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Research Article

Production of Kombucha SCOBY Cellulose by Using Tea and Pumpkin Peel Waste as Fermentation Substrates: A Comparison Study

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ABSTRACT

A market research survey by Technavio has estimated that the global market size of kombucha is projected to grow by USD 3.56 billion from 2021 to 2026 with a Compound annual growth rate (CAGR) value of 19.36% due to its recognition as a 'functional beverage' among consumers, leading to a significant growth in the global market. Kombucha can generate a new pellicle Symbiotic Culture of Bacteria and Yeast (SCOBY) that produces a biopolymer of cellulose. The production of cellulose from fermentation of Kombucha SCOBY has received great attention from scientists due to the global goal of developing more sustainable procedures for a greener and bio-based future. The main objective of this preliminary study was to produce kombucha SCOBY cellulose (KSC) from tea and pumpkin peel waste as fermentation substrates. The carbohydrate and protein contents of both tea and pumpkin peel waste were determined by proximate analysis. The physicochemical properties of KSC were studied using two spectroscopic techniques, namely, Fourier Transform Infrared (FTIR) and X-Ray Diffraction (XRD). FTIR spectra revealed the existence of functional groups in KSC. Meanwhile, XRD spectra presented the crystallinity of diffraction peak and crystal lattice of KSC. Overall, results from FTIR and XRD analyses suggest that both Kombucha SCOBY cellulose obtained from tea and pumpkin peel fermentation substrates possess similar physicochemical properties of cellulose. In conclusion, the application of tea and pumpkin peel waste as fermentation substrates in the production of kombucha SCOBY cellulose is feasible.

Keywords: Kombucha, SCOBY, Cellulose, Biopolymer, Tea, Pumpkin Peel

1. INTRODUCTION

Kombucha is a bittersweet and carbonated beverage, produced by the fermentation of sweetened green or black tea by the activity of a Symbiotic Culture of Bacteria and Yeasts called SCOBY (Chakravorty et al., 2016). Through symbiotic relationships, these bacteria produce metabolites and organic acids such as acetic acid, gluconic acid, citric acid, and other acids; water-soluble vitamins like B1, B2, B6, and C; ethanol; carbon dioxide; and cellulose (Bauer-Petrovska & Petrushevska-Tozi, 2000; Gaggìa et al., 2019; Leonarski et al., 2021).

The SCOBY ecosystem is a symbiosis that is beneficial to both bacteria and yeasts. Yeasts produce invertase, which releases monosaccharides into carbon-rich media that are accessible to all microorganisms. Bacteria swiftly metabolise released sugars, resulting in an environmental depletion of monomers and an increase in the frequency of invertase-producing yeast. By acidifying the medium and forming a physical barrier, microbes protect themselves from external adversaries by producing organic acids and a surface deposit, respectively. In addition, yeast-produced ethanol stimulates the bacterial cellulose synthase mechanism to produce cellulose film (Gullo et al., 2017; May et al., 2019).

Kombucha Brewers International (KBI), a non-profit organization dedicated to promoting the commercialisation of kombucha and supporting members with the regulations regulating the beverage, was created in 2014 as kombucha's popularity expanded. Kim and Adhikari (2020) report that 235 firms registered with the research publications in 2019, including 134 from the United States, in agreement with the results of scientific studies. In 2021, more than 300 firms were listed on the KBI website kombuchabrewers.org (Kombucha Brewers International, 2022). The kombucha industry is projected to expand by 20% by 2025 (Expert Market Research, 2020). Many waste items include valuable nutrients that can be used as growth media for microorganisms and as raw materials for the production of other products. Several low-cost resources and waste byproducts that can be used in place of manufactured commercial media. Multiple independent reports have documented the utilisation of alternative sources in the production of kombucha SCOBY cellulose (Ul-Islam et al., 2020).

A microfibril cellulose from plants and wood, it is a non-toxic polymer that can be used for energy storage and fuel cell membranes. It is a promising source of biopolymer materials that can replace petroleum-based polymers. The benefits of cellulose from natural sources were bio-renewability and biodegradability (Shaghaleh et al., 2018). Furthermore, the rapid increase of biobased material manufacturing and research has sparked significant interest in exploring cellulose as an abundant natural resource for many applications, especially in the field of medicine. Hence, it gives an impact on insufficient resources of cellulose (Naomi et. al., 2020). Due to its characteristics, the price of cellulose on the worldwide market is continuously rising. The global market price for cellulose is expected to reach USD 305 billion by 2026 (Grand View Research, 2016). In addition, the production of cellulose from plants produces low formation crystallinity 40%-60% of crystals (Zeng et al., 2017; Huang et al., 2013) and the production of plant cellulose also produces 40%-50% of cellulose (Peelman et al., 2013). Besides the production of cellulose from plants, cellulose is also coming from algae, oomycetes, and bacteria (Aditiawati et. al., 2023).

Hestrin Schramm method is widely used in the production of cellulose from bacteria worldwide. Utilising biowastes from food processing industries or agriculture as nutrient sources could greatly reduce the cost of bacterial cellulose (BC) production and facilitate the treatment of substantial amounts of waste produced by food industries. This approach indirectly decreases environmental pollution and promotes sustainability (Nguyen et. al., 2021). Besides, statistical data on food waste, specifically on pumpkin waste as substrate, reveals that waste materials pose significant economic and environmental risks worldwide. SWCorp reported that Malaysia produced a total of 38,219 tonnes of solid waste per day in 2021. Out of the total, 76% of the items were not suitable for consumption, such as bones and fruit skin. However, the remaining 24% were still edible, including leftover meat and vegetables (The STAR, 2022). In a study by Yok et al. (2016), it was found that the composition includes approximately 79-82% flesh, 13-17% peel and pulp, and 4-6% seeds. In a comparable way, Brian (2008) documented a waste rate of pumpkin peel waste that was 25%. According to Department of Agriculture (DOA), Malaysia (2023), the production of pumpkin in 2021, 2022, and 2023 was recorded as 21,759, 20,369 and 21,897 tonnes of pumpkin, respectively. Means that the 25% waste total generated from 2021-2023 was 16,006.25 tonnes pumpkin peel waste. In order to reduce the cost of cellulose, production was enhanced by utilising a cost-effective medium. However, the high-cost nutrient sources in media Hestrin Schramm limits the commercial production. Due to examining cellulose production in non-optimised, optimised, and commercial media in Hestrin Schramm Broth, further research was identified as the best economically efficient medium for cellulose production (Avcioglu et al., 2021). This production process does not necessitate the presence of particular bacterial strains and can be easily carried out even in home environments. For this study, we selected to utilise bacterial cellulose (BC) generated by a symbiotic colony of bacteria and yeasts (SCOBY) (Bryszewska et al., 2023).

Consequently, numerous investigations have been conducted to enhance the production of BC using inexpensive media (Avcioglu et al., 2021). This research aim used substrate black tea and pumpkin peel waste with kombucha SCOBY cellulose (KSC). Cellulose synthesis was enhanced using a cost-effective black tea and pumpkin peel waste. Black tea is used in kombucha as a traditional way. Moreover, the nutritional contents of pumpkin peel waste are the same characteristics with black tea, enabling its use as an alternative nutrient source for kombucha broth fermentation. Moreover, pumpkin peel is a rich source of carotenoids, pigment agents with health promoting potential. Pumpkin peel is a good source of biologically active compounds (Cuco et al., 2019). Presence of flavonoids, phenolic compounds, carotenoids, and β-carotene in both methanolic and ethanolic extracts of pumpkin peel has been reported during phytochemical screening of pumpkin peel (Chonoko & Rufai, 2011; Aziz et al., 2023).

The nutritional composition of black tea and pumpkin peel waste such as carbohydrate and protein were an additional component that influenced the choice. Pumpkin peels waste an abundant and low-cost byproduct of the fruit industry and easy to access. These preliminary studies involved three parts, namely: (1) the proximate analysis of tea and pumpkin peel waste, (2) analysis of kombucha fermentation broth, and (3) characterisation of kombucha SCOBY cellulose.

2. MATERIALS AND METHODS

2.1. Materials

Tea powder and pumpkin peel waste were used in fermentation broth. A commercial tea powder was purchased from a local supermarket, meanwhile pumpkin peel waste was obtained from a traditional chip factory in Selangor.

2.2. Sample Preparation of Pumpkin Peel Waste

After the pumpkin peel wastes are cleaned, the peel was cut into 2-3 mm cubes. Later, pumpkin peel was kept in the container and kept in a refrigerator for 24 h at -2ºC. The cube of pumpkin peel waste was freeze-dried at -97ºC and 0.001 mbar pressure using a Scanvac Coolsafe Freeze Dryer. Then, the samples were ground by using a laboratory blender.

2.3. Preparation of Kombucha Fermentation Broth

First, 1 L of mineral water was poured into a 2 L beaker and heated on a hot plate until boiled. Later, 6% and 12% (w/v) of sugar concentration was added to the same beaker and stirred until dissolved. Then, 0.4% (w/v) of substrates for black tea (T) or pumpkin peel (P) waste powder in the beaker of boiled water, stirred and left to infuse for 20 min. The residues were then removed by filtration. The solutions were left to cool down for 20 min. Then, SCOBY was added to the solution at 10% (w/v). The beaker was covered with cheese cloth and incubated at room temperature for 21 days. The growth progress of KSC was monitored.

2.4. Pre-treatment of Kombucha SCOBY Cellulose

The KSC was purified by immersing it in deionised water for a few hours. Later, purified with 1.0 M NaOH for 1 h. The KSC layers were rinsed with deionised water and ethanol and then refrigerated for 24 h at -2°C. Later dried for 96 h at -97°C and 0.001 mbar pressure using a Scanvac Coolsafe Freeze Dryer.

2.5. Proximate Analyses in Substrates Used on Kombucha Broth

In this study, several analyses were conducted to analyse kombucha broth and characterise the properties of substrates and KSC. The analyses were conducted to determine the pH analysis of fermentation broth. Meanwhile, the growth of the new layer film of cellulose was determined by the weight of the total container during fermentation growth. Proximate analysis was used to determine the amount of different components in food and food substances. These components include moisture, carbohydrates, protein, total ash, and dietary fiber (Ibiam et al., 2022). The carbohydrate content of the substrates was analysed by using phenol chemicals used in the identification of tea and pumpkin peel powder from carbohydrate with taken 0.1 g of sample added 5 mL of 2.5 M HCI. Then, the determination of carbohydrates present in the samples were further carried out by mixing the sample with 1 mL phenol and 5 mL of concentrated sulphuric acid 98%. The solution was left to stand for 10 min at 28ºC. After shaking, the solution was placed in a water bath at 30°C for 20 min. The sample of carbohydrate content was then measured by using Agilent Technologies carrying a 60 UV-Visible spectrophotometer at wavelength 490 nm (Biocyclopedia). Besides, protein content was determined by using a bicinchoninic acid (BCA) protein assay kit (Novagen®Merck) that changes in color proportional to the amount of protein present in the sample. A BCA reagent standard curve was prepared by measuring the absorbance of the sample with known amount of Bovine Serum Albumin (BSA). The samples were incubated for 30 minutes and the protein content in the samples was determined using a UV-Visible spectrophotometer by Agilent Technologies Cary 60 UV-Vis spectrophotometer at a wavelength of 562 nm (Sigma).

2.6. The Physiochemical Properties of KSC

In this study, physicochemical properties were conducted to characterise the properties of substrates and KSC. The physicochemical properties were conducted to determine the new pellicles produced in fermentation. FTIR and XRD techniques were used in order to characterise the functional groups and crystallinity of KSC.

2.6.1. FTIR Analysis of Kombucha SCOBY Cellulose

The instrument used for the characterization of kombucha SCOBY cellulose is Fourier-transform infrared spectroscopy (FTIR). The chemical characteristics of chemical compounds are determined by their functional groups. On an IRTracer-100 Shimadzu FTIR Spectrometer, FTIR spectra were recorded by adhering the compartments to a metallic slit in air medium. The transmission mode is configured at 2°C with a wavenumber range of 4000-400 cm^{-1} and over 32 cumulative scans.

2.6.2. XRD Analysis of Kombucha SCOBY Cellulose

The crystallinity of KSC samples was studied by using of X-ray diffractometer (XRD). XRD spectrum was used to detect the crystallinity index of samples. Sample was conducted by using XRD model Rigaku, Japan Tabletop equipped with copper anode Cu-Kα (λ = 1.54 nm) radiation operating at 40 kV/15 mA. The detector angle 2θ was set between 3° and 50° with a scan rate of 0.01°/min. XRD pattern was then analysed using the Bragg's Law given in Eq. (1):

$$nk\lambda = 2d \sin\theta$$

where:
n – integer
k – constant value (k = 0.89)
λ – wavelength of the X-ray
d – interplanar spacing generating the diffraction
θ – diffraction angle

The peak height calculations for the cellulose samples were determined using Expert Highscore software (Philips). The crystallinity index (XC) was calculated manually using Segal method (Oudiani et al., 2011). The formula is shown as Eq. (2):

$$XC = [(I_{002} - I_{am}) / I_{002}] \times 100$$

where:
I_{am} – diffraction intensity of the amorphous phase area (intensity at the minimum level)
I_{002} – maximum intensity of diffraction peak crystallinity area

3. RESULTS AND DISCUSSION

3.1. Carbohydrate and Protein Analysis of Tea with Pumpkin Peel Compared Substrates

The identification proximate analysis of pumpkin peel waste results show the properties of pumpkin peel is relevance as a substrate used during fermentation. Cellulose production in non-optimized, optimised, and commercial media (Hestrin Schramm Broth) to identify the best economical medium for cellulose production (Avcioglu et al., 2021). Some of the dominant bacterial and yeast groups were studied for their function in fermentation and cellulose production (Chakravorty et al., 2016). Even though the previous study did not mention the exact amount of chemical and nutritional content in substrates, however, substrates, such as carbon and nitrogen supplies, are essential for the proliferation of nearly all microorganisms (Ojo & de Smidt, 2023). The growing medium for substrates in kombucha requires nitrogen supplies derived from tea extract and a carbon source, typically sucrose, fructose, glucose, ethanol, or mannitol (Yim et. al., 2017). Cellulose synthesis was enhanced using a cost-effective black tea and pumpkin peel waste. The nutritional content, in pumpkin peel waste content carbohydrate and protein is the same characteristics with black tea, enabling pumpkin peel waste used as an alternative nutrient source for kombucha broth fermentation. Table 1 shows the percentage of carbohydrates and protein. The identification and presence of carbohydrates and protein in pumpkin peel waste results show the properties of pumpkin peel can be used as a substrate.

Table 1. Proximate analysis of tea (T) and pumpkin (P) peel waste as fermentation substrates

Composition	Tea	Pumpkin Peel
Carbohydrate (%)	4	74.0
Protein (%)	13	1.13

3.2. Analyses of Kombucha Broth of Kombucha SCOBY Cellulose

3.2.1. pH analysis of Fermentation Broth

The condition in fermented broth media has occurred the interaction between sugar concentration, substrate, and SCOBY that made change the environment. One of the important parameters to be measured is pH in broth media. From the results, the levels observed after fermentation between pH 4.51 to 2.61 exhibited a similarity between the fermentations using tea and pumpkin peel waste. The comparison between pH of the tea and pumpkin peel waste with different between 6% and 12% of sugar concentration (w/v), exhibited decreased values during the fermentation time, as depicted in Figure 1 shows the 3 stages of fermentation during the new pellicle growth in broth media.

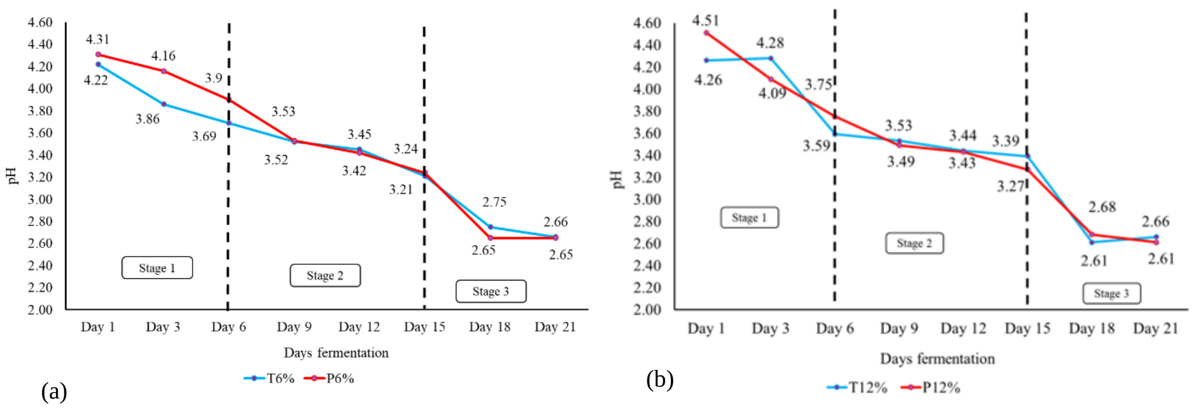

Figure 1. pH analysis of fermentation broth using tea (T) and pumpkin (P) peel wastes as fermentation substrates at (a) 6% (w/v) and (b) 12% (w/v) sugar concentrations.

Figure 1(a) presents T6% and P6%, meanwhile Figure 1(b) depicts T12% and P12%. From Figure 1(a) and Figure 1(b), three distinct growth stages were observed. Stage 1 starts from day 1 until day 6, stage 2 from day 9 until day 15, and stage 3 from day 18 until day 21. The 3 stages in Figures 1(a) and (b) show studies from Coton et al. (2017), investigating the rate of change of microbial communities in Kombucha produced in a period of time (in 3 days). So that, in this research took the day's period of fermentation in every day 3 period 21 days. As shown in Figure 1(a), stage 1 occurs from pH 4.31 to 3.69, stage 2 from pH 3.53 to 3.21, and stage 3 from pH 2.75 to 2.65. Meanwhile, in Figure 1(b), stage 1 starts from pH 4.51 to 3.59, stage 2 from pH 3.53 to 3.27, and stage 3 from pH 2.68 to 2.61. It is clear that from Figures 1(a) and 1(b), the pH broth was decreased in tea and pumpkin peel waste substrate. For example, for pumpkin peel waste at 12% (w/v) sugar concentration, the percentage decrease in pH broth from day 1 to day 21 was 42.13% as shown in Figure 1 (b). This percentage of reduction was slightly higher than 38.52% determined for pumpkin peel waste used as substrate at 6% (w/v) sugar concentration. The decrease pH broth in Figures 1 (a) and (b) happen at stage 1, the oxygen in the container and kombucha broth interacts with bacteria and yeast, then the acidity of the media can be generated and produce an organic acid (Kumar and Joshi et al., 2016). The process of fermentation results at stages 2 and 3 show the generation of byproducts like organic acids such as acetic acid, glucuronic acid, citric acid, gluconic acid. It has been observed that as the duration of fermentation grows, there is a corresponding increase in acidity levels (Ahmed et al., 2020; Laanvanya et al., 2021). The concentrations of organic acids are elevated. It has been observed that as the duration of fermentation grows, there is a corresponding increase in acidity levels. So that, the pH decreased. The production of cellulose has increased (Neera et al., 2015).

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3.2.2. Total Weight of Container during Fermentation Growth

Figure 2 shows the comparison of the total weight of the container during the fermentation process for both tea and pumpkin peel waste at a) 6% (w/v) and b) 12% (w/v) sugar concentrations. The interaction of broth fermented made change the environment so that the total weight container to be measured. In Figure 2, presented in 3 stages. From here relatable to the growth of Kombucha SCOBY Cellulose.

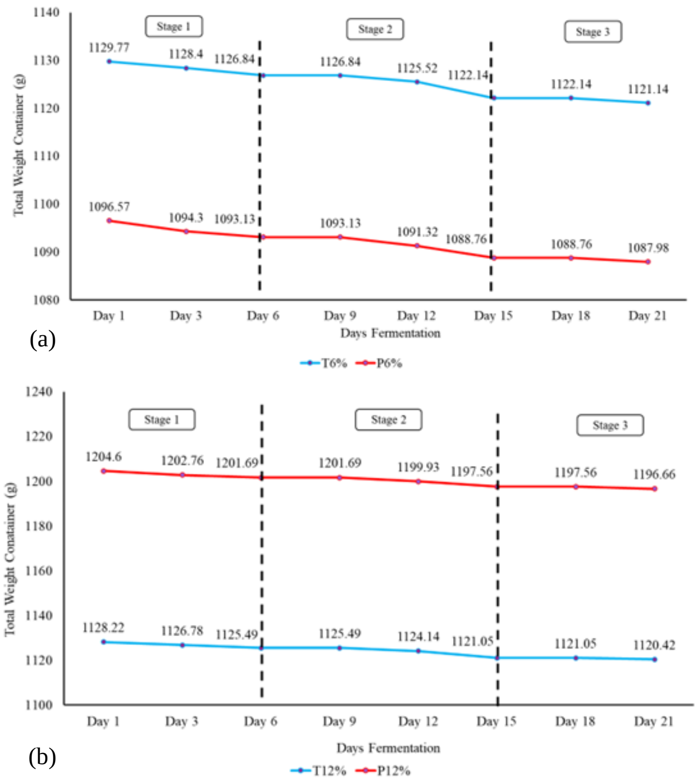

Figure 2. Total weight of container (g) during fermentation using tea (T) and pumpkin (P) peel wastes as fermentation substrates at (a), 6% (w/v) and (b) 12% (w/v) sugar concentration.

Figure 2(a) presents T6% and P6%, meanwhile Figure 2(b) depicts T12% and P12%. From Figure 2(a) and Figure 2(b), three distinct growth stages were observed. Stage 1 starts from day 1 until day 6, stage 2 from day 9 until day 15, and stage 3 from day 18 until day 21. As shown in Figure 2(a), for tea and pumpkin peel waste substrates, stage 1 occurs from 1129.77 g to 1093.13 g, stage 2 from 1126.84 g to 1088.76 g and stage 3 from 1122.14 g to 1086.98 g. Meanwhile, in Figure 2(b), for tea and pumpkin peel waste substrates, stage 1 starts from 1128.22 g to 1201.69 g, stage 2 from 1125.49 g to 1197.56 g and stage 3 from 1121.05 g to 1190.66 g. It is clear that from Figures 2(a) and 2(b), the total weight container was decreased in tea and pumpkin peel waste substrate. For example, for pumpkin peel waste at 12% (w/v) sugar concentration, the percentage decrease in the total weight container from day 1 to day 21 was 1.16% as shown in Figure 3 (b). This percentage of reduction was slightly higher than 0.8% determined for pumpkin peel waste immersed at 6% (w/v) sugar concentration. The total weight container for pumpkin peel waste at 6% (w/v) sugar concentration, the total weight container was found to decrease from 1096.57 g (day 1) to 1086.98 g (day 21) in Figure 3 (a).

The physical SCOBY changes according to the reaction between sugar concentration, SCOBY and substrates. As shown in Figure 2, the results show that at the 1st stage, the acidity of the media can be clarified by the amount of oxygen to the bacteria. At the 2nd and 3rd stage shows the generation of byproducts, such as glucuronic acid, acetic acid, carbon dioxide, ethanol, and layer biofilm which is cellulose (Chakravorty et al., 2016).

From the result show, the container between bacteria and yeast inside the SCOBY made the total weight container become a decrease because of the sugar concentration and nutrients in the kombucha broth transform into new compounds such as organic acid and formation layer of film which is cellulose (Chang et al., 2016; Hopfe et al., 2017). According to Gargey et al. (2019); Goh et al. (2012), the growth medium for SCOBY must contain a nutrient such as carbohydrate composition as the carbon source and tea extract as the nitrogen source so that it can be utilised in the fermentation process for cellulose production and microbial growth. So, the carbohydrate and protein content in tea and pumpkin peel assists the growth of SCOBY to generate a new layer. Therefore, the total weight container decreased. The production of cellulose has increased (Neera et al., 2015; Sari et al., 2023).

3.2.3. Relationship Studies between pH and Total Weight Container for Sugar Concentration 6% and 12% in 21 days Fermentation Growth

It is important that the relationship between 2 parameters pH and the total weight container. The trends in Figures 3 (a) and (b) showed that when the pH decreased, the total weight container also decreased. However, this is not sufficient between the 2 parameters pH and total weight container, therefore the relationship was evaluated in terms of correlation.

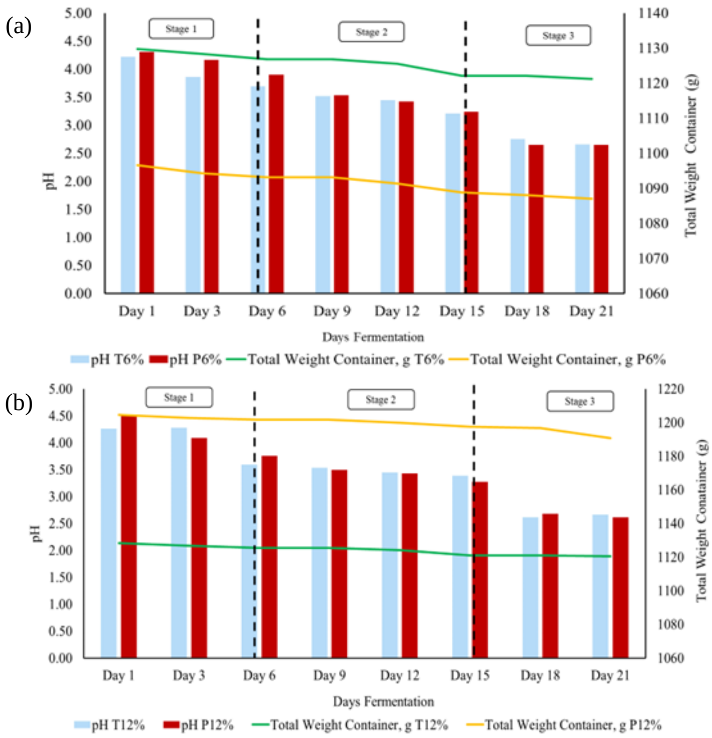

Figure 3. Comparison Relationship between pH analysis and Total Weight Container (g) for tea (T) and pumpkin peel (P) wastes as fermentation substrates at (a) 6% (w/v) and (b) 12% (w/v) sugar concentrations.

Figures 4 and 5 show the correlation relationship between pH and total weight container (g), the results show that between tea (T) and pumpkin (P) with sugar concentration 6% and 12%. Figure 4 shows for the T6% and P6%, meanwhile Figure 5 shows for T12% and P12%. Results in Figure 4 present same value, the R^2 = 0.922 for T6% and P6%. Results in Figure 5 present for T12% R^2= 0.817 and R^2 = 0.824. Based on Table 2, from the results, T6% and P6% present the same value R^2 = 0.922 close to 1, which is perfectly positive compared to R^2 for T12% present R^2 = 0.817 and P12% present R^2 = 0.824. Furthermore, in Figure 4 the outliers points are closer to each other in the linear graph compared to Figure 5 outlier points.

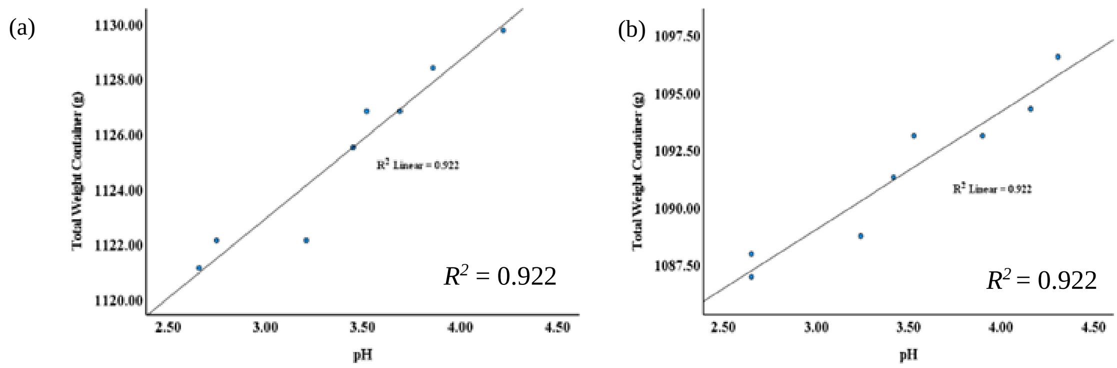

Figure 4. Correlation Relationship between pH and total weight Container (g/L) for tea (T) and pumpkin (P) peel waste at (a) T6% (w/v) and (b) P6% (w/v) sugar concentration.

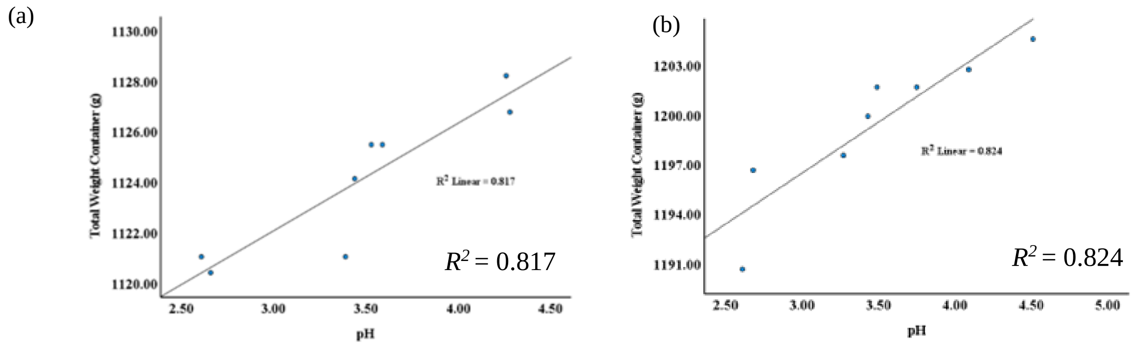

Figure 5. Correlation Relationship between pH and Total Weight Container (g/L) for tea (T) and pumpkin (P) peel waste at (a) T12% (w/v) and (b) P12% (w/v) sugar concentration.

Table 2 represents that the correlation between pH and total weight container (g) has interrelated with each other. Besides, tea substrate and pumpkin peel substrate show the same characteristics between correlations 6% and 12%. Furthermore, tea and pumpkin peel waste are interrelated substrates which are the chemical and nutritional contents of pumpkin peel waste and that content carbohydrate and protein is the same characteristics with black tea, enabling pumpkin peel waste used as an alternative nutrient source for kombucha broth fermentation. Moreover, the sugar concentrations with 6% reliance had more interaction between pH and total weight container in fermentation growth compared to 12%.

Table 2. Reference Table Correlation for 2 Variables

Correlation Coefficient Value (r)	Direction and Strength of Correlation
-1	Perfectly negative
- 0.8	Strongly negative
-0.5	Moderately negative
-0.2	Weakly negative
0	No association
0.2	Weakly positive
0.5	Moderately positive
0.8	Strongly positive
1	Perfectly positive

Source: Ratnasari et al. (2016), Baker (2018)

3.2.4. *Comparison Yield Kombucha SCOBY Cellulose Weight (g/L) in 21 Days for Tea (T) and Pumpkin (P) Substrates of Sugar Concentration 6% and 12%*

Figure 6 shows the comparison of dried yield KSC weight (g) between substrate tea (T) and pumpkin (P) with sugar concentration 6% and 12%. Results from Figure 6 show the substrates T12% and T6% higher than P12% and P6%.

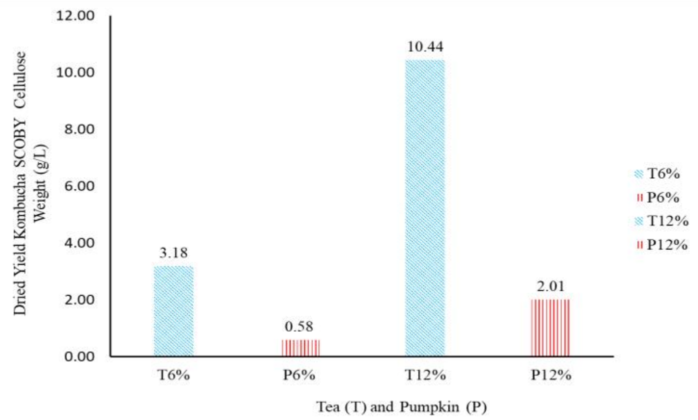

Figure 6. Comparison Dried Yield Kombucha SCOBY Cellulose Weight (g/L) in 21 Days for Tea (T) and Pumpkin (P) peel wastes of Sugar Concentration 6% (w/v) and 12% (w/v)

The physical structure of the SCOBY undergoes modifications as a result of the interaction between sugar, mineral water, SCOBY, and pumpkin peel powder. Research findings from Balentine et al. (1997) and Costa et al. (2016), the presence of caffeine, theophylline, and theobromine in tea extract has been found to accelerate cellulose formation by bacteria through the activation of cellulogenic complexes. Bacterial cells can be influenced by vitamins and other nutrients that are produced when yeast cells undergo death and autolysis (Chakravorty et al., 2016). Research by Aditiawati et al. (2023), the primary source of phenolic compounds (antioxidant activity) in kombucha is the breakdown of complex phenolics from lemongrass. This degradative process can potentially be accelerated, bacterial cellulose produces cellulose actively. During the time spanning from day 15 to day 18 of the incubation phase, a modest augmentation in phenolic metabolism was seen in lemongrass kombucha. Research from Sathiya Mala et al. (2016) mentioned that the proximate composition is high in pumpkin peel with β-carotene (mg/100 gm) 11.89 ± 0.10 and total phenols 5.19 ± 0.05. So that, it will help to generate cellulose during fermentation kombucha SCOBY cellulose.

Based on the conducted experiments, it has been observed that the inclusion of pumpkin peel in kombucha serves as a stimulant for the SCOBY, leading to the generation of a new layer during the kombucha production process. The nutritional composition of pumpkin peel, including carbohydrates and proteins, as well as the presence of phenolic compounds, has an impact on the process of generating a new layer of SCOBY. This specific composition of the growth medium is essential for facilitating the fermentation process required for cellulose production and promoting microbial development. The presence of carbohydrates and proteins in pumpkin peel facilitates the growth of the symbiotic culture of bacteria and yeast (SCOBY), enabling the formation of a new layer. The nutrients presence in tea and pumpkin influence the product yield of KSC. Tea is less carbohydrate and rich in protein, meanwhile pumpkin peel waste rich in carbohydrates and less in protein. In fermentation, growth has substrate and sugar concentration. When the sugar concentration is high effect, the KSC actively growth. Sugar as a carbon source to generate cellulose. The concentration of carbon source can affect the cellulose production. Thus, the excess of carbon sources from the substrate could lead to decreased KSC yield that showed in pumpkin peel as substrate.

3.3. Chemical Properties of Kombucha SCOBY Cellulose

3.3.1. Moisture content in Kombucha SCOBY Cellulose

After freeze-drying by using a freeze dryer, the KSC product was ground by using a laboratory blender. The transformation of texture sample will cause easier to further analyses. The result shows the kombucha SCOBY cellulose after drying with a freeze dryer. The samples were taken from standard and average data. From the results, the Tea (6%) and Pumpkin (6%) were higher moisture content than Tea (12%) and Pumpkin (12%) as shown in Table 3.

Table 3. Percentage of Moisture Content in sample Kombucha SCOBY Cellulose (KSC)

Samples	Moisture content (%)
KSC T6%	95.46
KSC P6%	97.24
KSC T12%	90.59
KSC P12%	95.04

Note: KSC is kombucha SCOBY cellulose, T and P symbol for Tea and Pumpkin, respectively, 6% and 12% are sugar concentrations (w/v).

3.3.2. Chemical Properties of Kombucha SCOBY Cellulose by FTIR Analysis

FTIR analysis was determined functional groups in the compound sample. From FTIR spectrum of the cellulose, Figure 7 shows the comparison analysis of Kombucha SCOBY Cellulose with different substrates tea (T) and pumpkin (P) used in fermentation with different sugar concentrations 6% and 12%.

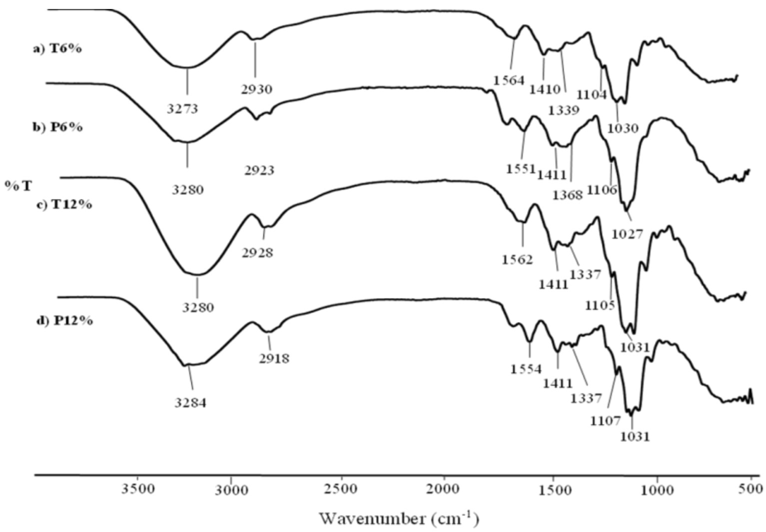

Figure 7. FTIR spectra of Kombucha SCOBY Cellulose determined from different substrates of tea and pumpkin peel wastes: (a) Tea (T) Substrate 6%, (b) Pumpkin (P) Substrate 6%, (c) Tea (T) Substrate 12% and (d) Pumpkin (P) Substrates 12%

All samples exhibited a wide hydrogen bonding range between 3284-3273 cm^{-1}, which can be attributed to the O-H stretching vibration (Zhu et al., 2014). The transmittance bands occurring between 2918-2930 cm^{-1} are associated with the stretching of the C-H groups found in the glucose unit of alkanes, as reported in the study by Treu-Filho et al. (2014). These bands are characterized by a high peak accompanied by a weaker peak for T6%, P6, T12%, and P12%. Furthermore, the presence of multiple transmittance bands within the range of 1410-1411 cm^{-1} substantiated the existence of C-H bending vibrations in all samples. The frequency range of 1309-1310 cm^{-1} corresponds to the bending motion of the C-H bonds. The spectral range of 1160-1150 cm^{-1} corresponds to the stretching of the C-O-C bond. The frequency of 1100 cm^{-1} corresponds to the stretching of the C-O-C bond (Rusdi et al., 2022; Zeng et al., 2014). The presence of transmittance bands in the range of 1027-1060 cm^{-1} in all samples indicates the stretching vibration of C-O-C and C-O-H in cellulose (or the sugar ring), proving the purity of the generated cellulose (Song & Kim, 2019). The presence of a transmittance band within the range of 1050-10650 cm^{-1} suggests a combination of cellulose I and cellulose II types (Tyagi & Suresh 2016). From the previous studies showed that FTIR spectrum shows a peak detected functional group (Engel et al., 2019; Lambert 2011). Results represent the cellulose structure as shown in the list functional groups with wavenumbers in Table 4. Overall, all spectra exhibited a consistent pattern, validating the fundamental composition of bacterial cellulose (Ul-Islam et al., 2013; Sezali et al., 2021; Wu et al., 2015).

Table 4. List of Peak Detected Functional Groups in Figure 7.

Wavenumbers (cm^{-1})	Functional group
3273-3284	O-H stretching
2918-2930	CH stretching
1551-1564	COO peak
1410-1411	CH_2 stretching
1337-1368	CH bending
1104-1107	C-O-C stretching
1027-1030	C-O stretching for β 1,4-glycosidic bond

3.3.3. Chemical Properties of Kombucha SCOBY Cellulose by XRD Analysis

The diffraction peaks observed in the diffractogram are caused by scattering from crystalline structures, whereas the diffuse background is a result of scattering from amorphous regions. The International Centre for Diffraction Data® (ICDD®) states that the diffraction peaks of cellulose Iβ can be found at approximately 2θ = 14.52°, 16.82°, and 22.78°. These values correlate to the (10-1), (11-1), and (002) crystal lattices (ICDD No. 00-060-1502). XRD patterns for KSC are shown in Figure 8. From the previous studies shows that XRD spectrum of cellulose in Figure 8 analysis of Kombucha SCOBY Cellulose (KSC) by different substrates tea (T) and pumpkin (P) used in fermentation with different sugar concentration 6% and 12%. Research findings from Rusdi et al. (2022) and Zeng et al. (2014) showed that the XRD peaks of bacterial cellulose (BC) sheets are observed at 2θ angles of 14.15°, 16.25°, and 22.46°. Similarly, the microcrystalline cellulose (MCC) exhibits peaks at 2θ angles of 14.72°, 16.33°, and 22.79°, whereas cotton fiber shows peaks at 2θ angles of 14.46°, 16.80°, and 22.85°. These peaks exhibit a characteristic pattern of diffraction peaks for cellulose Iβ. Oudiani et al. (2011) and Klemm et al. (2011) observed comparable peaks in their studies on cellulose.

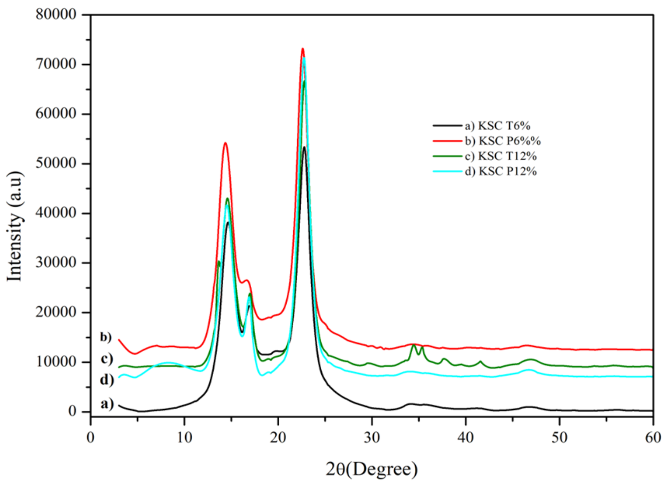

Figure 8. XRD spectra of Kombucha SCOBY Cellulose determined from different substrates of tea and pumpkin peel wastes: (a) Tea (T) Substrate 6%, (b) Pumpkin (P) Substrate 6%, (c) Tea (T) Substrates 12% and (d) Pumpkin (P) Substrates 12%.

In Table 5, all results show the same as ICDD peak data. Moreover, the high crystallinity of KSC T6% and KSC P6% were 88.67% and 88.01%, respectively. Meanwhile, for KSC, T12% and P12% were 87.51% and 87.60%, respectively.

Table 5. List of XRD peaks appear in KSC for all samples in Figure 8

KSC T6% 2θ	KSC P6% 2θ	KSC T12% 2θ	KSC P12% 2θ
14.66	14.37	14.59	14.53
16.95	16.63	16.99	16.90
22.78	22.62	22.81	22.76

4. CONCLUSION

Based on this, preliminary studies show that a new layer of SCOBY produced from broth fermented substrate, tea, and pumpkin peel waste are determined and confirmed as Kombucha SCOBY Cellulose (KSC). The production of yield kombucha SCOBY Cellulose is affected by many factors. Factor 1 of the type of substrates used during fermentation growth. The nutrient inside the substrates influences the growth of KSC. By using tea was good, comparing pumpkin peel as fermentation substrates. Factor 2 of the sugar concentration (w/v) during the fermentation broth. Sugar concentration 12% (w/v) was good compared to 6% (w/v). Hence, the sugar concentration provides the element carbon sources as providing the formation of cellulose layer time to time. From the FTIR spectra, the functional group of cellulose was determined as the exact cellulose structure. Then, it was supported by XRD analysis was determined cellulose crystal structure Iβ by ICDD reference. Overall, the research findings highlight the applicability of tea and pumpkin peel waste as fermentation substrates in the production of Kombucha SCOBY cellulose.

Declaration of Interest

The authors declare that there is no conflict of interest.

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