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Environmentally Friendly Bacterial Cellulose Films for Food Packaging

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Abstract

Use of biodegradable polymer films such as polyhydroxybutyrate (PHB), chitosan and cellulose as packaging materials in food storage has become an important issue in the storage of food. The aim of this study was to assess the effect of bacterial cellulose films in terms of extending the shelf life of food storage will be determined compared to other petrochemical materials. Bacterial cellulose films were formed by *Gluconacetobacter hansenii* HE1 strain. The cellulose layer was dried, sterilized and used as packaging material. Cling wrap was used as a positive control. Sausage specimens purchased from the market were kept in the fridge at 0, 2, 4 and 6 days at +4ºC without wrapping, wrapped with a cling wrap, wrapped with bacterial cellulose film. Colony counts for microbial load were determined by the arithmetic mean, with 3 replicates among each sample group. At the end of the sixth day, the microbial load of the sausage wrapped with bacterial cellulose was found to be as $1{,}2x10^4$ cfu/mL. However, the microbial load of the sausage wrapped with cling wrap was found to be as $2{,}7x10^5$ cfu/mL while the microbial load of the non-wrapped sausages was found to be as $1{,}0x10^6$ cfu/mL. It has been observed that microbial contamination with airborne filtration through porous, thin, web-like structure, which bacterial cellulose has, can filtrate air-borne contamination better than petrochemical-derived cling wrap. For this reason, bacterial cellulose can be used as a packaging material to store foodstuffs and to extend shelf life.

Keywords: Bacterial cellulose, food packaging, cling wrap, microbial load

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INTRODUCTION

It is an important issue for people to be able to take and consume food products in a safe and sufficient amount in order to maintain their lives and physical development in a healthy way. The processes of food from the manufacturing stage to the marketing stage are as important as the microbiological problems that may arise during the waiting times of the food by the consumer in the refrigerator (Brooks and Flint, 2008).

Food safety is known as taking precautions by obeying the necessary rules during the acquisition, production, processing, storage, transportation, distribution and consumption of food raw materials in order to ensure healthy and flawless food production. In food safety, it is very important that the food does not lose its nutritional value, that the food is clean and intact in physical, chemical and biological terms. Foreign particles such as soil, wood, metal, glass parts, hair, nails, flies, insects that should not be present in food are physical hazards.

Chemical hazards are the presence of metals such as mercury, lead, cadmium, detergent wastes and pesticides in foods. These chemical hazards are mainly caused by containers or petrochemical-legal bags in which food is stored or packaged. Among the biological hazards, they are the bacteria that threaten food safety the most and cause food poisoning (Borchers et al., 2010).

Many methods have been developed such as heat treatment and cold application, drying, salting and sealing protection, vacuuming protection, from the manufacture of food to consumption. Especially in recent years, it is very common for food to be vacuumed and delivered to consumers in this way. By vacuuming, the contact of the food with air is prevented and the growth of microorganisms on the food is prevented. However, even if a vacuumed food package is opened and stored in the refrigerator, it tends to deteriorate after a certain period of time. If the foods such as salami, sausage, soudjouk and cheese purchased in vacuumed packages are wrapped with materials such as refrigerator bag, cling film and stored in storage containers after the package is opened, it does not prevent food spoilage (Arvanitoyannis and Kotsanopoulos, 2014).

Petrochemical origin plastics such as polyethylene, polyester, polyolefin, and polyamides, which we use to store the remaining part of vacuumed foods in the refrigerator after opening, have been used a lot in recent years. Because such materials have a high tension and high tear strength, they also have important features in terms of air tightness, liquid leakage and heat retention. However, components such as styrene, 1,3-butadiene, melamine, formaldehyde, acrylamide, di-2-ethylhexyl phthalate, di-2-ethylhexyl adipate, vinyl chloride and bisphenol which may be present in petrochemical-based food packages can cause serious problems on human health (Durusoy and Karababa, 2011; Raheem, 2012). Besides, there is very low evaporation transition from such materials, which causes moisture to form in the food package and cause microorganism reproduction. In addition, since petrochemical-based materials used as food packaging materials don't have a biodegradable property, the waste remains in the environment for many years and causes serious ecological problems (Siroli et al., 2017). Therefore, the use of biodegradable polymer films such as polyhydroxybutyrate (PHB), chitosan and cellulose as packaging materials in food storage has become an important issue in the storage of food. Bacterial cellulose also has a biodegradable, flexible, porous structure that can be used as environmentally friendly packaging material (Aider, 2010; Khosravi-Darani and Bucci, 2016; Wu et al., 2016; Atares and Chiralt, 2016).

Cellulose and its derivatives are also used as packaging material in foods, including textile, paper and food industry. Although it is structurally similar to plant cellulose, the fact that bacterial cellulose is completely pure, very fine-pored and flexible is an important feature and has many industrial uses (Shah et al., 2013; Keshk, 2014).

In this study, we aimed the use of bacterial cellulose films as food packaging material. Therefore, we determined the microbiological load formed in different medium at specific time intervals.

MATERIAL AND METHODS

Material

Sausage samples sold in vacuum packages were used as material. Bacterial cellulose and cling wrap were used to store the food after the food sample was unpacked. *Gluconacetobacter hansenii* HE1 strain used for bacterial cellulose production was provided from Aydın Adnan Menderes University Microbiology Laboratory stock cultures.

Production and purification of cellulose

G. hansenii HE1 strain was inoculated in HS (Hestrin-Schramm) (2% glucose, 0.5% yeast extract, 0.5% polypeptone, 0.675% Na_2HPO_4, 0.115% citric acid) broth and was allowed to produce cellulose after an incubation period of 10 days at 30ºC (Hestrin and Schramm, 1954). 4% NaOH and 6% acetic acid solutions were used for purification and dried by lyophilisation (Çoban et al., 2017; Çoban et al., 2020).

Preparation of bacterial films as packaging material

The cellulose layer used as packaging material was sterilized at 121ºC for 20 min. Stretch films were used as a positive control. Stretch films were sterilized by standing under UV for 3 hours. Sausage specimens purchased from the market were kept in the fridge at 0, 2, 4 and 6 days at +4ºC without wrapping, wrapped with a cling wrap, wrapped with bacterial cellulose film.

Microbiological Analysis

A 10 g of sausage samples were aseptically added with 90 mL of sterile 0,85 % NaCl (physiological salt water) solution. After, the mixture was homogenized in a Stomacher for 2 min. Then, serial dilutions up to 10^{-4} were prepared and carried out following standard methodologies (Ferna´ndez-Lo´pez et al., 2008).

A 0,1 mL sample from each dilution was plated onto selective media. Plate Count Agar (Merck) was used to determine total mesophile aerobes at dilutions of 10^{-1}-$10^{-4.}$ PCA plates were incubated aerobically at 30°C for 72 h. Violet Red Bile Agar (VRBA) (Merck) was used to determine coliform bacteria at dilutions of 10^{-1}-$10^{-4.}$ VRBA plates were incubated aerobically at 37°C for 24 h. Baird Parker Agar (Merck) was used to determine *Staphylococcus aureus* at dilutions of 10^{-1}-10^{-4}. BPA plates were incubated aerobically at 37°C for 24-48 h. Selenite Cystine Broth (SCB) (Merck) was used to detect the presence of Salmonella as an enrichment medium at dilutions of 10^{-1}-10^{-2}. SCB tubes were incubated aerobically at 37°C for 18-24 h. A 0,1 mL sample from each SCB tubes was plated onto Bismuth Sulfite Agar (BSA) (Merck) for Salmonella. BSA plates were incubated aerobically at 37°C for 24 h. Rose-Bengal Chloramphenicol Agar (RBCA) (Merck) was used to determine microfungi at dilutions of 10^{-1}-10^{-4} . RBCA plates were incubated aerobically at 25°C for 5 days (Drosinos et al., 2005; Ferna´ndez-Lo´pez et al., 2008).

Following the incubations, colonies were counted as colony morphology and microbial load calculated as cfu/mL. Colony counts for microbial load were determined by the arithmetic mean, with 3 replicates among each sample group.

RESULTS and DISCUSSION

Production of purification of bacterial cellulose

Bacterial cellulose was obtained by *Gluconacetobacter hansenii* HE1 strain and purified and dried (Fig. 1a, b).

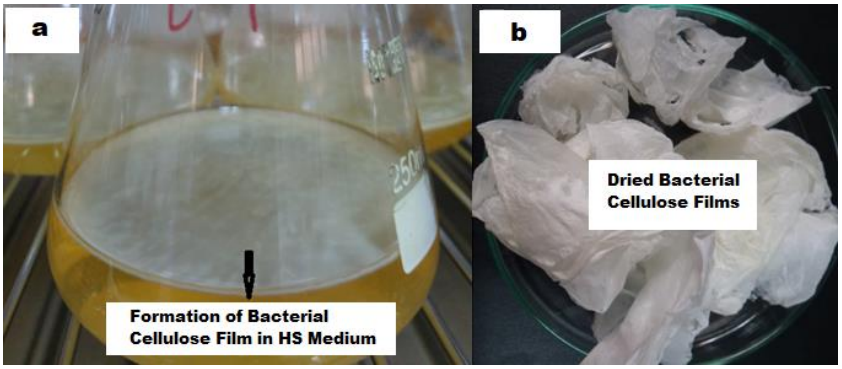

Figure 1. a. Formation bacterial cellulose film in HS Medium **b.** Dried bacterial cellulose films

Microbiological Analysis

Plate Count Agar (PCA) medium for the general viability count, Violet Red Bile Agar (VRBA) medium for the coliform bacteria count, Baird Parker Agar (BPA) for the *Staphylococcus aureus* count, Selenite Cystine Broth and Bismuth Sulfite Agar (BSA) for the presence of Salmonella and Rose-Bengal Chloramphenicol Agar for the microfungus count were used in the study.

According to study results, no microorganism growth was observed in the Violet Red Bile Agar (VRBA), Baird Parker Agar (BPA), Bismuth Sulfite Agar (BSA) and Rose-Bengal Chloramphenicol Agar (RBCA) media on day 6th (Fig. 2a, b, c; 3a, b, c; 4a, b, c). However, microorganism growth was seen on Plate Count Agar (PCA) medium (Fig. 5a, b, c).

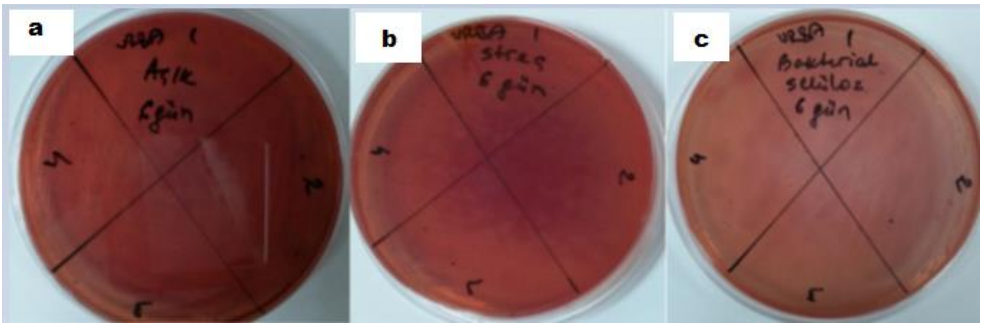

Figure 2. Bacteria growth in Violet Red Bile Agar Medium on day 6th **a.** without wrapping **b.**wrapped with a cling wrap **c.** wrapped with bacterial cellulose film (No growth)

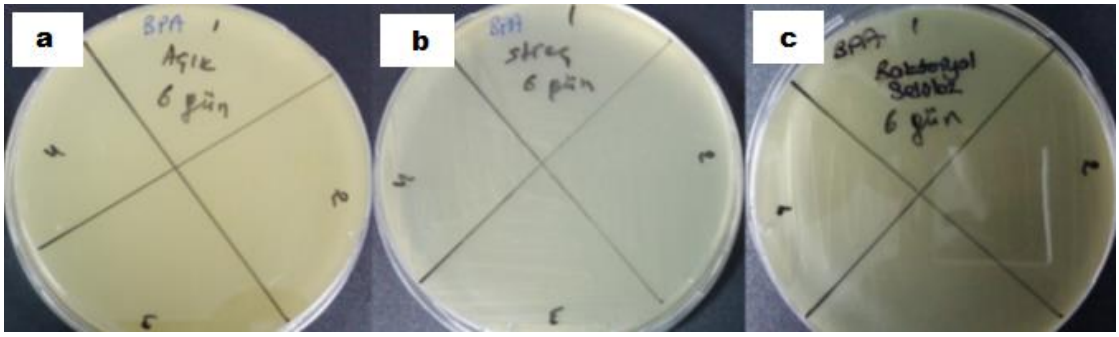

Figure 3. Bacteria growth in Baird Parker Agar Medium on day 6th **a.** without wrapping **b.** wrapped with a cling wrap **c.** wrapped with bacterial cellulose film (No growth)

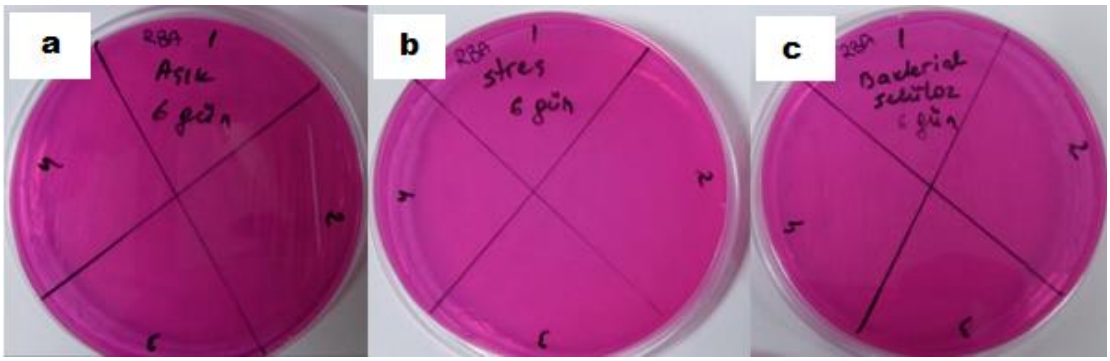

Figure 4. Bacteria growth in Rose-Bengal Chloramphenicol Agar Medium on day 6th **a.** without wrapping **b.** wrapped with a stretch film **c.** wrapped with bacterial cellulose film (No growth)

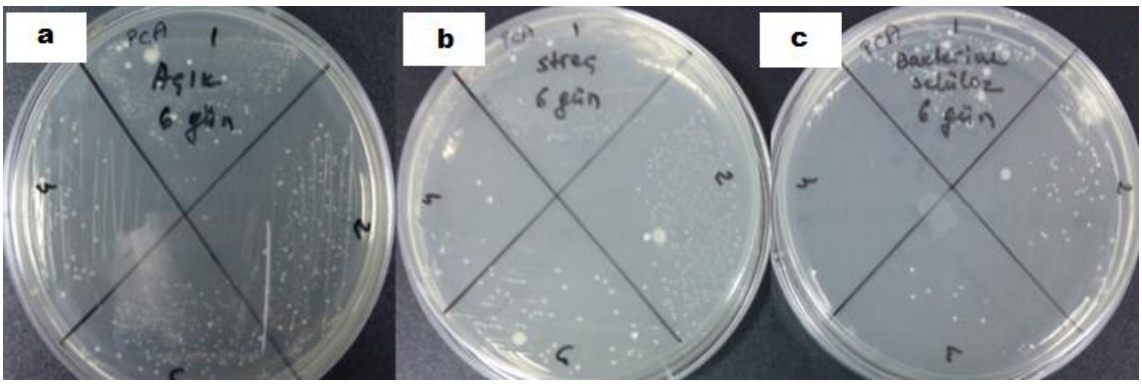

Figure 5. Bacteria growth in Plate Count Agar Medium on day 6th **a.** without wrapping **b.** wrapped with a cling wrap **c.** wrapped with bacterial cellulose film

The colonies formed on days 0, 2, 4, and 6th in PCA medium were counted and the results were given as cfu/mL in Table 1.

Table 1. Colony counts and microbial loads of microorganisms in PCA medium on days 0, 2, 4 and 6th

Medium	Dilution rates				Microbial load (cfu/mL)
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	
PCA	Colony Count				
Without wrapping					
0. day	-	-	-	-	-
2. day	62	15	1	-	$6,2x10^3$
4. day	≥300	86	12	-	$8,6x10^4$
6.day	≥300	≥300	102	24	$1,0x10^6$
Wrapped with a cling wrap					
0. day	-	-	-	-	-
2. day	23	6	-	-	$2,3x10^3$
4. day	≥300	55	6	-	$5,5\ x10^4$
6.day	≥300	270	53	2	$2,7\ x10^5$
Wrapped with bacterial cellulose film					
0. day	-	-	-	-	-
2. day	20	2	-	-	$2,0x10^3$
4. day	85	11	-	-	$8,5\ x10^3$
6.day	117	38	3	-	$1,2\ x10^4$

The sausages were kept in the refrigerator in 3 different ways for 6 days. At the end of the sixth day, the microbial load of the sausage wrapped with bacterial cellulose was found to be as $1,2x10^4$ cfu/mL. However, the microbial load of the sausage wrapped with cling wrap was found to be as $2,7x10^5$ cfu/mL while the microbial load of the non-wrapped sausages was found to be as $1,0x10^6$ cfu/mL.

Kuswandi et al. (2020) developed an edible pH sensor based on immobilized red cabbage anthocyanins into bacterial cellulose membrane for intelligent food packaging. It was reported that the edible pH sensor can distinguish fresh milk from spoilage, making it suitable to be used in an intelligent packaging system as a freshness sensor (Kuswandi et al., 2020). It showed that use of bacterial exopolysaccharides and their potential application are possible as food packaging materials, including edible coatings, intelligent films, and thermo-insulated aerogel packaging (Neši´c et al., 2020). Bandyopadhyay et al. (2020) researched use of bacterial cellulose based polymeric films for food packaging. They found that bacterial cellulose films preserved the freshness of fruits and vegetables for a long time (Bandyopadhyay et al., 2020). In another study, it was expressed that nanocomposites of bacterial cellulose nanofibrils and zein nanoparticles are useful for food packaging (Li et al., 2020). In a same study, it reported that bacterial cellulose is appropriate as a raw material for food and food packaging applications (Azeredo et al., 2019).

Padrao et al. (2016) showed that bacterial cellulose films modified by bovine lactoferrin. The films were applied as edible antimicrobial packaging on meat products such as fresh sausage. Bacteriocidal effect of the films were examined against *Escherichia coli* and *Staphylococcus aureus.* As a result, the films significantly hindered the specific growth rate of both bacteria (Padrao et al., 2016).

Also, Abdul Khalil et al. (2016) reviwed that the demand for exploring advanced and eco-friendly sustainable packaging materials with superior physical, mechanical and barrier properties is increasing. Therefore, it has been emphasized that cellulosic nanofibers can be used in food packaging applications (Abdul Khalil et al., 2016). In another review, it was remarked that use of natural polymers such as protein based edible films and films from cellulose and its derivatives are available as cling films for food packaging (Malhotra et al., 2015). Besides, Pawar and Purwar (2013) reported that bacterial cellulose can be used as a biodegradable food packaging material in the protection and storage of foods from microbial contaminants.

CONCLUSION

Bacterial cellulose has a porous, thin, reticulated structure. With this structure, it filters the air and keeps small particles such as dust, microorganisms and fungus spores that may be present in the air. However, microorganism growth is observed more in foods wrapped with petrochemical-derived stretch film and food spoilage happens more quickly.

Therefore, it is appropriate to use bacterial cellulose films as food packaging material in the storage of foods and extend their shelf life. We think that it should be evaluated as a new and alternative food packaging material of biological origin in the food industry. The fact that bacterial cellulose is non-toxic, compatible with living tissue, edible and environmentally friendly shows that it can be used safely in the food industry. Therefore, the use of bacterial cellulose as food packaging material has a high added value.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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