PREPARATION AND CHARACTERIZATION OF THE CHITOSAN FILM FOR PACKAGING MATERIAL

Sandar Win[1], Khin Than Yee[2], Sandar Tun[3], Kyaw Myo Naing[4]

ABSTRACT

This research work was concerned with the preparation, characterization and application of biodegradable chitosan (C) film to be used as a packaging materials. A series of chtisoan (C) film was prepared using different amounts of chitosan by solvent evaporating method. In this preparation 1%, 2%, 3% and 4% of chitosan solutions kept in an autoclave under 0.1 MPa pressure at 121°C for 20 min were used. The homogeneous chitosan solutions after autoclaving were casted on melamine plate and left for a few days. The chitosan films obtained were characterized according to physicomechanical and physicochemical properties. The prepared C films were characterized by mechanical properties such as tensile strength, elongation at break and tear strength. According to the mechanical properties, 2 % w/v C was the most suitable for preparing C-2 film. Prepared C-2 film was characterized by modern techniques such as SEM, FT IR and TG-DTA. Antimicrobial activities of C-2 film was investigated by agar-disc diffusion method. Biodegradability of prepared C-2 film was studied by soil burial method. The quality controlling factors of mango fruits (water content, titratable acidity, reducing sugar content, crude fiber, total soluble solid, effect of pH, refractive index and weight loss) were investigated by using prepared film (C-2) as packaging materials. It was found that, for all unpackaged (control) mango fruits and packaged fruits, water contents, sugar contents, and crude fiber contents were slightly increased, but the weight loss percents were sharply increased (inverse of pH). One determining quality controlling factor was the total solubility that decreases for unpackaged (control) mango fruit, i.e, control where as it increases for packaged mango fruit, i.e, mangoes packaged by C-2 film. Moreover, unpackaged (control) mango fruits started to undergo spoilage after 10 days. However, it was found that package of mango fruits showed longer shelf life, that is, with a longer ripening time compared unpackaged (control) fruits.

Keywords*:* chitosan, biodegradable film, mango, packaging material

1. Demonstrator, Department of Chemistry, Maubin University
2. Lecturer, Department of Chemistry, University of Yangon
3. Lecturer (Retd.), Department of Chemistry, University of Yangon
4. Professor (Retd.), Department of Chemistry, Kyaingtong University

Introduction

Chitosan

Chitosan is white to ability light yellow color, insoluble in water but it is readily soluble in dilute aqueous organic acid such as acetic acid, propionic acid, formic acid and lactic acid (Dutta *et al*., 2004). These properties include: biodegradability, lack of toxicity, antifungal effects, wound healing acceleration, and immune system stimulation. (Fangkanwanwong *et al*., 2006). Most important feature of chitosan of biodegradable ability flexibility and high resistance to heat due to intra-molecular hydrogen bonds formed hydroxyl and amino group. Chitosan offers a wide range of unique applications in the food industry including preservation of foods from microbial deterioration, formation of biodegradable films, and recovery of material from food processing discards (Aranaz et al., 2009)

Biodegradation

Biodergadability depends not only on the origin of the polymer but also on its chemical structure and the environmental degrading conditions (Vroman and Tighzert, 2009). The three main sectors where biodegradable polymers have been introduced include medicine, packaging and agriculture. The development of biodegradable packaging materials has received in increasing attention. Natural biopolymer usually have good biocompatibility as well as biodegradability (Jamaluddin, 2009).

Food Packaging

The requirements for food packaging, the keeping find food fresh, enhancing organoleptic characteristics of food such an appearance , order, and flavor, and providing food safety. In everyday life, packaging is another important area where biodegradable polymers are used. In order to reduce the volume of waste, biodegradable polymers are often used. Biodegradable polymers used in packaging require different physical characteristics, depending on the product to be packaged and the store conditions. Several polysaccharide-based biopolymers chitosan have been investigated as packaging films (Shahidi *et al*., 1999).

Materials and Methods

Commercial chitosan was purchased from Shwe Poe Company, Hlaing Tharyar Township, Yangon Region, Myanmar. The chemicals (acetic acid) used in the experimental work were from the British Drug House (BDH) Chemicals Ltd., Poole, England. The chemicals were used as received unless stated otherwise. All specific chemicals used are described in detail in each experimental section.

In all the investigations, the recommended standard methods and techniques involving both conventional and modern methods were provided on the statistical basis. The apparatus used in this work were conventional labware and glassware, and modern equipments. These are cited in each experiment. The following were some of the instruments and equipments used in the experiments in this study.

Identification of Commercial Chitosan

Determination of moisture content

Material

Chitosan flakes

Apparatus

Porcelain crucible with cover

Mattler balance AE 160 (160 ± 0.1 mg), Gallenkamp, England

Electric furnance, 100-1100°C, Gallenkamp, England

Procedure

Chitosan 1g was added into a known weight of pure and dry porcelain basin. It was heated up to 105 ºC in an oven for two hours. Then the porcelain basin was placed into a desiccators for an hour to cool down the temperature. It was weighed again. Heating, cooling and weighing were repeated until the constant weight was achieved.

Determination of degree of deacetylation by titrimetric method

Materials

Chitosan flakes, Hydrochloric acid, Sodium hydroxide, Phenolphthalein indicator, Acetone, Methanol

Apparatus

Mettler balance (160 g ± 0.001 mg), Centrifuge, Burette, Pipette, Conical flask

Procedure

Chitosan (2g) was completely dissolved in 200 mL of freshly prepared 0.2 M HCl solution and 100 mL of concentrated hydrochloric acid was then added to the homogeneous chitosan solution with vigorous stirring to precipitate the hydrochloride salt. The resultant solution was centrifuged for 15 min and the supernatant was discarded. The chitosan hydrochloride salt was then filtered off and washed several times with methanol until filtrate was neutral to litmus. Residual moisture in the chitosan hydrochloride salt was removed by stirring for 6 h in acetone. After final filtration, the precipitate was dried in a vacuum desiccators for 12 h to yield white chitosan hydrochloride salt.

The resulted chitosan hydrochloride salt was divided into two portions, one portion was placed in oven at 105°C to determine moisture content. In the other portion, an accurately weighed (approximately 0.2 g) chitosan hydrochloride salt was dissolved in distilled water and the volume made up to 100 mL in the volumetric flask. The resulting solution (25 mL) was titrated against a standard 0.05 M sodium hydroxide solution using phenolphthalein as an indicator.

Antimicrobial Test of Composite Films by Agar Disc Diffusion Method

Materials

Chitosan Film

Chemicals

1% meat extract, 0.5 % sodium chloride, 1% peptone, 2% agar

Bacterials

Bacillus *subitilis* (IFO-3080), *Staphylococcus aureus* (IFO-12732), *Pseudomonas aeruginosa* (IFO-3080), *Bacillus pumilus* (IFO-1210), *Candida ablicans* (IFO-1060), E.coli

Apparatus and Equipments

1) Automatic high speed autoclave, Model S-90N, Tomy Seiko Co.Ltd., Tokyo, Japan.
2) Hot oven Model, GM-10E (DEWG, No.9 B-81051)
3) Clean bench, Hitachi Ltd, Japan
4) Water bath, Yamoto Model BT-18 No. 157
5) Incubator box, Sanyo Co., Ltd.
6) Refrigerated centrifuge, Tomy Seiko, Co., Ltd., Tokyo, Japan.
7) Petridishes, Flat bottle, conical flask, pipette, test tube
8) Balance

Procedure

The Chitosan fim, tested with Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus Pumilus, Candida albicans and E.coli species to investigate the nature of antibacterial activity.

After preparing the bacteriological medias, the dried films were placed on the agar with flamed forceps and gently pressed down to ensure proper contact. The plates were inclubated immediately or within 30 min after

incubation. After overnight incubation at 37 °C, the result were shown in Table 4 and Figure 5.

Analysis of Soil

Biodegradations was determined by soil burial test examining and morphology changes. The soil sample was obtained from waste disposal area. Three types of soil sample were taken and dried in the shade. After all the soils had been dried the sample was ground and sifted.

Determination of Biodegradability of C Film

The nature of biodegradability of films was determined by soil burial test examining morphology changes.

Materials

Chitosan Film

Apparatus

Balance (Mettler)

Procedure

The chitosan film was cut into 1" × 1" dimension. The films were then accurately weighed and buried in soil at a depth of 5 cm. They were taken out from the soil at an interval of three days. Sample geometry on degradation was also recorded with photos which are presented in Figure 6.

Preparation of chitosan (C) films

A series of chitosan solutions 1 % w/v to 4 % w/v were prepared by using various weight percent of chitosan in 100 mL of 1 %v/v acetic acid and then autoclaved for 20 min at 121 ºC and 0.1MPa to get a clear solution. The prepared films were designated as C-1 for 1 % w/v chitosan, C-2 for 2 % w/v chitosan, C-3 for 3 % w/v chitosan and C-4 for 4 % w/v chitosan. The solutions were then made into films by pouring onto a melamine plate and allowed to take place at room temperature for 15 days.

Determination of Physicomechanical Properties of Chitosan Films

Determination of thickness

Materials

C-1, C-2, C-3, C-4

Apparatus

(NKS) micrometer, slide clipper

Procedure

Thickness of the prepared chitosan film was measured by using (NSK) micrometer. The thickness of the film was measured at 5 locations (centre and four corners) using digital micrometer.

Determination of tensile strength and percent elongation at break

Materials

C-1, C-2, C-3, C-4

Apparatus

Tensile testing machine, (Hounsfield. 5000 E), Cutter (Wallace)

Procedure

The prepared chitosan film was cut off according to JISK 7127 (1987). The shape and dimension of the test pieces were described in Appendix-IV. Both ends of the test piece were firmed clamped in the jaws of a testing machine. One jaw was fixed and the other was movable. The movable jaw moved at a rate of 100 mm/min. The recorder of the machine showed the tensile strength in MPa. The procedure was repeated three times for each result.

The resulting data was presented in Table 1 and Figure 1.

Determination of tear strength

Materials

C-1, C-2, C-3, C-4

Apparatus

Wallace cutter, tensile testing machine (AJ 100 mettler)

Procedure

Test specimen was cut out by a die from prepared chitosan film. Specimen was cut with a single nick (0.05 mm) at the centre of the inner concave edge by a special cutting device using a razor blade. The clamping of the specimen in the jaws of a testing machine was aligned with travel direction of the grip. The speed of the moving grip is 100 mm/min. The recorder of the machine showed the highest force to tear from a specimen nicked. The procedure was repeated three times for each result.

The resulting data was presented in Table 1 and Figure 1.

Results and Discussion

Aspect of Characterization of Pure Chitosan Films

The mechanical properties such as tensile strength, elongation at break (%), tear strength are important parameters which revealed the nature of films. The mechanical property of pure chitosan film as a function of chitosan content are presented in Table 1 and Figure 1. The thickness of pure chitosan film is about 0.10 mm.

It was found that the tensile strength was drastically increased to 23.4 MPa with chitosan content 2% w/v (i.e C-2). Similarly, the percent elongation at break also increased for C-2 film, however it was significantly decreased with increasing chitosan concentration. The maximum tensile strength and the percent elongation at break of C-2 film were found to be 23.4 MPa and 7.8 % respectively.

The tear strength is another mechanical property of the nature of films. It indicated that the tear strength of chitosan film was significantly increased

at C-2, however, it was significantly decreased beyond 2 % w/v of chitosan concentration. It can be concluded that according to the physicomechanical properties such as tensile strength, percent elongation and tear strength, the composition of chitosan for flexible film C-2 (2 % w/v) is the best.

Table 1: Physicomechanical Properties of A Series of Chitosan (C) Films

(%w/v) of Chitosan	Tensile strength(MPa)	Elongation at break (%)	Tear Strength (kN/m)
C - 1	19.1	3.5	26.7
C - 2	23.4	7.8	16.0
C - 3	20.1	2.5	11.4
C - 4	11.0	8.8	11.7

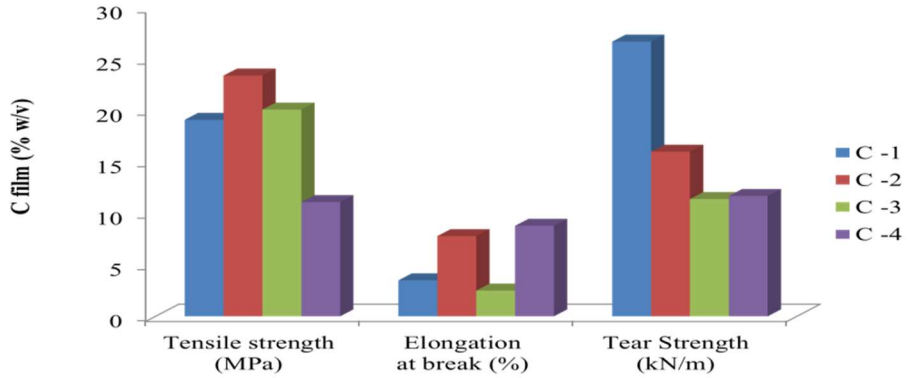


Figure 1: Tensile strength, elongation at break (%) and tear strength of C-2 film

SEM Analysis

Figure 2 shows the SEM photographs of C film (C-2), The surface morphology of pure chitosan film was relatively smooth, homogenous and continuous matrix without cracks with good structural integrity.

Figure 2: Scanning electron micrographs of C-2 Film

FT IR Spectrum of Chitosan C-2 Composite Film

FT IR spectroscopic studies allowed to analyze the characteristics bands corresponding to vibrations of the hydroxyl-, methyl-, methylene-, carbonyl- and amide groups. The FT-IR spectrum of C-2 film, is presented in Figure 3 and Table 2. The broad absorption band of N-H and OH stretching was between 3200 and 3600 cm^{-1}. The IR spectrum of chitosan at 3294 cm^{-1} was the OH stretching, which overlaped the NH stretching in the same region. The band at 1635 cm^{-1} represent C = O stretching ($\nu_{C=O}$) due to amide I band and the band at 1558 cm^{-1} represent (-NH-) amide II band due to N-H bending (δ_{NH}) vibration of secondary amide group chitosan . The band at 1411 cm^{-1} corresponds to the CH symmetrical deformation mode. The peak at 1149 cm^{-1} indicate the saccharide structure and a broad band at 1072 cm^{-1} was due to the C-O stretching vibration in chitosan (Salleh *et al*., 2009).

Table 2: FT IR Band Assignment for C-2 Film

Experimental	**Literature***	
C-2 film	**Frequency (cm^{-1})**	**Band Assignments**
3294	3200-3600	N-H, OH symmetric stretching vibration
2926-2891	2900-2950	Symmetric and asymmetric CH stretching of CH_2
1635	1649-1655	Amide I: C=O stretching vibration
1558	1515-1570	Amide II: NH deformation and C-N stretching vibration
1411	1400-1430	CH symmetrical deformation mode
1327	1322-1325	Deformation vibrations of CH group
1149	1153-1158	Bending vibration of hydroxyl group
1080	1080-1230	C-O stretching vibration

∗Silverstein 1991, Salleh 2009

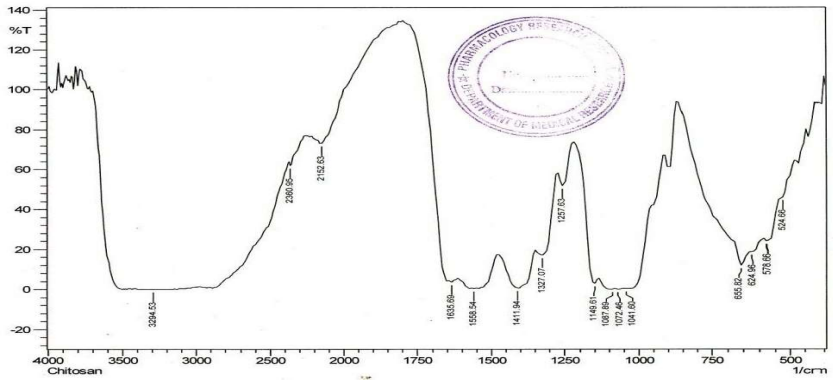


Figure 3: FT IR spectrum of C-2 film

TG-DTA Analysis

The nature and remarks regarding the thermogram profiles are presented in Table 3. The thermogram of uncross-linked chitosan film (C-2) is presented in Figure 4.

According to the TG-DTA thermogram profiles of chitosan film (C-2), three stages of weight loss were observed. In the first stage, the temperature range between 37 °C to 120 °C accompanied with 5.49 % weight loss due to

the dehydration of moisture and surface water. The slight weight loss approximately 17.09 % was observed in the temperature range between 120 °C and 280 °C in the second stage. This weight loss can be attributed to the decomposition of volatile materials. In the third stage, the obvious weight loss 51.08 % was found in the temperature range between 280 °C and 600 °C. In this stage, the weight loss was due to the degradation of polymer backbone and decomposed to monomer fragments up to the formation of residue.

Table 3: Thermal Analysis Data for C-2 Film

TG Thermogram			DTA Thermogram	TG and DTA Remarks
Temperature Range (°C)	**Break in Temperature (°C)**	**Weight loss (%)**		
37-120	-	5.49	-	Dehydration due to moisture and surface water
120-280	-	17.09	-	Loss of volatile materials
280-600	299.43	51.08	exothermic	Degradation of polymer backbone
-	525.94	-	endothermic	Decompose to monomer fragments up to the formation of residue

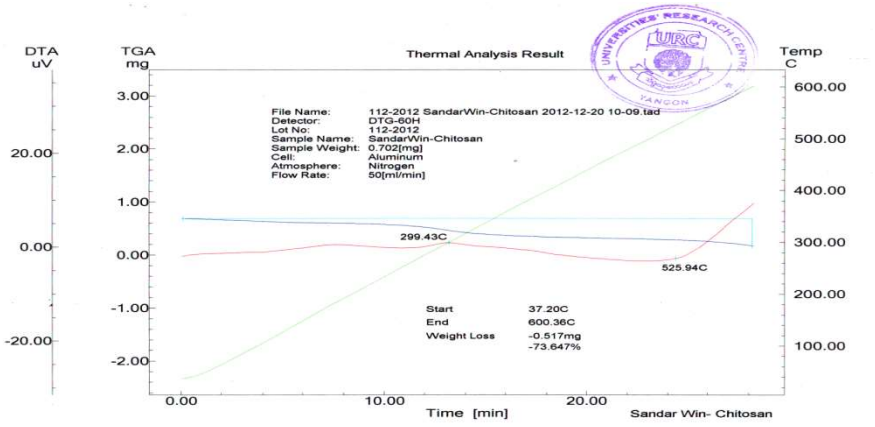


Figure 4: TG-DTA thermogram for C-2 film

Antimicrobial Activity C-2 Film

Antimicrobial activity of C-2 film is shown in Table 4 and Figure 5. The tested organisms were *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida ablicans and E.coli.* As seen in Figure 5 antimicrobial test of C-2 film was used in the agar medium cultivation. C-2 Film showed the antimicrobial activity such as *Bacillus subtilis, Staphylococcus aureus* and *E.coli* but the other three organisms did not show the activity.

Table 4. Antimicrobial Activity of C-2 Films by Agar Well Diffusion Method

No.	Microorganisms	Antimicrobial Activity of C-2 Film
1.	*Bacillus subtilis*	+
2.	*Staphylococcus aureus*	+
3.	*Pseudomonas aeruginosa*	-
4.	*Bacillus pumilus*	-
5.	*Candida albicans*	-
6.	*E.coli*	+

Agar Well- 10 mm (-), 10 mm ~ 14 mm (+)

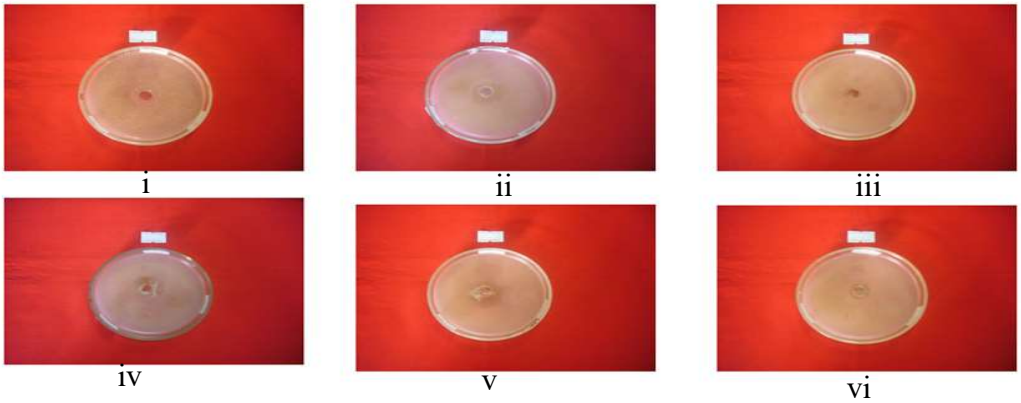


Figure 5: Antimicrobial activity of prepared C-2 film with *(i) Bacillus subtilis (ii) Staphylococcus aureus (iii) Pseudomonas aeruginosa (iv) Bacillus pumilus (v) Candida albicans (vi)E.coli*

On the Aspect Biodegradation

The environmental friendly degradable plastic has been developed by compositing the natural polymer chitosan .One of the objectives of development natural polymer chitosan is to make easy throw away materials from degradable plastic to alleviate waste disposal problems by means of environmental degradation. In this work, biodegradation of chitosan films such as C-2 film was tested by soil burial method. Soil burial is a traditional way to test samples for degradation because of its similarity to actual condition of waste disposal. Uniformly sized samples were buried in the soil from waste disposal. The physical appearance of film by soil burial method were shown in Figure 6. In the dry sand, there were not significantly difference in biodegradability. The biodegradability of C-2 film was found to be more biodegradable in sandy soil, soil and humus soil than the dry sand.

Soil Burial Test

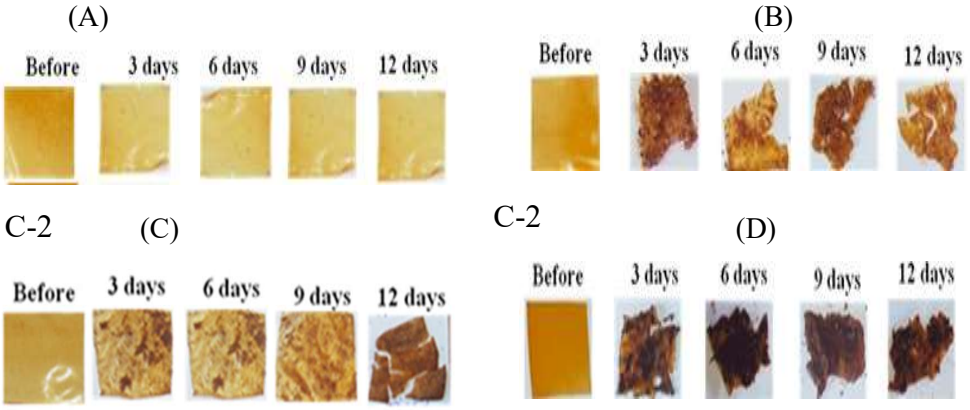


Figure 6: The physical appearances of films by soil burial method (A) Dry sand (B) Sandy soil(C) Soil (D) Humus soil

Packaging of Prepared C-2 Film on Mango

Packaging of prepared C-2 film on mango are shown in Figures 7 and 8. The film packaging is known to have the potential to prolong the storage life and control the decay of fruits. The processed mangoes were packaged in chitosan C-2 film boxes with these film. Packaging fruits with chitosan film

helps the long term storage of fruit because chitosan C-2 film provides a type of active package, which are released from the film deposited on the surface of the fruit. Citrus and other fruits can be stored for long period once they have been packaged with C-2 film which decreases respiration rates and inhibits fungal development and delays ripening by suppressing the evolution of ethylene and carbon dioxide (Dutta *et al*., 1997).

The effectiveness of C-2 film and control (unpackaged mangoes), the weight loss and maintaining the quality of mangoes were also investigated. Weighed mangoes were packaged with C-2 film. The control (unpackaged mangoes) and packaged mangoes were stored for 20 days at room temperature (≈ 28 ± 2 °C). The fruit quality such as water content, titratable acidity (TA), reducing sugar content, crude fibre, total soluble solid , pH , weight loss and refractive index of the unpackaged mangoes and packaged mangoes were also determined within 20 days of storage time.

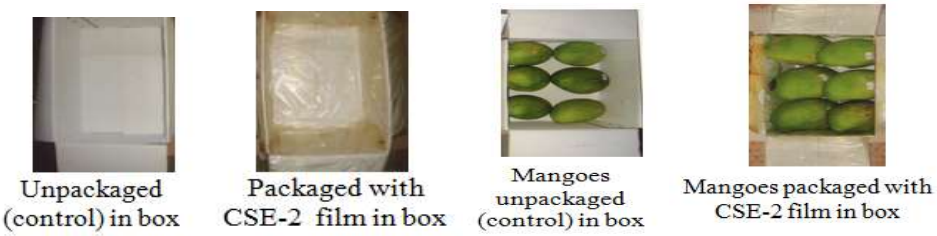


Figure 7: Photographs of film packaging treatments on mangoes

(A) **(B)**

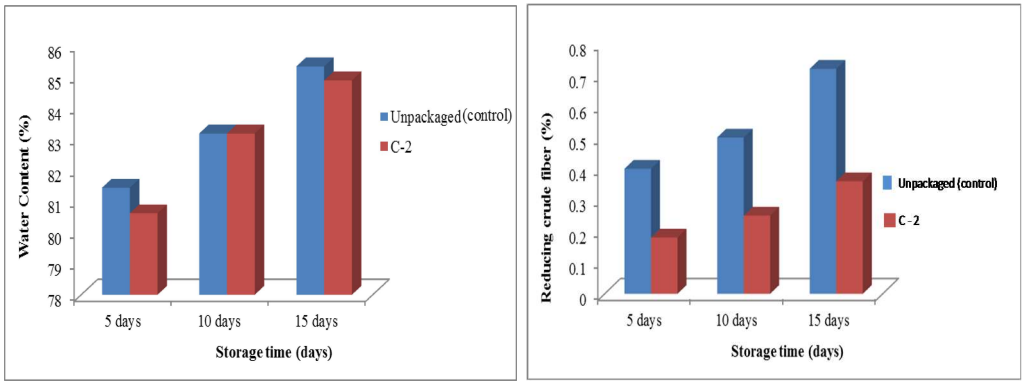
(A) = Unpackaged (control) Mangoes
(B) = Mangoes, packaged with C-2 film
Figure 8: Physical appearance of unpackaged(control) and packaged mangoes

Effect on Water Content and Titratable Acidity

Tables 5 and Figure 9 show that the increase in water content of mangoes packaged with chitosan films as a function of time at room temperature. In this study C-2 beneficially affected postharvest mango and retarded ripening, water loss and decay. Therefore reducing water loss from fruit during storage or ripening helps to maintain the quality of fruit. According to experimental results C-2, promoted the retention of firmness and increased water content. The titratable acidity was significantly decreased during the storage time. The titratable acidity value of C-2 packed fruit were lower than control. The decreasing acidity at the end of storage might be due to the metabolic changes in fruits.

Table 5: Water Content and Titratable Acidity of Unpackaged (control) and C-2 Packaged Mangoes as a Function of Times at Room Temperature

Sample	Water Content (%)			Titratable Acidity (g/100 g)		
	5 days	10 days	15 days	5 days	10 days	15 days
Unpackaged (control)	81.43	83.17	85.32	99.75	10.88	9.32
C-2 Film	80.61	83.17	84.87	108.54	14.04	10.85



(a) (b)

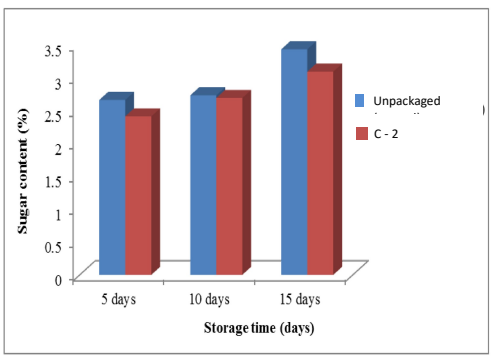
Figure 9: Variation of (a)water content and(b) titratable acidity of unpackaged (control) and packaged mangoes with storage time at room temperature

Effect on Sugar Content and Crude Fibre

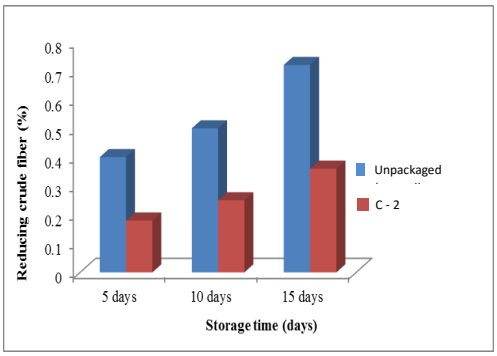
The changes in the sugar content and crude fiber content of packaged and unpackaged fruits are shown in Table 6 and Figure 10.The reducing sugar content and crude fiber content of C-2 packaged fruits were lower than and control. The increasing sugar content was due to breakdown of cell structure in order to senescence phenomena during storage. The crude fibre content of C-2 slightly increases from 0.18 to 0.36 within 15 days at room temperature. But crude fibre content of unpackaged (control) fruits obviously increased from 0.40 to 0.72 during 15 days. So C-2 can control the increasing of crude fibre content in a mango.

Table6: Sugar Content and Crude Fibre Content of Unpackaged (control) and C-2Packaged Mangoes as a Function of Times at Room Temperature

	Sugar content (%)			**Crude fiber (%)**		
Sample	**5 days**	**10 days**	**15 days**	**5 days**	**10 days**	**15 days**
Unpackaged (control)	2.66	2.73	3.43	0.40	0.50	0.72
C-2 Film	2.41	2.69	3.09	0.18	0.25	0.36



(a)



(b)

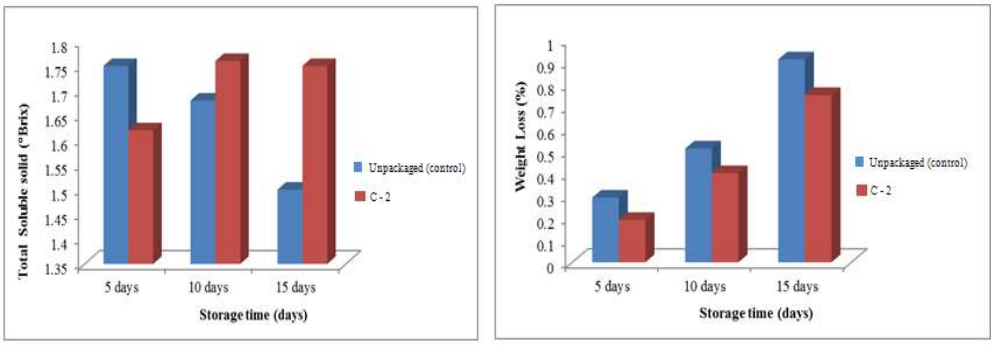
Figure 10: Variation of (a) sugar content and (b) Crude Fibre Content of unpackaged (control) and packaged mangoes with storage time at room temperature

Effect on Total Soluble Solid and Weight Loss

The changes in the total soluble solid content and weight loss in mangoes are shown in Table 7 and Figure 11. Total soluble solid (TSS) of packaged fruits was gradually increased during the storage except the unpackaged (control) fruit. No significant differences were found packaged with prepared C-2 sample and control fruits (unpackaged mango). There were significant differences about 15 days of storage in C-2 film. The increase packaged mango in TSS contents during storage might be due to the respiration rate and conversion of sugars to carbon dioxide and water. The weight loss associated with C-2 packaged with mangoes were slower than that unpackaged mangoes. Weight loss was lower in coated fruit with C-2 (0.19% to 0.75 %) as compared to control having higher percent weight loss (0.29 % to 0.91%).

Table 7: Total Soluble Solid and Weight Loss of Unpackaged (control) and C-2 Packaged Mangoes as a Function of Times at Room Temperature

Sample	Total Soluble solid (°Brix)			Weight Loss(%)		
	5 days	10 days	15 days	5 days	10 days	15 days
Unpackaged(control)	1.75	1.68	1.50	0.29	0.51	0.91
C-2 Film	1.62	1.76	1.75	0.19	0.40	0.75



(a) (b)

Figure 11: (a)Total soluble solid and (b) weight loss of unpackaged (control) and packaged mangoes with storage time at room temperature

Effect on pH and Refractive Index

The pH of mangoes were gradually increased during storage time. There were no significant differences between treated and control fruits. These data were shown in Table 8. According to the experimental results, the pH values were gradually increased during storage intervals. It might be due to decrease in acidity through the biochemical changes within the fruits during storage. The refractive index values of unpackaged mangoes and packaged mangoes are presented in Table 8. It was observed that there was no significant changes in the refractive index of the unpackaged mangoes and packaged mangoes were also determined within 15 days of storage time.

Table 8: pH and Refractive Index of Unpackaged (control) and C-2 Packaged Mangoes as a Function of Times at Room Temperature

Sample	pH			Refractive Index		
	5 days	10 days	15 days	5 days	10 days	15 days
Unpackaged(control)	4.04	5.85	6.09	1.3365	1.3365	1.3365
C-2 Film	3.85	5.78	5.93	1.3345	1.3345	1.3345

Conclusion

In this study, chitosan (C) films (C-1, C-2, C-3, C-4) were prepared by solvent evaporating method under autoclaving conditions of 0.1 MPa in a time frame of 20 min at 121°C. The mechanical properties of prepared C-2 film was found to possess the tensile strength (23.4 MPa), elongation at break (7.8 %) and tear strength (16.0 kN/m). According to the FT IR spectrum, the broad absorption band of N-H and OH stretching was between 3200 and 3600 cm^{-1}. The IR spectra of chitosan at 3294 cm^{-1} was the OH stretching, which overlaps the NH stretching in the same region. The band at 1635 cm^{-1} represent C=O stretching ($\nu_{C=O}$) due to amide I band and the band at 1558cm^{-1} represent (-NH-) amide II band due to N-H bending (δ_{NH}) vibration of secondary amide group chitosan . The band at 1411 cm^{-1} corresponds to the CH symmetrical deformation mode. The peak at 1149 cm^{-1} indicate the saccharide structure and a broad band at 1072 cm^{-1} was due to the C-O stretching vibration in chitosan. From the SEM analysis, the pattern of C-2 film was found to be relatively smooth, homogeneous and a continuous matrix

without cracks with good structural integrity. The TG-DTA, in the temperature range between 37°C and 120°C, the weight loss is 5.49% which is due to the dehydration of moisture and absorbed water. The slight weight loss of about 17.09 % was observed in the temperature range between 120 °C and 280 °C in the second stage. This weight loss can be attributed to the decomposition of volatile materials. In the third stage, the significant weight loss 51.08 % was found in the temperature range between 280 °C and 600 °C. In this stage, the weight loss was due to the degradation of polymer backbone and decomposed to monomer fragments up to the formation of residue.

The biodegradability of C-2 film was found to be more biodegradable in sandy soil, soil and humus soil than the dry sand. From the microbial activities, C-2 film showed significant antimicrobial activity such as *Bacillus subtilis, Staphylococcus aureus* and *E-coli* but not on the other three organisms. Application of C-2 as packaging film for mango fruits delayed physicochemical changes such as water content, titratable acidity, sugar content, crude fibre, total soluble solid, effect of pH, refractive index and weight loss. By comparing the unpackaged and packaging films, the mango fruit packaged by C-2 film show 15 days ripening film and can sustain better quality.

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