principle of gelatin preparation remains almost the same. The raw material is pretreated with either acid or alkaline solution, gelatin is then fractionally extracted by heating process at increasing temperatures and the resulting bouillon is treated by some classic techniques such as settling, filtration, centrifugation, etc. to obtain the final product.[2,6,13] The acid-extracted gelatin is designated "Type A" , whereas the product of the alkaline method is referred to as "Type B". In his investigations conducted on the properties and functions of gelatin, Kobayashi[14] introduced the use of dilute acid and saturated lime solution as media of pretreatment for gelatin type A and B, respectively. Traditionally, hydrogen chloride and lime or sodium hydroxide are used for the types A and B methods, respectively.

The clarity, transparency, and degree of purity especially for food- and pharmaceutical-grade gelatins are of great significance. Differences in these fundamental characteristics of commercial gelatins can arise not only from the differences in the two procedures (acid and alkali methods), but also from the history of the raw material, the heating conditions during extraction, and the care exercised in subsequent processing. The clarity of gelatin can be achieved by several methods.

The use of decolorizing agents to prepare pigment-free gelatins has been suggested. Apart from filtration as demonstrated by Saunders and Ward,[15] the use of adsorbents like aluminum sulfate and aluminum hydroxide have been claimed by Jacquet[16] to be effective for clarifying gelatin solutions. Ames[17] achieved interesting results when he proposed the treatment of the raw limed-stock with dilute solution of aluminum, presumably with the objective of precipitating aluminum hydroxide in the fibers to act as an adsorbent.

Monocalcium has been identified to be very effective in enhancing gelatin transparency especially in slight acidic solutions at a temperatures between 15 to 20°C.[2]

In 1946, Shepperd and Houck[18] introduced the use of good egg albumen as a clarifying agent and obtained impressive result.

More recently, Ozols et al.[19] have proposed the successive use of sodium carbonate neutralized with hydrogen chloride and disodium phosphate dodecahydrate during pretreatment procedure as a method for the improvement of transparency. The results were reported to be satisfactory, but no precision was given concerning the chemical alteration of the gelatin product.

Gu Xianjun and Liu Suqin[20] established a process for making gelatin with high transparency by introducing a refining step consisting of the addition of 1 to 35% phosphate as a clarifying agent prior to the concentration process.

It should be noted that the ion-exchange procedure and some special centrifugation techniques have seen rapid development in recent years and have also found applications in the clarification of gelatin.

III. CHEMICAL COMPOSITION OF GELATIN

The significant gelling property of gelatin has attracted many food scientists around the globe. In order to elucidate the mechanism of gelation and explain its other functional attributes, understanding of the structure involving the chemical composition has become imperative. The chemical composition of gelatin is well documented through research works carried out by several investigators. The most accurate and reliable pictures of the chemical composition of gelatin have been reported by Ward,[10] Eastoe,[12] Neuman,[21] and Tristram,[22] however, the previous works carried out by Bowes and Kenten,[7] and Chibnall with his co-workers[23] remain valuable references. They built up accurate data for gelatin and ox-hide collagen using a wide variety of analytical techniques.

Gelatin is reported to contain some 18 amino acids linked together in a partially ordered fashion. Three groups of amino acids are predominant in the gelatin molecule. Glycine or alanine accounts for about one-third to half of the total amino acid residues.[2,3] Glycine is the predominant *N*-terminal residue of alkali processed gelatin, whereas alanine tends to be larger in acid processed gelatin. Approximately one-fourth of the amino acid residues is either proline or hydroxyproline, and nearly one-fourth is basic or acidic. The absence of tryptophan, an "essential" amino acid and aromatic acid residue has been emphasized.[24,25]

In his investigations, Eastoe[12] demonstrated that only few differences exist in the composition of placental land mammals, ox, and pig, except for the low value of isoleucine in pig skin gelatin. The whale and fish especially show striking increases in the hydroxyamino acid serine and threonine compared with the land mammals. The wallaby was also found to differ from other land mammals in having increased levels of serine and threonine but with only slight effect compared with the whale and the other classes of marine vertebrates.

No significant differences in the value of N-terminal residues as well as in the amino acid composition have been mentioned relating to the origin of gelatin. However, an overall difference in amino acid sequence with respect to the ends of the polypeptide chains resulting from a shift in the side of bond breaking remains a plausible fact.

Chemical composition studies have revealed the presence of about 1% of sugar in gelatin. The type, nature, and amount of sugars vary with different authors, depending on the source of the gelatin and the method of determination. The reported sugars are galactose, glucose, mannose, lactose, and Xylose.[26,27,28,28,30] The sugars are deemed to arise from a cementing substance known as mucopolysaccharides and can be present in the form of amino-sugars.[7,31,32] The mucopolysaccharides are ascribed to play the role of cementing substances in the gelation and other chemical activities.

IV. STRUCTURE OF GELATIN

Several investigations have been carried out to clarify the structure of gelatin, and definite evidence has been achieved concerning the arrangement of a small number of peptide fractions through chromatographic separations. However,

the real molecular structure of this gelling substance still remains a matter of speculations.

In 1940, Astburg[33] proposed a single-chain model in which the repeating unit was the three amino acid sequence -P-G-R, where P represents the proline or hydroxyproline and G, glycine. This sequence was also used by Pauling and Corey[34] to explain the helical arrangement of polypeptide chains in collagen, but was later disapproved as an essential requirement of the structure of collagen and gelatin by Schroeder and coworkers,[35] and Kroner and associates.[36] Schroeder and co-workers speculated sequences of the type gly-pro-hypro-gly or gly-pro-hypro-gly-pro-hypro-gly, which may have interesting structural implications. These sequences got their confirmation through the works of Kroner and associates, who demonstrated the existence of prolyl-hydroxyproline linkage in collagen.

Following the works of Astburg[33] and those of Pauling and Corey,[34] and prior to the findings of Schroeder et al. [35] and Kroner et al.,[36] Ames[17] established with some degree of accuracy two structural models of collagen. He then explained the nature of the transformation of this substance to gelatin by various procedures. The first model (multichain model), built in conformity with the general agreement that collagen, consists of polypeptide chains that may be bonded together by cross-links of various kinds, gives a satisfactory interpretation of the conversion of collagen to a linear structured gelatin through breakage of cross-links between polypeptide chains. However, results of heat degradation of alkaline-prepared gelatin in the presence of alkali, and acid-prepared gelatin in the presence of acid, led him to the construction of a second structural model consisting of a single chain. This model gives the possibility that collagen may consist of a long single polypeptide chain, coiled in a random fashion and linked to itself. Therefore, gelatin may be obtained through breakage of the polypeptide chain and cross-linkages.

So far, it is well accepted that gelatin presents the structure of a linear chain with very little ramifications.[37] The linear chain is characterized by a chemical heterogeneity and some dynamic properties, depending on the preparation procedure involving different phenomena, such as rupture of peptide chains, breakdown or disorganization of lateral bonds between chains, modification of the gelatin chain configuration,[38] etc. However, recent advances in gelatin studies have given interesting insight of the structure of gelatin through new techniques such as laser light scattering and nuclear magnetic resonance and set basic data for elucidating the mechanism of gelatin interaction with other compounds. Laser light scattering studies on gelatin-glutaraldehyde supramolecular structure conducted by Sharma and Bohidar[39] showed the existence of two distinctive supramolecular structures for gelatin-glutaraldehyde complexes in solution: a random coil configuration and a double-strand conformation. Bocquier and associates[40] have also used NMR in structural investigation of gelatin and suggested that the binding region fibronectin interacts with the gelatin via a small hydrophobic interlace. Nuclear magnetic resonance relaxation studies have also made clear the state of water in gelatin gels,[41] which is an important step toward the understanding of interactions between gelatin and other macromolecules in model and food systems. The understanding of the structure of the gelatin molecule is the preliminary step toward the comprehension of its physicochemical properties.

V. PHYSICOCHEMICAL PROPERTIES OF GELATIN

Pure, dry commercial gelatin is generally a tasteless, odorless, transparent, brittle, glass-like solid, very faint yellow to amber in color.[42] The molecular weight of commercial edible gelatin is around 40,000 to 90,000 D. The presence of both amino and carboxylic groups in the amphoteric compounds building up the protein molecule makes it a necessity to consider the equilibria with acids and bases that may be very important for giving a comprehensive explanation of the nature of polypeptides and determining the amino acid composition. Furthermore, information concerning the electrically charged groups and their ionization constant would be useful for elucidating the stabilization of the structure and the nature of the reaction of gelatin with other substances.

The ionizable groups found in gelatin are the carboxyl groups of aspartic acid and carboxylic acids, the ε-amino group of lysine, the guanidinium group of arginine, the imidazolium group of histidine, and the terminal α-carboxyl and α-amino groups. The characteristics of these functional groups are well detailed in the works of Bernard and Emorey.[2]

Type A gelatin has been reported to have an isoionic point of 7 to 9, and the isoionic point for lime (alkali) processed gelatin falls in the range of 4.8 to 5.1.[14] The dispersion and gelation, intimately related to the isoionic point, are the most important properties determining the use of gelatin, especially in the food industry.

Gelatin is a water-soluble protein. However, adequate care must be taken to effect its dispersion. The dissolution of gelatin can be achieved through a preliminary soaking of the granules for a short time in adequate amounts of cold water followed either by heating or stirring and addition of hot water to the hydrated gelatin to reach a final temperature of at least 35°C.

The viscosity of gelatin varies widely with the type of gelatin, the concentration, temperature, and time. In general, acid-processed gelatin appears to have a slightly greater intrinsic viscosity than alkali-prepared gelatin, but no apparent difference had been found for melting points of gels.

Gelation of gelatin may involve several mechanisms, which are up to date not well elucidated. A number of suggestions, however, allow a general view of the interactions leading to the formation of a gel. It is theorized that small sections of a number of gelatin molecules unite to form cristallites, offering a structure of a highly ramified three-dimensional network capable of immobilizing the liquid. The fluid sol is then converted into an elastic "solid" or gel. Ferry[5] suggested the implication of both hydrogen bonds and van der Waals forces in the binding of gelatin molecules to form a fragile architecture of the gel while Bello and Vinograd[43] have related the gel formation to peptide linkage. The properties of gelatin gels are intimately dependent on the speed of cooling the gelatin sols and the degree of acidity. Slow cooling would permit better orientation of gelatin molecules for gel formation, while acid prolongs the setting time and lowers the liquefying temperature of a gel. Most edible gelatin gels liquefy at an extremely low temperature (28°C).

The other functional attributes of gelatin of interest in the food industry are foam and film-forming abilities.

Gelatin is one of the rare proteins known to have good foaming properties.[44] Gelatin sols cooled to 10°C to reach the consistency of thick egg white and can be whipped to yield foams at least double the volume of the initial sols.

The ability of gelatin to form a film has been the central focus of several investigations. The most recent works to confirm this property of gelatin are those of Pelaez and Karel,[45] Kamper and Fennema,[46,47] Kester and Fennema,[48] and Vojdani and Torres.[49] The effectiveness of moisture impermeable edible films, moisture content-water activity and moisture barrier properties of edible films and coatings seem to be the attractive points of various researches, but information on experimental details and quantitative results are indeed very few.

Gelatin is a kind of protein that contributes to an increase in the viscosity of the continuous phase of an emulsion, causes delay in flocculation and coalescence, and enhances the stability of oil-in-water emulsions. However, gelatin by nature has poor emulsifying properties. Unfortunately, none of the methods employed to improve the functional properties of ingredient proteins has been reported to give a satisfactory result in improving the emulsifying properties of proteins. The availability of a method to improve this functionality therefore would be useful to ensure the use of gelatin for multifunctional purposes in food and pharmaceutical systems.

After the most important functional properties characterizing gelatin for food applications have been discussed, the functional differences arising from the gelatin source may serve as an additional information to our knowledge about gelatin and constitute an important basis for extending the gelatin market and field of utilization. From various investigations, it appears that aquatic source gelatins have very similar functional behavior like land-based gelatins.[50,51] Gel strengths and melting points of both pork and fish gelatins have been shown to follow the same patterns with

increased maturation time, except that fish gelatins usually have lower melting points and the time needed for melting points to reach constant value seems longer than pork-derived gelatins. Fish gelatins and pork gelatins exhibit the same pH stabilities, but sodium chloride seems to depress more readily gelatins made from fish. Also, sucrose has similar effects on pork and fish gelatins by increasing their gel strengths and melting points.

The interactions between gelatin and other substances, especially macromolecules, may also be of great significance for elucidating the use of gelatin in various food and pharmaceutical systems as most of these systems are complex mixtures of biopolymers. The study of the compatibility of gelatin with major food components has been the central part of many investigations.[52–56]

Starch–protein interactions are stipulated to be a combination of gluten formation due to mixing, and starch gelatinization and protein denaturation due to heating in the presence of water. However, the exact nature of the interactions in food systems remains unclear due to the inherent difficulties of studying the interactions of two unlike macromolecules.[54] It is hypothesized that, during extrusion cooking of starch–gelatin blends, the gelatin acts as a lubricant, protecting the starch from being converted because more mechanical energy is dissipated in the gelatin phase than the starch phase.[55]

In the study of the effect of structural features of gelatin on its thermodynamic compatibility with locust bean gum, Alves et al.[56] found that it was difficult to understand the thermodynamic behaviors of biopolymer mixtures due to the complicated structure of the individual polymers. This, according to the authors, is in perfect accordance with what was previously reported on the complicated phase behavior of acid or neutral polysaccharides with gelatin. It is generally accepted that the degree of interaction between gelatin, and other biopolymers depends on pH and ionic strength of mixtures, ionogenic properties of gelatin and gelatin-solvent interactions.

Interactions between protein and fat known to take place in many food and biological systems, especially at the cellular and intracellular levels, have also received great attention. The protein-lipid interactions that exist in food systems do not involve covalent bonds but are basically related to hydrophobic interactions between apolar aliphatic chains of the lipid and the apolar regions of the protein. Although the real nature of protein-lipid interactions remains a matter of speculations, Brake and Fennema[57] found the interaction between gelatin and fat to exert inhibitory effect on lipolysis in both finely minced and coarsely mackerel.

As a general observation, the interactions between protein and other macromolecules (polysaccharides and fat) are still not well elucidated. The development of experimental tools that will allow such investigations in both model systems and real food would be desirable.

VI. THE BIOLOGICAL ACTIVITY OF GELATIN

The biological activity of a protein can be defined as its ability to provide after digestion, a complete set of all the amino acids necessary for the synthesis of a biological protein. According to Bender and Miller,[25] the absence of tryptophan, an "essential" amino acid, voids gelatin as a biologically complete protein. Prior to their publication, some investigations had reported the biological value of gelatin to be 21, 23, 25, or 29.5. Indeed, in view of the fact that one of the essential amino acids, tryptophan, is completely absent from gelatin, it therefore cannot provide a complete set of the amino acids essentially required for the synthesis of tissue proteins. Obviously, the biological activity of gelatin should be zero in conformity with Bender and Miller. However, gelatin has been reported to have some beneficial biological functions that justify its use in food and pharmaceuticals as illustrated below.

VII. THE USES OF GELATIN

Clarification and stabilization can be regarded as the major classical uses of gelatin in the food industry. Gelatin is used to obtain clearness of a solution and the stability of this clearness by inducing a complete or partial flocculation or sedi-

mentation of dissolved substances or particles in suspension for turbidity. The use of gelatin for such purposes is indicated only in drinks and beverages containing tannins. Gelatin reacts with the tannin to give flocculates or sediments as the result of tannin-gelatin complex formation. Concentration is an important factor for achieving efficiency or good results during gelatin application. The use of excessive amounts as well as insufficient quantities of gelatin should be avoided during clarification processes in order to prevent overgluing or stabilization of the colloidal substances to be removed.

It has been reported that tonnage of gelatin is used annually for the manufacture of gelatin desserts in recent years. In the United States of America, more than 50% of the edible gelatin goes into this type of product. The most important factor that can affect the formulation and development of gelatin dessert is the pH, which should be maintained between 3.0 and 3.5 for palatable tartness.

Gelatin also finds its application in the manufacture of marshmallow, a colloidal dispersion of gas within a liquid, commonly found in the American diet. The foaming ability of gelatin helps in producing a stable foam that gives the product a light and airy texture.

In bakery products, gelatin is used extensively as a setting agent, stabilizing substance or foam-producing material in pies, breads, and cakes. It is also used in icings of various types as a stabilizing agent. The amount of gelatin required differs with the wide range of these bakery commodities.

The meat industry is one of the major sources of food where considerable quantities of edible gelatin find their application, especially in the preparation of boned-cooked hams, meat loaves, sausages, cheese, canned hams, and meat jellies. Gelatin is used with the ultimate aim of absorbing juices, which separate out during cooking processes and serve for coating purposes.

The use of gelatin in frozen fruit purée deserts and frozen turkey products as well as in the production of jellied tomato consommé, jellied aspic, and jellied beef consommé must be pointed out due to the important position they occupy in the food industry.

Another field of interest besides the food industry, where the use of gelatin cannot be neglected, is the pharmaceutical industry. In developed countries, almost 10% of the edible gelatin goes into this sector for use essentially in capsules and emulsions. Despite the fact that Bender and Miller[25] had demonstrated the biological value of gelatin to be zero, several medicinal effects, including serological specificity and surgical characteristics, have been associated with this protein. Thus, gelatin has been claimed to have some oncotic effects that make it a valuable material for preparing plasma substitutes.[58] Also, highly purified gelatin hydrolyzates are claimed for co-administration with products normally used either to compensate calcium deficiency during childhood and adolescence, pregnancy, and lactation or to treat calcium deficit associated with osteoporosis in the elderly.[59,60] Moreover, there are reports indicating a positive benefit of gelatin on bone turnover makers and joint health. Collageneous connective tissue is one of the important constituents of bones, dentine of teeth, and tendons that attach muscles to bones. The amino acids building up collagen and the derived gelatin, methionine, and cystine especially, are known as carriers of sulfur that is involved in the structure of hair, skin, nail, bone, and connective tissue. This may explain why collageneous materials are also reported to be beneficial in treating connective tissue diseases such as rheumatoid arthrits, scleroderma, and erythematosus responsible for nephrotic syndrome.[61] The use of hydrogels, particularly gelatin, in studying bone regeneration has made rapid progress during recent years, and it has been demonstrated that TGF beta 1-gelatin hydrogel is a promising surgical tool for skull defect repair and skull base reconstruction.[62] It is therefore not surprising that gelatin is used in preparing injectable biomaterials for bone surgery and oral capsules.[63,64] Furthermore, gelatin has been used in addition to other substances to prepare "blood-lipid lowering agent", breathe freshener microcapsules, oral dissolvable medicaments, and cosmetics.[11,65,66] Elsewhere, prosthetic heart valves for intravascular applications as well as artificial skin developed using gelatin as the principal raw material have been suggested.[67,68]

From various literature, it appears that gelatin has a very wide range of utilization. This has brought about the necessity of improving the functional properties of gelatin through modification procedures in order to effect and extend its field of utilization.

VIII. MODIFICATION OF FUNCTIONAL PROPERTIES OF GELATIN

Protein modification, in general, is referred to as the modification of the conformation, structure, and consequently functional properties of protein through physical, chemical, and/or biological treatments.

For the simple fact that most food-processing technologies involve heat treatment, the study of the effect of heat on proteins has received much attention. From the many research works conducted on the physical modification of protein, it is well accepted that moderate heating can enhance solubility, whereas severe heat treatment may lead to aggregation or partial hydrolysis of proteins, including gelatin.[69,70,71,72,73,74,75,76,77]

More recently, the use of irradiation to extend the shelf life of food commodities has dictated the need for investigating whether irradiation could provoke protein degradation but was found to have no effect either on viscosity, solubility, or stability.[78,79,80,81]

Chemical modification of protein is one of the many available methods to produce food-grade and pharmaceutical ingredients with improved functional properties of the protein.[82,83] Intensive investigations have been carried out on the modification of many plant and animal sources of proteins with various chemical reagents.[84,85,86,87,88,89,90,91,92,93]

Among the many chemical modification techniques, acylation with acid anhydrides is the most widely studied with proteins from many sources. The use of succinic anhydride as a modifying agent in the study of protein was first described by Habeeb et al.[84] and since the technique has been evaluated and discussed by many other scientists. According to reports, succinic anhydride reacts with free amino groups, tyrosyl hydroxyl groups, and sulfhydryl groups in proteins, forming, respectively, amide ester, and thioester linkages that are spontaneously hydrolyzed in aqueous media. Acetic anhydride has been reported to have the same effects on protein as succinic anhydride.

Other chemical agents used in protein modification are guanidine hydrochloride and benzonyl chloride that seem to exhibit large increase in intrinsic viscosity and decrease in sedimentation constant of the modified proteins.[94,95] Unfortunately, there is no work that has been reported regarding the chemical modification of gelatin because these methods cannot improve emulsification properties, which seem to be the major problem with this protein.

Although chemical modified proteins, except in some few cases, are functionally superior to native proteins, food application of the modified proteins would need clear regulatory and safety guidelines. Moreover, chemically modified proteins may show a decrease in their nutritional profile because of the possible formation of bonds that would not be hydrolyzed by gastric and pancreatic proteases. Thus, enzymatic modification without safety and nutritional concern has been introduced and studied on a large scale.

The introduction of protein hydrolyzates can be traced to ancient times. The first commercial production of enzymatic protein hydrolyzates took place in 1914 and was for non-food application.[1] Initially, non-food enzymatic protein hydrolyzates were used mainly as fermentation media in which peptone received great attention. Due to the increasing cost and limited supply of animal and vegetable proteins, coupled with the in demand of the growing world population, enzymatic modification with less safety and nutritional concern has been widely investigated to improve the functional and nutritional properties of proteins from various sources.[96,97,98,99,100,101,102,103,104,105,106] The method today can be considered mature enough, although review of the literature has revealed that few investigations have been conducted on gelatin as a source of protein for functional and nutritional purposes. It is only when Cooperman and Johnson[107] showed in 1973 that gelatin hydrolyzates can strengthen the hair by penetrating the hair cuticle and depositing in the cortex that gelatin hydrolyzates became popular and were applied in shampoos and hair

care. However, when their nutritional profile was fully understood, gelatin hydrolyzates finally found their use in the food area even if not extensively.

Acknowledged to be non-bitter but highly nutritious, containing several essential amino acids, enzymatic gelatin hydrolyzates were first combined with casein hydrolyzates to make a nutritionally adequate beverage and later found their application in slimming diets.[1,108] Nedkov and co-workers[109] also demonstrated that enzymatic gelatin hydrolyzates contain considerable amounts of amino acids and therefore constitute a potential food ingredient, confirming Appleman's finding. A year latter, Takahashi et al.[110] reported fractionated gelatin containing tyrosine, histidine, methionine and also high amounts of iron (Fe^{3+}) and calcium (ca^{2+}), thus, highly purified gelatin hydrolyzates are administered with some products to compensate calcium deficit during childhood and adolescence, pregnancy, and lactation. They are also used in treating calcium deficiency associated with osteoporosis in the elderly.

CONCLUSION

A review of literature has revealed gelatin as a highly potential functional protein. It is not only a valuable substance for investigating the fundamental functional attributes in colloid studies, but it also exhibits relatively impressive biological functions of great interest in food and pharmaceutical studies. However, during the last 20 years while social and economic factors have obliged scientists to initiate intensive research work to improve proteins, ensure their availability, and extend their field of utilization, gelatin seems to be in oubliettes. In order to make clear the picture of the functional properties of gelatin, show its true potential, and enable its large-scale utilization in food and pharmaceuticals, it is necessary to reinforce the research in progress and undertake profound discussions on the chemical and enzymatic modifications on this unique protein.

REFERENCES

1. Adler-Nissen, J., *Enzymic Hydrolysis of Food Proteins*, London and New York: Elsevier Applied Science Publishers Ltd., 1985.
2. Bernard Idson and Emory Braswell, *Gelatin, Adv. Food Research*, 1957, 235–338.
3. Charley, H., Gelatin. In: *Food Science*, 2nd ed., New Jersey: Prentice-Hall, inc., 1992, 443–447.
4. Bello, J. and Vinigrad, J. R. [1958] The Biuret Complex and the Mechanism of Gelation: Importance of Peptide Groups in the Formation of Gel by Gelatin, *Nature*, 1958; 181: 273–274.
5. Ferry, J. D., Protein Gels: Interpretation of gelation as network formation, *Advance in Protein Chemistry*, 1948; 4:40–47.
6. Salgues, J., and Bidan, P., Les Agents de Clarification et de Stabilization des Boissons. In: *Additifs et Auxilliaires de Fabrication dans les Industries Agro-alimentaires*. Tec et Doc Lavoisier, 1984, 391.
7. Gordon, A. H., Martin, A. J. P., and Synge, R. L. M., A Study of the Partial Hydrolysis of cow-hide Gelatin: Determination of Order of Amino Acid Residues, *Biochem. J.*, 1942, 37(79): 92–101.
8. Bowes, J. A. and Kenten, R. H., The Amino Acid Composition and Titration Curve of Collagen, *Biochem. J.*, 1948; 43: 358–365.
9. Ward, A. G., Structure and Properties of Gelatin, *Food*, 1951; 20: 255.
10. Ames, W. M., The Conversion of Collagen to Gelatin and their Molecular Structures, *J. Sci. Food Agric*., 1952; 3: 454–463.
11. Yamagami, Y., Ooguru, Y., and Muto, T., Blood Lipid lowering Agent Containing Hyaluronates, *Jpn. Kokai Tokkyo Koho JP*, 1993; 05: 320, 058.
12. Eastoe, J. E., The Amino Acid Composition of Mammalian Collagen and Gelatin, *Biochem. J.*, 1955; 61: 589–600.
13. Salo, T. P., Degradation of Collagen into a Parent Gelatin, *J. Am. Chem. Soc*., 1949; 71: 2276.
14. Kobayashi, T., Properties and Functions of Gelatin, *Foods Food Ingredients J. Jpn*., 1996; 170: 82–88.
15. Saunders, P. R. and Ward, A. G., The Color and Clarity of Gelatin and Glue Solutions, *J. Sci. Food Agric*., 1953; 4: 523.
16. Jacquet, R. J. F., Process for the Treatment of Gelatin and Glue, U.S. Patent, 1939; 2: 166, 297.
17. Ames, W. M., The Manufacturing of Hide Glue and Gelatin, *J. Soc. Leather Trades Chemists*, 1949; 33: 407.
18. Sheppard, S. E. and Houck, R. C., Method of Refining Gelatin, U.S. Patent, 1946; 2: 400.
19. Ozols, A., Sidorovs, L., and Zavalnijs, V. L., Methods for Improvement of Transparency and Viscosity of Gelatin, Chemical Abstract: General Subject Index, 1996; 125:343155b.
20. Gu X-J. and Liu S-Q., Process for Making Gelatin with High Transparency, *Faming Zhuanli Shenqing Gongkai Shuomingshu*, CN. 1994; 1(082): 585.
21. Neuman, R. E., Amino Acid Composition of Gelatins, Collagens and Elastins from Different Sources, *Arch. Biochem.*, 1949; 24: 289
22. Tristram, G. R., *The Proteins*, Neurath and Bailey, Eds., New York: Academic Press, 1953; 1(A): 221.

23. Chibnall, A. C., Rees, M. W., and Williams, E. F., The Total Content of Egg Albumen and Other Proteins, *Biochem. J.*, 1943; 37: 354–372.
24. Bender, A. E., Miller, D. S., and Elizabeth, J. T., *The Biological Value of Gelatin, Chemistry and Industry*, 1953, 799.
25. Loofbourow, J. R., Gould, B. S., and Sizer, I. W., Studies on the Ultraviolet Absorption Spectra of Collagen, *Arch. Biochem.*, 1949; 22: 406.
26. Grassmann, W., and Schleich, H., Collagen II. Carbohydrate Content of Collagen, *Biochem. J.*, 1935; 277:320
27. Beek, J., The Carbohydrate Content of Collagen, *J. Am. Leather Chemists' Assoc.*, 1941; 36: 696.
28. Kulonen, E., *Experiment on Partial Hydrolyzates of Gelatin*, Suomen Kemistiliehti, 1955; 28(A): 107.
29. Landucci, J. M., The Occurrence of Carbohydrates in Proteins, *Bull. Soc. Chem., France*, 1953; 1072.
30. Eastoe, J. E., and Eastoe, B., The Organic Constituents of Mammalian Compact Bone, *Biochem. J.*, 1954; 57: 453.
31. Jackson, D. S. [1952] The Solubilization of Collagen, *Biochem. J. (London)*, 1952; 52: 29.
32. Loeven, W. A., Effect of Alkali Treatment of Collagen on the pH Swelling Curve of Collagen and of Gelatin Products Prepared from it, *J. Soc. Leaather Trades' Chemists*, 1954; 38: 117.
33. Astbury, W. T., The Molecular Structure of the Fibers of the Collagen Group, *J.Intern. Soc. Leather Trades' Chemists*, 1940; 24: 69.
34. Pauling, L. and Corey, R. B., The Structure of Fibrous proteins of the Collagen-Gelatin Group, *Proc. Natl. Acad. Sci. U.S.A.*, 1951; 37: 272.
35. Schroeder, W. A., Kay, L. M., LeGette, J., Honnen, L., and Green, F. C., The constitution of Gelatin. Separation and Estimation of Peptides in Partial Hydrolyzates of Gelatin, *Proc Natl. Acad. Sci. U.S.A.*, 1954; 39: 23.
36. Kroner, T. D., Tabroff, W., and McGorr, J. J., Peptides Isolated from Hydrolyzates of Steer Hide Collagen II. *J. Am. Chem. Soc.*, 1955; 77: 3356.
37. Pouradier, J., Structure of Gelatin, Chem. *Industry*, 1955; 74 (6): 75–84.
38. Sheppard, S. E., and Houck, R. C., The Structure of Gelatin Sols and Gels, *J. Phys. Chem*., 1930; 34: 273.
39. Sharma, J. and Bohidar, H. B., Gelatin-Glutaraldehyde Supramolecular Structures Studied by Laser Light Scattering, *European Polymer Journal*, 2000; 36(7): 1409–1418.
40. Bocquier, A. A., Potts, J. R., Pickford, A. R., and Campbell, I. D., Solution Structure of a Pair of Modules from the Gelatin –Binding Domain of Fibronectin, *Structure with Folding &Design*, 1999; 7(12): 1451–1460.
41. Vackier, M. C., Hills, B. P., and Rutledge, D. N., An NMR Relaxation Study of the State of Water in Gelatin Gels, *J. of Magnetic Resonance*, 1999; 138(1): 36–42.
42. Anding, C. E., Jr., *The Encyclopedia of Chemical Technology*, Othmer and Kirk, Eds., New York: Interscience, 1951, 7: 145.
43. Bello, J. and Vinograd, J. R., The Biuret Complex and the Mechanism of Gelation: Importance of the Peptide Groups in the Formation of a Gel by Gelatin, *Nature*, 1958; 181: 273–274.
44. Chaftel, J. C., Cuq, J-L., and Lorient, D., *Amino Acids, Peptides and Proteins.* In: Fennema, O. R., Ed. *Food Chemistry*, 2nd ed., New York and Basel: Marcel Dekker, Inc., 1985, 317–369.
45. Pelaez, C., and Karel, M., Improved Method for Preparation of Fruit-stimulating Alginate Gels, *J. Food Processing and Preservation*, 1980; 5: 63–81.
46. Kamper, S. L., and Fennema, O., Water Vapor Permeability of Edible Bilayer Films, *J. Food Sci*., 1984; 49: 1478–1485.
47. Kamper, S. L., and Fennema, O., Use of Edible Film to Maintain Water Vapor, Gradients in Food, *J. Food Sci.*, 1985; 50: 382–384.
48. Kester, J. J. and Fennema, O. R., Edible Films and Coatings: A Review, *Food Technology*, 1986; 40 (12): 47–59.
49. Vojdani, F., and Torres, J. A., Potassium Sorbate Permeability of Methylcellulose and Hydroxypropyl Methylcellulose Multi-layer Films, *J. Food Processing and Preservation*, 1989; 13: 417–430.
50. Leuenberger, B. H., Investigation of Viscosity and Gelation Properties of Different Mammalian and Fish Gelatins, *Food Hydrocolloids*, 1991; 5: 353–361.
51. Choi, S-S., and Regenstein, J. M., Physicochemical and Sensory Characteristics of Fish Gelatin, *J. Food Science*, 2000; 65(2): 194–199.
52. Liu, H. and Eskin, N. A. M., Interactions of Native and Acylated Pea Starch with Yellow Mustard Mucilage, Locust Bean Gum and Gelatin, *Food Hydrocolloids*, 1998; 12(1): 37–41.
53. Saxena, A., Antony, T., and Bohidar, H. B., Dynamic Light Scattering Study Gelatin-Surfactant Interactions, *J. of Physical Chemistry B*, 1998; 102(26): 5063–5068.
54. Whistler, R. L. and Daniel, J. R., Carbohydrates: Starch-Protein Interactions. In: Fennema, O. R. Ed., *Food Chemistry*, 2nd ed., Revised and Expanded, New York and Basel: Maecel Dekker, inc., 69–137.
55. Wulansari, R., Mitchell, J. R., and Blanshard, J. M. V., Starch Conversion During Extrusion as Affected by Added Gelatin, *J. Food Science*, 1999; 64(6): 1055–1058.
56. Alves, M. M., Antanov, Y-A., and Gonçalves, M. P., The Effect of Structural Features of Gelatin on Its Thermodynamic Compatibility with Locust Bean Gum in Aqueous Media, *Food Hydrocolloids*, 1999; 13: 157–166.
57. Brake, N. C. and Fennema, O. R., Lipolysis and Lipid Oxidation in Frozen Minced Mackerel as Related to Tg', Molecular Diffusion and Presence of Gelatin, *J. of Food Science*, 1999; 64(1): 25–32.

58. Parkins, W. M., Perlmutt, J. H., and VARS, h. M., Dextran, Oxypolygelatin and Modified Fluid Gelatin as Replacement Fluids in Experimental Hemorrhage, *Am. J. Physiol.*, 1953; 173: 404.
59. Pilar, Q. G., Jaime, M. B., and Quilez, B., Gelatin Hydrolyzates as Coadjuvant in Treatment of Calcium Deficit, Chem. Abstract Gal. Subject Index, 1996; 125: No.257239a.
60. Matsuno, Y., Nakajima, H., Izumi, K., and Okamoto, J., Calcium Enriched Fermented Protein Composition for Food and Pharmaceuticals, *Japan Kokai Tokkyo Koho Jp.*, 1993; 05 (268): 907.
61. Williams, S. R., Renal Disease. In: *Basic Nutrition and Diet Therapy*, 9th ed., St. Louis, Baltimore, Boston, Chicago, London, Philadelphia, Sydney, Toronto: Mosby Year Book, 1992, 330–347.
62. Hong, L., Tabata, Y., Miyamoto, S., Yamamoto, M., Yamada, K., Hashimoto, N., and Ikada, Y., Bone Regeneration at Rabbit Skull Defects Treated with Transforming Growth Factor-beta 1 Incorporated into Hydrogels with Different Levels of Biodegradability, *J. of Neurosurgery*, 2000; 92(2): 315–325.
63. Zahraoni, C. and Sharrock, P., Influence of Sterilization on Injectable Bone Materials, *Bone*, 1999; 25(2 suppl S): 63S–65S.
64. Herbeb, V. M. M., Rosing, H., Huinink, W. W. T., van Zomeran, D. M., Batchelor, D., Doyle, E., Beusenberg, F. O., Beijnen, J. H., and Schellens, J. H. M., Oral Topotecan: Bioavailability and Effect of Food Coadministration, *British Journal of Cancer*, 1999; 80(9): 1380–1386.
65. Stanley, T. H., and Hague, B., Composition of Oral Dissolvable Medicaments, US Appl., 1985; 7(29): 301.
66. Noël, H. and Callegari, J-P. (Jouvance Daniel), Carrangeenan Gel Grains Containing Gelatin for Cosmetics, *Fr. Demande FR*, 1994; 2(683): 720.
67. Hoffman, D., Gong, G., Pinchuk, L., and Sisto, D., Safety and Intercardiac Function of Silicone-polyurathane Elastometer Designed for Vascular Use, *Clin. Mater*, 1993; 13(1–4): 95–100.
68. Yoshizato, K., and Yoshikawa, E., Development of Bilayer Gelatin Substrate for Bioskin, *Mater Sci. Eng.*, 1994; C: 95–105.
69. Schmidt, R. H., Illingnorth, B. L., and Ahmed, E. M., Heat Induced Gelation of Peanut/Whey Protein Blends, *J. Food Sci.*, 1978; 43: 613–621.
70. Schmidt, R. H. and Ahmed, E. M., Differential Centrifugation of Peanut and Soybean Protein Concentrates as Influenced by Preparation Technique and Heat Treatment, *Peanut Sci.*, 1979; 6: 65–69.
71. Schmidt, R. H., Sistrunk, C. P., Richter, R. L., and Cornell, J. A., Heat Treatment and Storage Effects on Texture Characteristics of Milk and Yogurt fortified with Oilseed Protein, *J. Food Sci.*, 1980; 45: 471–475.
72. Schmidt, R. H. and Padua, M. R., Effect of Heat Treatment and Carrengeenan Addition on Protein Solubility and Viscosity of milk Protein/Peanut Flour Blends. *J. Food Proc. And Pres.*, 1982; 6:243–252.
73. Beas, V. E., Wagner, J. R., Crupkin, M., and Anon, M. C., Thermal Denaturation of Hake Myofibrillar Proteins — A Differential Scanning and Electrophoretic Study, *J. Food Sci*., 1990; 55:683–687.
74. Xiong, Y. L., and Blanchard, S. P., Myofibrillar Protein Gelation: Viscoelastic Changes Related to Heating Procedures, *J. Food Sci*., 1994;.59: 734–738.
75. Vanugopal, V. and Shahidi, F., Thermostable Water Dispersions of Myofibrillar Protein from Atlantic Mackerel, *J. Food Sci*., 1994; 59: 265–268.
76. Northrop, J. H., Comparative Hydrolysis of Gelatin by Pepsin, Trypsin, Acid and Alkali, *J. Gen. Physiol*., 1921; 10 (803): 905.
77. Greenberg, D. M., and Burk, N. F., The Rate of Hydrolysis of solution of Protein in Acid as Measured by the Formation of Amino Nitrogen, *J. Am. Chem. Soc*., 1927; 49:275.
78. Vanugopal, V., Quality of Proteins in Radurized Indian Mackerel: Physicochemical Evaluation, *J. Food Biochem*., 1981; .5:145–154.
79. Nickerson, J. T. R., Licciardello, J. J., and Ronsivalli, L. J., Radurization and Radication: Fish and Shellfish. In: Josephson, E. S., and Peterson, M. S., Ed., Preservation of Food by Ionizing Radiation. Florida: CRC Press, Inc., 1983, vol.3,.36.
80. Dymsza, H. A., Lee, C. M., Saibu, L. O., Huan, J., Silverman, G. J., and Josephson, E. S., Gamma Irradiation Effects on Shelf-life and Gel Forming Properties of Washed Red Hake Fish Mince, *J. Food Sci*., 1990; 55:1745–1746.
81. Diehl, J. F., *Safety of Irradiated Foods*, New York: Marcel Dekker, 1995.
82. Franzen, K. L., and Kinsella, J. E., Functional Properties of Succinylated and Acetylated Soy Protein, *J. Agric. Food Chem.*, 1976; .24:788.
83. Beuchat, L. R., Functional and Electrophoretic Characteristics of Succinylated Peanut Flour Protein, *J. Agric. Food Chem.*, 1977; 25: 256.
84. Hass, L. R., Aldolase Dissociation into Subunits by Reaction With Succinic Anhydride, *Biochemistry*, 1964; 3:535
85. Riordan, J. F., and Vallee, B. L., Succinylcarboxypeptidase, *Biochemistry*, 1967; 3: 1768.
86. Gounaris, A. D., and Perlmann, G. E., Succinylation of Pepsinogen, *J. Biol. Chem.*, 1967; 242:2739
87. Choi, Y. R., Kusas, E. W., and Rhee, K. C., Succinylation of Cottonseed Flour: Effects on the Functional Properties of Protein isolates Prepered from Modified Flour, *J. Food Sci.*, 1981; 46:954.
88. Choi, Y. R., Lusas, E. W., and Rhee, K. C., Effect of Acylation of Defated Cottonseed Flour with various acid Anhydrides on Protein Isolates, *J. Food Sci.*, 1982; 47:1713.
89. Choi, Y. R., Lusas, E. W., and Rhee, K. C., Molecular Structure and functionalities of Protein isolates Prepared from Defated Cottonseed Flour Succinylated at Various Levels, *J. Food Sci.*, 1983; 48:1275.
90. Shukla, T. P., Chemical Modification of Food Proteins. In: *Food Protein Deterioration: Mechanism and*

Functionality, Cherry, J. P., Ed., Washington, DC, Am. Chem. Soc., 1982, 275.
91. Johnson, E. A., and Brekke, C. J., Functional Properties of Acylated Pea Protein Isolates, *J. Food Sci.*, 1983; 48:722.
92. Kim, S. H., and Kinsella, J. E., Surface Active Properties: Effects of Progressive Succinylation on Film Properties and Foam Stability of Glycinin, *J. Food Sci.*, 1987; 52: 1341.
93. Habeeb, A. F. S. A., Cassiay, H. G., and Singer, S. J., Molecular Structure Effects Produced in Protein by Reaction with Succinic Anhydride, *Biochem. Biophys. Acta*, 1958; 29:587.
94. Mellon, E. F., Korn, A. H., and Hoover, S. R., [1947] Water Absorption of Proteins. I. The Effect of Free Amino Groups In Casein, *J. Amer. Chem. Soc.*, 1947; 69: 827.
95. Monti, J. C. and Jost, R., Enzymatic Solubilization of Heat-denatured Cheese Whey Protein, *J. Dairy Sci.*, 1978; 61:1233–1237.
96. Phillips, R. D. and Beuchat, L. R., Enzyme Modification of Proteins. Protein Functionality in Foods, Cherry, J. P. Ed. ACS Symposium Series 147, Washington, D.C: American Chem. Society, 1981; 275–298.
97. Saint- Paul, F., Humbert, G., Pâquet, D., and Linden, G., Enzymatic Hydrolysis of Industrial Dairy Proteins: Incidences on their Solubility (French), *Sci. Aliments*, 1984; 4:259–264.
98. Beuchat, L. R., Cherry, J. P., and Quinn, M. R., Physicochemical Properties of Peanut Flour as Affected by Proteolysis, *J. Agric. Food Chem.*1975; 23 (4): 616–620.
99. Chobert, J-M., Bertrand-Harb, C., and Nicolas, M. G., Solubility and Emulsifying Properties of Caseins and Whey Proteins Modified Enzymatically by Trypsin, *J. Agric. Food Chem.*, 1988; 36:883–892.
100. Chobert, J-M., Sitohy, M. Z., and Whitaker, J. R., Solubility and Emulsifying Properties of Caseins Modified Enzymatically by Staphylococcus aureus V8 Protease, *J. Agric. Food Chem.*,1988; 36:220–224.
101. Kim, S. Y. (Lee), Functional Properties of Proteolytic Enzyme Modified Soy Protein Isolate, *J. Agric. Food Chem.*, 1990; 38:651–656.
102. Slattery, H. and Fitzgerald, R. J., Functional Properties and Bitterness of Sodium Caseinate Hydrolyzates Prepared with a Bacillus Proteinase, *J. Food Sci.*, 1998; 63 (3): 418–422.
103. Mahajan, A. and Dua, S., Improvement of Functional Properties of Rapeseed Meal by Reduicing Antinutritional Factors Employing Enzymatic Modification, *Food Hydrocolloids* 1998; 12:349–355.
104. Dzwolak, W., and Ziajka, S., Enzymatic Hydrolysis of Milk Proteins Under Alkaline and Acidic Conditions, *J. Food Sci.*, 1999; 64 (3): 393–395.
105. Bai, H., Ge, S-J., and Zhang, L-X., Total Hydrolysis of Food Proteins by the Combined Use of Soluble and Immobilized Protease, *International Journal of Food Science and Technology*, 1999; 34: 95–99.
106. Allaoua, A., Wang, Z., and Xu, S-Y., Enzymatic Hydrolysis of Soy Protein isolate and Effect of Succinylation on the Functional Properties of resulting Protein Hydrolyzates, *Food Research International*, 1998; 31 (9): 617–623.
107. Cooperman, E. S., and Johnson, V. L., Penetration of Protein Hydrolyzates into Human Hair Strands. Cosmt. *Perfumery*, 1973; 88 (7): 19–22.
108. Appleman, M. D., High Protein Fruit-type Beverage and Processing Therefore, Br. Patent, 1973; 1(308): 690.
109. Nedkov, P. T., Bozhkova, D. K., and Dalev, P. G., Preparation of Gelatin and Enzymic Hydrolyzates from Sheep Skin Scraps, *Bulg. Chem. Commn.*,1995; 28 (1):125–132.
110. Takahashi, S., Ishikawa, T., Okawa, Y., Kobayashi, H., Itoh, N., and Ohno, Studies on the Properties of Fractinated Gelatins, *J. Photogr. Sci.*, 1996; 44:136–146.