Introduction

In the past few years, fine fibers (nano and submicron) have attracted significant scientific interest mostly because of their high surface area to volume ratio and porous structure within and among the developed nanofibers. These properties have given rise to numerous promising applications such as filtration, smart textiles, sensors, solar cells, fuel cells, batteries, etc. In the biomedical area, nano fiber mats have received increasing attention due to potential to solve a variety of existing challenges in burn and wound care, tissue regeneration and treatment for a variety of diseases given its ability to enhance adhesion of drugs, proteins, and cells [1]. The focus of published nano fiber studies pertains to the development and production of nanofibers and investigation of their chemical, electrical, thermo-physical, rheological, and mechanical properties, correlated to the applications mentioned above. Few studies have related the use of nanofibers in food science and engineering fields. There are a variety of applications to explore ranging from food preservation and packaging to more complex ones such as nutrient delivery systems. Recently, the Forcespinning® technology has garnered significant attention for nano, submicron, and single digit microfiber development. The reason behind this tendency is due to its unprecedented yield when compared to other methods such as electro spinning and wet chemistry methods. Forcespinning® is a novel fine fiber production method where the electrical forces used in electro spinning are replaced by centrifugal forces. The Forcespinning® technique provides ample flexibility in materials selection since electric fields are not needed; therefore no need to select solvents with appropriate dielectric parameters. Water based systems can be effectively utilized as well as melt spinning, further lowering production costs by removing the need to budget solvent recovery steps. In Forcespinning®, centrifugal force is used to extrude the solution/melt through small orifices with a controlled length to diameter ratio [2]. As the extruded fibers exit the rotating spinneret; air drag forces elongation of the fibers and reduces their diameter to the nanometer scale. At the same time that fibers are being stretched the solvent evaporates (or melt cools down) leaving solidified fibers. The fibers can then be collected by different methods allowing the creation of non-woven mats, aligned fiber mats, or yarns. The system has spinnerets specifically...
Materials and Methods

Materials

Pullulan (PULL) powder was purchased from Tokyo Chemical Industry Co., LTD. Glandless cottonseed meal (GCSM) was provided by Cotton Inc. (Texas, USA) containing 48.5 percent of crude protein and 11.8 percent of lipids. It was stored at 4°C until used. Protein was extracted utilizing a 0.1 M KOH solution at a 1:12 ratio (w/v; pH 12) via a thermal process at 65°C for 40 minutes. The liquid was centrifuged at 3500 rpm for 20min (RCF 4793g) at 4°C. Recovered supernatant was acidified to pH 4.5 with 0.5MHCl and distilled water was used to precipitate the protein. It was then centrifuged at 3500rpm for 20min at 4°C to recover protein solids. Protein solids were re-suspended in distilled water for storage and transportation in between laboratories [19]. Figure 1 shows the several steps used to prepare the fibers. The process started with developing pullulan solutions. Pullulan in its powder form was mixed with distilled water; 2.13g of PULL were used to prepare a 21 wt percentage PULL solution. The samples were prepared at room temperature and magnetically stirred for 2 hours. GCSM protein powder was obtained through vacuum filtration and added to the PULL solutions, two samples were prepared containing 5 and 8 wt. percentage of GCSM. The PULL solutions with the added GCSM were magnetically stirred in an oil bath for 2 hours at room temperature.

Experimental

PULL and PULL/GCSM nanofibers were produced by the Forcespinning® technique, using a CycloneL-1000M (manufactured by Fibero Technology, Corp. USA). The spinneret equipped with 30G half-inch needles (Becton Dickinson and Company) at each end was filled with 1 mL of the developed solutions. The solutions were spun at different RPM (4500-8500) at room temperature and distilled water was used to precipitate the protein. It was then centrifuged at 3500rpm for 20min (RCF 4793g) at 4°C. Recovered supernatant was acidified to pH 4.5 with 0.5MHCl and distilled water was used to precipitate the protein. It was then centrifuged at 3500rpm for 20min at 4°C to recover protein solids. Protein solids were re-suspended in distilled water for storage and transportation in between laboratories [19]. Figure 1 shows the several steps used to prepare the fibers. The process started with developing pullulan solutions. Pullulan in its powder form was mixed with distilled water; 2.13g of PULL were used to prepare a 21 wt percentage PULL solution. The samples were prepared at room temperature and magnetically stirred for 2 hours. GCSM protein powder was obtained through vacuum filtration and added to the PULL solutions, two samples were prepared containing 5 and 8 wt. percentage of GCSM. The PULL solutions with the added GCSM were magnetically stirred in an oil bath for 2 hours at room temperature.

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Fiber development was conducted at angular velocities starting from 4500 RPM up to 10000 RPM. It was observed that successful fiber production started at velocities higher than 5000 RPM, specifically at 5500 RPM and up to 8000 RPM. These range was considered the optimum for the pure pullulan, 5 and 8 weight percent GCSM solutions, respectively. Fibers were produced for all systems within the angular velocity range of 4500 to 8500 RPM though to determine optimum angular velocity, a synergy between fiber output and fiber diameter was considered. For example, fibers with diameters of less than 100 nm were observed though the fiber output was not significant. It was observed that the optimal RPM was around 5000-6000 RPM, the yield fiber output was the highest. Surpassing the previously stated speeds, the fiber output would significantly drop. At lower (< 5000) the fiber output was severely impacted; the fiber production was low, with beady morphology. Figure 2 shows scanning electron micrographs of the developed fiber mats with its corresponding fiber diameter statistical analysis. Figure 2a shows results for pure pullulan fibers, 2b for fibers prepared with 5 weight percent of GCSM, and 2c for fibers with 8 weight percent of GCSM. As observed, homogeneous, long, and continuous fine fibers were developed. Bead formation was not observed. The addition of the GCSM resulted in a small increase in fiber diameter being statistically insignificant for the different concentrations. The average fiber diameters were 720 nm respectively with a standard deviation of 200 nm. The developed nonwoven fiber mats are as shown in Figure 1, homogeneous, flexible and easy to handle.

Figure 2: (A) Pure pullulan fibers spun at 5500 rpm, (B) composite fibers containing 5 wt percentage of GCSM spun at 7000 rpm, (C) composite fibers containing 8% wt. of GCSM spun at 8000 rpm, and (D) statistical analysis of fiber diameter.
DSC analysis was performed in order to assess the effect of molecular elongation on the microstructure of the polymer. As can be seen in Figure 3, the DSC thermographs of the pullulan in bulk state and fiber state differ significantly. Pullulan powder is an amorphous polymer. As depicted in Figure 3, there was no melting transition, but subtle endothermic (Figure 3a) and exothermic (Figure 3b) transitions were observed attributed to the glass transition temperature (T_g). Upon spinning the polymer into fiber form, a clear change in the location and magnitude of the glass transition temperature is observed. First of all, the location of the T_g is shifted to lower temperature implying a plasticizing effect upon fiber formation, alignment of polymer molecules. Also, for the nonwoven fiber mats, the T_g depicted as a step in the bulk pullulan sample is now seen as an endothermic T_g peak. There is no evidence of cold crystallization (considering the great chain flexibility of pullulan) though, fiber formation certainly affected chain mobility and was manifested in the development of the observed T_g peak attributed to enthalpy relaxation or enthalpy recovery. This endothermic peak (in the fiber sample) allows to quantitatively analyze relaxation kinetics of amorphous systems, often conducted with moisture absorption tests [20]. In this case, the decrease in T_g and presence of these broad transition peaks suggests certain degree of molecular alignment and the tendency of the largely flexible chain to attain equilibrium. The fiber samples mimic a plasticized system. The observed plasticizing effect implies an increase in free volume and segmental movements, where consequently, water molecules can easily diffuse. These results could provide attractive opportunities to the use of pullulan fiber systems and GCSM/pullulan composites in edible systems and drug delivery applications. It is to be noted that the exothermic transitions presented in Figure 3b occur all at the same temperature, the thermal history of the polymer due to fiber processing has been erased and now the bulk systems present a transition similar to the one obtained for the raw pullulan.

Figure 3: Dynamic scanning calorimetry thermo grams for the pullulan in bulk state (powder), and fiber form for pullulan and its GCSM composites. Figures 3a and 3b, show first heating and first cooling, respectively, of the analyzed fibers.

Figure 4: Weight loss as a function of temperature for the GCSM, pullulan in powder and fiber form and the two (5 and 8 wt percentage) composite fiber mats.
Thermogravimetric analysis of the developed systems is shown in Figure 4. In this graph, it is clear that fiber samples absorb more humidity compared to the pullulan in powder form as can be seen in the weight loss occurring at around 100°C. The hydrophilicity of the polymer coupled with increased surface area due to fiber formation promotes a higher absorption of humidity, about 10 weight percent for all fiber samples. The fiber samples containing GCSM show a small reduction in thermal stability when compared to the pure pullulan fibers, this driven by the initial weight loss of the GCSM protein occurring at 211°C and attributed to rapid decomposition of small molecules (glycerol and protein domains) [20,21]. The main degradation occurs at 342°C and 347°C for the pullulan powder and pullulan fibers, respectively; and at 343°C for 5 weight percent and 340°C for 8 weight percent GCSM composite fibers.

Conclusion

Developing of nano and submicron fibers containing GCSM protein opens an opportunity to better utilize this abundant source of protein. Fine fibers containing pullulan as the polymer precursor reinforced with GCSM protein were developed. The developed pullulan and pullulan/GCSM solutions showed ease of fiber processing at angular velocities in between 5500 and 8000RPM. The collected fibers were homogenous, long and continuous, with average diameters in the range of 650nm. Fine fiber diameters such as less than 100nm were also obtained though not is sufficiently high yield as to promote practical applications. The PLL/GCSM composite fibers showed a decrease in Tg when compared to the pullulan in its powder form. This decrease is attributed to induced alignment of the pullulan molecules. Further studies will focus on the viability of using these fibers as products with high nutritional protein content for human consumption and other potential applications such as in drug delivery and novel snacks to mention some.

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