Deacetylation of Chitin and the Properties of Chitosan Films with Various Deacetylation Degrees

Thipwimon Potivas[1] **and Thunnop Laokuldilok**[2*]

[1]*Department of Food Science and Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai 50100, Thailand*
[2]*Department of Marine Product Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai 50100, Thailand*

**Corresponding author. E-mail: kopy081@hotmail.com*

ABSTRACT

The degree of deacetylation is the proportion of glucosamine monomer residues in chitin. It affects many properties of chitosan. The objectives of this research were to study the deacetylation of chitin by alkaline and to investigate chitosan film properties with different degrees of deacetylation. Chitin was deacetylated by boiling (95°C) in concentrated sodium hydroxide (50%) solution for 120-300 minutes. The degree of deacetylation of chitosan was determined by potentiometric titration method. Chitin possessed a degree of deacetylation of 7.74%. The alkaline deacetylation produced chitosan with a degree of deacetylation of 15.24-70.19%. We found a linear relationship between the degree of deacetylation and deacetylation time (degree of deacetylation = 2.476 + 0.230t, $R^2 = 0.915$). The deacetylation time had no effect on the yield and whiteness of the obtained chitosans. Film was formed from the obtained chitosan at each deacetylation time and their properties investigated. Increasing the deacetylation time increased the tensile strength, elongation and water vapor transmission rate of the films. In contrast, redness (a*) and yellowness (b*) of the films decreased.

Keywords: Chitosan, Degree of deacetylation, Deacetylation of chitin, Linear potentiometric titration, Chitosan film

INTRODUCTION

Chitin is a copolymer of N-acetyl-D-glucosamine and D-glucosamine units linked with a β-(1-4) glycosidic bond, where N-acetyl-D-glucosamine units are predominant in the polymeric chain (Kim, 2011). It is a highly insoluble material with low chemical reactivity. Chitosan is the N-deacetylated derivative of chitin, although this N-deacetylation is almost never complete (Kumar, 2000). Chitosan has a high density of amino groups and is soluble in weakly acidic solvents such as acetic or formic acid. It appears that the physicochemical properties of chitin and chitosan are widely different, which are governed by three principal factors: source of raw material, molecular weight and degree of deacetylation (Chandumpai et al., 2004). Chitosan can be obtained by alkaline deacetylation of chitin.

The process of deacetylation involves the removal of acetyl groups from the molecular chain of chitin, leaving behind a complete amino group (NH2); chitosan versatility depends mainly on this high degree chemically reactive amino group (Khan et al., 2002). However, in order to exploit the utility of chitin and chitosan, it is imperative to determine the degree of deacetylation – the average number of D-glucosamine units per 100 monomers expressed as a percentage (Sabnis and Block, 1997). The majority of commercial chitosan samples had an average degree of deacetylation of 70-90%, and never more than 95% (Balázs and Sipos, 2007). Alkaline deacetylation has been widely used to produce commercial chitosan. While different authors have proposed various alkaline methods, most involve the use of sodium or potassium hydroxide solutions (Tolaimate et al., 2003; Galed et al., 2005). The effectiveness of deacetylation is influenced by various parameters, including time, temperature, concentration and relation of alkali/chitin solution utilized in the deacetylation reaction (Methacanon et al., 2003). To produce chitosan with different degrees of deacetylation in this study, we investigated the effects of deacetylation time on the degree of deacetylation.

Replacing synthetic packaging films with edible and degradable films has drawn increasing attention. Chitin and chitosan can be readily converted to fibers, film coating, beads and powders. However, the low solubility of chitin in common solvents limits its chemical modification and applications. Chitosan is soluble in aqueous acids because of the protonation of amino groups (Feng et al., 2012). Because of its nontoxic, biocompatible, mucoadhesive and biodegradable properties, Chitosan has been evaluated for various uses in the food, medical, pharmaceutical, agricultural and chemical industries (Zivanovic et al., 2007). Chitosan is mostly applied as a food additive or preservative and as a component of packaging material, not only retarding microorganism growth in food, but also improving the quality and shelf life of food. Various kinds of chitosan-based packaging films modified with new polymeric material have been developed. The process endows these films with antimicrobial properties as well as advantageous mechanical characteristics (Kong et al., 2010). However, the degree of deacetylation of chitosan affects chitosan film properties. The tensile strength and elongation of chitosan films have been reported to be related with the degree of deacetylation of chitosan (Feng et al., 2012). Moreover, its high moisture permeability might limit the use of chitosan films in some applications. The designation of chitosan with an appropriate degree of deacetylation is desirable for processors and industries to know the suitable degree of deacetylation for their use. Thus, this study aimed to produce different degrees of deacetylation of chitosan by using concentrated sodium hydroxide (50%) at high temperature (95°C) with various deacetylation times (120-300 minutes) and to characterize the properties of the corresponding chitosan films.

MATERIAL AND METHODS

Deacetylation of chitin

Chitin from shrimp (Marine Bio Resources Co., Ltd) was deacetylated with 50% NaOH (ratio 1:50, w/v) at high temperature (95°C) (Benhabiles et al., 2012) with various times – 120, 150, 180, 210, 240, 270 and 300 min. After deacetylation, each product was filtered and washed with distilled water until neutral. All products were dried at 80°C to dryness.

Characterization of chitosan

Determination of degree of deacetylation of chitosan by potentiometric titration method. The obtained chitosan (0.2 g) was dissolved in 20 ml of 1M HCl. The volume was adjusted to 100 ml with distilled water. The solution was adjusted to pH 2 by adding 0.1M NaOH and was titrated with 0.1M NaOH. The degree of deacetylation of chitosan was calculated using (Chungsiriporn et al., 2010):

$$\text{Degree of deacetylation} = \frac{\Delta V \times C_{NaOH} \times 10^{-3} \times 16}{M \times 0.0994}$$

(C_{NaOH}: concentration of NaOH (M), M: weight of sample (g) and ΔV: volume of NaOH between the inflexion point (ml))

Whiteness index of chitosan. A colorimeter was used and the whiteness index of chitosan was calculated using (Li and Lee, 1996):

$$\text{Whiteness index (WI)} = 100 - \sqrt{\left[(100 - L^*)^2 + a^{*2} + b^{*2}\right]}$$

Yield of chitosan. The yield of chitosan was calculated using:

$$\text{Yield} = \frac{weight\ of\ chitosan\ (after\ deacetylation)}{weight\ of\ chitin} \times 100$$

Characterization of chitosan film

Film formation. Chitosan (1%) was dissolved in 1% acetic acid (Leceta et al., 2013). The solution was stirred using a magnetic stirrer at room temperature for 1 hour, and then filtrated with a cotton sheet to remove all residues. Ten g of film solution was poured into a 9 cm diameter Petri dish and dried at 55°C.

Film characterization. A universal testing machine H1KS (Hounsfield, England) was used to test the tensile strength and elongation of chitosan film with 5 cm initial grip and 25 mm/min crosshead speed. Film thickness was measured in millimeters with thickness testing equipment, model GT-313-A (Mitutoyo, Japan). The method of water vapor transmission rate followed Ekthamasut (2010) with 15% RH condition at 70°C for 5 hours. The film color was measured in CIELAB (*L**, *a** and *b**) by using ColorQuest XE HunterLab (Virginia, USA)

RESULTS

Characterization of chitosan

Chitin was deacetylated using 50% NaOH with high temperature at different times to produce different degrees of deacetylation of chitosan. The degree of deacetylation of original chitin was 7.74%. After deacetylation, the degree of deacetylation of the produced chitosan was measured by potentiometric titration method. The degree of deacetylation of chitosan significantly increased with increasing deacetylation time ($p < 0.05$) (Figure 1 and Table 1). At 120 minutes of deacetylation, chitosan showed a degree of deacetylation of 15.24%. The highest degree of deacetylation (70.19%) was found in chitosan produced from 300 minutes of deacetylation. This study found a linear relationship between degree of deacetylation and deacetylation time (degree of deacetylation = 2.476 + 0.230t, $R^2 = 0.915$) (Figure 1). The whiteness of chitosan (77-79) was significantly higher than chitin (72.82). The yield of produced chitosan ranged from 74-76%. This was not affected by deacetylation time (Table 1, $p \geq 0.05$).

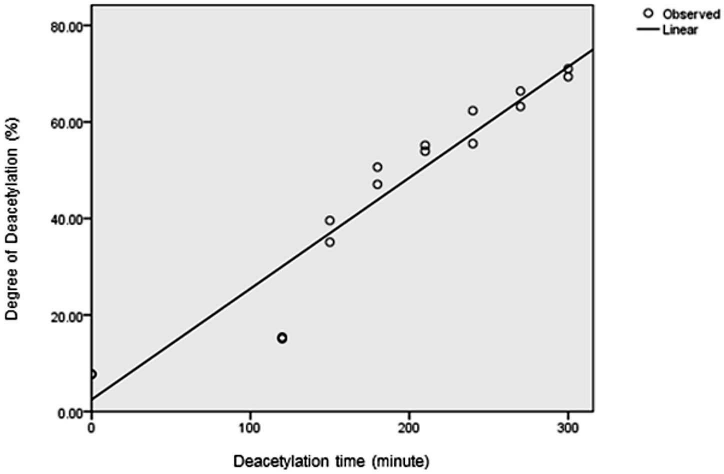

Figure 1. The relationship between degree of deacetylation of chitosan and deacetylation time, Regression DD = 2.476 + 0.230(t), $R^2 = 0.915$ DD; Degree of Deacetylation, t; deacetyltion time (minute).

Table 1. Degree of deacetylation, yield and whiteness of chitin and chitosan with different deacetylation time.

Deacetylation times of chitsan samples	%DD	Whiteness Index	Yield (%)^{NS}
0 min (Chitin)	7.74±0.04^{f}	72.82±0.11^{b}	-
120 min	15.24±0.23^{e}	77.21±0.88^{a}	74.96±1.99
150 min	37.33±3.17^{d}	78.20±0.47^{a}	76.12±0.35
180 min	48.86±2.52^{c}	78.04±0.58^{a}	75.43±0.82
210 min	54.55±0.86^{b}	79.28±1.27^{a}	76.87±1.70
240 min	58.92±4.82^{b}	79.02±0.72^{a}	74.99±1.29
270 min	64.79±2.26^{a}	78.88±1.53^{a}	75.85±0.79
300 min	70.19±1.10^{a}	78.33±1.48^{a}	74.64±0.51

Note: $^{a\text{-}f}$Means with different superscripts within a column significant differences ($p < 0.05$)
^{NS}Mean with superscript within a column indicates non-significant ($p \geq 0.05$)

Film characterization

Tensile strength, elongation, water transmission rate, thickness and color of chitosan films are shown in Table 2. Deacetylation of chitin improved the physical properties of chitosan films. The film produced from the higher degree of deacetylation chitosan possessed the higher tensile strength and elongation. The highest tensile strength (18.28 N/mm^2) and elongation (2.12%) was found in the chitosan film with a 70.19% degree of deacetylation (300 minutes of deacetylation time). However, the water transmission rate of chitosan films increased when deacetylation time increased. The water transmission rate of chitosan film with a 70.19% degree of deacetylation (182.62 g/m^2.day) increased by 2.1 times compared with the 15.24% degree of deacetylation chitosan film (85.33 g/m^2.day). The average thickness of chitosan films ranged from 0.01-0.03 mm. The chitosan films were rather transparent and slightly yellow. The L* values of chitosan film ranged from 96.11-96.37. Redness (*a**) and yellowness (*b**) of chitosan films were very low (0.03-0.17 and 0.65-1.14, respectively). This decreased with increased deacetylation time.

DISCUSSION

The highest degree of deacetylation (70.19%) of chitosan produced in this study was slightly lower that other reports. Behabiles et al. (2012) reported that chitosan produced from chitin (35% degree of deacetylation) by concentrated alkaline reagent at high temperature (90-100°C) for 3-5 hours showed an 80% degree of deacetylation on average. Moreover, Ocloo et al. (2011) produced local shrimp shell chitosan compared with commercial crab chitosan by using 50% NaOH at 100°C for 5-6 hours. They found that the commercial chitosan had a higher degree of deacetylation (82%) than the local chitosan (76%). The source of chitin affected the degree of deacetylation of chitosan. After alkaline deacetylation, the lower degree of deacetylation of the original chitin obtained a lower degree of deacetylation of chitosan. However, increasing either the temperature or NaOH concentration enhanced the degree of deacetylation (Hussain et al., 2013). The linear relationship between degree of deacetylation and deacetylation time found in this study can predict the degree of deacetylation of the obtained chitosan.

Chitosan showed a higher whiteness index than the original chitin. Chitin appeared as orange flakes before deacetylation. After deacetylation, the flakes of obtained chitosan were smaller and the color faded. Astaxanthin, an orange pigment in shrimp shells, was destroyed by oxidation reaction during high temperature deacetylation. Yen et al. (2009) found a similar result; after deacetylation, the whiteness index of chitosan was higher than the original chitin.

Films produced from the chitosan with a higher degree of deacetylation had higher tensile strength, elongation and water transmission rates. Moura et al. (2011) found similar results; higher tensile strength and water transmission rates were found in the higher degree of deacetylation chitosan films, but the elongation value was slightly affected by the degree of deacetylation of chitosan. Tensile strength and elongation of chitosan films increased with increasing degree of deacetylation, due to changes in the molecular arrangement structure in the film state. The chitosan with a high degree of deacetylation showed regular molecular structure and high crystallinity, because a high percentage of amino groups seemed to benefit the formation of intramolecular and intermolecular hydrogen bonding (Feng et al., 2012). The water transmission rate of chitosan films has been reported in the range of 6.7-1146 g/m^2.day over a range of water vapor pressure from 2.68 to 19.19 mmHg (Wiles et al., 2000). The degree of deacetylation, molecular weight and measurement conditions, such as RH gradient, temperature and film thickness, affect chitosan film (Kim et al., 2006). The result indicated that chitosan film with a higher degree of deacetylation had a lower water vapor barrier property.

The thickness of chitosan film in this study (0.01-0.03 mm) was lower than other studies, which reported thicknesses in the range of 0.032-0.056 mm (Rêgo et al., 2013; Peng and Li, 2014; Yoshida et al., 2014). The lower thickness of our chitosan films compared with other studies may be due to differences in the concentrations of film solution, volume of film solution and diameter of the plate used. The redness and yellowness of chitosan film decreased with increasing deacetylation time due to the destruction of astraxanthin during deacetylation.

Table 2. Tensile strength, elongation, water vapor transmission rate (WVTR), thickness and color of chitosan film.

Deacetylation times of chitsan samples	Tensile (N/mm^2)	Elongation (%)	WVTR (g/m^2.day)	Thickness (mm)	Film color		
					*L**	*a**	*b**
120 min	$7.11±0.95^{d}$	$0.80±0.18^{b}$	$85.33±2.67^{f}$	0.03±0.01	$96.30±0.04^{a,b}$	$0.17±0.03^{a}$	$1.01±0.09^{a,b}$
150 min	$11.89±1.41^{c}$	$0.91±0.74^{b}$	$97.61±6.14^{e}$	0.03±0.00	$96.11±0.07^{d}$	$0.13±0.02^{b}$	$1.14±0.14^{a}$
180 min	$12.06±1.96^{c}$	$1.00±0.90^{b}$	$126.55±3.18^{d}$	0.02±0.00	$96.13±0.05^{d}$	$0.13±0.01^{b}$	$1.03±0.09^{a,b}$
210 min	$13.61±0.40^{b,c}$	$1.09±0.29^{b}$	$128.65±2.01^{d}$	0.01±0.00	$96.15±0.04^{c,d}$	$0.07±0.01^{c}$	$0.96±0.06^{b}$
240 min	$15.61±0.08^{b}$	$1.46±0.30^{a,b}$	$149.97±6.49^{c}$	0.02±0.00	$96.32±0.02^{a,b}$	$0.04±0.01^{d}$	$0.76±0.06^{c}$
270 min	$15.84±0.23^{b}$	$1.70±0.40^{a,b}$	$170.62±2.97^{b}$	0.01±0.00	$96.37±0.02^{a}$	$0.03±0.01^{d}$	$0.65±0.06^{c}$
300 min	$18.28±0.55^{a}$	$2.12±0.24^{a}$	$182.62±6.54^{a}$	0.01±0.00	$96.25±0.12^{b,c}$	$0.04±0.01^{d}$	$0.72±0.13^{c}$

Note: $^{a\text{-}f}$Means with different superscripts within a column indicate significant differences ($p < 0.05$).

CONCLUSION

In deacetylation of chitin using a concentrated alkaline solution at high temperature, the degree of deacetylation of the obtained chitosan increased with increasing deacetylation time. The relationship between the degree of deacetylation and deacetylation time was linear. The equation could forecast the degree of deacetylation of the obtained chitosan. Moreover, the degree of deacetylation of chitosan affected the chitosan film properties. The film produced from the chitosan with a high degree of deacetylation had higher tensile strength and elongation; these are preferable properties. However, these films also had a high water vapor transmission rate, which may not suitable for some applications.

ACKNOWLEDGEMENTS

This research was supported by grants from Chiang Mai University and the Graduate School of Chiang Mai University. The authors thank the Faculty of Agro-Industry, Chiang Mai University for providing instruments.

REFERENCES

Balázs, N. and P. Sipos. 2007. Limitations of pH-potentiometric titration for the determination of the degree of deacetylation of chitosan. Carbohydrate Research. 342(1): 124-130. DOI: 10.1016/j.carres.2006.11.016

Benhabiles, M.S., R. Salah, H. Lounici, N. Drouiche, M.F.A. Goosen, and N. Mameri. 2012. Antibacterial activity of chitin, chitosan and its oligomers prepared from shrimp shell waste. Food Hydrocolliods. 29: 48-56. DOI: 10.1016/j.foodhyd.2012.02.013

Chandumpai, A., N. Singhpibulporn, and D. Faroonsarng. 2004. Preparation and physico-chemical characterization of chitin and chitosan from the pens of the squid species, Loligo lessoniana and Loligo formosana. Carbohydrate Polymers. 58: 467-474. DOI: 10.1016/j.carbpol.2004.08.015

Chang, K.L.B., G. Tsai, J. Lee, and W.R. Fu. 1997. Heterogeneous N-deacetylation of chitin in alkaline solution. Carbohydrate Research. 303: 327-332. DOI: 10.1016/S0008-6215(97)00179-1

Chungsiriporn, J., P. Pongyeela, and J. Inthamanee. 2010. Cost reduction of chitosan preparation from shrimp waste by recycle of alkaline solution. In Proceedings of the Prince of Songkla University on Engineering, Songkla, Thailand.

Ekthamasut, K. 2010. Effects of zein hydrolysate on physical properties of edible konjac film. Academic Journal of University of the Thai Chamber of Commerce. 30(1): 81-89.

Feng, F., Y. Liu, B. Zhao, and K. Hu. 2012. Characterization of half N-acetylation chitosan powders and films. Procedia Engineering. 27: 718-732. DOI: 10.1016/j.proeng.2011.12.511

Galed, G., B. Miralles, I. Panõs, A. Santiago, and A. Heras. 2005. N-deacetylation and depolymerization reactions of chitin/chitosan: influence of the source of chitin, Carbohydrate Polymers. 62: 316-320. DOI: 10.1016/j.carbpol.2005.03.019

Hussain, M.R., M. Iman, and T.K. Maji. 2013. Determination of degree of deacetylation of chitin and their effected on the release behavior of essential oil from chitosan and chitosan-gelatin complex microcapsules. International Journal of Advanced Engineering Application. 2(4): 4-12.

Khan, T.A., K.K. Peh, and H.S. Ch'ng. 2002. Reporting degree of deacetylation values of chitosan: the influence of analytical methods. Journal of Pharmacy and Phamarceutical Science. 5: 205-212.

Kim, K.M., J.H. Son, and S.K. Kim. 2006. Properties of chitosan films as a fuction of pH and solvent type. Journal of Food Science E: Food Engineering and Physical Properties. 71(3): 119-124. DOI: 10.1111/j.1365-2621.2006.tb15624.x

Kim, S.K. 2011. Chitin and chitosan from terrestrial organism. In N. Nwe, T. Furuike and H. Tamura (Eds.). Chitin, chitosan, oligosaccharides and their derivatives biological activities and applications (pp. 3-10). Taylor and Francis, Florida.

Kong, M., X.G. Chen, K. Xing, and H.J. Park. 2010. Antimicrobial properties of chitosan and Mode of action: A state of the art review. International Journal of Food Microbiology. 144: 51-63. DOI: 10.1016/j.ijfoodmicro.2010.09.012

Kumar, M.N.V.R. 2000. Areview of chitin and chitosan applications. Reactive and Functional Polymers. 46(1): 1-27. DOI:10.1016/S1381-5148(00)00038-9

Leceta, I., P. Guerrero, I. Ibarburu, M.T. Dueñas, and K. Caba. 2013. Characterization and antimicrobial analysis of chitosan-based films. Journal of Food Engineering. 116: 889-899. DOI: 10.1016/j.jfoodeng.2013.01.022

Li, M., and T.C. Lee. 1996. Effect of cysteine on the functional properties and microstructure of wheat flour extrudates. Journal of Agricultural and Food Chemistry. 44: 1871-1880. DOI: 10.1021/jf9505741

Liu, M., Y. Zhou, Y. Zhang, C. Yu, and S. Cao. 2013. Preparation and structural analysis of chitosan films with and without sorbitol. Food Hydrocolloids. 33: 186-191. DOI: 10.1016/j.foodhyd.2013.03.003

Methacanon, P., M. Prasitsilp, T. Pothsree, and J. Pattaraarchachai. 2003. Heterogeneous N-acetylation of squid chitin in alkaline solution. Carbohydrate Polymers. 52: 119-123. DOI: 10.1016/S0144-8617(02)00300-4

Moura, C.M., J.M. Moura, N.M. Soares, and L.A. Almeida Pinto. 2011. Evaluation of molar weight and deacetylation degree of chitosan during chitin deacetylation reaction: used to produce biofilm. Chemical Engineering and Processing. 50: 351-355. DOI: 10.1016/j.cep.2011.03.003

Ocloo, F.C.K., E.T. Quayson, A. Adu-Gyamfi, E.A. Quarcoo, and D. Asare. 2011. Physicochemical and functional characteristics of radiation-processed shrimp chitosan. Radiation Physics and Chemistry. 80: 837-841. DOI: 10.1016/j.radphyschem.2011.03.005

Peng, Y., and Y. Li. 2014. Combined effects of two kinds of essential oils on physical, mechanical and structural properties of chitosan films. Food Hydrocolloids. 36: 287-293. DOI: 10.1016/j.foodhyd.2013.10.013

Rêgo, T.V., Jr, T.R.S. Cadaval, G.L. Dotto, and L.A.A. Pinto. 2013. Statistical optimization, interaction analysis and desorption studies for the azo dyes adsorption onto chitosan films Journal of Colloid and Interface Science. 411: 27-33. DOI: 10.1016/j.jcis.2013.08.051

Sabnis, S. and L.H. Block. 1997. Improved infrared spectroscopic method for the analysis of degree of N-deacetylation. Polymer Bulletin. 39: 67-71.

Suyatma, N.E., L. Tighzert, and A. Copinet. 2005. Effects of hydrophilic plasticizers on mechanical, thermal and surface properties of chitosan films. Journal of Agicultural and Food Chemistry. 53(10): 3950-3957. DOI: 10.1021/jf048790+

Thirathumthavorn, D., and S. Charoenrein. 2007. Aging effects on sorbitol and non-crystallizing sorbitol-plasticized tapioca starch films. Starch Stärke. 59(10): 493-497. DOI: 10.1002/star.200700626

Tolaimate, A., J. Desbrières, M. Rhazi, and A. Alagui. 2003. Contribution to the preparation of chitins and chitosans with controlled physic–chemical properties, Polymer. 44: 7939-7952. DOI: 10.1016/j.polymer.2003.10.025

Wang, L., Y. Dong, H. Men, J. Tong, and J. Zhou. 2013. Preparation and characterization of active films based on chitosan incorporated tea polyphenols. Food Hydrocolloids. 32: 35-41. DOI:10.1016/j.foodhyd.2012.11.034

Wiles, J. L., P. J. Vergano, F. H. Barron, J. M. Bunn, and R. F. Testin. 2000. Water transmission rates and sortion behavior of chitosan films. Journal of Food Science. 7: 1175-1179.

Yen, M.T., J.H. Yang, and J.L. Mau. 2009. Physicochemical characterization ofchitin and chitosan from crab shell. Carbohydrate Polymers. 75: 15-21. DOI: 10.1016/j.carbpol.2008.06.006

Yoshida, C.M.P., V.B.V. Maciel, M.E.D. Mendonça, and T.T. Franco, 2014. Chitosan biobasedand intelligent film: monitoring pH variations. LWT –Food Science and Technology.55: 83-89.

Zivanovic, S., J. Li, P.M. Davidson, and K. Kit, 2007. Physical, mechanical and antibacterial properties of chitosan/PEO blend films. Biomacromolacules. 8: 1105-1510. DOI: 10.1021/bm061140p

NONE