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3,433,864

METHODS OF EXTRUDING COLLAGEN

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ABSTRACT OF THE DISCLOSURE

Process for making shaped collagen products by extruding an aqueous suspension of acid swollen collagen fibers in which the collagen fibers are formed from untanned limed hide and the suspension contains at least 2% of soluble collagen in solution and has a salt content under about 1% to insure wet strength of the extruded material in the deswelling bath.

This invention relates to a method of extruding collagen articles.

Formed collagen articles such as filaments, e.g. surgical sutures, sheets and food casings, e.g. sausage casings, have been formed by reducing skins and hides to an aqueous suspension of swollen collagen fibrils, shaping this suspension by extrusion, to the desired cross section shape and deswelling the swollen collagen fibrils to lock them together into a coherent body approximating the shape in which the suspension was formed. Ordinarily this deswelling of the fibrils has been effected by introducing the extruded suspension directly into an aqueous bath of a deswelling or protein precipitating agent such as an aqueous solution of ammonium sulfate.

The most readily available skin material for such use is limed and dehaired cattle hide which is readily reduced to a swollen collagen fiber or fibril suspension for extrusion. However, the wet strength of the article formed by deswelling of the extruded suspension may be undesirably low so that special precautions, such as an undesirably low rate of extrusion have been required to avoid breaking or rupture of the extruded article in the deswelling bath prior to drying. This difficulty may be avoided or reduced by forming the collagen suspension from unlimed hide material; but such material is not as readily or as cheaply available as the limed hide material and its use requires special means for removing the hair.

We have now provided an improved extrusion process in which the addition of acid soluble collagen to an aqueous suspension of native collagen fibrils from limed or other unsatisfactory skin or hide material markedly increases the early wet strength of the formed article, e.g. in the deswelling or coagulating bath.

In the coagulation of an aqueous suspension of collagen fibrous material it is believed that development of wet strength depends to a considerable extent on the coagulation and precipitation of dissolved collagen in fibrous form and that the reconstituted collagen fibers being intimately interwoven through the mass of existing insoluble fibers help to hold the wet shaped mass of fibers together and give it the coherence necessary for handling through the wet processes.

It is also believed that acid soluble collagen normally present to some extent in the fresh hide material in the more readily accessible intercellular and interfibrillar spaces is destroyed by the strong alkaline reaction and the extended exposure period of the time liquors employed in conventional unhairing processes so that the deficiency in acid soluble collagen is a principal reason for unsatisfactory extrusion behavior of collagen fiber

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suspensions prepared from conventionally lime unhaired hides.

The mechanism involved in the association of the very fine collagen fibrous material which is in essentially fibrillar state and the reconstituted collagen fibrous material is understood to involve a deswelling of the existing collagen fibrils in a closely packed relationship and the formation of very thin reconstituted collagen fibers which exert a substantially greater linking action between the closely packed existing collagen fibers than the heat reconstituted collagen material could exert between the coarser, longer collagen fiber masses involved in the manufacture of open fibered leather-like materials in the patent to Highberger and Whitmore 2,934,446. That is, in the patented procedure, reconstitution was effected without deswelling and close packing of fibrous material in contrast to the reconstitution with deswelling and close packing of fibrous material in the present procedure. It has also been observed that there is a time factor in the progressive development of strength of an extruded suspension in the present procedure. This suggests that a supersaturated solution of the acid-soluble collagen is first formed and is held in close contact with the surfaces of existing closely packed fibers in the course of deswelling and that fiber growth occurs from this supersaturated solution. It is also believed that the collagen fibers formed from solution may grow on initiating sites on existing collagen fibers or fibrils.

The aqueous suspension of collagen fibrils for use in the present extrusion process may be made by any of a variety of known methods. In general the raw material such as limed unhaired hide, or limed unhaired and pickled hide will be washed, chopped, ground, e.g. in a meat grinder and brought to a desired pH which will effect at least limited swelling of the collagen fibers. Usually it is preferred to operate in the acid condition and if limed hide is used acid will be added to bring the pH to a suitable value. The chopped and ground hide material is then subjected to mechanical action to separate it into essentially fibrillar state. This may involve treating the material in a beater comparable to a paper beater and/or treating it in a homogenizer to tease the hide material into fibrils. This separation into fibrils is effected in the presence of water and the resultant product is an aqueous suspension of collagen fibrils.

Acid-soluble collagen for addition to the collagen fibril suspension may be prepared by acid treatment of young mammalian skin, suitably calfskin. Procedure for preparing soluble collagen is described in greater detail in U.S. Patent 2,934,447 of Apr. 26, 1960. In brief, the raw material, after washing to remove blood, dirt, or the like is minced and introduced into an acid bath having a pH of from about 2 to 4.5. After acid soaking for from 12 to 48 hours preferably at from 0 to 25° C. the skin material is treated in a mechanical device subjecting it to severe shearing action to aid in freeing dissolved acid-soluble collagen. Ordinarily the liquid acid solution of soluble collagen is removed from the undissolved fiber material although the undissolved fibers may be left with the collagen solution where their presence does not interfere with later use. The extracted collagen may be purified by precipitation and re-solution and the collagen solution may be concentrated, preferably by pervaporation, i.e. evaporation of moisture through a membrane capable of transmitting moisture.

Acid-soluble collagen may be combined with the collagen fibril suspension by addition and thorough mixing. It has been found that the addition of acid-soluble collagen in amount to insure at least 2% and preferably at least 5% by weight of acid soluble collagen based on the dry weight of the total solids of the suspension gives

an important increase in the wet strength of the articles formed from the suspension and subjected to deswelling or coagulation. Up to 50% soluble collagen by weight based on the dry weight of the total solids of the suspension may be useful with higher total solids content mixtures.

The solids content of the suspension of collagen fibrils in an aqueous solution of acid-soluble collagen is adjusted to provide a suitable consistency for extrusion. For shaping by extrusion, the suspension should have a content of collagen fibers in essentially the fibrillar state in the range of 1% to 10% by weight preferably from about 3% to about 6%.

The suspension prior to shaping by any of the selected methods is also adjusted to have a pH outside the isoelectric range of collagen but not so acid nor so alkaline as to cause rapid destruction of collagen material, i.e. the pH should be above about 2.5 and below about 12. For operation on the preferred acidic side of the isoelectric range it is preferred to use a pH of from about 3 to about 3.6. The salt content of the suspension should be kept under about 1%. It is not necessary that the suspension be kept cold; but the composition should be kept below about 20° C. to prevent damage to the collagen.

The coagulation of the suspension is effected promptly after extrusion. It is desirable that the extrusion orifice discharge the shaped suspension directly into a bath of liquid coagulant or deswelling agent which supports the extruded material until solidification to shape retaining condition has been effected.

An aqueous concentrated ammonium sulfate solution is the preferred coagulant; but other agents known to coagulate collagen in fibrous form may be used. Such agents include water-soluble ketones and concentrated, preferably at least half saturated, aqueous solutions of salts which are soluble in water to an extent greater than about 25% such as sodium acetate or ammonium chloride. Where an aqueous solution of a salt, e.g. of ammonium sulfate, is the coagulating agent, it is preferred that it be mildly alkaline so that the coagulation action is a combination of neutralization of the acid in the suspension which brings the suspension to the isoelectric range, and a salting out effect.

Following shaping and coagulation of the suspension to shape-retaining condition, the shaped material body may be subjected to further treatments which may include tanning, washing, plasticizing, drying, etc. Tanning may be effected by alum, formaldehyde or other tanning agent. The tanning agent will be selected with consideration of the intended use of the product and where the product is to be edible, a tanning agent such as alum, which is acceptable for use in connection with edible products will be used. For use in sutures, formaldehyde, chromium or other tanning agents may be used.

Following the tanning step the shaped material body will be washed to remove free tanning material and while still wet may be plasticized as by soaking in an aqueous solution of the plasticizing agent such as glycerol.

The following examples are given to aid in understanding the invention and it is to be understood that the invention is not restricted to the materials, times, temperatures or other conditions of treatment given in the examples.

Example I

Fresh steer hide was limed for nine days in a saturated lime solution and was then removed from the lime solution, unhaird by scraping on the beam and placed in a fresh lime solution one additional day. The grain surface was removed from the limed unhaird hide on a commercial splitting machine and the flesh side splits were placed in a tanning drum, neutralized with acetic acid and then washed in water.

To eight pounds of the washed splits about 51 grams of 85% lactic acid in 500 cc. of water was added to make a 1.2% lactic acid solution. The splits were allowed to

stand over a weekend, then washed to remove any calcium lactate, hair, or other material and were next passed through a meat chopper using a plate with one-half inch holes. The chopped material was washed, allowed to stand for three hours in water and drained and a further 50 grams of 85% lactic acid in 500 grams of water was added to the chopped hide.

The chopped material was passed through a meat grinder provided with a plate having $\frac{5}{32}$ " holes and then given a first homogenizing treatment by being passed through a valve type homogenizer using a pressure of 1500 to 3500 lbs. per square inch. The homogenized material had a solids content of 10.8% and a pH of 3.4. The material was diluted with water to a 9.5% solids content and again passed through the homogenizer at 3500 lbs. per square inch to form a suspension consisting essentially of collagen fibrils in water.

25 lbs. of salted calfskin scrap were washed and placed in 100 lbs. of water, acidified with acetic acid to about pH 3.5 together with ice to cool the batch. After standing for 20 hours with occasional stirring, the pH of the liquid was about 4 and this liquid was drained off and discarded. The calfskin material was then placed in 120 lbs. of water containing 500 cc. of glacial acetic acid and allowed to stand in a cooler at about 5° C. for one week with occasional stirring. The liquor was drawn off and stored. A second extract was made by putting the calfskin material in 60 lbs. of water containing 500 cc. of acetic acid. The liquor was drawn off after two days. A third extract was made in which the calfskin was placed in 90 lbs. of water containing 500 cc. of glacial acetic acid. The liquid was drawn off after three days.

The three extracts were combined and filtered to remove gross impurities. Sodium chloride was added to the filtrate with stirring to a concentration of about 10% in order to precipitate the dissolved collagen. The precipitate was filtered off, re-dissolved in dilute acetic acid and dialyzed against a large volume of dilute acetic acid to remove the salt. After removal of the salt the solutions were concentrated by pervaporation in large dialysis bags in front of an electric fan. The pervaporation was carried to a solids content of 1.74% and by determination of the hydroxyproline content of the solids it was determined that the soluble collagen content was about 0.71%.

A series of batches were prepared for testing in extrusion as shown in the following table. These batches were prepared by combining portions of the collagen fiber suspension with collagen solution, mixing in a screw type mixer and evacuating to remove air.

TABLE I.—COMPOSITION OF EXTRUSION BATCHES 4% TOTAL SOLIDS

Components, grams.....	Added Soluble Collagen as Percent of Total Solids						
	0	0.59	1.88	2.95	5.89	8.87	11.78
Stock Slurry 9.5% Solids.....	421	208	204	200	189	179	168
Soluble Collagen Solution 0.71%.....	0	16.6	50	83	166	250	332
Water, Lactic Acid to pH 3.3.....	579	275.4	246	217	145	71	0
Total Weight, grams.....	1,000	500	500	500	500	500	500

The mixtures were placed successively in an extrusion apparatus in which a valve-type homogenizer setup was used to supply pumping pressure to force the mixture to an extrusion die comprising a 19 gauge stainless steel hypodermic needle cut to a length of one inch. The die was disposed with its exit directed to extrude the material upwards into a vertical glass tube filled with saturated ammonium sulfate solution adjusted to pH 8.4 with ammonium hydroxide. With extrusion pressure of about 50 to 100 lbs., a uniform filament was extruded into the ammonium sulfate solution and rose vertically through the solution because of density difference. After predetermined lengths of time of the filament extrudate in the coagulating ammonium sulfate bath, lengths of filament were removed for wet strength testing. The wet strength of ex-

truded filaments is recorded in the following table. The wet strength was determined by looping a 1½" length over a hook of a chatillon spring gauge and pulling to rupture. The gauge was graduated from 0 to 250 grams in 10 gram divisions and was read to the nearest 5 grams. At least 10 readings were recorded for each sample.

comprising a concentrated mildly alkaline aqueous solution of a salt and coagulating the extruded suspension by the liquid deswelling agent to form a product having early wet strength.

2. The process for the manufacture of continuous uniform cross section shaped products as defined in claim 1

TABLE II.—WET STRENGTHS OF EXTRUDED FILAMENTS BREAKING LOADS IN GRAMS																					
Percent Added Soluble Collagen																					
0		0.59		1.88		2.95		5.89		8.87		11.78									
Time in Ammonium Sulfate Bath																					
30 Sec.	15 Min.	1 Hr.	30 Sec.	15 Min.	1 Hr.	30 Sec.	15 Min.	1 Hr.	30 Sec.	15 Min.	1 Hr.	30 Sec.	15 Min.	1 Hr.	30 Sec.	15 Min.	1 Hr.	30 Sec.	15 Min.	1 Hr.	
30	30	30	20	40	30	25	40	35	30	45	45	35	45	50	45	50	50	40	55	60	
25	25	30	25	40	25	20	35	25	30	40	45	35	45	50	40	45	50	40	50	65	
25	30	30	25	40	30	25	40	30	35	40	40	35	45	50	40	50	50	40	55	65	
25	35	30	25	40	30	25	40	30	35	45	45	35	45	50	45	50	50	40	55	65	
25	35	30	20	35	35	25	40	30	35	45	45	35	45	50	40	50	50	40	50	65	
25	25	30	25	40	30	25	35	30	30	40	45	35	45	45	40	50	50	45	55	65	
30	30	30	20	40	40	25	40	30	35	40	40	35	45	50	40	40	50	45	60	65	
25	30	30	20	35	35	25	45	30	35	45	45	35	45	50	40	50	50	45	50	60	
30	30	30	20	35	35	25	40	30	35	40	40	35	45	50	45	50	50	45	55	70	
25	30	30	20	40	30	25	40	30	30	45	45	30	45	55	45	50	50	50	55	70	
25	30	-----	20	40	-----	25	35	30	-----	35	45	45	45	50	50	50	50	-----	60	65	
30	25	-----	-----	-----	-----	20	40	30	-----	35	45	50	45	60	50	-----	-----	60	65	-----	
-----	30	-----	-----	-----	-----	25	40	30	-----	35	45	55	45	50	50	-----	-----	55	60	-----	
-----	30	-----	-----	-----	-----	30	40	30	-----	35	45	50	45	55	50	-----	-----	55	65	-----	
-----	25	-----	-----	-----	-----	30	40	35	-----	30	45	50	45	50	50	-----	-----	60	60	-----	
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-----	-----	-----	-----	-----	-----	30	40	-----	-----	-----	-----	-----	-----	50	50	-----	-----	-----	-----	-----	
Average Load																					
27	29	30	22	39	32	26	39	30	33	43	44	34	45	50	44	51	50	43	56	64	

Example II

A batch of 4% by weight solids collagen fiber suspension (without added acid-soluble collagen) was prepared by a procedure similar to that described in Example I.

The suspension was placed in a commercial type extrusion device for extruding sausage casings, the extruded material being discharged directly into a coagulation bath comprising a saturated solution of ammonium sulfate. Extrusion was unsatisfactory due to rupture of the extruded casing material while wet and values for the breaking load of freshly extruded casing material was in the range of 175 to 236 grams.

Acid-soluble collagen solution prepared as described in Example I was added to collagen fiber suspension in amount to constitute about 5% of the total solids of the suspension the solids content of the mixture being adjusted to 4% by weight. This mixture was extruded and a wet breaking load of 600 grams was obtained.

Having thus described our invention what we claim as new and desire to secure by Letters Patent of the United States is:

1. The process for the manufacture of thin cross section shaped products comprising the steps of providing an acidic aqueous suspension of from about 1% to about 10% by weight of very fine acid swollen collagen fibers from untanned limed hides, said suspension containing at least about 2% of acid soluble collagen in solution based on the dry weight of the collagen fibers and soluble collagen in said suspension and having a salt content under about 1% extruding the suspension to a desired cross section directly into a body of liquid deswelling agent

in which the suspension has a collagen fiber content of from about 3% to about 6% by weight, said suspension containing at least about 5% of acid soluble collagen in solution based on the dry weight of the collagen fibers and soluble collagen in said suspension and having a pH of from about 3 to about 3.6.

3. The process for the manufacture of continuous uniform cross section shaped products as defined in claim 3 in which the liquid deswelling agent is an at least half saturated ammonium sulfate solution containing alkaline material in amount for reaction with acidic material in said aqueous suspension to give a pH in the isoelectric range of collagen.

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