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Incorporation of a High Concentration of Mineral or Vitamin into Chitosan-Based Films

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Mineral or vitamin E was incorporated into chitosan-based films: 10−200% (w/w chitosan) Gluconal Cal (GC), a mixture of calcium gluconate and lactate; 5−20% zinc lactate (ZL); and 5−20% α-tocopheryl acetate (VE) with acetylated monoglyceride (AM). The functionality of film-forming solutions and dried films was analyzed with standard procedures, and mathematical equations were developed to coordinate selected film functionality with the type and concentration of incorporated mineral or vitamin E. GC incorporation significantly increased pH and decreased viscosity of film-forming solutions, but not the addition of ZL or VE. The water barrier property of the films was improved by increasing the concentration of mineral or vitamin E in the film matrix. The tensile strength of the films was more significantly affected by GC or VE addition than film elongation, puncture strength, and puncture deformation. While a major endothermic peak around 200 °C was observed in DSC thermograms of chitosan-based films, only 200% GC incorporation altered this endothermic peak. This study demonstrated the capability of chitosan-based film matrix to carry a high concentration of mineral or vitamin E. Such films may be used for wrapping or coating to enhance the nutritional value of foods.

KEYWORDS: Chitosan films; calcium; zinc; vitamin E; water vapor permeability; mechanical property; thermal property

INTRODUCTION

Along with increased market demands on nutritionally fortified foods, edible coatings and films containing high concentration of nutraceuticals would provide alternative ways to fortify foods that otherwise cannot be accomplished with common processing approaches. This would be especially beneficial for unprocessed or fabricated foods, such as fresh or minimally processed fruits and vegetables. Products could be either coated or wrapped with nutritionally fortified coatings or films. Incorporation of a high concentration of nutraceuticals into the film matrix is one of the most challenging technologies in the field of edible films and coatings. A clear understanding of the interactions between the film matrix and the nutraceuticals is essential for developing such edible coatings and films. Our previous study demonstrated that the development of nutritionally fortified edible coatings and films strongly depends on the type of carriers (film-forming materials) and the type and concentration of nutraceuticals added into the film-forming solutions (*1*, *2*).

Chitin and its important derivative, chitosan, are abundant and renewable biopolymers found in nature. Chitosan has been well-known for its excellent film-forming property, antimicrobial activity, and unique coagulating ability with metal and other lipid and protein complexes from the presence of a high density of amino groups and hydroxyl groups in the chitosan polymer structure (*3*−*5*). The high binding ability of chitosan and its antimicrobial properties is the main driving force in developing new applications of this natural polymer in food preservation. For example, chitosan has been used as a semipermeable coating material for fresh fruits and vegetables, and been claimed as an excellent shelf life extender (*6*−*9*). The antifungal effects of chitosan against *Botrytis cinerea* and *Rhizopus* sp., the common post-harvest fungal pathogens in berries (*6*), have been demonstrated on chitosan-coated fresh strawberries and raspberries (*8*).

The chelating and coagulating ability of chitosan has expanded its applications to carry functional substances. Antimicrobials, antioxidants, nutrients, flavors, and colorants are possibly carried on chitosan-based films and released in a controlled manner (*4*). Chen et al. (*10*) incorporated potassium sorbate and sodium benzoate in a chitosan film matrix and reported that such films could inhibit the growth of microorganisms at the interface of film and medium without altering the tensile strength and elongation property of the films. Ouattara et al. (*11*) demonstrated the antimicrobial function of chitosan films incorporating with acetic or propionic acid on the surface spoilage bacteria of processed meat products.

Because of its excellent film-forming property, effective antimicrobial activity, and potential to carry functional sub-

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stances, it was hypothesized that chitosan can be used effectively as a film-forming material to carry a high concentration of nutraceuticals for developing nutritionally fortified edible coatings and films. In this study, chitosan-based films incorporated with a high concentration of active nutraceutical substances were prepared and characterized. The specific objectives include developing chitosan-based film formulations containing a high concentration of calcium, zinc, or vitamin E and evaluating the basic properties of film-forming solutions and functionalities of dried films.

MATERIALS AND METHODS

Materials. Chitosan from Vanson Inc. (Redmond, WA; 11 cps viscosity of a 1% w/w aqueous acetic acid solution at 25 °C and 89.9% deacetylation) was used without further purification. FCC (Food Chemicals Codex) grade DL-lactic acid (Mallinckrodt Baker, Inc., Phillipsburg, NJ) was used to make chitosan solutions instead of acetic acid because of its mild odor and shiny film or coating production. USP (United States Pharmacopeia) grade glycerol was obtained from EM Science (Darmstadt, Germany) and used as a plasticizer. Nutraceuticals incorporated into the film-forming solutions were Gluconal Cal (GC: Glucona America Inc., Janesville, WI), a mixture of calcium lactate and calcium gluconate with 10−11% pure calcium content, a water solubility up to 40 g/100 mL at 20 °C, and neutral taste; PURAMEX ZN (ZL: PURAC America, Lincolnshire, IL), a zinc-L-lactate with 23.5% zinc content; or α-tocopheryl acetate (VE: BASF Corporation, Mount Olive, NJ), a stable form of vitamin E with in vivo antioxidant activity. Acetylated monoglyceride (AM: Dansco, New Century, KS) was used as an emulsifier in the chitosan/VE films.

Preparation of Film-Forming Solutions. Film-forming solutions (FFS) were prepared by dissolving 2% chitosan in a 2% lactic acid solution with addition of 25% glycerol (w/w chitosan) in the mixture. Each of the following mineral or vitamin was incorporated into the chitosan solutions: 10−200% (w/w chitosan) GC, 5−20% ZL, or 5−20% VE with AM as an emulsifier. The amount of each mineral and vitamin added into the film-forming solutions was calculated based on an assumption to obtain at least a 10% dietary reference intake (DRI) value of each nutraceutical in 100 g of coated fruits or vegetables.

Chitosan/GC and chitosan/ZL films were prepared by directly dissolving a designated amount of GC or ZL in chitosan solutions and stirring the mixture for 1 h at room temperature. For chitosan/VE films, VE was first dissolved in melted AM at a ratio of 1:4 (w/w) and then added into chitosan solution that was preheated at 60 °C in a shaking water bath (Precision, Winchester, VA). The chitosan/VE mixture was homogenized at 3000 rpm for 1 min (PT 10-35, Kinematica, Switzerland) to ensure even distribution of VE droplets. All sample solutions were filtered through nylon cloth to remove insoluble residues under vacuum (Model 0211-P204, Gast Mfg. Corp., Benton Harbor, MI) at room temperature. The compositions of film-forming solutions are summarized in **Table 1**.

pH and Viscosity of FFS. The pH value of the FFS was measured with an IQ 240 pH meter (IQ Scientific Instruments, Inc., San Diego, CA), and the intrinsic viscosity of the solutions was determined on a Brookfield digital rheometer (DV-III+, Brookfield Engineering Laboratories, Inc., Middleboro, MA) with RV2 spindle at 30 rpm. All measurements were conducted at room temperature (24 ± 2 °C).

Film Formation. Two different sizes of films were formed in two consecutive studies. First, 91 mm diameter disposable polyethylene Petri dishes (Krackeler Scientific, Albany, NY) were used to cast small films for measuring basic film properties and establishing a film thickness control equation. Films thickness, density, and moisture content vary with the amount of nutraceuticals added into the FFS when the same weight of FFS was used for casting films. Since film thickness significantly affects the water barrier and mechanical properties of the films (*2*), it has to be controlled carefully. Ten grams of FFS was weighed, using an electronic balance (0.0001 accuracy) (TL-204, Denver Instrument Company, Arvada, CO), on a Petri dish, dried on a leveled surface at room condition (24 ± 2 °C and 40 ± 5% RH) for 2 days, and then conditioned in a temperature and humidity controlled environmental chamber (T10RS, Tenney Environmental, Williamsport, PA) set at 25 °C and 50% RH until equilibrium was reached, i.e., no weight change was observed. The Petri dish was then weighed again, and the percentage weight ratio of dried film to 10 g of FFS was calculated. Dried films were peeled out and cut into 25 × 25 mm^2 pieces for determination of film thickness, density, and moisture content.

Table 1. Composition of Chitosan-Based Film-Forming Solutions

film type	concn of nutrient (w/w chitosan)	glycerol concn (%, w/w chitosan)	concn of actual nutrient (w/w chitosan)
control	0%	25	0%
GC10	GC[a] 10%	25	calcium 1%
GC50	GC 50%	25	calcium 5%
GC100	GC 100%	25	calcium 10%
GC200	GC 200%	25	calcium 20%
ZL5	ZL[b] 5%	25	zinc 1.2%
ZL10	ZL 10%	25	zinc 2.4%
ZL20	ZL 20%	25	zinc 4.8%
VE5	VE[c] 5%	25	vitamin E 5%
VE10	VE 10%	25	vitamin E 10%
VE20	VE 20%	25	vitamin E 20%

[a] GC = Gluconal Cal, a mixture of calcium gluconate and lactate, containing 10–11% calcium. [b] ZL = zinc lactate, containing 23.5% zinc. [c] VE = tocopheryl acetate, a vitamin E acetate oil, 1 mg is equivalent to 1 IU of vitamin E. Acetylated monoglyceride at a ratio of 4:1 (w/w) to VE was added into VE formulations.

Large films were then formed based on the knowledge obtained from the small films. A Teflon-coated glass plate with an area of 260 × 260 mm^2 was used for film casting. To control the thickness of films containing different types and concentrations of nutraceuticals, the amount of FFS pouring on each plate was calculated as

$$W_s = \frac{T \times A \times D \times 10^{-2}}{W_{d/s}}$$

where W_s is weight of FFS (g), T is the targeting thickness of film (μm), A is the area of the casting plate (cm^2), D is the density of the film (g/cm^3), and $W_{d/s}$ is the percent weight of the film to FFS. The values of D and $W_{d/s}$ were obtained from the study of the small films.

A calculated amount of each FFS was cast on the leveled Teflon-coated glass plate with a designated film thickness of 100 μm, dried, and conditioned following the same procedures as used for the small films. The films were then used for the testing of opacity, water barrier, and mechanical and thermal properties within 14 days of storage at 25 °C and 50% RH.

Measurement of Film Thickness, Density, and Moisture Content. Film thickness was measured with a caliper micrometer (No. 293-766-30, Mytutoyo Manufacturing Co. Ltd., Japan). Film samples were cut into 70 × 70 mm^2 segments for the water vapor permeability test, 25 × 86 mm^2 for the tensile and elongation test, and 25 × 25 mm^2 for the puncture test. For each film specimen, five thickness measurements were randomly taken at different locations. The means were calculated and used in the determination of water barrier and mechanical properties.

Film density was calculated by dividing the film weight by the film volume, where the film volume was calculated by multiplying the film area by the thickness. The moisture content of the films was determined gravimetrically by drying the film specimen at 105 °C for 18 h in a forced-air oven (Precision Scientific Inc., Chicago, IL) (*2*). The percentage of moisture content was calculated in a wet base.

Film Color and Opacity. The color of the films was determined with a colorimeter (LabScan II, Hunter Associate Laboratory, Inc., Reston, VA). The film specimen was covered with a Hunterlab white standard plate (No. LS-12301, Hunter Associates Laboratory, Inc., Reston, VA) and the CIE (Commission Internationale de l'Eclairage) L^*, a^*, and b^* values were recorded (*12*). The total color difference ($[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$) was calculated, where the white standard plate was used as a reference ($L_{ref} = 93.49$, $a_{ref} = -0.25$, $b_{ref} = -0.09$). Three readings on different sites of each film were recorded.

The film opacity was determined by measuring the film absorbance at 600 nm with a spectrophotometer (UV160U, Shimadzu Corporation, Japan) (*13*). Results were reported as absorbance divided by film thickness (mm) based on three replications. Three film specimens were used for each replicate.

Water Vapor Permeability (WVP). WVP was determined by using a cup method at 25 °C and 100/50% RH gradient, following ASTM E 96 (*14*). Eleven milliliters of distilled water was placed in each test cup made of Plexiglas with an inside diameter of 57 mm and an inner depth of 15 mm. The distance between the water and the film was 10.686 mm and the effective film area was 25.5 cm^2. Test cup assemblies were placed in the same temperature- and humidity-controlled chamber conditioned at 25 °C and 50% RH. Each cup assembly was weighed every hour for 7 h on the electronic balance (0.0001 g accuracy) to record moisture loss over time. WVP was then corrected for resistance of the stagnant air gap between the film and the surface of water by using the WVP correction method (*15*). Three replicates of two film specimens were analyzed.

Mechanical Properties. Mechanical properties of the films were determined with a texture analyzer (TA.XT2i, Texture Technologies Corp., Scarsdale, NY). Prior to the measurements, film samples were conditioned in the same environmental chamber at 25 °C and 50% RH for at least 2 days. All property measurements were performed immediately after removing film specimens from the chamber to minimize moisture variances of these natural hydrophilic films.

The ASTM D882 method (*16*) was used for measuring tensile strength (TS) and percent elongation at the break (EL). Each film specimen (25 × 86 mm^2) was mounted between the grips (TA 96) of the texture analyzer and tested with an initial grip separation of 50 mm and crosshead speed of 1 mm/s. The TS value was reported as measured maximum load (*N*) divided by film cross-sectional area (mm^2) with units of MPa. EL values were obtained by recording elongation at the break divided by the initial length of the specimen and multiplying by 100.

The ASTM D 6241 method (*17*) was used with some modifications for the puncture strength and deformation measurement. The film specimen (25 × 25 mm^2) was mounted onto an extensibility fixture (TA-108S) with a circular opening 10 mm in diameter and uniformly secured with an upper clamping device to prevent slippage during the test. A 2-mm diameter puncture probe (TA 52) was compressed through the center of the film specimen at 1 mm/s. The applied force and the deformation length at the puncture point were recorded. The puncture strength (*N*) was calculated by dividing the applied force by the film thickness to eliminate the thickness variation effect (*18*). Three replicates of five film specimens were analyzed for all mechanical property measurements.

Thermal Analysis. Differential scanning calorimetry (DSC) measurements were performed on a Pyris 6 DSC system (Perkin-Elmer Instruments, Shelton, CT). About 5 mg of each film-forming component or film conditioned at 25 °C and 50% RH for 2 days was sealed in a standard aluminum pan and rapidly cooled to −10 °C with liquid nitrogen. The DSC curve was obtained in a temperature range of −10 to 360 °C at a heating rate of 20 deg/min under dry nitrogen flow. The first runs of scanning without any preheat treatments were used to observe the thermal properties of the films, including the effect of moisture trapped inside the film matrix. The miscibility of chitosan with incorporated mineral or vitamin and the thermal stability of the films were evaluated.

Statistical Analysis. A completely randomized design (CRD) was used as an experimental design, where 10 chitosan-based film-forming formulations containing different concentrations of calcium, zinc, or vitamin E were applied as treatments. Chitosan alone films were used as a control. All experiments were replicated three times. In each replication, 5 film specimens were prepared for mechanical property measurements and 3 film samples were used for density, moisture content, and color measurements. The general linear models (GLM) procedure was applied in testing differences among different films, using the SAS (Statistical Analysis System Institute Inc., Cary, NC). PROC GLM for analysis of variance (ANOVA) was performed for all treatments and PROC REG was performed to find the best fitting regression model for each measured response. Duncan's multiple-range test was used for the multiple means comparisons. A 95% confidence level was applied for all statistical analyses.

RESULTS AND DISCUSSION

Rheology Properties of FFS. The pH values and viscosity of FFS containing different concentrations of mineral or vitamin are reported in **Table 2**. The pH of GC-incorporated FFS significantly ($P < 0.05$) increased with increasing GC concentration, while the intrinsic viscosity decreased. These trends were also observed in ZL-incorporated FFS, but the difference was not statistically significant, which may be due to the small amount of ZL incorporation compared with that of GC addition. The addition of VE did not significantly change the pH and viscosity of FFS except for FFS with 20% VE showing a slight increase in viscosity ($P < 0.05$).

Addition of multivalent cations generally develops gel structure in protein and polysaccharide molecules (*2*, *18*). However, minerals tested in this study did not initiate gel formation in the chitosan molecule structure. The intrinsic viscosity of chitosan, which behaves as a polycationic electrolyte in acidic solution by the protonation of amine groups, is affected by pH and the ionic strength of solutions (*19*, *20*). Hydrogen bonding is a main binding force in chitosan film formation. The increasing ionic strength with addition of mineral salts may elicit additional ionic interaction between neighboring segments and weaken intermolecular hydrogen bond formation, thus resulting in decreased intrinsic viscosity. The pH increase in mineral-incorporated FFS resulted from the increased counterion associations, because calcium and zinc salts have higher pH values than that of chitosan alone solution (3.23 as shown in **Table 2**).

Calcium is the most difficult mineral to fortify because of its high DRI value (1000 mg/day for 25−65 years of age female) and low water solubility. Gluconal Cal (GC) has been commercially used in the calcium fortification of beverages because of its high water solubility, high nutritional value, and neutral taste. It was demonstrated in our previous studies that different film-forming materials have different interactions and carrying capabilities to high concentration of calcium. For example, a high concentration of GC was dissolved well in calcium caseinate and xanthan gum solutions without significant alternation of the basic function of FFS, but not whey proteins, where strong gels formed (*1*, *2*). This study verified the hypothesis of using chitosan to carry a high concentration of calcium. Zinc lactate is a commonly used form of zinc ester, and has a water solubility of 5.3% (w/w) at 25 °C. The relatively low DRI value (8−9 mg/day for adults) of zinc makes it less of a challenge to fortify zinc in edible films and coatings than that of calcium. The DL-α-tocopheryl acetate (vitamin E acetate) used in this study is a stable form of vitamin E, can be exposed to light, air, and heat, and has demonstrated its feasibility and stability when incorporated with xanthan gum and applied as edible coatings on peeled baby minicarrots (*1*).

Film Formation and Basic Physical Properties of Films. All films were easily peeled from the film-casting plates, except for the GC200 film, which was brittle at low RH (~30%) condition and became more coherent with increasing RH (Data not shown). Thin film formation was not easy for GC200 films due to the high surface tension on the Teflon-coated surface. To maintain a uniform film thickness of GC200 films, disposable polyethylene Petri dishes (Krackeler Scientific, Albany, NY) were used as a casting base instead of Teflon-coated plates. Peeled films were then conditioned at 50% RH to obtain soft, flexible, and easy to handle films. Chitosan/VE films were softer than other films, and have a yellowish color.

Table 2. The pH and Viscosity of Chitosan-Based Film-Forming Solutions Containing Different Concentrations of Mineral or Vitamin E[a]

Film type	PH	Viscosity (cp)
Control	3.23 ± 0.06	52.7 ± 0.75
GC10	3.27 ± 0.06	52.0 ± 1.30
GC50	3.43 ± 0.05	48.9 ± 2.03
GC100	3.55 ± 0.02	47.1 ± 2.03
GC200	3.74 ± 0.06	44.3 ± 0.65
	pH_{GC}=-7*10^{-6}GC^2+0.004*GC+3.23 R^2 = 0.998	V_{GC} = 2*10^{-4}GC^2-0.0749*GC+52.63 R^2 = 0.995
ZL5	3.24 ± 0.12	52.4 ± 1.50
ZL10	3.25 ± 0.12	51.1 ± 2.03
ZL20	3.29 ± 0.10	50.2 ± 0.81
	pH_{ZL} = 9*10^{-5}ZL^2+0.0011*ZL+3.23 R^2 = 0.998	V_{ZL} = 1.4*10^{-3}ZL^2-0.1608*ZL+52.83 R^2 = 0.951
VE5	3.24 ± 0.12	52.0 ± 2.31
VE10	3.27 ± 0.17	52.0 ± 2.31
VE20	3.27 ± 0.20	53.0 ± 2.55
	pH_{VE} = -10^{-4}VE^2+0.0051*VE+3.23 R^2 = 0.897	V_{VE} = 9*10^{-3}VE^2-0.163*VE+52.67 R^2 = 0.986

[a] Means of 3 measurements ± standard deviation for pH and viscosity of film=forming solutions. GC = Gluconal Cal, a mixture of calcium gluconate and lactate, containing 10–11% calcium; ZL = zinc lactate, containing 23.5% zinc; VE = tocopheryl acetate, a vitamin E acetate oil, 1 mg is equivalent to 1 IU of vitamin E. Acetylated monoglyceride at a ratio of 4 to 1 VE (w/w) was added into the chitosan/VE film formulations. GC, ZL, and VE in the equations represent the percentage of Gluconal Cal, zinc lactate, and vitamin E (w/w chitosan) added in film- forming solutions.

Table 3. Physical Properties of Chitosan-Based Films Incorporated with Mineral or Vitamin E[a]

film type[d]	solid matter[b] (%)	thickness[c] (μm)	density (g/mL)	moisture content (%)
control	4.15 ± 0.06	49.3 ± 7.1	1.35 ± 0.06	19.2 ± 1.55
GC10	4.27 ± 0.07	52.1 ± 5.2	1.36 ± 0.09	19.4 ± 1.48
GC50	5.29 ± 0.14	69.4 ± 4.8	1.38 ± 0.04	15.7 ± 1.66
GC100	6.27 ± 0.04	82.5 ± 2.3	1.44 ± 0.04	14.8 ± 0.97
GC200	8.20 ± 0.15	103.3 ± 8.1	1.42 ± 0.04	14.9 ± 0.79
ZL5	4.47 ± 0.10	55.7 ± 3.8	1.35 ± 0.07	20.1 ± 0.82
ZL10	4.58 ± 0.13	51.3 ± 4.1	1.37 ± 0.03	17.3 ± 4.21
ZL20	4.62 ± 0.05	54.1 ± 3.1	1.39 ± 0.03	17.8 ± 3.8
VE5	4.99 ± 0.10	63.2 ± 4.3	1.34 ± 0.03	21.0 ± 2.47
VE10	5.46 ± 0.19	68.5 ± 6.3	1.31 ± 0.01	20.2 ± 2.26
VE20	6.31 ± 0.21	76.7 ± 7.2	1.24 ± 0.01	20.3 ± 2.54

[a] Means of 9 measurements ± standard deviation. [b] Percent weight ratio between conditioned dry film to 10 g of film-forming solutions. [c] Uncontrolled thickness of films after drying 10 g of film-forming solutions. [d] GC Gluconal Cal, a mixture of calcium gluconate and lactate, containing 10–11% calcium; ZL = zinc lactate, containing 23.5% zinc; VE = tocopheryl acetate, a vitamin E acetate oil, 1 mg is equivalent to 1 IU of vitamin E.

Table 3 shows the basic film properties, including percent solid matter, film thickness, density, and moisture content. Percent solid matter and film thickness increased along with an increased amount of mineral or vitamin E added into the FFS, where linear equations can be used to describe the weight (W) and thickness (T) of chitosan/GC and chitosan/VE films as a function of GC or VE concentration.

$$W_{GC} = 0.0204GC + 4.1646,\ R^2 = 0.998;$$
$$T_{GC} = 0.2612GC + 50.667,\ R^2 = 0.975$$

$$W_{VE} = 0.1042VE + 4.316,\ R^2 = 0.972;$$
$$T_{VE} = 1.4187VE + 52.678,\ R^2 = 0.938$$

where W_{GC} and W_{VE} are the percentage of solid matter of chitosan/GC and chitosan/VE films, respectively (%); T_{GC} and T_{VE} are the thickness of chitosan/GC and chitosan/VE films, respectively (μm); and GC, ZL, and VE are the percentage of Gluconal Cal, zinc lactate, and vitamin E (w/w chitosan) added into FFS (%). However, such a linear relationship was not observed in chitosan/ZL films, which may be explained as the relatively small amount of ZL added into FFS is not sufficient to reflect its influence on these physical properties. The film thickness dependency on the concentration of mineral and vitamin induced the needs to control film thickness for the evaluation of film functionality, such as WVP and mechanical properties, because film thickness significantly affects these properties (*21*). In this study, film thickness was controlled in a range of 93 to 103 μm by using the equation described before.

Different impacts between mineral and vitamin E on the film density were observed, where the film density increased with increasing GC or ZL concentration, but decreased with increased VE concentration. The compactness of the film structure associated with the filling of small mineral molecules into chitosan molecules may cause the density increase, while the bulky structural nature of chitosan/VE films resulted in a decrease in the film density. Wong et al. (*22*) reported a loose and spongelike microstructure of acetylated monoglyceride incorporated chitosan film in their scanning electron microscopy study.

The moisture content of chitosan-based films decreased with mineral incorporation, but increased with the addition of VE. An increase in moisture contents by GC or VE incorporation into milk protein-based films was reported (*2*). It was hypothesized that calcium salts as a humectant absorb and keep the moisture inside the film matrix, thus increasing the moisture content of protein-based films. Chitosan-based film did not seem to form the three-dimensional cross-linking bridges with GC addition found in milk protein films. The occupation of

Table 4. Color and Opacity of Chitosan-Based Films Incorporated with Mineral or Vitamin E[a]

film[d]	*b**	total color diff (ΔE)[b]	opacity (Abs_{600}/mm)[c]
control	15.43 ± 2.32	16.65 ± 2.33	0.79 ± 0.03
Ca10	12.70 ± 0.43	13.92 ± 0.43	0.78 ± 0.17
Ca50	10.97 ± 0.66	12.16 ± 0.65	0.61 ± 0.01
Ca100	9.19 ± 0.71	10.48 ± 0.78	0.50 ± 0.08
Ca200	6.31 ± 0.44	7.58 ± 0.47	0.42 ± 0.11
ZL5	13.59 ± 0.68	14.72 ± 0.69	0.57 ± 0.07
ZL10	13.86 ± 1.44	14.99 ± 1.45	0.64 ± 0.03
ZL20	13.51 ± 1.22	14.72 ± 0.69	0.78 ± 0.11
VE5	21.48 ± 1.34	22.52 ± 1.39	8.36 ± 2.69
VE10	22.29 ± 1.70	23.27 ± 1.78	10.92 ± 3.01
VE20	21.71 ± 0.97	22.61 ± 1.07	13.09 ± 1.57

[a] Means of 9 measurements ± standard deviation. [b] White color standard plate was used as a reference (L = 93.49, a = –0.25, b = –0.09). [c] Absorbance at 600 nm divided by film thickness (mm). [d] GC = Gluconal Cal, a mixture of calcium gluconate and lactate, containing 10–11% calcium; ZL = zinc lactate, containing 23.5% zinc; VE = tocopheryl acetate, a vitamin E acetate oil, 1 mg is equivalent to 1 IU of vitamin E.

hydrophilic portions of the chitosan molecule by counterion interactions and the increased compactness of the film matrix with mineral incorporation may cause limited access of water molecules to hydrophilic chitosan polymer chains, thus resulting in less moisture in the film matrix. The bulky spongelike structure of chitosan/VE films (*22*) may enhance the entrapment of water molecules in the film structure, causing a slight increase in moisture content despite the increased hydrophobicity with AM addition. In addition, the moisture content may be overestimated with evaporation of AM and VE along with water vapor during drying of the film specimen at 105 °C.

Film Opacity and Color. The b^*, ΔE, and opacity of chitosan/GC films significantly ($P < 0.05$) decreased with increasing GC concentration, while incorporation of VE resulted in increased b^* and ΔE values (**Table 4**). Hence, chitosan films incorporated with GC or ZL were less yellow and more transparent than chitosan alone films, while the films with VE were more yellow and less transparent. The b^* value is probably the most important parameter describing the color of chitosan-based films due to the natural yellowish color of chitosan, and is the predominant parameter influencing the total color difference (ΔE) of the films studied here. The even yellowish color distribution in chitosan/VE films demonstrated that small oil droplets are evenly distributed and emulsified into film-forming matrix by homogenization process. Chitosan/VE films are emulsion films formed with adding emulsifying agent and applying vigorous mechanical work to reduce interfacial tension between two phases. This heterogeneous matrix of emulsion film and solidification of AM at ambient drying temperature may cause a significant yellowish color and increased opacity of chitosan/VE films.

Water Vapor Permeability. Water vapor permeability (WVP) of chitosan-based films incorporated with mineral or vitamin E is shown in **Figure 1**. Film thickness was controlled in the range of 93−101 μm for all the films. The water vapor barrier property of the films was significantly improved by incorporation of mineral or vitamin E in the film matrix ($P < 0.05$). The WVP of the films can be described by the following secondary polynomial equations:

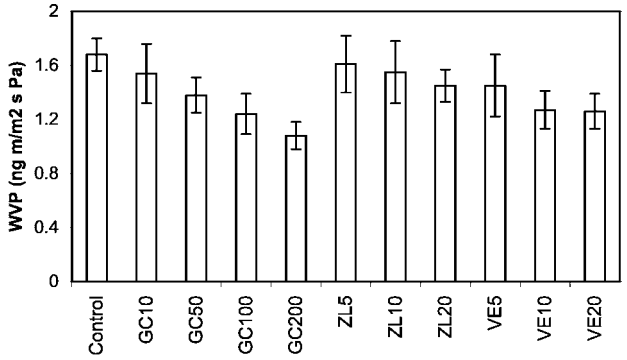

Figure 1. Water vapor permeability (WVP) of chitosan-based film incorporated with mineral or vitamin E (n = 6). Film thickness was controlled in a range of 93 to 101 μm for all films tested. GC = Gluconal Cal, a mixture of calcium gluconate and lactate; ZL = zinc lactate; and VE = tocopheryl acetate, a vitamin E acetate oil.

$$WVP_{GC} = 10^{-5}GC^2 - 0.0056GC + 1.6376,\ R^2 = 0.981$$

$$WVP_{ZL} = 0.0002ZL^2 - 0.0146ZL + 1.6797,\ R^2 = 1.00$$

$$WVP_{VE} = 0.0019VE^2 - 0.0595VE + 1.6855,\ R^2 = 0.997$$

where WVP_{GC}, WVP_{ZL}, and WVP_{VE} are the water vapor permeability of chitosan/GC, chitosan/ZL, and chitosan/VE films (ng m/m^2 s Pa).

The effects of GC or ZL addition on the WVP may be explained by the similar theory developed in moisture content evaluations. By adding a high concentration of mineral salts into the film matrix, counterion interactions increase among adjacent molecular structures and small mineral ions act as fillers, thus resulting in a decrease in diffusivity of water vapor through a film matrix and a decrease in hydrophilic tendency of chitosan films. Chitosan films, like many other protein or polysaccharide edible films, exhibited relatively low water barrier characteristics (*23*). The influence of hydrophobic additives, such as VE and AM, on the WVP of films is generally expected to improve the water vapor barrier properties by providing hydrophobicity and increasing film resistance to water transmission. Similar WVP reduction trends were observed in a previous study on calcium caseinate-based films containing a high concentration of GC or VE (*2*).

Mechanical Properties. The tensile strength of chitosan-based films was significantly affected by the addition of GC or VE, while the effect of zinc was ignorable (**Table 5**). The higher the GC or VE concentration in the film matrix, the lower the tensile strength. Secondary polynomial equations can be used to describe the tensile strength (TS) of chitosan films containing different concentrations of GC or VE:

$$TS_{GC} = 0.0001GC^2 - 0.0456GC + 8.1868,\ R^2 = 0.994$$

$$TS_{VE} = 0.0103VE^2 - 0.4216VE + 7.9412,\ R^2 = 0.989$$

where TS_{GC} and TS_{VE} are the tensile strength of chitosan/GC and chitosan/VE films, respectively (MPa).

The percent elongation of the films decreased with increasing GC concentration, but slightly increased with the addition of ZL or VE at 5 and 10% levels ($P < 0.05$). Further increase in ZL or VE concentration did not increase the elongation values. Puncture strength were significantly increased in ZL incorporated films, but decreased in VE films. GC additions did not significantly ($P > 0.05$) alter the puncture strength of the films

Table 5. Mechanical Properties of Chitosan-Based Films Incorporated with Mineral or Vitamin E[a]

film type[b]	thickness (μm)	tensile strength (MPa)	elongation at break (%)	puncture strength (N/mm)	puncture deformation (mm)
control	93.4 ± 6.7	8.04 ± 2.02	81.33 ± 18.93	71.90 ± 14.04	5.46 ± 0.42
GC10	95.1 ± 10.6	7.96 ± 1.31	75.38 ± 16.49	73.92 ± 25.04	4.89 ± 0.51
GC50	96.7 ± 9.9	6.10 ± 0.91	66.69 ± 13.38	72.37 ± 12.89	5.15 ± 0.43
GC100	95.5 ± 5.1	4.87 ± 1.05	70.79 ± 18.50	78.80 ± 25.46	5.83 ± 2.48
GC200	103.5 ± 10.2	3.88 ± 0.45	60.23 ± 10.77	63.76 ± 13.20	6.33 ± 1.44
ZL5	93.1 ± 6.6	8.15 ± 1.78	92.71 ± 28.36	83.06 ± 24.38	5.43 ± 0.30
ZL10	92.3 ± 8.6	7.78 ± 1.09	94.93 ± 25.40	80.85 ± 17.07	5.36 ± 0.20
ZL20	96.4 ± 4.7	7.71 ± 1.22	76.73 ± 16.55	87.38 ± 15.47	5.09 ± 0.34
VE5	96.9 ± 9.7	5.83 ± 2.01	92.30 ± 21.60	43.30 ± 23.08	5.15 ± 0.73
VE10	99.2 ± 7.5	4.95 ± 0.81	92.28 ± 26.34	32.60 ± 11.61	5.23 ± 0.72
VE20	94.8 ± 7.9	3.59 ± 1.10	83.65 ± 12.25	31.29 ± 11.74	4.94 ± 0.50

[a] Means of 15 measurements ± standard deviation, except thickness data which were means of 30 measurements ± standard deviation. [b] GC = Gluconal Cal, a mixture of calcium gluconate and lactate, containing 10–11% calcium; ZL = zinc lactate, containing 23.5% zinc; VE = tocopheryl acetate, a vitamin E acetate oil, 1 mg is equivalent to 1 IU of vitamin E.

except for the GC200 film, where the value decreased. The puncture deformation was not significantly ($P > 0.05$) changed by the addition of mineral or vitamin E.

The tensile strength is the measurement of maximum strength a film can withstand against applied tensile stress, and percent elongation represents the ability of films to stretch. While the puncture strength is the measurement of hardness of a film under the stress applied at right angles to its surface that generates multidirectional forces (*17*), the puncture deformation is the measurement of film elasticity under vertically loaded stress. Both tensile and puncture strength values can be used as measurement of hardness of films. No significant correlations between these values were observed in this study.

Film strength strongly depends on the crystallites created in the film structure. The mineral and its salt molecules possibly disrupt the crystalline parts of the films. Our results demonstrated that the chitosan molecule could withstand a high load of mineral or vitamin but resulted in a decrease in tensile strength. These results were consistent with previous findings in milk protein-based films (*2*). Reduction in both tensile and puncture strength indicated that introduction of GC or VE/AM into the film matrix could interrupt the crystalline structure formation in the chitosan matrix and weaken the intermolecular hydrogen bonding. The increased percent elongation in the chitosan/ZL film may be explained by the plasticization effects of small molecules acting as fillers to reduce molecular reactions and cohesion of films. However, films with a high concentration of ZL became less stretchable because of the saturation of the fillers. AM and VE may act as plasticizers in film structure, thus resulting in increased elongation of films. AM, the ester form of monoglyceride, is one of the most often used plasticizer in the area of lipid-based edible coating and films (*24*).

Thermal Properties. DSC thermograms of chitosan films are shown in **Figure 2**. Most of the chitosan films showed an endothermic peak around 100 °C associated with the dehydration of absorbed water in film matrix and a major thermal degradation exothermic peak around 325 °C. A distinct endothermic peak around 34 °C was observed in chitosan/VE films, which represents the melting of AM in the film matrix. The peak heights were increased with increased VE/AM concentration. The thermal degradation temperature of chitosan/VE films was lowered to about 286 °C with VE addition, resulting in a decrease of thermal stability. The addition of VE also shifted the water evaporation endothermic peak to below 100 °C. These results verified the plasticizing activity of VE and AM in the film matrix. Changes in the thermal properties of chtiosan/VE films also reflected their effects on the mechanical properties

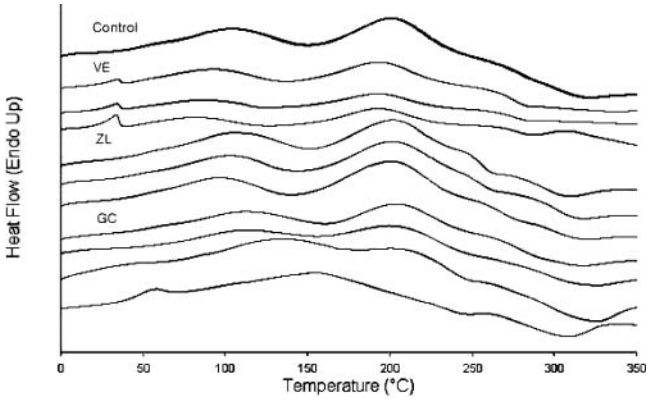

Figure 2. DSC thermograms of chitosan-based films incorporated with mineral or vitamin E. From the top to bottom: control, VE5 to VE20, ZL5 to ZL20, and GC10 to GC200. GC = Gluconal Cal, a mixture of calcium gluconate and lactate; ZL = zinc lactate; and VE = tocopheryl acetate, a vitamin E acetate oil.

of films, i.e., reduced film strength and increased film elongation. There were no significant differences in DSC thermograms among chitosan alone films and chitosan/ZL films except for a slight decrease in two major endothermic peak temperatures with increased ZL addition.

Chitosan/GC films showed the most significant differences among tested chitosan films. These differences may be caused by the complicacy of GC, a mixture of calcium lactate and calcium gluconate, and its relatively high incorporation levels compared to those of ZL or VE. With increasing GC concentration, two major endothermic peaks were merged around 150 °C. The thermograms of pure GC powder showed a sharp exothermic decomposition peak at around 200 °C (data not shown), where most films showed strong endothermic relaxation. This overlap at 200 °C might diminish the endothermic peak, resulting in merged endothermic peak formation in GC200 films. An endothermic peak at 58 °C in GC200 film could be caused by the thermal relaxation of calcium lactate. The pure calcium lactate showed a strong melting peak at 79.9 °C with onset temperature at 73.8 °C.

One significant observation in this study was the large endothermic peaks around 200 °C in all film types except GC200 films. These peak temperatures tend to shift to lower temperatures with increased mineral or vitamin concentration. This endothermic peak might associate with the lactic acid or lactate salts in film matrix since the peak at 200 °C appeared only in chitosan films prepared from lactic acid solution. No

peak was observed at 200 °C in the DSC curve of pure chitosan powders used in this study and the peak was observed at around 240 °C in the chitosan film made with acetic acid solution (data not shown). Sakurai et al. (*25*) estimated the α-relaxation of pure chitosan at 205 °C.

CONCLUSIONS

This study quantified the basic functionality of chitosan-based films containing different types and concentrations of mineral or vitamin. Mathematical equations were developed to coordinate the selected film functionality with the type and concentration of mineral and vitamin. These equations can be used as a tool to predict the functionality of such films. The study has clearly demonstrated that chitosan is a good film-forming material for developing nutritionally fortified edible films and coatings. Such films or coatings would provide alternative ways to fortify foods that otherwise cannot be fortified, such as fresh fruits, vegetables, and other unprocessed food items. The specific formulation of such films or coatings strongly depends on their intended applications, in which the concentration of mineral or vitamin E incorporated into the films needs to be carefully selected to meet required water barrier, mechanical, and thermal properties of the films. The feasibility and stability of these coatings applied to fresh fruits for enhancing storability and nutritional values are under evaluation now.

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