

Review

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Review

# Kombucha Bacterial Cellulose: A Promising Biopolymer for Advanced Food and Nonfood Applications

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Abstract: The pellicle is a coproduct from kombucha beverage production without economic value. This material is based on cellulose produced from bacteria and it has better physical properties than cellulose isolated from plants. This review analyzed systematically the research literature about pellicle (KBC – kombucha bacteria cellulose) valorization. In general, KBC has been used in food applications, especially as a packaging ingredient to improve the physical properties of biopolymer-based films, as well as to manufacture packaging materials based on KBC. In addition, some studies have investigated the potential of KBC to encapsulate food ingredients or as a food additive. Furthermore, KBC has been used in nonfood applications with a special interest in the development of materials for textile and medical applications, as well as to substitute disposable materials (*e.g.* spoons). Although the literature shows promising results, it is necessary to increase the production scale of this material, as well as to analyze its economic viability. It is also necessary to establish quality standards and international regulations for KBC with respect to its different food and nonfood applications.

**Keywords:** macromolecule; food ingredient; packaging material; pellicle; SCOBY.

### 1. Introduction

Kombucha is a carbonated beverage produced by tea fermentation from a symbiosis of bacteria and yeasts, this beverage has a slightly sweet and citrusy taste, with low alcohol content and a vinegar-like taste [1]. Kombucha was originally consumed in China, but recently this product has been commercialized worldwide due to the increase in the market and the potential health benefits associated with the presence of bioactive molecules and probiotics [2–4].

The kombucha production process involves two steps. Firstly, a sweet tea is produced with filtered herbal infusion where an inoculum is added (Figure 1). The fermentation time oscillates between 7 and 15 days, depending on temperature  $(18 - 26 \, ^{\circ}\text{C})$  [5]. Ethanol, acetic, lactic, glucuronic acids, as well as a large quantity of tea-derived polyphenols are the most common products found in kombucha tea after fermentation [6,7]. In sequence, the fermented beverage in step 1 is blended with fruit juice or another herbal infusion to flavor kombucha (Figure 1). The resulting blended beverage is packed and again fermented at the same conditions. After this second step, a flavored kombucha is obtained and commercialized under refrigeration [8].

Another product of kombucha fermentation is the pellicle (Kombucha Bacterial Cellulose – KBC, Figure 1) produced by the microbial consortium which consists of pure cellulose fibrils [9,10]. A small number of researchers have focused on the characterization and potential applications of KBC, probably because kombucha tea has high economic value and KBC is considered a residue [11]. However, KBC does not contain hemicellulose and lignin, having higher purity, degree of

polymerization, and crystallinity than cellulose isolated from plants. Furthermore, materials based on KBC have better mechanical strength, water holding capacity, chemical stability, and biological adaptability than their counterparts using plant cellulose [5,12]. In addition, the processes to purify plant cellulose demand high energy consumption and has negative impacts on cellulose chains (polymer degradation) and the environment since chemical residues are discarder in the processes [13]. In this scenario, the valorization of KBC involving a circular economy concept is fundamental to the development of a sustainable industry to achieve the 2030 Agenda for Sustainable Development adopted by all United Nations has focused on the 17 Sustainable Development Goals (SDGs) [14].

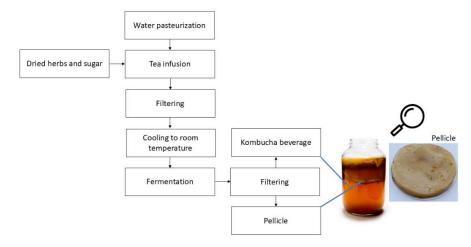


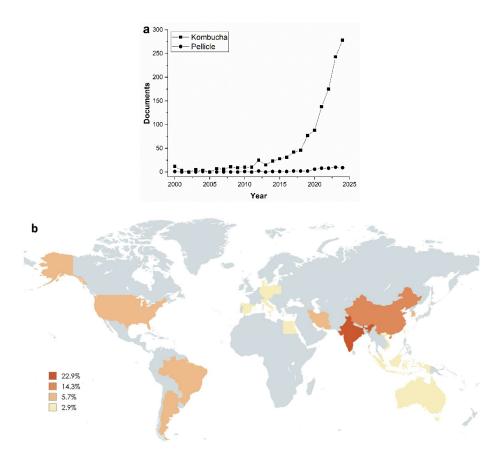
Figure 1. Kombucha production flowchart.

Previously Laavanya et al. [5] reviewed the KBC production, its biochemical composition, and explored some potential applications. It should, however, be noted that to our current knowledge, no review paper has comprehensively analyzed and reviewed the recent advances about KBC applications. Therefore, the objective of this review was to comprehensively analyze the potentiality of KBC for industrial applications.

## 2. Research about kombucha bacterial cellulose

A search using the SCOPUS database revealed that the number of scientific manuscripts on "kombucha" has increased in the last 24 years (1,287 documents) (Figure 2a). A new search using the same database and the keywords "kombucha AND pellicle AND scoby" revealed 50 research papers in the last 24 years (Figure 2a). Between them, only 25 manuscripts focused on valorization and application of the pellicle obtained after kombucha production. These results confirmed that research on kombucha is an important topic with expressive publication rate in the last years, however, pellicle from kombucha has been little explored.

Analyzing the global production of scientific articles on KBC is possible to conclude that several countries are researching this topic, having India as the country that most research about BKC (22.9 %), followed by China (14.3 %), Argentina, Brazil, Iran, South Korea, and USA (5.7 5 each), and Austria, Australia, Czech Republic, Denmark, Egypt, Germany, Italy, Indonesia, Malaysia, Poland, Spain, Taiwan, and Viet Nam (2.9 % each) (Figure 2b).



**Figure 2.** (a) A search of the SCOPUS database for scientific production using the keywords "kombucha" versus "kombucha AND pellicle AND scoby" between the years 2000 and 2024; (b) Global production of scientific articles on kombucha bacterial cellulose. The world map was made using MapChart (https://www.mapchart.net/).

# 3. Application of kombucha bacterial cellulose

#### 3.1. Food applications

Packaging is important for the food processing, preservation, supply, and distribution chain. Its main function is to contain and protect food material, but it is also essential to ensure food quality and safety; while also extending shelf life and minimizing food loss and waste [15,16].

KBC has been used as an additive or as the main macromolecule to manufacture food packaging materials (Figure 3). The drying method impacts on the physicochemical properties of KBC and its application as packaging material. Dey et al. [17] investigated different drying methods on the physicochemical properties of KBC. In this research, KBC was washed with distilled water and then soaked with NaOH (0.1 N) to remove tannins and microbial residues. In sequence, cleaned KBC was dried using microwave drying (180–900 W), hot air oven drying (30–70 °C), and shade drying (25 °C). The authors observed that elevated temperatures and power levels during the drying processes increased the formation of inter-fiber hydrogen bonding of KBC. Therefore, elastic modulus (EM) increased from 980 to 1972 MPa as the power level increased from 180 to 900 W (microwave drying) and from 529 to 1518 MPa as the drying temperature increased from 30 °C to 70 °C in oven drying. In contrast, KBC with higher water absorption ( $\approx 55$  %) values were obtained when this material was dried in oven drying at 30 °C or in shade drying. Packaging materials must have high mechanical parameters and low water sensitivity. This research demonstrated that KBC must be dried using drying technologies (microwave and hot air oven drying) at elevated power levels and temperatures to produce dried KBC with high EM and less water absorption.

In another research, Ramírez Tapias et al. [18] studied the production of KBC (pellicle) using different herbs (black tea, green tea, yerba mate, Patagonian lavender, oregano, and fennel) and concluded that in all cultures, pellicle production resulted in a maximum after 21 days. Native pellicles from yerba mate had a remarkable antioxidant activity of 93 ± 4% of radical inhibition due to plant polyphenols, which could prevent food oxidation. Results revealed that pellicles retained natural bioactive substances preserving important physicochemical properties, essential for developing active materials in packaging applications.

Table 1 summarizes the use of KBC in packaging applications. Most researches have used KBC as a filler to reinformed biopolymer-based films manufactured by casting and extrusion (Figure 3). In these studies, the use of KBC improved mechanical, optical, and barrier properties and imparted antioxidant and antimicrobial activities in the developed materials (Table 1). However, this macromolecule also can be used as polymeric matrix to manufacture packaging materials or as an encapsulating material of bioactive molecules (e.g. anthocyanins and essential oils) in packaging applications (Table 1 and Figure 3).

	<b>Table 1.</b> Application of kombucha bacterial cellulose (KBC) in food packaging materials.							
	KBC application and	Production method	Major findings	Ref.				
and ingredients	treatment							
CMC and anthocyanins extract	KBC was used as a filler (1 – 15 wt%). KBC was cleaned with deionized water and sodium hydroxide (1 M), and dried at 50 °C for 20 h	Casting method at 40 °C for 18 h	The incorporation of KBC increased TS from 1.28 to 18.51 MPa and improved UV-barrier (200 – 400 nm) properties in CMC films containing anthocyanin extract. Films incorporated with KBC increased red grapes and plums' shelf life by up to 25 days	[11]				
PLA plasticized with maleinized linseed oil	KBC was used as a filler (3 – 5 wt%). KBC was sterilized, cut into small pieces, and dispersed in deionized water in a 1:2 proportion. Dispersions were homogenized in 4 cycles of 30 s at 30,000 rpm by using an Ultra-turrax. Finally, KBC wad dried at 60 °C for 2 days	Films were produced using a conical twinscrew microextruder. The temperature profile was set up at 195–190–190 °C in the three extrusion areas and screw speed was established at 25 rpm. Formulations were mixed during 3 min. The die temperature was set up at 180 °C and the film drawing speed at 1200 mm/min. Films with a thickness of 100–200 μm were obtained	The incorporation of KBC produced a reduction of film transparency and reduced the transmittance in the UV region of the spectra. Furthermore, EM (1308 → 1639 MPa) and TS (13 → 31 MPa) increased with 5 wt% KBC. Unfortunately, WVTR increased from 82 → 116 g/m²-day with KBC incorporation	[19]				
KBC	KBC was used as the polymeric matrix. KBC was washed with deionized water (2 ×1.0 L) and pat dried with Kleenex tissues. In sequence, KBC was purified by immersion into a NaOH solution at 90 °C for 1.0 h. Finally, KBC was dried at 50 °C for 20 h to obtain	Films were produced by the casting method at room temperature for 24 h	Citric acid cross-linking resulted a decrease in TS (25.3 → 7.8 MPa). Whereas carbamate crosslinking with hexamethylene, toluene, methylene di- <i>p</i> -phenyl and 4,4'-methylene-bis(cyclohexyl) linking groups by treatments with corresponding diisocyanates resulted improvements in TS (25.3 → 44.1	[20]				

	films. Finally, films were modified using		$\pm$ 7.1 MPa), thermal stability (Tonset 215 $\rightarrow$ 281.5 $\pm$ 33.5 $^{\circ}$ C) and	
	citric acid and		reduction in water retention (100 $\rightarrow$ 60 ± 20 %) properties in KBC	
	carbamate groups		films	
Chitosan	KBC was used as a filler (1 – 3 wt%). No kombucha treatment was reported by the authors	Casting method at 50 °C for 24 h	The incorporation of KBC reduced WVP from 256.7 to 132.1 g·mm/cm²·h·KPa and enhanced the antioxidant activity (59% DPPH), and the protective effect of the film against ultra violet. Furthermore, active films reduced lipid oxidation and microbial growth in minced beef during storage	[21]
Agar and alginate	KBC was used as a filler (2.5 wt%). KBC was cleaned by stirring distilled water for 48 h, filtered, and then heated at 50 °C for 12 h with 1 M NaOH, followed by 1 h treatment with 1 % glacial acetic acid. KBC was washed with distilled water until the pH reached 7. Finally, KBC was freeze-dried. KBC was treated enzymatically with cellulase	Casting method at 45 °C for 20 h	TS of control films (agar and alginate) decreased from 9.98 MPa to 7.69 MPa with the incorporation of unhydrolyzed KBC, however, TS increased to 18.18 MPa when KBC was incorporated into the polymeric matrix. This result was due to the better uniformity and particle size distribution of KBC	[22]
Alginate and anthocyanins	KBC was used as encapsulating material (0.1 – 0.4 wt%). KBC was ground with a crusher for 4 min at 8000 rpm and then centrifuged at 6000 rpm for 10 min. KBC was hydrolyzed using a 50 % (w/v) sulfuric acid solution in a water bath at 45 °C for 6 h, followed by cleaning with ultra-pure water, centrifugation, and filtering. Hydrolyzed KBC was dialyzed and freeze-dried	Casting method at 45  °C for 20 h.  Oil in water (O/W)  Pickering emulsions were produced with camelia oil, water, and KBC as emulsifier, using an ultrasonic dispersion method	The incorporation of Pickering emulsions containing KBC increased TS from 12 to 33 MPa, reduced transmittance at 280 nm (52 → 3 %) and 660 nm (70 → 5 %), and increased WCA from 31 to 63°. Films containing Pickering emulsions displayed antioxidant activity	[23]
КВС	KBC was used as the polymer matrix. KBC was crushed in sterile deionized water and then homogenized at	N.i.	Materials based on KBC had elongation at break of 2 % and antimicrobial activity against <i>S. aureus</i> and <i>E. coli</i>	[24]

		T		
	10,000 rpm by Ultra- Turrax			
KBC, KBC and glycerol or KBC with chitosan	KBC was used as the polymer matrix. KBC was cleaned with NaOH (2 M) at 90 °C for 2 h, and then washed with deionized water 5–6 times. In sequence, KBC was treated with NaClO (2 M) at room temperature for 2 h and finally washed with deionized water for 1 h	Films were produced by drying KBC with hot air (temperature not informed). Furthermore, KBC was immersed in glycerol of chitosan solutions for 10 min at room temperature, followed by drying to obtain KBC plasticized with glycerol and composite KBC/chitosan films	The incorporation of glycerol and chitosan increased film thickness $(45 \rightarrow 130 \ \mu m)$ , density $(6 \rightarrow 15 \ g/m^2)$ , and TS $(50 \rightarrow 110 \ MPa)$ . KBC, KBC with glycerol, and KBC/chitosan films extended the shelf life of tomatoes by 12, 13, and 15 days when compared with uncoated tomatoes $(7 \ days)$	[25]
Gelatin	KBC was used as the encapsulating material (0.1 – 1 wt%). KBC was cleaned with NaOH (0.1 M) and then washed with distilled water	and KBC. Gelatin films were produced by the casting method with 1	Gelatin films containing 1% of Pickering emulsion had yellow color, homogeneous visual aspect, and antibacterial activities against <i>S. aureus</i> and <i>E. coli</i>	[26]
PLA and PHBV	KBC was used as a filler (5 wt%). KBC was homogenized with distilled water at 25000 rpm and treated by adding NaOH to the dispersion. The resulting mixture was centrifuged, washed, and freeze dried	Films were produced by extrusion (twin- screw microextruder) at 180 °C and 100 rpm for 2 min	Mechanical properties of PLA (EM ≈ 1.7 GPa, TS ≈ 61 MPa, and EB ≈ 4.2 %) and PHBV (EM ≈ 2.2 GPa, TS ≈ 31 MPa, and EB ≈ 9.0 %) were not altered with the incorporation of KB, however, the film biodegradability increased with the incorporation of KBC. Furthermore, KBC incorporation resulted in a ~23 % and ~45 % decrease in O₂ permeability for PLLA and PHBV, respectively	[27]

CMC: Carboxymethyl cellulose; DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate; EM: elastic modulus; N.i.: not informed; PLA: Polylactic acid; TS: tensile strength UV: ultraviolet; WVP: water vapor permeability; WVTR: water vapor transmission rate.

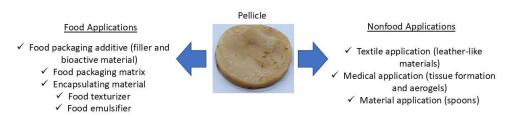


Figure 3. Main food and nonfood kombucha bacterial cellulose applications.

Pickering emulsions utilize solid particles to stabilize the interface between the two immiscible liquids, avoiding the use of classic surfactant [28,29]. Recently, KBC has been used to stabilize O/W emulsions where this biopolymer had reduced lipid oxidation and increased emulsion stability [28,30].

Finally, KBC has been used as an additive in mango jam. The incorporation of KBC between 20 to 100 g/kg reduced water activity up to 22.2% (0.68), moisture content from 37.1% to 19.9% and pH from 5.9 to 3.2. Furthermore, texture of jam with KBC gave higher gel strength and adhesiveness. Overall acceptability in sensory test scoring was above 70% on a nine-point hedonic scale with the 40 g/kg KBC jam chosen as the most preferred [31].

#### 3.2. Other material applications

KBC-based materials have potential nonfood applications (Figure 3). KBC has been used to develop materials with promissory industrial applications. In this way, KBC was incorporated with gold nanoparticles (AuNP), silver nanoparticles (AgNP), and graphene oxide (GO). The incorporation of nanomaterials increased glass transition (Tg =  $41 \rightarrow 50$  °C), tensile strength (TS  $63 \rightarrow 94.1$  MPa) and improved hydrophobicity (WCA =  $31 \rightarrow 81^{\circ}$ ), probably by the formation of noncovalent interactions. Furthermore, the presence of AgNP inhibited the growth of *S. aureus*. Although it was not informed the nanomaterial concentration added in KBC. The authors concluded that this nanocomposite can be used as a leather-like material with antimicrobial activity [32].

In another research, Candra et al. [33] synthetized AuNPs using a composite matrix based on chitosan and KBC. The obtained nanocomposite displayed antimicrobial effect against *Pseudomonas aeruginosa* and *Streptococcus mutans*, as well as in-vivo wound healing in mice, encompassing granulation tissue formation, reepithelization, reduced inflammation, and collagen fiber formation. Based on these results, KBC incorporated with AuNPs in a suitable candidate for medical applications.

Plastic spoons contribute significantly to environmental problems because they are typically made from non-degradable polymers. With this in mind, Muralidharan et al. [34] developed multi-layered composite spoon based on KBC and gelatin. KBC was cleaned with NaOH (0.5 N) at 90 °C for 1 h, followed by distilled water to until pH 7. Bilayer materials were produced by the layer-by-layer method where KBC and gelatin sheets were hot-pressed under a pressure of 2500 psi for 5 min at 90 °C. The resulting materials had high tensile strength of 47.7 MPa and a flexural strength of 117.27 MPa indicating high mechanical stiffness with the least density value of 0.71 g/cm³. In another research, Nguyen et al. [35] modified KBC with different silanes (dimethyldichlorosilane, hexadecyltrimethoxysilane, vinyltriethoxysilane and 3-aminopropyltriethoxysilane) followed by mixing with polyurethane (PU) and polylactic acid (PLA). According to the authors, the optimal biocomposite formulation was KBC 13.74% w/w, PU 73.89% w/w, and PLA 12.50% w/w compressed at 155 °C for 5 min. The KBC modification with silanes increased the WCA from 82° to 95° and reduced the water permeation through the material. In this way, WCA after 5 min was of 63° and 92° in untreated and treated KBC, respectively. Results from both studies suggest that disposable materials can be manufactured using KBC as matrix.

Aerogels are nanostructured materials with low bulk density and open porosity. Recently, they have been broadly investigated due to their capacity to load vitamins, chemical compounds and oils for food and material applications [36]. Aerogels based on KBC were produced after purification of KBC with KOH, followed by an acidic hydrolysis (H<sub>2</sub>SO<sub>4</sub> and HCl) and lyophilization. The obtained nanofibers presented thickness between 50 and 110 nm with high crystallinity (90%) [37]. KBC aerogels also have been applied 3D bioprinting in tissue engineering [38] and as acoustic foams [39].

#### 4. Future trends

#### 4.1. Food and pharmaceutical applications

Future research should investigate the biodegradability and toxicity of KBC. Regarding toxicity, both kombucha tea and KBC are not toxic when produced under controlled conditions and they are considered safe for consumption and use [40]. However, they should be used in moderation due to contraindications in infants, children under 4 years old, patients with renal, hepatic, and pulmonary diseases, as well as with the human immunodeficiency virus. Furthermore, this product is not recommended for pregnant women due to the interference with the coagulation process, which may be harmful to fetal development [40]. According to the literature, continuous consumption above 355 mL may cause internal organ perforations, resulting in renal lesions and necrosis in the duodenum, pancreas, and intestines [41]. The effects associated with kombucha consumption are still unclear, requiring further investigations. Currently, the regulations in effect for kombucha production address criteria such as pH and alcohol content to ensure food safety and quality for consumers.

The production conditions of kombucha, such as the type of tea used, fermentation time, and the microbial composition of the scoby are determining factors in its physicochemical, microbiological, and bioactive properties [42,43]. There is no doubt that both kombucha and scoby contain significant bioactive compounds, including polyphenols, organic acids, amino acids, vitamins, and minerals [41]. According to the literature, the use of green tea results in a quantitatively richer microbial composition, with free amino acids, reducing sugars, and increased antiproliferative activity against cancer cell lines, attributed to the presence of catechins [42–44]. Kombucha based on black tea, in turn, has demonstrated greater diversity and abundance of phenolic compounds, resulting in superior antioxidant capacity, presenting 70.2% flavonoids, 18.3% phenolic acids, 8.4% other polyphenols, 2.3% lignans, and 0.8% stilbenes [44]. These findings highlight that the choice of tea significantly influences the antioxidant properties of kombucha and KBC, indicating the need to study blends and the addition of new unconventional herbs to kombucha to evaluate their overall properties.

#### 4.2. Animal feed

In the animal feed industry, both kombucha and KBC have emerged as potential functional additives. Their biochemical composition, rich in probiotics, organic acids, and bioactive compounds, suggests significant benefits for animal nutrition, especially in the context of the growing demand for sustainable alternatives that repurpose organic waste [45]

Studies have already demonstrated these benefits in different species. Ramadhan et al. [46] supplemented the diet of catfish (*Clarias* sp.) with kombucha and observed a positive impact on growth, with increased weight gain, improved absolute growth rate, and better feed efficiency. These findings indicate that kombucha and KBC could be viable alternatives in formulating feed for aquatic animals. For terrestrial animals, Afsharmanesh and Sadaghi [47] investigated the use of probiotics, including kombucha, in the diet of broiler chickens. The results were promising: fermented tea exhibited growth-promoting effects comparable to traditional antibiotics, reinforcing its potential as a natural alternative in poultry farming.

In addition to lactic acid bacteria, yeasts play a fundamental role in KBC, actively participating in the fermentation of kombucha. In this context, some fungi have been studied as nutritional supplements due to their probiotic and enzymatic potential. Singh et al. [48] investigated the supplementation of white button mushrooms (*Agaricus bisporus*) in the diet of *Penaeus vannamei* (Pacific white shrimp) and observed a significant improvement in survival rate, specific growth rate, feed conversion efficiency, and protein efficiency ratio, along with increased average weight gain. These findings suggest that the presence of beneficial microorganisms in animal feed can contribute to better performance and improved nutrient absorption efficiency.

The probiotics present in kombucha play a crucial role in modulating the intestinal microbiota. Research indicates that its consumption can help balance gut microbiota in animals, promoting better digestibility and absorption of essential nutrients [49]. Additionally, antioxidant compounds such as polyphenols help reduce oxidative stress, strengthen the immune system, and increase resistance to infections, which directly impacts the physiological performance of animals [50]. These benefits, combined with the fact that the KBC, a by-product of kombucha fermentation, can be repurposed as an ingredient in animal feed, make it a promising alternative aligned with the global trend toward more sustainable and natural practices in animal nutrition [51].

Despite its great potential, more studies are still needed to validate the efficacy of kombucha on a large scale. The composition of the beverage can vary considerably due to factors such as fermentation time and the ingredients used, making standardization and the determination of ideal dosages challenging. However, with growing interest in sustainable solutions and the search for alternatives to synthetic antibiotics and additives, kombucha is likely to gain more visibility in research and the animal feed industry. Its potential to enhance animal performance and reduce environmental impact makes it a strong candidate to establish itself as a viable functional feed additive for the future of animal nutrition.

#### 4.3. Nonfood applications

As discussed in Section 3.2, some studies have explored the use of KBC in textile, medical, and material applications. It is necessary to explore new KBC applications in these fields, such as in the production of porous materials to be used as scaffolds and adsorbents, as well as for 3D printing. Furthermore, this macromolecule can be used to manufacture disposable plates and packaging for nonfood applications. However, before an industrial application, it is necessary to establish regulatory frameworks with the intention of standardizing the physicochemical properties of this macromolecule and its production at an industrial scale.

#### 5. Conclusions

Pellicle or kombucha bacterial cellulose (KBC) is a coproduct of the kombucha beverage production without economic value. Recent studies demonstrate that KBC has potential food and nonfood applications. KBC is composed of cellulose of high purity and it has been used to manufacture or reinforce food packaging, as well as to encapsulate active molecules or stabilize oil in water emulsions. Other applications range from the production of porous material for tissue engineering to the production of spoons. Future research studies must standardize KBC production at an industrial scale and establish international legislation about KBC. New KBC applications are expected to be explored since this material has antioxidant properties and it can be used to produce functional foods and feeds or to manufacture active materials. Furthermore, KBC can be used to produce nanocellulose and porous materials for culture meat and adsorbents.

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#### **Abbreviations**

The following abbreviations are used in this manuscript:

AgNPs Silver nanoparticles
AuNPs Gold nanoparticles
CMC Carboxymethyl cellulose

EM Elastic modulus
PLA Polylactic acid
PU Polyurethane
TS Tensile strength

SDGs Sustainable Development Goals

WCA Water contact angle
WVP Water vapor permeability
WVTR Water vapor transmission rate

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