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INVESTIGATING NITROGEN SOURCES FOR BACTERIAL CELLULOSE BASED VEGAN LEATHER

Chehak Bansal [1], Divyansha Sharma [2], Nidhi Sisodia [3], Chanchal [4]

[1] Research Scholar, Department of Fabric & Apparel Science, Institute of Home Economics, University of Delhi, New Delhi, India
[2] Assistant Professor, Department of Fabric & Apparel Science, Institute of Home Economics, University of Delhi, New Delhi, India
[3] Senior Scientist, Northern India Textile Research Association, Ghaziabad, India
[4] Professor, Department of Fabric & Apparel Science, Institute of Home Economics, University of Delhi, New Delhi, India

Corresponding Author
Chehak Bansal,
chehak.bansal@ihe.du.ac.in

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ABSTRACT

Background: A vegan alternative to leather is a novel concept, and substitutes are coming up from the most unlikely sources, like mushroom caps, pineapple, seaweed, etc. Bacterial cellulose is a leather-like material produced during the production of kombucha. The aim of the present study was to evaluate the proper conditions for the growth of bacterial cellulose and its after treatments for making vegan leather.
Methods: For effective biosynthesis of bacterial cellulose, a sufficient amount of carbon and nitrogen is required. Tea, coffee, and dried orange peels were taken as nitrogen sources, while various concentrations of edible crystal sugar were taken as carbon sources and were evaluated to obtain the best results. After treatment of bacterial cellulosic layers was done using various methods, results were evaluated. After drying the bacterial cellulosic layer, vegan leather was obtained. Functional properties of vegan leather were tested as per industry standards and compared on the basis of various nitrogen sources used for production.
Result and Conclusion: The present study indicated that vegan leather made from tea as a nitrogen source had the highest tensile strength, the lowest thickness, and very good water repellence. All three, tea, coffee, and sugar, can be used as good nitrogen sources for the propagation of bacterial cellulose. All of these could be used as economic as well as eco-friendly sources for making vegan leather.

Keywords: Bio- Material, Kombucha, Leather, Microbial Cellulose, Sustainable

1. INTRODUCTION

Consumer attitudes toward sustainable purchase have changed as a result of the recent surge in concerns about climate change, resource scarcity, ecosystem overuse, and pollution from non-biodegradable or hazardous materials. This entire scenario has impacted the consumer goods business as well as the developers of novel materials who aim to substitute fossil-based materials with naturally produced and fully biodegradable materials that are animal-free and do not contain any toxic elements. Ideally, sawdust, organic matter, or household waste is used to make the new materials (Meyer et al., 2021).

The heritage of leather is extensive and vivid. Its usage goes back to pre-historic times when people used to wrap raw animal hides as clothes and protect themselves from external environment. With time, advances in the leather production and processing took place and became a sequence of complex mechanical processes and chemical reactions (liming, batting, tanning, etc.). But these processes also became toxic with evolving times and proved to be really harmful for the environment. Hence, there aroused a need to find alternatives for leather which are both sustainable and environment friendly which led to the invention of various types of vegan leather. There are many companies which are producing plant-based leather from various parts of plants. Researchers are also trying to grow leather in lab to make leather processing an eco- friendly process (Fernandes et al., 2019).

Bacterial cellulose (BC), a natural polymer that comes from microorganisms, is safe for the environment and has gotten a lot of attention as a possible future material because of its unique mechanical and physicochemical properties. Bioprinting, cosmetics, synthetic paper, biosensors, antimicrobial wound dressings, and cosmetics are just a few of the applications of bacterial cellulose (Mohammad et al., 2014). Malai.eco is an Indian brand which is working forward to produce vegan leather from bacterial cellulose using coconut water as a propagation medium for cellulose producing bacteria. Komagatiebacter xylinus (also known as Acetobacter xylius) is used for biosynthesis of cellulose. Traditionally, Hestrin-Schramm (HS) medium was used for production of bio cellulose in laboratory, but due to its high-cost researchers are trying to find economical mediums on which these bacteria could be grown (Wood et al., 2023). Some studies used sour whey for the development for bacterial cellulose (Nguyen et al., 2022) while other used waste apple juice extract as a medium (Saha et al., 2020). Cellulose synthesizing bacteria grow rapidly when given the sufficient amount of carbon and nitrogen along with proper temperature and pH. Therefore, this study will aim at extracting various carbon and nitrogen rich mediums for the fabrication of bacterial cellulosic leather and assess its functional properties with respect to product development. The objectives of the study could be understood as follows:

- To identify various biomaterials for propagation of bacterial cellulose
- To develop and optimize recipe for vegan leather using different mediums
- To assess the functional properties of vegan leather

2. MATERIALS AND METHODS

The present study was entitled to find sustainable alternative to animal leather in the form of vegan leather produced by bio-cellulose.

2.1. MATERIAL COLLECTION

A Kombucha starter pack was bought online from Amazon.com as a bacteria source. It consists of SCOBY which stands for Symbiotic Culture of Bacteria and Yeast, which leads to fermentation of media and formation of cellulosic layer. As carbon source, crystal sugar was brought from local market. For nitrogen source, three different substrates were used: (1.) a packet of TATA tea was brought from local market, (2.) Nestle coffee powder pack was bought from local market, and (3.) oranges were brought from local market and its peels were sun dried and powdered in a mixer grinder.

2.2. METHODOLOGY

The methodology of the present study is divided into three phases:

Phase I: To identify biomaterials and propagation of bacteria

To identify various biomaterials for propagation of bacterial cellulose as nitrogen source, a culture media was prepared that consist of a nitrogen source, a carbon source and kombucha tea as innoculum. As nitrogen source, tea, coffee, and dried orange peels were taken and boiled in water separately for 1 hour. Sugar was dissolved separately in water and added into the three solutions. Little amount of kombucha was introduced as starter and kept in incubator for 14 days.

Phase II: Recipe Optimization

To develop and optimize the recipe for vegan leather production using different mediums, carbon source was taken as variables. Three concentrations of sugar (carbon source) were taken into consideration i.e., 10%, 20% and 30% with respect to volume of the media and the results were observed after 14 days. After the formation of bio-cellulosic layer, three methods of after-treatments were employed to neutralize the bio-cellulosic layer as it was produced from bacteria. First method was treatment with NaOH. Bio-cellulosic layer obtained was boiled in 0.5% NaOH solution for one hour. Second method was treatment of bio-cellulosic layer with propanol. For this, two methods were employed, one was boiling the layer in propanol for 40mins. Another method was to leave the bio-cellulosic layer overnight in propanol. Both the methods gave same results. Third method of after-treatment was to boil the bio-cellulosic layer in 0.5% NaCl solution for one hour.

Phase III: Assessment of functional properties

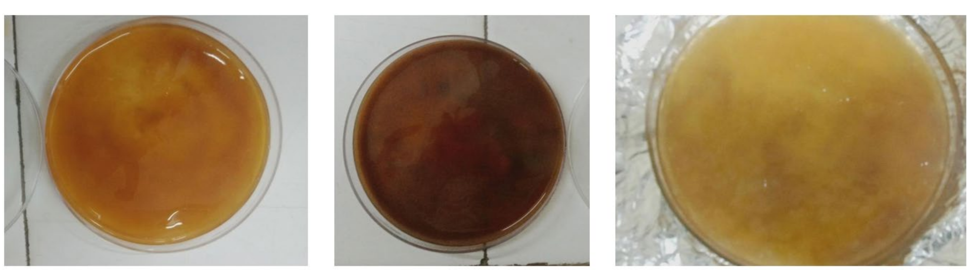
To assess the functional properties of vegan leather obtained from all three sources of nitrogen, tests like thickness, tensile strength, water repellency, and flexing endurance were performed. Thickness was measured using Dial Micrometer Gauge, according to standard BS EN ISO 2589:2016, Testometric Tensile Strength Tester was used to measure tensile strength as per standard BS EN ISO 3376:2020, Surface Wetting Spray Tester was used to analyze the water repellency of vegan leather as per standard BS EN ISO 9865:1991.

3. RESULT AND DISCUSSION

In the first phase of the study, three kinds of biomaterials were used, i.e., tea leaves, coffee, and orange peel as potential nitrogen sources for the growth of bacterial cellulose. All the three sources showed good support to cellulose synthesis capacity of Komagataeibacter xylinus. After 14 days, all three-culture media kept in the incubator produced an approximately 0.4 mm thick layer of cellulose as shown in Figure 1.

Figure 1 Bio- Cellulosic Layer Above Culture Media After 14days

In the previous studies, it was found that thickness of cellulose layer varied depending on the pH and temperature of the tea. Specifically, it was found that a pH of 4.5 and a temperature of 28oC resulted in the thickest cellulose layer, with the thickness of approximately 360μm (Lahiri et al., 2021). In this study, almost same results were obtained at same conditions in all the three mediums. It was observed that all the cellulosic layers had the colour of the medium on which they were grown, as in Figure 2. The observed phenomenon is likely due to the absorption of the medium's colour by the cellulosic layers. Cellulose is generally colourless and transparent, but it can take on the colour of substances it encounters. When cellulose is grown in a medium, it absorbs the components of the medium, including any pigments or dyes that give the medium its colour. Hence, the cellulosic layers take on the same color as the medium.



(a) (b) (c)

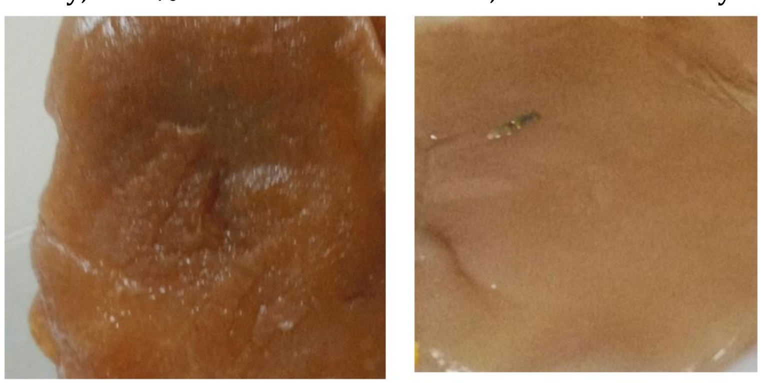
Figure 2 Bio-Cellulosic Layer Produced in Different Mediums (A)Tea, (B)Coffee, and (c)Orange Peel

In the second phase of the study, it was observed that sugar concentration affects the bio-cellulose production, and after testing, the best results were obtained at 20% sugar concentration, compared to both 10% and 30%. The results are summarised in Table 1. One possible reason is that at lower sugar levels, the bacteria may not receive enough glucose to synthesize cellulose effectively, which slows their growth and reduces yield. In addition, low sugar concentration can also lead to changes in the pH of the fermentation medium. Kombucha fermentation is a difficult process that makes lots of different organic acids. These acids can include lactic acid, acetic acid, and many other acids like gluconic acid. The medium's pH may be lowered by these acids, which can inhibit bacterial growth and bio-cellulose production. When the sugar concentration is low, this effect is more pronounced as it limits the buffer capacity of the bacteria against the acidification of the medium. Likewise, high sugar concentration has an adverse effect on bio-cellulose growth as it interferes with the osmotic equilibrium in the growth medium. If the sugar concentration is too high, it increases osmotic pressure, which hinders the ability of the bacteria to take up nutrients from the medium (Molina-Ramírez et al., 2017). All in all, the optimal sugar concentration for bio-cellulose production in Kombucha is between too high and too low. The study found 20% with respect to the volume of the culture media to be optimum.

Table 1 Weight of Bacterial Cellulose Layer at Different Sugar Concentrations

Nitrogen source used	Weight obtained at 10% sugar (in grams)	Weight obtained at 20% sugar (in grams)	Weight obtrained at 30% sugar (in grams)
Tea	26.30	34.09	26.96
Coffee	27.68	34.04	24.24
Orange peel	27.04	34.65	25.95

As a bio-cellulosic layer was produced from the bacteria, it is essential to neutralise it by means of boiling or chemical treatment. Three methods were used to treat the vegan leather afterwards. One way was to soak the bio-cellulosic layer in a 0.5% NaOH solution for an hour. Boiling in NaOH makes the layer smoother in appearance and lighter in colour (Figure 3). NaOH is a strong base that can break down the structure of bio-cellulose or at least part of it by disruption of hydrogen bonding between cellulose fibres. NaOH treatment increases bio-cellulose surface area by partial dissolution of the outer layer, thereby improving the absorption capacity and biocompatibility. This process will bring in functional groups like carboxyl (–COOH) and hydroxyl (–OH), which may further undergo chemical modification. On the other hand, NaOH in higher quantities or under longer exposure conditions can completely dissolve bio-cellulose. So, you have to control the concentration and duration of NaOH carefully to avoid damage to the bio-cellulose structure (Choi & Shin, 2020). When exposed to NaOH, bio cellulose can take on a lighter colour or sometimes even turn white. The reason for this is that cellulose's hydrolysis destroys the chromophore groups that cause bio-cellulose to be coloured. The amount of NaOH used determines how much the colour will change. This colour change will generally become more prominent in higher concentrations. In this study, a 0.5% NaOH solution was used, which caused only a slight loss of colour.



Before After

Figure 3 Effect of NaOH Treatment on Bio-Cellulose

Another method used for after-treatment was treatment with propanol. When propanol is added to the bacterial cellulosic layer, it acts as a solvent and causes the bio-cellulose to swell, which changes the physical structure of the layer. This results in changes in thickness and porosity of the bio-cellulosic layer, which can have implications on its mechanical properties and potential applications (Revin et al., 2022).

The third method used for after-treatment was boiling the bio-cellulosic layer in 0.5% NaCl solution for one hour. Since the concentration of NaCl used was low, it did not affect the physical structure and colour of the cellulosic layer. When the concentration of NaCl is high, it causes the aggregation and densification of bio-cellulose membrane and therefore affects its porosity and mechanical behaviour (Jiang et al., 2015).

Final Optimized recipe: Based on above observations, a recipe was concluded in which 20% of sugar with respect to volume of culture media was used, as it was optimum concentration which supported the good growth of bacterial cellulosic layer. 10ml kombucha was added as starter in 250ml of culture media. The layer was extracted after 14 days. For after treatment, sample was boiled in 0.5% NaOH, which helps in increasing the surface area. Both the other methods increase water retention of the cellulosic layers, and which was not required for this study.

In the third phase of the study, functional properties of the developed vegan leather were evaluated. The thickness of the bio-cellulosic layer obtained from the medium was approximately 0.4mm, but upon drying, the value decreased significantly due to the high-water retention of bio-cellulose, as presented in Table 2. The thickness of the dry state was taken to draw out the inference. As observed, the leather produced from tea has less thickness compared to the leather obtained from coffee and orange peel. This variation is because of the presence of catechins, which are a kind of polyphenol. These catechins can crosslink with the bio-cellulose fibers to form strong bonds that increase the strength of the material (Yu et al., 2014).

Table 2 Thickness of Vegan Leather

Leather	Thickness in wet state (in mm)	Thickness in dry state (in mm)
Tea	0.365	0.073
Coffee	0.627	0.113
Orange Peel	0.480	0.096

All three types of vegan leather were analysed for their tensile strength at a test speed of 25.00 mm/min, with the jaw distance being 50.00 mm. Results indicated that the leather produced from black tea exhibited greater tensile strength than the other two variants, as shown in Table 3. Tea contains certain compounds that can enhance the strength of bio-cellulose. In particular, tea has catechins, a form of polyphenol. These catechins can bind with the bio-cellulose fibres and strengthen the binding capacity, forming strong bonds that increase the strength of the material. Also, tea has high acidity that can also create the most suitable environment for the growth of the bacteria. This will enhance the yield of bio-cellulose and improve the quality of the material (Yu et al., 2014).

Table 3 Tensile Strength of Vegan Leather

Leather	Tensile Strength (in CN)
Tea	97.3
Coffee	40.17
Orange Peel	60.03

Water repellency of vegan leather was tested using spray method (gravimetric method) and the reading obtained was 90 (ISO 4) which shows slight random sticking or wetting of the upper surface. Vegan leather produced have very good water repellency because dried cellulose forms a porous structure that traps air pockets between the fibres. This porous and the hydrophobic nature of cellulose make vegan leather resistant to wetting by water. Also, surface of cellulose fibres is covered with a thin layer of waxy substances called cutin and suberin, which also contribute to the water repelling properties of dried cellulose (Szlek et al., 2022).

Due to very small samples obtained during the research timeline, flexing endurance test could not be performed. As this test is performed to evaluate the durability and resistance to cracking or creasing of leather used in footwear. The end use of leather produced was not decided and layers obtained were also very thin, hence this test could not be performed on vegan leather.

4. SUMMARY AND CONCLUSION

In this study, an exploration was done to observe the feasibility of utilizing kombucha leather as an environment friendly alternative to traditional animal leather. The finding of this study indicates that nitrogen source, sugar concentration and aftertreatments does influence the properties of resulting kombucha leather. It was observed that kombucha leather produced using different nitrogen sources exhibited variations in texture, color and tensile strength. This suggests that the selection of nitrogen source can be tailored to meet specific design and application requirements within the fashion and textile industry. This presents an opportunity to upcycle the waste material like coffee grounds and dried orange peel and lessen the environmental impact.

The research area holds multiple exploration opportunities, such as:

1) A thorough quality check of kombucha leather could be performed to determine its usability in fashion, accessories, and upholstery-making. They may check durability, flexibility, and biodegradability.
2) The environmental effects, carbon footprints, and water consumption of kombucha leather could be evaluated through a life cycle assessment. This may help in comparing the environmental impact of kombucha leather production and use against traditional leather and synthetic alternatives.
3) A study on scalable techniques for mass-producing kombucha leather could be done, which focuses on the consistency, cost efficiency and effective production.
4) A study that explores the consumer preferences and attitudes towards kombucha leather products could also be done.

Kombucha leather has a unique ability to promote sustainable fashion and innovative materials. However, continuous research and development are critical to realise its full potential. With the world increasingly embracing eco-friendly alternatives, it seems that kombucha leather can turn into a highly valuable and viable choice in the transition towards a more sustainable fashion industry.

CONFLICT OF INTERESTS

None.

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REFERENCES

Choi, S. M., & Shin, E. J. (2020). The nanofication and functionalization of bacterial cellulose and its applications. Nanomaterials, 10(3), 406. https://doi.org/10.3390/nano10030406

Fernandes, M., Gama, M., Dourado, F., & Souto, A. P. (2019). Development of novel bacterial cellulose composites for the textile and shoe industry. Microbial Biotechnology, 12(4), 650–661. https://doi.org/10.1111/1751-7915.13387

Jiang, Z., Yi, J., Li, J., He, T., & Hu, C. (2015). Promoting Effect of Sodium Chloride on the Solubilization and Depolymerization of Cellulose from Raw Biomass Materials in Water. ChemSusChem, 8(11), 1901–1907. https://doi.org/10.1002/cssc.201500158

Lahiri, D., Nag, M., Dutta, B., Dey, A., Sarkar, T., Pati, S., Edinur, H. A., Kari, Z. A., Noor, N. H. M., & Ray, R. R. (2021). Bacterial cellulose: production, characterization, and application as antimicrobial agent. International Journal of Molecular Sciences, 22(23), 12984. https://doi.org/10.3390/ijms222312984

Meyer, M., Dietrich, S., Schulz, H., & Mondschein, A. (2021). Comparison of the Technical Performance of Leather, Artificial Leather, and Trendy Alternatives. Coatings, 11(2), 226. https://doi.org/10.3390/coatings11020226

Mohammad, S., Author, C., Abd Rahman, N., Sahaid Khalil, M., & Rozaimah Sheikh Abdullah, S. (2014). An Overview of Biocellulose Production Using Acetobacter xylinum Culture. Advances in Biological Research, 8(6), 307–313. Molina-Ramírez, C., Castro, M., Osorio, M., Torres-Taborda, M., Gómez, B., Zuluaga, R., Gómez, C., Gañán, P., Rojas, O., & Castro, C. (2017). Effect of different carbon sources on bacterial nanocellulose production and structure using the low pH resistant strain Komagataeibacter medellinensis. Materials, 10(6), 639. https://doi.org/10.3390/ma10060639

Nguyen, H. T., Ngwabebhoh, F. A., Saha, N., Zandraa, O., Saha, T., & Saha, P. (2022). Development of novel biocomposites based on the clean production of microbial cellulose from dairy waste (sour whey). Journal of Applied Polymer Science, 139(1), 51433. https://doi.org/10.1002/app.51433

Revin, V. V., Liyaskina, E. V., Parchaykina, M. V., Kuzmenko, T. P., Kurgaeva, I. V., Revin, V. D., & Ullah, M. W. (2022). Bacterial Cellulose-Based Polymer Nanocomposites: A Review. Polymers, 14(21), 4670. https://doi.org/10.3390/polym14214670

Saha, N., Ngwabebhoh, F. A., Nguyen, H. T., & Saha, P. (2020). Environmentally friendly and animal free leather: Fabrication and characterization. 020049. https://doi.org/10.1063/5.0028467

Szlek, D. B., Reynolds, A. M., & Hubbe, M. A. (2022). Hydrophobic molecular treatments of cellulose-based or other polysaccharide barrier layers for sustainable food packaging: A Review. BioResources, 17(2), 3551–3673. https://doi.org/10.15376/biores.17.2.szlek

Wood, J., Verran, J., & Redfern, J. (2023). Bacterial cellulose grown from kombucha: Assessment of textile performance properties using fashion apparel tests. Textile Research Journal, 93(13–14), 3094–3108. https://doi.org/10.1177/00405175231152668

Yu, S., Tsai, M., Lin, B., Lin, C., & Mi, F. (2014). Tea catechins-cross-linked methylcellulose active films for inhibition of light irradiation and lipid peroxidation induced β-carotene degradation. Food Hydrocolloids, 44, 491–505. https://doi.org/10.1016/j.foodhyd.2014.10.022