Bacterial Cellulose Based Facial Mask With Antioxidant Property and High Moisturizing Capacity

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Research Article

Keywords: Bacterial cellulose, Facial mask, Taurine, Long term stability

Posted Date: May 10th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-438989/v1

Version of Record: A version of this preprint was published at Cellulose on September 13th, 2021. See the published version at https://doi.org/10.1007/s10570-021-04106-z.

Abstract

Bacterial cellulose (BC) produced by certain bacteria has the potential to be used in many different areas. Despite its advantageous properties 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 vegetable cellulose in terms of cost-effectiveness. Therefore, studies associated with BC can be divided in two categories which are development cost effective BC production methods and culture media, and 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), Differential Scanning Calorimetry (DSC), Liquid Chromatography–Mass spectrometry (LC-MS), microbial, and mechanical stability tests. According to our results, produced facial mask samples do not show any cytotoxic effect on neither human keratinocyte (HS2) nor mouse fibroblast (L-929) cell lines, it has high thermal stability which making it suitable for different sterilization techniques including sterilization by heat treatment. Taurine release (over 2µg/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.

1. Introduction

Cellulose, which is the main component of the plant cell wall, is capable of forming one third to half of the plant mass and is found in annual or perennial plants such as wood, cotton, linen, poppy and wheat, is the most produced and found biopolymer in nature due to several billion tones biosynthesis per year (Sun et al. 2004; McNamara et al. 2015). The first isolation of cellulose from green plants dates back to the 1850s (O'sullivan 1997). Cellulose, used as 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* (Iguchi et al. 2000; Cheng et al. 2011; Wei et al. 2011; Vitta and Thiruvengadam 2012; Reiniati et al. 2017; Ye et al. 2019; Ahmed et al. 2020). 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 distinguishable properties against plant cellulose such as high mechanical strength, high water holding capacity, biocompatibility, producibility with desired shape, and nanofiber network structure (<130 nm), make BC 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, cornea replacement etc. (Yamanaka et al. 1989; Fu et al. 2013; Picheth et al. 2017; Azeredo et al. 2019; Gorgieva and Trček 2019).

The skin is a complex organ that serves as the interface separating the organs from the outside and has the ability to reflect many features of the body's internal structure. In addition to great importance in terms of appearance, the skin protects the organism from external adversities, provides homeostasis and helps to adapt to the environment, and acts as a barrier to harmful external factors such as microorganisms and UV radiation (Tur 1997; Hussain et al. 2013). Since skin contain different stem cell types, maintenance of its physiology is crucial in adulthood (Blanpain and Fuchs 2009). One of the major obstacles in skin protection is the 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 malondialdehid (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 effect of these radicals is a naturally occurring process. With increasing OS, this balance shifts towards ROS and FR. Antioxidant supplement is a useful approach to prevent body from harmful effects of ROS and FR.

There are studies in the literature on the development of a facial mask by adding various herbal extracts or active agents used for moisturizing, to the bacterial cellulose structure (Amnuaikit et al. 2011; Pacheco et al. 2018; Perugini et al. 2018; Bianchet et al. 2020). Also some studies focused on using BC to transdermal drug delivery (Almeida et al. 2014; Morais et al. 2019). In this study we aimed to develop a BC based facial mask which has moisturizing feature with high water content (>%99 w/w) and antioxidant property with the addition of taurine. Taurine (2-aminoethanesulfonic acid), one of the common metabolites of cysteine, is known to act as a neurotransmitter and has been shown that it can reduce MDA levels (Perugini et al. 2018), has ability to nourishing and supporting cells and can be act as an antioxidant (Almeida et al. 2014; Bianchet et al. 2020). To carry out a cost effective production, carob and haricot bean (CHb) medium (Bilgi et al. 2016) was used to produce BC instead of standard Hestrin & Schramn medium (Hestrin and Schramm 1954).

2. Materials And Method

2.1. Activation and Cultivation of Microorganism

Gluconacetobacter xylinus (ATCC 700178) was activated in agitated culture (150 rpm, 30 °C) for 24-48 h. Activation of microorganism was done 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.

2.2. Production of BC

Activated culture was used in the production of BC based facial mask. In order to that our previously developed medium (CHb) was used with optimized parameter, namely 10 % v/v inoculum, 2.5% w/v carbon source –obtained from carob extract, 2.75% w/v protein source –obtained from haricot bean, 1.15 ‰ w/v citric acid, 2.7 ‰ w/v Na_2HPO_4, 1.5 cm^{-1} surface/volume ratio, pH 5, 30°C incubation temperature, and 7 days of incubation (Bilgi et al. 2016).

2.3. Purification of BC

The purification of BC samples was performed by a slightly modified alkali treatment method (Bilgi et al. 2016). To remove culture medium and bacterial residues, harvested BC membranes were rinsed in distilled water and kept in 0.1 M NaOH (Merck, 106498, USA) 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 within 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.

2.4. Modification of BC with Taurine

In preliminary studies BC was dried by both freeze drying and heat. After drying the samples lost more than 99% of their weight (from 90±4.5 g to 500±13.5 mg). When the 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 lyophilization-dried samples,

respectively. For this reason, drying process was not applied to the samples in BC modification to form a facial mask. Sterilized BC membranes immersed in sterile 50 mM taurine containing 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 should also be considered while preparing the taurine solution. Phenostat (1%) was added as a protetive agent to the solution, which is safe to use up to 3% and does not contain paraben, and the samples were stirred at 80 ° C for 2 hours on a magnetic stirrer, and then packaged.

2.5. Characterization of BC Based Facial Masks

2.5.1. 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 7^{th}, 14^{th} and 28^{th} day.

2.5.2. Cytotoxicity Tests

Two separate 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% confluence. 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. For this method, after the cells reached confluency the cell culture media were solidified with agar [1% (w/v)] and to prevent direct contact of the samples to the cells. BC samples, which were cut in appropriate sizes (1x1 cm^2), were placed on the agar. After 24 and 72 hours, the cells were stained with Giemsa (Semple et al. 1978). Briefly in this method, the cells were rinsed first with PBS then PBS-Methanol (1: 1.5) solution after removing the BC samples and the agar medium. Cells were fixed with methanol for 10 minutes, and then stained with Giemsa for 5 minutes. The morphology of the cells was examined under an inverted microscope.

2.5.3. 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), one of the *in vitro* endotoxicity measurement method, was used. For the sample preparation the international standard ISO 10993-12:2009 procedure was followed.

2.5.3. Bio-adhesion Tests

Samples were placed into a beaker filled water at 37° C, forced applied with the apparatus on which rabbit skin implemented, after removing force attachment of BC samples to skin was measured.

2.5.5. Differential Scanning Calorimeter (DSC)

Thermal properties of different BC samples –containing taurine, Phenostat or not- were measured with DSC below the melting temperature of taurine which is 300° C.

2.5.6. Taurine Release

Taurine release assays were performed using the LC/MS to determine whether the produced BC absorbed taurine and whether or not the taurine was released with an appropriate characteristic. Considering storage and application conditions, products kept for 6 months in incubators at 25-40 °C and containing 60% humidity. Taurine release was measured every month.

2.5.7. Fourier Transform Infrared Spectroscopy (FT-IR)

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

2.5.8. 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 analyze was performed in Ege University Central Research Test and Analysis Laboratory Application and Research Center (EGE-MATAL).

3. Results And Discussion

In our previous study, the optimum incubation time and protein concentration for production of BC 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 days to 7 days to produce lighter and thinner BC. With the production 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.

3.1. Endotoxicity tests

BC is widely used in the food industry as Nata de Cocco (Bernardo et al. 1998) and is generally recognized as safe (GRAS) by the Food and Drug Administration (FDA) (Lin et al. 2013). Since the lipopolysaccharide found in the cell wall of Gram-negative bacteria is an endotoxin (Fang et al. 2009) and *G. xylinus* is a Gram-negative bacterium (Chiaoprakobkij et al. 2011), the potential endotoxin content should be considered. 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, the effect of lipopolysaccharide residues on cells after washing should be evaluated, especially in biomedical, medical and cosmetic applications.

The results of the endotoxicity test with different dilutions of purified BC samples (1:1, 1:10, 1:100, 1:1000 and 1:10000) 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 0.05 EU / ml of endotoxin. However, there is no clear limit for the skin. The

reason is that skin protects our body against endotoxins and other different chemicals. Therefore, the endotoxin limit is high for the substances to be used on the skin surface as in the digestive system. When the test results of the samples produced in the 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 low concentration of 0,6 EU / ml or lower.

3.2. Bio-adhesion Tests

In previous studies Guilherme et al. used sensory tests (fragrance, skin adhesion and color, etc.) to qualitatively evaluate the performance of their BC masks (Pacheco et al. 2018). In another study using a BC based skin mask, Thanaporn Amnuaikit et. al. qualitatively measured user satisfaction and the decrease in sebum level. In their results after second application showed a decrease in sebum level (Amnuaikit et al. 2011). In our study, bio-adhesion test, which allows quantitative measurement of adhesion data, was preferred. How much distance the BC samples adheres to the apparatus after the applied force is shown in Table 2. During the experiment it appears that the highest strength was obtain with the products of CHb (0.102 kg sample) and HS (0.119 kg sample) media and contain neither Phenostat nor taurine. Taking into account the adhesion distances, the taurine and protective agent addition decrease the distance from 3.483 to 3.058 mm in products which are obtained in CHb medium, and from 2.950 to 2.133 mm in the products obtained in HS medium. In general, the addition of taurine and protective agent show less change in products of CHb (2.754-3.483) than in products of HS (2.133-3.208).

3.3. Cytotoxicity Results

Attachment tests were performed to determine the attachment time of HS2 cells to culture vessels, before 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 1. a) and the 12-day-old cells began to die as the culture medium did not replace during the experiment. (Fig 1. b).

To see if cell concentration under BC samples is decreased and/or morphology of cells changed, cells were dyed with Giemsa and investigated under light microscopy (Fig 2. and Fig 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 substance is placed are sparse or do not proliferate at all. When the images are examined no dilution is observed compared to the control. No difference is observed in the 72^{nd} hour photographs taken at wide angle (4X) compared to the control (Fig 3.). BC did not show toxic effects on cells, these results are consistent with literature studies carried out *in vitro* or *in vivo* with BC or BC based products [(BC-alginat, BC-polyethylenglycol, BC-hydroxapatite, BC-gelatin, BC-Poly(3-hydroxybutyric acid-co-4 hydroxybutirate)) composites] (Bäckdahl et al. 2006; Fang et al. 2009; Cai and Kim 2010; Kim et al. 2010; Chiaoprakobkij et al. 2011). Indirect cytotoxicity tests were performed after cells reached to confluence. If the test is continued for a long time, the cells will soon die because they cannot find the surface area to attach. For this reason, the test was terminated after 72 hours 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). Based on these results, it was decided that BC could be used as a mask and samples were packaged by loading protective agent and taurine after characterization.

3.4. 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 (Russell 2003). In this way, the effectiveness of the protective agent can be tested. According to the European Union cosmetic laws, 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).

These results show that 1% addition of preservative (Phenostat) allowed to be used in the range of 0.5-2% provides the desired effect. Protective 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). These results shows that 1% addition of protective agent (Phenostat) allowed to be used in the range of 0.5-2% provides the desired protection effect. 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 time. It is seen that the effect of the 1% added protective agent is above the legal limit of 3 log to eliminate certain microorganisms ($1x10^7$-$1x10^8$ cfu/ml *E. coli, S. aureus, P. aeruginosa*, $1x10^6$-$1x10^7$ cfu/ml *C. albicans*, $1x10^6$ - $1x10^7$ *A. niger*) (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 none of the studies using BC as a facial mask or drug delivery system had long-term microbial stability analysis(Amnuaikit et al. 2011; Almeida et al. 2014; Pacheco et al. 2018).

3.5. DSC Analysis

BC is a highly crystalline and thermal stable 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 (Badshah et al. 2018; Altun et al. 2019). 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, since autoclave was used for the sterilization of BC samples, it was also investigated whether taurine loading had a negative effect on thermal stability. 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 out 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 had a glass transition that was expected to be seen in the pure state of the products. This may be a sign that the material obtained is structurally different from the original material. It appears that addition of taurine and preservative shift degradation peaks in DSC plots. This also supports the conclusion that these additives contribute not only to microbiological and oxidative stress, but also to the thermal degradation as a result of dissolution in the material.

3.6. 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.

3.7. FT-IR analysis

FT-IR spectra of BC samples were given in Fig 6. in which, 2895, 1315, 1162, 1056, 665, 611 cm^{-1} and 3040, 2970, 1616, 1584, 1511, 1458, 1427, 1388, 1344, 1304, 1204, 1177, 1110, 1030, 962, 890, 848, 735, peaks were observed. In the FTIR spectrum, for pure cellulose, the peak at 3344 cm^{-1} and the peaks in the range of 3400-3500 cm-1 show OH

stretching, strong absorption peak C-H binding at 2895 cm^{-1}, 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 cm^{-1}, 1335 cm^{-1}, 1427 cm^{-1}, indicating the presence of crystalline region and pure cellulose, correspond to C-H bending (C-H bending), O-H bending and CH2 stretch bonds, respectively which are correlated with literature (Wan et al. 2006; Castro et al. 2011; Liu et al. 2011; Halib et al. 2012). The peaks for BC-taurine (purple) around 3214, 3040, 2970, 1204 (S=O) and 962 (C-S-O) corresponds to taurine (Wang et al. 2015) which show that taurine loading to BC has been successfully completed.

3.8. Taurine Release Experiments

The formation and destruction of ROS and FR in the body occurs in a variety of ways lipid oxidation, the formation of ROS and FRs using oxygen which is between hypoxanthine and xanthine as a cofactor by xanthine oxidase (Quinlan et al. 1997; Finaud et al. 2006). Taurine has been shown to be effective in cell renewal and proliferation, to be a nutritive and supportive for cells, and helped to reduce or eliminate the harmful effects of ROS and FR (Devamanoharan et al. 1997; Chen et al. 1998; Guerin et al. 2001; Değim et al. 2002). Fig. 7 shows the taurine release characteristics which are profiled in different storage conditions namely 25 or 40 °C and 40 or 60% humidity.

The highest value was observed at the end of the 5^{th} minute and the taurine concentration remained unchanged for the remaining 3 hours. A similar profile has been obtained in the following months Taurine concentrations 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 hydrogen bonding capacity of the hydrogen in the nitrogen and hydroxyl groups with unpaired electrons in each of the oxygen and nitrogen is present. 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 minutes at 30 ± 3.5 mg (when it is thought that the final product produced is 90 ± 4.5 g). Studies in the literature of antioxidant activity of taurine indicate that taurine is capable of 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 with *in vitro* study that taurine protects the cells from toxic damage even at low concentrations such as 1-10 mM (Timbrell et al. 1995). The ability to achieve the highest release value of taurine in as little as five minutes indicates that the facial mask will have a short application period. This situation is considered to be good for user comfort.

4. Conclusion

Although BC is distinguished from plant cellulose with its unique properties such as high-water holding capacity, nanofiber network structure, mechanical strength and high purity, it cannot find much place in the market. Two approaches can be taken to overcome this problem: to produce BC more cost-effectively and/or to obtain a high value-added end product from the produced BC. In this study, these two approaches were used together with the use of the cost-effective medium we had previously designed and the development of a BC-based taurine-containing facial mask, which is a high value-added product.It has been shown that the product, which is characterized by extensive tests, can be used as a facial mask with its antioxidant and moisturizing properties. Based on this study, BC-based facial masks can be developed in a wide range of products that serve different purposes by loading other active substances instead of taurine.

Declarations

Acknowledgements

This work is supported by Republic of Turkey, Ministry of Industry and Technology and BioRed Laboratory Products Company (SANTEZ Project Number: 0198-STZ-2013-1 and Ege University Science and Technology Center (EBILTEM Project Number: 2014/BIL/015).

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This work is supported by Republic of Turkey, Ministry of Industry and Technology and BioRed Laboratory Products Company (SANTEZ Project Number: 0198-STZ-2013-1 and Ege University Science and Technology Center (EBILTEM Project Number: 2014/BIL/015).

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).

Conflicts of interest/Competing interests: The authors declare no conflict of interest.

Availability of data and material: Not applicable.

Code availability: Not applicable.

Authors' contributions: Conceptualization, collecting the data, performing the analysis, and writing Eyup B.; data analysis and interpretation, E.H.G.; 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.

Ethics approval: Not applicable.

Consent to participate: Not applicable.

Consent for publication: Not applicable.

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Tables

Table 1. Semi quantitative endotoxicity results of BC samples with different dilutions. (+) gel formation, (-) no gel formation

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	(-)	1:10000	(-)
0.0150 EU/ml	(-)		

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

Samples		Force (kg)					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: 50mM taurine; Phe: %1 phenostat

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	³3 log
S. aureus ATCC 6538	>4.10	>4.10	>4.10	³3 log
P. aeruginosa ATCC 15442	>4.14	>4.14	>4.14	³3 log
C. albicans ATCC 10231	>3.04	>3.04	>3.04	³1 log
A. brasiliensis ATCC 16404	1.18	2.48	2.81	³1 log

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

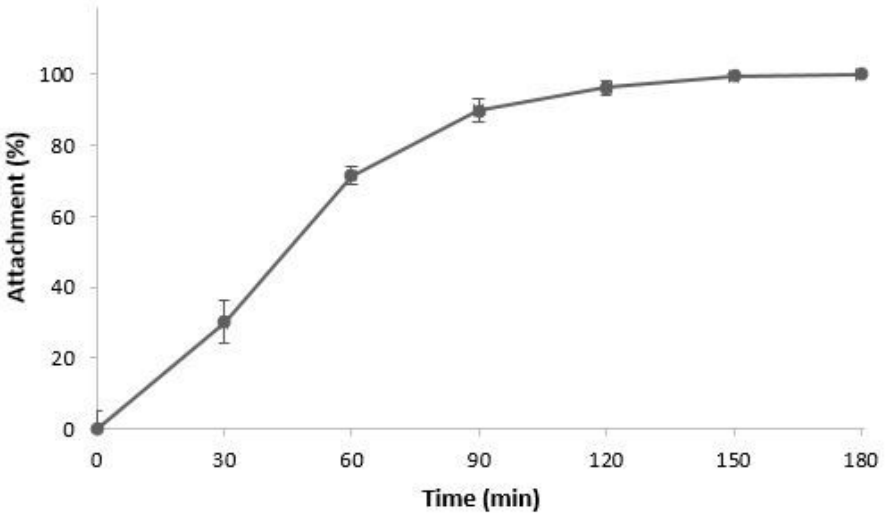
Element	Weight %	Atomic %
C	6.73	9.26
N	13.9	16.41
O	64.46	66.64
S	14.91	7.69

Element	Weight %	Atomic %
C K	21.71	26.97
O K	78.29	73.03

Element	Weight %	Atomic %
C K	1.9	2.8
N K	10.69	13.5
O K	64.04	70.81
S K	23.36	12.89

Figures

a)



b)

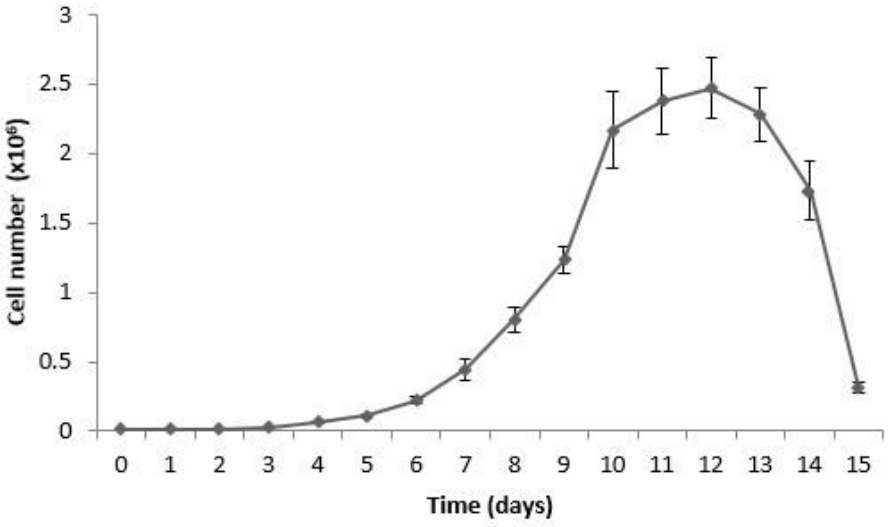


Figure 1

a) Attachment and b) growth kinetics of HS2 cells

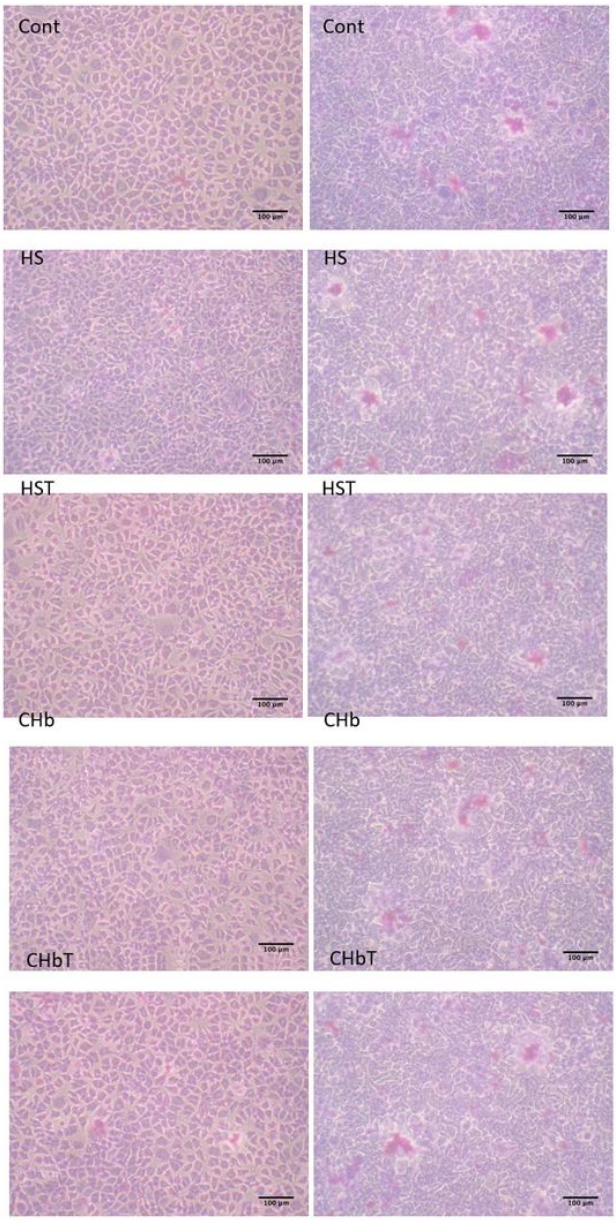


Figure 2

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 50mM taurine solution, CHb: BC samples produced with Carob Haricot bean (CHb) medium, and CHbT: BC samples produced with CHb medium and treated with 50mM taurine solution (10x Magnification)

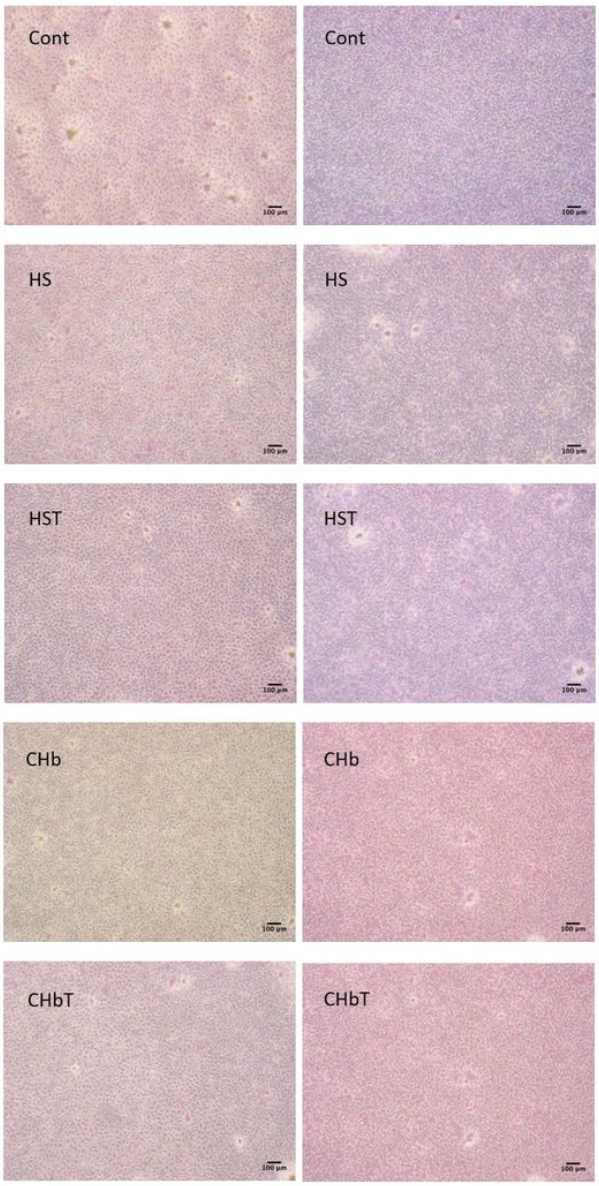


Figure 3

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 50mM taurine solution, CHb: BC samples produced with Carob Haricot bean (CHb) medium, and CHbT: BC samples produced with CHb medium and treated with 50mM taurine solution (4X magnification)

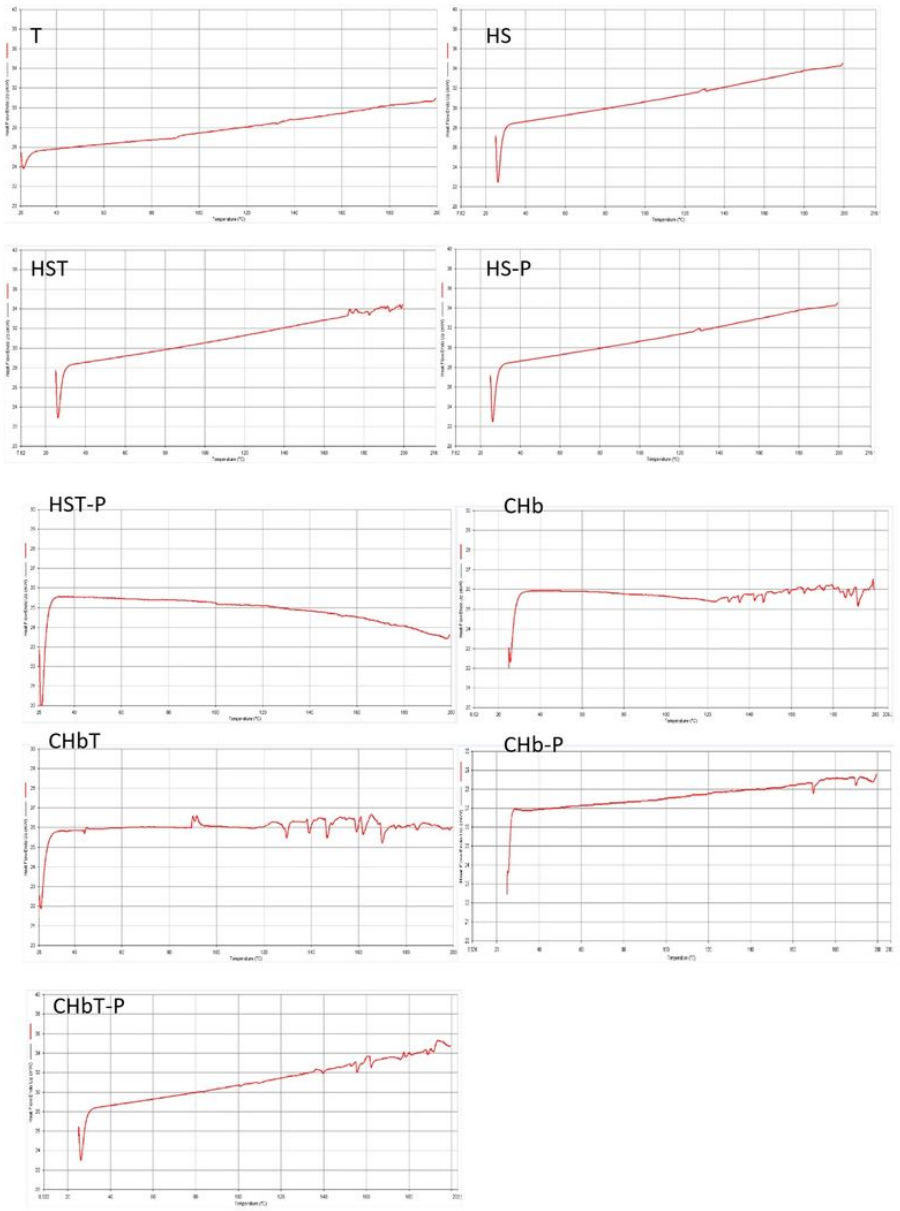


Figure 4

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

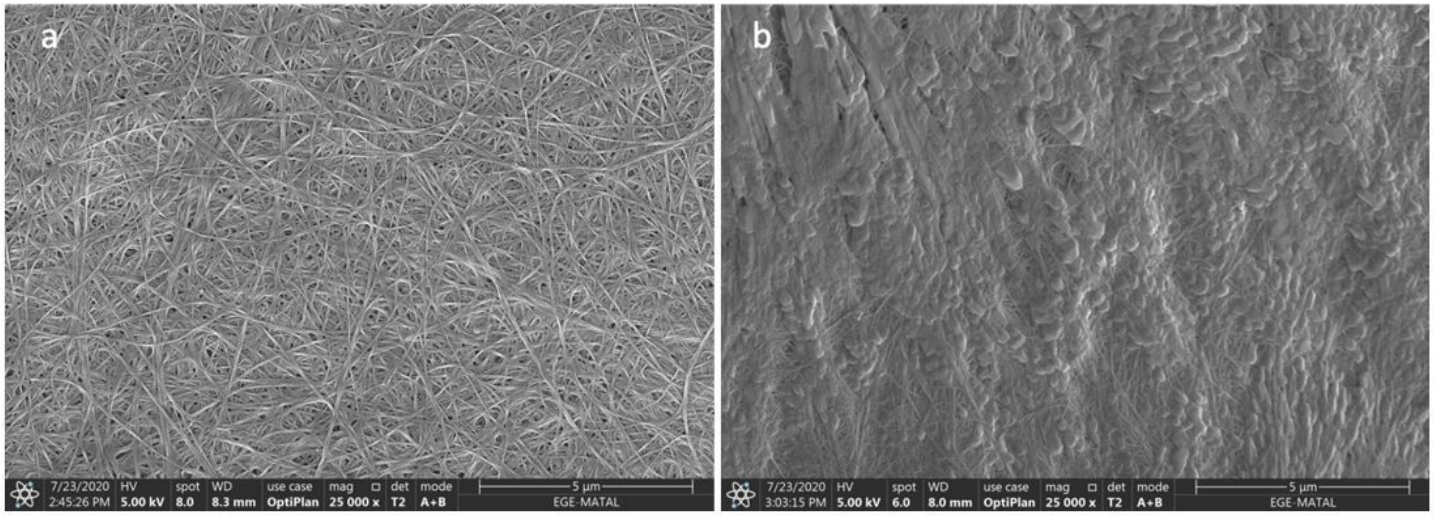


Figure 5

SEM images of BC; a) w/o taurine and b) with 50mM taurine loading

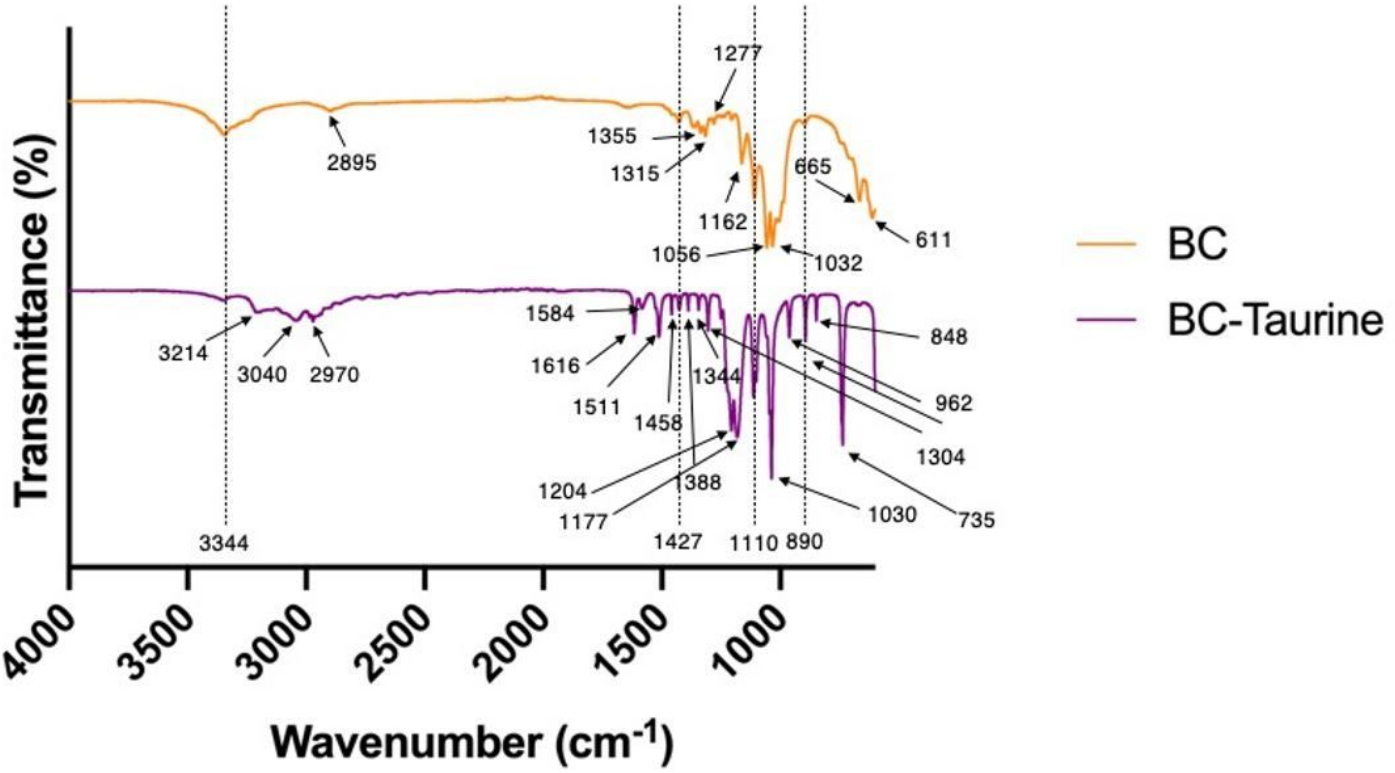


Figure 6

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

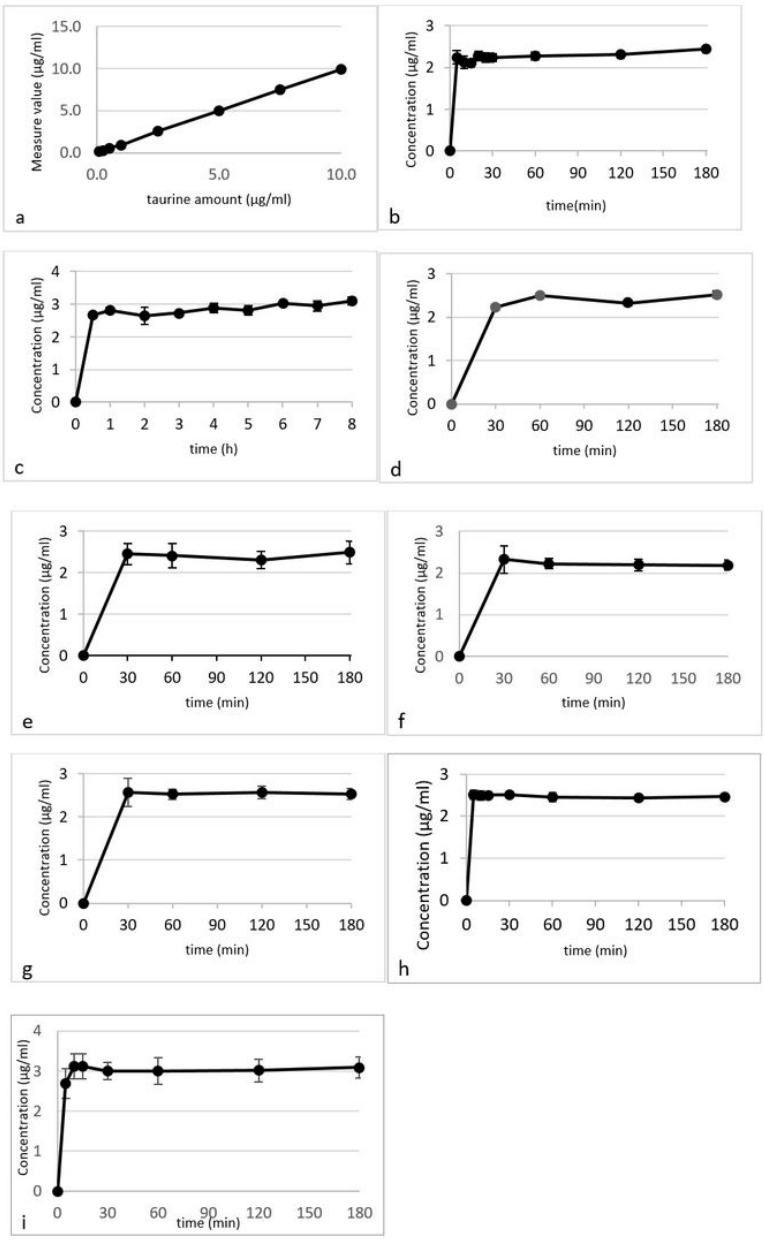


Figure 7

Taurine release profiles of BC samples produced with CHb medium and treated with 50mM 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