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Turning Waste into Fiber: Creating a Sustainable Alternative with Casein Protein from Expired Milk

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SUMMARY

The milk and clothing industries produce substantial waste. However, this wasted or expired milk can be reused due to its composition, specifically a milk protein called casein. The milk fiber created from casein is a sustainable alternative to other textiles. This research aims to find the optimal process for making a strong milk fiber. Following environmentally friendly steps in creating the milk fiber, various additives were used in the dope for fiber extrusion. These additives or predictors, assigned with different weight concentrations, include casein powder, cellulose nanofibril, beeswax, and corn protein zein. An orthogonal array-based maximin design was identified for this project. Experiments were conducted following this plan. Qualitative observations of the extruded fiber were recorded and classified as an ordinal categorical response with three levels for strength. Under ordinal logistic regression, beeswax and casein have a greater effect on the strength, while cellulose nanofibrils exhibit a quadratic relationship with the strength of the fiber.

Keywords: Beeswax; Casein; Cellulose Nanofibrils; Ordinal Logistic Regression; Orthogonal Arrays.

1. INTRODUCTION

Reducing waste is an ongoing goal. Food and clothing production are among the major industries that generate significant waste. Dairy farmers in the United States are forced to dump as much as 3.7 million gallons of milk every day (Townley, 2020). From the fashion industry, an estimated 17 million tons of textile waste ended up in landfills in 2018 (Agency, 2021). Due to these extensive landfills, advocates are emphasizing the importance of recycling milk and creating sustainable clothing with shorter decomposition times. These environmental issues give rise to research on milk fiber as a sustainable alternative for clothing textiles. The milk protein called casein, extracted from waste or expired milk, is the primary constituent of milk fiber (Di Ciancia, 2017). Milk fiber has been developed since the early 21st century (Domske, 2013); however, the fiber spinning process involved formulations that were harmful to the environment, thereby prohibiting the sustainability of this type of natural fiber. To combat the ongoing urgent issues of milk waste and environmental concerns, researchers are seeking a more sustainable milk fiber spinning process (Bier *et al.,* 2016). The milk fiber is created through a spinning process that begins with mixing a dope, or mixture, containing casein and other additives, then aging the dope, and subsequently extruding the fiber from the dope for further processing.

This research aims to optimize the fiber spinning process by using statistical methods to analyze which additives in the dope are most effective in producing strong milk fiber. Additionally, this research employs sustainable methods to produce the fiber. Previous research on milk fiber has focused on observations of the created milk fiber using different additives;

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however, the statistical analysis of the additives' effects has not been examined. The other additives and their corresponding weight concentrations (%w/w) in the total weight of the dope can characterize the resulting milk fiber. The chosen additives are protein casein, cellulose nanofibril, beeswax, and corn protein zein. Cellulose nanofibrils are a bio-based reinforcement that enhances the stiffness of fibers (Nechyporchuk & Kohnke, 2019). Beeswax is considered for its fiber-stabilizing properties (Bier *et al.,* 2017), and corn protein zein aids the fiber's elasticity and resistance to heat (Zhang *et al.,* 1997). By applying an optimal design, this research aims to investigate the effects of the four additives in the dope on the strength of the milk fiber.

2. MATERIALS AND METHODS

2.1 Materials

The following materials are needed to proceed with the experiment. Monoject 6ml syringes are used for loading the dope and extruding the fiber. The extruded fiber is dried on a PTFE Teflon sheet. To measure the pH of the dope and control its pH, a pH meter is utilized. Glass beakers, glass stirring rods, a stirring magnet, and a hot plate stirrer are used to prepare the dope before loading it into the syringe. To make the dope, casein powder from bovine milk, 9% cellulose nanofibrils, beeswax, and corn protein zein powder are added according to their assigned concentration for each experimental trial. Glycerol acts as a plasticizer for fiber formation at a concentration of 50% (Domske, 2013). Sodium hydroxide ensures a pH above 6.4, at which point casein becomes soluble in water (Bier *et al.,* 2017).

Casein powder is ground into a finer powder using a blender and filtered through a 200-micrometer sieve. Cellulose nanofibrils, beeswax, and corn protein zein are chosen for the experiment based on their properties that can influence the characteristics of textile fiber. Nanocellulose, also known as cellulose nanofibrils and cellulose nanocrystals, can be extracted from cell walls of different plants, and these nanofibrils are biodegradable and nontoxic (Dufresne, 2017). Studies on applying nanocellulose reinforcement to fiber fabrication are limited; cellulose nanocrystals have been utilized in dope formulation. A possible quadratic relationship between the concentration of cellulose nanocrystals in the dope and maximum tenacity can be observed from the rheological measurements, where the maximum tenacity is greatest when the dope consists of 3.5% and 7.0% cellulose nanocrystals (Nechyporchuk & Kohnke, 2019). In a study using paraffin oil, glucose, and wax in a casein fiber dope formulation, both wax and paraffin oil support water resistance (Bier *et al.,* 2017). Beeswax is a biodegradable alternative to dope formulation for casein fiber. Corn protein zein has been utilized in textile production (Zhang *et al.,* 1997) and is claimed to exhibit good elongation, elastic recovery, and heat resistance(Veatch, 1941).

2.2 Experimental Procedure

- Grind casein powder in a mortar and filter it through a 200-micrometer sieve.
- Prepare a 50% glycerol solution by mixing 5 ml of glycerol with 5 ml of distilled water in a small beaker.
- Combine the designated amount of distilled water for each trial with 4 grams of the 50% glycerol solution in a beaker at room temperature.
- Stir the solution using a glass stirring rod and add the specified amount of casein powder.
- Continue stirring and add the required amounts of cellulose nanofibrils, beeswax, and corn zein powder according to the trial specifications.
- Stir the prepared dope at 500 revolutions per minute (rpm).
- Make a 0.5M Sodium Hydroxide solution by dissolving 0.2 grams of NaOH in 10 ml of distilled water.
- Add 0.3 grams of the 0.5M Sodium Hydroxide solution to the dope.
- Age the dope for 48 hours while spinning at 500 rpm at room temperature.
- After aging, heat the dope for 15 minutes at 75°F.
- Load the dope into a syringe and extrude the fiber onto a Teflon sheet.
- Air-dry the fiber at room temperature for 48 hours.
- Observe the fiber's qualitative characteristics:
- **Crumbling:** Fiber breaks easily and shows visible crumbling parts.

- **Solid:** Fiber holds its shape and breaks without crumbling.
- **Solid-moldable:** Fiber is solid but also flexible enough to bend without breaking.

2.3 Method

The total weight of the dope is set at 15 grams, and the additives are assigned weight concentrations within the total dope. The corresponding grams for each level of the additive are shown in Table 1, and the corresponding weight concentrations of each level are shown in Table 2.

Table 1. Predictors and levels in grams

Predictors	Level 0	Level 1	Level 2
Casein	1.35	2.70	4.05
CNF	0.00	0.15	0.30
Beeswax	0.00	0.60	1.20
Zein	0.00	0.60	1.20

Table 2. Predictors and levels in % w/w

Predictors	Level 0	Level 1	Level 2
Casein	9.0	18.0	27.0
CNF	0.0	1.0	2.0
Beeswax	0.0	2.0	4.0
Zein	0.0	2.0	4.0

Each trial follows a set procedure derived from a literature review. The casein is ground and filtered through a sieve before mixing the dope (Bier *et al.,* 2017). Following suggestions from various research studies, the procedure for this research uses a set amount of 50% glycerol (26% w/w) and sodium hydroxide (2% w/w) to form the fiber (Bier *et al.,* 2017). Subtracting the assigned weight of predictors, 50% glycerol, and sodium hydroxide for each trial from the total weight, this is the amount of distilled water for the dope for that trial. Following each experiment, the observations of the extruded fiber are then recorded, which are categorized as "crumbling", "solid", and "solid-moldable"."Crumbling" means that the fiber does not break clean, and it does not hold its shape."Solid" means that the fiber breaks clean, but it lacks fiber flexibility."Solid-moldable" means that the fiber breaks clean, and it exhibits flexibility or malleability, a characteristic that allows elasticity and elongation for future fiber-making.

The procedure for each trial includes first mixing distilled water and 50% glycerol with the assigned level of the predictors at 500 revolutions per minute (rpm) and then adding sodium hydroxide during mixing. After the dope is aged for 48 hours of incubation while spun at 500 rpm at room temperature, the dope is heated at 75°F for fifteen minutes before extrusion. Then, the dope is hand extruded and air-dried at room temperature for 48 hours. Observations of the extruded fiber or matter are then recorded. After the data collection, it is manually entered into the statistical software R. The independent variables, or predictors, are casein, cellulose nanofibrils, beeswax, and zein, and they are treated as continuous variables since these variables can take any value from zero grams to a set, limited amount in grams. The response or dependent variable is categorical with three levels: "crumbling", "solid", and "solid-moldable". They are ranked from fiber that was crumbling being the least desirable to fiber that was solid-moldable being the most desirable ("crumbling" <"solid" <"solid-moldable").Since the response is an ordered categorical variable, an ordinal logistic regression model (proportional odds model) will be fitted to see the effects of the independent variables on the strength of the fiber.

2.4 DESIGN OF EXPERIMENT

This experiment utilizes orthogonal arrays (Hedayat *et al.,* 1999) with three levels and strength two, as there are three levels for each of the factors: casein, cellulose nanofibrils, beeswax, and zein. With limited resources, an efficient experimental design was employed to extract valuable information from the data with a minimal number of runs. As shown in Table 1, this experiment has four factors, each at three levels. We considered an orthogonal array of strength 2 with 18 runs. Sloane (http://neilsloane.com/oadir/) provides two suitable designs that accommodate four factors.

Since the experiment has only four factors, four columns of orthogonal arrays need to be chosen. It is desirable to have the design points or level combinations spread out in the design space for better exploration of the design space. Hence, the maximin distance criterion is used to measure the space-filling property of an experiment design (Jin *et al.,* 2005). The maximin distance criterion is evaluated by a scalar φ_p (Morris & Mitchell, 1995) as follows:

$$\varphi_p = \left\{ \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} d_q\left(x_i, x_j\right)^{-p} \right\}^{\frac{1}{p}}, \tag{1}$$

where n is the total number of design points (that is, the number of rows of the orthogonal array), $1 \leq i < j \leq n$, and m represents the number of columns for each combination. In equation (1), $d_q(x_i, x_j)$ measures the distance between the i^{th} and j^{th} design points. It is given by

$$d_q\left(x_i, x_j\right) = \left\{ \sum_{k=1}^{m} \left| x_{ik} - x_{jk} \right|^q \right\}^{\frac{1}{q}}.$$

Here, x_{ik} represents the entry at the ith row and kth column of the orthogonal array. Each term of $d_q(x_i, x_j)$ measures the distance between two rows of the same column. For this experiment, $n = 18$ and $m = 4$. In this formulation, q is an integer with values 1 and 2, representing the Manhattan distance and Euclide an distance, respectively. Finally, p is a tuning parameter, and as p approaches infinity, the φ_p criterion is asymptotically equivalent to the maximin distance criterion. In our experience, $p = 15$ is sufficiently large for this approximation to be accurate. Minimizing the φ_p criterion maximizes the distance between the design points.

Table 3. 4-Factor combinations and maximin distance criterion

Combinations of Arrays	φ_p	
	Manhattan Distance	Euclidean Distance
2,3,4,6 (ugly version)	0.6127581	0.8694688
2,3,4,5 (ugly version)	0.6127581	0.8694688
2,4,6,7 (ugly version)	0.6127581	0.8694688
2,5,6,7 (ugly version)	0.6127581	0.8694688
3,4,5,7 (ugly version)	0.6127581	0.8694688
3,5,6,7 (ugly version)	0.6127581	0.8694688
2,3,4,5 (regular version)	0.6181835	0.8768515

For each of the two orthogonal arrays considered here, since there are seven columns in each version, there are thirty-five combinations of four columns to be considered. The criterion is evaluated for every combination to identify the optimal design. There were some extra physical constraints for this experiment. To avoid the dope being too viscous or too dry, the following combinations of levels for the trials should be avoided: (0,0,0,0), (0,1,0,0), (0,2,0,0), (2,2,2,2), (2,1,2,2), (2,0,2,2). As shown in Table 3, the combination of arrays with the smallest criterion corresponds to $\varphi_p \approx 0.6128$ using Manhattan distance and $\varphi_p \approx 0.8696$ using

Table 4. From Sloane Orthogonal Array oa.18.7.3.2. ugly after permutation

Vector2	Vector3	Vector4	Vector 6
0	0	2	0
2	2	1	2
1	1	0	1
0	2	0	1
2	1	2	0
1	0	1	2
0	1	1	0
2	0	0	2
1	2	2	1
0	1	0	2
2	0	2	1
1	2	1	0
0	0	1	1
2	2	0	0
1	1	2	2
0	2	2	2
2	1	1	1
1	0	0	0

Table 5. Experimental trials (in grams)

Trial	Casein	CNF	Beeswax	Zein	0.5M NaOH (2% w/w)	50% Glycerol (30% w/w)	Distilled Water
1	1.35	0.00	1.20	0.00	0.30	4.00	8.15
2	4.05	0.30	0.60	1.20	0.30	4.00	4.55
3	2.70	0.15	0.00	0.60	0.30	4.00	7.55
4	1.35	0.30	0.00	0.60	0.30	4.00	8.45
5	4.05	0.15	1.20	0.00	0.30	4.00	5.30
6	2.70	0.00	0.60	1.20	0.30	4.00	6.20
7	1.35	0.15	0.60	0.00	0.30	4.00	8.60
8	4.05	0.00	0.00	1.20	0.30	4.00	5.45
9	2.70	0.30	1.20	0.60	0.30	4.00	5.90
10	1.35	0.15	0.00	1.20	0.30	4.00	8.00
11	4.05	0.00	1.20	0.60	0.30	4.00	4.85
12	2.70	0.30	0.60	0.00	0.30	4.00	7.10
13	1.35	0.00	0.60	0.60	0.30	4.00	8.15
14	4.05	0.30	0.00	0.00	0.30	4.00	6.35
15	2.70	0.15	1.20	1.20	0.30	4.00	5.45
16	1.35	0.30	1.20	1.20	0.30	4.00	6.65
17	4.05	0.15	0.60	0.60	0.30	4.00	5.30
18	2.70	0.00	0.00	0.00	0.30	4.00	8.00

Euclidean distance. Therefore, the selected four array combinations are vector 2 for the factor casein, vector 3 for cellulose nanofibril, vector 4 for beeswax, and vector 6 for zein from the ugly version, and the columns are permuted with level 1 to 2, level 2 and to 1, and level 0 to 0. The level combinations after permutation are shown in Table 4, and Table 5 shows the weight in grams for the constituents in each trial. Trials are randomized before conducting the experiment and are not directly listed in Table 5.

3. RESULTS

Before fitting the ordinal logistic regression model, we note that the dependent variable is measured on an ordinal scale, meaning the levels of the response are ranked. The ranking for this research response is "crumbling", "solid", and "solid-moldable" in ascending order. To have a better understanding of the categorical responses, Figure 1(b) shows an extruded fiber with a "crumbling" characteristic. Figure 1(c) shows a fiber with "solid" characteristics, and the extruded fiber in Figure 1(d) is classified in the "solid-moldable" category. To have a fiber with a "solid-moldable" characteristic, the dope for making the fiber should have a honey-like viscosity, as shown in its visual representation in Figure 1(a).

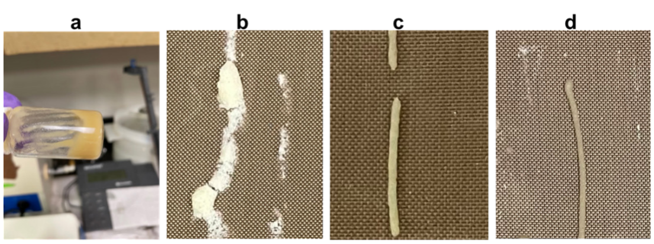

Fig. 1. Sample of dope and a visual representation of the categorical responses

To check the assumptions for the ordinal logistic model, the independent variables must be either continuous, categorical, or ordinal. In this case, the four independent variables stated in the experiment are treated as continuous variables. No multicollinearity has been detected between independent variables. After fitting the ordinal logistic model, the Brant Test was used to check the validity of the proportional odds model assumptions, for which the null hypothesis states that each independent variable has an identical effect at each cumulative split of the ordinal dependent variable. We used the function **polr** from the **MASS** package in R to fit our model, which following the modeling convention of **polr**, the following ordinal regression is parametrized as:

$$\text{logit}\,(P(Y \le j)) = \alpha_j - (\beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4)\ , \quad j=1,2$$

$$\text{where log it}\,(P(Y \le j)) = \log\left(\frac{P(Y \le j)}{1 - P(Y \le j)}\right).$$

Our model has different intercepts for different categories of the response, but independent variables influence the response in the same way, as coefficient *β's* do not change across the groups. Logit, or logit ($P(Y \le j)$), is the log odds. To identify the odds, a transformation using the exponential constant is applied for interpretations. The explanatory variables are x_1 for case in powder, x_2 for cellulose nanofibril, x_3 for beeswax, and x_4 for corn protein zein. Here, α_j when $j = 1$ is the threshold for comparing "crumbling" to "solid", and when $j = 2$, it is the threshold for comparing "solid" to "solid-moldable". In this case, β_1 is the log-odds coefficient for casein, and the odds ratio for casein is calculated as $\exp(\beta_1)$. *Similarly,* β_2 is the log-odds coefficient for cellulose nanofibrils, and the odds ratio is calculated as $\exp(\beta_2)$*;* β_3 is the log-odds coefficient for beeswax, and the odds ratio is $\exp(\beta_3)$*;* and β_4 is the log-odds coefficient for zein, and the odds ratio is $\exp(\beta_4)$. The estimated coefficients are shown in equations (2) and (3).The odds ratios and calculated p-value for each coefficient is shown in the summary output of the fitted model in Table 6. Following the summary output from R, the estimated models are:

$$\text{logit}(P(\hat{Y} \le 1)) = -5.2683 + 1.735\,x_1 + 2.505\,x_2 - 2.606\,x_3 + 2.160\,x_4, \quad (2)$$

$$\text{logit}(P(\hat{Y} \le 2)) = -1.8544 + 1.735\,x_1 + 2.505\,x_2 - 2.606\,x_3 + 2.160\,x_4. \quad (3)$$

The independent variables in the logit model, as described in Table 6, are **casein**, **cellulose nanofibrils (CNF), beeswax**, and **zein**. These variables represent the additives used in the dope mixture for producing milk fiber, and their effects on the fiber's strength are analyzed using an ordinal logistic model. We provide a brief explanation of each variable for clarity: Casein (x_1) is the primary protein extracted from expired milk and serves as the base material for milk fiber production;

cellulose nanofibrils(x_2) are bio-based reinforcement material derived from plant cell walls, enhancing the stiffness and mechanical properties of fibers. Beeswax (x_3) is a biodegradable additive that supports fiber stability and water resistance. Zein (x_4) is a corn protein that contributes to the fiber's elasticity and heat resistance.

Table 6. Estimated coefficients of the statistical model

Parameters	Estimate	Std. error	Z value	P-value	Odds Ratio
casein(β_1)	-1.735	0.7252	-2.3920	0.0168	0.1765
cellulose nanofibrils(β_2)	-2.505	4.7352	-0.5289	0.5968	0.0817
beeswax(β_3)	2.606	1.3214	1.9718	0.0486	13.5385
zein(β_4)	-2.160	1.2853	-1.6806	0.0928	0.1153
crumbling|solid (α_1)	-5.268	2.4081	-2.1878	0.0287	
solid|solid-moldable(α_2)	-1.854	2.0202	-0.9179	0.3587	

The Brant test indicated that the proportional-odds assumption of our fitted model was not violated (p-value> 0.05). Whether the predictors are statistically significant is determined by a significance level of 0.05. If the corresponding p-value for the predictor is less than the significance level, then the predictor is useful for predicting the strength of the fiber. Casein (p-value of 0.0168) and beeswax (p-value of 0.0486) were statistically significant, whereas cellulose nanofibrils (p-value of 0.5968) and zein (p-value of 0.0928) were not. In this ordinal logistic model, a positive coefficient increases the log-odds of being in a higher (more solid or solid-moldable) category, and a negative coefficient decreases those odds. We report odds ratios (ORs) for interpretability. For every 1-gram increase in casein powder, the odds of being in a higher category (solid or solid-moldable vs. crumbling) are multiplied by 0.1765 (i.e., decrease by about 82%), holding other variables constant. For every 1-gram increase in beeswax, the odds are multiplied by 13.5384 (i.e., about a 13.5-fold increase), holding other variables constant.

Precedent research suggests a possible quadratic relationship between cellulose nanofibrils and the strength of milk fiber, based on the observed qualitative data (Nechyporchuk & Kohnke, 2019). Thus, a quadratic term of cellulose nanofibrils is added to test whether it is statistically significant. Following the modeling convention of the function **polr**, the new ordinal regression is parameterized as:

$$\text{logit}\,(P(Y \leq j)) = \alpha_j - (\beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_5 x_2^2),\ j=1,2$$

where x_2^2 is the quadratic term of cellulose nanofibrils, and β_5 is the log odds of the term. The estimated coefficients and the corresponding p-value for the significance of the statistical model are shown in Table 7. The Brant test indicated that the proportional-odds assumption of our newly fitted model was not violated (p-value > 0.05). After the quadratic term of cellulose nanofibrils is added, if a significance level is assigned at 0.1, then there exists a relationship between the quadratic term of cellulose nanofibrils and the strength of the fiber.

The inclusion of the quadratic term allows the model to capture a potential nonlinear (inverted-U-shaped) relationship between CNF content and fiber strength. When the quadratic term of cellulose nanofibrils I(CNF^2) was added, the significance levels of other predictors changed slightly (Table 7). This behavior is expected because CNF and CNF^2 are mathematically related, and introducing both terms increases multicollinearity and increases the standard errors of the coefficients. Consequently, the p-value of beeswax increased above 0.05, although the direction and magnitude of its effect remained similar to that in the model without the quadratic term (Table 6). The casein variable continued to be significant (p-value of 0.0257) under a significance level of 0.05, indicating that its effect on fiber strength is consistent regardless of the functional form of CNF. The inclusion of the quadratic CNF term thus improves model flexibility but reduces the apparent precision of other estimates due to the shared variance among predictors.

Table 7. Estimated coefficients of the statistical model with quadratic term of cellulose nanofibrils (CNF)

Parameters	Estimate	Std. error	Z value	P-value
casein(β_1)	-2.729	1.2234	-2.2309	0.0257
cellulose nanofibril(β_2)	41.633	23.2922	1.7874	0.0739
beeswax(β_3)	3.915	2.0382	1.9209	0.0547
zein(β_4)	-3.358	2.0056	-1.6745	0.0940
I(CNF^2)(β_5)	-153.257	81.1059	-1.8896	0.0588
crumbling|solid(α_1)	-6.906	3.5761	-1.9312	0.0535
solid|solid-moldable(α_2)	-2.509	2.8971	-0.8660	0.3865

4. CONCLUSION

Utilizing an optimal design, this research examines the impact of various additives on the strength of milk fiber. The effective constituents include casein, beeswax, and cellulose nanofibrils. Holding other variables constant, a one-gram increase in casein powder reduced the odds of the fiber being in a higher solidity category (solid or solid-moldable vs. crumbling) by approximately 82% (odds ratio approximately 0.18). In contrast, a one-gram increase in beeswax increased those odds by about 13.5-fold, indicating a much stronger effect. The substantially higher odds ratio for beeswax suggests that a smaller amount of beeswax is sufficient to increase fiber strength and achieve a solid or solid-moldable texture, compared with casein.

After fitting the model with a quadratic term for cellulose nanofibrils, although we noted that a quadratic relationship exists between cellulose nanofibrils and the fiber strength, the evidence is not as strong as we expected. A modified study with additional trials at various levels outside the range of variables considered here should be conducted next to obtain more substantial evidence for this quadratic relationship. Through analyzing the milk fiber data using statistical methods, we have evidence to limit and use appropriate constituents in the dope mixture for further investigation of the milk fiber strength. Using a lower weight concentration of beeswax, such as around 1 to 2 % w/w, and paying attention to cellulose nanofibrils weight concentration, would be reasonable since too much or too little compared to the total weight concentration can influence the strength of the fiber.

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