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Preparation of Starch and Cellulose-Based Edible Films Incorporated with Propolis Extract and Their Physical and Antimicrobial Properties

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Abstract

Starch (SBEF) and cellulose-based edible films (CBEF) incorporated with propolis extract were prepared depend on the film thickness. The WVTR of CBEF with propolis extract decreased as their thickness increased from 0.06 to 0.163 mm, meanwhile the CBEF with propolis extract showed a percent reduction in WVTR at different thickness 0.092, 0.1 and 0.145 mm as 70, 33 and 65.6%, respectively. The G' of the SBEF incorporated with propolis extract (SP1 and SP2) maintained lower value until around 60, and then increased slightly when compared to those control of SC1 and SC2. However, in comparison of SC3 and SP3, the SP3 showed a higher value clearly in modulus change when compared to the SC3, and then maintained almost flat with increasing temperature.

From the beginning of temperature scanning, there was an exponential drop in G’ and peaking in tan δ in the CBEF incorporated with or without propolis extract. Especially, the thin films CC1 and CP1 exhibited a great difference in G’ change at approximately 25 to 50°C, simultaneously, the modulus value decreased and then increased until the second transition at approximately 60°C. However, for films thicker than CC1 and CP2, even though they showed a similar pattern of G’ to those, the range of modulus did not change significantly. When CBEF without propolis extract as a control was used for the fresh noodle package, total microorganisms was 1.1×10^6 CFU/g, which is over safety standard, after 4 weeks storage at 10°C, while it was 7.7×10^4 CFU/g when CBEF incorporated with propolis extract.

Key words: Edible films, starch, cellulose, propolis extract, physical and antimicrobial properties

Introduction

Edible films can regulate undesirable water vapor transfer in food products, thus improving food quality and extending shelf life. Edible films may be used as food coatings or as stand-alone film wraps and pouches to supplement synthetic packaging. The major portion of food spoilage originates from microbial contamination on the food surfaces. Active packaging is one of the innovative food packaging concepts that has been introduced as a response to the continuous changes in current consumer demands and market trends. Major active packaging techniques are concerned with substances that absorb oxygen, ethylene, moisture, carbon dioxide, flavours/odours and those which release carbon dioxide, antimicrobial agents, antioxidants and flavours. The incorporation of antimicrobial substances into polymer films for food packaging to inhibit the growth of microorganisms on the food surface has been developed (Weng *et al*., 1999; Hong *et al*., 2000). There are many types of antimicrobial substances has been introduced in the food industry including Ag-substituted zeolite (Ishitani, 1995), organic acids e.g. sorbate, propionate and benzoate (Han and Floros, 1997), bacteriocins e.g. nisin and pediocin (Ming *et al.*, 1997), enzymes such as lysozyme (Padgett *et al.*, 1998), metals (Ishitani, 1995), fungicides such as benomyl (Halek and Garg, 1989) and imazalil (Weng and Hotchkiss, 1992),

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grape fruit seed (Katz, 1998) and chitosan (Padgett *et al.*, 1998). Propolis is a mixture of various amounts of beewax and resins collected by the honeybee from plants, particularly from flowers and leaf buds. The antioxidant, antimicrobial and antifungal activities of propolis offer scope for application in food industry. Mizuno (1989) registered a patent which includes propolis as a preservative in food packaging material. A frozen storage life of fish could be extended by 2-3 times with pretreatment of propolis (Donadieu, 1979). Özcan and Ayar (2003) reported that the propolis extracts had effects on peroxide and free fatty acid value of butter during storage, and might be considered as a new source of natural antioxdants of moderate efficiency. Kim *et al.*, (1997) studied that the added propolis inhibited the growth of fungi in white bread, and the more propolis extract was added, the higher degree of inhibition of fungal growth. However, there has been no report on the edible films from cellulose and starch-based material incorporated with propolis extract and the physical and antimicrobial properties. The objectives of this study were to: (1) investigate the physical and antimicrobial properties of edible films of cellulose and starch-based incorporated with propolis extract, and (2) evaluate the ability of these films maintain desirable storage quality in fresh noodle package.

Materials and Methods

Materials

Crude propolis was purchased from Iljin Pharm. Co., Korea. Potato starch, methyl cellulose (MC), hydro xypropylmethylcellulose (HPMC), polyethylene glycol 400 (PEG), stearic acid, palmitic acid, glycerin and alginic acid were purchased from Sigma Co., USA.

Methods

Preparation of Starch-Based Edible Films (SBEF) Incorporated with Propolis

Potato starch was dissolved into a distilled water with stirring and heating at 68 for 14 min, and then glycerin, alginic acids, propylene glycol were added directly (Table 1). The propolis was added at 0.4 % (w/w) relative to the original weight of the mixture of emulsion films and then the mixture homogenized using an Ultra-Turrax T-25 homogenizer (Ultra-Turrax, Model T25, IKA-Works, Inc., Cincinnati, OH, USA). The mixed solution was added to a spreader for thin layer chromatography (TLC) and plated onto 8" × 8" glass TLC plates at a thickness of 1.0, 1.5 and 2.0 mm. The coated plates were placed in an oven at approximately 90°C. After drying for approximately 15 min, the plates were cooled and the films were peeled from the plates.

Table 1. Composition of starch-based edible films

Component	Composition
Starch	8g
Glycerin	2g
Propylene glycol	2g
Alginic acid	4g
Distilled water	75ml
Propolis	0.6

Preparation of Cellulose-Based Edible Films (CBEF) Incorporated with Propolis

MC and HPMC were dissolved into an aqueous ethanol (EtOH : Water = 2 : 1) with stirring at 65°C for 30 min, and then fatty acids (stearic-palmitic acid blend) were added directly (Table 2). The propolis was added at 0.4% (w/w) relative to the original weight of the mixture of emulsion films and then the mixture homogenized using an Ultra-Turrax T-25 homogenizer (Ultra-Turrax, Model T25, IKA-Works, Inc., Cincinnati, OH, USA). The mixed solution was added to a spreader for thin layer chromatography (TLC) and plated onto 8" × 8" glass TLC plates at a thickness of 1.0, 1.5 and 2.0 mm. The coated plates were placed in an oven at approximately 90°C. After drying for approximately 15 min, the plates were cooled and the films were peeled from the plates.

Table 2. Composition of cellulose-based edible films

Component	Composition
Methyl cellulose	7g
Hydroxypropylmethylcellulose	3g
Polyethyleneglycol 400	6g
Stearic-palmitic acid blend (50 : 50)	9g
Ethanol (95%)	100ml
Distilled water	100ml
Propolis	0.6g

Film Thickness Measurement

Films specimens (8 × 8 cm) were cut from oven-dried films. Seven thickness measurements were taken on each specimen, two at the center and five around the perimeter.

Water Vapor Transmission Rate (WVTR) Measurement

For SBEF and CBEF after storage at 33% of relative humidity, WVTR was determined gravimetrically using a modification of ASTM standard method F 1249-90, known as the “modulated infrared sensor”. For this, the apparatus was used with a Permatran-W1A (Mocon, MN, USA).

Dynamic Mechanical Analysis(DMA) of Edible Films

The film for DMA analysis was cut into 10 × 5 mm strips after storage at 33% of relative humidity. The strips were pressed together manually and then clamped into the DMA furnace at both ends. The sample was heated from 25 to 120°C at 5°C/min. Simultaneously, a sinusoidal force was applied to the center of the strip (three-point bending) at 1 Hz frequency with 120 mN of static force and 100 mN of dynamic force. Storage modulus (G'), loss modulus (G'') and loss angle (tan δ) were recorded and plotted.

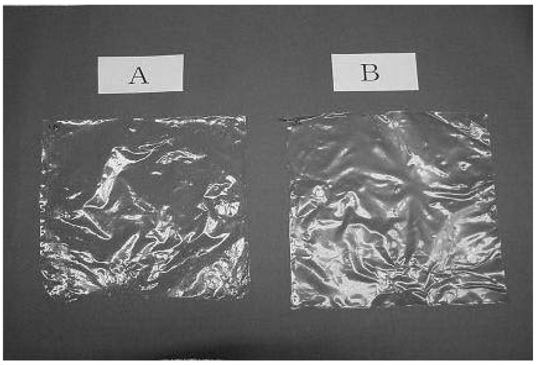
Antimicrobial Effect of CBEF on Noodle

The fresh noodles were packed with CBEF and stored at a different storage temperature (0, 5, 10, 25°C) for 4 weeks. The noodles were cut into small pieces. 5 g of noodles were added to 90 ml of the 0.85% saline water and shaken for 60 sec, and then the suspension was taken for viable cell counts. The viable cell counts were measured by plating 0.1 ml of the samples on the corresponding agar.

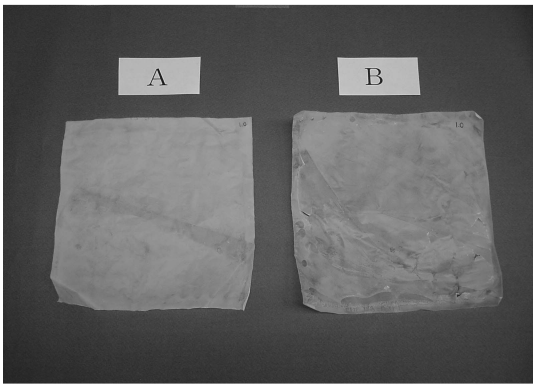
Results and Discussion

Physical Properties of SBEF and CBEF

Fig. 1 shows the SBEF and CBEF incorporated with or without propolis extract. The edible films incorporated with propolis appeared yellowish in color, it might be due to the flavonoids containing in propolis. The SBEF formed more transparent and glossy than CBEF, due to the difference of materials between starch and cellulose. The thickness of SBEF and CBEF were indicated in Table 3. Both SBEF and CBEF ranged from 0.063~0.163 mm depend on the loading thickness during spreading. The WVTR of SBEF and CBEF were also shown in Table 3. The WVTR of CBEF with propolis extract decreased as their thickness increased from 0.06 to 0.163 mm, meanwhile the CBEF with propolis extract showed a percent reduction in WVTR at different thickness 0.092, 0.1 and 0.145 mm as 70, 33 and 65.6%, respectively. Therefore, the CBEF incorporated with propolis extract must be effective moisture barriers. Similar result also observed in the edible film of whey protein with beewax which exhibited lower WVTR than other lipid emulsion films with increasing lipid concentration, fatty acid and fatty



(SBEF)



(CBEF)

Fig. 1. SBEF and CBEF incorporated without propolis extract(A) and with propolis extract(B).

Table 3. Water vapor transmission rate for SBEF and CBEF.

Edible films		Thickness (mm)	WVTR (g/cm^2/day)
SBEF-C[1]	SC1	0.083±0.0004	<1,200
	SC2	0.117±0.0002	<1,200
	SC3	0.163±0.0003	<1,200
SBEF-P[2]	SP1	0.081±0.0002	<1,200
	SP2	0.114±0.0003	<1,200
	SP3	0.188±0.0002	<1,200
CBEF-C[3]	CC1	0.063±0.0002	47.30
	CC2	0.102±0.0003	41.28
	CC3	0.117±0.0004	22.28
CBEF-P[4]	CP1	0.092±0.0001	31.27
	CP2	0.101±0.0002	27.74
	CP3	0.145±0.0005	13.45

[1] SBEF-C; Starch-based edible films without propolis extract, [2] SBEF-P; Starch-based edible films incorporated with propolis extract, [3] CBEF-C; Cellulose-based edible films incorporated without propolis extract, [4] CBEF-P; Cellulose-based edible films incorporated with propolis extract.

alcohol chain length (McHugh and Krochta, 1994). In generally, the propolis can be collected from a mixture of various amounts of beewax and resins, and during process of collecting they are mixed with some saliva and other secretions of the bees as well as with wax. The WVTR of SBEF were much higher than that of CBEF, it might to be due to the poor properties of starch such as brittles, water sensitive and readily disintegrate in water. Many researchers have indicated relationship between film thickness and permeability properties in hydrophilic film systems. Ideal polymeric film exhibits no thickness effect on WVTR; however, hydrophilic films often exhibit positive slope relationships between thickness and WVTR (McHugh *et al.*, 1993). The use of starch for the edible film processing has been limited in commercial production (Kotnis *et al.*, 1995), therefore, the addition of plasticizers overcomes the starch films brittles and improves their durability against water. Hydrophilic compounds such as polyols (glycerol, sorbitol and poly (ethylene glycerol)) are commonly used as plasticizer in hydrophilic film formation (Laohakunjit and Noomhorm, 2004).

DMA Characteristics

DMA thermograms of SBEF and CBEF are shown in Fig. 2. The plot of storage modulus (G') and tan δ as a function of temperature showed changes in these

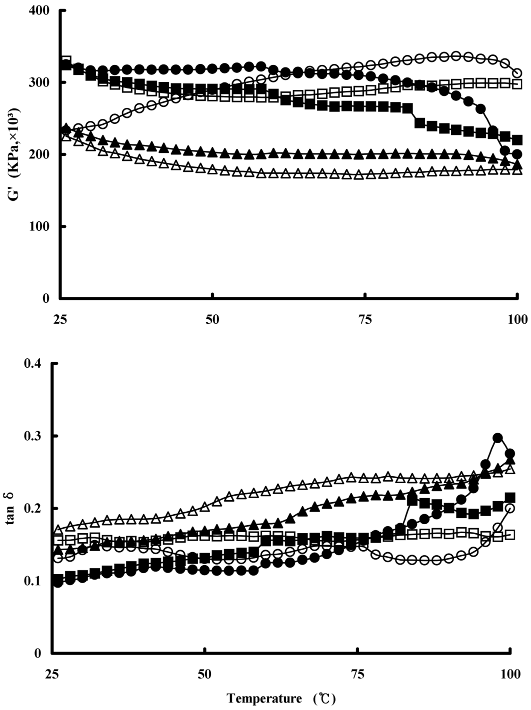


Fig. 2. DMA tensile modulus and tan δ as a function of temperature for SBEF.
● ; SC1, ○ ; SP1, ■ ;SC2, □ ; SP2, ▲ ; SC3, △ ; SP3

parameters as the films of different thickness underwent thermal transitions. The G' of the SBEF incorporated with propolis extract (SP1 and SP2) maintained lower value until around 60, and then increased slightly when compared to those control of SC1 and SC2. This might to be the inflection point of the films which ranged 280,000~320,000 KPa, is called glass transition. At the glass-to-rubber transition around 81~85°C, the modulus value observed in the range of 260,000~300,000 Kpa. However, in comparison of SC3 and SP3, the SP3 showed a higher value clearly in modulus change when compared to the SC3, and then maintained almost flat with increasing temperature. From the tan δ peak, we could be recognized the characteristics of SBEF as displays clearly in Fig. 2, indicating that the glass region, the transition region and a rubbery plateau. As the humidity is increased from 20 to 90% RH, the modulus measured for the film increased five times, and also the relative crystallinity of plasticized starch films increased with increasing air humidity during film formation (Rindlav-Westling *et al.*, 1998). The water content and thickness are affecting the mechanical properties such as strength and strain in starch films, therefore those parameters have been used as a quality index during processing of starch films. In Fig. 3, the DMA graphs showing G' and tan δ as a function of temperature. From the beginning of temperature scanning, there was an exponential drop in G' and peaking in tan δ in the CBEF incorporated with or without propolis extract.

Especially, the thin films CC1 and CP1 exhibited a great difference in G' change at approximately 25 to 50°C, simultaneously, the modulus value decreased and then increased until the second transition at approximately 60°C. However, for films thicker than CC1 and CP2, even though they showed a similar pattern of G' to those, the range of modulus did not change significantly. In this case, the reduction in G' may be due to polymer-polymer hydrogen bonds being replaced by labile polymer-water hydrogen bonds (Kalichevsky *et al.*, 1992). The data from tan showed a more distinguishable patterns between CBEF depend on the thickness and composition of the films than that of G' data. The tan δ peak of CP2 and CP3 ranged from 25 to 60°C, which is more differed than CP1 in phase transition and could be mainly due to the amount of plasticizers depending on the thickness. Therefore, these CP2 and CP3 seem to be possesses a more flexibility of film than CC2 or CC3. In generally, it is well known that the size of the tan δ is regard to reflect the volume fraction of the material undergoing the thermal transition (Wetton, 1986), and also is similar to the typical tan of other totally amorphous biopolymers such as elastin, amylopectin, gelatin, or native wheat gluten (Kalichevsky *et al.*, 1993).

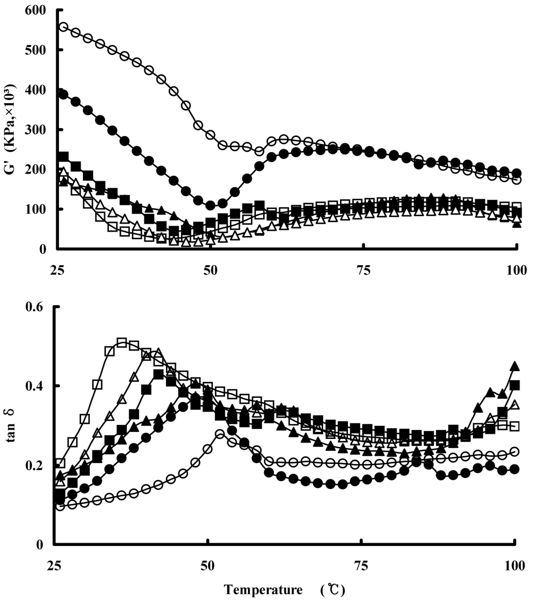


Fig. 3. DMA tensile modulus and tan δ as a function of temperature for CBEF.
● ; CC1, ○ ; CP1, ■ ;CC2, □ ; CP2, ▲ ; CC3, △ ; CP

Application of CBEF for Fresh Noodle Package

Fig. 4 showed a change in total microorganisms for wet noodle packed in CBEF during storage at 0, 5°C, 10 and 25°C for 4 weeks. In the application test of CBEF for fresh noodle package, the incorporation of propolis extract inhibited the growth of microorganisms during storage. The total microorganisms were decreased after storage of 1 and 2 weeks as 30% and 29%, respectively, for fresh noodle packed with CBEF incorporated with propolis extract. When CBEF without propolis extract as a control was used for the fresh noodle package, total microorganisms was 1.1×10^6 CFU/g, which is over safety standard, after 4 weeks storage at 10, while it was

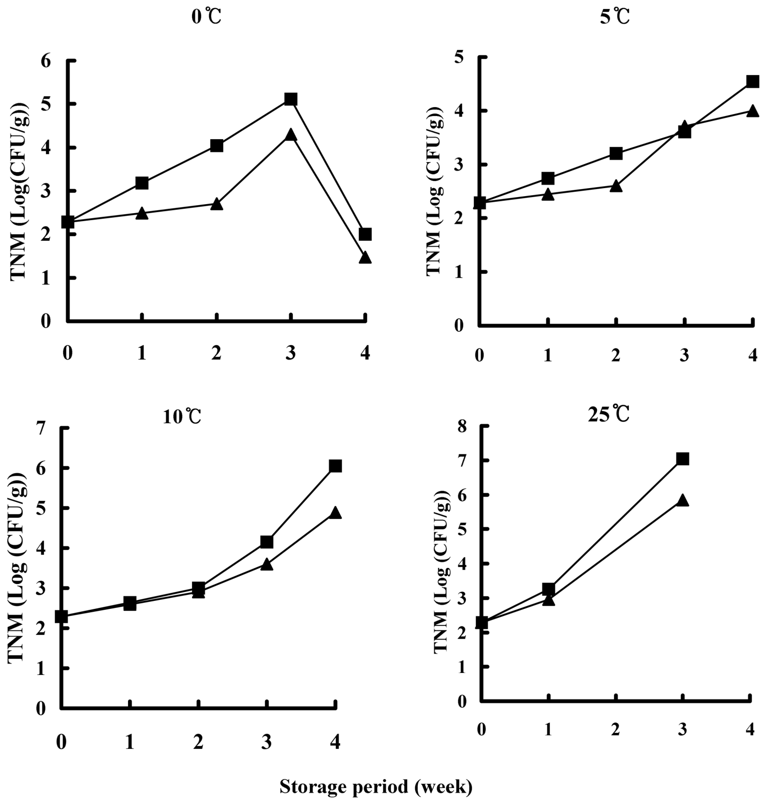


Fig. 4. Changes in total number of microorganisms(TNM) for wet noodles packed in CBEF during storage at 0, 5, 10 and 25 for 4 weeks.
■ ; CBEF without propolis extract, ▲ ; CBEF with propolis extract

7.7×10^4 CFU/g when CBEF incorporated with propolis extract. However, total microorganisms of the fresh noodle packed with CBEF incorporated with and without propolis extract could not determined due to the contamination and spoilage of wet noodle stored at 25°C after 2 weeks. From this result, we may expect that CBEF incorporated with propolis extract could be used as an active packaging in the food industry.

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