Author(s): Ottenhall, Anna; Seppänen, Tiinamari; Ek, Monica

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ORIGINAL PAPER

Water-stable cellulose fiber foam with antimicrobial properties for bio based low-density materials

Anna Ottenhall · Tiinamari Seppänen · Monica Ek

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Abstract New bio-based packaging materials are highly interesting for replacing conventional fossil based products for a more sustainable society. Water-stable cellulose fiber foams have been produced in a simple one-batch foam-forming process with drying under ambient conditions. The cellulose fiber foams have a low density (33–66 kg/m^3) and can inhibit microbial growth; two highly valuable features for insulating packaging materials, especially in combination with stability in water. Cationic chitosan and/or polyvinylamine have been added during the foam-forming process to give the foams water-stability and antimicrobial properties. The structural and mechanical properties of the cellulose fiber foams have been studied and the antimicrobial properties have been evaluated with respect to both *Escherichia coli*, a common model bacteria and *Aspergillus brasiliensis*, a sporulating mold. The cellulose foams containing chitosan had both good water-stability and good antibacterial and antifungal properties, while the foams containing PVAm did disintegrate in water and did not inhibit fungal growth when nutrients were added to the foam, showing that it is possible to produce a bio-based foam material with the desired characters. This can be an interesting low-density packaging material for protection from both mechanical and microbial damage without using any toxic compounds.

Keywords Antibacterial · Antifungal · Cellulosic · Chitosan · Citric acid · Insulation · Packaging · Polyvinylamine · Wet-stable

A. Ottenhall · T. Seppänen · M. Ek (✉)
Department of Fibre and Polymer Technology, School of Engineering Science in Chemistry, Biotechnology and Health, KTH Royal Institute of Technology, Teknikringen 56-58, 114 28 Stockholm, Sweden
e-mail: monicaek@kth.se

T. Seppänen
Department of Chemistry, Nanoscience Center, University of Jyväskylä, P.O. Box 35, 40014 Jyväskylä, Finland

Introduction

Interest in cellulose-based packaging materials is steadily growing as we are trying to replace petroleum-based plastics with materials from renewable resources (Makaremi et al. 2017; Svagan et al. 2011). Cellulose fiber materials have many advantages in disposable packaging, as cellulose is an abundant resource that is affordable and light weight. The packaging material can be recycled or possibly decomposed after use, as cellulose is biodegradable (Klemm et al. 2005). Many customer products like food, glassware or electronics are sensitive for mechanical damage and need to be protected during transportation. Shock adsorbing materials with low

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density, e.g. polystyrene foam, are therefore often used for protection and insulation of sensitive products. However, bio-based alternatives for low-density packaging materials are needed to replace the fossil based ones. Foam forming of papers was first developed in the 1960s to reduce the amount of pulp fibers needed compared to traditional wet-forming of paper products (Bernardin 1969; Chung 1974). The pulp fibers were often mixed with a foaming agent to obtain a lightweight paper with a low density but the foam papers were mechanically pressed with a high load to increase the strength of the paper and this also increased the density (Li et al. 2017; Madani et al. 2014). With the development of cellulose nanofibril (CNF) production, research into highly porous bio-based foam materials has exploded (De France et al. 2017). In the case of CNF foam materials, CNF is still expensive to produce, due to the high energy consumption during defibrillation, and the foams often require freeze-drying to preserve the porous structure during drying (Nechyporchuk et al. 2016; Nemoto et al. 2015). From an economic and industrial point of view, it would be a great advantage to be able to create foam packaging materials directly from cellulose pulp fibers, rather than advanced CNF foams.

The challenges when using cellulose-based products for packaging are mainly their sensitivity to moisture and water as well as to microbial growth (Peelman et al. 2013; Petersen et al. 1999). Most of the commercial available antimicrobial materials are based on a leaching approach where biocides are incorporated in the material and are leached into the environment to kill the microorganisms. Silver nanoparticles are commonly used for this purpose, even though silver ions are known to be toxic for mammalian cells and can lead to silver-resistant bacteria (Greulich et al. 2009; Kittler et al. 2010; Silver 2003).

An alternative for antimicrobial materials is to rely on a contact-active approach, where the microorganisms are inactivated upon contact with the material (Illergård et al. 2012). It is known that several cationic polymers have antibacterial properties and this is often attributed to the strong interactions between the positively charged groups on the polymer and the negative charges on the bacterial cell wall (Lichter and Rubner 2009; Lichter et al. 2009). Both gram-positive and gram-negative bacteria have a negatively charged cell surface and the cationic polymers bind to the anionic components, e.g. lipopolysaccharides and proteins, on the microorganisms cell walls, which change the permeability of the cell wall and leads to cell death (Vaara 1992). Polyvinylamine (PVAm) is a cationic polymer that is used in the paper industry to increase the strength of paper (BASF 2010). PVAm has good antibacterial properties, due to its primary amine groups, and has been used in several studies to create contact-active antibacterial cellulose using Layer-by-Layer (LbL) modification (Chen et al. 2017; Henschen et al. 2016; Illergård et al. 2011, 2015; Westman et al. 2009). Chitosan is a cationic polysaccharide derived from naturally occurring chitin through deacetylation, and it is used in cosmetic, food and pharmaceutical products because of its antimicrobial properties (Rinaudo 2006; Sebti et al. 2005). Chitin is the second most common naturally occurring polymer in the world after cellulose and it can be found in many different animals; it acts, for example, as reinforcement in crab shells. Chitosan has primary amine groups on a polysaccharide chain that provide the polymer with its positive charge and antimicrobial properties (Tegl et al. 2015). A great advantage of chitosan compared to other antimicrobial polymers is that chitosan is biocompatible and nontoxic for mammalian cells (Regiel-Futyra et al. 2015).

An important issue that needs to be addressed when using low-density cellulose based materials is the stability in wet conditions. Both cellulose fiber foams and CNF foams have a tendency to disintegrate in water but this can be avoided by crosslinking of the fibers to create water-stable structures. Polycarboxylic acids, especially butanetetracarboxylic acid (BTCA), can be used in polysaccharide based materials to increase the stability in water, it have e.g. been used as crosslinking agents in cotton textiles to reduce wrinkles (El-Tahlawy et al. 2005; Yang and Xu 1998). BTCA has also been used to improve the wet strength of paper (Gu and Yang 1998). An option to BTCA could be citric acid (CA) as it is an affordable and non-toxic polycarboxylic acid that is able to crosslink chitosan (Varshousaz and Alinagari 2005).

The novel idea presented in this paper is to combine cellulose pulp fibers with cationic polymers to create lightweight and low-density foam materials with good water-stability and good antimicrobial properties, using citric acid to evaluate if it improves the water-stability of the foams. To the authors' knowledge, it is

the first time citric acid and chitosan has been used to produce a water-stable low-density cellulose fiber foam material with antimicrobial properties, using sodium dodecyl sulfate (SDS) as a foam forming agent. This simple production process facilitates large-scale production, as it does not include costly steps like freeze-drying or organic solvents, as many other processes do (Abraham et al. 2017; Heydarifard et al. 2016). The antimicrobial effect of the cellulose fiber foams towards both bacteria and fungi have been evaluated, as both bacteria and fungi play an important role in microbial degradation.

Experimental

Chemicals and materials

Imperial Anchor®, a paper grade bleached kraft cellulose pulp with a brightness of 89% (ISO 2470), 0.1% ash content and $< 0.05\%$ extractives, was supplied by Holmen AB (Iggesund, Sweden). The raw material for the cellulose pulp was a mixture of Scots Pine and Norway Spruce, and the pulp had been elementary chlorine free (ECF) bleached.

Cationic polyvinylamine Lupamin® 9095 was supplied by BASF SE (Lundwigshafen, Germany). Deacetylated chitosan ($> 75\%$) with a molecular weight of 310–375 kDa, citric acid (CA) and sodium dodecyl sulfate (SDS) were obtained from Sigma-Aldrich (Stockholm, Sweden).

The model bacteria *Escherichia coli* ATCC 11775 (Biorad, Solna, Sweden) and black sporulating fungi *Aspergillus brasiliensis* (previously *A. niger*) ATCC 16404 (Sigma-Aldrich, Stockholm, Sweden) were used for the antimicrobial evaluation (Varga et al. 2007).

Methods

Foam preparation

The fiber size of the bleached Kraft pulp was measured using a L&W Fiber Tester Plus (ABB Lorentzen & Wettre products, Kista, Sweden) and the pulp was refined for 6 min in a PFI-mill (HAM-JERN, Hammar, Norway) before foam formation. Two types of cationic polymer, PVAm and chitosan, were used in four different combinations and CA was added to some of the foams (Table 1). The chitosan stock solution was mixed by dissolving 10 g/L chitosan in a 100 g/L CA solution, as the chitosan is only soluble at low pH.

All cellulose fiber foams were prepared by mixing the refined pulp fibers, at a dry weight consistency of 3 w/w%, in deionized water (dH_2O) together with 3 g/L SDS as a foam-forming agent and 0.1 M NaCl. CA and cationic polymer i.e. PVAm and/or chitosan were added to the different foams according to Table 1. The pH of the foam-forming mixture was set to 3 and the suspension was mixed for 3 min using a blender (Braun, Aschaffenburg, Germany). Excess liquid was removed from the fiber foam by draining it through a metal mesh mold (190 × 120 × 60 mm). All samples were cured at 150 °C for 5 min after drying at room temperature.

Both non-washed and washed foams were analyzed to determine their chemical composition and antimicrobial efficiency. The foams were washed in dH_2O to evaluate the leaching properties of the materials. Two different washing schemes were used; 5 × 30 min washing with agitation (× 5 washed) and washing 2 × 30 min followed by 1 × 18 h with agitation (19 h washed). The samples where thereafter dried at room temperature.

Structural analysis

The volume of the foams was determined by a Vernier caliper, as an average of five measurements to enable the density to be calculated. The water-stability was evaluated by shaking 0.1 g of foam material in 10 mL of dH_2O in a shaking incubator at 130 rpm for 18 h.

Scanning electron microscope

The structure of the non-washed cellulose foam samples was shown by scanning electron microscope (SEM) images, using a tabletop SEM (Hitachi TM-1000, Tokyo, Japan). The average distance between the pore walls in the foams was estimated by measuring the distance in the images using the software TM-1000, version 03-02-01.

Nitrogen analysis

Both PVAm and chitosan contain amine groups that are responsible for the antimicrobial properties of the

Table 1 Composition of the foam-forming mixture for preparing the four different cellulose fiber foams

Foam material	Pulp fibers (g/L)	NaCl (g/L)	SDS (g/L)	PVAm (P) (g/L)	Chitosan (Ch) (g/L)	Citric acid (CA) (g/L)
Foam P	30	5.8	3.0	0.1	–	–
Foam P–CA	30	5.8	3.0	0.1	–	50
Foam Ch–CA	30	5.8	3.0	–	1	50
Foam P–Ch–CA	30	5.8	3.0	0.1	1	50

polymers. No other nitrogen-containing compounds were added to the foams during the foam-forming process. The nitrogen content of the foam samples was evaluated using an ANTEK MultiTek analyzer (PAC, Huston, TX, USA) before and after washing of the foams, to detect whether the foams release nitrogen-containing polymers during the washing.

FTIR

The chemical compositions of the cellulose foams were analyzed before and after washing using FTIR spectra obtained using a Perkin-Elmer Spectrum 2000 (Specac LTHD, London, UK) with a MKII Golden Gate Single-Reflection ATR system. A total of 16 scans ranging from 4000 to 600 cm^{-1} were recorded for each sample. The FTIR spectra were normalized with respect to the signal at 1317 cm^{-1}.

Adsorption test

The water absorption capacity of the foam materials was evaluated by soaking 0.1 g of the foam samples in dH_2O and gravimetrically measuring the amount of water adsorbed after 1 min. The results are presented as an average of four measurements. The dry content of the samples was determined gravimetrically by drying at 105 °C for 20 h.

Reducing bacteria in water

The bacterial-reducing effect of the foams was examined by a reduction test. Specimens, 0.1 g of foam material, were placed in cultivation flasks containing 10 mL of 10^6 CFU/mL *E. coli* suspension in quarter strength Ringer's solution with 100 mM tris-(hydroxymethyl)-aminomethane (Tris) buffer (Scharlab, Barcelona, Spain). The test was performed in duplicates. Reference samples with cellulose pulp fibers with and without the addition of CA or SDS were evaluated. All samples were incubated for 4 h at 37 °C under agitation. The number of viable bacteria remaining after 4 h was evaluated by cultivation on Petrifilm (3MTM PetrifilmTM Plates, 3M Svenska AB, Sollentuna, Sweden). The number of colony-forming units (CFU) was counted after 2 days incubation at 37 °C using the image-analysis tool ImageJ (Schneider et al. 2012).

Bacterial growth inhibition

The bacterial-growth-inhibition of the foams was studied by adding nutrients to the bacterial suspension. After incubating samples, 0.1 g, in 10 mL of 10^6 CFU/mL *E. coli* suspension in quarter strength Ringer's solution with 100 mM TRIS buffer for 4 h at 37 °C and 130 rpm; 1 mL nutrition broth medium (Sharlab, Barcelona, Spain) was added before incubation overnight at 37 °C and 130 rpm. The increase in optical density (OD) of the bacterial suspensions was measured with a Multiscan FC Microplate reader (Thermo Scientific, Shangai, China). The material samples were thereafter removed from the flasks and the remaining bacterial suspension was incubated for a further 18 h at 37 °C under agitation, before the increase in optical density was measured to determine the extent to which the material leaches growth-inhibiting compounds into the suspension.

Agar diffusion test with bacteria

The bacterial inhibitory effect through compounds being leached from the foams was evaluated using an agar diffusion test on agar plates inoculated with *E. coli*. Samples of non-washed and 5 × 30 min washed foam materials was tested using circular discs with a diameter of 10 mm. Agar plates, prepared with nutrition broth, were inoculated with 100 µL of

10^6 CFU/mL *E. coli* suspension and the material discs were wetted with sterilized dH_2O before they were placed on the inoculated agar plates. Duplicate samples were tested for each material. Plates were incubated for 3 days at 37 °C before the zone of inhibition (ZOI) around the discs was measured.

Fungal resistance

Specimens of foam materials, approx. size 2 × 2 × 1 cm, were inoculated with 100 µL of 10^6 spores/mL of *A. brasiliensis*, prepared according to the spore suspension preparation described in ASTM-1338-14; the standard test method for determining fungal resistance in insulating materials. The inoculated samples were incubated for 28 days at 30 °C and 95% humidity to evaluate the foams ability to resist fungal growth at humid conditions. A sample of wood was used as a positive reference.

Fungal growth inhibition

Non-washed specimens of material, 0.1 g, were incubated with 10 mL of 10^6 spores/mL of *A. brasiliensis* and Sabouraud dextrose (SD) broth (Sigma-Aldrich, Stockholm, Sweden). The samples were incubated for 23 days at 30 °C under agitation. All materials were tested in duplicates.

Agar diffusion test with fungi

The fungal inhibitory effect resulting from leaching of compounds from the foams was evaluated using an agar diffusion test on agar plates inoculated with *A. brasiliensis*. Samples of non-washed and 5 × 30 min washed foam materials were tested by cutting circular discs with a diameter of 10 mm. Agar plates, prepared with SD nutrition broth, were inoculated with 100 µL of 10^7 spores/mL *A. brasiliensis* suspension. The material discs were wetted with sterilized dH_2O and placed on the inoculated agar plates. Duplicate samples were tested for each material. Plates were incubated for 2 days at 30 °C and the zone of inhibition (ZOI) was measured.

Results and discussion

Low-density cellulose fiber foams, containing cationic polymers, PVAm and/or chitosan were prepared, using SDS as a foam forming agent. The materials were mixed in a single batch, dried under ambient conditions, and thereafter cured at 150 °C for 5 min. The foam samples were characterized through structural and chemical analysis. The antimicrobial properties of the foams were evaluated through several microbiological assays using both bacteria and sporulating fungi. The average length of the pulp fibers used in the foams was measured to 1.95 mm and the average width was 29 µm, both weighted with respect to fiber length.

Structural analysis

Water-stability of the foams has been achieved by mixing the cellulose pulp fibers with cationic polymers during the foam-forming process. Both PVAm and chitosan have been reported as possible environmentally friendly wet strength additives for papermaking, as they can improve the interfiber bonding within the paper (BASF 2010; Chen et al. 2013; Kamel et al. 2004). Several research studies have also reported that it is possible to cross-link chitosan with the cellulose in cotton textiles using citric acid (CA), to obtain a fabric that inhibits bacterial and fungal growth (Alonso et al. 2009; El-Tahlawy et al. 2005). Here, CA has been added to the foams to improve the water-stability, using a short curing for 5 min at 150 °C after the foams were dried in ambient conditions.

The foam containing PVAm was flexible and soft, but the addition of CA makes the foams more brittle. It is well known that paper treated with polycarboxylic acids has improved wet strength but it also makes the paper more brittle (Yang and Xu 1998). It can be seen in the images of the cross-section of the foam materials that the foam P–CA is much denser than the other three foams (Fig. 1). The foams containing chitosan were less dense than those without it. No chitosan-containing foam was created without the addition of citric acid as the chitosan is only soluble at low pH. The amine groups on chitosan are protonated and positively charged at low pH, e.g. in the presence of CA, which makes the polyelectrolyte water soluble (Pillai et al. 2009; Yi et al. 2005).

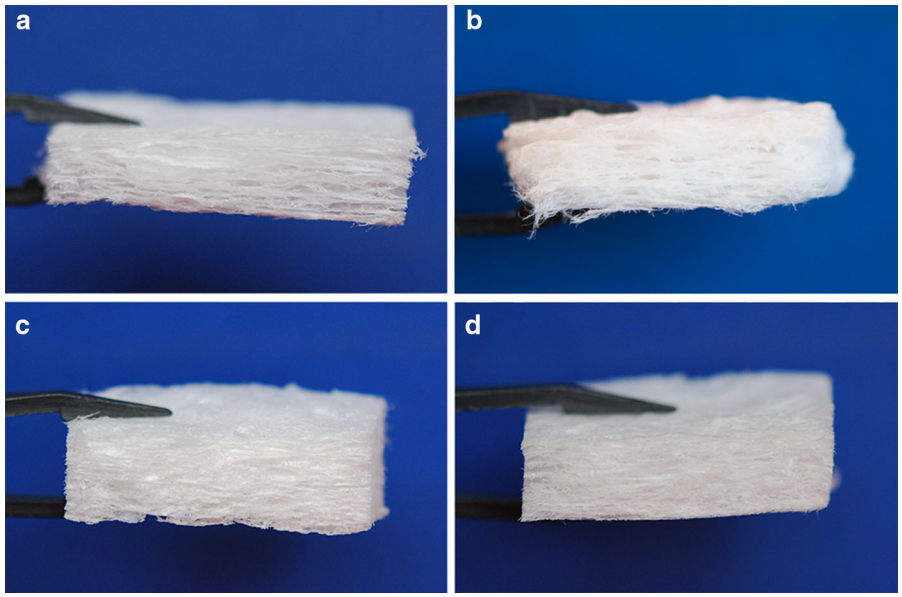

Fig. 1 Images of cross-section of the cellulose fiber foam materials. **a** Foam P, **b** foam P–CA, **c** foam Ch–CA and **d** foam P–Ch–CA

All produced cellulose foams had a relatively low density (33–66 kg/m^3), the density of the foam containing PVAm increased drastically when CA was introduced (Table 2). The lowest density was however achieved for the foam containing PVAm, chitosan and CA. These values can be compared to expanded polystyrene (EPS) foam, a low-density fossil based material commonly used for packaging materials. The density of EPS foam can vary between 12 and 48 kg/m^3 depending on application (ASTM 2004).

The stability of the cellulose fiber foams in water was evaluated by shaking the foams in dH_2O. The foam containing PVAm easily disintegrated in the water and the foam containing PVAm and CA was completely disintegrated into fibers (Fig. 2). It is not determined if the increased water-stability is caused by physical crosslinking, due to increased interaction between the cellulose fibers, or by chemical cross-linking through ester-bond formation. However, the foams containing chitosan and CA performed very well in water, they did not disintegrate even after vigorous agitation on a shaking table for 18 h. It can be concluded that it is possible to produce bio-based water-stable cellulose foam using cellulose, chitosan and CA.

Scanning electron microscope

The macro-structure of the foams can be seen in the SEM micrographs in Fig. 3. The pores in all the foams are irregular and the average distance between the pore walls vary between the analyzed foams (Table 3). Foam P–CA had the highest density and it also had the smallest average distance between the pore walls. Foam P had the largest average distance between the pore walls, while foam Ch–CA and foam P–Ch–CA had roughly the same average distance between the pore walls.

Table 2 Density of the cellulose fiber foams

Material	Foam P	Foam P–CA	Foam Ch–CA	Foam P–Ch–CA
Density (kg/m^3)	35	66	43	33

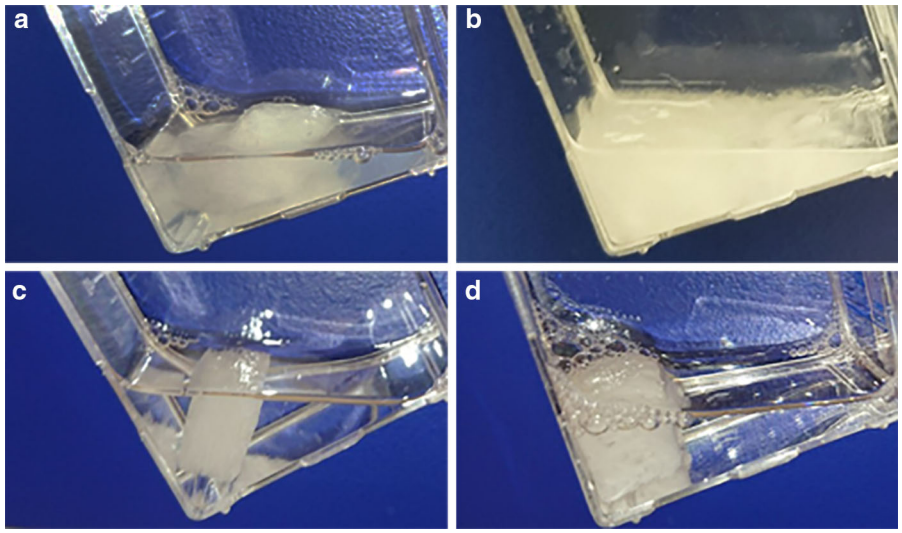

Fig. 2 Images of the cellulose fiber foams after 18 h shaking in water. **a** Foam P, **b** foam P–CA, **c** foam Ch–CA and **d** foam P–Ch–CA

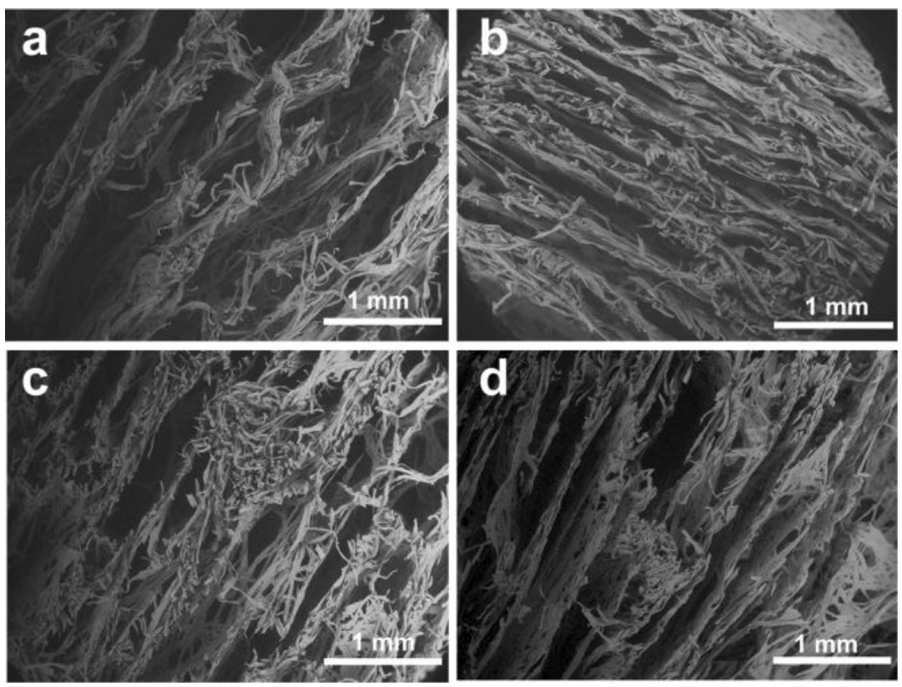

Fig. 3 SEM micrographs of cellulose foams cross-sections of the a foam P, **b** foam P–CA, **c** foam Ch–CA and **d** foam P–Ch–CA

Nitrogen-content

The nitrogen analysis showed that the foams containing chitosan had a nitrogen content twice as high as that of the foams prepared only using PVAm (Fig. 4). It was expected that the chitosan-containing foams would have higher nitrogen content, as the total amount of nitrogen-containing compounds added during foam preparation was higher for the foams containing chitosan than for the foams with only PVAm as the nitrogen-containing component. No significant difference was observed between the foam containing only chitosan and CA and to the foam containing PVAm, chitosan and CA. No great

Table 3 Average distance between the pore walls, estimated through SEM imaging

Sample	Average distance between pore walls (μm)
Foam P	440 ± 140
Foam P–CA	160 ± 30
Foam Ch–CA	220 ± 80
Foam P–Ch–CA	240 ± 110

The showed errors represent the standard deviation

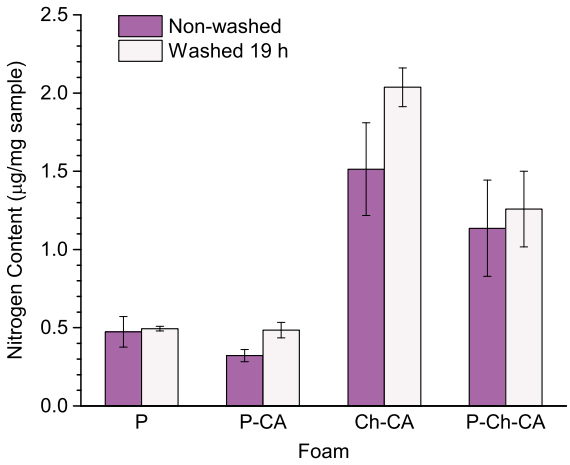

Fig. 4 Result of the nitrogen analysis for the non-washed and washed cellulose fiber foams. Error bars show the 95% confidence interval

difference could be seen in nitrogen content before and after washing of the foam, however it seems like foam P–CA and foam P–Ch–CA have a slightly higher nitrogen content after washing. This may be caused by an uneven distribution of chitosan within the foam or a loss of CA and NaCl from the foam causing a higher nitrogen content. The nitrogen analysis shows no difference in nitrogen content before and after washing of foam P and foam P–Ch–CA, which indicates that no nitrogen-containing component, i.e. PVAm or chitosan, was leached from these foams during washing.

FTIR

The spectra from the FTIR analysis are complex to analyze due to overlapping signals from the different components and the similar structures of cellulose and chitosan, but it was used to evaluate the presence of CA the foams.

Primary amine groups show a double peak at 3550–3330 cm^{-1}, due to the asymmetrical and symmetrical N–H stretching, and the N–H bending can be observed as a medium strong peak at 1650–1580 cm^{-1} (Socrates 2001). Primary amines also absorb at 1295–1145 cm^{-1} due to rocking and twisting of the amine group. Carboxylic acids, such as citric acid, absorbs strongly at 1740–1700 cm^{-1} due to the C = O stretching in the carboxyl groups (Socrates 2001) and the carboxylate anion stretching give rise to a strong peak at 1550–1600 cm^{-1} and a weak peak near 1400 cm^{-1}(Nunthanid et al. 2001).

Foam P contains very little PVAm, 0.5 μg/mg sample, and it is difficult to see any difference between the foams containing PVAm and the reference pulp sample. The curve changes when CA is introduced to the foam P–CA, it can be seen at the medium intense peaks at 1715 and 1580 cm^{-1}. These peaks do however decrease significantly when the foam has been washed, indicating that most citric acid is removed during washing (Fig. 5).

The presence of citric acid is clearly visible in the foam Ch–CA with chitosan and CA, and it is removed after washing. A possible crosslinking of chitosan and citric acid would result in an ester group, the C–O–C asymmetrical stretching occurs at 1275–1185. However, it is not possible to determine whether the CA and the chitosan is crosslinked or not through the FTIR analysis as the primary amine group and the ester group overlaps at the large peak at 1210 cm^{-1}. The chitosan is partially acetylated, > 75%, and the amide I band, due to C = O stretching in the remaining amide groups, can be seen in the spectra with a peak at 1650 cm^{-1} (Socrates 2001), indicating that chitosan is still present after washing (Fig. 5).

Absorption

A high water absorption capacity is both an advantage for cellulose-based materials and one of the more problematic features, since water swells the structure and can reduce the mechanical strength of the material and promote microbial growth. The adsorption test showed that all the foams adsorbed 12–18 times their own dry weight of water, in contrast to the reference pulp that absorbed 9.5 times its own weight (Table 4). This increase can be explained by the porous structure

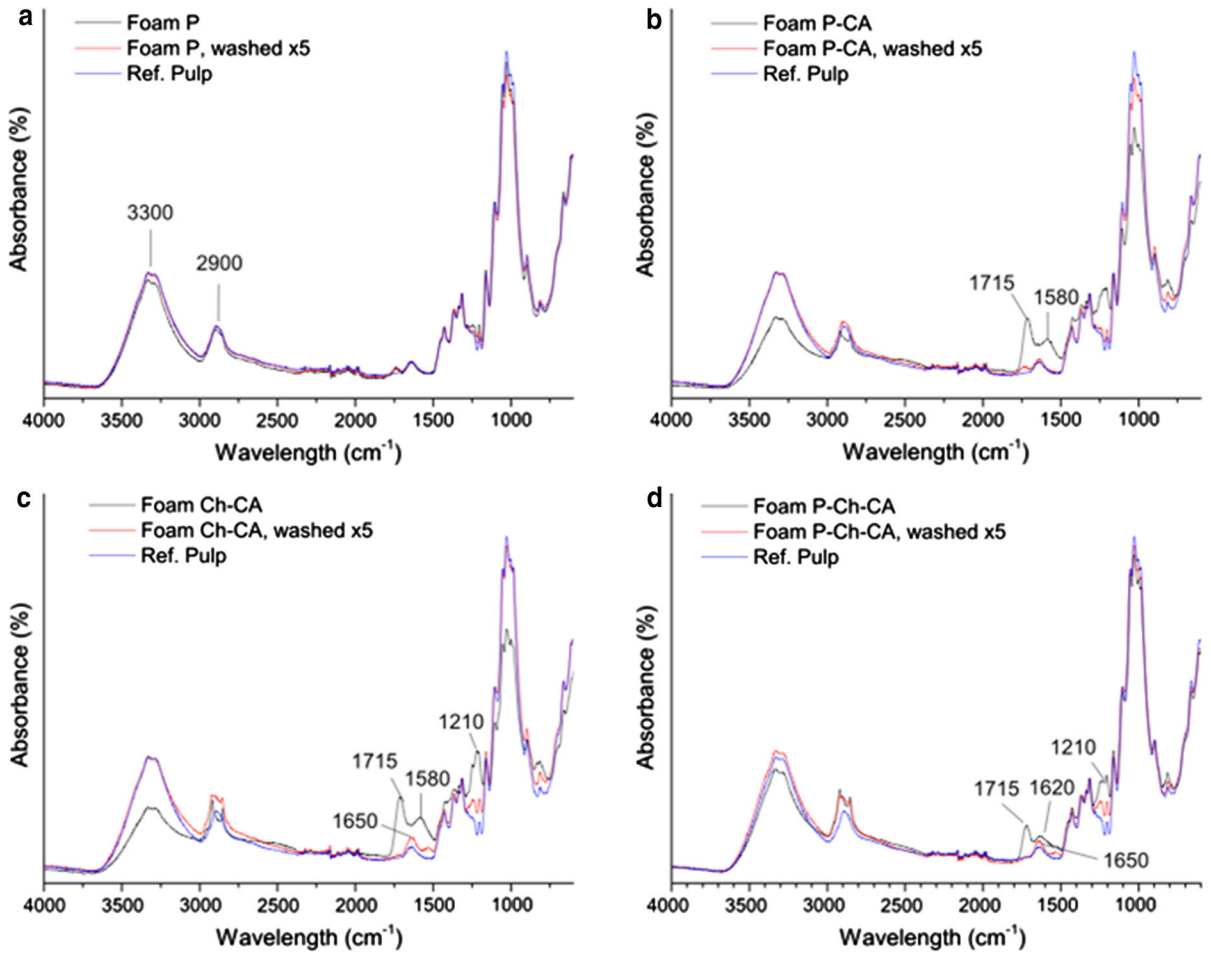

Fig. 5 FTIR spectra for non-washed and washed x5 foam materials. **a** Foam P, **b** foam P–CA, **c** foam Ch–CA and **d** Foam P–Ch–CA

Table 4 Water absorption capacity of the cellulose foam materials, mean and the 95% confidence interval

Sample	Water absorption (g water/g dry weight)
Reference pulp	9.5 ± 0.7
Foam P	18.3 ± 3.2
Foam P–CA	12.3 ± 1.9
Foam Ch–CA	15.8 ± 1.6
Foam P–Ch–CA	12.8 ± 3.3

of the foam materials. The foam with PVAm and CA had the lowest absorption capacity, and this was also the most brittle of the samples with the highest density.

Antimicrobial assays

In this study, the antibacterial effect of the cellulose fiber foams has been evaluated using *E. coli*, and the antifungal effect has been evaluated using *A. brasiliensis*, which is closely related to *Aspergillus niger* that is a common contaminant that can cause food spoilage (Varga et al. 2007). CA was originally extracted from lemons but today it is industrially produced by fermentation using *A. niger* (Papagianni 2007). *A. niger* can grow at pH as low as 1.4 and should not therefor be affected by the excess CA in the foams (Schuster et al. 2002).

Reducing bacteria

The non-washed samples containing CA have all a bacterial-reducing efficiency of 100%, no viable bacteria were detected after incubation (Fig. 6). Of the washed foam samples, foam Ch–CA washed 5 times had the best bacterial reduction of 99%. The result for foam P–Ch–CA was very similar, indicating that the addition of PVAm did not improve the antibacterial effect. Thus, it is possible to produce a water-stable foam with good antibacterial effect using only bio-based polymers. Foam P–CA had a bacterial reducing effect after washing similar to that of foam P, approximately 50% reduction. This supports the result of the FTIR analysis, showing that CA is removed from the foam during washing. The great bacterial-reducing effect of the non-washed foam P–CA is ascribed to the leaching of CA. However, the foams containing chitosan still had a large bacterial-reducing effect of 99% for the 5 times washed foams and 90% for the 19 h washed foams. The reference sample containing pulp and SDS had a bacterial-reducing effect of approximately 45%, while the reference sample containing pulp and CA had a bacterial-reducing effect of 90%, again showing that excess CA has a good bacterial-reducing effect. It can be concluded that the non-washed samples reduce the amount of viable bacteria by leaching CA while the foams containing chitosan also have a contact active antimicrobial effect that are still active after the CA is removed during the washing step.

Several studies have been using chitosan as an antimicrobial agent to create antimicrobial materials as it is an environmentally sustainable alternative to harmful biocides. However, many of them combine chitosan with other bactericides like silver ions or silver nanoparticles to get a leaching antibacterial effect (Guibal et al. 2013; Ma et al. 2008; Vimala et al. 2010), which makes it difficult to compare the results with the cellulose foams, especially since the testing procedure varies. The studies using PVAm in LbL modified antibacterial cellulose have shown a bacterial reduction greater than 99.9% when using pulp modified with PVAm and polyacrylic acid in similar reduction tests (Illergård et al. 2012, 2015). However, the materials are produced in completely different manner compared to the cellulose foams and it is not possible to compare the antibacterial effect of PVAm as the foam forming process involve other components like CA and SDS.

Bacterial growth inhibition

The bacterial growth inhibition test confirms that CA inhibits bacterial growth, as no increase in optical density (OD) was observed for the non-washed samples containing CA or for the reference sample with pulp and CA when *E. coli* were incubated

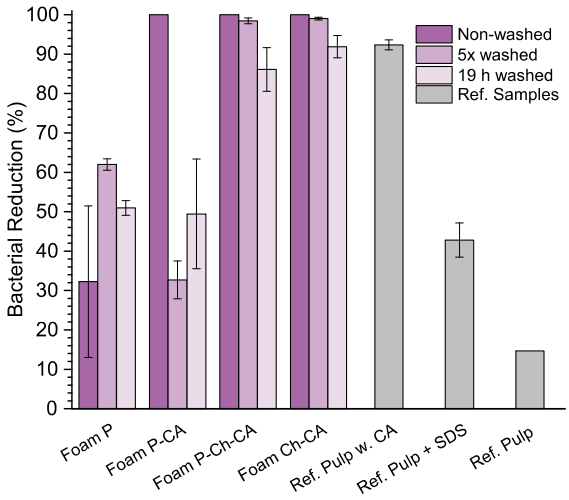

Fig. 6 Bacterial reduction after 4 h incubation with foam materials and reference samples. The error bars show the standard deviation

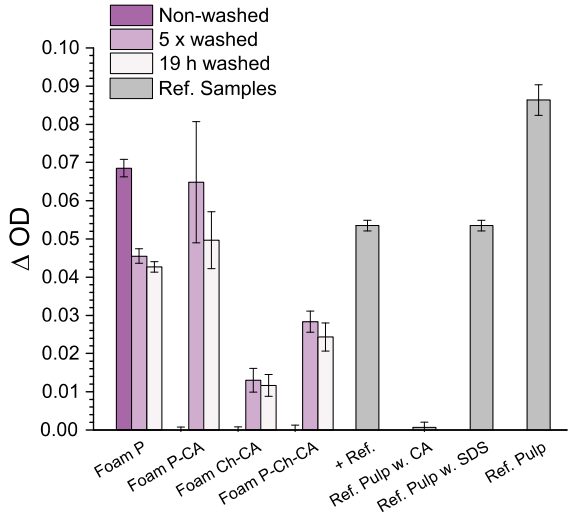

Fig. 7 Bacterial growth shown as increase in OD after cultivation with nutrient at 37 °C for 19 h. The error bars show the standard deviation

overnight with the materials (Fig. 7). The OD value for the non-washed foam P increased more than that for the positive reference, containing only *E. coli* and nutrient, but less than for the pulp reference, indicating that foam P had no growth-inhibiting effect on bacteria. The OD values for the foams containing chitosan had increased less than that for the reference samples, indicating that the chitosan-containing foams had a bacterial-growth inhibiting effect. The chitosan-containing foams had a growth-inhibiting effect even after the CA was washed away, indicating that the chitosan containing foams had a contact-active effect due to the non-leaching chitosan within the foam, shown by the nitrogen analysis.

The increase in the OD values after the material was removed from the bacterial suspension showed whether the materials were releasing any growth-inhibiting agent into the suspensions (Fig. 8). The increase in OD of the suspensions incubated after removing the non-washed samples containing CA was very low, showing that the non-washed samples were releasing growth-inhibiting compounds. However, the increase in OD after removing non-washed foams containing chitosan was higher than that of the suspensions incubated with foams without chitosan, indicating that these materials are releasing less growth-inhibiting agents by a leaching process. The increase in OD value for the 5 times washed samples were similar to that for the positive reference sample

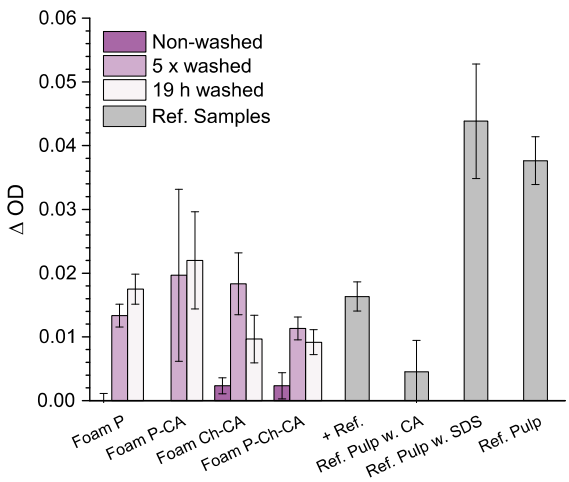

Fig. 8 Bacterial growth shown as increase in OD after the samples has been removed to determine whether the materials are leaching growth-inhibiting compounds. The error bars show the standard deviation

indicating that these do not leach growth-inhibiting compounds. The OD value increased much less for the suspension where the pulp with CA reference had been removed than for the reference incubated with only pulp. For the pulp reference and the pulp with SDS, the OD value increased significantly. Other studies have shown a similar pattern when cellulose pulp is incubated with *E. coli*. One reason could be that the pulp serves as nutrient for the bacteria (Illergård et al. 2012; Ottenhall et al. 2017). The results of the leaching test confirm that CA leaching from the non-washed foams leading to a bacteria-reducing effect, but the excess CA is removed after washing.

Agar diffusion test with bacteria

The leaching effect of the materials was studied in an agar diffusion test. The zone of inhibition around the materials was measured and compared between non-washed and washed samples (Table 5). All the non-washed samples inhibited bacterial growth around the material, indicating that the materials were releasing growth-inhibiting compounds. The boundaries of the zone of inhibition were uneven for the foams, but a clear circle could be observed surrounding the pulp with CA as reference sample. A very small zone of inhibition was observed around the 5 times washed samples. No zone of inhibition was observed for the SDS reference sample, which indicates that the leaching compound that inhibits bacterial growth around the non-washed samples is CA.

Fungal resistance

No fungal growth was observed on the foams after 28 days incubation in the humidity chamber. Fungal growth was observed on the positive reference made of wood but no fungal growth was detected on the reference pulp sample. It could be that the cellulose pulp itself does not contain sufficient nutrient for *A. brasiliensis* grow. Similar results have been observed for cellulosic insulating materials tested under the same conditions (Zheng et al. 2017).

Fungal growth inhibition

Non-washed cellulose foam samples were incubated with nutrient for 23 days. Heavy fungal growth was observed on the reference pulp sample, foam P and

Table 5 Results of the agar diffusion test with bacteria

Material	Non-washed sample, inhibition zone (mm)	Washed sample, inhibition zone (mm)
Reference pulp	No ZOI	n.a.
Reference pulp with SDS	No ZOI	n.a.
Reference pulp with CA	7.6	n.a.
Foam P	4.8	No ZOI
Foam P–CA	3.7	No ZOI
Foam Ch–CA	7.4	No ZOI
Foam P–Ch–CA	5.3	No ZOI

The zone of inhibition (ZOI) around the foam samples are expressed as the distance measured from the edge of the disc sample

foam P–CA (Fig. 9) All samples with fungal growth had turned lightly yellow. No fungal growth or discoloration was observed on foam Ch–CA or on foam P–Ch–CA, indicating that the foams containing chitosan had a strong fungal-growth-inhibiting effect even when nutrient was present. Sebti et al. showed that chitosan, even at low concentrations, in films and coatings, can inhibit growth of *A. niger* in the presence of nutrients, which corresponds well with the results from the fungal growth test (Sebti et al. 2005).

Agar diffusion test with fungi

An agar diffusion test with *A. brasiliensis* was performed using the foam samples containing chitosan, as these were the samples with a successful performance in the fungal growth inhibition test with added nutrient. The diffusion test showed that the leaching effect of the cellulose foam samples decreased after washing. Non-washed foam Ch–CA had the largest zone of inhibition around the material, followed by non-washed foam P–Ch–CA (Table 6).

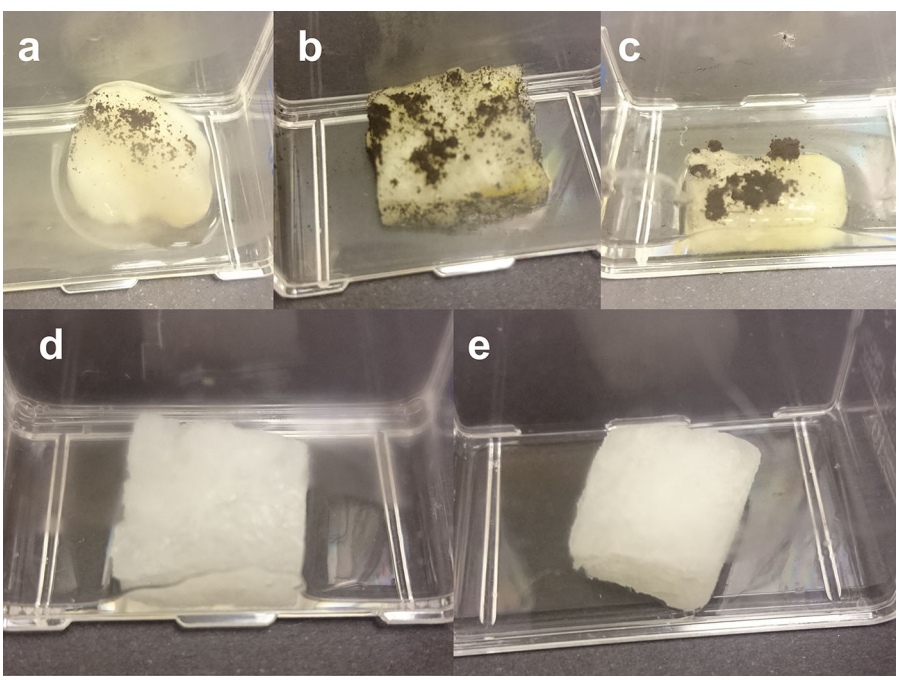

Fig. 9 Cellulose foam samples after 23 days of incubation with *A. brasiliensis* and nutrition at 30 °C. **a** Reference pulp, **b** foam P, **c** foam P–CA, **d** foam with Ch–CA and **e** foam P–Ch–CA

Table 6 Results of the fungal agar diffusion test

Material	Non-washed sample, inhibition zone (mm)	Washed sample, inhibition zone (mm)
Reference pulp	No ZOI	No ZOI
Foam Ch–CA	6.5	No ZOI
Foam P–Ch–CA	3	No ZOI

The zone of inhibition (ZOI) around the foam samples is expressed as the inhibition zone distance from the edge of the disc sample

No zone of inhibition was detected with the reference pulp sample or with the washed foam samples. The FTIR analysis indicated that the CA was removed during the washing, but the nitrogen analysis showed no great decrease in nitrogen-containing polymers. The leaching antimicrobial effect is ascribed to the CA, while chitosan inhibits fungal growth on the materials.

Conclusions

It is possible to create water-stable and low-density cellulose fiber foams with antimicrobial properties by adding chitosan and CA to the fiber suspension during the foam-forming process. The foam material containing only bio-based polymers, i.e. cellulose fibers and chitosan, had both good water-stability and good antimicrobial properties. This bio-based foam material would be a good alternative for the packaging of fragile customer products that need to withstand both moisture and microbial attack, as the one-batch production process is simple and contains no harmful substances. All the foams were resistant to fungal growth under humid conditions, but only the foams containing chitosan inhibited fungal growth when nutrients were present. The cationic chitosan provides both antibacterial and antifungal properties, and it can be seen as a contact-active antimicrobial component as the chitosan is not leached from the foam during washing. All the non-washed foams containing CA showed good antibacterial effect, due to the leaching of excess CA that contributes to acidic conditions. Analysis of the washed cellulose foams showed that excess CA was removed from the foams during washing, while the cationic polymers were not leached from the foam. This could be the future for environmentally sustainable low-density packaging material that can withstand microbial growth while protecting sensitive products from mechanical damage.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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