Asian Journal of Pharmaceutical and Health Sciences
www.ajphs.com

Bacterial cellulose production by Acetobacter xylinum and Saccharomyces cerevisiae in green tea leaves and fruits juice medium.

DC Moretti Vieira*, BTG Senna, M Ishii, TC Vessoni Penna

Department of Pharmaceutical Technology, Pharmaceutical Science College, University of São Paulo, Brazil

ARTICLE HISTORY

Received: 18.12.2012

Accepted: 18.01.2013

Available online: 10.05.2013

Keywords:

cellulose; fruits residues; *Camellia sinensis* (green tea); *Gluconacetobacter xylinus*

*Corresponding author:

Email : vieirade2003@yahoo.com.br
Tel : 55 11 99309 7005

ABSTRACT

The biomembrane (bacterial cellulose) was developed by *Gluconacetobacter xylinus* ATTC 23769, associated to *Saccharomyces cerevisiae*, for 7-10 days cultivation, from residual fruit-vegetable juices added to green tea broth, was studied and hydroalcoholic *Calendula officinalis* extract (1:1) was incorporated into biomembrane. Cultures were grown 1200 mL flasks with 80% v/v broth (Green Tea, Orange, Papaya, Wine, Collagen and Beet broths) and 20% v/v inoculum, incubated for 7-10 days at 28±2°C under static conditions, pH initial 3.5 4.0. The biomembrane thickness, diameter and weight were measured. The DSC, FTIR, BET analyses were performed and Water and Marigold absorption capacity were calculated.The highest bacterial cellulose yield (6.1±0.5 g cellulose/day) was obtained in green tea medium with 40% v/v papaya juice and also 10% v/v orange juice. Moreover, the addition of 1.2% w/v collagen to the green tea medium increased by 1.5 times the biomembrane weight. The developed biomembrane is composed by cellulose (confirmed by infrared spectroscopy (FTIR) at λ_{max} = 1644 cm^{-1}), with a total surface of 2.07 m^2/g, an average porous size of 158 Å (BET), strength and elasticity between 0.09-0.5 MPa, (similar to Young's model for indentation) and thermal stability up to 137 °C (DSC). The biomembrane absorption capacity for water and hydroalcoholic *Calendula officinalis* extract (1:1), was six and five times higher than its dry weight, respectively. The Marigold extract was incorporated into the biomembrane due to its suitable topical properties, such as antioxidant, anti-inflammatory and wound healing.

INTRODUCTION

Cellulose is the most abundant, renewable, biodegradable and sustainable biopolymer. It is a polysaccharide, consisting of linear β-1,4-D-glucose units [1], which can be obtained from plants or be synthesizing by different types of microorganisms such as algae (*Vallonia*), fungi (*Saprolegnia*) and bacteria (*Acetobacter, Agrobacterium, Rhizobium*).

Plant cellulose and bacterial cellulose (BC) have a similar chemical structure. However, bacteria cellulose (BC) is different from plant cellulose in some aspects, including finer structure (nanoscale microfibrils < 10 nm in width), higher purity (hemicellulose and lignin free), longer fiber length (polymerization degree between 2000 6000), higher crystallinity, better morphological control (it can be grown into many shapes under static cultivation conditions in different fermentation vessels) [1], strong biological adaptability, nontoxic and non-allergenic [2 - 4].

The increasing demand for cellulose has resulted in extreme negative pressure on the delicate ecological balance of the plant world. One approach to reduce the demand for cellulose of plant origin is the production of cellulose using a microbial system [5].

The Gram-negative bacterium *Acetobacter xylinum is the most studied and promising microorganism that produce BC*. Two culture methods can be used to synthesize the extracellular cellulose by *Acetobacter xylinum*. One is the static culture, which is used to create a gelatinous membrane of cellulose on the interface between the air and the liquid medium. The other is the agitated culture, where the synthesized cellulose is distributed

throughout the culture medium as a fibrous structure [1]. The BC produced in these agitated systems exhibits a lower degree of polymerization (DP), crystallinity, and Young's modulus than that produced under static cultivation. The less-organized form of BC may result from shear stress during agitation [5].

Bacterial cellulose biosynthesized by *Acetobacter xylinum* was first discovered in the late nineteenth century and has been used in practical applications for several decades. In the 80s, a pure microfibrilar biosynthetic cellulose membrane was developed through a fermentative process and since then, the BC has been utilized as skin substitute for the recovery of burned and injured skin, to repair defects of the abdominal wall in aponeurotic muscles, bandages for periodontal surgery, artificial blood vessel, tourniquets, dura mater substitute, stent coating, food packaging, dietary fibers, culture substrate for mammalian cells, biofuel and others [6 - 9].

In Brazil, 13 million tons of food/year is wasted by Markets. These fruit wastes have abundant sugars, such as glucose and fructose that could be bio-converted into useful products with high aggregate value as BC by acetic acid bacterium such as *A. xylinum.* Although coconut water is known to produce bacterial cellulose [10], there is a lack of articles about BC by fruits-vegetable wastes.

Green tea (*Camellia sinensis*), also known as *bancha*, has been a leading beverage in the Far East for thousands of years. Green tea contains a high percentage of polyphenol (potent antioxidant and antimicrobial agents), catechins, methylxanthines, manganese, potassium, acid folic and vitamins (C, K, B1 and B2) and has the local ability to induce apoptosis in oral cancer cells [11 - 12].

Calendula officinalis (Asteraceae) is an Egyptian ornamental and medicinal plant. The Marigold tincture, gel, infusion, cream and ointment are widely used as an anti-inflammatory and healing agent for skin and mucous membranes [13- 15]. The Marigold flower contains flavonoids, essential oils, sesquiterpene, triterpenes and saponins [16]. The main marigold components with anti- inflammatory activity are faradiol (aromatic rings A, D and E l), arnidiol and calenduladiol [17].

In this article the biomembrane weight (g cellulose), productivity (g cellulose/day) and conversion factor (g cellulose/g sugar) were evaluated in different culture medium (green-tea, fruit juices (orange, papaya), and vegetable (beet) and wine), as well as was studied the effect of collagen on the biomembrane production. Marigold hydroalcoholic extract was added into the BC due it healing, anti-inflammatory and analgesic propriety. The biomembranes produced in the different media were also characterized.

MATERIALS AND METHODS

Inoculum

The strains of *Acetobacter xylinum* (ATCC 23769) were obtained from Fundação André Tosselo located in Campinas, São Paulo.

Lyophilized cells of *A. xylinum* (ATCC 23769) (10 mg) and dry cells of *S. cerevisiae* (bakery source) (2.5 g) were dissolved in 250 mL of green tea (17.5 g green tea leaves in 250 mL distilled water) with 25 g sugar, and kept in static condition at 28 ± 2°C for 10 days; after this period, a pellicle was formed on the surface. After this time, 250 mL of supernatant and the pellicle were used as pre-inoculum for 600 mL of green tea medium (7% w/v green tea leaves, 10% w/v sugar and distilled water qsp 1L). The media inoculated with the pre-inoculum was kept in static condition at 28 ± 2°C for 10 days and used as the inoculum.

Medium

In this study, a very ripe *Citrus simensis* (orange), *Carica papaya* (papaya) and *Beta vulgaris L* (beet) were provided by a local market in São Paulo. The fruits and vegetables were washed, crushed and squeezed to prepare the juices.

The medium used to produce BC were*:* Green tea medium (GT) (7% w/v green tea leaves, 10% w/v sugar and distilled water qsp 1L); Orange medium (80% v/v GT and 20% v/v orange juice); Papaya medium (40% v/v GT , 50% v/v papaya juice and 10% v/v orange juice); Wine medium (33% v/v GT , 34% v/v wine and 33% v/v water); Collagen medium 0.5% (100% v/v GT, 0.5% w/v collagen); Collagen medium 1.0% (100% v/v GT, 1.0 % w/v collagen); Collagen medium 1.2% (100% v/v GT, 1.2 % w/v collagen); Beet medium 8 % (92% v/v green tea (7% w/v green leaves, and distilled water qsp 1L) and 8% v/v beet juice) and Beet medium 17 % (85% v/v green tea (7% w/v green leaves, and distilled water qsp 1L) and 17% v/v beet juice).

Culture Condition

Cultures were grown in 1200 mL flasks containing 600 mL of media (Green tea medium, Orange medium, Papaya medium, Wine medium, Collagen medium, Beet medium) and 150 mL of inoculum. Cultures were incubated for 10 days at 28 ± 2°C under static conditions, pH: 3.5 ± 0.5. A pellicle (biomembrane) was formed at the air/surface interface.

Bacterial Cellulose Purification and Dryness

After the cultivation period, the harvested biomembrane was washed with distilled water to remove medium components, dried until constant weight by lyophilization or oven at 50°C and autoclaved by 30 minutes at 121°C. After this, the biomembrane was weighed in a semi-analytic scale and the diameter and thickness were measured using digital calipers [18 - 19]. Productivity (g cellulose/day) and conversation factor (g cellulose/g sugar) were then calculated.

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy was used primarily to identify the chemical structure of the membrane. The FTIR spectra of the membranes were measured at wave numbers ranging from 4000 to 400 cm^{-1} with a Bomem MB100 FTIR spectrometer [20].

Mechanical Properties

The indentation tests were performed on a Stable Microsystem TA-XT2 texturometer. The device consists of a spherical probe 5 mm in diameter and a film holder. The samples were put in the holder between the two plates.

The probe was driven through the sample at a speed of 0.2 mm/s. The tests were done in triplicate of 3 cm^2. The equipment determined the load required for indentation (F1), the displacement of the probe from the point of contact to the point of indentation (D1) and the area under the curve related to energy to the indentation. Then, the indentation strength (F1/Acs, where Acs is the cross-sectional area of the film located in the cylindrical opening of the film holder) and the elongation to the indentation ([{(R2 + D1 2)1/2 - R}/R].100, where R is the radius of the film exposed in the cylindrical hole of the film holder) were calculated [21].

Scanning Electron Microscopy (SEM)

BC samples after cell removal were lyophilized and then coated with a thin Platinum film of around 5 nm. A SEM-FEI field emission scanning electron microscope (Leo Co., Oberkochen, Germany) was used, operating at 4 kV 10 kV and imaging magnification about 5,0 00 30,000 was used for examination of BC samples.

Brunauer-Emmett-Teller (BET) Surface Analysis

The pore size and surface area of the membranes were determined with a BET surface area analyzer. To remove moisture from the film samples, the samples were placed in sample cells, which were then heated up to 348 K (75ºC) for 3 h and cooled down to room temperature before the BET analysis. The BET pore size and surface area were determined with N_2 adsorption at 77 K (-196ºC) in a Micromeritics (Atlanta, GA) ASAP 2020. [20]

Water Absorption Capacity (WAC) and Marigold Absorption Capacity (MAC)

To determine the WAC and MAC, the dried biomembranes (surface area 1 cm^2 , thickness 1.5 mm) were immersed respectively in distilled water and Marigold hydroalcoholic extract (diluted with alcohol 70%, 1:1), at room temperature until equilibration. After that, the biomembranes were removed from the water/Marigold hydroalcoholic extract; the excess on the surface of the biomembranes was blotted out with Kim wipes paper.

The weights of the swollen biomembranes were measured, and the procedure was repeated until no further weight change was observed. The WA/MAC was calculated according to the following formula:

WAC (%) or MAC (%) = (Mh - Md / Md).100

Where: Mh and Md represent the weight of the hydrated and dry membrane, respectively [20].

Marigold Transfer Capacity (MTC)

The MTC was performed to verify the deliverable capacity of the biomembrane. The lyophilized biomembrane was put in contact with the Marigold biomembrane (lyophilized green tea biomembrane rehydrated with 3 mL of Marigold hydro-alcoholic extract) at room temperature until equilibration. The Marigold biomembrane weight was measured before and after the contact. The transference capacity was calculated using the following formula

MTC (%) = (W1a W1b / W1a).100

Where: W1a and W1b are the Marigold biomembrane weight before and after the transference, respectively.

RESULTS AND DISCUSSION

Cellulose production (weight, productivity and thickness)

The culture was carried out in static condition around 28 ± 2ºC by adding an aliquot of activated seed medium to the culture medium. The system becomes turbid and after 3-5 days, a pellicle (biomembrane) appears on the surface.

The mechanism of biomembrane formation was considered as follows. In the initial stage, the bacteria increase their population by taking dissolved oxygen and producing a certain amount of cellulose in the entire liquid phase, as observed by the appearance of turbidity. When the dissolved oxygen is used up, only bacteria existing in the surface vicinity can maintain their activity to produce cellulose. The bacteria below the surface are not 'dead' but 'asleep', so they can be reactivated and used as the seed for new culture operation [1].

A general trend observed is that the thickness, as well as the yield of cellulose, increases sharply after a few days of induction period, until the rate starts to slow down after a week or ten days. It was considered as glucose the kind of saccharide that was digested by bacteria and converted to cellulose [2]. The pH value during the fermentation varied ± 0.5, because gluconic acid is not produced during cultivation [2].

As observed in Figure 1, the heaviest biomembrane is the one produced in the papaya medium (61.4 g), followed by the wine (35.0 g) and orange biomembrane (20.5 g). The weight of green tea and papaya biomembranes is respectively 3.6 and 61.8-fold greater than the weight of the biomembrane produced by Iguchi [2] in coconut medium. The biomembrane weight produced in the papaya medium was 12.5-fold greater than the bacterial cellulose produced by Cheng [5] in corn steep liquor with fructose with 0.5% CMC in agitated culture and 6 times greater than BC obtained by Ruka [2] in yamaka-manitol medium in static condition. The orange biomembrane weight was 17 greater than the BC produced by Kuroshimi in orange medium without a nitrogen [5].

The biomembrane produced in the green tea medium (0.6 mg/mL) and papaya medium (10.23 mg/mL) were respectively 1.5 and 25.6-fold greater than that obtained by Hu, 2010 [1], during cellulose sphere production by *Acetobacter xylinum* NCIMB(ATCC 23769) in agitated culture.

Under the studied conditions, the most productive medium was the papaya medium (6.14 g cellulose/day), followed by wine (3.50 g cellulose/day) and orange media (2.93 g cellulose/day). The BC productivity in the papaya medium was 2.1-fold and 12.80-fold greater than that in the orange and in green tea media, respectively. The BC productivity in the orange medium was 7.3-fold greater than that in green tea medium. Kurosumi [18] described that the orange medium is the most productive in comparison of other media studied by his group [pineapple, apple, Japanese pear and grape medium].

These results show that adding orange and papaya to the green tea medium increased the biomembrane productivity and weight. Also, the acetic acid present in the wine improved the biomembrane weight. The substitution of sugar by beet juice 17% (v/v) increased 3.0-fold the BC productivity and 4.3-fold the biomembrane weight, while adding 8% beet juice (v/v) increased 2.1- fold the BC productivity and 3.0-fold biomembrane weight in relation to green-tea medium, probably because the beet juice contains nutrients that enhance cellulose production. This fact will be further evaluated in a future study.

During the cultivation, a pellicle appeared on the surface and its thickness increased steadily with time, reaching over 7 mm in 10 days (papaya biomembrane). It is important to note that during the pellicle growth process, the aerobic bacteria generate cellulose only in the surface vicinity. As long as the system is kept unshaken, the pellicle is suspended by cohesion to the inner container wall and slides steadily downwards as it thickens. Iguchi [2] showed that the continuous growth of the pellicle layer tended to fail if a container with a tapered wall, such as a conical flask, was used. The pellicle thickness increases until all sugar is

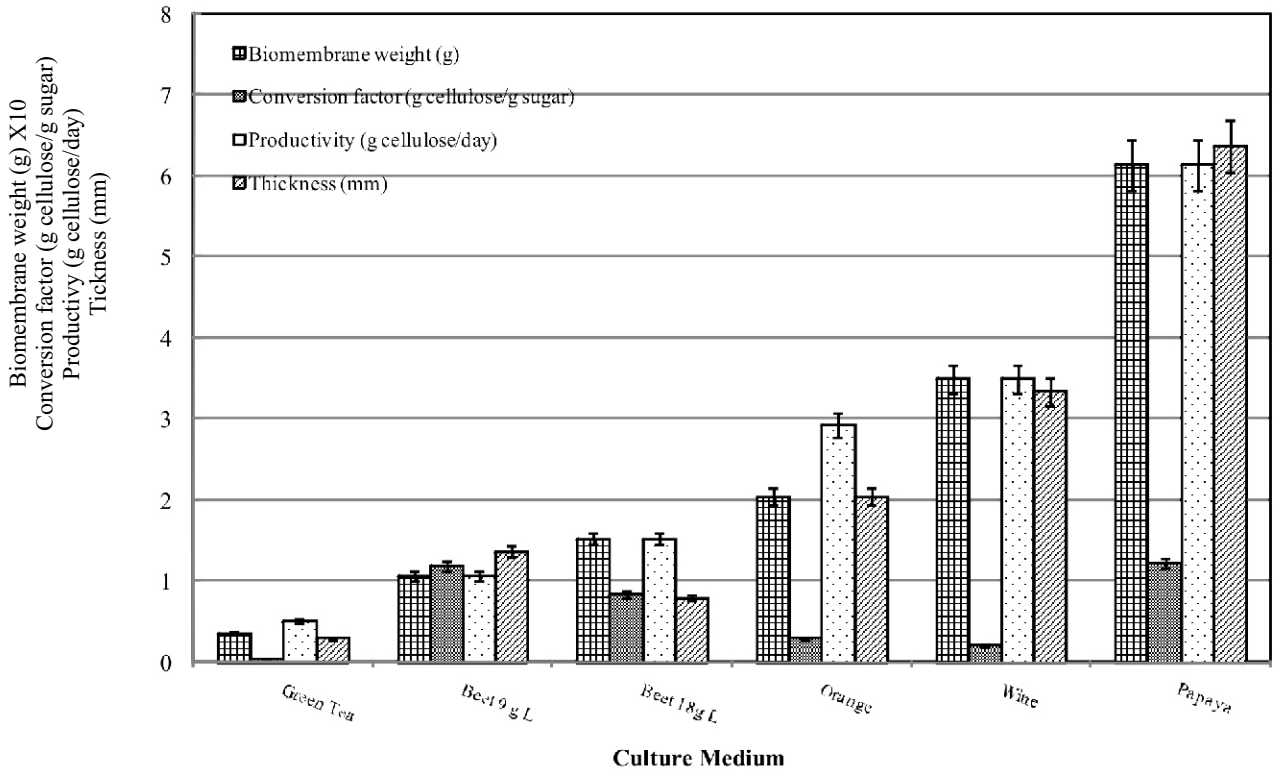

Figure 1: Biomembrane weight (g); productivity (g cellulose/day); conversion factor (g cellulose/ g sugar) and thickness (mm) in the green tea medium (green tea), orange medium (orange), wine (wine medium); papaya (papaya medium); beet 8% (beet medium with 8% v/v beet juice) and beet 15% (beet medium with 15% v/v beet juice), 10 days of cultivation, initial pH = 3.5.

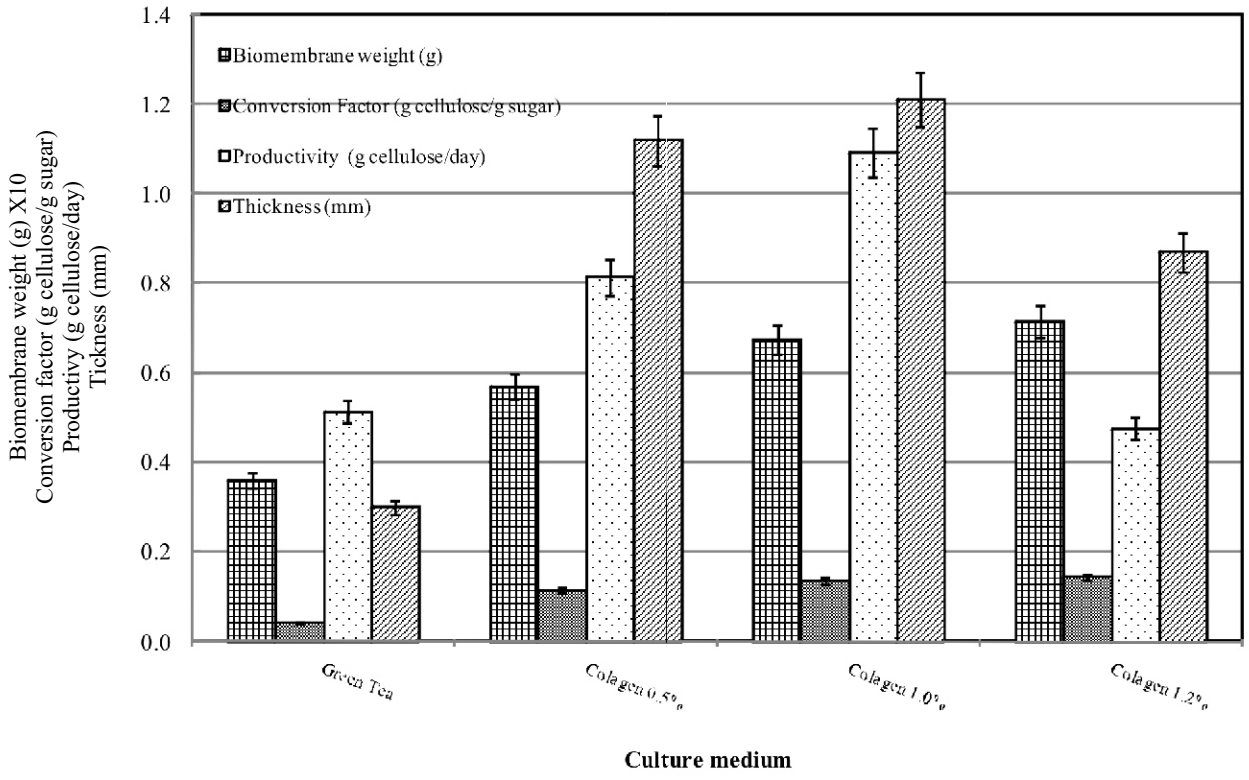

Figure 2: Biomembrane weight (g); productivity (g cellulose/day); conversion factor (g cellulose/ g sugar) and thickness (mm) in the green tea medium (green tea) , Collagen 0.5% (collagen medium 0.5%); Collagen 1.0% (collagen medium 1.0%); Collagen medium 1.2% (collagen medium 1.2%); 10 days of cultivation, initial pH = 3.5.

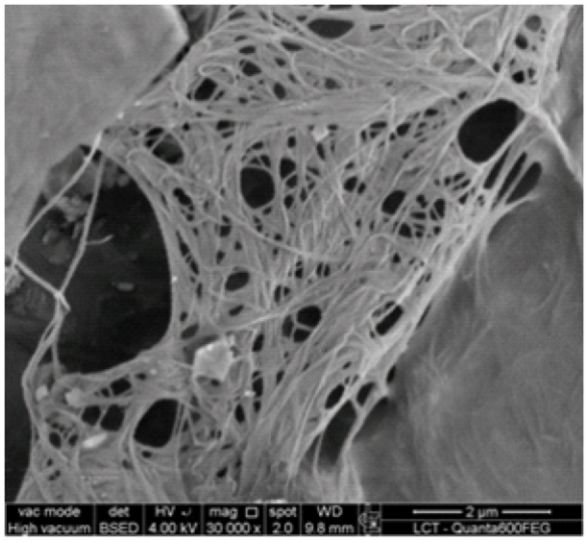

Figure 3a: Green tea biomembrane, 10-day cultivation.

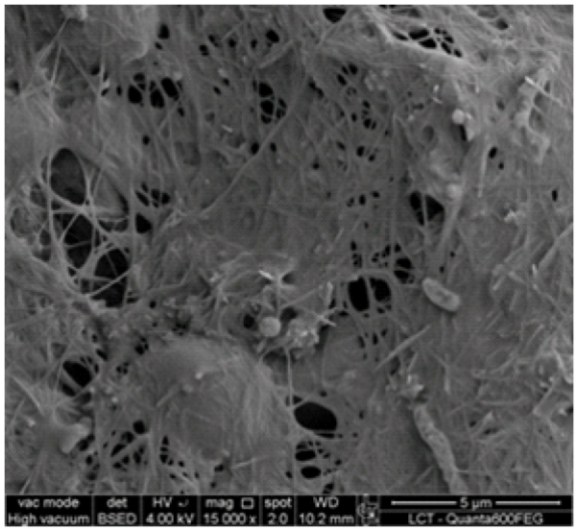

Figure 3b: Orange biomembrane, 10-day cultivation.

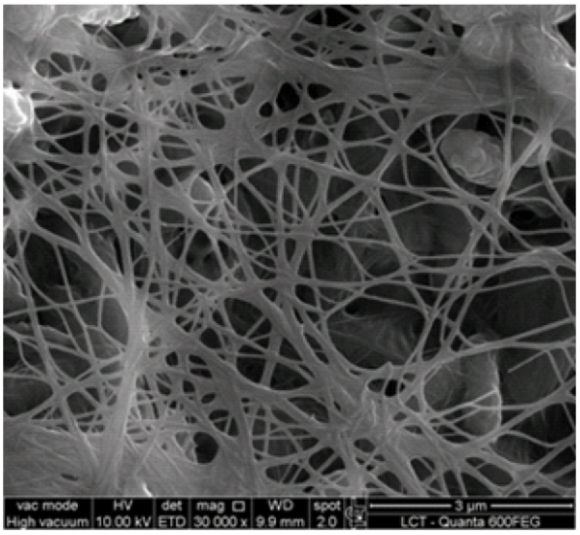

Figure 3c: Papaya biomembrane, 10-day cultivation

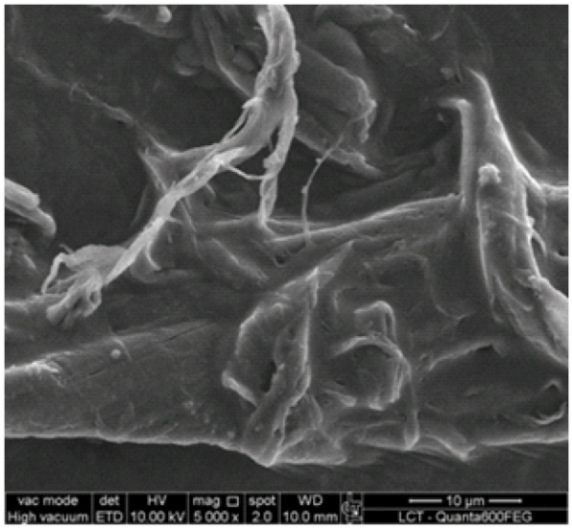

Figure 3d: Wine biomembrane, 10-day cultivation

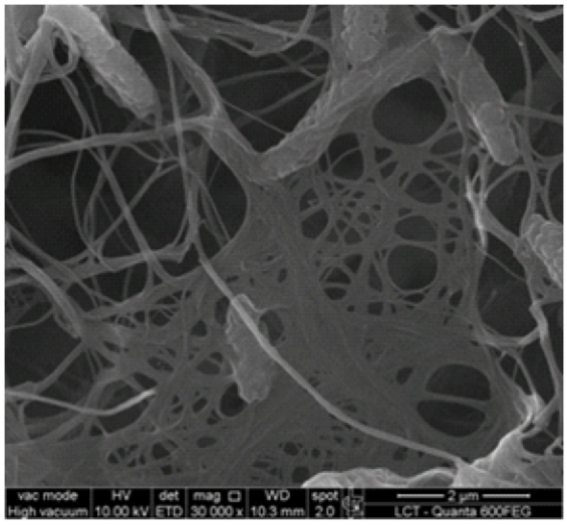

Figure 3e: Beet biomembrane, 10-day cultivation

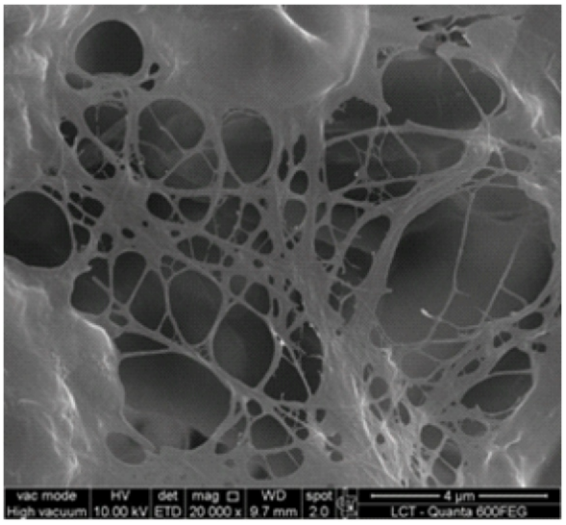

Figure 3f: Collagen biomembrane, 10-day cultivation

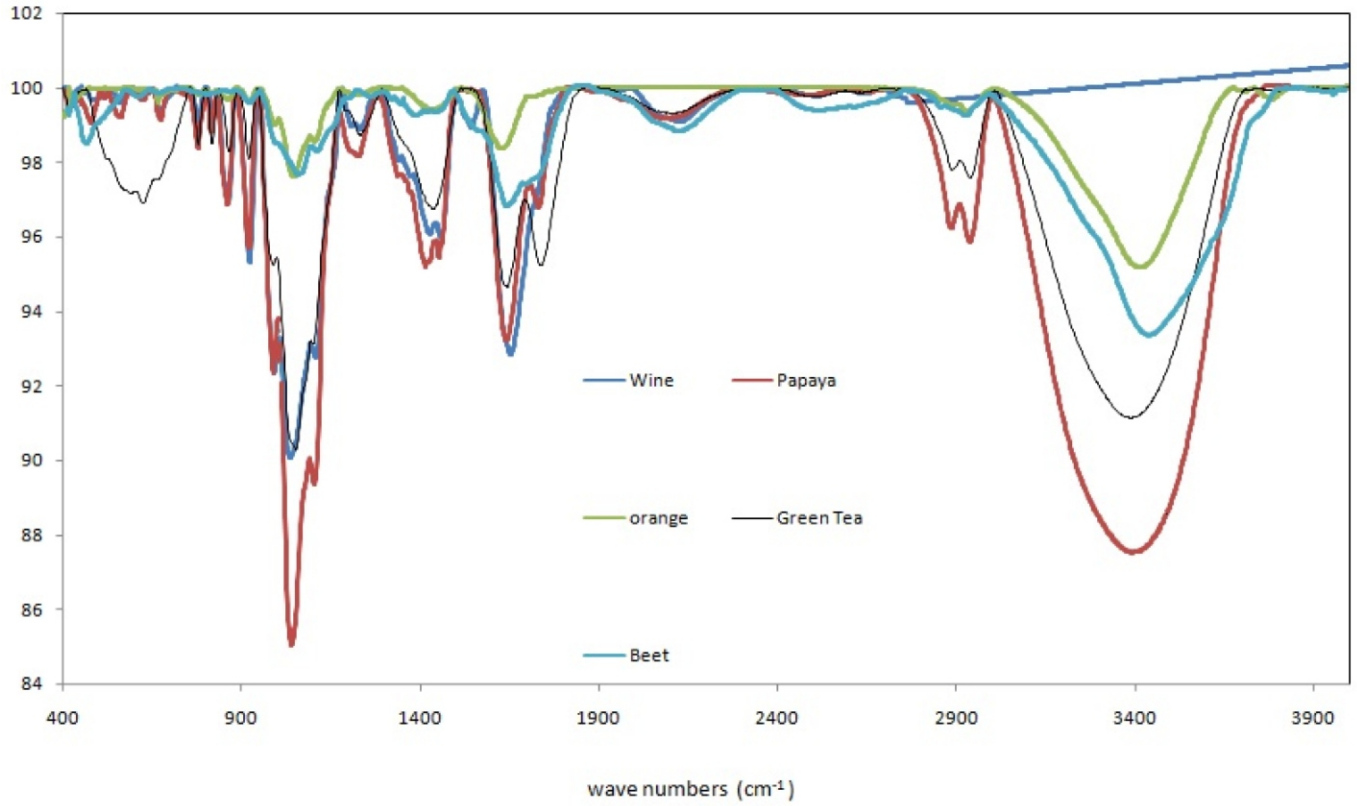

Figure 4: The FTIR spectra of biomembranes, after 7 days of cultivation and initial pH = 3.5, at wave numbers ranging from 400 to 4000 cm^{-1} in green tea (green tea medium); orange medium (orange medium); papaya (papaya medium); wine (wine medium); Marigold (green tea biomembrane rehydrated with 3 mL of Marigold hydroalcoholic extract)

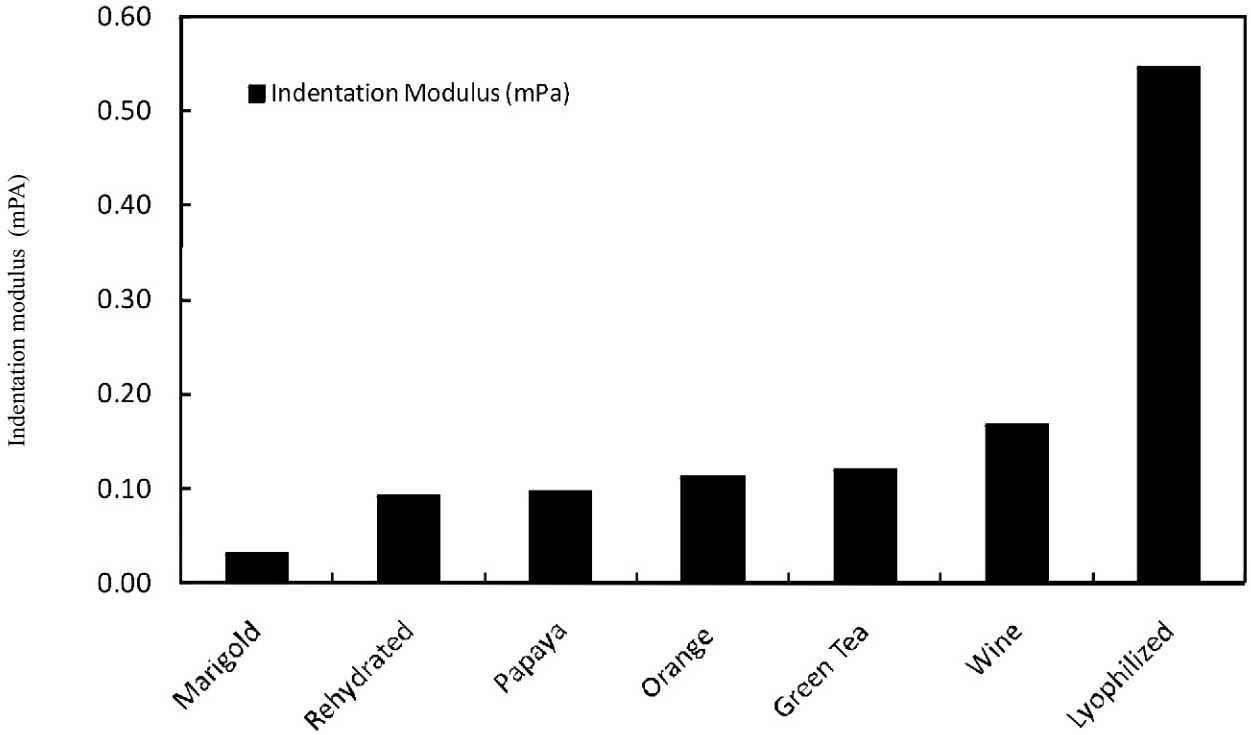

Figure 5: Indentation module of biomembrane, after 7 days of cultivation, initial pH = 3, in green tea medium (green tea); orange medium (orange medium); papaya medium (papaya); wine medium (wine), Marigold (GT biomembrane rehydrated with 3 mL Marigold hydroalcoholic extract), Rehydrated (GT biomembrane rehydrated with 3 ml water) and Lyophilized (lyophilized green tea biomembrane).

TABLE 1 : WAC, MAC and MTC

Biomembrane	WAC	MAC
Beet	73	24
Papaya	197	68
Wine	227	11
Green tea	525	400
Orange	663	125
Capacity Transfer	27%	
Absorption	0.25 g of Marigold/g cellulose	

consumed by *A. Xylinium.*

The addition of papaya with orange juice (papaya: 50% v/v and orange: 10% v/v), wine (34% v/v) and orange juice (20% v/v) increased green tea biomembrane thickness 21.3, 11.1 and 6.3-fold, respectively and it is 3.13-fold thinner than the one produced by Iguchi [2] in coconut medium. The substitution of sugar by beet juice 8% v/v (9 g/L) and 17% v/v (18 g/L), increased the biomembrane thickness 2.8 and 2.7-fold, respectively (Figure 1). Probably these results are due to the fact that wine, orange, papaya and beet contain substances that reinforce the biomembrane structure, consequently improving thickness.

The adding of papaya with orange juice (papaya: 50% v/v and orange: 10% v/v), wine (34% v/v) and orange juice (20% v/v) increased the conversion factor 30.7, 5.25 and 7.43-fold, respectively. The substitution of sugar by beet juice 8% v/v (9 g/L) and 17% v/v (18 g/L), increased the conversion factor 29.72 and 21.25-fold, respectively (Figure 1). The ethylic alcohol and sugars present in the wine, the citric acid present in the orange juice, and sugars that compose the papaya and beet juices are easily converted into cellulose, consequently improving the conversion factor.

Collagen Effect

As other polymeric additives such as agar, acetan, xanthan and CMC, collagen exhibited its ability to hinder formation of large clumps of BC and enhanced BC production during fermentation.

The Figure 2 is observed that the collagen addition to the green tea medium has a tendency to increase the biomembrane weight, probably due to collagen incorporation into the biomembrane, producing a more robust biomembrane. This probably occurs because collagen has affinity with the biomembrane during the BC formation.

In this study, different collagen concentrations (0.5%, 1% and 1.2%) were added to the green tea medium, and their BC production (weight, productivity, conversion factor and thickness) were compared with the BC production in the green tea medium. BC production in the control case (green tea medium) was 6 g/L, 4.3-fold greater than that found by Cheng [5]. BC production with 0.5% w/v collagen (9.7 g/L) and 1.0% w/v collagen (11.3 g/L) were 1.6 and 1.8-fold larger than control, respectively and somewhat greater than that found by Cheng [5] with 0.5% (w/v) CMC (7.2 g/L), and approximately 2.1 - 2.5-fold greater than that found by them when microcrystalline cellulose (0.5% - 4.37 g/L) and agar (0.2% - 4.49 g/L), were added, respectively.

Productivity in the medium containing 1.2% w/w collagen was very similar to the control medium (0.5 g cellulose /day) and the addition of 1.0% w/w collagen improved the productivity by 2.1 fold. The addition of 0.5% w/w, 1% w/w and 1.2% w/w of collagen in the green tea medium increased the thickness 3.7, 4.0 and 3-fold, respectively.

The conversion factor was also improved 2-fold and 1.6-fold when 0.5% w/ and 1.0% w/w collagen was added to green tea medium, respectively. The conversion factor in green tea medium with 1.2% w/w collagen was very similar to control (green tea medium).

Scanning Electron Microscopy (SEM)

Biomembranes seem to be composed of piles of thin cellulose layers, regardless of the direction in which it is analyzed.In the BC, the density of interfibrillar hydrogen-bonds must be much higher, as the diameter of fibrils is much smaller [2] than pulp paper. The fiber structure and homogeneity of the biomembrane are showed in Figure 3. Similar structure was found by Saibuatong [20] for the production of BC with aloe vera.

The formation of BC pellicle may be impacted by the overall medium composition, carbon source, medium viscosity *A. xylinum* strain and factors related to the operational conditions (eg. pH, temperature, agitation or not of the medium, size and shape of the container, etc.) [1]. As shown in figure 3, the biomembrane produced in orange medium has smaller pores than the ones produced in green tea medium and are also more compacted. In the biomembranes produced using collagen, it can also be observed that the collagen molecules are involved/ intertwined with cellulose fibers. All produced biomembranes have similar structures.

Brunauer-Emmett-Teller (BET) Surface Analysis and DSC Analyses

The total surface area and average pore size determined by BET for Green Tea biomembrane were 2.07 m^2/g and 158.2 Å (1 Å = 0.1 nm) in dry form. It means the network resulted in a mean porosity of 20 nm. The porous size was a little small than the value obtained for dry biomembrane and similar to the rehydrated membrane with 10% of aloe vera developed by Saibuatong [20], but the area is about 26 times smaller than the dried membrane developed by Saibuatong [20]. The BET result were in accordance with the observations from the SEM micrographs.

The DSC analyses showed that the glassing point is around -20°C, the crystallization points is around 10°C and there is no thermal degradation until 137°C. These results confirm that the biomembrane is composed by cellulose and can be sterilized by autoclaving.

FTIR Analysis

The cellulose absorption spectrum is the band at 1642.9 cm^{-1}, which has been assigned to carbonyl groups and the band of 1090 cm^{-1} that has been assigned to the ligation between C-O groups.

FTIR spectroscopy of BC films developed in different media were carried out in order to detect the occurrence of new peaks or any peak shift that could be attributed to interactions between cellulose and medium components.

The FTIR spectra of all samples were detected at

wavenumbers ranging from 400 to 4000 cm^{-1} as shown in Figure 4. In the region from 1800 to 1500 cm^{-1}, the intense absorption in the cellulose spectrum was the band at 1642.9 cm^{-1}, which has been assigned to the carbonyl groups [1]. The bands at 16501578 cm^{-1} were assigned to C-O stretching, which overlaps with NH bending. The absorption band at 1565 1540 cm^{-1} was NH deformation. All biomembranes have a similar spectrum (Figure 4) that shows that the biomembrane (1600-4000 cm^{-1}) is composed by cellulose and there is no interaction between the medium components and biomembrane. Similar data was found by Saibuatong [20] for the production of bacterial cellulose with aloe vera.

Mechanical Properties

Although bacterial cellulose is obtained in the form of a highly swollen gel, the texture is quite unique and different from typical hydro-gels. The original elasticity would never recover once the gel is crashed. This is due to the fact that the elements that constitute the gel are microfibrils, not the segments of chain molecules, such as in agar or gelatin gels, which can take a thermodynamically stable form [2]. Figure 5 shows that the elasticity of the rehydrated and marigold biomembranes (0.05 MPa) is higher than in the others. On the other hand, the lyophilized biomembrane showed to be more rigid (0.55 MPa) than the others. Therefore, the addition of Marigold to the lyophilized biomembrane improved its flexibility from 0.09 to 0.03 MPa, probably because of the interaction between the fibril network and marigold extract, resulting in improvement of biomembrane flexibility. The wine biomembrane increased the indentation module from 0.09 to 0.27 MPa. The orange supplement increased the indentation module from 0.09 to 0.11 MPa. The indentation module was similar to that obtained with the orange, green tea and papaya biomembranes. The most flexible membrane was the Marigold membrane. Similar data were found by Saibuatong [20] for the production of BC with aloevera.

Water Absorption Capacity (WAC), Marigold Absorption Capacity (MAC) and Marigold Transfer Capacity (MTC)

The effect of the medium components on the biomembrane WAC was analogous to the medium effect on the mechanical proprieties (Table 1). The papaya medium decreased the WAC of the biomembrane from 525 % to 197% (328%), which is approximately 2.7- fold lower than the green tea biomembrane. The degree of water swelling and WAC can be increased with the introduction of a hydrophilic component to the culture medium. The WAC of the wine biomembrane is 2.3-fold lower than the green tea biomembrane. The WAC value for the orange biomembrane (663%) is similar to that of the cellulose membrane with 30% of aloe vera (around 700%) [20]. The orange biomembrane has the highest WAC (663%), which is 1.3-fold greater than that of the green tea biomembrane.

The MAC of green tea biomembrane is 400%, which means that the biomembrane absorbs 1.3 times more water than marigold and this probably happens due to the hydrophilic proprieties of Marigold extract. The green tea biomembrane absorbed 0.25 g of Marigold/g cellulose. The Marigold transfer capacity to dry biomembrane is 27%. It means that the biomembrane can transfer marigold extract through concentration gradient. This is an indication that the biomembrane can transfer marigold and other hydrophilic extracts. Moreover, the values of MAC and MTC indicate that the marigold biomembrane could have a therapeutic effect, as according to the Brazilian regulation, it is necessary to have a marigold concentration of 8.8 to 17.6 mg of flavonoids for topic use. Additionally, one can consider that these WAC and MAC values indicate that the different biomembrane may have different uses, such as the orange biomembrane can be applied to the region with more exudates and the green tea biomembrane use to absorb more hydrophilic compounds.

CONCLUSION

It is possible to produce cellulose by using fruit (papaya and orange) wastage and green tea. The best cellulose production was obtained with the papaya medium (6.14 g cellulose/day). The addition of 1.0% (w/v) collagen increased 2.1-fold the cellulose productivity. The green tea biomembrane has good water absorption capacity (6 times the dry weight) and Marigold absorption capacity (5 times the biomembrane dry weight) and the green tea biomembrane has the capacity to transfer Marigold extract to other dry biomembranes (27%). The incorporation of Marigold hydroalcoholic extract into the biomembrane improved its flexibility and added antioxidant, anti-inflammatory and wound healing topical effects to the green tea biomembrane. The developed biomembrane is suitable to be used in the treatment of skin burns.

ACKNOWLEDGMENT

The authors would like to thank CAPES for financial support.

REFERENCES

1. Klemm A, Schumann D, Kramer F, Hessler N, Hornung M, Schmauder HP. Nanocelluloses as innovative polymersin research and application. *Adv. Polym. Science.* 2006: 205: 4996.
2. Iguchi M, Yamanaka S, Budhiono A. Bacterial cellulose a masterpiece of nature's arts. *J. Mat. Science*. 2000: 35: 261-270.
3. Moon SH, Park JM, Chun HY, Kim SJ. Comparison of physicalproperties of bacterial celluloses produced in different culture conditions using Saccharified food wastes. *Biot. Biop. Eng.* 2006:11:2631.
4. Backdahl H, Helenius G, Bodin A, Nannmark U, Johansson BR, Risbergb B. Mechanical properties of bacterial cellulose and interactions with smooth muscle cells. *Biomater.* 2006: 27: 2141-2149.
5. Cheng KC, Catchmark JM, Demirci A. Effect of different additives on bacterial 430 cellulose production by Acetobacter xylinum and analysis of material property. *Cellul.* 2009: 16(6): 431: 1033-1045.
6. Iguchi M, Yamanaka S. Industrial use of bacterial cellulose A review. Proceedings of International Workshop Green Polymer, Bandung-Bogor. 1997: 4754.
7. Klemm D, Schumann D, Udhardt U, Marsch S. Bacterial synthesized cellulose-artificial blood vessels for microsurgery. *Progr. Polym. Science*. 2001:26:1561-1603.
8. Setyawati MI, Chien LJ, Lee CK. Expressing Vitreoscilla hemoglobin in statically cultured *Acetobacter xylinum* with reduced O(2) tension maximizes bacterial cellulose pellicle production. *J. Biotech.* 2007:123:38-43.
9. Okiyama A, Motoki M, Yamanaka S. Bacterial cellulose

III. Development of a new form of cellulose. *F. Hydroc*. 1993:6:493-501.

10. Adejoye OD, Adebayo-Toyo BC, Ogunjobi AA, Olaoye OA, Fadahunsi FI. Effect of carbon, nitrogen and mineral sources on growth of Pleurotusflorida, a Nigeria edible mushroom. *Afric. J. Biotech.* 2006:5:13551359
11. Narotzki B, Reznick AZ, Aizenbud D, Levy Y. Green tea: A promising natural product in oral health. *Arch. Or. Biol*. 2012. Available online 5 January 2012.
12. Staszewski M, Pilosof AMR, Jagus RJ. Antioxidant and antimicrobial performance of different Argentinean green tea varieties as affected by whey proteins. *F. Chem.* 2011:125(1):186-192.
13. Brown D, Dattner A. Phytoterapeutic approaches to common dermatologic conditions. Archive Dermato. 1998:134:1401-1404.
14. Zitterl-Eglseer K, Sosa S, Jurenitsch J, Schubert-Zsilavecz M, Della Loggia R, Tubaro A, Bertoldi M, Franz C. Anti-oedematous activities of the main triterpendiol esters of marigold (*Calendula officinalis L.*). *J. Ethnopharm.* 1997:57:139144.
15. Blumenthal M, Busse WR. The complete german commission e monographs: therapeutic guide to herbal medicines. Boston: American botanical Council, 1998.
16. Bruneton. Pharmacognosy, phytochemistry, medicinal plants, Paris: Lavoisier Publishing, 1995
17. Akihisa T, Yasukawa K, Oinuma H, Kasahara Y, Yamanouchi S, Takido M, Kumaki K, Tamura, T. Triterpene alcohols from the flowers of compositae and their anti-inflammatory effects. *Phytochem*. 1996:43:1255-1260.
18. Kurosumi A, Sasaki C, Yamashita Y, Nakamura Y. Utilization of various fruit juices as carbon source for production of bacterial cellulose by *Acetobacter xylinum* NBRC 13693. *Carboh.Polym*. 2009:76:333-335
19. Nguyen T, Gidley MJ, Dykes GA. Potential of a nisin-containing bacterial cellulose film to inhibit *Listeria monocytogenes* on processed meats. *F. Microbiol.* 2008:25:471478.
20. Saibuatong O, Phisalaphong M. Novo aloe vera bacterial cellulose composite film from biosynthesis. *Carboh. Polym.* 2010:79:455460.
21. Borgognoni FC, Maizato M.JS, Leirner AA, Polakiewicz1 B, Beppu MM, Higa OZ, Pitombo RNM. Effect of freeze-drying on the mechanical, physical and morphological properties of glutaraldehyde-treated bovine pericardium: evaluation of freeze-dried treated bovine pericardium properties. *J. Appl. Biomater. Biomech.* 2010:8(3):186-190.