USE OF COLLAGEN IN SAUSAGE CASINGS*

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Introduction--Collagen

In animals, collagen is the major fibrous element of the extracellular connective tissues and probably the most abundant protein. It is found in skin, bone, tendon, teeth, blood vessels, intestines and even in the eye's cornea.

Because collagen exists mostly in an insoluble fiber form, early studies on this protein consisted of x-ray diffraction, electron-microscopy and chemical analyses. More recently, with the development of various physico-chemical methods, the molecular structure of collagen has been elucidated.

A collagen molecule, termed tropocollagen, is in a rigid rod shape with the length of 2800 A^{o}, the diameter of 15 A^{o} and the molecular weight of 300,000[1 2 3]. This rod is made up of three chains of polypeptides[4], which are associated with each other through a number of hydrogen bonds for structural reinforcement.[5]

Because of its rigid, rod structure, materials that are reconstituted from a solution or a dispersion of collagen have generally superior mechanical properties. For example, the tensile strength of collagen suture which has been reconstituted from a solution of solubilized collagen is about 3 g/denier. This is stronger than suture reconstituted from soy protein or casein and is between wool and silk in tensile strength ranking.[6]

Intestinal Casings

Traditionally, intestines of sheep and hogs have been used as edible sausage casing. After removing submucosa, these natural intestines are cleaned to become sausage casings or tubes of collagen fibers that make up the wall of the intestines. The collagenous structure in the intestine wall has not been clarified as much as that in the cornea, but it has a laminated structure with each layer being a network of randomly oriented collagen fibers.

The natural sausage casings have demonstrated their excellent elasticity, appetizing appearance for packaged sausages and tenacious stability against punishing cooking. All of these advantages are believed to originate from the above mentioned structure that is responsible for the mechanical strength and elasticity of intestines, even though collagen fibers themselves are not elastic.

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However, there are several serious shortcomings inherent to natural sausage casings. First of all, they must undergo a thorough cleaning and still require preservation. Furthermore, their lack of uniformity in thickness, color and in diameter are definite disadvantages for modern, high-speed stuffing. Lastly, if one must depend on the import to obtain large quantities of animal intestines, it would be another handicap.

There have been only a few patented improvements[7,8,9,10,11,12] in recent years on natural casings in the area of color control, uniform diameter, reinforcement and shirring. These products are probably most useful in specialized sausage applications, but not readily manufacturable in an efficient manner.

Reconstituted Collagen Casings

Attempts to make a better edible casing from various collagen sources have been made. Out of these attempts, a number of manufacturing processes and products have been invented. The majority of inventors in this area appear to have a common raw material for collagen--beef hide.

In general, to manufacture reconstituted collagen casing, this collagen raw material is comminuted, mixed with a swelling agent to produce a uniform dispersion from which a continuous tube is formed. The tube undergoes, if it is a wet process, several treatments before it becomes shirred slugs of uniform size, length and strength. In a dry process, a swollen collagen dispersion may contain a tanning agent and a higher collagen solids. From this dispersion, a tube is extruded into air and is simply dried.

The first important step in the manufacture of reconstituted collagen casings is to prepare a uniform dispersion for tube formation. This is particularly critical for the extrusion process which is the most popular method to form collagenous tubes. Other methods of apparently minor importance are electrodeposition,[13,14] dip coating,[15] and coextrusion[16,17].

Uniform, collagen dispersions for tube extrusion are prepared, for example, by swelling collagen fibrils obtained from fresh corium layers of steer hide with lactic acid followed by homogenization and deaeration[18,19,20]. Some collagen dispersions are made from corium layer which has undergone short liming with[21] or without[22] subsequent deliming. Hide collagen can be modified, prior to the preparation of dispersions, through strong alkali treatment[23,24] or mild proteolytic enzyme treatment[25,26]. Some inventions use mixtures of steer hide collagen and pigskin collagen[15,27] mixtures of steer hide collagen and casein,[28] and mixtures of beef tendon collagen and gelatin[29]. Also, there is a group of inventions which are based on a logical principle of having two distinct components in dispersions, i.e., heavily limed collagen fibers and enzymatically solubilized tropocollagen solution[30,31]. A most recent patent[32] describes a simple method of completely liming collagen fibers followed by acid soaking and water washing before a dispersion is formed.

The second important consideration for the manufacture of high-quality, reconstituted collagen casings is the alignment of collagen fibers or fibrils within uniformly formed tubes. Unlike some plastic materials, collagen tube, after it is formed and especially after it is coagulated, cannot readily change fiber or fibril orientation. Therefore, any kind of orientation to optimize mechanical properties of collagen casing film must be done almost entirely in the extruder.

There have been only a few developments that have been published in the area of extruders. To design an extruder for the manufacture of reconstituted collagen casing, it is vital to understand the physico-chemical nature, especially the rheological properties, of a given swollen collagen dispersion. One patent[33] teaches us that a dispersion of collagen fibrils swollen with a dilute acid solution can be subjected to two directional flows which will be later combined to form cross-orientation of fibrils in a casing film. More simply constructed and probably more commonly used than the afore-mentioned extruder, is the one in which the nozzle has two counter-rotating walls to effect similar cross-orientation of collagen fibers and fibrils.[34]

In addition to the diameter of an extruder's nozzle, the pressure difference between inside and outside of the extruded tube will have an important effect on the control of tube diameter. The pressure difference is created by either liquid[35] or gaseous[20] coagulant.

After collagen tubing is reconstituted, it is usually subjected to a strength development process commonly called "tanning" or "cross-linking" or "hardening." This process takes advantage of one of collagen's unique chemical properties, i.e., its affinity to heavy metal ions and to aldehydes.

Similar to the chrome tanning in the leather industry, aluminum tanning has been popular in the hardening of edible collagen casings[36,37]. Iron can also be used to effectively crosslink collagen, especially that from limed hide[38], but it should be followed by a discoloration step for better appearance[39].

A number of aldehydic crosslinking methods have been successfully tried to strengthen reconstituted collagen casings. The simplest of these is to use dextrose[40] and, with better control, liquid smoke[41,42].

Probably the most effective and controllable aldehyde is glutaraldehyde[43], which is also used in combinations with other reagents [44,45,46,47]. On a more complicated side, one process is designed to form a reactive aldehyde[48] and another requires a fixing step following aldehyde tanning[49].

Interestingly, there are hardening processes that do not use the so-called crosslinking agent, but depend on strong alkali media[24,50]. All in all, the tanning of reconstituted collagen casings is highly desirable to make casing products withstand punishment during sausage manufacture. However, if it is overdone, casings could become too brittle and/or too tough.

Generally, prior to drying, in a wet process collagen casings undergo a plasticization step where a desired amount of plasticizer, such as glycerin, is absorbed by casings. Sometimes, this step is modified so that reagents other than plasticizer can be taken up by casings to impart additional desirable properties to the final products. The drying method of plasticized collagen tubes appears to be rather conventional, i.e., the tube is inflated and passed through dry air-flow at a temperature sufficiently high, but not too high to denature the collagen. One recent patent[51] explains that improved performance of casing during cooking is obtained by controlling the inflation pressure during drying to a moderate level. In some cases, however, the diameter control must be watched closely with the adjustment of inflation pressure.

In the area of functional modification of reconstituted collagen casings, most of them seem to aim at improvements of appearance, texture, machinability and cooking performance. One method[52] makes use of caramel, which is blended in swollen collagen mass to be extruded for the purpose of improving stuffing responses, improving pigtail link retention, and improving the stability of non-smoked areas. In order to minimize the shrinkage of collagen during sausage cooking, several methods have been reported. The basic idea is to add to the collagen matrix a substance that does not shrink upon heating to cooking temperatures. Albumin[53] and albumin-carboxymethylcellulose combinations[54] were used in a solution form in which wet collagen tubes are passed. Also, sodium alginates were mixed in various proportions with swollen[55] or unswollen[56] collagen fibers and extruded tubes therefrom are treated with a setting bath for alginates.

Even though most casings are plasticized with glycerin, it is highly desirable in many cases that casings contain a hydrophobic material for further softening, improved appearance, lubrication, and wet strength retention. Hydrophobic materials, unless applied directly to the surface of casings, are emulsified in aqueous systems, such as a plasticizer bath, with a surface active agent and absorbed by passing collagen tubes. Edible oil is used in one method[57], whereas monoglycerides[58] and fatty acid esters of propylene glycol[59] are used in others.

Conclusions

To design an ideal casing for a given sausage application, it is necessary to understand the composition of meat emulsions, the mode of stuffing and linking, the conditions of further processing, if any, the desired shelf-life, and the methods of cooking by consumers. Any sausage casing manufacturer would like to produce universal casings which only by changing the diameter, can satisfy worldwide sausage industry needs. Collagen sausage casing technology has made tremendous progress in the last twenty years or so, but there seems to be a long distance separating the present state of art and the universal casing. There is no doubt that more improved products and processes will be researched and developed at an everaccelerating frequency during the next several years. These will have to be built on deeper understanding of collagen raw materials, as well as sausage technology.

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Dennis Campion: Now, if all four of the speakers would please come forward, we will entertain questions.

Al Pearson, Michigan State: I'd like to ask a couple of questions. I think Thayne might be able to answer the first one which is relative to the formation of collagen and the nutritional factors that are involved. I don't believe you covered that.

T. R. Dutson: You're speaking of the co-factors necessary, this sort of thing?

Al Pearson, Michigan State: And where do they fit into the picture on the biosynthesis of collagen? That's what I'd like to know.

T. R. Dutson: Well, primarily the co-factors necessary for synthesis of collagen are similar to those required for synthesis of other proteins. However, it hasn't really been investigated to the extent that some of the other proteins have. The co-factors necessary for hydroxylation, in particular, involve ascorbic acid as one, ferrous iron as another, molecular oxygen, and α-ketoglutarate. α-ketoglutarate is necessary and it is decarboxylated in the process. Ferrous iron seems to be necessary for, probably binding of the oxygen. Ascorbic acid probably acts like a reducing agent in the sequence of hydroxylation. In glycosylation, this is generally a transferrence type of reaction. In the literature, I found no specific mention of micronutrients or co-factors that are necessary for this reaction.

Al Pearson, Michigan State: Okay. The other question I'd like to ask deals with the type I and III collagen. If you look at the composition of type I and III collagen, they're both low in carbohydrate material as compared to the other two types of collagen, that is types II and IV. Do you think that this is of significance in regard to their properties?

T. R. Dutson: I think certainly so. The amount of carbohydrate has been alluded to as being important in both the formation of the types of fibrils and the strength of the fibrils. From some of the research we've done, and in looking at other research, I feel that the amount of carbohydrate is particularly important in the association of the collagen with the ground substance matrix. If you have a sugar moiety on collagen, you can see where this would be a mechanism in linking to mucopolysaccharides of the ground substance, which themselves are then bound to proteins of the ground substance. So you have really sort of a three phase structure: the collagen with its carbohydrate moiety probably bound to the mucopolysaccharide of the ground substance; then the mucopolysaccharide of the ground substance being bound to the protein moiety of the ground substance. Also, I think the amount of carbohydrate is important in fiber formation, particularly in type IV collagen. The high amount of carbohydrate in this collagen type seems to be very important in its amorphous structure because it does not form typical fibers.

P. E. McClain: The carbohydrate moieties are, in a number of cases, associated with the lysines that are also involved with cross-linking. Now, the mechanism here is not yet clear either, but there is a relationship.

Al Pearson: While you've got the microphone, let me ask you a question, Phil. In your patterns of pro-crosslinking materials you spoke of histidino-hydroxydesmosine. Of course, desmosine is an amino acid that is commonly found in the connective tissues of a component.

P. E. McClain: It's found as a crosslink in elastic tissue. This histidino product, though, is a real weird one. Histidine has to be positioned in the right spot. Now, there's a school of thought that says, "This compound is an artifact produced during reduction." But, it's not the same as the one found in elastin. They've searched high and low for it in collagen because this is the one that will tie up four different molecules.

Al Pearson: One other question while I've got you, Phil. You mentioned the fact that nutrition may play a role in the relative proportion of type I and III collagen. I don't think you elaborated on that. Would you mind doing so?

P. E. McClain: This is a real exciting new area of collagen research. They're finding that conditions such as athrosclerosis, for example, may be a function of the type of collagen present. Arthritis is presently thought to be a result of type III/type I interchange of some sort. It's a brand new area. We don't really know what the significance of it is as yet.

Al Pearson: You're talking about the nutritional aspects, what are these?

P. E. McClain: Nutrition is affecting collagen directly. There are several things that will. The type of carbohydrate might. Now, I don't know whether this again goes back to this carbohydrate moiety and hydroxylysine. But, we fed fructose and glucose diets and there was a very marked alteration in the type of collagen we found. Starvation or food restriction has been long recognized as influncing longevity in experimental animals. McKay, back in the 30's reported that food restricted rats would live up to twice as long as the rats that were eating ad-libitum diets. Our work indicates that there may be a chemical basis for this. If food restriction inhibits crosslinking, then our arteries are going to be more pliable. The wrinkles that some of the ladies don't like will be later in coming about.

Al Pearson, Michigan State: There is another thing that Thayne didn't mention, copper.

T. R. Dutson: Well, copper seems to be important in lysyloxidase the enzyme that oxidizes the epsilon amino group of lysine. The primary position of action of this micronutrient on crosslinking is at this point because this enzyme does require copper for action. You can inhibit this enzyme by having copper deficient rations.

On the type III collagen, one thing that I think is very significant is in fetal skin; type III collagen is there in higher amounts. The collagen in fetal skin is very, very insoluble. Then shortly after birth, there is a shift from type III collagen back to type I collagen. Then as Phil showed, the collagen solubility then increases. Now, Phil showed it in the muscle, but typical research was originally done in fetal skin. So, there's a shift in total collagen solubility related to a shift in collagen types. Some researchers feel that collagen solubility, at least in some cases, might be almost entirely due to the type of collagen that is present. Possibly, later on in maturation, an increase in the amount of crosslinks that Phil talked about decreases the solubility of the type I collagen.

Dr. Greaser, Wisconsin: Would one of you like to comment on whether there is such a thing as a protein reticulin?

Thayne Dutson: By a protein reticulin, you mean the traditional protein that's supposed to be some kind of--

Dr. Greaser: In the textbooks, we list collagen and reticulin.

Thayne Dutson: I don't know. My feeling is that this could be the type IV collagen that we see in the basement membrane. My basis for saying this, in looking at longitudinal sections of electronmicrographs, if you look through numerous sections, you very seldom ever find any definite structural connective tissue fibers right down when you get right down to the endomysium. Now, occasionally you do find

them there, but I think they're still ramifications of the perimysium. As you come down to the actual point where one fiber connects onto the other, about all you can see is the plasmalemma, the basement membrane, some space but very little, then another basement membrane, and another plasmalemma. That's my basis for saying it could very well be the basement membrane type collagen. Another thing is that basement membrane type collagen could be binding the silver stain that has been shown classically. That's where the reticular fibers come from, was the staining with silver. This collagen could be a silver staining type of collagen, particularly with its high amount of carbohydrates. As Howard said, if you remove the lipid, which could be associated with some of the carbohydrates, you reduce the staining. Well, maybe the stain could be binding just to that.

H. J. Swatland: You can dissect the endomysium or reticular fibers and pull them out of the muscle and stand them up by themselves. Now, that's not a membrane you're pulling out, those are distinct fibers. I agree, there's some considerable overlap in dimension and staining properties of the small diameter fibers. In instances like wound healing in the skin, you can find distinct types of fibers: there are small ones that stay small, always stained with silver; there are some collagen fibers that never stain with silver, and yet get bigger and bigger in a man's wounds and form scar tissue; there are organs which are held together, such as the spleen, by reticular fibers. You can find reticular fibers in all sorts of other tissues.

Thayne Dutson: Of course, in the type of graphs that you showed of the fat cells, this was a fairly fine network and could be basement membrane of the fat cells, also. Does that follow?

H. J. Swatland: Yes, fat cells, conceivably. But the silk stocking around the muscle fiber, that's a really substantial silk stocking.

Dr. Allen, Minnesota: I guess this is for Phil or Thayne. Is there any evidence of the hormonal changes in the animal? I'm thinking now of cycling females, for example, cows, if this results in changes in the connective tissues collagen that might affect tenderness. I'm thinking about some of the old cows having very tender meat. Do we have any evidence for that? Is there anything that might be tied up with collagen turnover essentially?

P. E. McClain: I know there's a change in the uterus, but I don't know whether there's any relationship to the muscle or not. Do you know, Thayne?

Dr. Allen: Do you have any hormonal things you can relate to collagen synthesis crosslinking?

P. E. McClain: I know that cortisone affects collagen. Now whether collagen itself or the ground substance is altered is not known.

Dr. Allen: Now, is that used in crosslinking?

P. E. McClain: I don't think so. I don't know what the affect is. I'm sorry, I can't help you.

Dr. Allen: While I've got the floor, Phil, I'd like for you to summarize the effects of marinates, long time, low temperature cooking, so we have in our minds exactly what's going on here on collagen. For example, does salt have any affect on the marinate, or is it entirely the acid?

P. E. McClain: Yes, salts could affect it. Collagen is salt soluble, especially the early collagen, recently synthesized material. Acids are certainly going to work on the aldamine bonds. It's long been known that if you store collagen at room temperature it forms crosslinks. Now, the only study that I've ever seen is that one we did and this was 100 hours at 4° C. It would be interesting to see what happens at higher temperatures. It may alter the ultimate characteristics of the meat by the aging process.

Dr. Fields, Wyoming: I just wanted to follow up about your comment about the old cow, or your question about the old cow being tender, a certain percentage of them. The thing I thought of, Phil in connection when you were talking, was the study you quoted showing that fasting, even for 48 hours or so, could reduce the β crosslinks especially. Certainly, many of these cows aren't in too good of condition to start with, and if fasted a little bit longer might these be the more tender cows? I don't know if anyone has followed up on this. I would appreciate a comment on that.

P. E. McClain: Well, this fasting is another interesting thing. I think in these fasting animals it would be a matter of accelerated turnover. Now, if this were a turnover of collagen, it would be nice to have this process working for us, wouldn't it? There is a need for more work in this area.

Dr. Wierbicki: Are there going to be differences in collagen composition with ages?

P. E. McClain: I think you're asking are there any changes in amino acid composition especially hydroxyproline? I think Thayne will agree that there is. Not a whole lot, but young immature type collagen is somewhat under hydroxylated.

Thayne Dutson: I think, probably, at the point that you're measuring it in an animal, say two years of age or 18 months, something like this, I think there probably wouldn't be. But, if you had a shift or difference in the type of collagen, there would be a slight amount of difference in hydroxyproline. But even in those situations you find little difference unless you go to the type II or type IV collagen, there's where you would get a large difference in the amount of hydroxyproline. Now, in muscle tissue it's primarily type I and type III which have about the same amount of hydroxyproline in there. I think you will find very little difference, really, unless you're looking at very immature collagen which hasn't been completely hydroxylated.

John Romans, Illinois: This is for Phil McClain. Is crosslinking related to animal age in such a way that we might use crosslinking to estimate the animal's age?

P. E. McClain: I think very much so. I think it would follow a very typical pattern, especially with animals under the same nutrition regime. I wonder what these grass fed cattle look like as compared to feed lot cattle of the same age? We see this definite protein effect on collagen crosslinking.

John Romans: How long does it take to get that data? If we took a sample how long would it take, two or three days, to work to get that slide you had there, or one day or two?

P. E. McClain: It depends on how much you want to know. I'm pretty curious all the way down the line. Dr. Wierbicki is using heat label cards and it's probably as good as any. It tells you how much of that is permanent crosslink.

Dennis Campion: I think that will conclude then the session on Connective Tissue. I'd like to thank you four gentlemen for your fine work.

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