

Biodegradable film from kombucha SCOBY culture using different types of tea extracts with/without glycerol

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ABSTRACT

The use of single-use plastic goods causes serious pollution in many countries, contributing significantly to environmental waste. This environmental crisis creates a necessity for developing sustainable, biodegradable packaging materials. Furthermore, in the rapidly growing kombucha industry, the symbiotic culture of bacteria as well as yeast produces an additional cellulose layer that is usually discarded, which adds to the waste generation. While previous research has established the use of symbiotic culture of bacteria and yeast (SCOBY) as a biopolymer and demonstrated that plasticizers can improve film flexibility, there was a lack of comparative data on how different tea types impacted the physical, thermal, and mechanical properties and antioxidant activity of SCOBY films after modification with glycerol. Hence, this study aimed to fabricate biodegradable SCOBY films using three different tea extracts ((black (BT), green (GT), and combination (BTGT)) with/without glycerol coating, determine the physical properties (thickness, color, water solubility, degradation rate, and thermal properties) and mechanical properties, and antioxidant capacity (DPPH activity) of the developed SCOBY films. GT produced the thickest SCOBY films (0.9 mm), significantly lighter ($L = 71.34$) and exhibited a greener hue, while BT films were darker ($L = 46.50$). SCOBY films coated with glycerol increased both water solubility (from ~6.6% to ~54%) and biodegradability rate (e.g., GT from 19.81% to 33.50%). This effect is linked to glycerol's hydrophilic nature and its role in forming hydrogen bonds, which enhances water absorption and promotes breakdown. There was a greater degradation rate (19.81%) of GT films than BT (16.00%), which was possibly attributed to the preservative property of tannins in black tea. Uncoated BT films had the highest tensile strength (0.27 MPa) and the elongation at break (34.48%), but tannins were used as natural reinforcing agents. Glycerol coating was a plasticizer, which had a major reduction in tensile strength (e.g., BT decreased from 0.27 MPa to 0.17 MPa) and at the same time an increase in flexibility (elongation). The DPPH antioxidant activity (38.03% uncoated and 59% with glycerol) was also highest in GT films. The activity of DPPH was supported by glycerol, which stabilized and solubilized these antioxidants. To sum up, tea type is the main determinant of color and antioxidant activity, whereas glycerol is the most effective plasticizer and has a considerable influence on solubility, flexibility, and thermal stability.

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1. INTRODUCTION

The traditional, disposable use of plastic containers has brought about extreme environmental problems that have caused great pollution to both landfills and the oceans. Such an environmental crisis calls for an immediate change in attitude to sustainable, biodegradable packaging materials based on renewable sources. Biodegradable films have become the focus of recent research as they naturally break down with the help of microorganisms and create the least possible impact on the environment (Birania et al., 2021).

Kombucha is a fermented, sweetened tea with the

help of a symbiotic culture of bacteria and yeast (SCOBY), which has a slightly sweet, citrusy taste, originally consumed in China or Japan (Abaci et al., 2022; Crum & Lagory, 2016). The SCOBY is very important in the fermentation process, as it transforms the nutrients in the tea into organic acids and other compounds, which later form a new layer of cellulose (Martinez et al., 2018).

The resulting cellulose mat from the SCOBY, commonly called a pellicle or bacterial cellulose (BC), is a high-density network that is produced largely due to *Gluconacetobacter xylinus*, a bacterium known to be robust,

having a large water-binding capacity, and having high potential for application in sustainable materials due to its nanoscale size (Laavanya et al., 2021). BC has higher purity, degree of polymerization and crystallinity, and is structurally finer and unbranched than plant cellulose, as it lacks hemicellulose and lignin (Gagliardi et al., 2025; Tapias et al., 2020). The development of this SCOBY layer that may be discarded by manufacturers is an effective approach that can be used in waste valorization to produce environmentally friendly packaging films (Charoenrak et al., 2023).

Past studies revealed that the kombucha fermentation of BC or SCOBY could be turned into biodegradable films that are compatible with contact food and could be used to preserve the quality of the food product (Cazon et al., 2017; Aduri et al., 2019; Marques-Reyes et al., 2022). Research also indicates that the type of tea used to make kombucha affects the formation of SCOBY, with both black and green tea having certain benefits (Khasnabis et al., 2015; Jayabalan et al., 2008; Hsieh et al., 2021). SCOBY has also been used in non-food applications such as textiles, medical and material (Gagliardi et al., 2025). Regardless of these results, there is still a gap in research that directly compares SCOBY films prepared with other teas, especially in the presence of glycerol as a plasticizer, even though there is uncertainty about how these variables can affect the strength of the film and antioxidant activity. Thus, the gap to be addressed by this study is to evaluate the physical characteristics and radical scavenging capacity of SCOBY films pre-prepared using different types of tea, with or without glycerol.

2. MATERIALS AND METHODS

2.1. Materials

The kombucha starter cultures (SCOBY), black tea (Boh), green tea (Boh), and granulated sugar (sucrose) used in fermentation were obtained locally in Terengganu, Malaysia.

2.2 Preparation of kombucha tea

All equipment was thoroughly cleaned and sanitized to ensure a sterile fermentation environment. One liter of water was boiled at 90°C, and six tea bags (black and/or green tea) were steeped for 15 min (Hsieh et al., 2021). After removing the tea bags, 100 g of sugar was added to the hot tea and dissolved, and the solution was then allowed to cool (Sederaviciute et al., 2019). The cooled tea was transferred to a clean container, inoculated with a freshly grown SCOBY, 60 mL of sour broth, along with 50 g of the cellulosic layer (Neffe et al., 2017). The container was sealed with a cotton cloth and

kept at 21°C to 27°C, away from direct sunlight, for a 14-day fermentation period (Watawana et al., 2015).

2.3 Preparation of kombucha SCOBY films

The kombucha SCOBY formed needs to be pre-treated by washing and immersed in a 1N NaOH solution for 24 h. The SCOBY was then immersed in distilled water overnight, with the washing solution replaced after 1, 2, and 4 h (Marquez-Reyes et al., 2022).

The SCOBY films were dried in a forced convection oven at 55°C for 24 h. The yield was determined by comparing the initial fresh weight to the dry weight after pretreatment. Films were then immersed in 5 mL of glycerol for 30 min and dried again at 55°C until a constant weight was reached. SCOBY films without glycerol were used as a control (Aduri et al., 2019).

2.4 Thickness of SCOBY films

The thickness of the SCOBY films was measured at six different positions using a digital micrometer (Mitutoyo QuantuMike) with a measurement range of 0 to 25 mm and a precision of 0.01 mm. The average thickness (in mm) was calculated for each treatment (Tapias et al., 2023).

2.5 Color analysis

The color of the SCOBY films made from different types of tea was analyzed using a colorimeter (Chroma meter CR-400 Konika Minolta Sensing, Inc., Japan). The CIELAB color parameters L* (lightness, 0 black to 100 white), a* (-green to +red), and b* (-blue to +yellow) were utilized to represent the results (Marquez-Reyes et al., 2022). Prior to measurement, the colorimeter was calibrated using a white tile.

2.6 Water solubility tests

The dried SCOBY films were split into 1×4 cm rectangles, and their initial weight was recorded. The rectangular films were then put in conical tubes containing 50 mL of distilled water and agitated for 24 h. After agitation, the final mass was measured, and the water solubility of the films in water was calculated using Equation 1 (Marquez-Reyes et al., 2022):

$$\text{Solubility (\%)} = [(W_1 - W_2) / W_1] \times 100 \quad \text{Equation 1}$$

Where;

W_1 = The initial dry weight of the film sample before immersion in the water.

W_2 = The final dry weight of the film sample before immersion in the water.

2.7 Biodegradability test

Biodegradability was evaluated using a soil burial method. SCOBY films were cut into 1×2 cm, dried in a desiccator until a constant weight (W_1) was achieved, and then buried in 250 g of soil for 14 days. The samples were weighed at 0, 5, 7, 10, and 14 days. After the burial period, the films were re-dried to a constant weight (W_2). Biodegradability was calculated as percentage weight loss (%W), similar to Equation 1.

2.8 Thermal properties (DSC)

The melting point of SCOBY films was determined using DSC (Q 2000, TA Instrument, USA). Samples weighing between 5.3 and 5.9 mg were heated from 25 to 300°C at a rate of 10°C/min under a nitrogen flow of 50 mL/min. The melting point was defined as the highest endothermic temperature peak (Ramirez et al., 2020; Arteaga et al., 2020).

2.9 Mechanical properties

Tensile strength and elongation at break of the SCOBY films were measured using a Texture Analyzer TA.XT Texture (Stable Microsystem Ltd., U.K.) with a 100 kg load cell. Dried samples were split into rectangles measuring 80×10 mm and secured in the pulling clamp (ATG). Tensile test was carried out at a head speed of 10 mm/sec, with a return distance of 50 mm, a return speed of 10 mm/sec, and a contact force of 5 g. Tensile strength (kPa) and breaking strain were measured, with the measurements being repeated for each of the treatments three times (Peng et al., 2020). The mechanical characteristics of SCOBY films were measured according to ASTM D882-02 /TG.

2.10 Antioxidant activity tests (DPPH radical scavenging activity)

Macerating 0.5 g of bacterial cellulose (BC) films in 10 mL of methanol and incubating them in complete darkness over the course of 24 h were the conditions that determined the free radical-scavenging activity of the SCOBY films (Ashrafi et al., 2018). The solution of 2, 2- diphenyl-1-picrylhydrazyl (DPPH) in methanol (1 mM) was developed by dissolving 11.83 mg of DPPH powder in 30 mL of methanol. The SCOBY films were added to the DPPH solution (1 mL). The solution was left to stand at RT for 30 min, after which the absorbance at 517 nm was measured with a UV-Vis spectrophotometer (UV-1900i Shimadzu, Japan). The absorbance changes were used to obtain the percentage of DPPH radical scavenging activity. It is represented by the following formula in Equation 2 (Marquez-Reyes et al., 2022):

$$\% \text{ of DPPH} = [(A_c - A_s) / A_c] \times 100 \quad \text{Equation 2}$$

Where;

A_c = absorbance of DPPH solution

A_s = absorbance of the sample

3. RESULT AND DISCUSSION

Figure 1 demonstrates the thickness of the SCOBY films prepared using various kinds of tea covered with/without glycerol. Films that SCOBY made with green tea yielded the highest thickness films (0.9 mm), followed by the combination of black and green tea films (0.8 mm), and then black tea films (0.6 mm). This variation could be due to the lower tannin content in green tea, and thus it can undergo fast fermentation. The levels of tannin content in black tea were reported to be 11.76% to 15.14% with green tea samples reported to contain a lower concentration of tannin content of about 3.11% (Bishop et al., 2022; Khasnabis et al., 2015). There was no noticeable difference ($p > 0.05$) in the thickness of the fermented SCOBY films with/without glycerol coating under various tea types (black and/or green tea). The thickness values in this research are significantly higher compared to the same variables in other studies, such as Amarasekara et al. (2020) and Marquez-Reyes et al. (2022), where the range of values lies at about 0.4 mm (Looi et al., 2022).

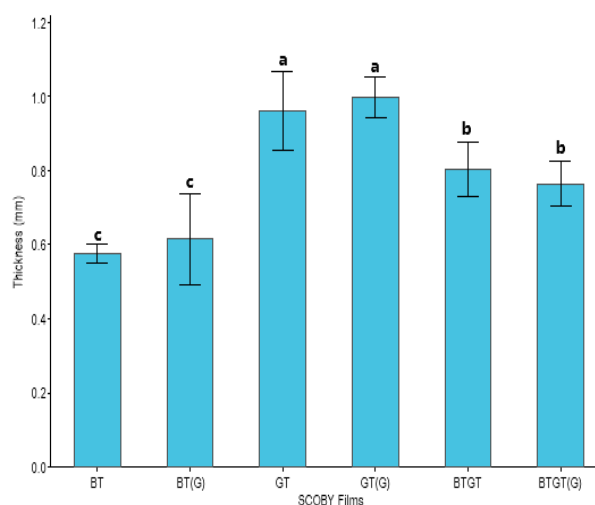


Figure 1: Thickness of SCOBY films made from different types of tea coated with/without glycerol. Values with different letters (a-c) were significantly different ($p < 0.05$).

Where,

- BT = SCOBY film fermented with black tea
- BT(G) = SCOBY film fermented with black tea coated with glycerol
- GT = SCOBY film fermented with green tea
- GT(G) = SCOBY film fermented with green tea coated with glycerol
- BTGT = SCOBY film fermented with black and green tea
- BTGT(G) = SCOBY film fermented with black and green tea coated with glycerol

Table 1 indicates that the color of the SCOBY film produced using various varieties of tea (black tea and/or green tea), with/without glycerol, is significantly different. SCOBY films made out of green tea were much lighter ($L = 71.34$) than the black tea ($L = 46.50$). Such disparity may be attributed to the processing methods and chemical characteristics of the two teas, since black tea undergoes prolonged oxidation, generating dark, intricate polymeric compounds (theaflavins and thearubigins) that are highly chromophoric and are trapped in the BC matrix, thereby reducing the L values. On the other hand, green tea is hardly oxidized, leaving behind the lighter-colored catechin compounds. The small decrease in greenness ($-a^*$) found in the GT films is typical of the remaining chlorophyll and the absence of any oxidized catechin (Peng et al., 2020). By contrast, the brown and yellow shades (Gramza et al., 2016) are typical of the oxidized tea compounds (Bishop et al., 2022). At the same time, the incorporation of glycerol considerably lowered L^* values (lightness) of all SCOBY films, whereas increasing the redness (a^*) and yellowness (b^*), agreeing with its impact on light scattering (Ben et al., 2022). This finding concurs with Marquez-Reyes et al. (2022) and Ashrafi et al. (2018). There was also an increase in the a^* and b^* values of SCOBY films treated with glycerol.

The water solubility of SCOBY films of the various kinds of tea with/without glycerol is also summarized in Table 1. No significant difference ($p > 0.05$) was observed in the water solubility of the films fermented using either type of tea, which might have come up as a result of cellulose fibril formation (Laavanya et al., 2021). A coating of SCOBY with glycerol, however, significantly increased solubility by 6.82% to 50.82% as glycerol is hydrophilic in nature, which enhances water absorption (Arham et al., 2018). Glycerol is a plasticizer that enhances the capacity of the film to hold water (Ulfah et al., 2018). This finding is congruent with Marquez-Reyes et al. (2022), who similarly discovered that solubility increased by 40% when pretreatment agents were applied to SCOBY films.

Table 1: Colors and water solubility of SCOBY films made from different types of tea with/without glycerol.

SCOBY films	L^*	a^*	b^*	Water solubility
BT	46.50 ± 0.77^a	6.70 ± 2.80^b	18.74 ± 2.22^{ab}	6.82 ± 0.43^c
BT (G)	38.91 ± 0.37^f	20.44 ± 0.96^a	23.97 ± 1.69^a	50.82 ± 0.70^{ab}
GT	71.34 ± 0.36^a	1.63 ± 1.23^{cd}	13.90 ± 3.51^{bc}	6.99 ± 0.60^c
GT (G)	61.56 ± 1.93^b	0.22 ± 1.17^d	25.50 ± 4.74^a	54.92 ± 1.95^a
BTGT	58.27 ± 0.53^c	4.21 ± 0.41^{bc}	10.48 ± 0.33^c	6.64 ± 0.94^c
BTGT (G)	53.03 ± 0.38^d	-1.02 ± 0.05^d	22.78 ± 0.61^a	48.82 ± 4.35^a

Data values are expressed in mean $\pm$ standard deviation for three replications ($n=3$). Values with different superscript letters (a-d) in the same column indicate significant differences ($p < 0.05$).

Figure 2 displays the biodegradability rate (%) of SCOBY films made from different types of tea coated with/without glycerol. In general, the biodegradability rate of the SCOBY films is substantially ($p < 0.05$) affected by the choice of tea. SCOBY films fermented using green tea present a higher percentage rate of degradation (19.81%) compared to a combination of black and green tea films (19.11%) and black tea (16.00%). This may be due to the lower nutrient and tannin content in green tea, making the films less durable and more prone to breakdown (Bishop et al., 2022). Tannins in black tea act as natural preservatives, enhancing film stability. SCOBY's natural origin contributes to its biodegradability, breaking down into non-toxic substances (Atiweh, 2021). SCOBY films coated with glycerol increased ($p < 0.05$) the degradation rates compared to non-coated films, regardless of the different types of tea used. These findings are consistent with those reported by Muchtar et al. (2023) and Aduri (2019), where an increase in degradation rate was observed in glycerol-coated films and pre-treated SCOBY films in moist conditions. Glycerol's hydrophilic nature enhances water solubility and promotes biodegradability by forming hydrogen bonds with water (Ulfah et al., 2018).

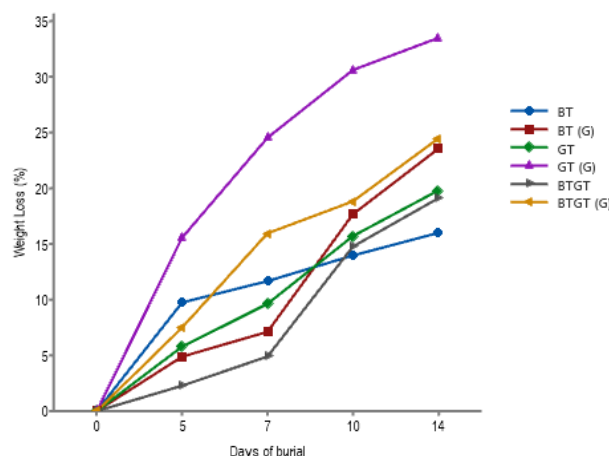


Figure 2: Biodegradability of SCOBY films made from different types of tea coated with/without glycerol.

Table 2 outlines the thermal analysis of the SCOBY film made from different types of tea coated with/without glycerol. The thermal analysis confirmed the dramatic effect of plasticizer, indicating a significant reduction in melting point T_m (e.g., GT reduced from 171.83°C to GT(G) at 150.36°C). The loss of thermal stability is directly linked to changes in mechanical properties (refer to mechanical properties). Coating the SCOBY films with glycerol reduced their melting points since glycerol has a plasticizing effect, which increases film flexibility and lowers molecular tension (Farrag, 2022). Arteaga et al. (2020) found the above findings, as they found that plasticization caused a loss of thermal stability as the

relations between polymers were broken. Such disruption is an effective way to increase the free volume in the film structure, requiring less thermal energy to break molecular forces and to cause a polymer chain movement or degradation. Black tea (175.39°C) SCOBY films had higher T_m values than green tea (171.83°C). This observation can probably be attributed to the composition of tea extracts, which have a higher concentration of complex, highly oxidized polymers (theaflavins and thearubigins) in black tea than the simple catechins in green tea. During fermentation, the presence of polyphenolic compounds becomes entangled in the BC network. This may form additional cross-linking points or serve as micro-fillers, requiring more energy to break, which increases the thermal resistance of the film compared to the formulation (green tea) (Lin et al., 2019).

Table 3 outlines the mechanical properties of SCOBY films fermented using various kinds of tea that were coated completely with glycerol, and also those that were not coated with glycerol. Fermented SCOBY with black tea had the highest tensile strength (0.27 MPa) and break elongation (34.48%) compared to the uncoated SCOBY films. This observation indicates that black tea has a high tannin and polyphenol level that is mostly oxidized and therefore a probable natural reinforcing agent. These particles were incorporated into the BC matrix during fermentation and might create physical cross-linkage that enhanced the strength and the ability to stretch before breaking (Bishop et al., 2022).

Table 2: Thermal properties of SCOBY film made from different types of tea with/without glycerol.

SCOBY films	T_o (Onset temperature)	T_m (Melting temperature)	ΔH (Enthalpy changes)
BT	174.45°C	175.39°C	417.7 J/g
BT (G)	168.62°C	172.36°C	746.9 J/g
GT	170.20°C	171.83°C	890.4 J/g
GT (G)	146.84°C	150.36°C	532.0 J/g
BTGT	180.53°C	182.73°C	522.0 J/g
BTGT (G)	139.92°C	158.43°C	1414 J/g

Values are expressed in mean $\pm$ standard deviation for three replications ($n = 3$).

Table 3: Tensile strength and elongation at break of SCOBY film made from different types of tea with/without glycerol.

SCOBY films	Tensile strength (MPa)	Elongation break (%)
BT	0.27 $\pm$ 0.05 ^a	34.48 $\pm$ 3.90 ^{cd}
BT (G)	0.17 $\pm$ 0.01 ^b	37.26 $\pm$ 10.14 ^{bc}
GT	0.18 $\pm$ 0.03 ^b	21.78 $\pm$ 0.28 ^d
GT (G)	0.13 $\pm$ 0.01 ^{bc}	52.21 $\pm$ 3.44 ^a
BTGT	0.14 $\pm$ 0.00 ^{bc}	33.88 $\pm$ 2.40 ^{cd}
BTGT (G)	0.10 $\pm$ 0.01 ^c	49.76 $\pm$ 3.03 ^{ab}

Values are expressed in mean $\pm$ standard deviation for three replications ($n = 3$). Values with different superscript letters (a-d) in the same column indicate significant differences ($p < 0.05$).

A large ($p < 0.05$) reduction in tensile strength was found when glycerol was used as a coating, and elongation at break improved in all types of films (e.g., the tensile strength of BT(G) reduced to 0.17 MPa, and elongation at break rose to 37.26%). This is in line with glycerol application in improving flexibility in films. It is possible that uncoated films can have higher values of intermolecular forces, which leads to an increase in tensile strength (Farrag, 2022). The same results were observed by Sederavičiute et al. (2019), who established that pretreatment on the SCOBY films enhanced ($p < 0.05$) the tensile strength (27.91 MPa up to 75.75 MPa) and diminished ($p < 0.05$) the elongation at break values (45.18% to 8.36%).

Table 4 presents the DPPH values of SCOBY films. SCOBY fermented with green tea was the most active in terms of DPPH, as it is the least oxidized (38.03%), thus retaining antioxidants such as catechins. This is consistent with Looi et al. (2022), who indicated that black tea contains a lesser amount of flavonoids, suggesting reduced radical-scavenging activity. Therefore, the minimum DPPH radical scavenging activity (33.12%) is observed in SCOBY fermented with black tea. SCOBY films coatings with glycerol significantly increased DPPH values (e.g., increased GT 38.03% to 46.53%), possibly by means of solubility and stabilization of antioxidants within the films by glycerol. This effect enhances antioxidant activity, as reported in a previous study by Marquez-Reyes et al. (2022), which demonstrated that chitosan-coated films had higher DPPH values than the control sample (between 13.56% and 38.02%). Green tea exhibited a radical scavenging activity of 95.3%, which was notably higher than that of black tea (38.7%) (Fu et al., 2014).

Table 4: Antioxidant activity (DPPH) of SCOBY films made from different types of tea with/without glycerol.

SCOBY films	DPPH radical scavenging activity (%)
BT	33.12 $\pm$ 0.49 ^f
BT (G)	40.53 $\pm$ 0.54 ^e
GT	38.03 $\pm$ 0.71 ^d
GT (G)	46.53 $\pm$ 0.85 ^a
BTGT	34.95 $\pm$ 0.77 ^e
BTGT (G)	44.32 $\pm$ 0.92 ^b

Values are expressed in mean $\pm$ standard deviation for three replications ($n=3$). Values with different superscript letters (a-f) in the same column indicate significant differences ($p < 0.05$).

According to Ashrafi et al. (2018), with longer fermentation times, kombucha tea became more effective at scavenging DPPH. The scavenging abilities of kombucha are directly dependent on the components created during fermentation, as well as the types of tea used. The type of tea utilised also reveals variations in the DPPH value within the SCOBY film. This is due to the minimal oxidation green tea undergoes during processing, which helps retain natural ingredients like epigallocatechin gallate, which is one of the

catechin polyphenols. Thus, SCOBY films coated with glycerol and fermented with green tea demonstrate the largest DPPH radical scavenging activity (up to 59%).

4. CONCLUSION

In conclusion, results revealed that GT fermentation produced the thickest SCOBY film (0.9 mm), and glycerol coating had minimal impact on thickness, though it enhanced the redness of the films. The water solubility of uncoated films was low since bacterial cellulose was not soluble. The tensile strength of the films was decreased by the coating of glycerol, but their plasticity was increased, as reflected in the increased values of elongation at break. The DPPH antioxidant activity (38.03% uncoated, reaching 59% with glycerol) was also highest in GT films. The activity of DPPH was supported by glycerol, which stabilized and solubilized these antioxidants. To sum up, tea type is the main determinant of color and antioxidant activity, whereas glycerol is the most effective plasticizer, and it has a considerable influence on solubility, flexibility, and thermal stability. The next step in the research should involve enhancing the odour of SCOBY films to eliminate the residual fermentation odours. Also, it would be valuable to investigate the potential of the film in the food packaging industry and its implications for shelf life, food moisture content, and microbiological resistance. Microbiological analysis of the films is also recommended to identify and quantify microbial content.

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