Cellulose (2021) 28:10399–10414
https://doi.org/10.1007/s10570-021-04106-z

ORIGINAL RESEARCH

Bacterial cellulose based facial mask with antioxidant property and high moisturizing capacity

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Received: 18 April 2021 / Accepted: 23 July 2021 / Published online: 13 September 2021

Abstract Bacterial cellulose (BC) produced by certain bacteria has the potential to be used in many different areas. Despite its advantageous properties compared to plant cellulose, such as high purity, mechanical strength, nanofiber mesh structure, and high-water holding capacity, its production through a biotechnological process prevents it from competing with plant counterparts in terms of cost-effectiveness. Therefore, studies have focused on the development of culture media with cost-effective BC production methods and the production of high value-added products from BC. In this study, it was aimed to develop a taurine-loaded moisturizing facial mask with antioxidant properties based on BC's high-water retention and chemical retention capacity. BC facial mask samples were characterized by Scanning Electron Microscopy (SEM) imaging, Fourier Transform Infrared (FTIR) Spectroscopy, Differential Scanning Calorimetry (DSC), Liquid Chromatography–Mass spectrometry (LC–MS), microbial and mechanical stability tests, as well as cytotoxicity tests. According to our results, produced facial mask samples did not show any cytotoxic effect on human keratinocyte (HS2) or mouse fibroblast (L-929) cell lines; it has high thermal stability, which makes it suitable for different sterilization techniques including sterilization by heat treatment. Taurine release (over 2 µg/mL in 5 min) and microbial stability tests (no bacterial growth observed) of packaged products kept at 40 and 25 °C for 6 months have shown that the product preserves its characteristics for a long time. In conclusion "bacterial cellulose-based facial masks" are suitable for use as a facial mask, and they can be used for moisturizing and antioxidant properties by means of taurine.

Keywords Bacterial cellulose · Facial mask · Taurine · Long term stability

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Introduction

Cellulose, which is the main component of the plant cell wall, forms one third to half of the plant mass and

is found in annual or perennial plants, such as wood, cotton, linen, poppy and wheat. It is the most produced and found biopolymer in nature due to several billion tones biosynthesis per year (McNamara et al. 2015; Sun et al. 2004). More than 180 years have passed since the first isolation of cellulose from green plants by Anselma Payen (Payen 1838). Cellulose, used as a chemical raw material for the last 150 years, has been used for thousands of years in the making of clothing goods, as an energy source for heating and in the construction of shelters (Klemm et al. 2005; Moran-Mirabal and Cranston 2015). In addition to plants, cellulose, which is a high molecular weight polymer formed by linking of D-glucopyranose units with β-1,4-glycosidic bonds, can be synthesized by several microorganisms, such as *Gluconacetobacter xylinus* (formerly *Acetobacter xylinum*), *Achromobacter, Alcaligenes, Aerobacter, Agrobacterium, Azotobacter, Pseudomonas, Rhizobium* and *Sarcina* (Ahmed et al. 2020; Cheng et al. 2011; Reiniati et al. 2017; Vitta and Thiruvengadam 2012; Wei et al. 2011; Ye et al. 2019). Even though bacterial cellulose (BC) has same molecular formula with plant cellulose $(C_6H_{10}O_5)_n$ (Vitta and Thiruvengadam 2012), since it is does not contain lignin, hemicellulose or pectin in its structure, it can be produced with high purity via relatively easy procedures (Pang et al. 2020). Having properties distinguishable from plant cellulose, such as high mechanical strength, high water holding capacity, biocompatibility, producibility in desired shape, and nanofiber (< 130 nm diameter) network structure, make BC the material of choice for many applications like tissue engineering, artificial organ development, wound dressing, membrane production, enzyme immobilization, polymer-metal composite production, electronic paper production, food packaging, dental implants, and cornea replacements (Azeredo et al. 2019; Fu et al. 2013; Gorgieva and Trček 2019; Picheth et al. 2017; Yamanaka et al. 1989).

The skin is a complex organ that serves as an interface separating the organs from the outside. It protects the organism from external adversities, regulates homeostasis and helps to adapt to the environment, and acts as a barrier to harmful external factors, such as microorganisms and UV radiation. In addition to all these, today, where cosmetic concerns are at a high level, it is also of great importance in terms of physical appearance (Hussain et al. 2013; Tur 1997). The high amount of water contained in the skin enables it to perform many important functions for the body. Skin aging, which is a natural process, accelerates due to external factors that the skin is exposed to and water loss. Therefore, moisturizing the skin has a priority in providing skin care today (Spada et al. 2018) and this is often done by wearing a mask with moisturizers. One of the major obstacles to skin protection is the negative effects of oxidative stress (OS) caused by external or internal factors (Trouba et al. 2002). Lipid oxidation is a reaction in which free radicals like ROO– and ROOH, and substances such as malondialdehyde (MDA) and conjugated dienes are produced (Finaud et al. 2006). The formation of reactive oxygen species (ROS) and free radicals (FR) in the body, and the removal of the effects of these radicals are natural processes. With increasing OS, the balance shifts towards ROS and FR. Antioxidant supplement is a useful approach to protect body from harmful effects of ROS and FR.

In the literature, there are studies on the development of facial masks by adding various herbal extracts or active agents to the BC structure as a moisturizers (Amnuaikit et al. 2011; Bianchet et al. 2020; Pacheco et al. 2018; Perugini et al. 2018). Also, some studies focus on using BC for transdermal drug delivery (Almeida et al. 2014; Morais et al. 2019). In this study, it was aimed to cost-effectively produce BC, a natural and biocompatible polymer with high water holding capacity (> %99 w/w), and then to develop a BC-based facial mask with antioxidant and moisturizing properties by loading taurine. Taurine (2-aminoethanesulfonic acid), derived from methionine and cysteine metabolism, plays a pivotal role in numerous physiological functions, among which is the protection of tissues and membranes caused by free oxygen radicals (Koyama et al. 1992; Nakamura et al. 1993; Perugini et al. 2018). Taurine has effects on osmoregulation, cell proliferation, inflammation control and collagen production. It has a unique ability to neutralize hypochlorous acid, which destroys cell membranes, leading to premature skin wrinkling. Thus, taurine is frequently added in energy drinks, infant formulas and cosmetics (Almeida et al. 2014; Bianchet et al. 2020; Rona et al. 2004; Wright et al. 1985).

Materials and method

Cultivation of microorganism

Gluconacetobacter xylinus (ATCC 700178) was activated in agitated culture (150 rpm, 30 °C) for 24–48 h. Activation of microorganism was performed in Hestrin & Schramm standard medium (Hestrin and Schramm 1954), in which 2.5 glucose (w/v %) (Merck, 108342, USA), 0.5 (w/v %) peptone (Merck, 107214, USA), 0.5 (w/v %) yeast extract (Oxoid, LP0021, England), 0.27 (w/v %) $Na_2HPO_4.2H_2O$ (Merck, 106580, USA) and 0.115 (w/v %) citric acid monohydrate (Merck, 100244, USA) were used, and the initial pH was adjusted to 5.5.

Production of BC

Activated culture was used in the production of BC based facial mask. For this, previously developed and optimized culture medium (Carob and haricot bean-CHb) was used. The medium content consists of 2.5% w/v carbon source (obtained from carob extract), 2.75% w/v protein source (obtained from dried beans), 1.15 ‰ w/v citric acid and 2.7 ‰ w/v Na_2HPO_4 (pH 5). The medium volume was adjusted to 1.5 cm^{-1} surface/volume ratio considering the production vessel and incubated at 30 °C for 7 days using 10% v/v inoculum (Bilgi et al. 2016).

Purification of BC

The purification of BC samples was performed by a slightly modified alkali treatment method (Bilgi et al. 2016). To remove the culture medium and bacterial residues, harvested BC membranes were rinsed in distilled water and kept in 0.1 M NaOH (Merck, 106498, USA) at 60–80 °C overnight. After boiling in freshly prepared 0.1 M NaOH (BC: NaOH, 1:5 w/v) for 20 min, BC samples were neutralized in 5% (v/v) acetic acid (J.T. Baker, 6052, Holland) for 3–5 s, and boiled in distilled water repeatedly. Finally, samples were autoclaved at 121 °C for 15 min.

Modification of BC with taurine

In the preliminary studies, the liquid absorption capacities of the BC samples were analyzed after freeze drying (frozen first at − 20 °C and then lyophilized in (Christ Freeze Dryer—Alpha 1–2 LD-Germany) at − 55 °C and 0.07 mbar) and heat drying (60 °C in Forced Convection Oven-JSR JSOF-100-Korea). After the two drying processes, the samples lost more than 99% of their weight (from 90 ± 4.5 g to 500 ± 13.5 mg). When dried samples were re-immersed in water, they were only able to reabsorb 10.5 ± 1.5 and 52 ± 8 times their dry weights for the heat-dried and lyophilized samples, respectively. For this reason, samples in BC modification to form the facial mask were not dried by either method. Sterilized BC membranes were immersed in sterile 50 mM taurine in distilled water (1-unit wet BC: 4 units of taurine solution—w/v). Since nearly the entire the weight of BC (> %99) is water, the weight of the samples were also considered while preparing the taurine solution. Phenostat (1%) was added as a preservative agent to the solution, which is safe to use for up to 3%, and does not contain paraben, and the samples were stirred at 80 °C for 2 h on a magnetic stirrer, and then packaged. The packaging process was carried out with a rail brought to 250 °C using packages made of non-woven aluminum added foils (17.5 × 12.5 cm). No leakage or interaction with the product was detected as a result of the packaging.

Characterization of BC based facial masks

Microbial stability and challenge tests

Samples were controlled via counting aerobic mesophilic bacteria, yeast, *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans*, every month for 6 months. Challenge tests were performed against *Escherichia coli* ATCC 8739, *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 9027, *C. albicans* ATCC 10231, *Aspergillus brasiliensis* ATCC 16404 at 7th, 14th and 28th day. The methods stated in FDA's Bacteriological Analytical Manual have been used for both microbial stability and challenge tests (FDA 1998).

For microbial stability test, in brief, 10 g of CHbT-P samples (BC samples produced in CHb and loaded with 50 mM taurine and 1% phenostat) were removed from their packages and homogenized in 90 mL Letheen Broth (Merck KGaA, Darmstadt, Germany). Serial dilutions were prepared and mixed with (1:15 mL) proper agar medium and incubated at 35 °C for 72 h and then formed colonies were counted. Tryptic Soy Agar, Sabouraud Dextrose

Chloramphenicol Agar, Candida Ident Agar, Cetrimide Agar and Bair Parker Agar (Merck KGaA, Darmstadt, Germany) were used for aerobic mesophilic bacteria, yeast, *C. albicans*, *P. aeruginosa*, and *S. aureus*, respectively.

For challenge tests 5.0×10^8 cfu/mL suspensions were prepared for *E. coli, S. aureus,* and *P. aeruginosa*, 5.0×10^7 cfu/mL suspensions were prepared for *C. albicans* and 5.0×10^7 spores/mL suspensions were prepared for *A. brasiliensis.* 0.2 mL of culture suspension were added to 20 g packaged BC samples and incubated at 25 °C for 7, 14, and 28 days. At the end of the incubation periods 1 g of sample was taken and neutralized by 9 mL neutralizer, and 30 min later inoculated onto petri dishes containing proper agar medium. Bacteria and *C. albicans* were incubated at 35 °C and *A. brasiliensis* at 25 °C. Calculations were performed according to the formula given below:

$$N = \frac{\sum c}{V(n_1 + 0,1\,n_2)d}$$

where N: number of colonies in the samples, $\sum c$: number of colonies in petri dishes for individual samples, V: inoculum amount in terms of mL, n_1: the number of petri dishes for the first dilution, n_2: the number of petri dishes for the second dilution and d: dilution factor (for the first dilution).

Cytotoxicity tests

Two cell lines were used for cytotoxicity assessments. The human keratinocyte (HS2) cell line was used because the final product would be used on the skin, while the mouse fibroblast (L929) cell line was used as standard cells according to ISO 10993–5:2009 (EN). Dulbecco's Modified Eagle's Medium F12 (DMEM-F12) supplemented with 10% (v/v) Fetal Bovine Serum (FBS), 1.0% (v/v) sodium pyruvate, 100 U/mL penicillin, and 100 µg/mL streptomycin was used as HS2 cell culture medium. Minimum Essential Medium (MEM) supplemented with 10% (v/v) Fetal Bovine Serum (FBS), 1.0% (v/v) sodium pyruvate, 100 U/mL penicillin and 100 µg/mL streptomycin was used as L929 cell culture medium. The cells were cultivated in a 37 °C, 5% CO_2 humidified incubator. The cell culture medium was replaced with fresh medium every other day. The cells were detached by trypsinization and subcultured before reaching 85–90% confluency. Cells were seeded at a concentration of 1×10^5 cells/well in a 6-well plate in three repetitions. The cytotoxicity of the four different BC samples (produced with HS or CHb medium, with or without taurine) were evaluated via indirect contact agar diffusion test (Gola 2019). For this method, after the cells reached confluency the cell culture media were solidified with agar [1% (w/v)] to prevent direct contact of the samples to the cells. BC samples (produced with HS or CHb medium, with or without taurine), cut to appropriate sizes (1×1 cm^2), were placed on the agar. After 24 and 72 h, the cells were stained with Giemsa (Sharma et al. 2021). Briefly in this method, the cells were rinsed first by PBS, then by PBS-Methanol (1:1.5) solution after removing the BC samples and the agar medium. Cells were fixed by methanol for 10 min, and then stained with Giemsa for 5 min. The morphology of the cells was examined under an inverted microscope.

Endotoxicity test

Since *G. xylinus* is a Gram-negative bacterium, prepared samples are likely to contain endotoxin despite washing and sterilization procedures. In this study, semi-quantitative gel-clotting method (Blechova and Pivodova 2001) was used to measure endotoxicity in vitro. For the sample preparation, the international standard ISO 10993–12:2009 procedure was followed.

Bio-adhesion tests

Bio-adhesion measurements were performed using the penetrometer TA-TX Plus equipped with a load cell of 500 g, a cylinder probe of 1 cm, and an A/MUC measuring system (Karavana et al. 2012). The A/MUC measuring system consisted of a ring, in which the biological support could be fixed. The samples were placed into a beaker filled with water at 37 °C, force was applied with the apparatus on which rabbit skin was implemented and attachment of BC samples to skin was measured after the force was removed. The samples were applied to the cylinder probe. The sample and biologic substrate were put in contact with a pre-load of 6000 mN for 3 min. The cylinder probe was moved upwards at a predetermined speed of 2.5 mm/min until the complete separation of the adhesive interface (skin–sample). Maximum

detachment force was obtained from the force–distance graph. The area under the curve as the muco-adhesion took place was calculated from the force–distance plot. Each experiment was carried out six times.

Differential scanning calorimeter (DSC)

Thermal properties of different BC samples –containing taurine, phenostat or not- were measured with DSC. The samples were sealed in aluminum pans (50 μL) and placed in DSC (Perkin Elmer DSC-8000). DSC analysis was conducted under nitrogen flow (20 mL/min) in a temperature range between 25 and 200 °C at a heating rate of 20 °C/min.

Taurine release

Taurine release assays were performed using the LC/MS to determine whether the produced BC absorbed taurine and whether the taurine was released with an appropriate characteristic. Considering storage and application conditions, products were kept for 6 months in incubators at 25–40 °C and containing 60% humidity. The samples were taken every month and taurine amount was measured. For the determination of taurine amount, separation was performed on Zorbax SB C18 (50 mm × 2.1 mm, 5 μm) analytical column (Agilent, USA) maintained at 25 °C. The mobile phase used was methanol: 0.5% formic acid (60:40, v/v) delivered at a flow rate of 0.4 mL/min with an injection volume of 5 μL. The total LC run time was 1 min. The mass spectrometer was operated at SRM mode. The transitions of mass to-charge ratio were m/z 124.1 → 80.0, m/z 126.350 → 44.820, m/z 126.350 → 108.340. The following parameters were set: Air (zero grade) as nebulizer gas, nitrogen as auxiliary gas pressure = 10 Arb, sheath gas pressure = 35 Arb, collision gas = 1.5 mmTorr, capillary temperature = 350 °C, vaporizer = 350 °C, positive polarity = 3000 V, negative polarity = 3000 V, discharge current = 4 μA ion source gas one and ion source gas two = 25 and 45 psi, respectively.

Fourier transform infrared spectroscopy (FT-IR)

FT-IR analysis of dried BC and taurine-BC samples were performed by FT-IR spectrophotometer (Perkin Elmer Spectrum Two, FT-IR Spectrophotometer, USA). Scans were obtained in 600–4000 cm^{-1} spectral region. This analysis was performed in Ege University Central Research Test and Analysis Laboratory Application and Research Center (EGE-MATAL).

Scanning electron microscopy–energy dispersive spectroscopy (SEM-EDS)

SEM-EDS (Thermo Scientific Apreo S, USA) analysis was performed to determine the modification of BC with taurine and to observe the morphology of the samples. Before the observation both BC samples were freeze-dried and coated by 8 nm gold–palladium. This analysis was performed in Ege University Central Research Test and Analysis Laboratory Application and Research Center (EGE-MATAL).

Results and discussion

In our previous study, the optimum incubation time and protein concentration (haricot bean extract used as a protein source) for production of BC from *G. xylinus* was found to be 10 days and 2.75% (w/v), respectively (Bilgi et al. 2016). However, the wet weight of the facial mask (BC) sample produced under these conditions was over 200 g. As this weight was considered unsuitable as a facial mask, the incubation time was reduced from 10 to 7 days to produce lighter and thinner BC. By shortening the incubation period, products with a wet weight of 90 g (± 4.5 g) and a dry weight of 500 mg (± 13.5 mg) were obtained.

Endotoxicity tests

BC is widely used in the food industry as Nata de Cocco (Piadozo 2016) and is generally recognized as safe (GRAS) by the Food and Drug Administration (FDA) (Gorgieva 2020; Lin et al. 2013). However, due to the lipopolysaccharide (also known as endotoxin) found in the cell wall of *G. xylinus* used for BC production, the potential endotoxin content of BC samples should also be considered (Chiaoprakobkij et al. 2011; Fang et al. 2009). Although there are no restrictions on the use of this polymer, which is in the GRAS category, in sectors where the endotoxin threshold is very high, such as food, food supplements or probiotics (Wassenaar and Zimmermann 2018), the

effect of lipopolysaccharide residues on cells after washing should be evaluated, especially in biomedical, medical and cosmetic applications (Gorbet and Sefton 2004).

The results of the endotoxicity test with different dilutions of purified BC samples (1:1, 1:10, 1:100, 1:1000 and 1:10,000) are given in Table 1. FDA states that biomedical devices that will come into direct contact with body fluids should not contain more endotoxin than 0.06 EU/mL (FDA 2012).There is no clear limit in terms of endotoxin for the skin. Since the skin protects the body against endotoxins and other chemicals, the endotoxin limit is higher for substances to be used on the skin surface, as in the digestive system. When the test results of the samples produced in this study were examined, no gel formation was observed in 1:10 dilutions. The absence of gel formation indicates that the endotoxin content in that sample is below the limit that the test can detect. Accordingly, the samples intended to be used as facial masks contain endotoxin at a concentration of 0.6 EU/mL or lower, which is lower than the suggested preclinical-clinical study dosage limits that are 3 and 5 EU/mL for mouse and human, respectively (Malyala and Singh 2008).

Bio-adhesion tests

In a previous study, Pacheco et al. used sensory tests (fragrance, skin adhesion and color, etc.) to qualitatively evaluate the performance of their BC masks (Pacheco et al. 2018). Amnuaikit et al. qualitatively measured user satisfaction of BC-based facial masks, and the change in sebum level showed a decrease after the second application of their masks (Amnuaikit et al. 2011). In this study, bio-adhesion test, which allows quantitative measurement of adhesion, was preferred. The distance at which the BC samples adhere to the apparatus after the applied force is shown in Table 2. The highest strength was obtained with the products of CHb (0.102 kg-f) and HS (0.119 kg-f) media and those containing neither Phenostat nor taurine. Addition of taurine to both preservative-containing and control BC samples reduced adhesion distances in all samples produced in CHb or HS medium (Table 2). In general, the decrease in adhesion distances after the addition of taurine showed less change in CHb samples.

Table 1 Semi quantitative endotoxicity results of BC samples with different dilutions

Standards		Samples	
0.25 EU/mL	(+)	1:1	(+)
0.5 EU/mL	(+)	1:10	(−)
0.125 EU/mL	(+)	1:100	(−)
0.06 EU/mL	(+)	1:1000	(−)
0.03 EU/mL	(−)	1:10,000	(−)
0.0150 EU/mL	(−)		

(+) gel formation, (−) no gel formation

Cytotoxicity results

Attachment tests were performed to determine the attachment time of HS2 cells to culture vessels prior to cytotoxicity analysis. Attachment and growth kinetics of HS2 cell are given in Fig. 1. The results indicate that almost all cells were able to attach to the plates after 180 min (Fig. 1a) and the 12-day-old cells began to die as the culture medium was not replaced during the experiment (Fig. 1b). The standard deviation, that is, how far the values diverged from the mean value, was calculated using Microsoft Excel (Microsoft Corporation, Washington, USA).

Cells were examined under a light microscope by staining with Giemsa to see if the cell concentration under the BC samples decreased and/or the cell morphology changed (Figs. 2 and 3). In the indirect cytotoxicity test, if the tested substance shows toxic effect, it is expected that the cells in the area under the part where the sample is placed are sparse or do not proliferate at all. When the images are examined for BC samples, no dilution in cell confluency is observed compared to the control. No difference is observed in the 72nd hour photographs taken at wide angle (4X) compared to the control (Fig. 3.). BC did not show any toxic effects on cells, consistent with literature studies carried out in vitro or in vivo with BC or BC based products [(BC-alginate, BC-polyethylenglycol, BC-hydroxapatite, BC-gelatin, BC-Poly(3-hydroxybutyric acid-co-4 hydroxybutirate, BC-poly (l-lactic acid), BC-cerium nitrate and silver nanoparticles BC-bioactive glass, BC-silk fibroin composites] (Abdelraof et al. 2019; Bäckdahl et al. 2006; Cai and Kim 2010; Chiaoprakobkij et al. 2011; Fang et al. 2009; Kim et al. 2010; Teixeira et al. 2019; Wang et al. 2020;

Table 2 Bio-adhesion results of different samples. CHb: Carob Haricot bean medium, T: Taurine, Phe: Phenostat, HS: Hestrin & Schramm medium

Samples		Force (kg-f)					Adhesion distance mm				
Production medium	BC and/or additions	1	2	3	Mean	Std	1	2	3	Mean	Std
CHb	BC	0.062	0.071	0.060	0.064	0.006	2.525	3.450	2.788	2.921	0.477
	BC + T	0.078	0.089	0.062	0.076	0.014	2.66	2.850	2.750	2.754	0.094
	BC + Phe	0.096	0.105	0.104	0.102	0.005	3.187	3.537	3.500	3.483	0.192
	BC + T + Phe	0.066	0.078	0.067	0.070	0.007	2.725	3.125	3.325	3.058	0.306
HS	BC	0.081	0.069	0.075	0.075	0.008	3.138	3.125	3.362	3.208	0.133
	BC + T	0.106	0.106	0.107	0.106	0.001	2.850	2.425	2.463	2.579	0.235
	BC + Phe	0.126	0.115	0.115	0.119	0.006	3.013	2.825	3.013	2.950	0.109
	BC + T + Phe	0.108	0.121	0.119	0.116	0.007	1.800	2.038	2.562	2.133	0390

T: 50 mM taurine; Phe: %1 phenostat

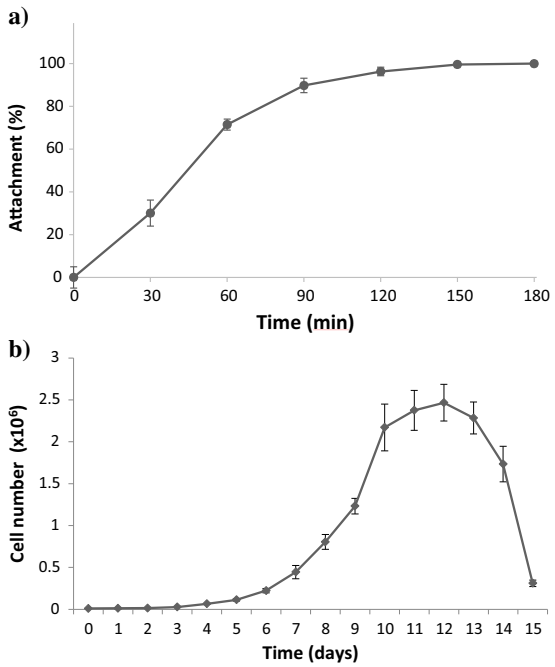

Fig. 1 **a** Attachment and **b** growth kinetics of HS2 cells

y Pérez et al. 2014; Zhang et al. 2020). Indirect cytotoxicity tests were performed after cells reached to 100% confluency. If the test is continued for a long time, the cells will die, because they cannot find a surface area to attach. For this reason, the test was terminated after 72 h to avoid false negative results. Long term in vivo biocompatibility studies on BC have also shown positive results (Klemm et al. 2001; Pértile et al. 2012). Considering these results, it was decided that BC could be used as a mask, and samples were packaged by loading preservative agent and taurine after characterization.

Microbial stability

It is difficult to accurately predict the microbial stability and efficacy of a cosmetic product. Therefore, cosmetic products need to be tested against certain Gram-negative and Gram-positive bacterial strains that they may encounter during their shelf life (Dao et al. 2018). In this way, the effectiveness of the preservative agent can be tested. According to the European Union cosmetic regulations, it is prohibited to subject cosmetic products to animal tests. Therefore, products must be tested without using animals and risking human health (Pauwels and Rogiers 2010). Although there are some difficulties, in vitro laboratory trials are proposed as an alternative to animal tests that was prohibited in 2013 (Adler et al. 2011).

Bacterial stability results show that the preservative (Phenostat^{TM}, INOLEX, Europe) used at a rate of 1% and allowed to be used in the range of 0.5–2%, provides the desired effect. Preservative stability and microbial stability tests were performed for products that were found to have no toxic effects on cells. After certain incubation periods, it was determined that there was no microbial load on the samples tested (Table 3). Since the contamination of *S. aureus, P. aeruginosa* and *C. albicans* was not present initially, their subsequent occurrence was not expected, and these microorganisms were not sought after the initial

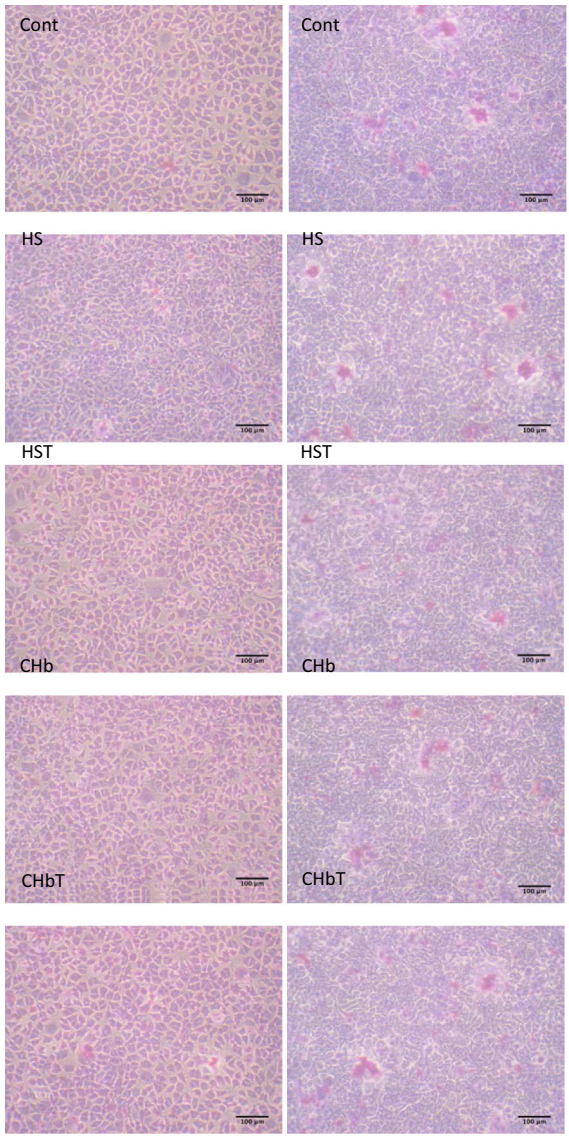

Fig. 2 Microscope images of L929 (left) and HS2 (right) cells after 24 h. Cont: Control, HS: BC samples produced with Hestrin&Schramm (HS) medium, HST: BC samples produced with HS medium and treated with 50 mM taurine solution, CHb: BC samples produced with Carob Haricot bean (CHb) medium, and CHbT: BC samples produced with CHb medium and treated with 50 mM taurine solution (10 × Magnification)

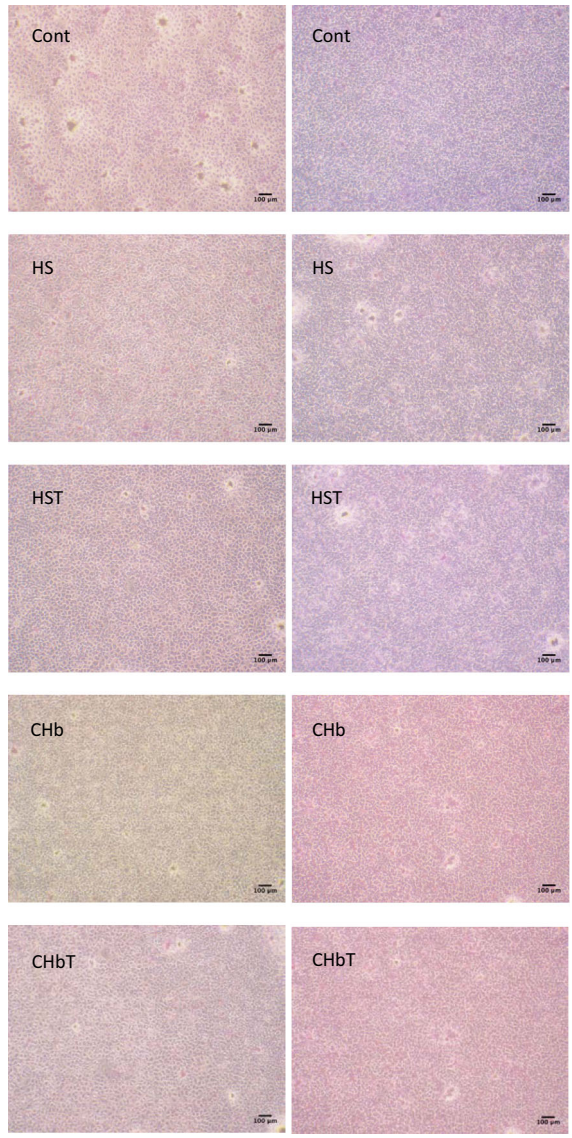

Fig. 3 Microscope images of L929 (left) and HS2 (right) cells after 72 h. Cont: Control, HS: BC samples produced with Hestrin&Schramm (HS) medium, HST: BC samples produced with HS medium and treated with 50 mM taurine solution, CHb: BC samples produced with Carob Haricot bean (CHb) medium, and CHbT: BC samples produced with CHb medium and treated with 50 mM taurine solution (4X magnification)

time point. It is seen that the effect of the 1% preservative agent is above the legal limit of 3-log reduction to eliminate certain microorganisms (Table 4). These results show that the product is effectively protected against microbial load for a long time (5 months), and microorganisms are effectively eliminated in case of contamination. According to the literature review, no long-term microbial stability analysis was performed in studies using BC as a facial mask or drug delivery system (Almeida et al. 2014; Amnuaikit et al. 2011; Pacheco et al. 2018).

Table 3 Microbial stability results

Microbial analysis	Day 1	1st month	2nd month	3rd month	4th month	5th month
Aerobic bacteria count	None	None	None	None	None	None
Yeast-mold	None	None	None	None	None	None
S. aureus	None					
P. aeruginosa	None					
C. albicans	None					

Table 4 Microbial challenge (screening) results

	7th day	14th day	28th day	Limit
E. coli ATCC 8739	> 4.04	> 4.04	> 4.04	≥ 3 log
S. aureus ATCC 6538	> 4.10	> 4.10	> 4.10	≥ 3 log
P. aeruginosa ATCC 15,442	> 4.14	> 4.14	> 4.14	≥ 3 log
C. albicans ATCC 10,231	> 3.04	> 3.04	> 3.04	≥ 1 log
A. brasiliensis ATCC 16,404	1.18	2.48	2.81	≥ 1 log

DSC analysis

BC is a highly crystalline and relatively thermally stable as a natural polymer with inter and intramolecular hydrogen bonds. Although the thermal decomposition temperature of BC is 250 °C, in surface-modified BC samples or BC-polymer composites, this value may drop to 121 °C or even below 110 °C, the temperature used for steam sterilization (Altun et al. 2019; Badshah et al. 2018). The water holding capacity of BC is mostly provided by the hydrophilicity of the high amount of hydrogen bonds in its structure. In this study, it was also investigated whether taurine loading has a negative effect on thermal stability, since autoclave is used for the sterilization of BC samples. DSC, a widely used method for determining the thermal properties of biological molecules (Spink 2008), allows the thermal capacity of the biological molecule in liquid solution to be obtained as a function of temperature (Lopez and Makhatadze 2002). The melting temperature of taurine is above 300 °C (White and Fishman 1936). At this temperature, disruption peaks are observed in biological polymers and studies have been conducted in a range where we can observe possible glass transition temperatures. DSC plots of different BC samples are given in Fig. 4. None of the formulations showed a glass transition within the tested temperature range. The thermal responses were similar for HS, HS-T and HS-P especially in the range where the biological materials were not affected from heat. The additives have changed the thermal behavior in HST-P samples, degradation peaks were less likely to occur. In HS samples additives contribute not only to the resistance against microbiological contamination and oxidative stress, but also to the thermal degradation. However, the same result could not be gathered from CHb group. These observations were attributed to the solubilization of additives and structural differences of the media.

SEM-EDS

Structural difference can easily be seen in SEM images of BC samples with and without taurine loading (Fig. 5). While the fibrous structure is preserved in freeze-dried BC samples without taurine, it is seen that the fibers are coated when taurine is added. EDS results of taurine (Table 5), dried BC and taurine on freeze dried BC indicate that taurine incorporation to BC samples were achieved successfully.

FT-IR analysis

FT-IR spectra of BC samples were given in Fig. 6. in which, 3344, 2895, 1427, 1335, 1162, 1056, and 1032 cm^{-1} peaks were observed for BC, and in addition to these peaks 3214, 3040, 2970, and 962 cm^{-1} peaks were observed for taurine loaded BC. In the FTIR spectrum, for BC, the peak at 3344 cm^{-1} and the peaks in the range of 3400–3500 cm^{-1} show –OH stretching, strong

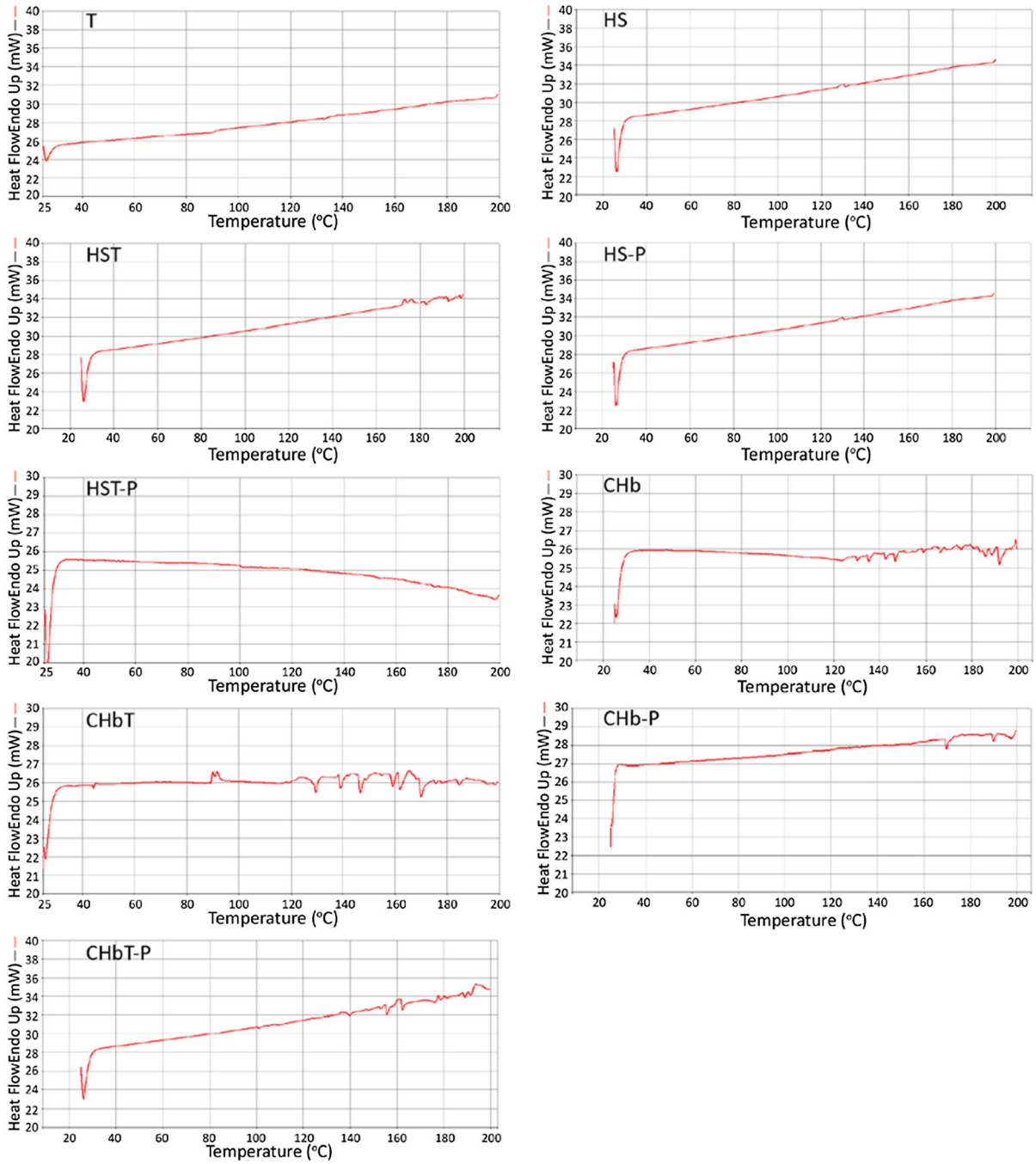

Fig. 4 DSC plots: T; taurine, HS; BC samples produced with HS medium, HST; BC samples produced with HS medium and treated with 50 mM taurine solution, HS-P; BC samples produced with HS medium and treated with 1% Phenostat, HST-P; BC samples produced with CHb medium and treated with 50 mM taurine and 1% Phenostat solution CHbT; BC samples produced with CHb medium and treated with 50 mM taurine solution, CHb-P; BC samples produced with CHb medium and treated with 1% Phenostat, CHb; BC samples produced with CHb medium, CHbT-P; BC samples produced with CHb medium and treated with 50 mM taurine and 1% Phenostat solution

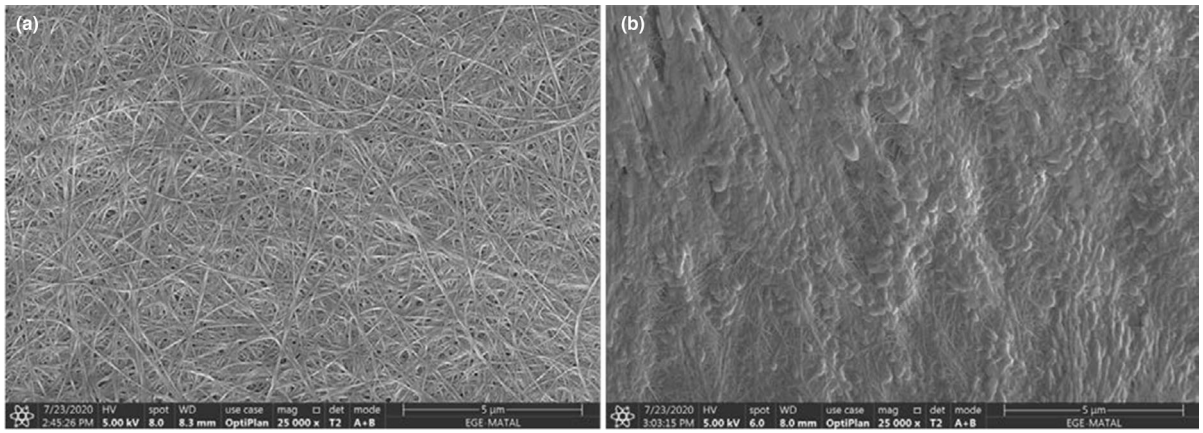

Fig. 5 SEM images of BC; **a** w/o taurine and **b** with 50 mM taurine loading

Table 5 EDX analysis of taurine, dried BC, and taurine section at freeze dried BC

Element	Taurine		Dried BC		Taurine + BC	
	W	A	W	A	W	A
C	6.73	9.26	21.71	26.97	1.9	2.8
N	13.9	16.41	–	–	10.69	13.5
O	64.46	66.64	78.29	73.03	64.04	70.81
S	14.91	7.69	–	–	23.36	12.89

*W: Weight (%), A: Atomic (%)

absorption peak at 2895 cm^{-1} shows C–H binding, peak at 1162 cm^{-1} shows C–O–C stretching, peaks at 1032 and 1056 cm^{-1} correspond to C–O bonds, the peaks at 1277, 1335 and 1427 cm^{-1}, indicating the presence of crystalline region and pure cellulose, correspond to C–H bending, O–H bending and $–CH_2–$ stretch bonds, respectively, which are correlated with literature (Castro et al. 2011; Halib et al. 2012; Liu et al. 2011; Wan et al. 2006). The peaks for BC-taurine (purple) around 3214, 3040, 2970, 1204 (S=O) and 962 (C–S–O) corresponds to taurine peaks (Wang et al. 2015), which show that taurine loading to BC has been successfully achieved.

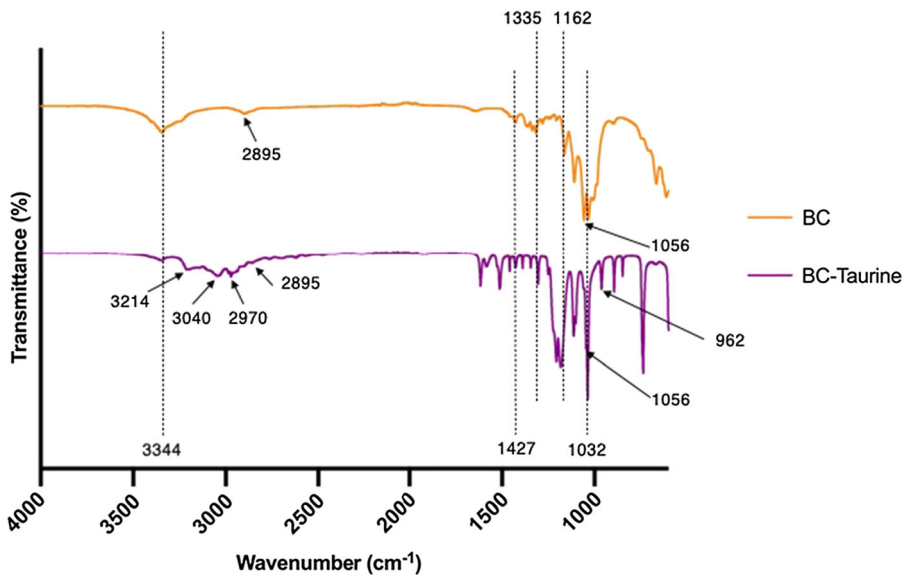

Fig. 6 FT-IR images of BC; orange w/o taurine and purple with 50 mM taurine loading

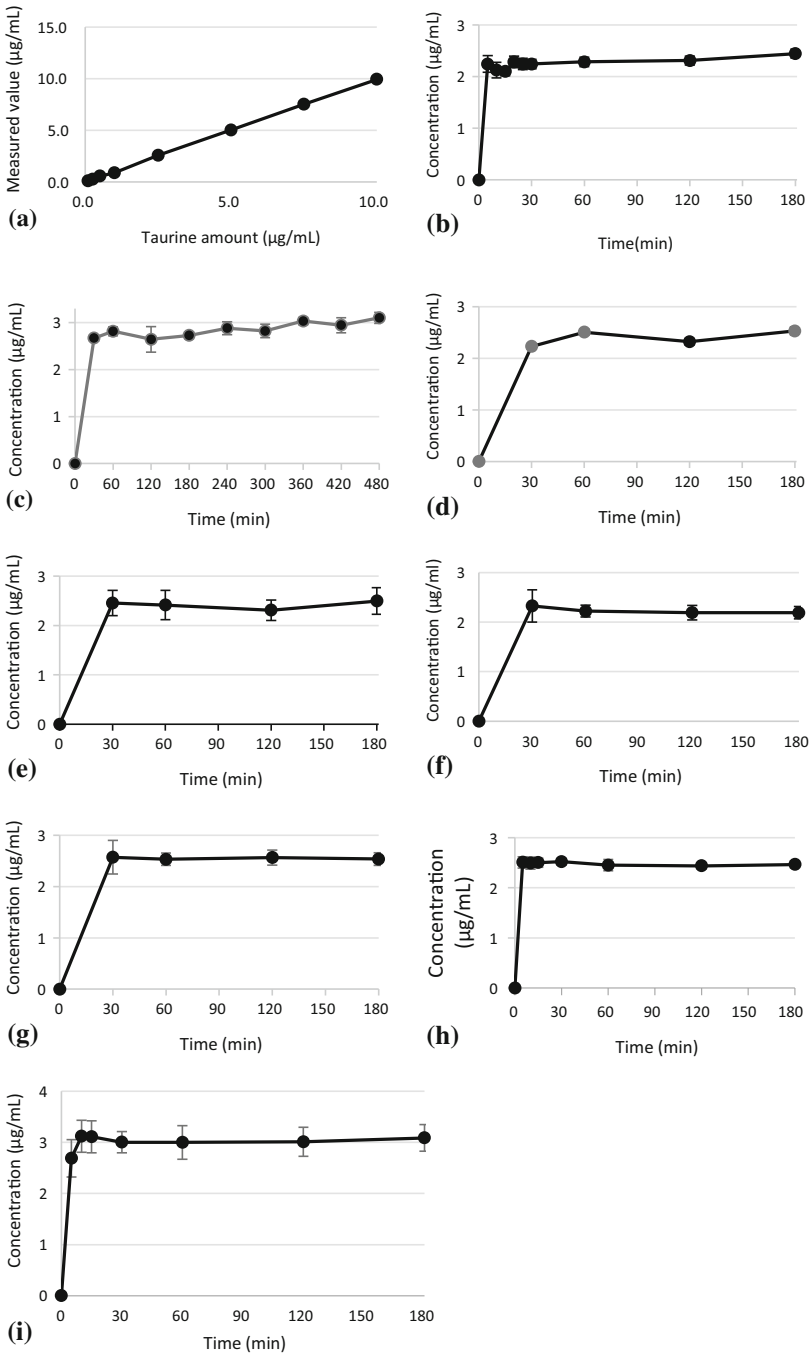

Fig. 7 Taurine release profiles of BC samples produced with CHb medium and treated with 50 mM taurine and 1% Phenostat solution: **a** standard graphic for taurine, **b** day 0, **c** 1 month 25 °C, **d** 2 months 25 °C, **e** 2 months 40 °C, **f** 4 months 25 °C, **g** 4 months 40 °C, **h** 6 months 25 °C and **i** 6 months 40 °C

Taurine release experiments

The formation and destruction of ROS and FR in the body occurs in a variety of ways of lipid oxidation, the formation of ROS and FRs using oxygen is between hypoxanthine and xanthine as a cofactor by xanthine oxidase (Finaud et al. 2006; Quinlan et al. 1997). Taurine has been shown to be effective in cell renewal and proliferation, to be nutritive and supportive for cells, and to help to reduce or eliminate the harmful effects of ROS and FR (Chen et al. 1998; Değim et al. 2002; Kim et al. 2017; Lee and Cheong 2017; Lou et al. 2018). Figure 7 shows the taurine release profiles of BC samples stored for up to 6 months at different storage conditions (25–40 °C and 40–60% humidity).

The highest release value was observed at the end of the 5th minute and the taurine concentration remained unchanged for the remaining 3 h. A similar pattern has been obtained in the following months. Taurine concentrations were stabilized at 2.5 and 3.0 μg/mL initially and gave the same result at the end of the following 1st and 6th months. Considering the limits that the LC–MS device can detect taurine linearly (Fig. 7a), BC samples were cut to 0.15 g/piece. BC samples immersed in 50 mM taurine solution were expected to contain 900–1000 μg taurine. During analysis the BC samples were kept in 20 mL PBS solution. Taurine concentration was predicted to be around 45–50 μg/mL. However, although the characteristic of release was consistent each month, the measured taurine concentration was found to be lower (2.5–3.5 μg/mL) than expected. BC is known to have high hydrogen bonding capacity (Hakeem et al. 2016). When the molecular structure of taurine is examined, it is seen that the unpaired electrons on each of the oxygens and on the nitrogen and the hydrogens in the nitrogen and hydroxyl group have the capacity to make hydrogen bonds.

Hydrogen bonds that may have occurred between taurine and BC may have caused the taurine release amount to be low. As a result, it is expected that a facial mask will release taurine after 5 min at 30 ± 3.5 mg (when it is thought that the final product produced is 90 ± 4.5 g). Studies of antioxidant activity of taurine in the literature indicate that taurine has 11% reactive oxygen scavenging capacity at 10 μg/mL and 25% at 250 μg/mL concentration (Ripoll et al. 2012). It has also been shown by an in vitro study that taurine protects the cells from toxic damage even at low concentrations, such as 1–10 mM (Timbrell et al. 1995) Accumulation of taurine in keratinocyte cells prevent the cells from osmotic stress and ultraviolet radiation triggered apoptosis (Siefken et al. 2003). In their study Farzamfar et. al used up to 10% w/w taurine to modify poly (ε-caprolactone)/gelatin electrospun wound dressing and showed that using 5% w/w taurine loading accelerates wound closure (Farzamfar et al. 2018). The ability to achieve the highest release value of taurine in as little as five minutes in our study indicates that the facial mask will have a short application period. This feature is important for user comfort because it does not require the mask to be kept on the face for a long time.

Conclusion

BC is distinguished from plant cellulose with its unique properties, such as high-water holding capacity, nanofiber network structure, mechanical strength, and high purity. However, it cannot find much place in the market due to mainly the constraints on large-scale production. To overcome this problem, BC needs both the development of cost-effective production systems and purposeful modifications, especially to produce high value-added products. In this study, taurine loaded BC-based face mask, a high value-added product, was developed using a previously developed cost-effective medium. In addition to antioxidant and moisturizing property, this study can form the basis for the development of BC face masks serving different purposes by loading other active substances instead of taurine. For this intent, researchers need to demonstrate the suitability of the relevant active substance by performing the necessary physical and chemical characterization tests, namely physical loading, chemical binding, adsorption, release, endotoxin content, cytotoxicity, thermal and microbial stability.

Acknowledgements This work is supported by Republic of Turkey, Ministry of Industry and Technology (SANTEZ Project Number: 0198-STZ-2013-1) and Scientific Research Foundation of Ege University (Project no. 2014/BIL/015).

Authors' contributions Conceptualization, collecting the data, performing the analysis, and writing Eyup B.; data analysis and interpretation, E.H.G.; conceptualization performing the analysis and writing Ece B.; conceptualization, data analysis and interpretation, and editing A.S.; data analysis and interpretation K.Ö.Ö.; conceptualization, data analysis and

interpretation, and editing E.E.H.T. All authors have read and agreed to the published version of the manuscript.

Funding Republic of Turkey, Ministry of Industry and Technology and BioRed Laboratory Products Company (0198-STZ-2013-1); Ege University Science and Technology Center (2014/BIL/015).

Declaration

Conflict of interest The authors declare no conflict of interest.

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