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Edible Collagen Films

Packaging meat to present a readily apparent and pleasing appearance despite production, handling, shipping and display conditions, is a problem to which much attention has been directed. Despite commercial precautions in controlling atmospheric conditions, degree of refrigeration and humidity, such methods are only partially successful in preserving qualities such as freshness, texture and colour. The problem is greatly magnified in the case of small cut portions of meat.

Films formed as chilled protein gels were thought to have promise as protective coatings, but are found to be readily destroyed by normal handling. Many resultant films were opaque and provided poor product visibility. These films had a propensity to crack upon storage, particularly at freezer temperatures, providing limited product protection.

Edible films

Increased consumer demands for high quality, longer-shelf-life foods have led to increased interest in edible film research. Currently, edible films and coatings find use in a variety of applications, including casings for sausages, chocolate coatings for nuts and fruit, and wax coatings for fruits and vegetables. Edible coatings and films can prevent quality changes in foods by acting as barriers to control moisture transfer, oxygen uptake, lipid oxidation and loss of volatile flavours and aromas.

Edible films can be made from proteins and polysaccharides or a combination of these materials. Proteins for edible coatings may be derived from maize, wheat, soybeans, and collagen (gelatine). Protein-based films are good barriers to oxygen and carbon dioxide (when dry), but not to water. Edible films prepared from polysaccharides (eg starch and cellulose) and hydrocolloids (eg pectin, alginate, and carrageenan) form strong films but, because of their hydrophilic nature, exhibit poor water-vapour-barrier properties.

By themselves, protein and polysaccharide films are poor barriers to water vapour. However, combining proteins and polysaccharides to form films may offer better mechanical and water-vapour-barrier properties. Acidic polysaccharides, such as alginate, pectate and carboxymethyl cellulose, are known to form electrostatic complexes with proteins. Covalent bonding between protein and propylene glycol alginate (PGA) has been investigated. Interactions that form covalent bonds between protein and polysaccharide are particularly desirable, because the bonded complex will be more stable to heat, ionic effects and other conditions.

Collagen

Collagen is a major protein constituent of connective tissue. Gelatine is obtained from collagen by heating it above the helix-coil transition temperature. Collagen and proteoglycans (glycosaminoglycans) coexist in the extracellular matrix of the connective tissue. Glycosaminoglycans have a major role in the formation of collagen fibrils in vitro. When collagen and glycosaminoglycans interact in vitro, the binding occurs by electrostatic bonds. On this basis, other food hydrocolloids are expected to interact by charge interaction with collagen/gelatine.

A novel process has been trialed to develop a coating for fresh meat by preparing collagen films that will be:

- transparent
- have desirable wet or dry tensile strength
- resistant to heat during use
- possess a bland colour and flavour in the food products
- edible
- have a high degree of resistance to transmission of liquids, gases, and fat or oil.

In order to develop a collagen film with these desired properties, it was first necessary to investigate the various processes to prepare the acid- or alkaline-swollen collagen that could be easily extruded to form films.

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The successful efforts made to prepare edible films by interacting type A or type B gelatine either with PGA, kappa-carrageenan, xanthan, alginate or dermatan sulphate hydrocolloid at various pH values, are described below. Each film type produced has been investigated for a range of properties including:

- film-forming ability
- colour
- thickness
- mechanical properties (tensile strength and percentage elongation at break)
- water vapour permeability
- corrected water-vapour-transmission rate.

Preparation of acid-soluble collagen

Acid-soluble collagen was prepared from bovine hide using the following process.

Step 1: Acid de-hairing of bovine hide

The bovine hide is soaked in 3% acetic acid (pH 2.3) at 25°C. The pH of the hide is monitored by homogenising a small amount of hide in water and checking the pH of the homogenate. The hides are soaked in the acid bath with occasional agitation for 48 hours at 25°C. After the acid treatment, the hides are scraped on both sides to remove hair, epidermis and any residual flesh and fat, finally giving a collagen-rich corium.

Step 2: De-swelling the hide

In order to aid the milling process, it is necessary to de-swell the hide. To do this the hides are neutralised in 2M sodium carbonate at pH 8.0. The neutralised hides are then washed in water for 5 hours with 4 changes of water in order to remove the salts formed during neutralisation.

Step 3: Grinding the hide to smaller particle size

To achieve the necessary reduction of particle size of the hide, a comminuting machine is necessary. A Comitrol™ flaker or conventional mincer can be used. Equipment should be chilled to 10°C. and the hide pieces to –20°C, so as not to generate any heat during the grinding. The hides are ground to a small particle size using the comminuting equipment. The ground hide pieces are then further homogenised in 3% acetic acid.

Step 4: Preparation of acid-swollen collagen

Evaluations of a range of procedures to prepare acid-swollen collagen have been made. These involved taking portions of hide pieces and extracting as follows:

(i) 3% acetic acid at 4°C

(ii) 3% acetic acid at 25°C

(iii) 5% acetic acid at 25°C.

The hide:solvent ratio was 1:70 and the collagen was extracted at pH 2.3 for 25 hours. The pH of the solution was monitored by measuring the pH of the homogenised hide. The collagen fibrils were recovered by changing the pH of the solution to 5.0.

Enzyme-modified collagen

Evaluations of a range of procedures to modify the acid-swollen collagen have also been made. These involved taking portions of hide pieces and treating with the following enzymes:

(i) pepsin

(ii) aspergillus oryzae

(iii) *aspergillus saitoi.*

Pepsin-modified collagen

Evaluations with pepsin involved a series of other variables. These variables included:

- acetic acid concentration
- addition of sodium chloride
- extraction temperature
- pepsin inactivation by shifting the pH to 4.0 with 1M sodium hydroxide
- re-extraction of collagen 2 times by adding additional acetic acid and hide, but no additional pepsin, thereby minimising the usage of pepsin.

All suspensions were centrifuged at 3000 RPM for 30 minutes to remove most of the pepsin. The pellets were then suspended in 0.05M sodium phosphate buffer pH 7.0 and stirred for 10 minutes, after which the solutions were again centrifuged at 3000 RPM for 30 minutes to remove intact digested glycoprotein and proteoglycans. De-salting was achieved by rinsing the collagen fibrils in 1 litre of water, 3 times.

Preparation of gelatine

Type A gelatine was extracted from acid-soluble collagen by heating the collagen solution at 40°C in a water bath for 30 minutes. Type B gelatine from a commercial source was used as comparison.

Plasticisers

The addition of a plasticising agent to edible films is required to overcome film brittleness caused by extensive intermolecular forces. Glycerin or sorbitol was added to the film-forming solutions as plasticisers.

Preparation of film-forming solutions

The appropriate quantity of hydrocolloid was dissolved in water followed by gelatine and a plasticiser, to yield 1.5% (w/w) mixture. Various conditions were investigated to develop a process for reliable film formation including:

- pH – addition of either 1N NaOH or 1N HCl
- addition of salts
- temperature.

The mixture was stirred until all the solids were dissolved in solution and degassed to remove dissolved air.

Control films were prepared from film-forming solutions of either type A gelatine or type B gelatine. Complexed films were prepared from:

- type A gelatine mixed with either PGA, kappa-carrageenan, xanthan, alginate or dermatan sulphate hydrocolloid at various pH values;
- type B gelatine mixed with either PGA, kappa-carrageenan, xanthan, alginate or dermatan sulphate hydrocolloid at various pH values.

Casting of films

Film-forming solutions, while still hot, were cast on flat glass plates. Casting areas on the plates were 'framed' with strips to restrain the solution from spreading. Plates with cast solution were air-dried at room temperature for 48 hours before manually recovering the dry films from the plates.

Conditioning of films

Before measurements of thickness, tensile strength, percentage elongation at break and water-vapour permeability could be taken, the films were conditioned for three days at 75% relative humidity (RH) and 23°C.

Measurements

For each film type, four test pieces were taken from each of four separately cast films. The thickness, colour, tensile strength, percentage elongation at break, water-vapour permeability and water-vapour-transmission-rate properties were measured on each piece and the mean determined.

Edible film characteristics

The control type A and type B gelatine films and type A or type B gelatine:hydrocolloids films formed were smooth and transparent. The type A gelatine:hydrocolloids films were flexible; on the other hand, the type B gelatine:hydrocolloids films were less flexible, and some exhibited brittleness.

The resulting films varied depending on the pH. Stronger and clearer films were formed under alkaline conditions (pH 8.5), where the gelatine and hydrocolloids reacted to form a complex. Relatively low levels of hydrocolloids were needed and good quality films were obtained at ratios of gelatine to hydrocolloid as low as 10:1 (w/w). Film-forming properties decreased when plasticisers (glycerin or sorbitol) or salts were added to gelatine or to the gelatine-copolymer films.

Colour

Colour characteristics of edible films can be an important factor affecting consumer acceptance. The Hunter colour values and total colour difference for the type A or type B gelatine:hydrocolloid films are comparable. However, the 'a' value for the control type A or type B gelatine films was lower when compared to type A or type B gelatine:hydrocolloid films. The type A or type B gelatine:hydrocolloid films were transparent.

Potential preparation of gelatine:hydrocolloid films

The acid-swollen collagens prepared by the different processes were all easily extruded and could form a film. The enzyme-modified collagen mass had a lower viscosity as compared to those prepared in the absence of enzyme. By incorporating sodium alginate with collagen, the addition of plasticisers can be avoided since the salts act as lubricants for the collagen fibres, thus allowing the casting of thinner films.

Very viscous collagen masses could be easily extruded into thin films using a test extruder.

Films formed from type A or type B gelatine:hydrocolloids were thin and transparent. Mechanical properties of type A or type B gelatine:hydrocolloids films were affected by factors such as concentration, type of hydrocolloid and pH of interaction. Gelatine is an amphoteric electrolyte and its molecular dimensions are influenced by pH. Thus, the interaction between type A or type B gelatine and various hydrocolloids is affected by pH.

This study demonstrated that charge interaction can be utilised to form edible films with good mechanical and water-vapour-barrier properties by mixing type A gelatine with either PGA, xanthan or alginate. Thus, charge interaction between different hydrocolloids and type A gelatine may make them useful for improving the structural integrity of fragile products and may constitute a new category of edible films.

These results serve as a guide for the selection of gelatine and hydrocolloid mixtures to produce desired properties in a film. The hydrocolloids, PGA, kappa-carrageenan, xanthan and alginate, used in this study are all safe for human consumption.

The interaction of gelatine with different hydrocolloids could be applied to the food industry for the production of edible

films and coatings. Co-polymer gels can also be used as heat-stable gels for pet or human foods—which may improve the texture and consistency, and increase the shelf life.

Potential advantages of acid-swelling systems for commercial collagen-film production

The acid de-hairing process could be performed relatively quickly to facilitate the processing line as compared to the traditional liming process. Extracting collagen in 5% acetic acid with pepsin at 25°C was the most effective process, extracting 86% of the soluble collagen. 3% acetic acid with pepsin at 25°C, and 3% acetic acid with *aspergillus saitoi* at 25°C, were also effective, extracting 79.7% and 79% respectively of the soluble collagen.

Of the collagen-extraction processes, extracting collagen with enzyme in the presence of salt facilitates the enzyme to remove the non-helical extensions present at either end of the molecule without disassembling the collagen fibrils. Furthermore, the usage of the centrifugation step is avoided. The enzyme used has a higher affinity for solid collagen particles than for soluble collagen, therefore the enzyme can be reused several times before it loses its activity. These processes result in complete solubilisation of the hide in 24 hours at 25°C in acidic solutions and are relatively easy to control. The costs of collagen production can be minimised using this process.

Further reading

This information is a summary of information from the following project funded by the Meat Research Corporation:

- Project M218: Collagen Utilisation

Further detail is available from the final project report of Module B – Part 2 for this project which is available from Meat and Livestock Australia.

Related information is given in the MLA Co-product brochure:

- Bovine Collagen Isinglass.

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