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Preparation and characterization of PVA/starch blend composite film based on modified chicken feather protein and effect of plasticizer type on its properties

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ABSTRACT

Edible films were prepared from maleic anhydride modified alkaline hydrolyzed chicken feather protein, poly vinyl alcohol and starch. The effect of glycerol and 1,2- propandiol as plasticizers on protein based film was investigated. Intermolecular interaction between film components was investigated using FTIR. Morphology using scanning electron microscopy (SEM) reflects films of good appearances. Thermal analysis (TGA) of edible films indicates that glycerol – plasticized-PVA/starch/modified protein film is more thermaly stable than 1,2-propanediol-plasticized PVA/ starch/modified protein. Glycerol has significant effect on % elongation and oxygen permeability (OP) more than 1,2- propanediol. Edible films containing proteins have good tensile strengths. The lowest OP was obtained from protein based film without plasticizer, which can make these films suitable as sachets or pouches for dry foods, thus reducing the need for plastic materials Antimicrobial activity of selected edible films was studied.

KEYWORDS

Biopolymer;
Film;
Feather protein;
Oxygen permeability.

INTRODUCTION

Biopolymer-based packaging materials originated from naturally renewable resources such as polysaccharides, proteins, and lipids have become the focus of worldwide attention in recent years since such biopolymers offer favorable environmental advantages of recyclability and reutilization compared to conventional petroleum-based synthetic polymers. Biopolymer films may also serve as gas and solute barriers and complement other types of packaging by minimizing food quality deterioration and extending the shelf life of foods[1-3]. In particular, proteins derived from various animal and plant sources have successfully been formed into films and/ or coatings along with their film properties being quantified[4-6]. Moreover, biopolymer-based films and coatings can act as efficient vehicles for incorporating various additives including antimicrobials, antioxidants, coloring agents, and other[7,8]. The formation of edible films and coatings composed of whey is not only important in finding new uses for whey proteins, but also in improving the microbial stability of foods particularly in presence of preservatives in film formulations. Whey protein films produced without addition of any plasti-

cizers were very brittle, requiring a food grade plasticizer to add film flexibility[9,10]. Polyols including sorbitol and glycerol have been reported to decrease attractive intermolecular forces along protein chains, thus increasing flexibility and reducing brittleness However, starch as polysacharide which dissolve easily in water, do not present mechanical and shape stabilities in fluids. An effective method for overcoming this issue is to blend it with synthesized polymer gel networks in order to form natural and synthesized polymer blend hydrogels. Among the existing synthesized polymers, PVA possesses many useful properties, such as excellent chemical resistance, optical and physical properties[11], good film-forming capability, water solubility[12], and an excellent biocompatibility[13]. The strength, flexibility, and water resistance of starch productions improved when PVA aqueous solution was added[14]. The objectives of this research was preparing multicomponent films and investigate the effect of different constituents as plasticizer, and active edible protein extracted from chicken feathers on their mechanical properties, heat stability, oxygen permeability, and antimicrobial activity. By combining proteins, starch and PVA, the advantages of each may be exploited to form improved films.

EXPERIMENTAL

Materials

PVA [degree of polymerization:17,000–18,000; and hydrolyzed between 99.0% and 99.8% from poly (vinyl acetate) used in this study] was obtained from M/s. Loba Chem (India) and corn starch was obtained from M/s. Riddhi Siddhi Chemicals Ltd. (India). Analytical grade glycerol was purchased from Fluka Biochemika (ultra, > 99.5). 1,2-propandediol with 99 % purity was purchased from BDH Chemical LdDH Poole, England. Laboratory grade NaOH pellets. Maleic anhydride-modified alkaline hydrolyzed chicken feather protein was used as raw material and prepared as follows:

(a) Preparation of maleic anhydride modified alkaline hydrolyzed chicken feather protein

Alkaline hydrolyzed protein 10 grams in 140 ml water was stirred for 120 minutes at room temperature. The resulting mixture was adjusted to pH 10 and stirred at 50 ^{0}C for 120 minutes. Maleic anhydride 0.5 g was added to the alkaline hydrolyzed chicken feather protein. The reaction mixture was further stirred for one hour then diluted to 1000 ml with distilled water. Finally, the diluted solution was concentrated to 100 ml[15].

Film preparation

Formation of protein/PVA/starch films was based on a solution casting and evaporation method[16]. Where the solid content was 7.5% and plasticizer was added at 13 % based on solid content. A control film was formed using mixtures of starch and PVA weight ratio 1:1. Starch was dispersed in 50 ml of distilled water and stirred with magnetic bar at 60 ^{0}C until gelatination of starch occur. PVA (dissolved in 50 ml distilled water) was poured into starch solution, then the plasticizer. The solution mixture was then homogenized for about 15 min. Stirring and heating were ended when the solution reaches temperature of 80-86 ºC. The film forming solution was spread evenly into a Teflon coated metal plate with (14x 14 cm) and allowed to dry in an oven at 75 ºC. The films were then easily peeled from the plate. Sample film prepared from alkaline hydrolyzed chicken feather proteins, starch, PVA, and plasticizer. The three components of this sample film were added in equal ratios.

Film structural characterization

The FT-IR spectra were performed by a JASCO FT-IR-6100 Fourier transform infrared spectrophotometer using the KBr pellet disk method for transmittance measurements. The surface morphologies of film samples were characterized by scanning electron microscopy (SEM) using PROBE Micro Analyzer Microscope. The samples were prepared by deposition of a thin gold film, sputtered using a Balzers SCD 050 deposition system. The mechanical behavior of the prepared films was analyzed with a LLOYD Instrument, Model LR 10K with a load cell of 100 N. Experiments were performed with a crosshead speed and distance between jaws of 5 mm/min and 50 mm, respectively at room temperature, 25 ^{0}C. The dimensions of the test samples were: length 100 mm, width 16.5 mm and thickness 1.0 mm. Extension percent and tensile strength were calculated on the basis of initial sample dimen-

sions, and the results were averaged over four measurements.

Thermal stabilities of prepared films, TG and DTA analysis were carried out at heating rate 10 ^{0}C/ minute from 50- 650 ^{0}C on Perkin Elmer Thermo Gravimetric Analyzer. Oxygen permeability (OP)) of film was measured at 23 ^{0}C and 50 ± 1%RH and pressure 4 par using an Oxygen Permeability tester (Lyssy OPT 5000, United Kingdom).

Antimicrobial activity

(a) Microorganisms

The microorganisms used for the experiment were obtained from Microbiological Resources Center (Cairo MIRCEN). *Escherichia coli* O157:H7 and *Salmonella enteritidis* (as Gram negative bacteria), *Listeria monocytogenes, Bacillus cereus* and *Staphylococcus aureus* (as Gram positive bacteria).

(b) Antimicrobial substances

Antimicrobial substances used for the experiment (glycerol plasticized PVA/starch film and glycerol plasticized PVA-starch / modified alkaline hydrolyzed chicken feather protein with maleic anhydride blend films) were tested as diluted in water at concentration of 1%.

(c) Antibacterial assay using the disc diffusion method

The antibacterial activity of diluted substances (glycerol plasticized PVA/starch film and glycerol plasticized PVA-starch / modified alkaline hydrolyzed chicken feather protein with malei tose Soy Agar (TSA), c anhydride blend films) were tested by disc diffusion method on Tryp[17]. The concentration of pathogenic bacteria inoculated in TSA was 10^6 CFU/ml over night activated culture. All experiment was performed in duplicate. Sterilized filter paper discs (Whatman type 1, 0.6 cm in diameter) were placed on the surface of TSA and 20µl of the diluted substances were added to filter paper disc. Plates were incubated aerobically for 24h at the optimum temperature of the indicator bacteria (37°c for *Staphylococcus aureus, Salmonella enteritidis, Listeria monocytogenes* and *Escherichia coli* O157:H7) whereas 30°c for *Bacillus cereus*. The inhibition zone diameter was measured (including the filter paper disc).

RESULTS AND DISCUSSION

FTIR characterization

Figure 1 explain FTIR spectra of maleic anhydride modified chicken feather protein and glycerol-plasticized PVA/starch/ modified protein. The broad band at 3287 cm^{-1}, represents a typical -NH free of amino group, while in this spectrum part a shoulder at 3216 cm^{-1} which is a typical of -NH bonded is only slightly pronounced. The band at 1735 cm^{-1} was attributed to typically carbonyl group of ester bond indicating formation of ester between maleic anhydride and amino acids. The fact that, the absorption band at 1657 and 1549 cm^{-1} associate with –NH groups are in agreement with amide I and amide II bands of protein. The band is at 1084 cm^{-1} for -C-O stretching vibration. Glycerol-plasticized PVA/ Starch blend composite film based on modified alkaline hydrolyzed chicken feather protein with maleic anhydride showed a significant shift, in stretching vibration of carbonyl ester group between maleic anhydride and protein from 1735 cm^{-1} to 1718 cm^{-1} may this result suggests that the hydrogen bonding is mainly formed between the hydroxyl of glycerol and or hydrogen atom of amino group and -CO groups. These results indicated that main interaction between blend components occur through formation of hydrogen bonding has been suggested that contribute to the vibrational stretching associated to free and intermolecular bound hydroxyl and amino groups of starch, glycerol, PVA, and modified protein.

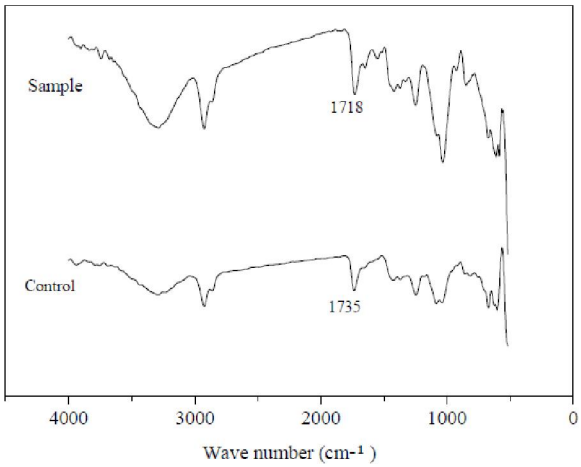

Figure 1 : FTIR spectra of maleic anhydeide modified chicken feather protein (control) and glycerol-plasticized PVA/starch/ maleic anhydrid modified chicken feather protein (sample)

FULL PAPER

Scanning electron microscope

Figure 2 shows microscope images of glycerol-plasticized PVA/ starch film (1), and glycerol-plasticized PVA/starch blend film based on modified chicken feather protein (2). Glycerol-plasticized PVA/ starch film surface appear to be relatively smooth, homogeneous and to be a continuous matrix with apparent regular starch cracks without any phase separation throughout the film surface. The surface of glycerol-plasticized PVA/starch blend composite based on modified chicken feather protein showed homogeneous, continuous smooth surface, and compact structure is also seen with relatively good interfacial adhesion between the film components indicating great compatibility between film components.

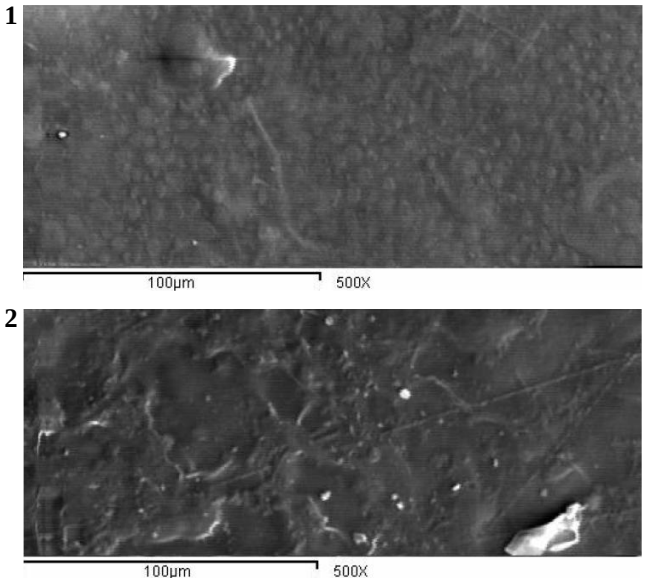

Figure 2 : SEM surface morphologies of glycerol-plasticized PVA/ starch film (1), and glycerol-plasticized PVA/starch blend film based on modified chicken feather protein (2)

Film mechanical properties

High tensile strengths are generally necessary for edible films in order to withstand the normal stress encountered during their application, subsequent shipping, and food handling. Tensile strength of films was changed by the incorporation of plasticizer together with polymeric materials used in film formation. From Figure 2 the film prepared from PVA/starch / based on modified chicken feather protein without plasticizer has a good tensile strength. This is in agreement with the properties of polyvinyl alcohol (PVA) which is a synthetic water soluble polymer with good film forming property, and offers good tensile strength. Moreover, the formation of hydrogen bonds between –OH of PVA and-OH of starch and –NH2 of protein. In presence of glycerol or 1,2- propane diole the tensile strength was decreased and % elongation was increased. Generally films plasticized with glycerol hasve remakely lower tensile strength and higher elongation at break values than plasticized 1, 2- propanediol, and non-plasticized films respectively, because of its low molecular weight that consequently decreasing the blend glass transition temperature thus decreasing tensile strength and increase elongation at break The low molecular weight plasticizer was able to insert itself into polmyer chains. Since glycerol molecule has three –OH groups, its polarity and ability to interact and forming hydrogen bonds with polymer segments is higher than 1,2 propane diole.

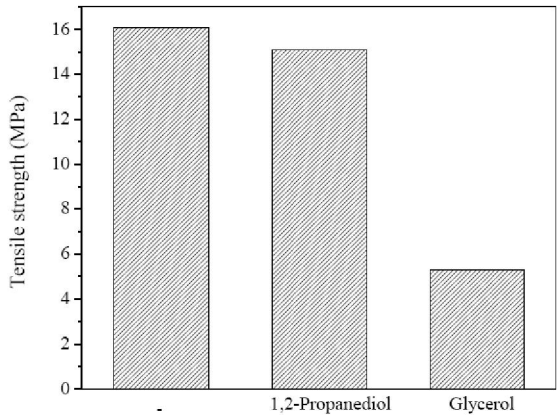

Figure 3 : Effect of plasticizer type on tensile strength of starch/PVA blend composite films based on modified alkaline hydrolyzed chicken feather protein

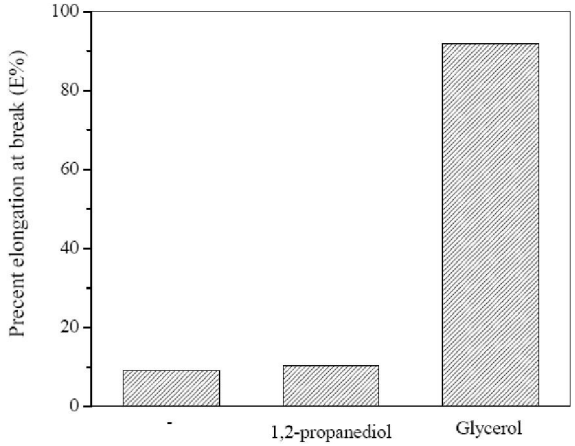

Figure 4 : Effect of plasticizer type on percent elongation at break of starch/PVA blend composite film based on alkaline hydrolyzed chicken featherprotein

Thermal stabilities

The prepared glycerol-plasticized PVA/starch (control film) and glycerol and 1, 2-propanediol-plasticized PVA/starch blend composite film based on maleic anhydride modified alkaline hydrolyzed chicken feather protein (sample films), thermal behavior is showed in Figure 5, control film degraded in three distinct weight loss stages. A typical weight loss occurred at about 100 °C due to moisture escape during the melting.[18] reported that the mass loss from 100 ºC to the onset decomposition temperature is related to the volatilization of both water and also glycerol. It can be seen that the major weight loss are observed at about 80-90 wt% in the range of 200-450ºC for all samples which correspond to the structural decomposition of PVA. At about 480 °C the graph shows complete decomposition of the sample. PVA/starch blend composite films based on maleic anhydride modified alkaline hydrolyzed chicken feather protein TG curves exhibit four –decomposition stages. First degradation temperature of sample films shifted to lower temperature as seen in TABLE 1. In addition, glycerol – plasticized-PVA/starch/modified protein film is more thermal stable than 1,2-propanediol-plasticized PVA/starch/modified protein. The results suggested that incorporation of modified chicken feather protein decrease thermal stability of prepared film.

TABLE 1: Thermal degradation measurements of plasticized -PVA/starch /and plasticized maleic anhydride modified feather protein PVA/ starch blend composite films

Sample identification	First degradation temp, ^{0}C	Steps	Temp. range, ^{0}C	Maximum weight loss temp., ^{0}C
Glycerol-plasticized	200.17	1st	79.38-00.17	126.06
VA/starch		2nd	200.17-340.55	248.13
		3rd	340.55-490.72	454.45
1,2-propanediol-	134.88	1st	50-134.88	-
plasticized		2nd	134.88-314.43	208.16
PVA/starch/ modified		3rd	314.43-530	-
protein		4th	530-660	484.81
Glycerol –plasticized-	153.33	1st	50-153.33	-
PVA/starch/modified		2nd	153.33-330	251.29
protein		3rd	330-448.28	-
		4th	448.28-591.92	531.16

Oxygen permeability

Oxygen is the key factor that might cause oxidation, which initiates several food changes such as odor, color, flavor and nutrients deterioration, so obtaining films with proper oxygen barrier can help improving food quality and extending food shelf life. It is found that film prepared from PVA/ starch/glycerol in absence of protein has very height OP. Film prepared from PVA/starch/ based on maleic anhydride modified alkaline hydrolyzed chicken feather protein have low OP (9ml/m^2/day). This may be due to increasing density of of film solution leading to increased film thickness and minimize the space between molecules of polymer film.. The effect of plasticizer on OP of protein based film was also examined. A significant increase in OP was observed in presence of glycerol reached to (32 ml/m^2/day).

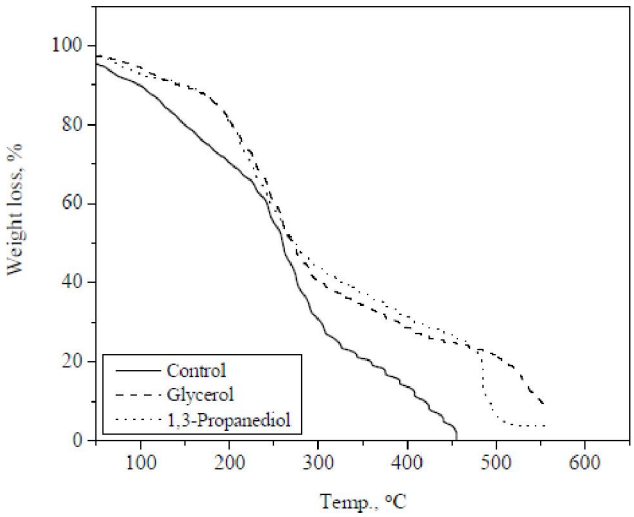

Figure 5 : TG curves of glycerol-plasticized PVA/starch blend composite film (control) and glycerol- plasticized and 1,2-propanediol-plasticized PVA/ starch based on maleic anhydride modified feather protein

Glycerol may compete with water for the active sites on the polymer, thus promoting water clustering and increased free volume in the polymers at low moisture levels. The effect of Glycerol on diffusivity was greater than that of 1,2 propane diole[20] This behavior can be attributed to the fact that water acts as plasticizer, increasing the polymer free volume and reducing the crystallinity of the polymeric chains[19,20]. Both effects facilitate the transport of oxygen molecule, leading to an increase in permeability.

Antimicrobial activity

All the diluted substances were not able to inhibit

the growth of pathogenic bacteria.

CONCLUSION

PVA–starch- maleic anhydride modified alkaline hydrolyzed chicken feather protein edible films were prepared and examined in absence and presence of plascticizers. FTIR results indicated that main interaction between blend components occur through formation of hydrogen bonding SEM illustrates that the surface of glycerol-plasticized PVA/starch blend film based on modified chicken feather protein showed homogeneous, continuous smooth surface, and compact structure is also seen with relatively good interfacial adhesion between the film components indicating great compatibility between these components. Plasticizer exhibit enhanced % elongation and reduced tensile strength. The effect of Glycerol on oxygen permeability was greater than that of 1,2-propane diole. Thermo gravimetric analysis reflects that glycerol – plasticized-PVA/ starch/modified protein film is more thermaly stable than 1,2-propanediol-plasticized PVA/starch/modified protein.. Blending modified protein with glycerol plasticized PVA/ starch decreased thermal stability of edible film. The films showed no antimicrobial activity against the previously mentioned organisms.

REFERENCES

[1] S.Guilbert, N.Gontard, L.G.M.Gorris; Prolongation of the shelf life of perishable food products using biodegradable films and coatings. LWT–Food Science and Technology, **29**, 10-17 **(1996)**.

[2] J.M.Krochta, C.De Mulder-Johnston; Edible and biodegradable polymer films: challenges and opportunities. Food Technology, **51(2)**, 61-74 **(1997)**.

[3] F.Debeaufort, J.A.Quezada-Gallo, A.Voilley; Edible films and coatings: tomorrow's packagings: A review. Critical Reviews in Food Science and Nutrition, **38**, 299-313 **(1998)**.

[4] J.M.Krochta; Proteins as raw materials for films and coatings: Definitions, current status, and opportunities. In: A.Gennadios (Ed); Protein-Based Films and Coatings. CRC Press, Boca Raton, FL, 1-41 **(2002)**.

[5] P.J.do A.Sobral, J.S.dos Santos, F.T.Garcýa; Effect of protein and plasticizer concentrations in film forming solutions on physical properties of edible films based on muscle proteins of a Thai Tilapia. Journal of Food Engineering, **70**, 93-100 **(2005)**.

[6] M.E.Gounga, S.Y.Xu, Z.Wang; Whey protein isolate-based edible films as affected by protein concentration, glycerol ratio and pullulan addition in film formation. Journal of Food Engineering, **83**, 521-530 **(2007)**.

[7] E.A.Baldwin; Edible coatings for fresh fruits and vegetables: past, present and future. In: J.M.Krochta, E.A.Baldwin, M.O.Nisperos-Carriedo (Eds); Edible Coatings and Films to Improve Food Quality. Technomic Publishing, Lancaster, PA, 25-64 **(1994)**.

[8] J.H.Han, A.Gennadios; Edible films and coatings: a review. In: J.H.Han (Ed); Innovations in Food Packaging. Elsevier Academic Press, San Diego, CA, 239-262 **(2005)**.

[9] T.H.McHugh, J.M.Krochta; Sorbitol- vs glycerol-plasticized whey protein edible films: integrated oxygen permeability and tensile property evaluation. J.Agric Food Chem., **42**, 841-45 **(1994)**.

[10] S.Özdemir, Ö.Zorba, A.Bodur; Some selected analytical and sensory data of frozen stored yoghurt. Food Science and Nutrition, 129-131 **(1999)**.

[11] Y.Nakayama, M.Takatsuka, T.Matsuda; Surface hydrogelation using photolysis of dithiocarbamate or xanthate: Hydrogelation, surface fixation, and bioactive substance immobilization. Langmuir, **15**, 1667-1672 **(1999)**.

[12] P.Cinelli, E.Chiellini, L.S.H.Gordon, L.E.Chiellini; Characterization of biodegradable composite films prepared from blends of poly (vinyl alcohol), cornstarch, and lignocellulosic fiber. Journal of Polymer Environment, **13**, 47-59 **(2005)**.

[13] H.J.Jiang, G.Campbell, D.Boughner, W.K.Wan, M.Quantz; Design and manufacture of a polyvinyl alcohol (PVA) cryogel tri-leaflet heart valve prosthesis. Medical Engineering and Physics, **26**, 269-277 **(2004)**.

[14] P.Cinelli, L.E.J.Chiellini, N.W.Lawton, M.G.Imam; Foamed articles based on potato starch, corn fibers and poly (vinyl alcohol). Polymer Degradation and Stability, **91**, 1147-1155 **(2006)**.

[15] Yuan Liu, Kaichang Li; Development and characterization of adhesives from soybean protein for bonding wood. International Journal of Adhesives & Adhesion, **27**, 59-67 **(2007)**.

[16] J.F.Su, Z.Huang, K.Liu, L.L.Fu, H.R.Liu; Mechanical properties, biodegradation and water vapor per-

meability of blend films of soy protein isolate and poly (vinyl alcohol) compatibilized by glycerol. Polym.Bull., **58**, 913-921 **(2007)**.

[17] G.-M.Kim, A.S.Asran, G.H.Michler, P.Simon, J.-S.Kim; Electrospun PVA/Hap nanocomposites nanofibers: biomimetics of mineralized hard tissues at a lower level of complexity. Bioinspiration & Biomimetics, **3**, 046003 **(2008)**.

[18] P.Kotzekidou, P.Giannakidis, Boulamatsis; Antimicrobial activity of some plant extracts and essential oils against foodborne pathogens in vitro and on the fate of inoculated pathogens in chocolate. Swiss Society of Food Science and Technology, **41**, 119-127 **(2008)**.

[19] E.G.Mahamadou, Shi-Ying Xu, Zhang W.Whey; Protein isolate-based edible films as affected by protein concentration, glycerol ratio and pullulan addition in film formation. Journal of Food Engineering, **83**, 521-530 **(2007)**.

[20] S.K.Mallapragada, N.A.Peppas; Dissolution mechanism of semicrystalline poly (vinyl alcohol) in water. J.Polym.Sci.Part B: Polym.Phys., **34**, 1339-46 **(1996)**.