MICROWAVE-ASSISTED CENTRIFUGAL SPINNING OF GELATIN MICROFIBERS

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ABSTRACT

MICROWAVE-ASSISTED CENTRIFUGAL SPINNING OF GELATIN MICROFIBERS. Herein, we disclose a method for preparing polymeric microfibers. According to an embodiment of the method, gelatin fibers were prepared from 50% aqueous gelatin solution by centrifugal spinning. The produced material feels similar to wool to the touch. Average fiber diameter was determined to be 40 μm. Thermal stability of the material is similar to that of gelatin fibers prepared by other techniques, e.g. electrospinning. Elastic modulus of the fibrous material was determined to be 230 MPa and its toughness was found to be 160 kJ/m$^3$. The described method is one of the fastest low-cost lab-scale fiber production techniques available (as a practical example, the speed of fiber production was roughly 5 g/s) and could be used to prepare various polymeric fibers in an eco-friendly way for a wide range of applications, including textile industry, tissue engineering and drug delivery.

Keywords: Centrifugal spinning, Fibers, Gelatin, Cross-linking, Eco-friendly

INTRODUCTION

Textile industry is one of the most polluting industries in the world, producing large quantities of toxic waste, in addition to using a lot of clean water. However, a lot of effort is made to make textile industry more sustainable and move towards circular economy. Some approaches are directed at improving existing textile production technologies, e.g. developing novel eco-friendly dyeing techniques (e.g. applying natural dyes extracted from grape pomace to cotton [1], treating textiles with chickpea husk [2]), using epigeic earthworms to treat textile mill wastewater sludge in order to remove heavy metals [3], using waste cow bones to develop novel membranes for textile wastewater treatment [4], etc. Other approaches are directed at reusing textiles, e.g. separating fibers in wool/polyester blends by selectively digesting wool fibers [5] so that the fibers can be reused to make new textiles, upcycling waste textiles into regenerated fibers [6], fabricating acoustic...
insulation materials from recycled textiles [7], etc. Furthermore, some approaches, including the current work, are aimed at developing fiber production techniques that do not require the use of toxic chemicals.

Numerous methods can be used to prepare micro- and nanofibers, including self-assembly [8], phase separation [9], interfacial polymerization [10], drawing [11], various spinning techniques, e.g. solution blow spinning [12], wet-spinning [13], melt-spinning [14], microfluidic spinning [15], electrospinning [16], biospinning [17], dry-spinning [18] and centrifugal spinning [19]. Among these, both dry- and centrifugal spinning allow rapid production of fibers in large quantities. Furthermore, gelatin fibers have already been prepared by dry spinning from water at elevated temperatures [20]. Nevertheless, centrifugal spinning was the method of choice in the current work, since preliminary tests indicated that compared to dry-spinning, centrifugal spinning allows the same degree of control over the spinning process and properties of the fibers with more simple and robust equipment.

Several polymers of natural origin could be used to prepare eco-friendly textiles. For example, agar [21], starch [22], chitosan [23], cellulose/curcumin [24], collagen [25], and gelatin [26], have already been used to prepare fibrous materials. In the current work, gelatin was chosen as the main component of the fibers for the following reasons. Firstly, since gelatin is derived by hydrolysis from collagen present in skin, bones, and connective tissue of animals, it can be produced from cheap and abundantly available waste of meat industry/slaughterhouse waste. Secondly, since chemical composition of gelatin fibers is similar to that of leather, we wanted to explore if it is possible to prepare textiles that feel leather-like to the touch. Gelatin fibers have been briefly manufactured industrially in the past as early as at the end of 19th century [27]. However, gelatin has some drawbacks (e.g. it is hydrophilic, it has low wet strength, etc.) and was quickly replaced by synthetic fibers. Recently, interest in gelatin fibers has started to grow again, e.g. researchers have fabricated a gelatin glove [28].

Typically, more or less toxic solvents and cross-linking agents have been used to prepare and cross-link gelatin fibers. In the current work, gelatin was spun from water. Subsequently, the fabrics were cross-linked thermally by glucose as we have previously described in case of electrospun gelatin [29]. Such cross-linking technique doesn’t have a considerable effect on morphology of the fibers, allows tuning the rate of degradation of the fibers, and considerably increases mechanical strength of the fiber material [30]. In short, the current work describes an eco-friendly technique to rapidly, controllably and cost-effectively produce gelatin microfibers without using any toxic substances.

**MATERIALS AND METHODS**

**Preparation of Gelatin Microfibers**

240 bloom pig skin premium grade gelatin was obtained from Classikool Ltd. Wilton® glucose was added to gelatin as a cross-linking agent so that the mixture contained 15% glucose and weighed 50 g. Deionized water was added to the mixture to obtain a 30%, 40% and 50% solutions. The mixture was placed in an 800 W commercially available microwave for 30 s and stirred vigorously for another 30 s. The obtained viscous solution (at about 50°C) was spun into fibers at room temperature using a self-built centrifugal spinning setup depicted in Figure 1.

![Figure 1. Centrifugal spinning setup used to prepare microfibers](image)

The centrifugal spinning equipment was built based on a cylinder (110 mm diameter). 20 openings were drilled in the sides of the cylinder with a 1 mm drill. The cylinder was closed from both ends with blind flanges and an opening (1 cm in diameter) was drilled in the center of one of the flanges to be used as an inlet for polymer solution. The cylinder was mounted on an electric motor (adjustable up to 3000 rpm). The collector was built by placing metal rods circularly around the cylinder. The distance between the rods and the center of the cylinder was kept at 35 cm during the experiments.

Gelatin solution was successfully prepared by placing a mixture of gelatin, glucose, which was used as a cross-linking agent, and water in a microwave for 30 s. There was no need to mix gelatin and water prior to placing the mixture in the microwave. The mixture was taken out of the microwave and stirred vigorously for 30 s, which was enough to obtain a clear solution at about 50°C. It should be noted that in case of preparing different amount of solution and/or using a microwave with different power, the duration of microwave treatment should be adjusted as well.

Depending on the preparation process parameters, either non-woven fabric or bunches of fibers were obtained and collected from the collector.
The material was thermally cross-linked by glucose by placing it in an oven at 175°C for 3h, which, as we have previously reported in case of electrospun gelatin fibers [29], are the optimal conditions for such cross-linking.

**Characterization of Gelatin Microfibers**

Morphology of the material was characterized and fiber diameters were measured by scanning electron microscopy (SEM). 40-5000 times magnified SEM images were recorded using Hitachi FlexSEM 1000 Scanning Electron Microscope. The obtained images were used to characterize the structure of the prepared fabrics and morphology of individual fibers as well as to determine fiber diameters. Fiber diameter distribution graphs were constructed by measuring the number of fibers with diameters ±10 μm from a certain fiber diameter. In particular, every point of the graph corresponding to a certain fiber diameter d was constructed by counting the number of fibers with diameters between d-10 μm and d+10 μm. Such technique allows obtaining smooth histograms, which facilitates visual appraisal of the graphs.

Fourier transform infrared microscopy (FTIR) was used to analyze changes occurring as a result of thermal treatment. The fabrics were dried in a critical point drier (Leica EM CPD300) and FTIR spectra between 400 cm\(^{-1}\) and 4000 cm\(^{-1}\) was recorded using Bruker Vertex 70 spectrometer equipped with an attenuated total reflection (ATR) accessory (diamond ATR-crystal). The spectra were converted to absorbance spectra after baseline correction and normalized to a constant penetration depth.

Thermal properties were characterized by thermogravimetric analysis (TGA). Thermogravimetric analysis was performed using TGA 4000 (Perkin Elmer, USA) apparatus. Small pieces (between 5-12 mg) of gelatin fabrics were placed in the apparatus and the samples were heated up to 1000°C at 10°C/min, recording the change in sample mass and the speed of weight loss. The experiment was carried out in nitrogen.

Mechanical properties of the material were characterized by tensile test, which was carried out using a self-built tensile testing station. The fibers were wound around each other, making it hard to separate individual fibers. Therefore, bundles consisting of up to about 50 fibers wound around each other were analyzed. Such bundles formed a larger fiber with diameters ranging between 500-1000 μm. These larger fibers were folded and glued together from both ends and the ends were fixed between clamps. Subsequently, the initial length of the sample was measured photographically. The samples were pulled at 10 mm/min, elongation and force were recorded. The results were used to study mechanical behavior of the bundled fibers.

**RESULTS AND DISCUSSION**

**Preparation of Gelatin Microfibers**

Gelatin microfibers were successfully spun from 30%, 40%, and 50% water solutions. At higher concentrations, the solution had high viscosity, it tended to stick to the cylinder and clog its openings. It is also possible to spin solutions at even lower concentrations (perhaps in order to obtain fibers with smaller diameters), but this requires modifying other fiber preparation process parameters as well to ensure evaporation of water. In the current work, all the analyzed fibers were prepared from 30%, 40%, and 50% water solutions. In case of solutions with lower concentration, the fibers were often merged together and formed a non-woven fabric instead of individual fibers (see the results of SEM analysis). In case of 50% solutions, the fibers were still somewhat wound around each other, forming larger, bundled fibers with 500-1000 μm diameter. Thus, 50% solution was deemed to be the best for preparing textiles. The speed of fiber production was determined to be about 5 g/s (100 g of 50% solution was spun into microfibers in roughly 10 s).

After thermal treatment, the fibers turned golden-brown (Figure 2) as a result of the Maillard reaction (between gelatin and glucose) and became insoluble in water. The material is easy to handle and feels similar to wool to the touch. Compared to using naturally occurring fibers (e.g. wool) and traditional eco-friendly textile production techniques, which also allow sustainable manufacturing of clothes and accessories, the artificial fibers production approach has two main advantages. Firstly, it gives us much greater control over the properties of the material, e.g. it is possible to tune the rate of degradation by modifying the degree of cross-linking. Secondly, it allows us to use low-value waste of meat industry in a useful way, allowing us to move towards a more circular economy.

Compared to other fiber production techniques, the described microwave-assisted centrifugal spinning method has the following advantages. Firstly, the cost of equipment needed to prepare the fibers is low, e.g.
just a small fraction of the cost of equipment needed for a simple electrospinning setup. Secondly, the used method is among the fastest state-of-the-art fiber production techniques, since both preparation of the spinning solution and the spinning process itself can be carried out quickly. For example, the cost of all the materials used to construct the first prototype of the centrifugal spinning apparatus used in the current study was below 100 EUR and it allowed preparation of gelatin microfibers at 5 g/s. As an example, 50 grams of gelatin microfibers were produced in less than 5 minutes altogether, including mixing gelatin with glucose, adding water, microwave treatment, and the spinning process. As comparison, we have previously prepared glucose-containing gelatin fibers by electrospinning from aqueous acetic acid using lab-scale equipment; in this case, the speed of electrospinning was 6-8 l/min (25% solution) [29]. The method proposed herein allows more than 200000 times faster production speed, while using more cost-effective equipment. Next, since only water is used as solvent, the whole process is eco-friendly and safe for the personnel. One of the disadvantages of the used system is wide fiber diameter distribution. However, this can probably be improved by modifying the used apparatus.

**Morphology**

As mentioned above, it was observed during preparation of the material that fibers spun from solutions with lower gelatin concentration tended to stick together and form a non-woven fabric instead of individual fibers. This was confirmed by SEM analysis. It can be seen (Figure 3a) that in case of the lowest tested concentrations (~30%) the structure of the resulting material resembled something between fibers and a film. The non-woven fiber-film structure probably formed, because the solvent (water) didn’t fully evaporate before reaching the collector. However, this material still looked like a non-woven fabric to the naked eye. In case of slightly higher concentrations (~40%), the fibers have merged together and form a non-woven fabric (Figure 3b). While this kind of structure might be suitable for some applications, it is undesirable for textile industry, since the individual fibers cannot be separated and processed further without braking the structure of the material. 50% solutions were spun into material which seems to consist mostly of individual fibers (Figure 3c). This could easily be seen visually as well – it was possible to separate individual fibers (the larger fibers consisting of smaller fibers wound around each other) by hand and, for example, subject them to tensile test. SEM was also used to analyze surface morphology of the fibers. It can be seen (Figure 3d) that the fibers are relatively smooth.

SEM images were used to determine the average fiber diameter and to analyze fiber diameter distribution (Figure 4). Significant difference between average fiber diameters was not detected in case of samples spun from solutions with different concentrations, probably because the range of fiber diameters was wide. Average fiber diameter was determined to be 40 μm in all three cases (samples prepared from 30%, 40%, and 50% solutions), although it varied between 7 μm and 114 μm. This was probably caused by the used centrifugal spinning setup and the range could possibly be decreased by modifying the setup, e.g. by using different kind of cylinder outlets (e.g. needles instead of openings). Thermal treatment didn’t have a detectable effect on fiber diameters.

![Figure 3](image-url)  
*Figure 3. Material prepared from 30% solution (a), 40% solution (b), 50% solution (c), and a close-up of an individual fiber prepared from 50% solution (d). All samples were analyzed after 3 h of thermal treatment.*

![Figure 4](image-url)  
*Figure 4. Fiber diameter distribution of samples prepared from 50% gelatin solution after 3 h of thermal treatment.*

**FTIR**

In the analyzed FTIR spectra, peaks were detected (in case of all the samples) at 3286 and 3074 cm⁻¹ (mainly OH and NH vibrations), 2935 and 2881 cm⁻¹ (mainly CH₂ asymmetric and symmetric vibrations, respectively), 1657, 1641, 1631 cm⁻¹ (amide I band, C=O vibrations), 1562, 1547, 1535 cm⁻¹ (amide II band, attributed to NH bending), 1240 cm⁻¹ (amide III band, attributed to in plane NH bending and CN stretching), 1450, 1406, 1334, 1203 cm⁻¹ (various in plane vibrations), 1082, 1030 cm⁻¹ (CO vibrations overlapping with other
vibrations in glucose) [29,31,32,33,34] (Figure 5). Notable differences between peak intensities of thermally treated and non-treated material were observed between 900 and 1700 cm\(^{-1}\). Due to the occurring Maillard reaction, the intensity of CO vibrations in glucose (especially the peak at 1030 cm\(^{-1}\)) decreased and the intensity of the amide bands increased. Caramelization also possibly contributes to the changes occurring as a result of thermal treatment. Both Maillard reaction and caramelization contribute to the formation of cross-links between gelatin molecules, making the material insoluble and increasing its strength.

TGA

TGA was used to examine thermal stability and confirm that the cross-linking reaction is complete. TGA results didn’t depend on the concentration of the used spinning solution. TGA curves of samples prepared from 50% gelatin solution after 3h of thermal treatment are presented in Figure 6. It can be seen that the material exhibits two stages of weight loss. The first stage (10-11% weight loss) with a maximum rate of weight loss at 84°C can be attributed to loss of absorbed water. The second stage of weight loss (65-66% weight loss) with a maximum weight loss rate at 343°C results from degradation of protein chains and decomposition of cross-links between them. Since the material is mostly comprised of amino acids, its pyrolysis can cause deamination, homolysis of the side chains, dimerization and decarboxylation reactions, yielding such volatiles as \(\text{H}_2\), \(\text{H}_2\text{O}\), \(\text{CO}, \text{HCN}, \text{NH}_3\), methane, \(\text{CO}_2\), etc [35,36] during this stage. The onset point of thermal degradation was determined to be 204°C, residual mass at 500°C and 1000°C were 29% and 18-19%, respectively. Temperature at 50% weight loss was 362°C.

This kind of behavior is similar to that of both pure gelatin and gelatin cross-linked in various ways. It is generally accepted that cross-linking stabilizes the protein chains to some degree and increases thermal stability of the material. The use of different cross-linking agents results in variations in thermal stability [37]. Additionally, if additives are used, more than two stages of weight loss might be observed [38]. Therefore, the existence of only two stages of weight loss confirms that the cross-linking reaction between gelatin and glucose is indeed complete.

Tensile Test

Mechanical behavior of the bundled fibers was studied by tensile test. Only samples prepared from 50% solution were tested, since material consisting mostly of individual fibers is more desirable for the intended applications and since it was difficult to separate sufficiently long bundled fibers in case of samples prepared from less concentrated solutions. Tensile test was carried out after thermal treatment. A typical tensile test graph is presented in Figure 7. The shape of the first part of the curve (up to 2.4% elongation) corresponds to the straightening of the microfibers forming the larger, bundled fibers. The smaller fibers are wound around each other. As the force is applied to them longitudinally, these smaller fibers are pulled straight. The second section (2.4-3.7% elongation) corresponds to the elastic deformation of the material. The final section of the graph (elongation over 3.7%) corresponds to plastic deformation and failure of the material. The average elongation at failure was 4.6%.

Elastic modulus of the fibers was calculated as the slope of the elastic (linear) region of the stress-strain curve, which is the most important part of the graph for practical applications. The average elastic modulus of the bundled fibers was determined to be 230 MPa. The elastic modulus of the fiber material is probably significantly higher because of the space between microfibers forming the bundled fibers. Toughness of the material was calculated by integrating the stress-strain curve while ignoring the first section of the curve by extrapolating the linear region until the stress-axis, since the slope of the first part of the graph is smaller due to the fibers not being straight. Hence, the first part of the stress-strain curve doesn’t characterize toughness. Toughness of the material was determined to be 160 kJ/m\(^3\). It is important to note that the fibers are easy to handle and mechanically sufficiently strong, although weaker than many currently used synthetic
fibers. Consequently, it would be desirable to further increase mechanical strength of the material by altering its composition or during post-treatment. Although the material is already easy to handle and can be used to prepare clothes and accessories, it is, in its untreated form, still mechanically weaker than currently used synthetic fibers. Additionally, gelatin is hydrophilic, tends to swell and has low wet strength. While this can be advantageous in some applications, e.g. drug delivery or tissue engineering, it is undesirable in case of textile industry. Therefore, the main challenge lies in making the material hydrophobic and waterproof and further increasing its mechanical strength in an eco-friendly way.

CONCLUSIONS

In the current work, we describe preparation of gelatin microfibers by microwave-assisted centrifugal spinning in a fast, robust, easily scalable way, using only water as a solvent. As a practical example, 50 grams of fibrous material was obtained in just 5 minutes, including preparation of the spinning solution and the production process itself, which is extremely fast compared to other methods used according to the state-of-the-art. The prepared material is easy to handle and feels like wool to the touch. While the properties of the obtained gelatin fibers are not outstanding and are similar to previously prepared materials, the value of the current work lies in the fact that the described method could be applied to prepare various other kind of polymeric fibers for a wide range of applications, allowing production of fibers in a fast, eco-friendly, cost-effective way.

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REFERENCES

[1]. N. Baaka, W. Haddar, M.B. Ticha, M.F. Mhenni. “Eco-friendly dyeing of modified cotton fabrics with grape pomace colorant: Optimization using full factorial design approach.” Journal of Natural Fibers, vol. 16(5), pp. 625-661, 2019.
[2]. S. Jose, P. Pandit, R. Pandey. “Chickpea husk – A potential agro waste for coloration and functional finishing of textiles.” Industrial Crops and Products, vol. 142, No. 11183, 2019.
[3]. A. Yuvaraj, N. Karmegam, S. Tripathi, S. Kannan, R. Thangaraj. “Environment-friendly management of textile mill wastewater sludge using epigeic earthworms: Bioaccumulation of heavy metals and metallothionein production.” Journal of Environmental Management, vol. 254, No. 109813, 2020.
[4]. S.K. Hubadillah, M.H.D. Othman, Z.S. Tai, M.R. Jamalludin, N.K. Yusuf, A. Ahmad, M.A. Rahman, J. Jaafar, S.H.S.A. Kadir, Z. Harun. “Novel hydroxyapatite-based bio-ceramic hollowfiber membrane derived from waste cow bone for textile wastewater treatment.” Chemical Engineering Journal, vol. 379, No. 122396, 2020.
[5]. L. Navone, K. Moffitt, K.-A. Hansen, J. Blinco, A. Payne, R. Speight. “Closing the textile loop: Enzymatic fibre separation and recycling of wool/polyester fabric blends.” Waste Management, vol. 102, pp. 149-160, 2020.
[6]. Y. Ma, L. Rosson, X. Wang, N. Byrne. “Upcycling of waste textiles into regenerated cellulose fibres: impact of pretreatments.” The Journal of The Textile Institute, vol. 111:5, pp. 630-638, 2020.
[7]. S. Islam, G. Bhat. “Environmentally-friendly thermal and acoustic insulation materials from recycled textiles.” Journal of Environmental Management, vol. 251, No. 109536, 2019.
[8]. X. Hu, M. Liao, H. Gong, L. Zhang, H. Cox, T.A. Waigh, J.R. Lu. “Recent advances in short peptide self-assembly: from rational design to novel applications.” Current Opinion in Colloid & Interface Science, vol. 45, pp. 1-13, 2020.
[9]. L. He, Y. Zhang, X. Zeng, D. Quan, S. Liao, Y. Zeng, J. Lu, S. Ramakrishna. “Fabrication and characterization of poly(L-lactic acid) 3D nanofibrous scaffolds with controlled architecture by liquid–liquid phase separation from a ternary polymer–solvent system.” Polymer, vol. 50, pp. 4128-4138, 2009.
[10]. A.B. El-Basaty, E. Moustafa, A.A. El-Moneim. “3D hierarchical graphene/CNT with interfacial polymerized polyaniline nanofibers.” Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, vol. 226, No. 117629, 2020.
C. Li, T. Lou, X. Yan, Y. Long, G. Cui, X. Wang.

J. Zhang, H. Kitayama, Y. Gotoh, A. Potthast, T. Rosenau. “Non-woven fabrics of fine regenerated cellulose fibers prepared from ionic liquid solution via wet type solution blow spinning.” Carbohydrate Polymers, vol. 226, No. 115258, 2019.

B.R. Lee, K.H. Lee, E. Kang, D.-S. Kim, S.-H. Lee. “Microfluidic wet spinning of chitosan-alginategullar fibers and encapsulation of HepG2 cells in fibers.” Biomicrofluidics, vol. 5, No. 022208, 2011.

M. Kanerva, A. Puolakka, T.M. Takala, A.M. Elert, V. Mylläri, I. Jönkkäri, E. Sarlin, J. Seitsonen, J. Ruokolainen, P. Saris, J. Vuorinen. “Antibacterial polymerfibres by rosin compounding and melt-spinning.” Materials Today Communications, vol. 20, No. 100527, 2020.

X. Hu, M. Tian, N. Pan, B. Sun, Z. Li, Y. Ma, X. Zhang, S. Zhu, Z. Chen, L. Qu. “Structure-tunable graphene oxidefibers via microfluidic spinningroute for multifunctional textiles.” Carbon, vol. 152, pp. 106-113, 2019.

J. Ding, J. Zhang, J. Li, D. Li, C. Xiao, H. Xiao, H. Yang, X. Zhuang, X. Chen. “Electrospun polymer biomaterials.” Progress in Polymer Science, vol. 90, pp. 1-34, 2019.

B.B. Mandal, S.C. Kundu. “Biospinning by silkworms: Silk fiber matrices for tissue engineering applications.” Acta Biomaterialia, vol. 6, pp. 360–371, 2010.

D. Ma, B. Liu, X. Xin, L. Zhu, X. Wang, G. Zhang, D. Xu. “Rheologic behaviors and continuously dry spinning of polyacetylatedaconitrozirconium fibers.” Materials Letters, vol. 258, No. 126824, 2020.

Z. Li, S. Mei, Y. Dong, F. She, L. Kong. “High Efficiency Fabrication of Chitosan Composite Nanofibers with Uniform Morphology via Centrifugal Spinning.” Polymers, vol. 11(10), No. 1550, 2019.

T. Chaochai, Y. Imai, T. Furuike, H. Tamura. “Preparation and Properties of Gelatin Fibers Fabricated by Dry Spinning.” Fibers, vol. 4(1), No. 2, 2016.

J. Liu, Z. Xue, W. Zhang, M. Yan, Y. Xia. “Preparation and properties of wet-spin agar fibers.” Carbohydrate Polymers, vol. 181, pp. 760–767, 2018.

X. Li, H. Chen, B. Yang. “Centrifugally spun starch-based fibers from amylopectin rich starches.” Carbohydrate Polymers, vol. 137, pp. 459–465, 2016.

C. Li, T. Lou, X. Yan, Y. Long, G. Cui, X. Wang. “Fabrication of pure chitosan nanofibrous membranes as effective absorbent for dye removal.” International Journal of Biological Macromolecules, vol. 106, pp. 768-774, 2018.
[35]. M.A. Ratcliff, E.E. Medley, P.G. Simmons. “Pyrolysis of amino acids. Mechanistic considerations.” The Journal of Organic Chemistry, vol. 39(11), pp. 1481–1490, 1974.

[36]. Q. Ren, C. Zhao. “NOx and N2O Precursors from Biomass Pyrolysis: Nitrogen Transformation from Amino Acid.” Environmental Science & Technology, vol. 46(7), pp. 4236–4240, 2012.

[37]. Y. Zhao, Z. Sun. “Effects of gelatin-polyphenol and gelatin–genipin cross-linking on the structure of gelatin hydrogels.” International Journal of Food Properties, vol. 20(sup3), pp. S2822–S2832, 2017.

[38]. J. Lin, D. Pan, Y. Sun, C. Ou, Y. Wang, J. Cao. “The modification of gelatin films: Based on various cross-linking mechanism of glutaraldehyde at acidic and alkaline conditions.” Food Science & Nutrition, vol. 7(12), pp. 4140–4146, 2019.