

Hydrogel-based bioactive food packaging material for agro products

Smarak Bandyopadhyay, M.Sc. Ph.D.

Doctoral Thesis Summary



Tomas Bata University in Zlín

Faculty of Technology

Doctoral Thesis Summary

Hydrogel-based bioactive food packaging material for agro products

Bioaktivní obalový materiál na bázi hydrogelu pro agro produkty

Author: **Smarak Bandyopadhyay, M.Sc. Ph.D.**

Degree programme: Chemistry and Materials Technology

Degree course: Technology of Macromolecular Compounds

Supervisor: Professor Petr Sába

Consultant: Assoc. Professor Nabanita Saha

External examiners: prof. Ing. František Buňka, Ph.D.
prof. Ing. Petr Slobodian, Ph.D.

Zlín, August 2020

© Smarak Bandyopadhyay

Published by **Tomas Bata University in Zlín** in the Edition **Doctoral Thesis**.
The publication was issued in the year 2020.

Klíčová: *biopolymer, hydrogel, balení potravin, aktivní balení, trvanlivost*

Key words: *biopolymer, hydrogel, food packaging, active packaging, shelf life*

Full text of the scientific publication is available in the Library of TBU in Zlín.

ISBN 978-80-7454-930-4

Abstrakt

Obalové materiály na bázi ropy zvyšují celosvětovou uhlíkovou stopu díky své biologické nerozložitelnosti. Jejich nadměrné používání a nesprávná likvidace navíc škodí vodním i suchozemským živočichům a obecně životnímu prostředí. Podle Evropského parlamentu by mělo být používání plastů na jedno použití do roku 2022 nahrazeno biologicky rozložitelným obalovým materiálem, a to zejména u potravinářských obalů. Alternativou k biologicky nerozložitelným obalovým materiálům jsou bio-hydrogely, které navíc mají také schopnost prodloužit skladovatelnost produktu.

Předložená disertační práce je zaměřena na produkci bakteriální celulózy (BC) z odpadní jablečné šťávy a její využití k vývoji nové biologicky rozložitelné hydrogelové fólie na bázi **PVP-CMC-BC-GG** s přísadkou éterického oleje a antokyaninu.

Pro hodnocení hydrogelové fólie byly použity metody SEM, FTIR, XRD, TGA, DMA, testy na změny zbarvení, mechanická a biodegradační analýza. Testovací přístroj Instron byl použit pro měření Youngova modulu, prodloužení při přetržení a pevnosti fólie v tahu, fyzikálně-chemická analýza nového obalového materiálu pak byla provedena pomocí FTIR a XRD. Metoda SEM umožnila znázornit strukturální orientaci fólie a pomocí TGA byla zjištěna tepelná stabilita materiálů. Důležitým kritériem pro hodnocení potravinářských obalových materiálů jsou bariérové vlastnosti. Proto byla, podle protokolů ASTM, stanovena propustnost kyslíku a rychlost propouštění vodních par. Analýza čerstvosti potravin (bobulí hroznového vína a sýru) zabalených do hydrogelového obalu byla hodnocena vizuálně za běžných laboratorních podmínek v různých časových intervalech.

Bylo zjištěno, že obaly na bázi PVP-CMC-BC-GG mají lepší bariérové a mechanické vlastnosti ve srovnání s běžně používanými potravinářskými obaly. Metoda SEM ukázala, že porozita hydrogelového obalu zůstala nezměněna po celou dobu hodnocení. Vhodnost jeho použití podporuje také prodloužení trvanlivosti bobulí hroznového vína a vzorků sýru v něm balených. Přidání éterického oleje do těchto fólií mělo za následek vznik inhibičních zón, tj. antimikrobiální vlastnosti proti řadě mikrobů znehodnocujících potraviny. Přídavkem antokyaninu do PVP-CMC-BC-GG vznikly fólie citlivé na pH, které detekují znehodnocení potravin v důsledku změn pH.

Nově vyvinutá hydrogelová fólie degraduje z 80% v kompostu za 30 dní a úplné degradace je dosaženo za 60 dní. Z výsledků práce je tedy možno vyvodit závěr, že hydrofilní fólie PVP-CMC-BC-GG hydrogelu mohou být vhodným řešením pro budoucí rozložitelné potravinové obaly.

Klíčová slova: *biopolymer, hydrogel, balení potravin, aktivní balení, doba použitelnosti*

Abstract

Petroleum based food packaging materials increase the global carbon footprint due to their non-biodegradability. Moreover, the excessive use and improper disposal have caused enough harm to the aquatic and terrestrial lives. According to the European Parliament, the usage of single-use plastics (mainly used for food packaging) should be replaced with biodegradable packaging materials by 2022. Bio-based hydrogels are not only an alternative to the non-biodegradable packaging materials but also have the capability to enhance the shelf life of the product.

This doctoral thesis is focused on the production of bacterial cellulose (BC) from waste apple juice and utilizing the BC to develop novel biodegradable hydrogel film (**PVP-CMC-BC-GG**). Furthermore, essential oils and anthocyanin were added to the films to make them functional packaging material.

Among the evaluations done for the hydrogel film, SEM, FTIR, XRD, TGA, DMA, color assay, mechanical and biodegradation analysis are the notable ones. Mechanical tester Instron gave the Young's modulus, elongation at break and tensile strength of the films. The physico-chemical analysis of the novel packaging material was done using FTIR and XRD methods. SEM provided an insight to the structural orientation of the film, and TGA gave the thermal stability of the materials.

The determination of barrier properties is also an important parameter for food packaging material, hence the oxygen permeability and water vapour transmission rate were calculated following ASTM protocols. The analysis of freshness of food (berries and cheese) were done by packing in the hydrogel film and monitoring them over a period of time. The results from SEM revealed that the final film structure has characteristic hydrogel pores. The FTIR spectra support the presence of PVP-CMC, BC and GG in the final structure.

PVP-CMC-BC-GG has the best barrier and mechanical properties among all the compositions tested. Further, the shelf life enhancement of berries and cheese by this film also supports its use as a packaging material.

The addition of essential oil to the films has resulted in inhibition zones (antimicrobial properties) against an array of food spoilage microbes. Further, the incorporation of anthocyanin has made the films pH-sensitive and successful in detecting pH changes due to food spoilage. Finally, the newly developed hydrogel film was 80% degraded in compost bed in 30 days and complete degradation in 60 days. Thus it can be concluded from the achievements of the thesis that PVP-CMC-BC-GG hydrogel dried films can be an alternative to the petroleum based food packaging materials.

Key words: *biopolymer, hydrogel, food packaging, active packaging, shelf life*

CONTENT

INTRODUCTION	1
1. MOTIVATION AND AIMS OF DOCTORAL THESIS	2
2. THEORETICAL PART	4
2.1 Present scenario of food packaging (for agro based products).....	4
2.2 Role of hydrogel in food packaging.....	5
2.3 Polysaccharide based hydrogels	7
2.4 Recent progress of polysaccharide based hydrogels.....	7
2.5 Essential oils in bioactive packaging.....	8
2.6 Hydrogel as a bioactive packaging material.....	8
2.7 Growth and progress in this field in past 10 years.....	8
3. EXPERIMENTAL DESIGN	10
3.1 Materials and methods of preparing bioactive food packages.....	10
3.2 Characterization methods of bioactive food packaging material.....	14
4. BRIEF DISCUSSION OF DOCTORAL THESIS RESULTS	18
5. CLOSING REMARKS AND CONTRIBUTION	24
5.1 Conclusion.....	24
5.2 Contribution to the society.....	24
5.3 Future plan	24
ACKNOWLEDGEMENTS	25
LIST OF FIGURES & TABLES	26
LIST OF SYMBOLS & ABBREVIATIONS	27
LIST OF PUBLICATIONS	28
CURRICULUM VITAE	29
REFERENCES	30

INTRODUCTION

The use of petroleum based packaging material for food packaging can be dated back to 1946, until then polyethylene based packaging was not popular ¹. In the later years the use of polyethylene (plastic) based material caused havoc harm to the terrestrial and marine lives ². The different conventional petroleum (plastic) materials for food packaging are polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC) and polyethylene terephthalate (PET)³. These polymeric materials are non-biodegradable thus increasing the global carbon foot print. Moreover, the ecological imbalances and environmental pollution caused by the expansive misuse of plastic has motivated the scientific community to think beyond the box and come up with new biodegradable polymer matrices. Figure 1 clearly shows the research inclination towards the biopolymers based food packaging in the past five years. Biopolymers has several advantages over other biodegradable polymers with their better biocompatibility, abundancy, renewability, pocket friendly, eco-friendly and last but not least biodegradability ^{4,5}.

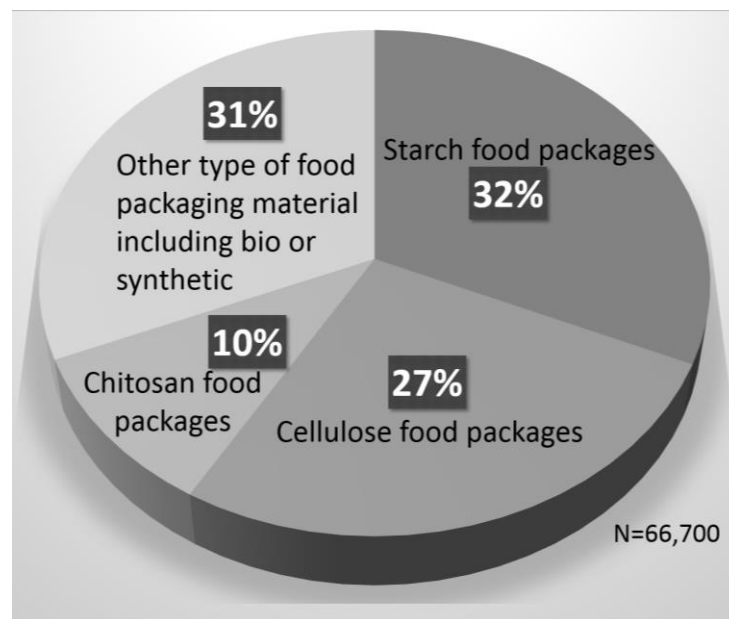


Figure 1: Graphical representation of the trend in food packaging research from Google Scholar database (as of 01.06.2018) for the past five years' research publication. The key words for the searches are mentioned in the pie segments (starch, cellulose, and chitosan), the keyword for the total number (N) of publication was 'Food Packages'.⁶

1. MOTIVATION AND AIMS OF THE DOCTORAL THESIS

The motivation for this work is mentioned below:

- The use of biodegradable alternative material for food packaging is the call of the time. Thus it is important to design and suggest a bio-based alternative to the petroleum based packaging material.
- The lack of thermoplasticity of BC based hydrogels can be achieved by mixing BC with other biopolymers, to use them further for food packaging application.
- Another biopolymer, GG has good water absorption rate but in cold water GG hydrates slowly⁷. This property can be utilized to design hydrogel films which will absorb water slowly from the food in touch, thereby preventing quick dehydration of the products and also maintaining lower A_w in the package.
- The base structure of PVP-CMC has thermoplastic properties but also has a drawback for using as a packaging material is its high oxygen permeability and water vapour permeability. This problem can be addressed by using BC and GG as filler with PVP-CMC to design a new hydrogel formulation, which has better mechanical properties and reduced permeability.
- There is a gap in the integration of active compounds to the hydrogel matrix, to use it as an active packaging material⁸. Therefore, considering all these aspects, the present doctoral study was motivated to design a novel bio-polymer based hydrogel and incorporating active packaging compounds in it. So that the material can be used to pack fresh fruits and vegetables and also enhancing the shelf life of the products.
- There is no report on PVP-CMC-BC-GG based hydrogel films and none of the similar formulation has never been used or evaluated as an active packaging material.

Thus the target to be achieved throughout the doctoral study was to develop a biodegradable hydrogel based packaging material for agro food products. The research was split into several sections to accomplish this purpose.

- 1. Evaluation of BC produced from waste apple juice medium.** This study helped to select the best medium for BC production using waste apple juice. (Publication I)
- 2. Preparation and characterization of bacterial cellulose integrated PVP-CMC hydrogels.** In this study, focus was given to prepare BC based hydrogel and to evaluate their morphological, physical, mechanical, colour and bio adhesion properties. (Publication II)
- 3. Shelf life evaluation of berries packed with GG incorporated in PVP-CMC-BC hydrogels.** The study included the shelf life analysis of fruits like berries and the biodegradability analysis of the hydrogel packaging material. Apart from morphological, mechanical, colorimetric assays, thermal and permeability analysis was also done. (Publication III)
- 4. Development of functional packaging from PVP-CMC-BC-GG based hydrogels.** The BC from the waste apple juice was use to prepare hydrogel films. The antimicrobial analysis of the active packaging materials was evaluated against an array of food spoiling microbes. Moreover, the effect of pH indicators on detecting the food spoilage was evaluated. (Publication IV)

2. THEORETICAL PART

2.1 Present scenario of food packaging (for agro based products)

The present research mainly focuses on the fitting of packaging materials used for agro based products in the circular economy model ⁹. Thus it also a research trend to utilize 100% biodegradable plastics (Table 1) and to remove the petroleum based outputs from the food packaging industry, so that the packaging system can fit in the proposed Figure 2.

Table 1: Overview of the current innovation status in the sustainable food packaging sector ⁹

Period 2010-2015	Active AND packaging	Biopolymers AND bio-based AND bioplastics
Nb of scientific publications *	8,250 (900 in 2015)	11,000 (1400 in 2015)
Nb of patents **	89 (11 in 2015)	754 (26 in 2015)
Nb of exploited patents ***	53 (6 in 2015)	452 (15 in 2015)
Current deployment ratio ****	1%	4%

* From the Web of Science

** Worldwide database

*** exactly referred from the paper of Guillard et.al ⁹

**** Ratio of exploited patents on papers

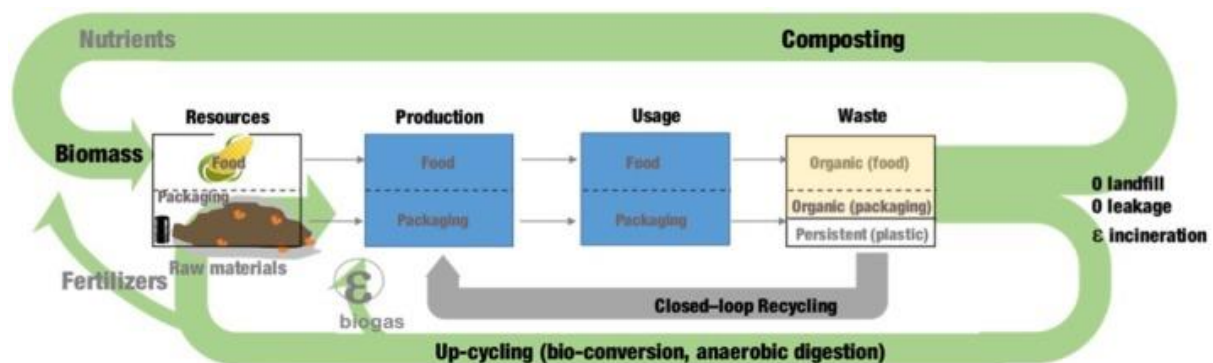


Figure 2: Unlocking the circular economy potential of the food packaging chain, a prospect for the future ⁹

The use of bioplastics has an advantage of reducing the carbon dioxide production as they are biodegradable and compostable, which will eventually reduce the waste amount in the landfills. Moreover, the EU Landfill Directive 99/31/EC in 1999 instructed that the quantity of biodegradable waste going to landfill “was to be reduced to 75% of 1995 levels by 2010 and 35% of 1995 levels by 2020” ¹⁰.

2.2 Role of hydrogel in food packaging

Hydrogels are 3-D structures made up of polymeric chains, crosslinked with physical or chemical bonds¹¹⁻¹³. The 3-D structure is made of two phases, a liquid phase comprising of water or any biological fluid and a solid phase made of the polymeric mixture. The hydrogel can absorb fluid and store in the space between its 3-D networks, without changing its structure^{14,15}. Mechanical resistance, swelling potential and moisture absorption capacity are some of the most important features of hydrogels, which makes them suitable for different industrial uses¹⁶.

Hydrogel finds its application in food packaging due to its ability to control humidity in the package. The hydrogel absorbs the excess water produced due to transpiration or water loss by physicochemical changes from the packed fruits and vegetables, thereby decreasing the water activity (A_w) inside the package. The water vapour permeation from the atmosphere may also be restricted by the hydrogel films. The overall decline in the A_w will keep the packaged fruits and vegetables from decaying by mold, yeast or spoilage bacteria⁸. Hydrogel can also keep hygroscopic products like powder food and crispy foods like chips and biscuits fresh for a longer time¹⁷.

The important characterization techniques for hydrogels as food packaging materials including mechanical, thermal, physical and degradability are mentioned in Table 2.

The cellulose based hydrogels (CBH) have the ability to keep fruits and vegetables fresh for a longer time as the excess moisture in the package is absorbed by the hydrogels³. Biodegradability of the CBH by microorganisms or under controlled environment are promising approach to establish them as environmental friendly packaging material^{18,19}. During degradation of CBH, the mechanical strength, functional properties, wettability, molecular weight and morphology changes with the storage period³.

The PVP-CMC hydrogel can absorb transpirants and release moisture simultaneously when use to pack fresh fruits and vegetables (Figure 3) thereby keeping the foods fresh for a longer time²⁰. The only drawback of PVP-CMC hydrogels for using as a packaging material is its high oxygen permeability and water vapour permeability²¹. This property can be improved with the addition of other bio filler components to the polymer structure.

Table 2: Important analysis of hydrogel for food packaging applications ⁸

Property	Main charecteristics	Analytical methodology
Morphological properties	Determination of appearance and arrangements of the polymer layers	SEM
Chemical & structural charecterization	Identification of main funtional groups, Identification of crystalline nature of the polymer	FTIR XRD
Mechanical properties	Tensile strength, elongation at break and Young's modulus	Instron or mechanical tester
Thermal resistance	Thermal stability of the material at specific conditions	TGA
Physical properties	Wettability, permeability of gases, colorimetric etc	Gravimetric method, O ₂ permeability tester, colorimeter
Biodegradation	Rate of degradation in soil	Soil burrial method following ASTM protocols



Figure 3: The food package prepared with “PVP-CMC” hydrogel film ²⁰

Biodegradation is an important part of designing any bio-based packaging material. The disposal of plastic waste has raised a serious concern worldwide leading to the environmental pollution. The previous studies have already established that BC degrades completely in soil within 180 days ²², followed by degradation of PVP-CMC hydrogels in Czapek-Dox solution within 8 weeks ²³ and in soil within 5 weeks ²¹, Guar gum (GG) based hydrogel has the least complete degradation time of 14 days in soil ²⁴.

2.3 Polysaccharide based hydrogels

Several natural biopolymers are used to prepare hydrogels but among them polysaccharides are most commonly used because of their ionic group which allows easy modifications of their physical and chemical properties by addition of new functional groups from another co-polymer ²⁵. Also the presence of large hydrophilic groups in the polysaccharides, hydrogels made from them shows good water holding and absorption capacity ²⁵. Thus making them suitable for food packaging applications. Among all the polysaccharides, hyaluronic acid (HYAL), carboxymethylcellulose (CMC), guar gum (GG) and chitosan (CHT) are the most popular for hydrogel preparations ^{25,26}. Although CMC and GG has been tried for hydrogel preparation but the end product has low elastic properties ²⁷. Both the components are non-toxic, easily available and low cost materials for hydrogel preparation which can be formulated in a different composition along with other polysaccharide or biocompatible compounds, to enhance their physical and mechanical properties.

2.4 Recent progress of polysaccharide based hydrogels

A significant number of articles covering preparation, characterization and application of polysaccharide based hydrogels have been reported with a majority of them focussing on the biomedical applications ²⁸⁻³⁰. The majority of the articles published with polysaccharide hydrogels for food packaging has used starch, alginates, carrageenan, pectin, xanthan gum, and cellulose derivatives, etc. ³¹. Although CMC coupled with PVA and silver nanoparticles showed antimicrobial properties against urinary tract pathogens ³² and BC has been evaluated for its application in food packaging ³³, but they have not been combined together to study the effect they can have on shelf life of foods. The work done by Y. Tang, Zhang, Zhao, Guo, & Zhang³⁴ has used nano-crystalline cellulose with GG but shelf life extension studies were not performed. CMC has also been used as a smart hydrogel in biomedical application ²⁶. Moreover the use of single polysaccharide for food packaging has drawbacks for their compromised mechanical properties ³⁵. Normal and edible packaging films are also being made from GG ^{36,37} but lower mechanical and poor barrier properties are the limitations to its application as a packaging material ³⁴. Although various efforts have been done to overcome these limitations by chemical modifications of the bio-polymers ³⁸ but they are not cost effective. Thus the aim of this thesis is to overcome the limitations generated from making films with any of these single component. There are very limited number of studies on biopolymer based hydrogels showing

shelf life of foods ⁸. As far as our best knowledge is concerned, no research was recorded on PVP-CMC-BC-GG film with the food packaging study.

2.5 Essential oils in bioactive packaging

EO activity can be found in both antimicrobial and antioxidant studies. In EOs, volatile compounds are responsible for their biological activity, including antimicrobial and antioxidant ability. Several manuscripts and patents have emerged with applications of EOs and their constituents, and their incorporation into food packaging ³⁹. These packages with EOs have shown efficiency against microorganisms and oxidants *in vitro*, in tests with food and/or food simulants evaluation tests ⁴⁰⁻⁴³. In line with this, chromatographic techniques has also been used to identify the main volatile compounds present in EOs or to determine the compounds that migrated from packaging to food or food simulants ⁴⁴.

2.6 Hydrogel as a bioactive packaging material

It is very nicely explained in a recent review on the “Hydrogel as an alternative structure for food packaging systems” that the gap to use hydrogel based food packaging can be addressed by two ways: 1) search for new materials and methodologies to obtain stable and bio-degradable hydrogels and 2) the use of hydrogels as delivery systems for active compounds, such as antimicrobials, antioxidants, flavourings, and colorants ⁸. Many studies have been done on the application of essential oil based hydrogels in medical fields ⁴⁵⁻⁴⁷. Although the use of hydrogel as an active packaging material has been reported by Otoni et.al ⁴⁸ and Peña et.al ⁴⁹ for using as an absorbent pads and to prevent oxidation of butter by molecularly imprinting ferulic acid, respectively but both challenges remain to be explored to a great extent. The mechanism of binding of hydrophobic EO to the hydrogel structure has been shown in Figure 4.

2.7 Growth and progress in this field in past 10 years

Although the use of hydrogel as an active packaging material has not been widely studied by the scientific community, the list of publications in the last decade has been discussed below:

2009-2011

The use of agar based liquid hydrogel with silver nanoparticles was evaluated for its antimicrobial properties against cheese spoilage microbes and the cheese were also assayed for sensory quality, showed the effectiveness of silver nanoparticles in shelf life elongation of cheese ⁵⁰.

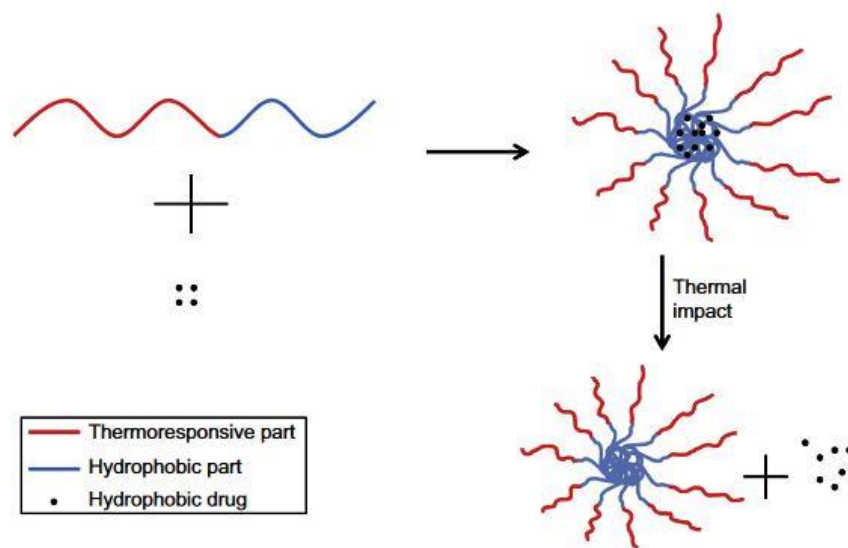


Figure 4: Mechanism of delivery of an oil-based drug in thermoresponsive hydrogel
45

2012-2015

Agar, alginate and collagen based hydrogel is prepared with silver nanoparticles and grapefruit seed extract and their antimicrobial performance is successfully evaluated against food borne pathogenic bacteria and prevention of moisture collection in the head space of potato packaging ⁵¹.

2016-2019

The development of sodium alginate-gelatin hydrogel was done followed by its antibacterial and swelling assays. The results suggested its further use as an active packaging material ⁵².

Carrageenan based hydrogels coupled with ZnO and CuO were evaluated for their physical, mechanical and antimicrobial properties and ZnO incorporated films were suggested for active packaging applications ⁵³.

Agar based hydrogels mineralized with Zn-carbonate and Zn-phosphate to evaluate their antimicrobial, optical and thermal properties. The results recommends its use as an eco-friendly active packaging material ⁵⁴.

Hydrogel made with PVA and poly (ethylene glycol) diglycidyl ether as a cross linker, was used to immobilize enzyme from hen's egg to study the antimicrobial activity of the composite and proposed as an food packaging material ⁵⁵.

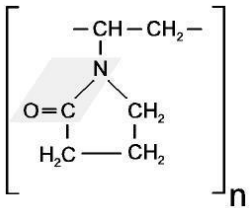
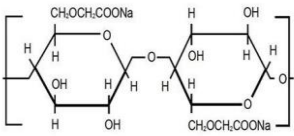
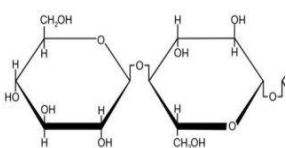
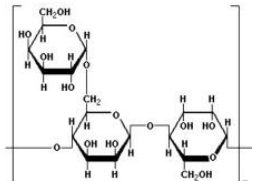
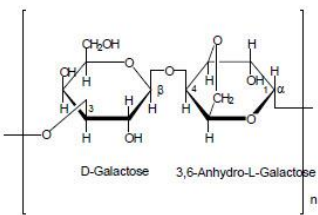
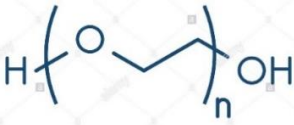
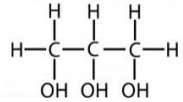
Active packaging hydrogel material developed from PVA and tannic acid with pineapple peel derived cellulose nanocrystals ⁵⁶.

3. EXPERIMENTAL DESIGN

3.1 Materials and methods of preparing bioactive food packages

Table 3 describes the products used for processing of Bio based packaging material.

Table 3: Description of materials used for hydrogel preparation ^{51,57-65}

Name of the compounds	Chemical structure of the compounds	Description about the compounds
Polyvinyl pyrrolidone (PVP)		PVP is a stabilizer as a food additive, and has E code E1201. This is also used as a fining agent for white wine and some breweries within the wine industry. It is a strong adsorbent but is not absorbed by the animal body itself. It is not only safe for human consumption but also used to avoid the production of aflatoxins in foods after harvest.
Carboxy methyl cellulose (CMC)		CMC is used as thickeners and stabiliser in fruit juices, meats and toothpastes. CMC is also applied as a texture reinforce to meat products (cooked sausage). It also adsorbs molecules of mycotoxins present in food.
Bacterial cellulose (BC)		BC is used as the raw material for food (Nata de Coco) and food packaging (edible films) uses. It is also used in the food industry as a fat replacer, meat analogs, stabilisers and rheological modifiers.
Guar gum (GG)		GG is primarily used in the food industry as a thickener and stabiliser spanning from bakery to beef. It is also effective in managing other health problems such as asthma, bowel motions, cardiac disease and colon cancer.
Agar		Agar melts on heating and set on cooling, and this process can be replicated continuously without affecting the gel's mechanical properties. These can be combined with other biopolymers to improve the mechanical properties of the blended hydrogels agar. It has extraordinarily high capacity for holding water, and film forming capability at room temperature under mild conditions.
Polyethylene glycol (PEG)		Many bio-based films use PEG as plasticizer, or as a polymer-forming material. The rate of movement of water vapor can be slightly reduced by using a plasticizer. PEG can be used in a variety of other items, including skin creams and personal lubricants, and as an anti-foaming food additive or in constipation medicines.
Glycerin		As reported, it provides a protective effect for UV in the films, preventing lipid oxidative deterioration of the packaged food.

a. Preparation of apple juice (nutrient medium for BC production):

Using a standard kitchen juicer and blender the whole apple was mashed. The mash was then centrifuged (Sorvall LYNX 4000 by Thermo Scientific, Germany) for 20 minutes at 7.5 K rpm followed by 20 minutes at 9 K rpm and then 45 minutes at 10 K rpm. In each phase the supernatant was extracted and the final buoyant was vacuum filtered using a filter paper (whatman, Grade I, 11µm pore, 25 mm diameter). Apple juice extracted (*Malus domestica*) has been adjusted with various sources of nitrogen and carbon (as shown in Table 4) and used as the nutrient medium for BC growth. The collection of nutrient based apple juice was labeled as A, B, C, and D respectively and consequently the initial pH of the nutrient based apple juice was modified with sodium hydroxide crystals. All four compositions (A, B, C and D) of freshly prepared apple juice were sterilized for 15 minutes in an autoclave under humid heat at 121°C.

Table 4: Composition of apple juice based nutrient medium (AJM and m-AJM) for BC biosynthesis ⁶⁶.

MEDIUM (index) →	Medium 'A' (AJM)	Medium 'B' (m-AJM)	Medium 'C' (m-AJM)	Medium 'D' (m-AJM)
MEDIUM (composition) ↓				
Apple juice * (ml)	100	100	100	100
Ethanol absolute (ml)	-	-	1	1
Ammonium sulphate (g)		0.8	-	0.8
Dipotassium phosphate (g)		0.2		0.2
Sucrose (g)		2.0		2.0
Acetic acid (ml)		0.5		0.5

*Apple juice contained **Fructose:** 7.5 g/100mL, **Glucose:** 3.3 g/100 mL, **Sucrose:** Nil (after sterilization) analysed by HPLC (Waters 1525, UK) using Luna NH2 column and RI detector (Waters 2414) operated at 30°C following the methods described by Chinnici et al. ⁶⁷ and Shui & Leong ⁶⁸.

The final pH of AJM and m-AJM was adjusted to 5.5.

b. Biosynthesis and purification of BC:

Gluconobacter xylinum inoculum (CCM 3611 T) 5mL enriched with Hestrin and Schramm medium ⁶⁹ for three days, was inoculated in each set of bottles (A, B, C and D) filled with AJM (apple juice medium) or m-AJM (modified – apple juice

medium). A volume of 100 mL AJM or m-AJM was used in each 250 mL glass bottle for BC biosynthesis. Each bottle of culture (A, B, C, D) was then sealed for aeration with perforated parafilm and incubated under static condition at 28°C for 15 days.

The BC pellicle obtained from AJM and m-AJM was soaked overnight in distilled water, accompanied by a wash at 80°C for one hour with 0.5N sodium hydroxide solution. The prepared BC is then thoroughly washed with distilled water till neutral pH of the BC pellicle is achieved (7.0).

c. Hydrolysis of BC:

BC hydrolysis was performed by two methods (a) hand blender grinding (b) procedure with ultrasound. To obtain an average particle size of 209 ± 72 nm, the BC mat was grinded in a hand blender (Bosch, MSM67140R, 750 watt) for 12 minutes and 30 seconds. The grinded BC (GBC) was processed overnight in a refrigerator for further use in ultrasonic therapy or immediate use in film preparation. The GBC was consequently subjected to ultrasound (Sonopuls Ultrasonic Homogenizers HD 2070, Bandelin, Germany) at 35 per cent capacity and 20 KHz frequency at a distance of 1 cm from the base for 30 minutes. Using ice bath with salt the temperature was kept at $5^\circ\text{C} \pm 1^\circ\text{C}$ during the ultrasound procedure. The ultrasound treated GBC (US-BC) is also used to render neat BC films and BC related films.

d. BC based hydrogel preparation:

Table 5: The composition of BC based polymeric films ⁶

Polymeric films	Components, %							
	PVP	CMC	Wet BC (99% water)		PEG	Agar	Gly	Water
			US-BC	GBC				
Neat BC	-	-	100	-	-	-	-	-
PVP-BC	0.67	-	66.67	-	1	2	1	28.66
PVP-CMC-BC	0.67	0.34	-	33.34	1	2	1	61.65

All the films are made using casting process. The neat BC films are made by casting 100 gm US-BC in a silicone tray (manufactured by Orion Group CZ) 28.8 cm in length x 12 cm in width x 6.5 cm in height. The US-BC film is allowed to

stand at 50 °C for 2 days, then peeled off with the aid of tweezers from the silicone tray. The hydrogel films based on the BC are prepared by mixing the BC with other additives as described in Table 5 and 6. All materials for the BC-based hydrogel films are well combined for 20 min with a magnetic stirrer at 500 rpm. In an autoclave, the mixture is then subjected to temperature at 121 °C for 30 minutes. Heat serves as a physical interconnection agent between the elements. Following heat treatment, the transparent solution of hydrogel components is casted in a tray made of polyvinyl chloride (PVC) with a length of 25 cm x 25 cm x 0.5 cm height. The cast hydrogels are left for 2 days at RT (23.85 °C) to dry out. Then removed off the PVC tray with a tweezer.

Table 6: Composition of hydrogels⁷⁰

Hydrogel films	PVP (g)	CMC (g)	GG (g)	BC (g)	PEG (g)	Agar (g)	Gly. (mL)	Water (mL)	Moisture content after preparation (%)	Water absorptivity after drying (%)
PVP-CMC	1	1	-	-	1.5	3	1.5	150	93 ± 2	1134 ± 125
PVP-CMC-BC	1	0.5	-	0.5	1.5	3	1.5	150	92 ± 4	826 ± 69
PVP-CMC-GG	1	0.5	0.5	-	1.5	3	1.5	150	93 ± 3	895 ± 143
PVP-CMC-BC-GG	1	0.5	0.35	0.15	1.5	3	1.5	150	94 ± 5	74111

e. Preparation of bioactive hydrogel films

The films are made in polyvinyl chloride trays using casting process. Composition of PVP-CMC-BC-GG is taken according to the previous section ⁷⁰ (Table 11) and treated as a control. The essential oils are applied before heat polymerization to the mixture at a rate of 2 per cent of the total amount. Two oils are applied to various PVP-CMC-BC-GG formulations separately at a rate of 2 per cent. The 2 per cent clove EO PVP-CMC-BC-GG film is referred to as CLEO and the 2 per cent cinnamon EO PVP-CMC-BC-GG film is listed as CiEO in later texts. While a synergistic film of the two oils is prepared by adding 1 per cent of each oils to the composition of PVP-CMC-BC-GG, specified as CLiEO. The films are air-dried at RT (23 ° C) for 24 hours, and then pulled out with tweezers. Both dried

films display hydrogel properties since they had 90 percent \pm 5 water before drying, and after drying they would consume 600 percent \pm 50 water.

f. Production of bio stickers or pH indicator films

The Red Cabbage Extract (RCE) was immobilized by modified absorption method reported earlier on the hydrogel films ⁷¹. The films have been sliced in circles with a diameter of 40 mm, and three circles are immersed in a Petri dish with a diameter of 90 mm and 5 mL RCE. The Petri dish is held at 100 rpm in a 3D shaker by BenchRocker™ (Benchmark, USA). They were transferred to the vacuum oven for 2 hours at 24-27 ° C after 30 minutes.

3.2 Characterization methods of bioactive food packaging material

g. *Bioadhesion studies*

The peel test (ASTM D903) used the tensile test instrument Instron 5567 (Instron, USA) to determine the bioadhesion of the films. All the films were held at room temperature (24 ° C) and 90 per cent RH for 48 hours. The films were trimmed to reach 80 mm and width 10 mm. The contact length between the films and the rubber probe was 60 mm located at 90 degrees ¹⁴⁵. Only difference from the method by Palacio and Bhushan ⁷² was that we didn't use any external adhesive since the adhesion was good and the films didn't dislocate during the test. The test was conducted with a static load of 5 kg and a test speed of 0.5 mm s⁻¹. All the results are an average of at least three replications. The adhesiveness of the films were determined from the maximum force (F_{max}) required to detach the films from the rubber surface ^{73,74}.

h. *The oxygen permeability (OP)*

It was tested using PERME™ VAC V1 (Labthink Instruments Co., Ltd., Jinan, China) according to the ASTM D1434 specifications. The films had been sliced into a circle of 38.48 cm² test area. During the test, temperature and humidity were maintained respectively at 30 ° C and 50 percent. The partial oxygen pressure was 1 atm. outgassing period was 4 h, while gas replacement time and test interval were 60 s, and 3600 s respectively.

i. *Weight loss analysis of berry packages*

Per cent of slopes (m) of the graphs for weight loss of the packages were calculated using the formula $(\text{Rise/Run}) * 100 = [(y_2 - y_1) / (x_2 - x_1)] * 100$ ⁷⁵. Where y_2

and y_1 are any two weights in the Y co-ordinate, x_2 and x_1 are the corresponding time intervals in the X co-ordinate.

j. *Antimicrobial activity*

The PVP-CMC-BC-GG films' antibacterial and antifungal effects (with and without EOs) were investigated against six specific pathogenic and spoilage microbes, typically present in dairy products. Two Gram negative bacteria (*Bacillus cereus* CCM 7934 and *Staphylococcus aureus* CCM 4516), two Gram positive bacteria (*E. coli* CCM 4517 and *Klebsiella pneumoniae* CCM 4415) and two fungi (*Aspergillus brasiliensis* CCM 8222 and *Candida albicans* CCM 8215) were used for Kirby–Bauer disc diffusion process. All the bacteria and fungi were pre-cultured at 35 ° C for 24 h in TSA, and at 35 ° C for 48 h in SDMA. The films were cut into circular disks with a diameter of 6 mm and put in HMA medium (bacteria) and HMA with 2 percent Glucose (fungi) on pre-inoculated colonies (diluted to 0.5 McFarland turbidity standard). They were then 24 hour incubated at 35 ° C. The zones for inhibition were determined using automated colony counters Scan ® 500 (interscience, FR).

k. *Migration test*

UV-Vis spectroscopy system tests the release of the antimicrobial EOs from the films⁷⁶. Oil-based films are sliced (30 mm x 10 mm) and soaked in 5 mL distilled water held for 24 h in a medium speed BenchRocker™ 3D shaker (Benchmark, USA). Upon immersion of the films the first O.D value (289 nm) of the water was registered at 2 h, 24 h accompanied by 48 h and 72 h, changing the water at each point. It is predicted that with time the O.D values would gradually decline as the film's release the oils in the water, but the goal is to find the film with the best balance of preservation and release rate (steady release).

l. *Proof-of-concept application of active packaging with cheese*

Using FRN-600 Plastic Film Sealer (HotAir ®, CZ) to sachet size 120 mm x 120 mm, the edges of the films are heat sealed for 7 sec and fresh Gouda cheese (average 18.5 gms) slice is stored inside each sachet / pack. Inside each sachet the bio-sticker is positioned maintaining in direct contact with the slice of cheese. Both trial packets (PVP-CMC-BC-GG hydrogel sachet with and without EO) and a standard polyethylene (PE thickness 0.03 mm) cheese packet are held at 4 ° C, as shown in Figure 2 a. The bio-sticker is checked at regular intervals for change of colour. The sign of spoilage on the cheese is also tracked. The weight loss percentage (W %) from each packaging system is calculated by the formula:

$$W\% = [(W_i - W_d)/W_i] \times 100$$

where, W_i is the weight of the package on the first day and W_d is the weight of the package on the day of reading.

m. *Biodegradation studies*

Biodegradability study for non-active films was done by soil burial method in vermicompost for 4 weeks. The vermicompost was produced by *Eisenia andrei* species of earthworm and partially digested kitchen waste used as a feeder. The fresh and active vermicompost was used (as a whole with the live vermis and other undigested food waste) to replicate natural soil. Moreover, vermicompost has better particle size and homogeneous texture than normal compost soil mixture⁷⁷ facilitating faster degradation. The pH of the vermicompost was recorded at 7.59 and 7.25 in deionized water and 0.01M CaCl_2 respectively, following ASTM D4972 standards. The initial moisture content of the compost was 73 % \pm 2. The average particle size of the compost analysed with Zetasizer (Malvern Instruments, UK) was 0.002 mm. The films were cut into rectangular pieces 30 mm x 50 mm and kept vertically in 50 g compost in a 250 ml beaker. The beakers were placed in a humidity chamber with temperature and humidity maintained at 30 °C and 68-70 % RH respectively. The buried films were removed from the compost every 7 days, washed with distilled water, dried to constant weight and weighed.

The soil burial analysis of the active films was carried out in compliance with ASTM G160-12 in order to assess the biodegradability of the hydrogel films based on EO. Rectangular film fragments (50 mm x 50 mm) is cut and held in horizontal location inside a 250 mL beaker with 215 g of soil. The soil had a pH of 6.7 – 7.0 and 20 % moisture. The experiment is conducted in a humidity chamber with humidity and temperature maintained at 85-90 % surrounding humidity and 30 °C respectively. The films were collected from the soil every fifteen days and cleaned with distilled water, drying to constant weight before measuring.

The degradation percentage of the films in the soil over 60 days was calculated using the formula⁷⁸:

$$D \% = (\Delta W/W) \times 100$$

where, 'D' is the degradation percentage, ΔW is the change in weight of the films at different buried time and W is the initial weight of the films'⁷⁰.

n. *Experimental design and statistical evaluation*

Single factor split plot-CRD (completely randomized design) was made on all microorganisms for each film, with five replicates on each microbe for each film. Using Windows software package SPSS Ver. 21.0, the data obtained were analysed by one-way ANOVA. Post-hoc pairwise analysis of mean was performed with General linear model check accompanied by Tukey HSD approach with covariate modification. The Shapiro-Wilk method was originally adopted to establish a normal distribution for each metric, and then logarithmic translation was carried out on the skewed variables. Mean and standard error (of mean) for the individual microorganism are measured for each film. For explanatory purposes the mean values are shown in untransformed ways. A two-tailed $P < 0.05$ value is considered statistically significant.

4. BRIEF DISCUSSION OF DOCTORAL THESIS RESULTS

a) Evaluation of Bacterial Cellulose produced from apple juice medium

As reported in **publication I**, we have selected 'AJM,' fruit juice from waste apples (collected from garden) as a medium of cellulose growth. Our investigations were primarily designed / focused on seeing the impact of AJM (apple juice medium) or m-AJM (modified – apple juice medium) on BC growth (Table 8). This segment reports on the BC generated in the presence of AJM and m-AJM (yield, physical appearance, composition, viscoelasticity, thermal stability, and water binding capacities).

The results of this investigation assures that the composition of nutrient media had great influence on structure and properties of BC. It was possible to produce more or less equal quantity of BC by modification of AJM with only absolute ethanol (1%) or with additional nutrient supplement but without ethanol. AJM is a cost effective nutrient source for production of BC from sustainable garden waste (apple). This study shows an avenue that according to requirement, it is possible to produce BC by modifying the media composition. The BC achieved from m-AJM B (Table 7) is recommended for its application as a biomaterial for food packaging.

Table 7a: Influence of drying on the dimensions of lyophilized BC sheet ⁶⁶

Medium (Index)	Initial diameter in wet state (mm)	Reduction of diameter after lyophilization (%)	Initial thickness in wet state (mm)	Reduction of thickness after lyophilization (%)	Initial volume* in wet state (mm ³)	Reduction of volume* after lyophilization (%)
A (AJM)	60±03	10	0.9±0.04	35.5	2543.4	47.02
B (m-AJM)	60±2.5	3.6	1.19±0.05	56.3	3362.9	59.50
C (m-AJM)	60±2.7	2.3	0.6±0.03	33.3	1695.6	30.04
D (m-AJM)	60±3.2	2.8	0.4±0.02	negligible	1130.4	05.58

*: Formula used to calculate the volume of the BC disk: $\pi r^2 h$ (where, 'r' is the radius and 'h' is the thickness of the BC)

Table 7b: Summary of the yield of BC before & after purification ⁶⁶

Medium (Index)	Biomass of BC (g/100 ml)		Biomass of bacteria (g/100mL)
	Before (NaOH treatment)	After (NaOH treatment)	
A (AJM)	6.28 ± 1.14	5.1±2.24	1.01 ± 0.56
B (m-AJM)	6.69 ± 1.54	6.2±2.52	2 ± 0.2
C (m-AJM)	8.17 ± 0.42	6.4±0.9	1.65 ± 0.48
D (m-AJM)	7.01 ± 0.48	5.4±0.5	4.1 0.2

b) Preparation and characterization of BC integrated PVP-CMC hydrogels

In **publication II**, we have made a novel composition for food packaging material by combining BC with polyvinylpyrrolidone (PVP) and carboxymethyl cellulose (CMC), two biodegradable and biocompatible ⁷⁹ food packaging materials which are already reported by Roy et al. ²³. In this segment, we have prepared and compared the characteristics of films prepared from BC, PVP, and CMC, in different combinations (Table 5).

Later, the analysis of the physicomechanical properties of the BC, PVP, and CMC films revealed the practical feasibility of the films to be used as a packaging material. The higher elongation at break and tensile strength for the PVP-CMC-BC film has proved its ability to resist the direct pull. Materials with better tensile strength can withstand more stress before breaking. Thus a higher tensile strength material is preferred for a wide variety of packaging applications. So PVP-CMC-BC film can be utilized as a wrapping or packaging tape which will ensure a better seal and reduce product damage while transport apart from increasing load stabilization. The optical property of the packaging material is an important factor in deciding the amount of light passing through the packaging material and reaching the food items. The consequence of exposing food to light results in photodegrading of the food products. The product is mainly affected due to the oxidation of fats and degradation of the protein, pigments, and vitamins by light causing in discoloration, nutrient loss and having an effect on customer's choice of the produce. Now the yellowish appearance of the PVP-CMC-BC film can resist in the transmission of light in comparison to the other two films. Therefore,

the limited entry of light through PVP-CMC-BC can reduce the light sensitivity of the food products and extend their shelf life. Moreover, all the films have shown very fewer bio adhesive properties. Also, PVP-CMC-BC can be an effective packaging material for both vegetables and meat products. Thus it can be concluded that PVP-CMC-BC films can be promoted as an alternative packaging material for its better mechanical, optical and biodegradable properties.

The next research is designed to test the shelf life promoting abilities and biodegradability of the PVP-CMC-BC film and modified PVP-CMC-BC films.

c) Shelf life evaluation of berries packed with GG incorporated in PVP-CMC-BC hydrogels

The goal of **publication III** was to prepare and evaluate PVP-CMC-BC hydrogel films (reported in earlier section) incorporated with GG for food packaging applications (Figure 5). Moreover, structural (XRD and FTIR), morphological (SEM), mechanical, dynamic modulus, fruit preservation, physical properties (WVP, OTR and solubility), adhesives, biodegradation, colour, gloss and hydrophobicity parameters were analysed for the films.

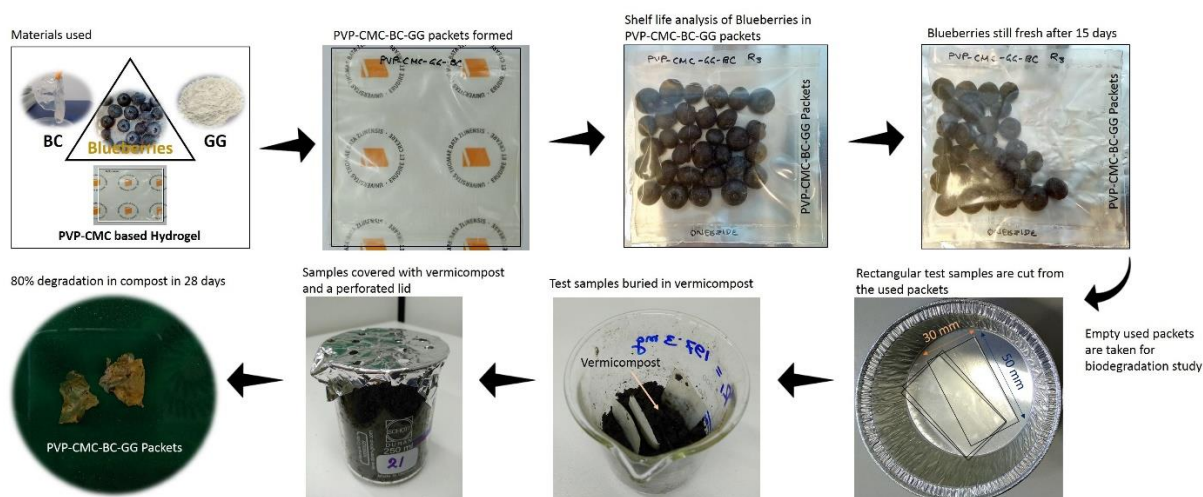


Figure 5: Graphical representation of the preparation, shelf life evaluation on berries and biodegradability analysis of the PVP-CMC-BC-GG films

Novel series of PVP-CMC based hydrogels are prepared from the natural biodegradable polysaccharides, BC and GG components. It was concluded from the results that the incorporation of GG to PVP-CMC-BC has increased its elasticity, tensile strength and elongation percentage. Thus, PVP-CMC-BC-GG

films can withstand more strength before breaking, making it suitable for food packaging with better load stabilization efficacy. Moreover, PVP-CMC-BC-GG based packaging films have the best resistivity to the permeability of oxygen and water vapour. The restricted entry of oxygen and reduced water vapour loss through the films will help to keep the packed fruits fresh for a longer time. The berries packed in PVP-CMC-BC-GG had the least weight loss with no moisture (A_w) in the headspace or free space in the packaging system. Since less water is absorbed by the PVP-CMC-BC-GG films, less water is lost from the system, but at the same time, the free space or headspace in the package is kept moisture-free thereby enhancing the shelf life of the berries. All the hydrogel packages had a reduction in the A_w which also helped the extension of shelf life of the berries. The berries in the perforated PET, with lowest A_w , was completely dried due to heavy loss of water vapour or exudates through the packaging material thereby losing its acceptability. The least hydrophilicity and water solubility of PVP-CMC-BC-GG films also suggest its use as a packaging material. It is also important to note that all the films have undergone structural degradation and 80% weight loss in 28 days during biodegradation study.

Therefore, the GG based modified PVP-CMC-BC hydrogel films can be one of the alternatives to conventional packaging material for berries. Next part involves the incorporation of active components to the films for the development of active packaging material.

d) Development of functional packaging from PVP-CMC-BC-GG based hydrogels

The justification for using PVP-CMC-BC-GG based hydrogel films as an alternative food packaging material for berries has been established in our previous segment. This study (**publication IV**) describes about preservation of 'cheese' using the same composition of hydrogel (i.e. PVP-CMC-BC-GG) but with and without essential oils (EO), as a food preserving agent and anthocyanin based pH sticker (as an indicator of food spoilage) (Figure 6).

The safest food preserving agents comes from the natural sources of plants. The EO/s extracted from different plant parts, apart from extending the shelf life of the food also, enhance the organoleptic acceptability of the packaged food among customers⁸⁰. Moreover, EOs are being classified safe under GRAS (*Generally Recognized as Safe*) and QPS (*Qualified Presumption of Safety*) in the EU and USA respectively^{80,81}. Among all the EOs, clove and cinnamon extract oils with

eugenol and cinnamaldehyde as the two main active compounds ⁸², are used from time immemorial for their antimicrobial properties. The shelf life quality of the cheese packed with hydrogels can be monitored with the change in pH inside the packaging system that is directly linked with the food quality change ⁸³. The anthocyanin from red cabbage (RC) was used for monitoring the pH for its significant colour range for a wide range of pH ⁸⁴.

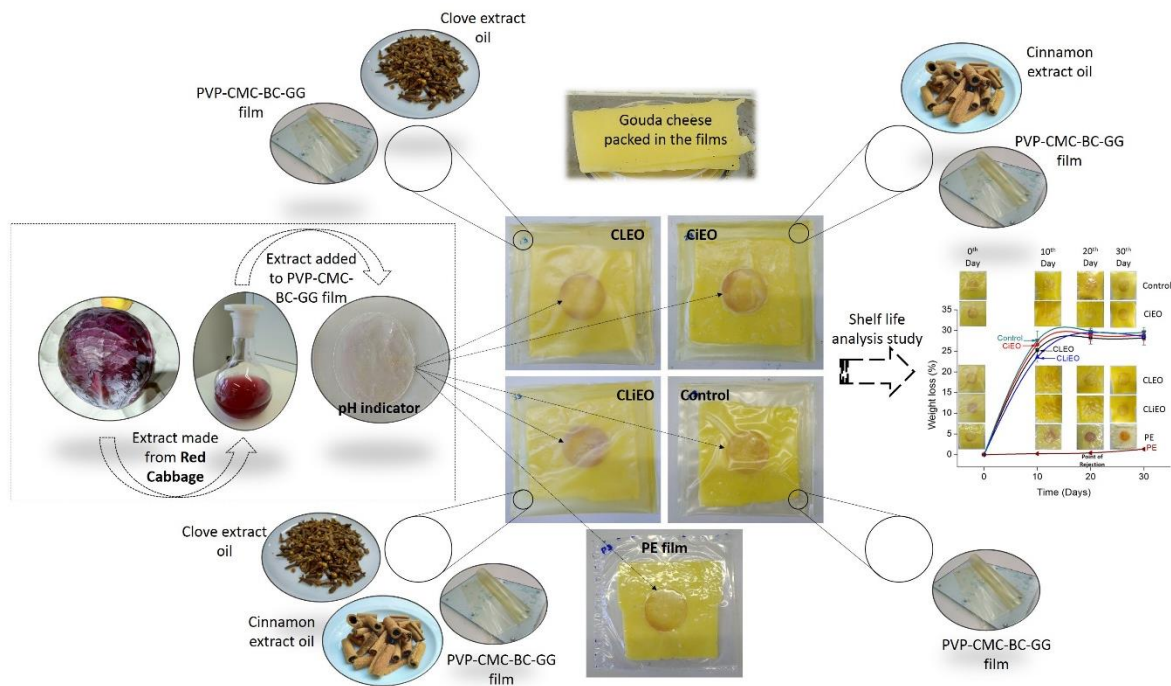


Figure 6: Graphical representation of the preparation and shelf life evaluation on cheese of the PVP-CMC-BC-GG based functional films

In addition, the increase in the world market for cheese has made it a good platform for the application of the innovative packages ⁸⁵ discussed above. ‘Cheese’ is selected in this study because of its popularity in daily life and its nutritional value comprising of fat, vitamins and inorganic salts.

It was evident from the results, that the addition of EOs to the PVP-CMC-BC-GG hydrogel films has enhanced its in-vitro antimicrobial properties, hydrophobicity and mechanical properties. The slow and steady migration rate of EOs from the hydrogel film also suggest its effectivity in microbial control for a wider span of time. The oxygen barrier properties of the control film (i.e. PVP-CMC-BC-GG) declined with addition of individual EOs but only enhanced when both the oils are added together in lower amounts which needs to be evaluated further under different conditions. The thermal properties remained unaffected with the addition of EOs to the PVP-CMC-BC-GG hydrogel film. CLiEO (PVP-CMC-BC-GG +

clove EO + cinnamon EO) stands best among the individual EO based PVP-CMC-BC-GG films when compared on antimicrobial properties and colour values. The freshness assessment of cheese is successfully confirmed by the anthocyanin based pH stickers placed inside the packages/ sachets (as shown in Figure 17). While the cheese in PE showed spoilage and quality deterioration after 18-20 days, the cheese packed in hydrogel films (with and without EO) remained fresh for 30 days under refrigerated condition (4 °C). Moreover, all the PVP-CMC-BC-GG films are $95\% \pm 5$ biodegraded within 60 days under moist soil conditions.

Thus the present work recommends the consideration of PVP-CMC-BC-GG hydrogel film (with EO) as an active and functional packaging material for cheese preservation and storage, which has successfully enhanced the shelf life of cheese than conventional packaging.

5. CLOSING REMARKS AND CONTRIBUTION

5.1 Conclusion

The work from the doctoral studies can be concluded with the development of PVP-CMC-BC-GG based hydrogel dried films with functional properties. The BC utilized in the preparation of PVP-CMC-BC-GG hydrogels was produced from waste apple juice. The complete biodegradation of the films was assayed in compost and soil. The shelf life enhancement of berries and cheese packed in PVP-CMC-BC-GG films, supports its application as a packaging material.

5.2 Contribution to the society

Through the entire work accomplished, a biodegradable and bioactive food packaging material is developed, which can also communicate with the consumers regarding the quality of the content. This hydrogel dried film can be an alternative to the single use plastic in some of the food packaging segments. Moreover, it can be an answer to the problems faced by mankind due to environmental plastic pollution.

5.3 Future plan

Apart from the goals of the doctoral study, which is already achieved, the next immediate focus will be on the following developments:

- a. Sensory evaluation of the foods packed in the functional PVP-CMC-BC-GG hydrogel films.
- b. Evaluation of pest repellent activities by the films, for a specific group of food items.
- c. Comparison of the physical and mechanical properties of the PVP-CMC-BC-GG films with the commercially available biodegradable packaging materials.
- d. Development of the composition according to the industry need for large scale production as the final achievement of **our research is to fit the 'lab to land' model.**

ACKNOWLEDGEMENTS

Firstly, I might prefer to express my sincere gratitude to my advisor **Prof. Petr Saha** and my consultant **Assoc. Prof. Nabanita Saha**, for the continual support of my Ph.D. study and related research, for his or her patience, motivation, and immense knowledge. Their guidance helped me during the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my Ph.D. study.

I would also share my respect to the **Monks, Teachers** and **the entire Environment** of my previous university (**Ramakrishna Mission Vivekananda University**) for building my character towards hard work and patience.

Besides my advisors, I would like to thank the others in my **thesis committee** for the insightful comments and encouragement, but also for the hard questions which incited me to widen my research from various perspectives.

My sincere thanks also goes to **Assistant Prof. Dr. Urška Vrabič Brodnjak**, who provided me a chance to work with her team on short term scientific mission, and who gave access to the laboratory and research facilities. I also bestow my gratitude to **Prof. Anežka Lengálová**, who taught me the science of expressing my thoughts into words. I shall always remain as a student to them.

I thank my fellow lab mates specially **Hau Trung Nguyen**, for the stimulating discussions, for the tiresome days we were working together before deadlines. Also I thank my friend **Dr. Lucina Yeasmin** in Institute of Life Sciences NALCO Square, Bhubaneswar, for her selfless assistance on the statistical analysis. Specifically, I am grateful to **Dr. Oyunchimeg Zandraa** for enlightening me the first glance of research.

I would also like to thank my family for supporting me spiritually throughout: my grandparents Late Smt. **Bandana** & Sri **Ashok Kumar Chakraborty**, Late Smt. **Jharna** & Sri **Kalipada Bandyopadhyay**, my parents Mrs. **Manashi** & Mr. **Mriganka Sekhar Bandyopadhyay**, and to the gentle hands of my wife Mrs **Payal Mitra Bandyopadhyay**. Without their blessings, sacrifices and tireless efforts, I could not have made this journey possible. I also humbly bestow this work to the confidence and trust my father-in-law Mr. **Subhas Mitra** and my mother-in-law Mrs. **Malati Mitra** had on me.

Last but not least, two persons, **Madhab** and **Chandra**, my brothers, deserve a very special word of love and gratitude for their inspiration and making me feel special whenever I went through my hard times.

LIST OF FIGURES & TABLES

Figure 1: Graphical representation of the trend in food packaging research from Google Scholar database (as of 01.06.2018) for the past five years research publication. The key words for the searches are mentioned in the pie segments (starch, cellulose, and chitosan), the keyword for the total number (N) of publication was 'Food Packages'.

Figure 2: Unlocking the circular economy potential of the food packaging chain, a prospect for the future

Figure 3: The food package prepared with "PVP-CMC" hydrogel film

Figure 4: Mechanism of delivery of an oil-based drug in thermoresponsive hydrogel

Figure 5: Graphical representation of the preparation, shelf life evaluation on berries and biodegradability analysis of the PVP-CMC-BC-GG films

Figure 6: Graphical representation of the preparation and shelf life evaluation on cheese of the PVP-CMC-BC-GG based functional films

Table 1: Overview of the current innovation status in the sustainable food packaging

Table 2: Important analysis of hydrogel for food packaging applications

Table 3: Description of materials used for hydrogel preparation

Table 4: Composition of apple juice based nutrient medium (AJM and m-AJM) for BC biosynthesis

Table 5: The composition of BC based polymeric films

Table 6: Composition of hydrogels

Table 7a: Influence of drying on the dimensions of lyophilized BC sheet

Table 7b: Summary of the yield of BC before & after purification

LIST OF SYMBOLS & ABBREVIATIONS

3-D	3 dimensional
ASTM	American standards testing methods
AJM	Apple juice medium
BC	Bacterial cellulose
CBH	Cellulose based hydrogels
CLiEO	Clove and cinnamon essential oil based PVP- CMC-BC-GG hydrogel
CMC	Carboxymethyl cellulose
CNC	Cellulose nano crystals
CZ	Czech republic
EO	Essential oils
FTIR	Fourier transform-infrared spectroscopy
GBC	Grinded BC
GG	Guar gum
HMA	Hinton mueller agar
OP	Oxygen permeability
PE	Polyethylene
PEG	Polyethylene glycol
PET	Polyethylene terephthalate
PP	Polypropylene
PVC	Polyvinyl chloride
PVP	Polyvinyl pyrrolidone
RH	Relative humidity
SDMA	Sabouraud dextrose maltose agar
SEM	Scanning electron microscope
TGA	Thermogravimetric analysis
TSA	Tryptone soy agar
US-BC	Ultrasound treated grinded BC
XRD	X-ray diffraction

LIST OF PUBLICATIONS

Publication I:

Characterization of bacterial cellulose produced using media containing waste apple juice. **Smarak Bandyopadhyay**, Nabanita Saha, Petr Saha. *Applied Biochemistry and Microbiology* 54(6):649–657 (2018)

<https://doi.org/10.1134/S0003683818060042>

Publication II:

Bacterial cellulose based greener packaging material: A bioadhesive polymeric film.

Smarak Bandyopadhyay, Nabanita Saha, Urška Vrabič Brodnjak, Petr Saha. *Mater. Res. Express* 5 115405 (2018) <https://doi.org/10.1088/2053-1591/aadb01>

Publication III:

Bacterial cellulose and guar gum based modified PVP-CMC hydrogel films: characterized for packaging fresh berries. **Smarak Bandyopadhyay**, Nabanita Saha, Urška Vrabič Brodnjak, Petr Saha; *Food Packaging and Shelf Life* 22 100402 (2019)

<https://doi.org/10.1016/j.fpsl.2019.100402>

Publication IV:

Development of essential oil based PVP-CMC-BC-GG functional hydrogel packaging material for cheese: confirmed with anthocyanin bio stickers. **Smarak Bandyopadhyay**, Nabanita Saha, Oyunchimeg Zandraa, Martina Pummerová and Petr Saha; *Foods* 22 9(3):307 (2020) <https://doi.org/10.3390/foods9030307>

CURRICULUM VITAE

Name:	Smarak Bandyopadhyay
Date of birth:	13 th November 1987
Place of birth:	Kolkata, India
Permanent address:	N353 B.P Township, Flat G13, Kolkata 700094
Affiliation:	Centre of Polymer system, Faculty of Technology, Tomas Bata University in Zlin, Nám. T. G. Masaryka 5678, 760 01 Zlín
Telephone:	(+420) 776643456
E-mail:	bandyopadhyay@utb.cz
Education:	2016 – to date Tomas Bata University in Zlin, Faculty of Technology, Ph.D. studies in Chemistry and Materials Technology, Specialization: Technology of Macromolecular Compounds 2010 – 2012 Ramakrishna Mission Vivekananda University (India), Master`s degree in Agricultural Biotechnology 2007 – 2010 Bangalore University (India), Bachelor`s degree in Biotechnology

References

1. Gilbert, M. Chapter 1 - Plastics Materials: Introduction and Historical Development. in *Brydson's Plastics Materials (Eighth Edition)* (ed. Gilbert, M.) 1–18 (Butterworth-Heinemann, 2017). doi:10.1016/B978-0-323-35824-8.00001-3.
2. Plastic in the ocean: the facts, effects and new EU rules | News | European Parliament. <http://www.europarl.europa.eu/news/en/headlines/society/20181005STO15110/plastic-in-the-ocean-the-facts-effects-and-new-eu-rules> (2018).
3. Ghosh, T. & Katiyar, V. Cellulose-Based Hydrogel Films for Food Packaging. in *Cellulose-Based Superabsorbent Hydrogels* (ed. Mondal, Md. I. H.) 1–25 (Springer International Publishing, 2018). doi:10.1007/978-3-319-76573-0_35-1.
4. Luckachan, G. E. & Pillai, C. K. S. Biodegradable Polymers- A Review on Recent Trends and Emerging Perspectives. *J Polym Environ* **19**, 637–676 (2011).
5. Tang, X. Z., Kumar, P., Alavi, S. & Sandeep, K. P. Recent advances in biopolymers and biopolymer-based nanocomposites for food packaging materials. *Crit Rev Food Sci Nutr* **52**, 426–442 (2012).
6. Bandyopadhyay, S., Saha, N., Brodnjak, U. V. & Saha, P. Bacterial cellulose based greener packaging material: a bioadhesive polymeric film. *Mater. Res. Express* **5**, 115405 (2018).
7. Thakur, S. *et al.* Recent approaches in guar gum hydrogel synthesis for water purification. *International Journal of Polymer Analysis and Characterization* **23**, 621–632 (2018).
8. Batista, R. A. *et al.* Hydrogel as an alternative structure for food packaging systems. *Carbohydrate Polymers* **205**, 106–116 (2019).
9. Guillard, V. *et al.* The Next Generation of Sustainable Food Packaging to Preserve Our Environment in a Circular Economy Context. *Front Nutr* **5**, (2018).
10. Byun, Y. & Kim, Y. T. Chapter 15 - Utilization of Bioplastics for Food Packaging Industry. in *Innovations in Food Packaging (Second Edition)* (ed. Han, J. H.) 369–390 (Academic Press, 2014). doi:10.1016/B978-0-12-394601-0.00015-1.
11. Chang, C., Duan, B., Cai, J. & Zhang, L. Superabsorbent hydrogels based on cellulose for smart swelling and controllable delivery. *European Polymer Journal* **46**, 92–100 (2010).
12. Ahmed, E. M. Hydrogel: Preparation, characterization, and applications: A review. *Journal of Advanced Research* **6**, 105–121 (2015).
13. Aminabhavi, T. M. & Deshmukh, A. S. Polysaccharide-Based Hydrogels as Biomaterials. in *Polymeric Hydrogels as Smart Biomaterials* (ed. Kalia, S.) 45–71 (Springer International Publishing, 2016). doi:10.1007/978-3-319-25322-0_3.
14. Bao, Y., Ma, J. & Li, N. Synthesis and swelling behaviors of sodium carboxymethyl cellulose-g-poly(AA-co-AM-co-AMPS)/MMT superabsorbent hydrogel. *Carbohydrate Polymers* **84**, 76–82 (2011).
15. Croisier, F. & Jérôme, C. Chitosan-based biomaterials for tissue engineering. *European Polymer Journal* **49**, 780–792 (2013).
16. Buwalda, S. J. *et al.* Hydrogels in a historical perspective: From simple networks to smart materials. *Journal of Controlled Release* **190**, 254–273 (2014).
17. de Azeredo, H. M. C. Antimicrobial nanostructures in food packaging. *Trends in Food Science & Technology* **30**, 56–69 (2013).

18. Alves, V. *et al.* Design of biodegradable composite films for food packaging. *Desalination* **199**, 331–333 (2006).
19. Iwata, T. Biodegradable and Bio-Based Polymers: Future Prospects of Eco-Friendly Plastics. *Angewandte Chemie International Edition* **54**, 3210–3215 (2015).
20. Saha, N., Das, M., Shinde, D. S., Minařík, A. & Saha, P. Moisture Sorption Isotherm and Isothermic Heat of Sorption Characteristics of PVP-CMC Hydrogel Film: A Useful Food Packaging Material. in *Cellulose-Based Superabsorbent Hydrogels* (ed. Mondal, Md. I. H.) 1–17 (Springer International Publishing, 2018). doi:10.1007/978-3-319-76573-0_36-1.
21. Roy, N., Saha, N. & Saha, P. Biodegradable Hydrogel Film for Food Packaging. in *Recent Researches in Geography, Geology, Energy, Environment and Biomedicine* 353 (WSEAS Press, 2011).
22. Schröpfer, S. B. *et al.* Biodegradation evaluation of bacterial cellulose, vegetable cellulose and poly (3-hydroxybutyrate) in soil. *Polímeros* **25**, 154–160 (2015).
23. Roy, N., Saha, N., Kitano, T. & Saha, P. Biodegradation of PVP–CMC hydrogel film: A useful food packaging material. *Carbohydrate Polymers* **89**, 346–353 (2012).
24. Kaith, B. S., Sharma, R. & Kalia, S. Guar gum based biodegradable, antibacterial and electrically conductive hydrogels. *Int. J. Biol. Macromol.* **75**, 266–275 (2015).
25. Camponeschi, F. *et al.* New Formulations of Polysaccharide-Based Hydrogels for Drug Release and Tissue Engineering. *Gels* **1**, 3–23 (2015).
26. *Polysaccharide Hydrogels: Characterization and Biomedical Applications*. (Jenny Stanford Publishing, 2016). doi:10.1201/b19751.
27. Barbucci, R., Giardino, R., Cagna, M. D., Golini, L. & Pasqui, D. Inter-penetrating hydrogels (IPHs) as a new class of injectable polysaccharide hydrogels with thixotropic nature and interesting mechanical and biological properties. *Soft Matter* **6**, 3524–3532 (2010).
28. Construction of ordered structure in polysaccharide hydrogel: A review. *Carbohydrate Polymers* **205**, 225–235 (2019).
29. Kabir, S. M. F. *et al.* Cellulose-based hydrogel materials: chemistry, properties and their prospective applications. *Prog Biomater* **7**, 153–174 (2018).
30. Zhu, T. *et al.* Recent Progress of Polysaccharide-Based Hydrogel Interfaces for Wound Healing and Tissue Engineering. *Advanced Materials Interfaces* **6**, 1900761 (2019).
31. Ali, A. & Ahmed, S. Recent Advances in Edible Polymer Based Hydrogels as a Sustainable Alternative to Conventional Polymers. *J. Agric. Food Chem.* **66**, 6940–6967 (2018).
32. Alshehri, S. M. *et al.* Development of carboxymethyl cellulose-based hydrogel and nanosilver composite as antimicrobial agents for UTI pathogens. *Carbohydrate Polymers* **138**, 229–236 (2016).
33. Shi, Z., Zhang, Y., Phillips, G. O. & Yang, G. Utilization of bacterial cellulose in food. *Food Hydrocolloids* **35**, 539–545 (2014).
34. Tang, Y., Zhang, X., Zhao, R., Guo, D. & Zhang, J. Preparation and properties of chitosan/guar gum/nanocrystalline cellulose nanocomposite films. *Carbohydrate Polymers* **197**, 128–136 (2018).

35. Rudin, A. & Choi, P. Chapter 13 - Biopolymers. in *The Elements of Polymer Science & Engineering (Third Edition)* 521–535 (Academic Press, 2013). doi:10.1016/B978-0-12-382178-2.00013-4.
36. Rao, M. S., Kanatt, S. R., Chawla, S. P. & Sharma, A. Chitosan and guar gum composite films: Preparation, physical, mechanical and antimicrobial properties. *Carbohydrate Polymers* **82**, 1243–1247 (2010).
37. Saberi, B. *et al.* Physical, Barrier, and Antioxidant Properties of Pea Starch-Guar Gum Biocomposite Edible Films by Incorporation of Natural Plant Extracts. *Food Bioprocess Technol* **10**, 2240–2250 (2017).
38. Biswas, A., Pal, S. & Udayabhanu, G. Effect of chemical modification of a natural polysaccharide on its inhibitory action on mild steel in 15% HCl solution. *Journal of Adhesion Science and Technology* **31**, 2468–2489 (2017).
39. Ribeiro-Santos, R., Andrade, M., Melo, N. R. de & Sanches-Silva, A. Use of essential oils in active food packaging: Recent advances and future trends. *Trends in Food Science & Technology* **61**, 132–140 (2017).
40. Fernández-Pan, I., Carrión-Granda, X. & Maté, J. I. Antimicrobial efficiency of edible coatings on the preservation of chicken breast fillets. *Food Control* **36**, 69–75 (2014).
41. Espitia, P. J. P. *et al.* Assessment of the efficiency of essential oils in the preservation of postharvest papaya in an antimicrobial packaging system. *Brazilian Journal of Food Technology* **15**, 333–342 (2012).
42. Sánchez-González, L., Cháfer, M., Chiralt, A. & González-Martínez, C. Physical properties of edible chitosan films containing bergamot essential oil and their inhibitory action on *Penicillium italicum*. *Carbohydrate Polymers* **82**, 277–283 (2010).
43. Wen, P. *et al.* Encapsulation of cinnamon essential oil in electrospun nanofibrous film for active food packaging. *Food Control* **59**, 366–376 (2016).
44. Fisher, K. & Phillips, C. A. The effect of lemon, orange and bergamot essential oils and their components on the survival of *Campylobacter jejuni*, *Escherichia coli* O157, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus* in vitro and in food systems. *Journal of Applied Microbiology* **101**, 1232–1240 (2006).
45. Syed, I., Garg, S. & Sarkar, P. 5 - Entrapment of essential oils in hydrogels for biomedical applications. in *Polymeric Gels* (eds. Pal, K. & Banerjee, I.) 125–141 (Woodhead Publishing, 2018). doi:10.1016/B978-0-08-102179-8.00005-3.
46. Low, W. L., Kenward, M. A. (Ken), Amin, M. C. I. M. & Martin, C. Ionically Crosslinked Chitosan Hydrogels for the Controlled Release of Antimicrobial Essential Oils and Metal Ions for Wound Management Applications. *Medicines (Basel)* **3**, (2016).
47. Kavooosi, G., Bordbar, Z., Dadfar, S. M. & Dadfar, S. M. M. Preparation and characterization of a novel gelatin–poly(vinyl alcohol) hydrogel film loaded with *Zataria multiflora* essential oil for antibacterial–antioxidant wound-dressing applications. *Journal of Applied Polymer Science* **134**, 45351 (2017).
48. Trends in antimicrobial food packaging systems: Emitting sachets and absorbent pads. *Food Research International* **83**, 60–73 (2016).
49. Benito-Peña, E. *et al.* Molecularly imprinted hydrogels as functional active packaging materials. *Food Chem* **190**, 487–494 (2016).

50. Incoronato, A. L., Conte, A., Buonocore, G. G. & Del Nobile, M. A. Agar hydrogel with silver nanoparticles to prolong the shelf life of Fior di Latte cheese. *Journal of Dairy Science* **94**, 1697–1704 (2011).
51. Wang, L.-F. & Rhim, J.-W. Preparation and application of agar/alginate/collagen ternary blend functional food packaging films. *International Journal of Biological Macromolecules* **80**, 460–468 (2015).
52. Acharya, C. *et al.* Physicochemical and antimicrobial properties of sodium alginate/gelatin-based silver nanoformulations. *Polym. Bull.* **74**, 689–706 (2017).
53. Oun, A. A. & Rhim, J.-W. Carrageenan-based hydrogels and films: Effect of ZnO and CuO nanoparticles on the physical, mechanical, and antimicrobial properties. *Food Hydrocolloids* **67**, 45–53 (2017).
54. Malagurski, I. *et al.* Mineralized agar-based nanocomposite films: Potential food packaging materials with antimicrobial properties. *Carbohydrate Polymers* **175**, 55–62 (2017).
55. Mirabelli, V. *et al.* Enzyme Crystals and Hydrogel Composite Membranes as New Active Food Packaging Material. *Global Challenges* **2**, 1700089 (2018).
56. Dai, H., Huang, Y. & Huang, H. Enhanced performances of polyvinyl alcohol films by introducing tannic acid and pineapple peel-derived cellulose nanocrystals. *Cellulose* **25**, 4623–4637 (2018).
57. 493. Polyvinylpyrrolidone (PVP) (WHO Food Additives Series 15). <http://www.inchem.org/documents/jecfa/jecmono/v15je08.htm>.
58. Ninjiaranai, P. Biopolymer Films Based on Chitosan and Polyethylene Glycol with Pineapple Leaf Fiber for Food Packaging Applications. *Macromolecular Symposia* **354**, 294–298 (2015).
59. Lizárraga-Paulín, E. G., Miranda-Castro, S. P., Moreno-Martínez, E., Torres-Pacheco, I. & Lara-Sagahón, A. V. Novel Methods for Preventing and Controlling Aflatoxins in Food: A Worldwide Daily Challenge. *Aflatoxins - Recent Advances and Future Prospects* (2013) doi:10.5772/50707.
60. Solís-Cruz, B. *et al.* Evaluation of Chitosan and Cellulosic Polymers as Binding Adsorbent Materials to Prevent Aflatoxin B1, Fumonisin B1, Ochratoxin, Trichothecene, Deoxynivalenol, and Zearalenone Mycotoxins Through an In Vitro Gastrointestinal Model for Poultry. *Polymers* **9**, 529 (2017).
61. Azeredo, H. M. C., Barud, H., Farinas, C. S., Vasconcellos, V. M. & Claro, A. M. Bacterial Cellulose as a Raw Material for Food and Food Packaging Applications. *Front. Sustain. Food Syst.* **3**, (2019).
62. CMC Functional Properties in Food Applications. <https://celluloseether.com/cmc-functional-properties-food-applications/>.
63. Mudgil, D., Barak, S. & Khatkar, B. S. Guar gum: processing, properties and food applications—A Review. *J Food Sci Technol* **51**, 409–418 (2014).
64. Cazón, P., Vázquez, M. & Velázquez, G. Cellulose-glycerol-polyvinyl alcohol composite films for food packaging: Evaluation of water adsorption, mechanical properties, light-barrier properties and transparency. *Carbohydrate Polymers* **195**, 432–443 (2018).
65. Guar Gum - Food Grade Guar Gum Powder, Industrial Guar Gum Powder, Cassia Tora Powder. *Agro Gums* <https://www.agrogums.com/applications/>.

66. Bandyopadhyay, S., Saha, N. & Saha, P. Characterization of Bacterial Cellulose Produced using Media Containing Waste Apple Juice. *Appl Biochem Microbiol* **54**, 649–657 (2018).
67. Chinnici, F., Spinabelli, U., Riponi, C. & Amati, A. Optimization of the determination of organic acids and sugars in fruit juices by ion-exclusion liquid chromatography. *Journal of Food Composition and Analysis* **18**, 121–130 (2005).
68. Shui, G. & Leong, L. P. Separation and determination of organic acids and phenolic compounds in fruit juices and drinks by high-performance liquid chromatography. *Journal of Chromatography A* **977**, 89–96 (2002).
69. Hestrin, S. & Schramm, M. Synthesis of cellulose by *Acetobacter xylinum*. 2. Preparation of freeze-dried cells capable of polymerizing glucose to cellulose. *Biochem J* **58**, 345–352 (1954).
70. Bandyopadhyay, S., Saha, N., Brodnjak, U. V. & Saha, P. Bacterial cellulose and guar gum based modified PVP-CMC hydrogel films: Characterized for packaging fresh berries. *Food Packaging and Shelf Life* **22**, 100402 (2019).
71. Kuswandi, B., Jayus, Oktaviana, R., Abdullah, A. & Heng, L. Y. A Novel On-Package Sticker Sensor Based on Methyl Red for Real-Time Monitoring of Broiler Chicken Cut Freshness. *Packaging Technology and Science* **27**, 69–81 (2013).
72. Palacio, M. L. B. & Bhushan, B. Bioadhesion: a review of concepts and applications. *Phil. Trans. R. Soc. A* **370**, 2321–2347 (2012).
73. Mohamad, N. *et al.* Characterization and biocompatibility evaluation of bacterial cellulose-based wound dressing hydrogel: effect of electron beam irradiation doses and concentration of acrylic acid. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **105**, 2553–2564 (2017).
74. Ataide, J. A. *et al.* Bacterial Nanocellulose Loaded with Bromelain: Assessment of Antimicrobial, Antioxidant and Physical-Chemical Properties. *Scientific Reports* **7**, 18031 (2017).
75. Perpiñá, C. *et al.* Methodology based on Geographic Information Systems for biomass logistics and transport optimisation. *Renewable Energy* **34**, 555–565 (2009).
76. Souza, A. C., Goto, G. E. O., Mainardi, J. A., Coelho, A. C. V. & Tadini, C. C. Cassava starch composite films incorporated with cinnamon essential oil: Antimicrobial activity, microstructure, mechanical and barrier properties. *LWT - Food Science and Technology* **54**, 346–352 (2013).
77. Hanc, A. & Dreslova, M. Effect of composting and vermicomposting on properties of particle size fractions. *Bioresource Technology* **217**, 186–189 (2016).
78. Yu, Z., Li, B., Chu, J. & Zhang, P. Silica in situ enhanced PVA/chitosan biodegradable films for food packages. *Carbohydrate Polymers* **184**, 214–220 (2018).
79. Saha, N. *et al.* PVP - CMC hydrogel: An excellent bioinspired and biocompatible scaffold for osseointegration. *Materials Science and Engineering: C* (2018) doi:10.1016/j.msec.2018.04.050.
80. Saeed, F., Afzaal, M., Tufail, T. & Ahmad, A. Use of Natural Antimicrobial Agents: A Safe Preservation Approach. *Active Antimicrobial Food Packaging* (2019) doi:10.5772/intechopen.80869.
81. Atarés, L. & Chiralt, A. Essential oils as additives in biodegradable films and coatings for active food packaging. *Trends in Food Science & Technology* **48**, 51–62 (2016).

82. Hoque, M. M. (University of D. (Bangladesh)), Bari, M. L., Juneja, V. K. & Kawamoto, S. Antimicrobial activity of cloves and cinnamon extracts against food borne pathogens and spoilage bacteria, and inactivation of *Listeria monocytogenes* in ground chicken meat with their essential oils. *Report of National Food Research Institute (Japan)* (2008).
83. Peralta, J., Bitencourt-Cervi, C. M., Maciel, V. B. V., Yoshida, C. M. P. & Carvalho, R. A. Aqueous hibiscus extract as a potential natural pH indicator incorporated in natural polymeric films. *Food Packaging and Shelf Life* **19**, 47–55 (2019).
84. Pourjavaher, S., Almasi, H., Meshkini, S., Pirsá, S. & Parandi, E. Development of a colorimetric pH indicator based on bacterial cellulose nanofibers and red cabbage (*Brassica oleraceae*) extract. *Carbohydrate Polymers* **156**, 193–201 (2017).
85. Costa, M. J., Maciel, L. C., Teixeira, J. A., Vicente, A. A. & Cerqueira, M. A. Use of edible films and coatings in cheese preservation: Opportunities and challenges. *Food Research International* **107**, 84–92 (2018).

Hydrogel-based bioactive food packaging material for agro products

Bioaktivní obalový materiál na bázi hydrogelu pro agro produkty

Doctoral Thesis

Published by: Tomas Bata University in Zlín,
nám. T. G. Masaryka 5555, 760 01 Zlín.

Edition: published electronically

Typesetting by: Smarak Bandyopadhyay, M.Sc. Ph.D.

This publication has not undergone any proofreading or editorial review.

Publication year: 2020

First Edition

ISBN 978-80-7454-930-4

