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(54) Title: CONTINUOUS PRODUCTION OF KERATIN FIBERS

(57) Abstract: This disclosure relates to processes for preparing a keratin fiber, for example, continuous process. In some embodiments, a process as described herein can comprise extruding a keratin solution into a first solution to form a first fiber; drawing the first fiber and oxidizing the first fiber to form a treated fiber; drawing the treated fiber and oxidizing the treated fiber one or more times; and setting the treated fiber to form the keratin fiber. Such processes can be useful for preparing a keratin fiber with a high draw ratio.

CONTINUOUS PRODUCTION OF KERATIN FIBERS

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under Grant No. 2019-67021-29940, awarded by the United States Department of Agriculture, National Institute of Food and Agriculture. The government has certain rights in the invention.

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application Serial No. 62/963,968, filed on January 21, 2020, the contents of which are incorporated by reference in their entirety herein.

TECHNICAL FIELD

The present disclosure relates to continuous processes for preparing a keratin fiber. Such processes can be useful for preparing a keratin fiber with a high draw ratio, for example.

BACKGROUND

Research on the preparation of high-end materials through green synthesis technology has received more and more attention [1-3]. At the same time, the preparation of common products for daily use via green and sustainable approaches is gaining popularity, such as the use of renewable monomers to prepare sustainable polyester [4, 5]. But many of these sustainable polymers are hardly degradable [6]. Long-term accumulation of such materials will inevitably affect the environment and human health. It is reported that synthetic polymers are circulating in the atmosphere and ocean after turning into nano- or microparticles [7]. For instance, precipitations have been found to contain polymer particles in many districts even in the Artic [8]. Recently people found that plastic particles fell out of the sky with snow in the Arctic. These plastic particles will inevitably enter the human body and accumulate [9]. Recent studies show that synthetic polymer particles can cause various hazards to human health such as cancer, chronical illness, and

damage to the reproductive system [10]. Therefore, the utilization of wastes to develop quality products with good degradability should also receive equal attention in the future [11], especially in the textile field which can be plagued by environmental pollution. In terms of textile fibers, more than 80 million tons of synthetic fibers are produced every year globally [12]. Almost all synthetic fibers are petroleum-based and hardly degradable in the environment [13]. Therefore, it is desirable to find alternatives to replace petroleum-based fibers [14, 15]. Alternatives should be sustainable, environmentally responsible and affordable.

Although studies for the utilization of keratinous wastes began decades ago, very few regenerated products with high quality have been developed due to damages to the primary structures during extraction and recovery of the secondary structures in the regeneration of keratin materials. Among the regenerated keratin products, fibers have high quality specifications, including resistance to repeated launderings and high toughness.

SUMMARY

Provided herein are processes for preparing a keratin fiber. In some embodiments, the methods comprise extruding a keratin solution into a first solution to form a first fiber; drawing the first fiber and oxidizing the first fiber to form a treated fiber; drawing the treated fiber and oxidizing the treated fiber one or more times; and setting the treated fiber to form the keratin fiber.

In some embodiments, the keratin fiber has a draw ratio of above about 500%. In some embodiments, the keratin fiber has a draw ratio of about 800% to about 1000%. In some embodiments, the keratin fiber has a draw ratio of about 1500%.

In some embodiments, the diameter of the keratin fiber is about 5 micrometers to about 30 micrometers. In some embodiments, the diameter of the keratin fiber is about 15 micrometers.

In some embodiments, the keratin fiber comprises at least about 70% keratin. In some embodiments, the keratin fiber comprises at least about 85% keratin.

In some embodiments, the keratin fiber has a tenacity greater than about 0.8 g/den. In some embodiments, the keratin fiber has a tenacity of about 1 g/den to about 2 g/den.

In some embodiments, the keratin fiber has a strain greater than about 5%. In some embodiments, the keratin fiber has a strain of about 10% to about 30%. In some embodiments, the keratin fiber has a strain of about 15%. In some embodiments, the keratin fiber has been dried prior to measuring the strain.

In some embodiments, the keratin fiber has a toughness higher than about 15 J/cm^3.

In some embodiments, the keratin solution has a consistency coefficient (K) of about 2 $Pa \cdot s^n$ to about 6 $Pa \cdot s^n$, wherein the keratin solution is at 25 °C and comprises keratin at about 18% w/w of the composition. In some embodiments, the keratin solution has a consistency coefficient (K) of about 4.2 $Pa \cdot s^n$, wherein the keratin solution is at 25 °C and comprises keratin at about 18% w/w of the composition. In some embodiments, the keratin solution has a flow behavior index of about 0.9 to about 0.94, wherein the keratin solution is at 25 °C and comprises keratin at about 18% w/w of the composition. In some embodiments, the keratin solution has a flow behavior index of about 0.91, wherein the keratin solution is at 25 °C and comprises keratin at about 18% w/w of the composition.

In some embodiments, the keratin solution comprises a reducing agent. In some embodiments, the keratin solution comprises an electrolyte. In some embodiments, the electrolyte is a sulfate, an acetate, a chloride, a citrate, a carbonate, a phosphate, or a combination thereof. In some embodiments, the keratin solution comprises sodium dodecyl sulfate (SDS). In some embodiments, the keratin solution is prepared from a keratinous material.

In some embodiments, the process further comprises preparing the keratin solution. In some embodiments, preparing the keratin solution comprises: extracting keratin from a keratinous material to form extracted keratin; and dissolving the extracted keratin in an aqueous solution comprising a reducing agent to form the keratin solution. In some embodiments, the aqueous solution further comprises SDS.

In some embodiments, the keratinous material comprises one or more of: animal hair, horn, and feather. In some embodiments, the hair is the hair is wool, camel hair, alpaca hair, rabbit hair, or a combination thereof. In some embodiments, the feather is a duck feather, a goose feather, a chicken feather, or a combination thereof.

In some embodiments, the keratin fiber comprises at least 70% of the disulfide crosslinkages compared to the amount of disulfide crosslinkages in the keratinous material. In some embodiments, the keratin fiber comprises at least 85% of the beta-sheet crystallinity compared to the amount of beta-sheet crystallinity in the keratinous material.

In some embodiments, the reducing agent comprises a thiol group. In some embodiments, the reducing agent comprises mercaptoethanol, cysteine, dithiothreitol, 1,2-ethanedithiol, 1,3-benzenedithiol, bis(2-mercaptoethyl) ether, ethylene glycol bisthioglycolate, or a combination thereof.

In some embodiments, the step of extruding a keratin solution into a first solution comprises using a spinneret to extrude the keratin solution. In some embodiments, the spinneret comprises a hole, wherein the hole has a diameter of about 50 micrometers.

In some embodiments, the first solution comprises sodium sulfate, zinc sulfate, and acetate buffer. In some embodiments, the pH of the first solution is about 2. In some embodiments, the first solution comprises sodium sulfate in an amount of about 15% w/w of the composition, zinc sulfate in an amount of about 5% w/w of the composition, and acetate buffer with a pH of 2.

In some embodiments, the step of oxidizing comprises exposing the fiber to an oxidizing solution comprising an oxidant selected from the group consisting of: a peroxide, a halogen oxoacid or salt thereof, a high-valent metal salt, and a combination thereof. In some embodiments, the peroxide is an alkali metal peroxide, an alkaline earth metal peroxide, or a combination thereof. In some embodiments, the oxidant is sodium periodate. In some embodiments, the oxidizing solution further comprises a buffer. In some embodiments, the oxidizing solution further comprises acetate buffer. In some embodiments, the pH of the oxidizing solution is about 2. In some embodiments, the temperature of the oxidizing solution is about 35 °C.

In some embodiments, the step of drawing the treated fiber and oxidizing the treated fiber is repeated two times. In some embodiments, the process further comprises drawing the treated fiber prior to setting the treated fiber.

In some embodiments, setting the treated fiber comprises exposing the treated fiber to a wash solution comprising a surfactant. In some embodiments, the surfactant is selected

from the group consisting of: ammonium lauryl sulfate, SDS, sodium laureth sulfate, sodium myreth sulfate, sodium stearate, sodium lauroyl sarcosinate, perfluorononanoate, perfluorooctanoate, (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate), cocamidopropyl hydroxysultaine, cocamidopropyl betaine, phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine, a sphingomyelin, cetrimonium bromide (CTAB), cetylpyridinium chloride (CPC), benzalkonium chloride (BAC), benzethonium chloride (BZT), dimethyldioctadecylammonium chloride, dioctadecyldimethylammonium bromide (DODAB), and a combination thereof.

In some embodiments, the wash solution further comprises an acetate buffer. In some embodiments, the wash solution has a pH of about 2. In some embodiments, the wash solution is at a temperature of about 40 °C.

In some embodiments, setting the treated fiber comprises winding the treated fiber and oxidizing the treated fiber.

In some embodiments, exposing the treated fiber to a wash solution comprising a surfactant is performed prior to winding the treated fiber and oxidizing the treated fiber. In some embodiments, winding the treated fiber is at a rate of about 15 meters/minute.

In some embodiments, the keratin fiber is dried at about 85 °C for about 1 hour. In some embodiments, the keratin fiber is annealed at about 125 °C for about 1 hour. In some embodiments, the keratin fiber is annealed after it is dried.

In some embodiments, the process is a continuous process.

In some embodiments, the process further comprises exposing the keratin fiber to a solution comprising an oxidized saccharide. In some embodiments, the keratin fiber is exposed to the solution comprising an oxidized saccharide for about 3 to about 25 hours. In some embodiments, the oxidized saccharide is a sucrose polyaldehyde.

In some embodiments, the step of exposing the keratin fiber to a solution comprising an oxidized saccharide is performed prior to exposing the treated fiber to a wash solution comprising a surfactant.

Also described herein are keratin fibers prepared by any of the processes described herein.

In some embodiments, the term "about" is used herein to mean approximately, in the region of, roughly, or around. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" is used herein to modify a numerical value above and below the stated value by a variance of 10%.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

DESCRIPTION OF DRAWINGS

FIG. 1 is a diagram of a wet spinning line employed with stepwise oxidation and drawing.

FIG. 2 is a reducing SDS-PAGE gel with Lane 1 containing standard protein markers; Lane 2 containing regenerated keratin; and Lane 3 containing chicken feathers.

FIG. 3A is a comparison of disulfide bonds in keratin between chicken feathers and spun keratin fibers using Raman spectra.

FIG. 3B shows recovery of disulfide bonds in keratin fibers measured by HPLC at different oxidation stages via controlled disulfide bond assembly.

FIG 3C shows the effect of controlled disulfide bond assembly on spinnability of keratin fibers. Fine control of disulfide bond assembly was achieved using stepwise oxidation and drawing on a continuous spinning line.

FIG. 4A shows the relationship between recovery ratio of disulfide bonds in fibers and highest draw ratio.

FIG. 4B shows the morphologies of spun keratin fiber from highest draw ratio (scale bar = 50 μm).

FIG. 5 shows a morphological change in keratin fibers on a continuous spinning line with controlled disulfide bonds assembly.

FIG. 6A shows a continuous line for keratin production.

FIG. 6B shows spun keratin fibers.

FIG. 7A is a plot showing typical stress-strain curves for feather barbs and continuously spun keratin fibers.

FIG. 7B is a keratin filament that endured a high degree of twisting.

FIG. 8A is an XRD spectra of chicken feathers and keratin fibers.

FIG. 8B shows deconvolutions of the ^{13}C NMR spectra (around 170 ppm) of chicken feathers and keratin fibers.

DETAILED DESCRIPTION

Keratinous wastes, especially poultry feathers, are abundant, safe, cost-effective and readily available materials that could be used to produce fibers [16]. Among all poultries, chicken has the largest consumption worldwide [17]. With an annual chicken consumption of about 65 million tons and a subsequent generation of chicken feathers of 5 million tons worldwide annually [18], the potential production of protein fibers from chicken feathers is already 2.5 times higher than the current output of both wool and silk. Chicken feathers are also rich in protein (keratin) content as high as 90-92% [19]. Having linear polymeric backbones and an average molecular weight higher than 10 kDa [20], feather keratin meets the molecular specifications for fiber spinning. With around 7% cysteine served as crosslinking sites, feather keratin is expected to possess good tensile properties and aqueous stabilities [21]. Fibers made of feather keratin are very likely to have smooth touch, moisture transmission and thermal insulation due to similar chemical structures to those of wool and silk [15].

Unsuccessful continuous fiber production can result from the difficulty in keratin extraction from feathers, incomplete re-dissolution of keratin, limited alignment of molecular chains of protein, and inefficient recovery of disulfide crosslinkages. For a long period of time, strong alkali solutions were used to dissolve and extract keratin [22].

However, high pH not only hydrolyzes the backbone of the protein but it can also reduce amounts of sulfhydryl groups on the keratin [23, 24]. Damaged protein backbones and a decline in sulfur groups can make it challenging to produce high-quality fibers. Ionic liquids have been used to extract keratin from wastes [25, 26]. Recently, ionic liquids were used to dissolve keratin for spinning. However, the properties of obtained fibers were not satisfactory [27]. One reason for such low mechanical property is poor dissolution of keratin. Liquids could break ionic interactions and hydrogen bonds; they are not able to interrupt disulfide bonds and hydrophobic interactions among keratin molecules. A non-destructive extraction system was developed and regenerated chicken keratin fibers on a lab-scale [28]. However, such fiber spinnings lacked techniques to efficiently recover the disulfide bonds and secondary structures. As a result, the spinnability of keratin fibers was not good. The stretchability of the regenerated fiber was also remarkably limited. The linearity of the regenerated fiber was poor, leading to long-distance between keratin backbones and low chances for the formation of intermolecular disulfide crosslinkages. The resulting fibers had a large diameter, low strength, and poor flexibility. The tenacity of the regenerated fiber was only 50% of the original chicken feathers and strain was only 4%. In addition, the regenerated keratin fiber did not inherit good wet properties of chicken feathers. Although the research regarding the fiber production from keratinous waste began in the 1940s [29], there are few efficacious processes developed to continuously produce regenerated pure keratin fibers with high quality. It is desirable for fibers to have stress higher than 100 Mpa and strain higher than 10%. To achieve this, most research focuses on either post crosslinking or addition of high-performance polymers such as PVA and cellulose into keratin fibers to improve the properties [27, 30-33].

Accordingly, the present application provides keratin fibers and processes for preparing the keratin fibers. Such fibers can include keratin fibers produced on a continuous line via stepwise oxidation and drawing. Stepwise oxidation and drawing of a fiber can result in one or more of: controlled assembly of disulfide crosslinkages, optimum recovery of secondary structures, satisfactory mechanical properties, and scalable production of keratin fibers. For example, the properties of the regenerated keratin fibers can be close to that of chicken feathers. Furthermore, through the use and recycling of safe

and inexpensive chemicals, the continuous keratin fiber production disclosed herein is sustainable, environmentally responsible, and affordable. Continuous keratin fiber production via efficient recovery of secondary structures and disulfide bonds can minimize the use and generation of hazardous substances in the manufacturing process and can open a new window for the utilization of keratinous wastes.

A “keratin fiber” as used herein is a fiber comprising at least about 70% keratin. As used herein, the term "fiber" or "textile fiber" means a unit of matter which is capable of being spun into a yarn or made into a fabric by bonding or by interlacing in a variety of processes including weaving, knitting, braiding, felting, twisting, or webbing, and which is the basic structural element of a textile product.

In some embodiments, a keratin fiber described herein has at least about 70% keratin. For example, the keratin fiber can have about 70% to about 99%, about 70% to about 95%, about 70% to about 90%, about 70% to about 85%, about 70% to about 80%, about 70% to about 75%, or about 95% to about 99%, about 90% to about 99%, about 85% to about 99%, about 80% to about 99%, about 75% to about 99%, or about 75% to about 95% keratin. For example, the keratin fiber can have about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 99% keratin.

In some embodiments, a process for preparing a keratin fiber includes extruding a keratin solution into a first solution to form a first fiber; drawing the first fiber and oxidizing the first fiber to form a treated fiber; drawing the treated fiber and oxidizing the treated fiber one or more times; and setting the treated fiber to form the keratin fiber. In some embodiments, the process is a continuous process.

A keratin solution as described herein includes keratin, e.g., extracted keratin. In some embodiments, the keratin solutions further includes sodium dodecyl sulfate (SDS), and/or a reducing agent. Reducing agents as described herein can include a thiol-based group, e.g., monothiols and dithiols. Non-limiting examples of such reducing agents include mercaptoethanol, cysteine, dithiothreitol, 1,2-ethanedithiol, 1,3-benzenedithiol, bis(2-mercaptoethyl) ether, and ethylene glycol bisthioglycolate. In some embodiments, the keratin solution includes a reducing agent at a concentration of about 0.5% to about 3% w/w of the keratin. For example, about 0.5% to about 2.5%, about 0.5% to about 2%, about

0.5% to about 1.5%, about 0.5% to about 1%, about 2.5% to about 3%, about 2% to about 3%, about 1.5% to about 3%, about 1% to about 3%, about 1.5% to about 2.5%, or about 1.75% to about 2.25% w/w of the keratin. In some embodiments, the keratin solution includes a reducing agent at a concentration of about 1.4%, about 1.6%, about 1.8%, about 2%, about 2.2%, about 2.4%, about 2.6%, or about 2.8% w/w of the keratin.

In some embodiments, the keratin solution includes extracted keratin at about 20% to about 35% w/w of the solution. For example, about 20% to about 25%, about 20% to about 30%, about 30% to about 35%, or about 25% to about 35% w/w of the solution. In some embodiments, the keratin solution include extracted keratin at about 25% to about 30% or about 26% to about 28% w/w of the solution. In some embodiments, the keratin solution includes extracted keratin at about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, or about 30% w/w of the solution.

In some embodiments, the keratin solution includes SDS. In some embodiments, the keratin solution includes SDS at about 5% to about 15% w/w of the solution. For example, about 5% to about 8%, about 5% to about 10%, about 5% to about 12%, about 12% to about 15%, about 10% to about 15%, about 8% to about 15%, or about 8% to about 12% w/w of the solution. In some embodiments, the keratin solution includes SDS at about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, or about 15% w/w of the solution.

In some embodiments, the pH of the keratin solution is adjusted to be about 7 to about 10. For example, about 7 to about 8, about 7.5 to about 8.5, about 8 to about 9, about 8.5 to about 9.5, or about 9 to about 10. Any buffer suitable for maintaining the desired pH can be used. Non-limiting examples of such buffers include carbonate-bicarbonate buffer, glycine-sodium hydroxide buffer, sodium borate buffer, TRIZMA® buffer (e.g., 2-Amino-2-(hydroxymethyl)-1,3-propanediol buffer), and diethanolamine buffer. In some embodiments, the keratin solution includes a buffer at about 0.1 M to about 0.3 M. For example, about 0.1 M to about 0.15 M, about 0.1 M to about 0.2 M, about 0.1 M to about 0.25 M, about 0.25 M to about 0.3 M, about 0.2 M to about 0.3 M, about 0.15 M to about 0.3 M, about 0.15 M to about 0.2 M, about 0.18 M to about 0.22 M, or about 0.2 M to about 0.25 M.

In some embodiments, the amount of reducing agent in a keratin solution described herein is optimized such that the keratin is fully dissolved and/or the molecular entanglement is low. In some embodiments, the degree of molecular entanglement is indicated by the flow behavior index (n), which can be determined by measuring shear stress using a rotational rheometer (see Equation 1). The consistency coefficient (K) for the keratin solution, which is directly proportional to polymer viscosity in solution, can also be determined by measuring shear stress using a rotational rheometer.

Equation 1: $\tau=K\dot{\gamma}^n$

where $\dot{\gamma}$ is the shear rate (s^{-1}) measured in the range of 0-1000 s^{-1}

In some embodiments, the keratin solution has a flow behavior index of about 0.8 to about 0.95. For example, about 0.8 to about 0.82, about 0.8 to about 0.84, about 0.8 to about 0.86, about 0.8 to about 0.88, about 0.8 to about 0.9, about 0.8 to about 0.92, about 0.8 to about 0.94, about 0.92 to about 0.95, about 0.9 to about 0.95, about 0.88 to about 0.95, about 0.86 to about 0.95, about 0.84 to about 0.95, about 0.82 to about 0.95, about 0.88 to about 0.92, or about 0.90 to about 0.94. In some embodiments, the keratin solution has a flow behavior index of about 0.88, about 0.89, about 0.9, about 0.91, about 0.92, about 0.93, about 0.94, or about 0.95. In some embodiments, the keratin solution is at about 25 °C and includes extracted keratin at about 18% w/w of the solution during the measurement of the shear stress.

In some embodiments, the keratin solution has a consistency coefficient (K) of about 2 $Pa•s^n$ to about 6 $Pa•s^n$. For example, about 2 $Pa•s^n$ to about 6 $Pa•s^n$, about 3 $Pa•s^n$ to about 6 $Pa•s^n$, about 4 $Pa•s^n$ to about 6 $Pa•s^n$, about 5 $Pa•s^n$ to about 6 $Pa•s^n$, about 4 $Pa•s^n$ to about 6 $Pa•s^n$, about 3 $Pa•s^n$ to about 6 $Pa•s^n$, about 3 $Pa•s^n$ to about 5 $Pa•s^n$, about 3.5 $Pa•s^n$ to about 5.5 $Pa•s^n$, about 3.5 $Pa•s^n$ to about 4.5 $Pa•s^n$, or about 4 $Pa•s^n$ to about 4.5 $Pa•s^n$. In some embodiments, the keratin solution is at about 25 °C and includes extracted keratin at about 18% w/w of the solution during the measurement of the shear stress.

In some embodiments, a process described herein further includes preparing the keratin solution, e.g,. any of the keratin solutions as described herein. In some

embodiments, preparing the keratin solution includes extracting keratin from a keratinous material to form extracted keratin; and dissolving the extracted keratin in an aqueous solution comprising a reducing agent, e.g., any of the reducing agents described herein to form the keratin solution. In some embodiments,

Keratin can be extracted from any keratinous material, e.g., a material comprising keratin. Non-limiting examples of keratinous materials include: hair, horn, and feather. In some embodiments, the keratinous material includes wool, camel hair, alpaca hair, rabbit hair, duck feather, goose feather, chicken feather, or a combination thereof. In some embodiments, the step of extracting keratin from a keratinous material to form extracted keratin includes exposing a keratinous material to an extraction solution. The extraction solution can include one or more of SDS, urea, and a reducing agent.

In some embodiments, the extraction solution includes SDS. In some embodiments, the extraction solution includes SDS at about 5% to about 15% w/w of the solution. For example, about 5% to about 8%, about 5% to about 10%, about 5% to about 12%, about 12% to about 15%, about 10% to about 15%, about 8% to about 15%, or about 8% to about 12% w/w of the solution. In some embodiments, the extraction solution includes SDS at about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, or about 15% w/w of the solution.

In some embodiments, the extraction solution includes urea at about 1 M to about 3 M. For example, about 1 M to about 1.5 M, about 1 M to about 2 M, about 1 M to about 2.5 M, about 2.5 M to about 3 M, about 2 M to about 3 M, about 1.5 M to about 3 M, about 1.5 M to about 2 M, about 1.8 M to about 2.2 M, or about 2 M to about 2.5 M.

In some embodiments, the extraction solution includes a reducing agent, e.g., any of the reducing agents described herein. In some embodiments, the extraction solution includes the reducing agent at about 5% to about 15% w/w of the solution. For example, about 5% to about 8%, about 5% to about 10%, about 5% to about 12%, about 12% to about 15%, about 10% to about 15%, about 8% to about 15%, or about 8% to about 12% w/w of the solution. In some embodiments, the extraction solution includes the reducing agent at about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about

12%, about 13%, about 14%, or about 15% w/w of the solution. In some embodiments, the reducing agent is cysteine.

In some embodiments, the pH of the extraction solution is adjusted to be about 9 to about 11.5. For example, about 9 to about 11, about 9 to about 10.5, about 9 to about 10, about 11 to about 11.5, about 10.5 to about 11.5, about 10 to about 11.5, about 9.5 to about 11.5, or about 10 to about 11. In some embodiments, the pH of the extraction solution is adjusted to be about 9, about 9.5, about 10, about 10.5, about 11, or about 11.5.

In some embodiments, the step of extracting keratin from a keratinous material to form extracted keratin includes exposing a keratinous material to an extraction solution for a period of time. In some embodiments, the period of time is about 8 hours to about 15 hours. For example, about 8 hours to about 9 hours, about 8 hours to about 10 hours, about 8 hours to about 11 hours, about 8 hours to about 12 hours, about 8 hours to about 13 hours, about 8 hours to about 14 hours, about 14 hours to about 15 hours, about 13 hours to about 15 hours, about 12 hours to about 15 hours, about 11 hours to about 15 hours, about 10 hours to about 15 hours, about 9 hours to about 15 hours, about 10 hours to about 14 hours, or about 11 hours to about 13 hours. In some embodiments, the period of time is about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, or about 15 hours.

In some embodiments, the temperature of the extraction solution is held at about 60 °C to about 80 °C. For example, about 60 °C to about 65 °C, about 60 °C to about 70 °C, about 60 °C to about 75 °C, about 75 °C to about 80 °C, about 70 °C to about 80 °C, about 65 °C to about 80 °C. In some embodiments, the temperature of the extraction solution is held at about 65 °C, about 66 °C, about 67 °C, about 68 °C, about 70 °C, about 71 °C, about 72 °C, about 73 °C, about 74 °C, or about 75 °C.

In some embodiments, the step of extracting keratin from a keratinous material to form extracted keratin further includes centrifuging the extraction solution comprising the keratinous material to form a centrifuged extraction solution comprising the keratinous material. In some embodiments, the supernatant of the centrifuged extraction solution comprising the keratinous material is adjusted to the isoelectric point using any suitable acid (e.g., hydrochloric acid). In some embodiments, sodium sulfate is added to the

supernatant of the centrifuged extraction solution comprising the keratinous material to precipitate the extracted keratin. In some embodiments, the extracted keratin is washed to remove impurities. In some embodiments, the extracted keratin is vacuumed dried.

A keratin solution as described herein can be installed on a spinning line prior to the step of extruding the keratin solution into a first solution to form a first fiber. The spinning line can be any suitable spinning line, e.g., a wet spinning line. In some embodiments, the step of extruding a keratin solution into a first solution comprises using a spinneret to extrude the keratin solution. For example, the keratin solution is extruded into the first solution using a spinneret. In some embodiments, the spinneret comprises one or more holes, wherein the holes have a diameter of about 50 micrometers.

In some embodiments, the first solution includes an electrolyte. Non-limiting examples of suitable electrolytes include sulfate, acetate, chloride, citrate, carbonate, and phosphate. The electrolytes can be paired with any suitable cation. Non-limiting examples of such cations include alkali metals and transition metals such as lithium, sodium, magnesium, and zinc. Accordingly, in some embodiments, the first solution includes lithium sulfate, sodium sulfate, sodium acetate, zinc sulfate, zinc acetate, zinc chloride, sodium carbonate, sodium phosphate, zinc carbonate, or a combination thereof.

In some embodiments, the first solution includes an electrolyte in an amount of about 10% to about 30% w/w of the composition. For example, about 10% to about 15%, about 10% to about 20%, about 10% to about 25%, about 25% to about 30%, about 20% to about 30%, about 15% to about 30%, about 15% to about 20%, or about 18% to about 22% w/w of the composition. In some embodiments, the first solution includes an electrolyte in an amount of about 10%, about 12%, about 14%, about 16%, about 18%, about 20%, about 22%, about 24%, about 26%, about 28%, or about 30% w/w of the composition. In some embodiments, the first solution includes sodium sulfate at 15% w/w of the composition, zinc sulfate at 5% w/w of the composition.

In some embodiments, the first solution further includes a buffer. Any buffer suitable for maintaining the desired pH can be used. Non-limiting examples of such buffers include hydrochloric acid (HCl)-potassium chloride buffer, glycine-HCl buffer, and acetate buffer. In some embodiments, the first solution includes a buffer at a concentration of about

0.1 M to about 0.3 M. For example, about 0.1 M to about 0.15 M, about 0.1 M to about 0.2 M, about 0.1 M to about 0.25 M, about 0.25 M to about 0.3 M, about 0.2 M to about 0.3 M, about 0.15 M to about 0.3 M, about 0.15 M to about 0.2 M, about 0.18 M to about 0.22 M, or about 0.2 M to about 0.25 M.

In some embodiments, the pH of the first solution is adjusted to be about 1 to about 4. For example, about 1 to about 1.5, about 1 to about 2, about 1 to about 2.5, about 1 to about 3, about 1 to about 3.5, about 3.5 to about 4, about 3 to about 4, about 2.5 to about 4, about 2 to about 4, about 1.5 to about 4, or about 1.5 to about 2.5. In some embodiments, the pH of the first solution is adjusted to be about 1, about 1.4, about 1.6, about 1.8, about 2, about 2.2, about 2.4, about 2.6, about 3, about 3.5, or about 4.

In some embodiments, the first solution includes sodium sulfate, zinc sulfate, and acetate buffer. In some embodiments, the first solution includes sodium sulfate at 15% w/w of the composition, zinc sulfate at 5% w/w of the composition, and acetate buffer.

Stepwise drawing and oxidizing after extruding a keratin solution into a first solution to form a first fiber can help establish disulfide bonds and ordered structures in the first fiber. In some embodiments, the step of oxidizing comprises exposing the first fiber to an oxidizing solution comprising an oxidant selected from the group comprising a peroxide, a halogen oxoacid or salt thereof, a high-valent metal salt, or a combination thereof. Non-limiting examples of a peroxide include alkali metal peroxides and alkaline earth metal peroxides such as sodium periodate, hydrogen peroxide, chlorite, hypochlorite, and sodium ferrate(VI). In some embodiments, the oxidant is present in an amount of about 2 g/L to about 6 g/L. For example, about 2 g/L to about 2.5g/L, about 2 g/L to about 3g/L, about 2 g/L to about 3.5g/L, about 2 g/L to about 4g/L, about 2 g/L to about 4.5g/L, about 2 g/L to about 5g/L, about 2 g/L to about 5.5g/L, about 5.5 g/L to about 6g/L, about 5 g/L to about 6g/L, about 4.5 g/L to about 6g/L, about 4 g/L to about 6g/L, about 3.5 g/L to about 6g/L, about 3 g/L to about 6g/L, about 2.5 g/L to about 6g/L, about 3 g/L to about 5g/L, or about 3.5 g/L to about 4.5 g/L. In some embodiments, the oxidant is present in an amount of about 2g/L, about 2.5g/L, about 3g/L, about 3.5g/L, about 4g/L, about 4.5g/L, about 5g/L, about 5.5g/L, about 6 g/L.

In some embodiments, the oxidizing solution further comprises a buffer. In some embodiments, the first solution further includes a buffer. Any buffer suitable for maintaining the desired pH can be used. Non-limiting examples of such buffers include HCl-potassium chloride buffer, glycine-HCl buffer, citrate buffer, and acetate buffer. In some embodiments, the first solution includes a buffer at a concentration of about 0.1 M to about 0.3 M. For example, about 0.1 M to about 0.15 M, about 0.1 M to about 0.2 M, about 0.1 M to about 0.25 M, about 0.25 M to about 0.3 M, about 0.2 M to about 0.3 M, about 0.15 M to about 0.3 M, about 0.15 M to about 0.2 M, about 0.18 M to about 0.22 M, or about 0.2 M to about 0.25 M.

In some embodiments, the pH of the first solution is adjusted to be about 1 to about 4. For example, about 1 to about 1.5, about 1 to about 2, about 1 to about 2.5, about 1 to about 3, about 1 to about 3.5, about 3.5 to about 4, about 3 to about 4, about 2.5 to about 4, about 2 to about 4, about 1.5 to about 4, or about 1.5 to about 2.5. In some embodiments, the pH of the first solution is adjusted to be about 1, about 1.4, about 1.6, about 1.8, about 2, about 2.2, about 2.4, about 2.6, about 3, about 3.5, or about 4.

In some embodiments, the temperature of the oxidizing solution is about 30 to about 40. For example, about 30 °C to about 35°C, about 35 °C to about 40°C, or about 33 °C to about 38 °C. In some embodiments, the temperature of the oxidizing solution is about 30°C, about 31°C, about 32°C, about 33°C, about 34°C, about 35°C, about 35°C, about 36°C, about 37°C, about 238°C, about 39°C, or about 40 °C.

In some embodiments, the step of drawing the treated fiber and oxidizing the treated fiber is repeated two or more times. For example, the step of drawing the treated fiber and oxidizing the treated fiber is repeated two, three, four, or five times. In some embodiments, the step of drawing the treated fiber and oxidizing the treated fiber is repeated two times. In some embodiments, the process further comprises drawing the treated fiber prior to setting the treated fiber.

Setting and enhancing the fiber

In some embodiments, setting the treated fiber comprises exposing the treated fiber to a wash solution comprising a surfactant. Non-limiting examples of suitable surfactants

include ammonium lauryl sulfate, SDS, sodium laureth sulfate, sodium myreth sulfate, sodium stearate, sodium lauroyl sarcosinate, perfluorononanoate, perfluorooctanoate, (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate), cocamidopropyl hydroxysultaine, cocamidopropyl betaine, phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine, a sphingomyelin, cetrimonium bromide (CTAB), cetylpyridinium chloride (CPC), benzalkonium chloride (BAC), benzethonium chloride (BZT), dimethyldioctadecylammonium chloride, and dioctadecyldimethylammonium bromide (DODAB). In some embodiments, the surfactant is present in an amount of about 0.2 to about 2 g/L. For example, about 0.2 g/L to about 0.6 g/L, about 0.2 g/L to about 1g/L, about 0.2 g/L to about 1.4 g/L, about 0.2 g/L to about 1.8 g/L, about 1.6 g/L to about 2 g/L, about 1.2 g/L to about 2 g/L, about 0.8 g/L to about 2 g/L, about 0.4 g/L to about 2 g/L, about 0.5 g/L to about 1.5 g/L, or about 0.8 g/L to about 1.2 g/L.

In some embodiments, the wash solution further comprises a buffer. Any buffer suitable for maintaining the desired pH can be used. Non-limiting examples of such buffers include HCl-potassium chloride buffer, glycine-HCl buffer, citrate buffer, and acetate buffer. In some embodiments, the wash solution includes a buffer at a concentration of about 0.05 M to about 0.3 M. For example, about 0.05 M to about 0.1 M, about 0.05 M to about 0.15 M, about 0.05 M to about 0.2 M, about 0.05 M to about 0.25 M, about 0.25 M to about 0.3 M, about 0.2 M to about 0.3 M, about 0.15 M to about 0.3 M, about 0.1 M to about 0.3 M, about 0.15 M to about 0.2 M, about 0.18 M to about 0.22 M, or about 0.2 M to about 0.25 M.

In some embodiments, the pH of the wash solution is adjusted to be about 1 to about 4. For example, about 1 to about 1.5, about 1 to about 2, about 1 to about 2.5, about 1 to about 3, about 1 to about 3.5, about 3.5 to about 4, about 3 to about 4, about 2.5 to about 4, about 2 to about 4, about 1.5 to about 4, or about 1.5 to about 2.5. In some embodiments, the pH of the wash solution is adjusted to be about 1, about 1.4, about 1.6, about 1.8, about 2, about 2.2, about 2.4, about 2.6, about 3, about 3.5, or about 4.

In some embodiments, the wash solution is at a temperature of about 35 °C to about 45 °C. For example, about 35 °C to about 40 °C, about 40 °C to about 45 °C, or about 38 °C to about 42 °C. In some embodiments, the wash solution is at a temperature of about 35

°C, about 36 °C, about 37 °C, about 38 °C, about 39 °C, about 40 °C, about 41 °C, about 42 °C, about 43 °C, about 44 °C, or about 45 °C.

In some embodiments, setting the treated fiber comprises winding the treated fiber and oxidizing the treated fiber.

In some embodiments, exposing the treated fiber to a wash solution comprising a surfactant is performed prior to winding the treated fiber and oxidizing the treated fiber. In some embodiments, winding the treated fiber is at a rate of about 15 meters/minute.

In some embodiments, the keratin fiber is dried at about 85 °C for about 1 hour. In some embodiments, the keratin fiber is annealed at about 125 °C for about 1 hour. In some embodiments, the keratin fiber is annealed after it is dried.

In some embodiments, the process further comprises exposing the keratin fiber to a solution comprising an oxidized saccharide. Non-limiting examples of suitable saccharides include glucose, sucrose, raffinose, cellobiose, dextran, and alginate. A saccharide can be oxidized using any of the oxidants described herein. In some embodiments, the keratin fiber is exposed to the solution comprising an oxidized saccharide for about 3 to about 25 hours. For about 3 to about 5, about 3 to about 10, about 3 to about 15, about 3 to about 25, about 20 to about 25, about 15 to about 25, about 10 to about 25, about 5 to about 25. In some embodiments, the keratin fiber is exposed to the solution comprising an oxidized saccharide for about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 15 hours, or about 20 hours. In some embodiments, the oxidized saccharide is a sucrose polyaldehyde.

In some embodiments, the step of exposing the keratin fiber to a solution comprising an oxidized saccharide is performed prior to exposing the treated fiber to a wash solution comprising a surfactant.

A keratin fiber produced using the processes described herein can have a high draw ratio. The draw ratio is the ratio of the drawing rate and extrusion rate. In some embodiments, the keratin fiber can have a draw ratio of at least about 500%, e.g., at least about 600%, about 700%, about 800%, about 900%, about 1000%, about 1100%, about 1200%, about 1300%, about 1400%, about 1500%, about 1600%, about 1700%, about

1800%, about 1900%, or about 2000%. In some embodiments, a keratin fiber produced using the processes described herein can have a draw ratio of about 500% to about 2500%. For example, about 500% to about 2300%, about 500% to about 2100%, about 500% to about 1900%, about 500% to about 1700%, about 500% to about 1500%, about 500% to about 1300%, about 500% to about 1100%, about 500% to about 900%, about 500% to about 700%, about 2300% to about 2500%, about 2100% to about 2500%, about 1900% to about 2500%, about 1700% to about 2500%, about 1500% to about 2500%, about 1300% to about 2500%, about 1100% to about 2500%, about 900% to about 2500%, or about 700% to about 2500%. In some embodiments, a keratin fiber produced using the processes described herein can have a draw ratio of about 500%, about 600%, about 700%, about 800%, about 900%, about 1000%, about 1100%, about 1200%, about 1300%, about 1500%, about 1600%, about 17000%, about 1800%, about 1900%, about 2000%, about 2100%, about 2200%, about 2300%, about 2400%, or about 2500%.

In some embodiments, a keratin fiber produced using the processes described herein can have a diameter of about 5 micrometers to about 30 micrometers. For example, about 5 micrometers to about 25 micrometers, about 5 micrometers to about 20 micrometers, about 5 micrometers to about 15 micrometers, about 5 micrometers to about 10 micrometers, about 25 micrometers to about 30 micrometers, about 20 micrometers to about 30 micrometers, about 15 micrometers to about 30 micrometers, or about 10 micrometers to about 30 micrometers.

Several processes can be used to assess the tenacity and strain of a fiber as described herein. Non-limiting examples of such processes include the ASTM standard D-3822 and the ISO 5079:1995. In some embodiments, the keratin fiber is equilibrated at 21 °C and 65% relative humidity for 24 h prior to the test. In some embodiments, the gauge length and extension speed are 1 inch and 18 mm/min, respectively.

In some embodiments, a keratin fiber produced using the processes described herein can have a tenacity at least about 0.8 g/den. For example, about at least about 1 g/den, about 1.2 g/den, or about 1.4 g/den. In some embodiments, a keratin fiber produced as described herein can have a tenacity of about 0.8 g/den to about 2.5 g/den. For example, about 0.8 g/den to about 2.4 g/den, about 0.8 g/den to about 2.2 g/den, about 0.8 g/den to

about 2.0 g/den, about 0.8 g/den to about 1.8 g/den, about 0.8 g/den to about 1.6 g/den, about 0.8 g/den to about 1.4 g/den, about 0.8 g/den to about 1.2 g/den, about 0.8 g/den to about 1 g/den, about 2.4 g/den to about 2.5 g/den, about 2.2 g/den to about 2.5 g/den, about 2 g/den to about 2.5 g/den, about 1.8 g/den to about 2.5 g/den, about 1.6 g/den to about 2.5 g/den, about 1.4 g/den to about 2.5 g/den, about 1.2 g/den to about 2.5 g/den, about or about 1 g/den to about 2.5 g/den.

In some embodiments, the keratin fiber has a strain of at least about 5%. For example, at least about 5%, at least about 6%, at least about 7%, or at least about 8%. In some embodiments, the keratin fiber has a strain of about 10% to about 30%. For example, about 10% to about 15%, about 10% to about 20%, about 10% to about 25%, about 25% to about 30%, about 20% to about 30%, or about 15% to about 30%. In some embodiments, the keratin fiber has a strain of 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, or about 20%. In some embodiments, the keratin fiber has been dried prior to measuring the strain.

Toughness of a keratin fiber described herein can be determined by measuring total area under the stress-strain curve of keratin fibers. Any method that can obtain a stress-strain curve can be used. In some embodiments, the keratin fiber is equilibrated at 21 °C and 65% relative humidity for 24 h prior to the test. In some embodiments, the gauge length and extension speed are 1 inch and 18 mm/min, respectively. In some embodiments, the keratin fiber has a toughness of at least about 15 J/cm^3. For example, at least about 20 J/cm^3, or at least about 25 J/cm^3. In some embodiments, the keratin fiber has a toughness of about 15 J/cm^3 to about 30 J/cm^3.

Beta-sheet crystallinity of a keratin fiber and/or keratinous material can be determined using, for example, X-ray diffraction. In some embodiments, the keratin fiber comprises at least 85% of the beta-sheet crystallinity compared to the amount of beta-sheet crystallinity in the keratinous material.

Disulfide bonds in a keratin fiber and/or keratinous material can be determined using, for example, using Raman spectroscopy. In some embodiments, the keratin fiber comprises at least 70% of the disulfide crosslinkages compared to the amount of disulfide crosslinkages in the keratinous material.

Also provided herein are keratin fibers prepared by any of the processes described herein. For example, provided herein is a keratin fiber prepared by extruding a keratin solution into a first solution to form a first fiber; drawing the first fiber and oxidizing the first fiber to form a treated fiber; drawing the treated fiber and oxidizing the treated fiber one or more times; and setting the treated fiber to form the keratin fiber. In some embodiments, the process is a continuous process. In some embodiments, provided herein is a keratin fiber prepared by extracting keratin from a keratinous material to form extracted keratin; dissolving the extracted keratin in an aqueous solution comprising a reducing agent to form the keratin solution; extruding a keratin solution into a first solution to form a first fiber; drawing the first fiber and oxidizing the first fiber to form a treated fiber; drawing the treated fiber and oxidizing the treated fiber one or more times; and setting the treated fiber to form the keratin fiber.

Additional details of the embodiments provided herein can be found, for example, in references 39 and 40, both of which are incorporated by reference in their entireties herein.

EXAMPLES

Example 1. Continuous production of keratin fiber from chicken feathers

Materials

Feather fiber corporation, Nixa, CO provided the chicken feather barbs. Other chemicals, with ACS reagent grade, such as sodium dodecyl sulfate (SDS), cysteine, mercaptoethnaol and urea, were purchased from VWR International (Radnor, PA). Chemical reagents used in SDS-PAGE analysis, including LDS sample buffer (4×), Nupage 20× MES running buffer and NuPAGE 4–12% Bis-Tris gel, were purchased from Invitrogen, Inc., Grand Island, NY.

Keratin extraction from chicken feathers

The extraction of keratin from chicken feathers was conducted using an aqueous solution containing different amounts of urea and SDS. To determine the optimal recipe,

viscosity of supernatant of extraction and keratin yield were measured. Detailed results are in **Table S1**. 2 M urea and 10% SDS based on weight of feathers were chosen. 10% of cysteine was used to fully cleave the disulfide bonds in feathers for optimal keratin dissolution. According to the previous work, the pH of the extraction solution was adjusted to 10.5 using 15 wt% NaOH solution. The extraction was held for 12 hours at 70 °C. After the extraction, the dispersion was centrifuged at 9800 rcf for 20 minutes to get the supernatant, which was adjusted to the isoelectric point using hydrochloric acid accompanied by sodium sulfate to precipitate the keratin inside. The precipitated keratin was further washed to remove other impurities before being vacuumed dried.

Table S1. Viscosity and keratin yield from various extraction systems

Extraction systems	Specific viscosity of supernatant	Yield
8M urea	2.52	68.64±2.52%
4M urea	2.14	58.40±3.42%
10% SDS	1.46	31.20±2.51%
2M urea 5%SDS based on feather	1.72	48.50±2.43%
2M urea 8%SDS based on feather	2.01	61.80±3.56%
2M urea 10%SDS based on feather	2.52	66.90±3.14%
2M urea 15%SDS based on feather	2.73	67.14±2.97%
2M urea 20%SDS based on feather	3.04	67.51±3.25%

Preparation of keratin spinning dope

Spinning dope for continuous pilot-scale spinning was prepared by dissolving 27% extracted keratin and 10% SDS based on weight of keratin in 0.2 M carbonate-bicarbonate

buffer at pH of 8. To fully dissolve keratin and obtain the optimal molecular entanglement in solution, different amounts of a reducing agent (mercaptoethanol), were added for controlled cleavage of disulfide bonds in keratin molecules.

Stepwise oxidation and drawing were applied to continuous wet spinning lines (ALEX JAMES AND ASSOC, US) for controlled assembly of disulfide bonds in keratin fibers. Stepwise oxidation and drawing can help improve the spinnability, keratin molecular alignment, degree of crosslinkages and fiber properties. Detailed design is demonstrated in **FIG. 1**. Prepared keratin spinning dope was centrifuged before being installed onto the spinning line. Keratin solution was extruded via a spinneret with multiple 50 μm-diameter holes to the coagulation bath containing 15 wt% sodium sulfate, 5 wt% zinc sulfate and acetate buffer with pH 2. Fibers from the coagulation bath were drawn for the first time before entering the first oxidation bath. The oxidation bath contained 4 g/L sodium periodate as the oxidation agents and acetate buffer with pH 2. Oxidation temperature was 35℃ to ensure the fast disulfide bond assembly and fine fiber stretchability. Fibers then went through multiple drawings and oxidations before going to the washing bath. Multiple oxidation and drawing steps can help the establishment of disulfide bonds and ordered structures in keratin fibers. The washing bath contained surfactants with concentrations from 1 g/L and acetate buffer with a pH of 2. The temperature was 40 °C to ensure high washing efficiency. Reaching the final winding roller, the fibers went through the oxidation bath once again for the immobilization of ordered molecular structures in keratin fibers. The final speed of fiber collecting was 15 meters/min. The dry fibers obtained were dried in an oven at 85 °C for 1 h and later annealed at 125 °C for about 1 h. Annealing was done to improve the mechanical properties of the fibers.

Characterizations

Rheological properties of spinning dope

Shear stress (τ, Pa) of keratin spinning dopes with various concentrations of reducing agents was measured using a rotational rheometer, R/S plus (Brookfield, U.S.A.) for determination of consistency coefficient (K, $Pa \cdot s^n$) and flow behavior index (n) based

on Equation 1. K is directly proportional to polymer viscosity in solution and n indicates the degree of molecular entanglement in solution. The smaller the value of n, the better the molecular entanglement. To precisely measure the rheological properties, 18 wt% of keratin solution was used.

$$\tau=K\dot{\gamma}^{n} \quad \text{Equation 1}$$

where $\dot{\gamma}$ is the shear rate (s^{-1}) measured in the range of 0-1000 s^{-1}.

Molecular weight of keratin backbones

About 1 mg of feather and keratin fiber was dissolved in 100 μL of NuPAGE LDS sample buffer (1 ×) with excess mercaptoethanol, heated at 70 °C for 5 h. The solution was centrifuged prior to loading. Each sample of 10 μL was loaded into an individual slot of the gel. A molecular marker from Spectra Multicolor Low Range Protein Ladder was used. The molecular weights of the protein standard mixture ranged from 4.6 to 42 kDa.

Mechanical properties

Keratin fibers were conditioned at 21 °C and 65% relative humidity for 24 h prior to tests. Tensile properties of keratin fibers were obtained according to ASTM standard D-3822 using an Instron tensile testing machine (Norwood, MA). Gauge length set for testing was 1 inch and crosshead speed was 18 mm/min. Denier of fibers was used to describe the fineness of keratin fibers. For each test, at least 20 specimens were used.

Qualitative measurement of disulfide bonds in keratin using Raman Spectroscopy

Feathers and keratin fibers were characterized on a Raman spectrometer (The DXR Raman microscope, Thermo, USA). The laser wavelength was set at 532 nm with a power of 10 mW. The sample collect exposure time was 15 seconds with 15 cycles of exposures per sample. To compare the disulfide bonds in keratin, the ratio of peak areas around 500 cm^{-1} (S-S) and 1450 cm^{-1} (C-H) was used.

Quantification of cystine in fibers

Fibers collected from each step of continuous spinning line were frozen immediately after washing in distilled water and then freeze-dried. The cystine content in fibers was determined based on the process developed by Campanella et al [34]. In detail, dried fibers were hydrolyzed using 6 N HCl under 110 °C for 24 hours to obtain amino acids. Phenylisothiocyanate was used for pre-column quantitative derivatization of amino acids by a HPLC, UltiMate 3000 series, USA equipped with a C-18 column (Acclaim 120, 120 Å, 4.6 × 250 mm, 5 μm) and UV detector with wavelength set at 254 nm. The flow rate was 1 mL/min and a ternary gradient was employed using 0.7 M sodium acetate with pH 6.4 (phase A), water (phase B) and acetonitrile/water with volume ratio of 8:2 (phase C). The gradient is shown in **Table 1**. Total retention time was 30 min with additional 10 min for column re-equilibration.

Table 1. Gradient for HPLC analysis

Time	Phase A	Phase B	Phase C
0	20	75	5
25	20	30	50
26	10	10	80

We used mercaptoethanol as the reducing agent in the spinning dope, therefore, newly formed cystine on regenerated fibers could be considered as intra or inter molecular crosslinkings. In addition, cystine was formed during the fiber drawing. Drawing could increase the degree of linearity of molecular chains. Therefore, majority of cystine came from intermolecular crosslinking.

Analysis of secondary structures

X-ray diffraction and solid ^{13}C NMR studies were carried out for secondary structure analysis of feathers and keratin fibers. X-ray diffraction was obtained using a Rigaku D/Max-B X-ray diffractometer with Bragg–Brentano parafocusing geometry, diffracted beam monochromator, and conventional copper target X-ray tube set (λ = 1.54 Å) to 40 kV and 30 mA at 26 °C. Diffraction intensities were recorded with 2θ ranging

from 3° to 40° at a scan speed of 0.05° per second. The degree of crystallinity was calculated using Jade 6.0 software (Materials Data Incorporated: Livermore, CA, USA) with Gaussian peak fittings. The ^{13}C solid-state NMR spectra were obtained using a triple-resonance (1H/13C/15N) magic angle spinning probe (3.2 mm) was equipped on the NMR spectrometer (Bruker, Avance 600, USA).

Statistical analysis

One-way analysis of variance using Scheffe test with a confidence interval of 95% was used for all obtained data. The statistical analysis was conducted on SAS 9.4 software (Cary, North Carolina) and survey procedures of PROC GLIMMIX.

Results and discussion

FIG. 2 compares molecular weights of protein backbones from regenerated keratin and the chicken feather. The results show that the damages to backbone of regenerated keratin is minimized. Compared to the chicken feather, contents of proteins with 21 kDa of regenerated keratin were slightly lower than that of the chicken feather while contents of protein with molecular weight from 8 kDa and12 kDa were higher than those of chicken feathers. The results in **FIG. 2** also indicate that the regenerated keratin contained a high amount of γ-keratin, which has a molecular weight of 11 kDa [35] and contains high sulfur content. γ-keratin would facilitate formation of disulfide bonds in the process of fiber regeneration. The above results indicate that molecular structures in keratin fibers could be optimized via controlled cleavages and assembly of disulfide bonds on continuous production line. As a result, the final properties of keratin fibers were improved.

Table 2. Rheological properties of 18% w/w keratin solution at 25 °C

Concentration of reducing agent (wt% based on keratin)	K value ($Pa•s^n$)	n value
0.5	18.90	0.97

1	14.23	0.97
2	4.19	0.91
3	1.50	0.95
4	1.31	0.96

K is the consistency coefficient, directly proportional to polymer viscosity. n is the flow behavior index. The smaller value of n indicates the better molecular entanglement.

Table 2 shows the effect of different concentrations of reducing agents on the rheological properties of keratin spinning dope. The results show that cleavage degrees of disulfide linkages in the spinning solution directly affect the viscosity and entanglement of the keratin molecule in the solution. Specifically, as the concentration of the reducing agent in dope increased, the viscosity of the solution gradually decreased, but the degree of molecular entanglement increased first and then decreased. Increase in concentrations of reducing agent led to cleavage of disulfide bonds. As a result, as the molecular weight of keratin gradually decreased, so did the viscosity. In terms of degrees of molecular entanglement, when the amount of the reducing agent increased from 0.5% to 2%, most ordered structures became uncoiled as the cleavage of disulfide bonds, leading to the better solubility of keratin molecules. Therefore, the entanglement of molecules increased. When the concentration of the reducing agent continued to rise, the molecular weight was further lowered while the solubility of keratin remained unchanged. As a result, degrees of molecular entanglement decreased.

In order to ensure better spinnability of keratin fibers on a continuous spinning line, the disulfide bonds in keratin should be partially retained because of the following three benefits: 1) ensuring that the protein still has good solubility; 2) ensuring that the protein molecules are most entangled; and 3) keeping the molecular weight of the protein high enough to allow the spinning dope to be solidified rapidly in the coagulation bath. Therefore, 2% reducing agent was selected to partially cleave the disulfide linkages in spinning dope.

FIG. 3 shows the efficient recovery of disulfide bonds in keratin fibers via controlled disulfide bond assembly on a continuous spinning line and resultant spinnability

of keratin fibers. The results show that the rapid establishment of disulfide crosslinkages in continuous spinning is an important factor ensuring the good spinnability of keratin fiber. **FIG. 3A** qualitatively compares disulfide bonds in keratin fibers and chicken feathers. Results show that keratin fibers recovered high degree of disulfide crosslinkages. The peaks at about 500 and 1450 cm^{-1} were considered to be S–S bonds and C–H bonds, respectively. The Raman spectra were normalized according to the C–H band, whose peak area was relatively large and not affected by the chemical treatments. Keratin fibers had an intensity of S–S band slightly lower than chicken feathers, demonstrating high recovery of disulfide crosslinkages. Disulfide linkages were quantified using HPLC. **FIG. 3B** shows that less than 10% of the crosslinkage bonds in fibers were recovered by stretching in the coagulation bath. After initial drawing and introduction of the first oxidation bath, nearly 20% of the disulfide bonds in the fiber were recovered. As further introductions of oxidation baths accompanied by constant fiber drawing, the degree of disulfide linkages recovery could further increase. When fibers reached the final collection roller on a spinning line, nearly 70% of the disulfide bonds have been recovered, with a degree of crosslinking of about 5%. The rapid and high recovery in degrees of disulfide linkages in the continuous spinning process ensured good fiber spinnability. As shown in **FIG. 3C**, the spinnability of the fibers was substantially higher via fine control of disulfide bond assembly than that via non-control and simple control of disulfide bond assembly. The only oxidation process for non-control of disulfide bond assembly is the air oxidation. Simple control only contained single-step oxidation. Via controlled disulfide bond assembly, the final collection speed of the fiber can reach 15 m / min, 160% of spinning using simple control of disulfide bond assembly, 300% of spinning with the air oxidation.

FIG. 4A shows that recovery of degree of crosslinkages in the keratin fibers determined the maximum draw ratio on the continuous spinning line. The results show that the high disulfide linkages recovery was the key to high drawing ratios of fiber. A high drawing ratio is useful for producing high-quality fibers. As shown in **FIG. 4A**, when the degree of the disulfide bond recovery was less than 10%, the drawing ratio of the fiber was only 2 times. As recovery degree of disulfide bonds increased, the maximum draw ratio of the fiber can be increased to 10 times. **FIG. 4A** also shows that draw ratio of fibers on

continuous spinning line had a linear relationship with the degree of recovery of the disulfide bond. Specifically Maximum drawing ratio = 0.2* Disulfide bond recovery ratio + 0.5 with R^2 0.98. The increase in draw ratio substantially reduced the diameter of spun fibers. As shown in **FIG. 4B**, fibers after 10 times of drawing had diameter of only 15 μm, which is lower than most natural wool fibers (30) and slightly larger than silk fibers (10). The fine fibers ensure a good hand, breathability and dyeability. The results further indicate that continuous production of quality keratin fibers was heavily dependent on the rapid formation of crosslinkages via controlled disulfide bond assembly. Without controlled disulfide bond assembly, the recovery degree of the disulfide bond was not more than 20%, resulting in a limited fiber draw ratio and subsequently poor fiber properties.

FIG. 5 is the description of how high degree of ordered protein structures was formed in keratin fibers via controlled disulfide bond assembly under external stretch force. The distance between protein backbones in newly solidified fiber can be reduced to some extent because of the existence of limited disulfide linkages inside. The reduced distance between protein backbones can facilitate the formation of intermolecular disulfide bond linkages during the first oxidation process. Then the formed crosslinkages in oxidation bath can help to improve the fiber stretchability and drawing ratios. High draw ratio can contribute to the linearity of molecular chains in keratin fibers and the decrease in distance between protein backbones. In turn, formation of intermolecular disulfide bonds was further facilitated. Via the controlled cleavage and assembly of disulfide bonds, intermolecular disulfide crosslinkages were gradually increased and secondary structures were gradually recovered in keratin fibers. At the last step of continuous spinning, collection step, fibers were once again oxidized to immobilize ordered structures of keratin.

Recovered secondary structure in keratin fibers

Table 3. Comparison of secondary structures between chicken feather and keratin fibers

Materials	Degree of crystallinity		
	Total crystallinity	Portion of α-helix	Portion of β-sheet
Chicken feathers	31%	11%	20%

Keratin fibers	24%	5%	19%

The data obtained from XRD and ^{13}C solid NMR. These spectra are shown in **FIG. 8A** and **8B**, respectively. In XRD, two peaks, a minor peak at around 9° and a major peak at around 19° are used to analyze the secondary structure. In NMR, the crystalline structures of chicken feathers and keratin fibers were analyzed using the chemical shift of carbonyl groups. The deconvolution of carbonyl groups usually results in two peaks at 176 ppm, attributed to α-helix and 172 ppm, attributed to both random coil and β-sheet conformations.

Table 3 compares the secondary structure of keratin fibers with chicken feathers. The results showed the beta-sheet secondary structure and total crystallinity of keratin fibers recovered 95% and 80%, respectively. The high crystallinity in keratin fiber is due to the high degree of ordered structures resulting from fast controlled disulfide bonds cleavages and assembly. The reason for high degree of beta-sheet recovery is as below. Controlled disulfide assembly contributed to the high stretchability of fibers and increase in the fiber drawing ratios. As a result, a portion of the alpha-helix structures in the fiber transformed into beta-sheet structures. The degree of crystallinity in keratin fiber was lower than that of the original chicken feathers mainly because the disulfide bond in the chicken feathers cannot be completely recovered, and the ordered structures in keratin fibers were less than those in chicken feathers. In addition, slight damages on keratin backbone also contributed to the lower degree of crystallinity in keratin fibers.

FIG. 6A shows the continuous production of keratin fiber was achieved via controlled cleavage and assembly of disulfide bonds. **FIG. 6B** shows a specimen of continuously spun keratin fibers. **FIG. 7A** compares stress-strain curves of the original feathers and keratin fibers from continuous spinning. The results show that although the feather barbs had a strain of about 10%, the feather barbs demonstrated a curve of brittle pattern. However, keratin fibers underwent a “strain hardening” stage before break. The reason is that chicken feathers had a high degree of cross-linkages and ordered molecular structure, providing a strong interaction between intermolecular chains. Therefore, under external forces, the molecular segment was unlikely to have dislocation. As a result, fibers

broken before yielding point. In keratin fibers, interaction between molecular chains was relatively weak but most of the protein backbones were connected via controlled recovery of disulfide crosslinkages. Weak interaction can help the movement of protein chains while long molecular chains could help increase the slip distance between two molecular chains. Under the external force, dislocation was likely to happen between molecular segments, resulting in the strain hardening. In addition, the keratin fibers were through high drawing ratios, leading to the molecular chains in stretched state. Therefore, the degree of molecular entanglement was high. Under the external force, the overall orientation of the molecular segment in fibers would increase, contributing to the strain hardening.

Keratin fibers possessed high ductility due to the strain hardening process. **FIG. 7B** shows that keratin fibers can endure a high degree of twisting. The other two Supporting videos further demonstrate the high ductility of keratin fiber. The fiber ductility was high because of the high toughness of the fiber, stemming from substantial restoration of the secondary protein structures via controlled disulfide bond cleavage and assembly in the continuous spinning process.

Table 4. Mechanical properties of keratin fibers compared to other common fibers.

Fiber source	Dry state			Wet state		
	Strength (Mpa)	Strain	Toughness (J/cm^3)	Strength (Mpa)	Strain	Toughness ($J.cm^3$)
Feather barbs	161±27.5	9.7±3.3%	21±2	127±24.5	18±3%	31.5±3
Keratin fibers	**138±30.5**	**11±2.8%**	**18.5±2**	**81±23**	**25±3%**	**28.7±4**
Wool	173±23	36±5%	32±5	140±22	46±5%	36±4
Cotton[13]	420±46	6.2±1.5%	10.5±5	472±34	9±1%	24.6±6
Line[13]	700±45	3.1±0.4%	5±1	800±40	5±1%	7±1
Viscose[13]	276±20	21±5.2%	24±6	120±20	25±4%	21.1±4.3

Our work is shown in **bold** font.

Table 4 shows the mechanical properties of the fibers with restored secondary protein structures via controlled cleavage and assembly of disulfide bonds in the continuous production process and compares properties with other commonly fibers. The results show that keratin fibers recovered 86% of stress properties at dry state, 64% of wet stress, 89% of dry toughness and 91.55 of wet toughness of original chicken feathers. The good properties of the feathers were preserved. Keratin fibers with restored secondary structures had a slightly lower strength than feather barbs because of damages of the protein backbone and a decrease in the degree of disulfide crosslinkages. The strain of keratin fibers was slightly higher than the original feathers because of easier dislocation of the protein segments. Compared with other commonly used fibers, keratin fibers had merits. For example, keratin strain and toughness were substantially higher than cotton and linen. The toughness was close to viscose fibers. The above results show that keratin fibers with restoration of secondary structures from continuous production meet the specificaitons for actual uses.

Table 5. Comparison of properties of regenerated keratin fibers developed from various approaches.

Approach to regeneration	Properties recovery	Continuous production on pilot scale	Reference
Controlled disulfide cleavage and assembly	**86% of tenacity 113% of strain**	**Yes**	**This work**
Control of disulfide cleavages	48% of tenacity 41% of strain	No	[28]
Regeneration from ionic liquids	14% of tenacity Strain not reported	No	[36]
Applied glycerol as plasticizer	4% of tenacity 280% of strain	No	[37]

Blend poly(ethylene oxide) (containing 10% keratin)	3% of tenacity 1100% of strain	No	[38]

Property recovery based on the original keratin materials, such as feathers and wool.

Table 5 compares the properties of keratin fibers from various regeneration approaches. All keratin fibers except this work were regenerated on a lab-scale. Results show that due to the low recovery of secondary structures, tenacity recovery was low, with the highest was less than 50%. With poor recovery of secondary structure, the strain of regenerated fibers was even poor. To increase the strain, incorporation of plasticizer or other polymers into keratin was developed. As a result, the strain increases at the cost of sacrifice of fiber tenacity. For example, after incorporation of glycerol, the tenacity of regenerated keratin fibers was only 4% of raw fibers.

Cost-effective production with minimal environmental impact

Continuous fiber production via controlled cleavage and assembly of disulfide bonds used non-toxic chemicals. Furthermore, baths such as coagulation and oxidation on spinning lines could be reused. Therefore, the discharges of continuous spinning have minimal discharges. Keratin content in continuous spun fibers was higher than 98%, indicating the full degradability of fibers. Our process to produce keratin fibers from chicken feathers does not contain toxic chemicals that impose negative impacts on the environment. In order to estimate market potential of the regenerated keratin fibers, the material consumption and costs in our process are assessed, as shown in **Table S2.**

Table S2. Material cost to produce 1kg of pure keratin fibers

	Materials & Chemicals	Unit price ($/kg) †	Consumption to produce 1 kg of keratin fibers (kg)	Cost ($)	Total material cost ($)
Extraction §	Chicken feathers	0 ¶	1.33	0	0.83
	Cysteine	5.5	0.09	0.495	

	Urea & SDS	0.39	0.28	0.11	
	Alkali	0.2	0.20	0.040	
	Hydrochloric acid (31%)	0.10	0.07	0.007	
	Sodium sulfate	0.06	0.08	0.005	
Spinning	Sodium carbonate	0.13	0.016	0.002	
	Sodium dodecyl sulfate	0.9	0.05	0.045	
	Mercaptoethanol	4	0.02	0.08	
	Acetic acid	0.2	0.02	0.004	
	Sodium sulfate	0.17	0.15	0.003	
	Zinc sulfate	0.62	0.05	0.03	
	Oxidants	0.8	0.004	0.003	
	Surfactant	0.3	0.001	0.003	

§ Calculation is based on our previous urea-cysteine based extraction method

† All the prices of chemicals were obtained from Alibaba.com or 1688.com (accessed on 9/30/2019).

¶ Chicken feathers are deemed as wastes and thus could be obtained at no cost.

As shown in **Table S2**, the total material cost to produce 1 kg of pure keratin fibers is about $0.83. Since it is unable for us to obtain the material cost of commercial protein fibers and compare with that of the regenerated keratin fibers, the retail prices of commercial proteins such as wools and silk are used. Comparing to the bulk price (metric ton scale) of wool at about $7-30/kg and silk at about $45-80/kg, keratin fibers from chicken feathers have its cost at least 91% and 99% lower than sale prices. Considering

other costs in large-scale production, the final price of keratin fibers from chicken feathers will be competitive. If keratin fibers from poultry feathers are sold at about $4 per kg, which is close to some natural cellulose fibers like linen, a ton of poultry feathers will produce fibers worth at least $3,000. If the 5 million tons of poultry feathers worldwide can be fully exploited, the market value of regenerated keratin fibers will exceed $15 billion.

Example 2. Chemical crosslinking using oxidized saccharides

Five percent of sucrose in water reacted with sodium periodate at room temperature for 5 h. The molar ratio of sucrose to periodate was 1:3. The pH of reaction medium was kept at 5.5 ±0.1. After the reaction, slightly excessive barium dichloride was added to completely precipitate the oxidation agents. The mixture was filtrated to obtain the polyaldehyde derivatives of sucrose. Spun fibers from chicken feathers were dipped in solutions containing sucrose polyaldehydes for 5 h at room temperature before the washing process. After washing, fibers were dried in an oven at 85 °C for 1 h and later annealed at 125 °C for about 1 h. The stress and strain of obtained fibers were 1.5±0.2 g/den and 16±2.1%.

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OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention which is defined by the scope of the appended claims. Other aspects, advantages, and modification are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A process for preparing a keratin fiber, comprising:
   (i) extruding a keratin solution into a first solution to form a first fiber;
   (ii) drawing the first fiber and oxidizing the first fiber to form a treated fiber;
   (iii) drawing the treated fiber and oxidizing the treated fiber one or more times; and
   (iv) setting the treated fiber to form the keratin fiber.

2. The process according to claim 1, wherein the keratin fiber has a draw ratio of at least about 500%.

3. The process according to claim 1 or 2, wherein the keratin fiber has a draw ratio of about 800% to about 2000%.

4. The process according to any one of claims 1-3, wherein the keratin fiber has a draw ratio of about 1500%.

5. The process according to any one of claims 1-4, wherein the diameter of the keratin fiber is about 5 micrometers to about 30 micrometers.

6. The process according to any one of claims 1-5, wherein the diameter of the keratin fiber is about 15 micrometers.

7. The process according to any one of claims 1-6, wherein the keratin fiber comprises at least about 70% keratin.

8. The process according to any one of claims 1-7, wherein the keratin fiber comprises at least about 85% keratin.

9. The process according to any one of claims 1-8, wherein the keratin fiber has a tenacity of at least about 0.8 g/den.

10. The process according to any one of claims 1-8, wherein the keratin fiber has a tenacity of about 1 g/den to about 2 g/den.

11. The process according to any one of claims 1-10, wherein the keratin fiber has a strain of at least about 5%.

12. The process according to any one of claims 1-10, wherein the keratin fiber has a strain of about 5% to about 30%.

13. The process according to any one of claims 1-10, wherein the keratin fiber has a strain of about 15%.

14. The process according to claim 13, wherein the keratin fiber has been dried prior to measuring the strain.

15. The process according to any one of claims 1-14, wherein the keratin fiber has a toughness higher than about 15 J/cm^3.

16. The process according to any one of claims 1-15, wherein the keratin solution has a consistency coefficient (K) of about 2 $Pa \cdot s^n$ to about 6 $Pa \cdot s^n$, wherein the keratin solution is at about 25 $^{\circ}C$ and comprises keratin at about 18% w/w of the composition.

17. The process according to any one of claims 1-16, wherein the keratin solution has a consistency coefficient (K) of about 4.2 $Pa \cdot s^n$, wherein the keratin solution is at about 25 $^{\circ}C$ and comprises keratin at about 18% w/w of the composition.

18. The process according to any one of claims 1-17, wherein the keratin solution has a flow behavior index of about 0.8 to about 0.95, wherein the keratin solution is at about 25 °C and comprises keratin at about 18% w/w of the composition.

19. The process according to any one of claims 1-18, wherein the keratin solution has a flow behavior index of about 0.91, wherein the keratin solution is at about 25 °C and comprises keratin at about 18% w/w of the composition.

20. The process according to any one of claims 1-19, wherein the keratin solution comprises a reducing agent.

21. The process according to any one of claims 1-20, wherein the keratin solution comprises sodium dodecyl sulfate (SDS).

22. The process according to any one of claims 1-21, wherein the keratin solution is prepared from a keratinous material.

23. The process according to claim 1, further comprising preparing the keratin solution.

24. The process according to claim 23, wherein preparing the keratin solution comprises:

i. extracting keratin from a keratinous material to form extracted keratin; and
ii. dissolving the extracted keratin in an aqueous solution comprising a reducing agent to form the keratin solution.

25. The process according to claim 24, wherein the aqueous solution further comprises SDS.

26. The process according to any one of claims 22, 24, and 25, wherein the keratinous material comprises one or more of: animal hair, horn, and feather including but not limited to wool, camel hair, alpacas hair and rabbit hair.

27. The process according to claim 26, wherein the feather is a duck feather, a goose feather, a chicken feather, or a combination thereof.

28. The process according to claim 26, wherein the hair is wool, camel hair, alpaca hair, rabbit hair, or a combination thereof.

29. The process according to any one of claims 23-28, wherein the keratin fiber comprises at least 70% of the disulfide crosslinkages compared to the amount of disulfide crosslinkages in the keratinous material.

30. The process according to any one of claims 23-29, wherein the keratin fiber comprises at least 85% of the beta-sheet crystallinity compared to the amount of beta-sheet crystallinity in the keratinous material.

31. The process according to any one of claims 18 and 24-30, wherein the reducing agent comprises a thiol group.

32. The process according to claim 31, wherein the reducing agent comprises mercaptoethanol, cysteine, dithiothreitol, 1,2-ethanedithiol, 1,3-benzenedithiol, bis(2-mercaptoethyl) ether, ethylene glycol bisthioglycolate, or a combination thereof.

33. The process according to any one of claims 1-32, wherein the step of extruding a keratin solution into a first solution comprises using a spinneret to extrude the keratin solution.

34. The process according to claim 33, wherein the spinneret comprises a hole, wherein the hole has a diameter of about 50 micrometers.

35. The process according to any one of claims 1-34, wherein the first solution comprises an electrolyte.

36. The process according to claim 35, wherein the electrolyte is a sulfate, an acetate, a chloride, a citrate, a carbonate, a phosphate, or a combination thereof.

37. The process according to any one of claims 1-36, wherein the first solution comprises sodium sulfate, zinc sulfate, and acetate buffer.

38. The process according to any one of claims 1-37, wherein the pH of the first solution is about 2.

39. The process according to any one of claims 1-38, wherein the first solution comprises sodium sulfate in an amount of about 15% w/w of the composition, zinc sulfate in an amount of about 5% w/w of the composition, and acetate buffer with a pH of 2.

40. The process according to any one of claims 1-39, wherein the step of oxidizing comprises exposing the fiber to an oxidizing solution comprising an oxidant selected from the group consisting of: a peroxide, a halogen oxoacid or salt thereof, a high-valent metal salt, and a combination thereof.

41. The process according to claim 40, wherein the peroxide is an alkali metal peroxide, an alkaline earth metal peroxide, or a combination thereof.

42. The process according to claim 41, wherein the oxidant is sodium periodate.

43. The process according to any one of claims 40-42, wherein the oxidizing solution further comprises a buffer.

44. The process according to any one of claims 40-43, wherein the oxidizing solution further comprises acetate buffer.

45. The process according to any one of claims 40-44, wherein the pH of the oxidizing solution is about 2.

46. The process according to any one of claims 40-45, wherein the temperature of the oxidizing solution is about 35 °C.

47. The process according to any one of claims 1-46, wherein the step of drawing the treated fiber and oxidizing the treated fiber is repeated two times.

48. The process according to any one of claims 1-47, wherein the process further comprises drawing the treated fiber prior to setting the treated fiber.

49. The process according to any one of claims 1-48, wherein setting the treated fiber comprises exposing the treated fiber to a wash solution comprising a surfactant.

50. The process according to claim 49, wherein the surfactant is selected from the group consisting of: ammonium lauryl sulfate, SDS, sodium laureth sulfate, sodium myreth sulfate, sodium stearate, sodium lauroyl sarcosinate, perfluorononanoate, perfluorooctanoate, (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate), cocamidopropyl hydroxysultaine, cocamidopropyl betaine, phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine, a sphingomyelin, cetrimonium bromide (CTAB), cetylpyridinium chloride (CPC), benzalkonium chloride (BAC), benzethonium chloride (BZT), dimethyldioctadecylammonium chloride, dioctadecyldimethylammonium bromide (DODAB), and a combination thereof.

51. The process according to claim 49 or 50, wherein the wash solution further comprises an acetate buffer.

52. The process according to any one of claims 49-51, wherein the wash solution has a pH of about 2.

53. The process according to any one of claims 49-52, wherein the wash solution is at a temperature of about 40 °C.

54. The process according to any one of claims 1-53, wherein setting the treated fiber comprises winding the treated fiber and oxidizing the treated fiber.

55. The process according to claim 54, wherein exposing the treated fiber to a wash solution comprising a surfactant is performed prior to winding the treated fiber and oxidizing the treated fiber.

56. The process according to any one of claims 1-55, wherein winding the treated fiber is at a rate of about 15 meters/minute.

57. The process according to any one of claims 1-56, wherein the keratin fiber is dried at about 85 °C for about 1 hour.

58. The process according to any one of claims 1-57, wherein the keratin fiber is annealed at about 125 °C for about 1 hour.

59. The process according to claim 58, wherein the keratin fiber is annealed after it is dried.

60. The process according to any one of claims 1-59, wherein the process is a continuous process.

61. The process according to any one of claim 1-60, wherein the process further comprises exposing the keratin fiber to a solution comprising an oxidized saccharide.

62. The process according to claim 61, wherein the keratin fiber is exposed to the solution comprising an oxidized saccharide for about 3 to about 25 hours.

63. The process according to claim 61 or 62, wherein the oxidized saccharide is a sucrose polyaldehyde.

64. The process according to any one of claims 61-63, wherein the step of exposing the keratin fiber to a solution comprising an oxidized saccharide is performed prior to exposing the treated fiber to a wash solution comprising a surfactant.

65. A keratin fiber prepared by the process of any of one claims 1-64.

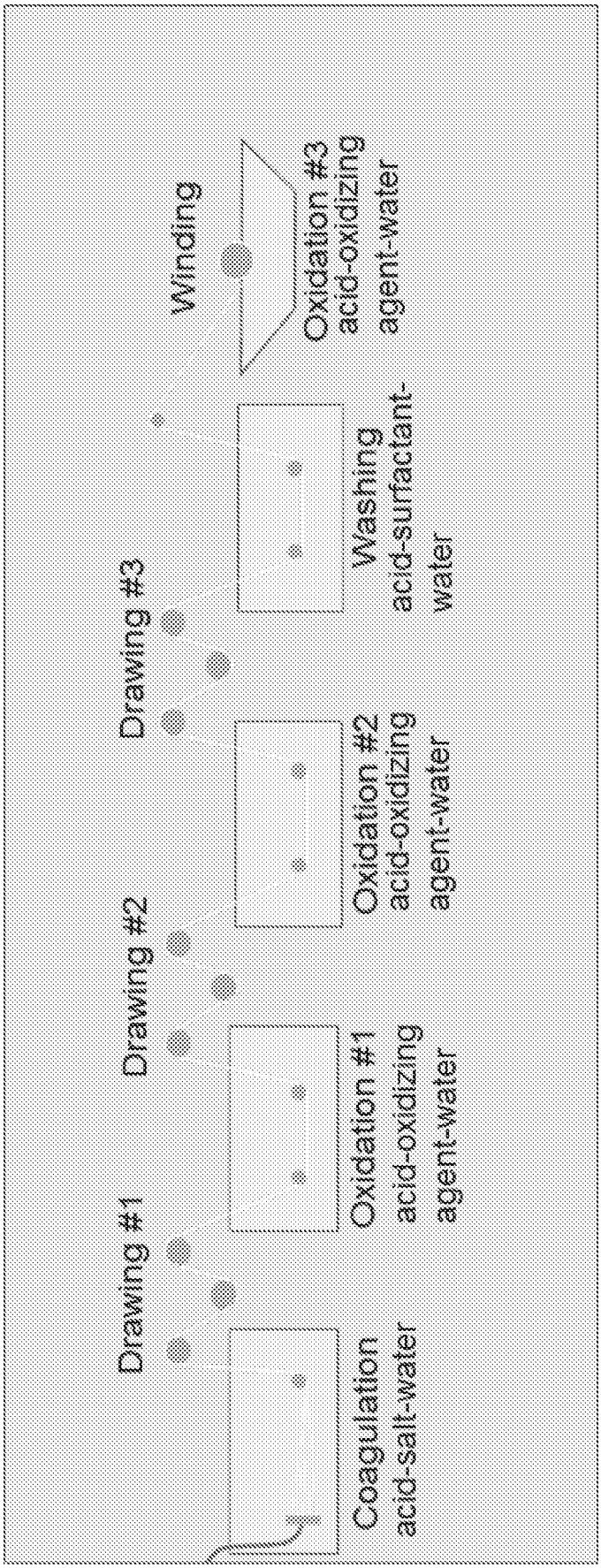

Coagulation
acid-salt-water
Drawing #1
Oxidation #1
acid-oxidizing
agent-water
Drawing #2
Oxidation #2
acid-oxidizing
agent-water
Drawing #3
Washing
acid-surfactant-
water
Winding
Oxidation #3
acid-oxidizing
agent-water

FIG. 1

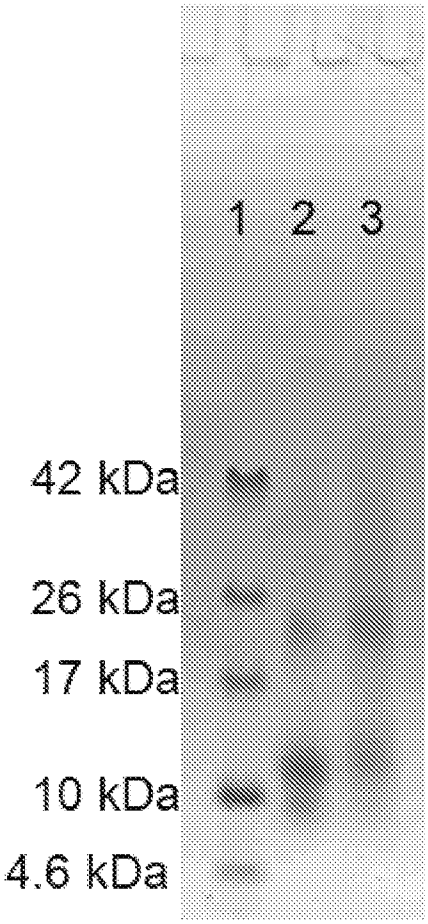


FIG. 2

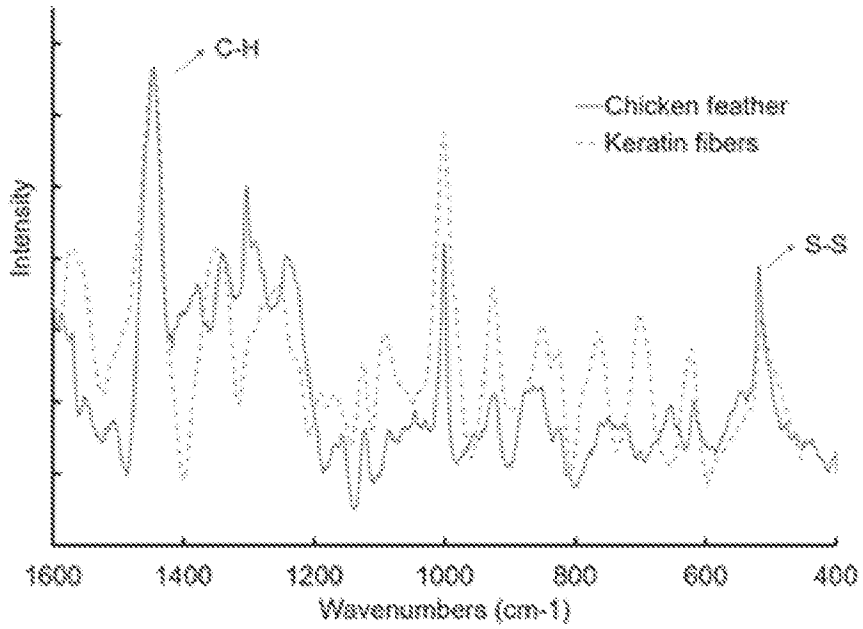

C-H
Chicken feather
Keratin fibers
S-S
Intensity
1600
1400
1200
1000
800
600
400
Wavenumbers (cm-1)

FIG. 3A

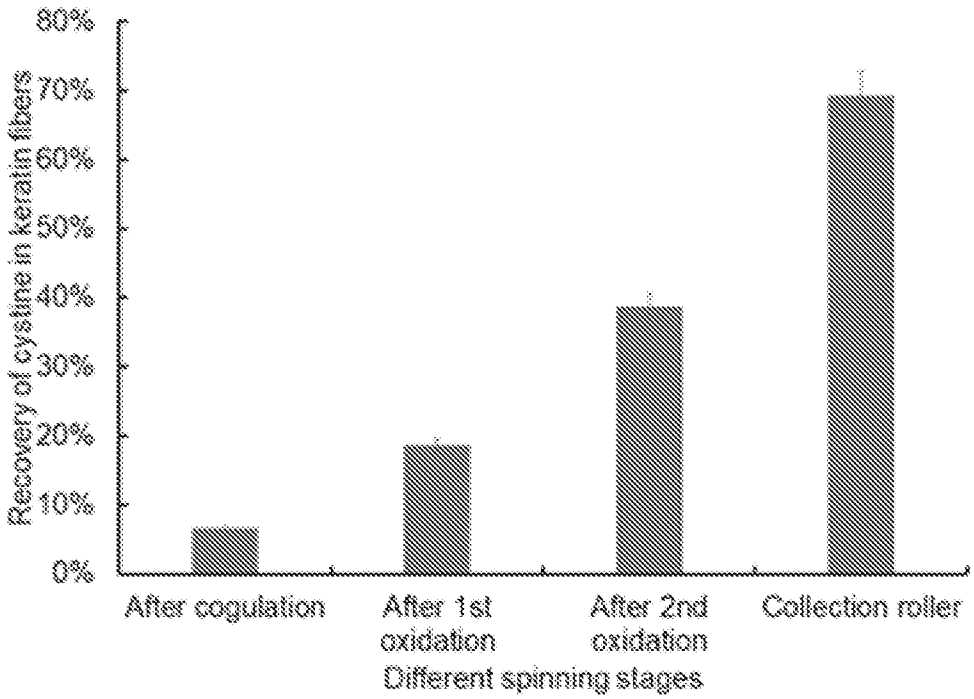

80%
70%
60%
50%
40%
30%
20%
10%
0%
Recovery of cystine in keratin fibers
After cogulation
After 1st oxidation
After 2nd oxidation
Collection roller
Different spinning stages

FIG. 3B

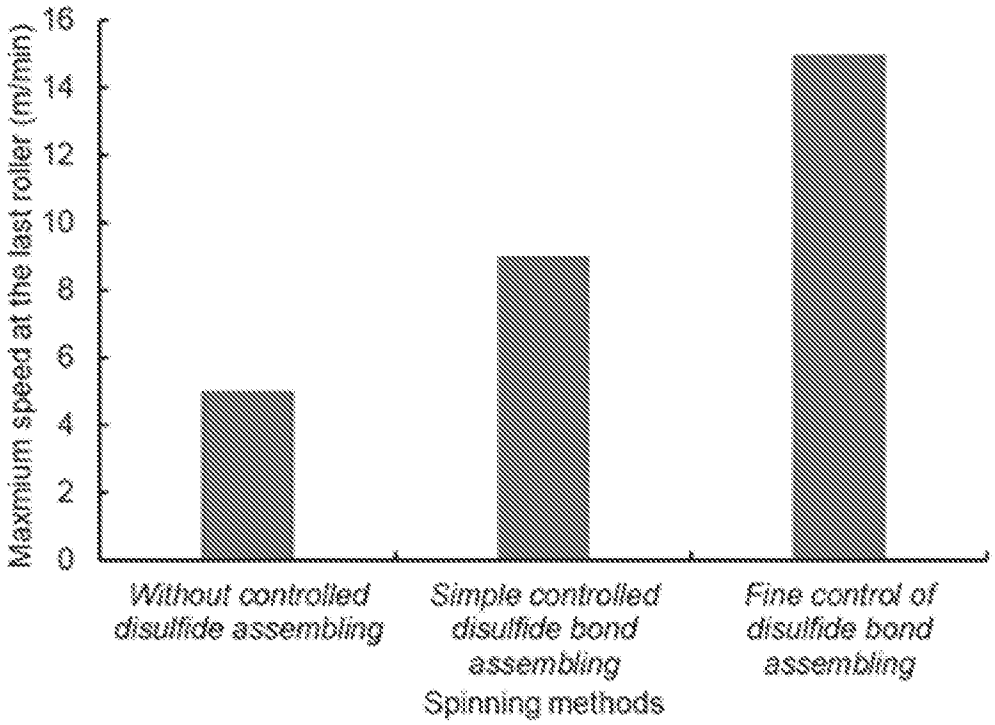

Maxmium speed at the last roller (m/min)
0
2
4
6
8
10
12
14
16
Without controlled disulfide assembling
Simple controlled disulfide bond assembling
Fine control of disulfide bond assembling
Spinning methods

FIG. 3C

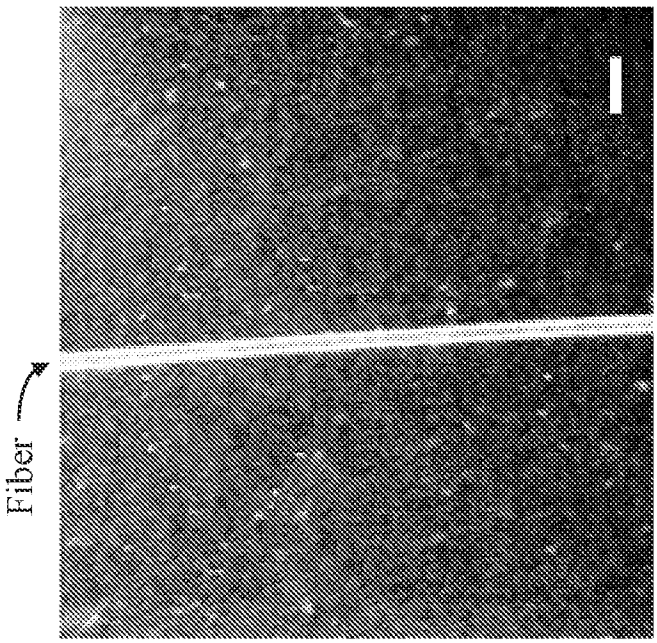

Fiber

FIG. 4B

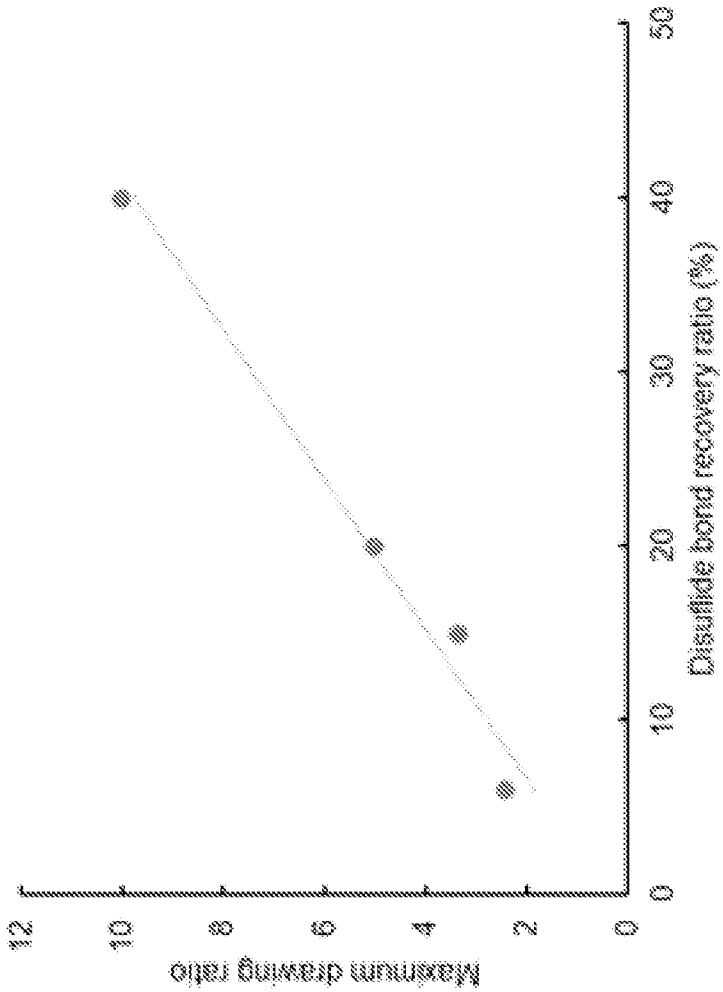

Maximum drawing ratio
0
2
4
6
8
10
12
Disulfide bond recovery ratio (%)
0
10
20
30
40
50

FIG. 4A

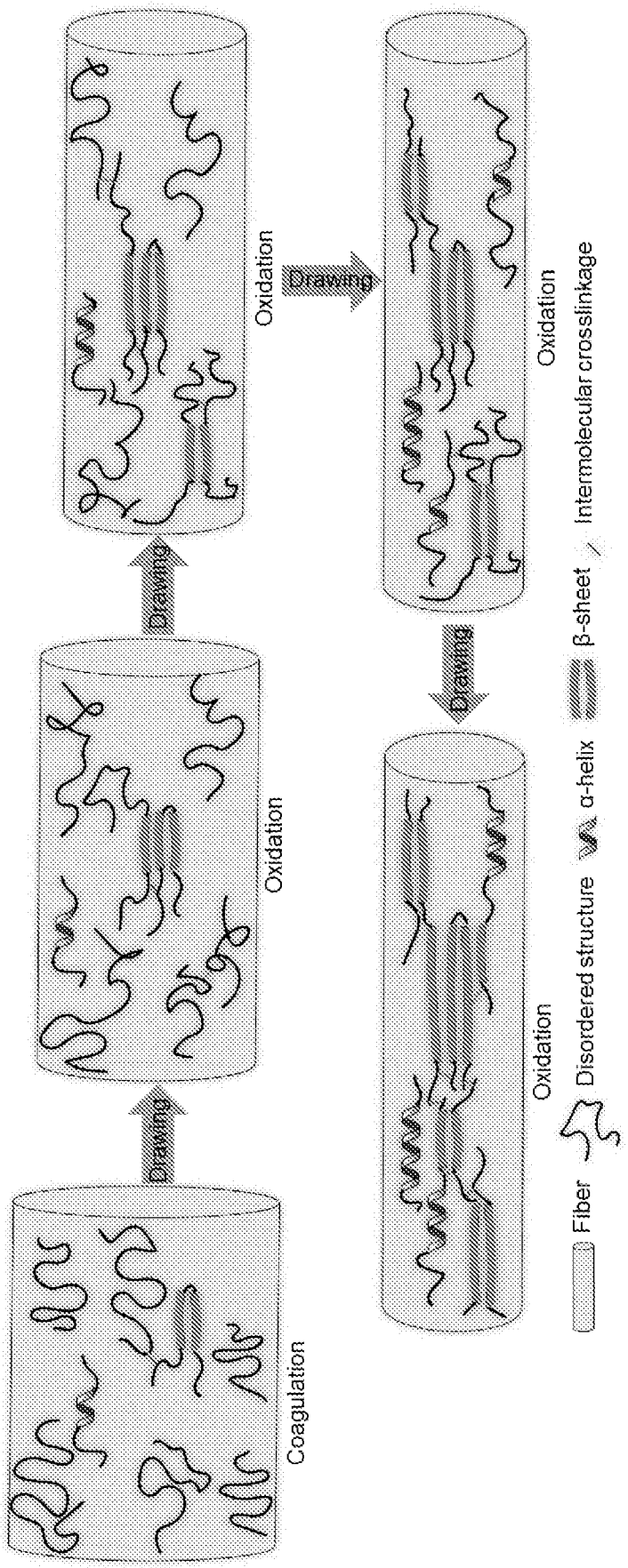

Coagulation
Drawing
Oxidation
Drawing
Oxidation
Drawing
Oxidation
Drawing
Oxidation
Fiber
Disordered structure
α-helix
β-sheet
Intermolecular crosslinkage

FIG. 5

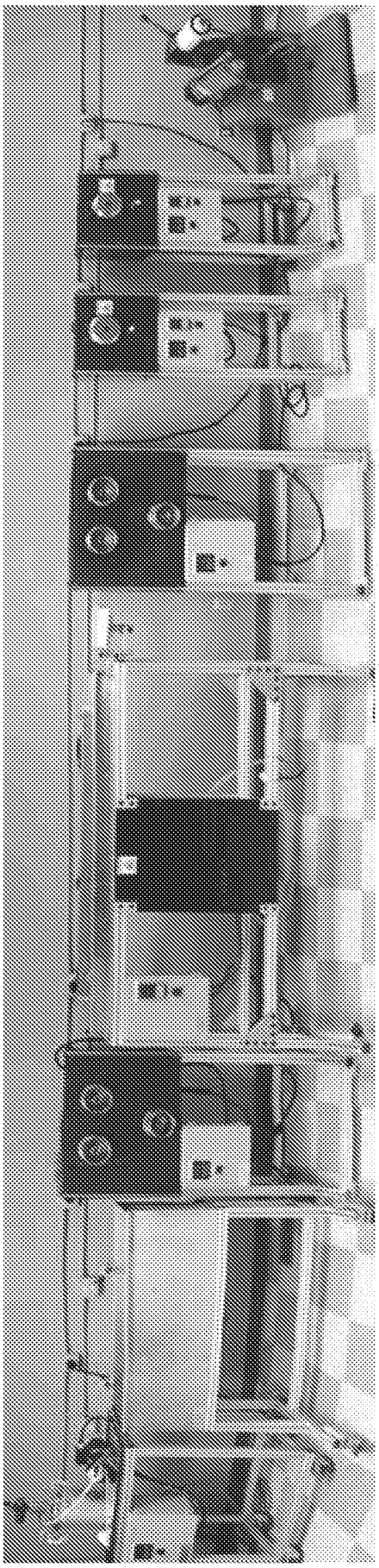


FIG. 6A

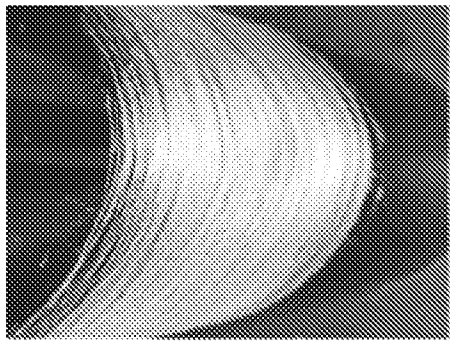


FIG. 6B

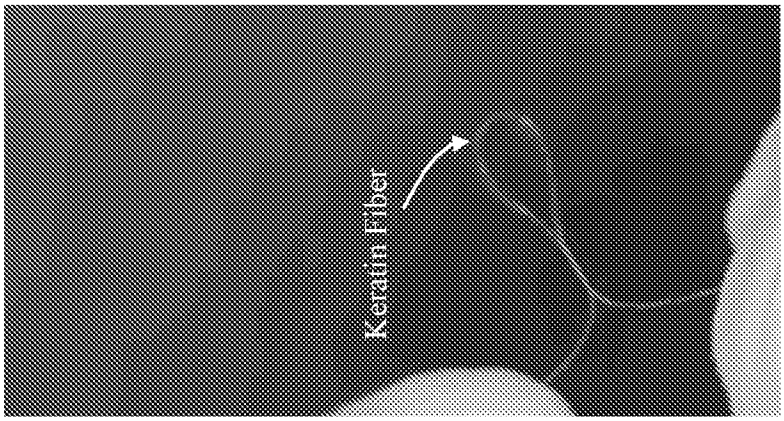

Keratin Fiber

FIG. 7B

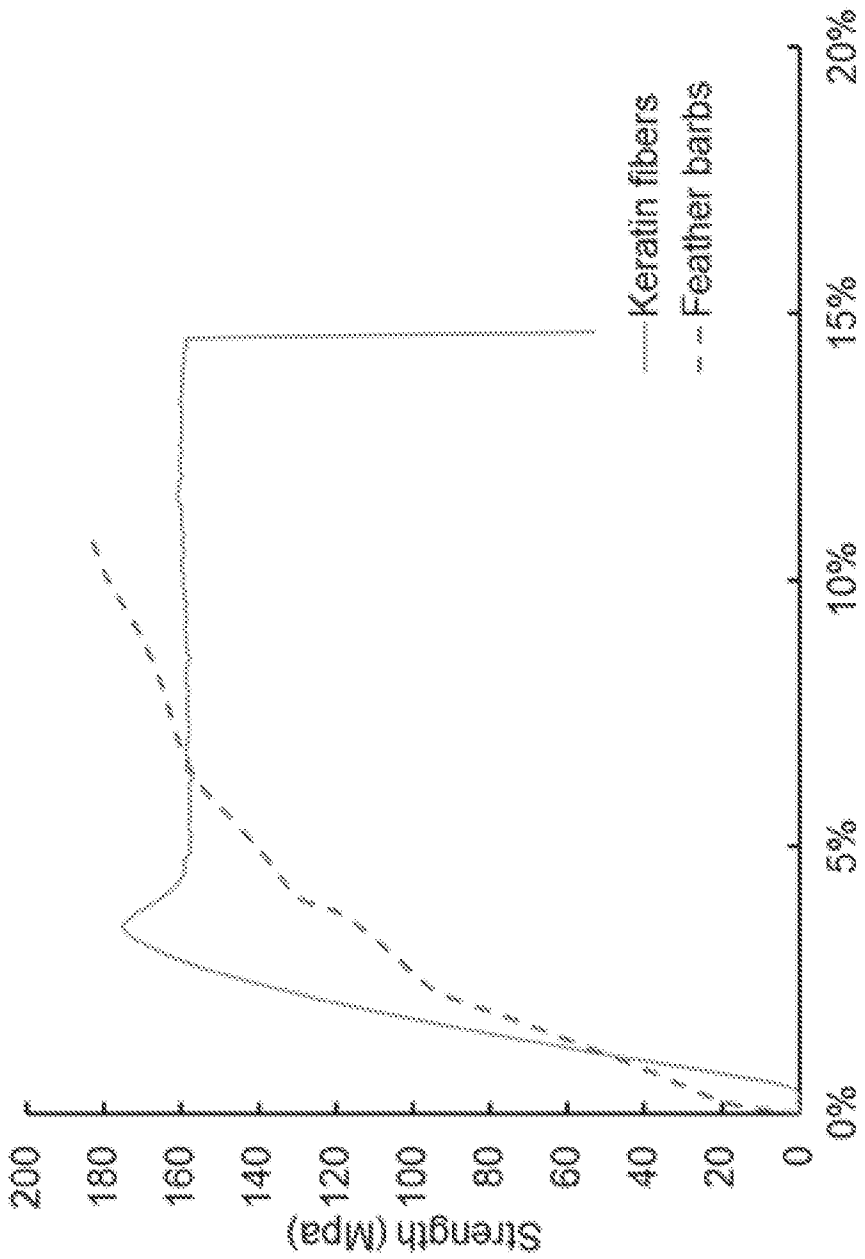

Strength (Mpa)
200
180
160
140
120
100
80
60
40
20
0
0%
5%
10%
15%
20%
Strain
Keratin fibers
Feather barbs

FIG. 7A

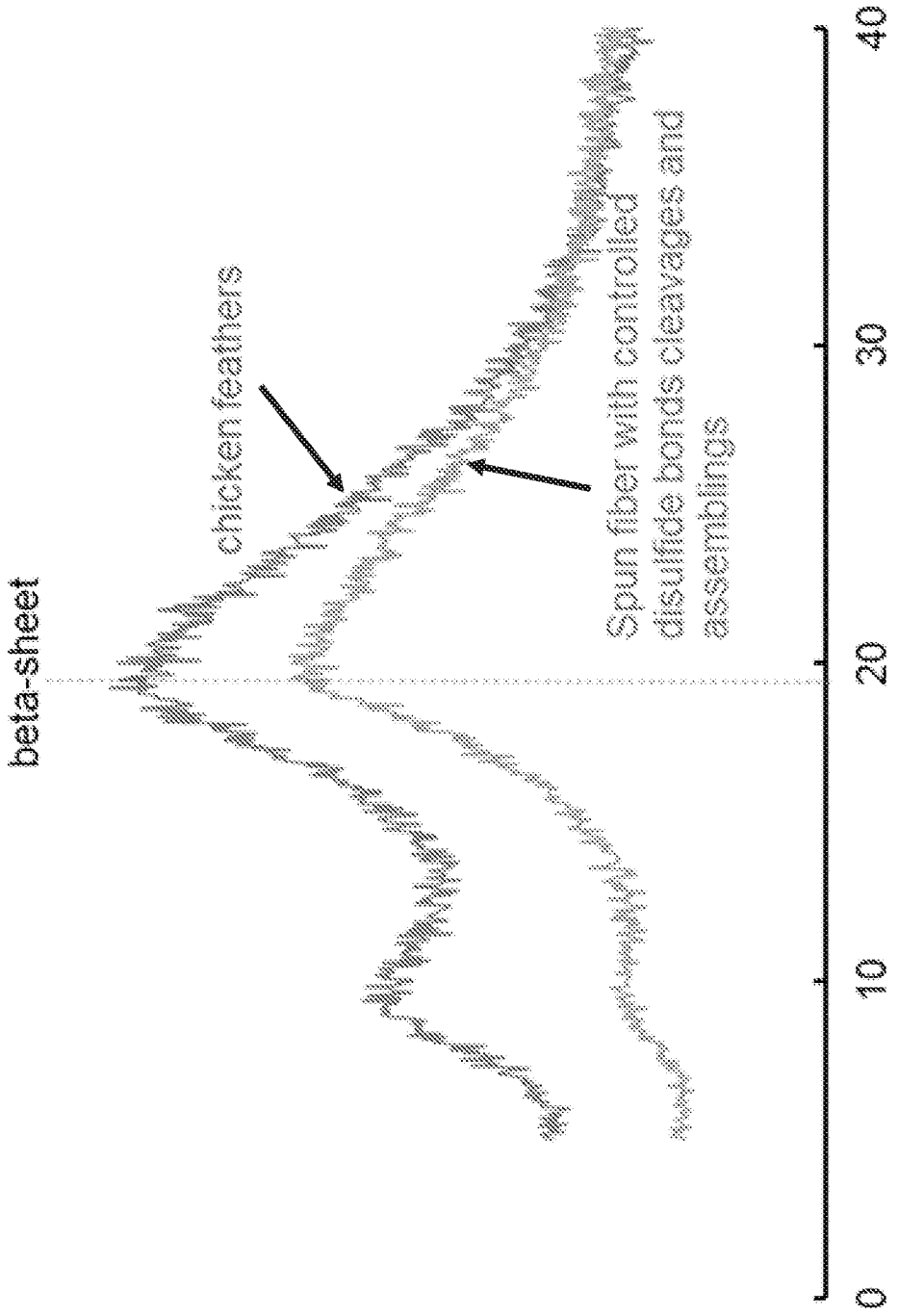

beta-sheet
chicken feathers
Spun fiber with controled disulfide bonds cleavages and assemblings
0
10
20
30
40
2θ

FIG. 8A

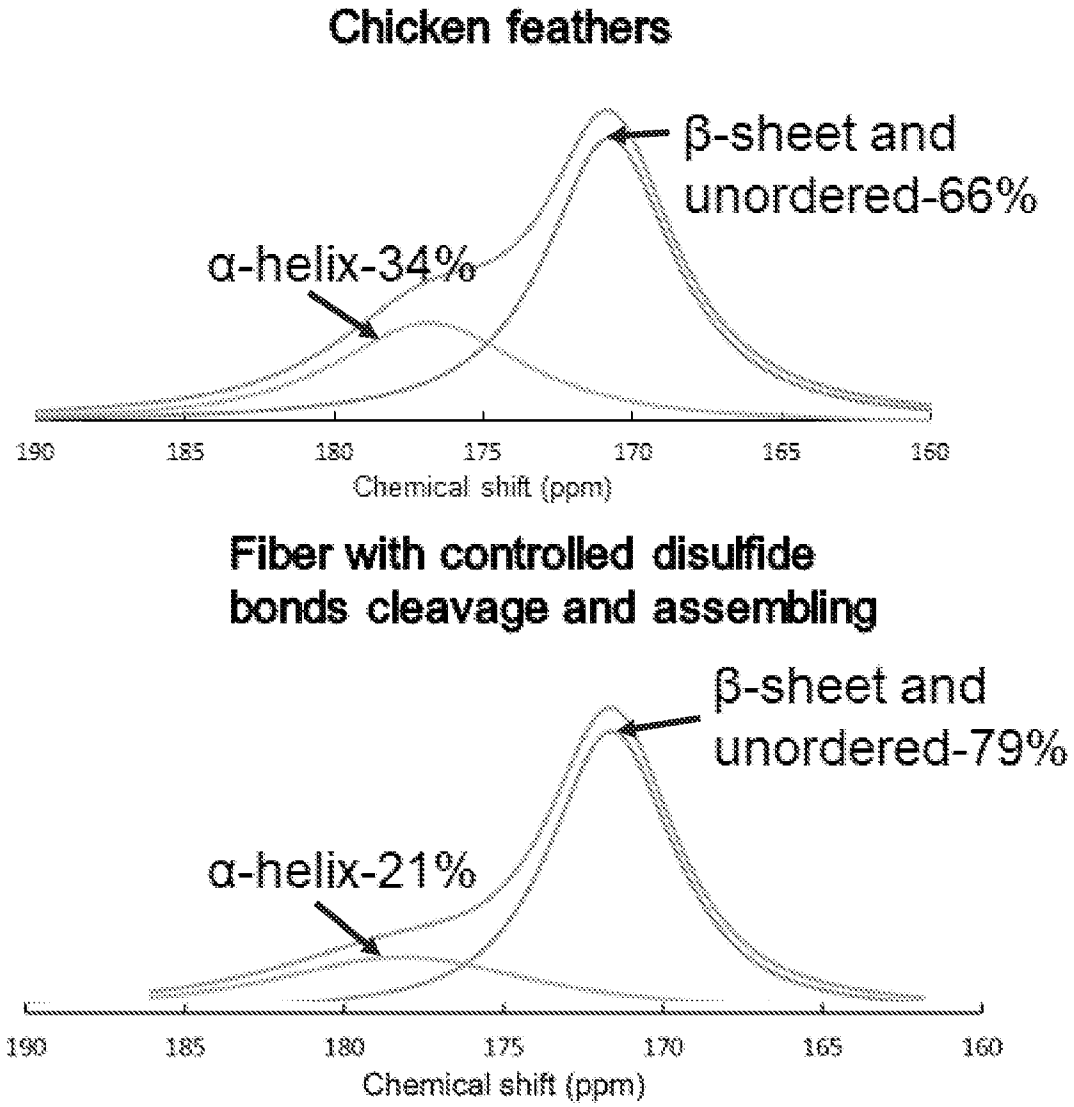

Chicken feathers
β-sheet and
unordered-66%
α-helix-34%
190
185
180
175
170
165
160
Chemical shift (ppm)
Fiber with controlled disulfide
bonds cleavage and assembling
β-sheet and
unordered-79%
α-helix-21%
190
185
180
175
170
165
160
Chemical shift (ppm)

FIG. 8B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/14401

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 8/04; A61K 8/39; A61K 8/86 (2021.01)

CPC - A61K 8/044; A61K 8/06; A61K 8/37; A61K 8/39

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	CN 110699855 A (Nantong University) 17 January 2020 (17.01.2020), entire document, especially page 2.	1-3 and 23-24 --- 25
Y	Xu eat al. "Controlled De-Cross-Linking and Disentanglement of Feather Keratin for Fiber Preparation via a Novel Process", ACS Sustainable Chemistry & Engineering 2014 2 (6), 1404-1410 [Retrived on 2021-03-17] Retrived from the Internet: <URL:https://pubs.acs.org/doi/pdf/10.1021/sc400461d> <DOI: 10.1021/sc400461d>, entire document especially page 1405 and 1409.	25
A	DeFrates, Kelsey G et al. "Protein-Based Fiber Materials in Medicine: A Review." Nanomaterials (Basel, Switzerland) vol. 8,7 457. 22 Jun. 2018, [Retrived on 2021-03-17] Retrived from the Internet: <URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6071022/> <DOI:10.3390/nano8070457>, entire document, especially page 11.	1-3 and 23-25
A	Tomisawa et al. "Effect of draw ratio on fiber structure development of polyethylene terephthalate", Polymer, Volume 116, 2017, Pages 357-366, ISSN 0032-3861, Retrived on 2021-03-17] Retrived from the Internet: <URL:https://doi.org/10.1016/j.polymer.2016.12.071.> entire document especially page 366.	1-3 and 23-25
A	CN 102817101 B (Shanghai Quanyu Biological Technology Suiping Co., Ltd.) 21 May 2014 (21.05.2014), entire document.	1-3 and 23-25
A	US 2003/0068493 A1 (Umehara et al.) 10 April 2003 (10.04.2003), entire document.	1-3 and 23-25

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 March 2021	APR 0 7 2021
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Lee Young Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/14401

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-22 and 26-65
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.