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Evaluation of molar weight and deacetylation degree of chitosan during chitin deacetylation reaction: Used to produce biofilm

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ABSTRACT

Chitosan is a polysaccharide derived from chitin, mainly of crustacean shells and shrimp wastes. The utilization of chitosan is related to the molar weight and deacetylation degree of the biopolymer. The aim of this work is to study the chitin deacetylation reaction, by the viscosity average molar weight and deacetylation degree of chitosan as a function of reaction time. Deacetylation was carried out in concentrated alkaline solution, 421 g L^{-1}, at 130 °C and the reaction occurred during 4 h. Chitosan paste obtained after 20, 90 and 240 min was used to produce biofilms, which were characterized according water vapor permeability and mechanical properties (tensile strength and percentage tensile elongation at break). During the reaction time deacetylation degree reached 93%, and a 50% reduction in the viscosity average molar weight value in relation to the value of the first 20 min of reaction was found Both reactions presented a kinetic behavior of the pseudo-first order. Biofilm produced from the paste of chitosan with high deacetylation degree showed higher water vapor permeability (WVP), tensile strength (TS) and elongation (*E*) when compared to films with a low deacetylation.

1. Introduction

Chitosan is a natural polysaccharide, obtained from the chitin hydrolysis in an alkaline medium. Usually, the deacetylation reaction is carried out with sodium hydroxide or potassium hydroxide at high temperatures. This deacetylation generally does not occur in a homogeneous and complete way throughout all of the chains, due to the fact that chitin is a semicrystalline biopolymer and there is limited accessibility to reactive sites contained in the crystalline domain, forming in this manner a copolymer composed of units of β-(1 → 4)-2-amine-2-deoxy-D-glucose and β-(1 → 4)-2-acetamine-2-deoxy-D-glucose [1].

Due to basic character of the chitosan, that is attributed to the presence of the amine grouping at the repeated monomeric units, and its biodegrability, this polymeric composition possesses many applications, such as water treatment, manufacturing of contact lenses and artificial membranes, preservation of fruits and vegetables, and gastric protection [2,3].

Commercial chitosan usually has a deacetylation degree (DD) ranging from 70 to 95% and molar weight in the range 10^4–10^6 g mol^{-1} [4]. In this manner, it is important to know the N-deacetylated groups (DD) and NH_2 groups content, in order to characterize the chitin deacetylation process, as well as any other chemical modification [5]. Therefore, chitosan samples may present different characteristics in terms of deacetylation degree and molar weight distributions, which define its applications and influence the final performance of the biopolymer [6,7]. The source of chitin extraction and its characteristics directly influence the velocity of the deacetylation reaction [5].

Various alkaline methods have been proposed by different authors, most involving the use of sodium or potassium hydroxide solutions [8,9] or enzymatic deacetylation [10]. In the chemical processes of chitin deacetylation, the distribution of viscosity average molar weight is influenced by various parameters, such as: time, temperature, concentration and relation of alkali/chitin solution utilized in the deacetylation reaction [11]. Generally, chitin is suspended in NaOH or KOH concentrated aqueous solutions (40–60%), during different times (0.5–24 h), at relatively high temperatures (50–130 °C).

Viscosity average molar weight of linear polymers, such as chitosan, is determined based on the empiric Mark–Houwink–Sakurada equation, which relates intrinsic viscosity to the polymer's molar weight [12]. The degree of deacetylation is determined by potentiometric titration according to method described by Tan et al. [13] and Jiang et al. [14].

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The aim of this work was the study of the chitin deacetylation reaction, by the behavior of viscosity average molar weight and deacetylation degree of chitosan, as functions of deacetylation reaction time. Chitosan pastes obtained in reaction time of 20, 90 and 240 min were used to produce biofilms, and then characterized according to water vapor permeability and mechanical properties.

2. Material and methods

2.1. Chitin extraction

Chitin utilized in the experiments was extracted from pink shrimp (*Farfantepenaeus brasiliensis*) wastes, through the chemical treatments to eliminate carbonates, proteins and pigments content. The demineralization, deproteination and deodorization/depigmentation steps were carried out according to Tolaimate et al. [8].

The steps for chitin extraction from shrimp wastes were carried out with diluted solutions at room temperature and short reaction periods, in order to avoid any degradation of the properties linked to the macromolecular character of chitin and hydrolysis of the acetamide groups during the extraction process.

2.2. Chitosan production

Deacetylation of chitin was carried out with 66 g of chitin and 4 L of concentrated sodium hydroxide solution (421 g L^{-1}) at 130 ± 1 °C, under constant agitation of 50 rpm [11].

2.3. Chitosan purification

Chitosan samples were purified through dissolution in diluted acetic acid (1%). The solutions were then centrifuged at 6650 × *g* in a centrifuge (model Sigma 6-15, D-37520 Osterode, Germany) for 30 min, for the removal of insoluble material. Total precipitation of chitosan occurred by addition of sodium hydroxide until pH 12.5, followed by neutralization until pH 7.0. The resulting chitosan suspension was centrifuged for separation of the supernatant.

2.4. Viscosity average molar weight

Viscosimetric measurements of the chitosan samples were carried out in a Cannon-Fensk (Schott Geraete, model GMBH – D65719, Germany) capillary viscosimeter at a temperature of 25.0 ± 0.1 °C.

Diluted solutions of the biopolymer were prepared, utilizing an aqueous system composed of acetic acid 0.1 M and sodium chloride 0.2 M as solvent. The solutions were filtered before the viscosity determinations, which were carried out at the lowest shear velocities permitted within the experimental error and the Newtonian plateau. Viscosities of the chitosan solutions were prepared at five different concentrations from 0.001 to 0.012 g mL^{-1}. Intrinsic viscosity, [η], was estimated by fit of the Huggins equation (Eq. (1)) to reach the obtained results [15].

$$\frac{\eta_{sp}}{c} = [\eta] + k[\eta]^2 c \quad (1)$$

where η_{sp}/c is the reduced viscosity, in mL g^{-1}, η_{sp} is the relation between viscosity of the polymer in solution and the solvent, dimensionless, *c* is the concentration of the solution, in g mL^{-1}, and *k* is a constant valid for each polymer, dimensionless.

Chitosan viscosity average molar weight, M_V, was calculated based on the intrinsic viscosity value utilizing the Mark–Houwink–Sakurada equation (Eq. (2)), cited by Roberts & Domsky [12].

$$[\eta] = KM_v^{\alpha} \quad (2)$$

where $K = 1.81 \times 10^{-3}$ mL g^{-1} and $\alpha = 0.93$ are constants that depend on the solvent-polymer system [9,16,17].

2.5. Potentiometric titration

The determination of the deacetylation degree of chitosan samples was carried out by the linear potentiometric method. This analysis occurred by dissolving 0.25 g of chitosan in 20 mL of HCl solution, 0.1 N, and completing until 100 mL with distilled water. The solution, measured for pH by pHmeter (model MB10-Marte-Brazil), was placed under constant agitation in a magnetic stirrer at 600 rpm and then adjusted to approximately 2.0 with standard NaOH solution, 0.1 M, under constant agitation, with this moment being considered as the titration starting point. Titration was performed until the chitosan solution reached a pH of approximately 6.5 (range of chitosan non-protonation).

An *f*(*x*) value corresponding to the utilized NaOH volume was calculated by Eq. (3).

$$f(x) = \left(\frac{V_0 + V}{N_B}\right) \cdot \left([H^+] - [OH^-]\right) \quad (3)$$

where V_0 is the volume of the chitosan solution, in mL; *V* is the volume of NaOH utilized in the titration, in mL; N_B is the concentration of NaOH, in meq L^{-1}; $[H^+]$ is the concentration of H^+, in mol L^{-1}; and $[OH^-]$ is the concentration of OH^-, in mol L^{-1}.

The linear titration curve was obtained plotting a graph of *f*(*x*) versus NaOH volume.

The NaOH volume at the end of titration, V_e, was found by extrapolating the linear titration curve as a function of the added NaOH volume. Deacetylation degree of the chitosan sample was calculated by Eq. (4):

$$DD(\%) = \frac{\phi}{[(W - 161\phi)/204 + \phi]} \cdot 100 \quad (4)$$

and

$$\phi = \frac{(N_A V_A - N_B V_e)}{1000} \quad (5)$$

where N_A is the HCl concentration, in meq L^{-1}; V_A is the HCl volume, in mL; N_B is the NaOH concentration, in meq L^{-1}; V_e is the volume of NaOH at the end of the titration, in mL, and *W* is the chitosan mass, in g [13,14].

2.6. Deacetylation kinetics

In the deacetylation reaction, samples were taken at regular intervals of 20 min, throughout four hours of experiment. These samples were analyzed in order to determine the behavior of viscosity average molar weight and deacetylation degree of the biopolymer throughout the reaction time.

Results were analyzed through the regression analysis, by pseudo-first-order equation for the viscosity average molar weight and deacetylation degree according to Eq. (6).

$$\frac{dX}{dt} = k'X \quad (6)$$

Thus with the slope of the neperian plot of *X* versus reaction time the pseudo-first-order kinetic constant, *k*′ was found by Eq. (7).

$$\ln X = \ln X_0 + k't \quad (7)$$

where *X* is the viscosity average molar weight, M_V, or the deacetylation degree, DD.

2.7. Biofilm production and characterization

The chitosan obtained in times 20, 90 and 240 min was used to produce a biofilm. The biofilm was produced from chitosan solution prepared in 0.1 M acetic acid, through dispersion of the chitosan paste by moderate stirring at room temperature (25 °C). Then the film-forming solution was filtered under vacuum. An appropriate volume (100 mL) of the film-forming solution was poured onto a level Plexiglas plate, in order to keep constant the total amount of polymer deposited. The films were obtained by solvent evaporation in an oven with air circulation at 30 °C for about 24 h. Finally, the film samples were removed from plates and conditioned in desiccators to 25 ± 1 °C for at least 48 h prior to testing. Before testing, the thickness of the film samples was measured by a digital micrometer (model MDC-25S, Mitutoya Corp., Tokyo, Japan). Mean thickness was calculated from ten measurements taken at different locations on film sample, according to Ferreira et al. [18].

The biofilm was characterized in relation to water vapor permeability [19] and mechanical properties (tensile strength and percentage of elongation). Mechanical properties were measured by using a Texture Analyzer TA-XT-2*i* (Stable Micro Systems, Surrey, UK) with a 50 N load cell equipped with tensile grips (A/TG model). Sample films were cut into 25 mm wide and 100 mm long strips [20].

Tukey test was used to verify the significant differences ($p > 0.05$) between the characteristics of the biofilm obtained from chitosan.

3. Results and discussion

After the demineralization, deproteination and deodorization steps, the chitin showed (in wet basis) 0.9% ashes, 0.1% protein and 5.0% moisture contents.

Figs. 1 and 2 show the graphs of viscosity average molar weight and deacetylation degree versus reaction time, respectively.

Different deacetylation conditions have been compared to allow the experimental determination to reach adequate molecular weight and deacetylation degree for required properties.

Fig. 1 shows that after 4 h of deacetylation reaction, the viscosity average molar weight value of chitosan decreased by approximately 50% in relation to the molar weight value of the first 20 min of reaction. In the presence of the alkaloid, the polysaccharide chains are submitted to degradation due to the elevated concentration of reagents and the prolonged times necessary for obtaining

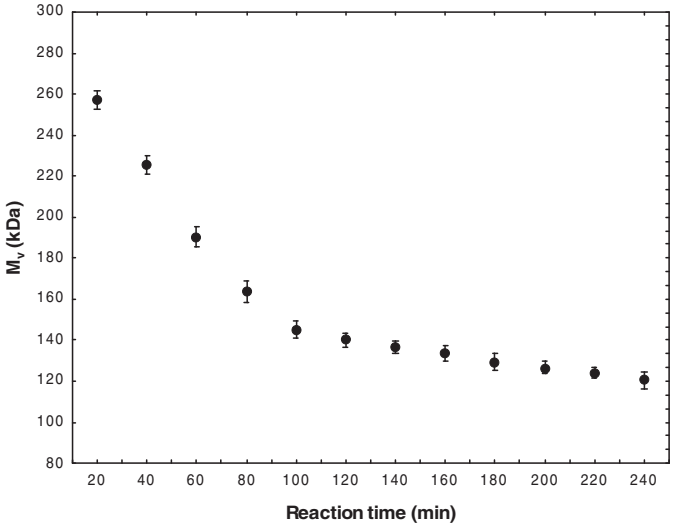

Fig. 1. Viscosity average molar weight values versus reaction time.

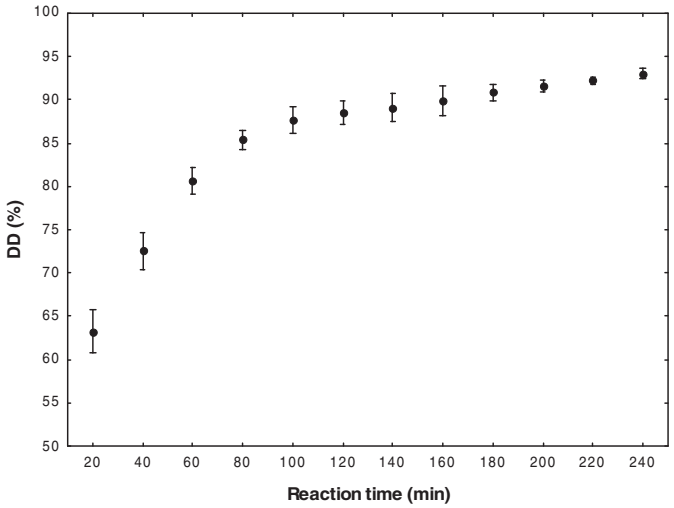

Fig. 2. Deacetylation degree values versus reaction time.

complete deacetylation [6]. It is known that chitin degradation occurs during the deacetylation reaction, although the molar mechanism has not yet been explained [21].

Hwang et al. [22], studied molar weight and deacetylation degree of chitosan in chemical processing through response surface methodology under determined conditions. Chitosan was widely depolymerized ranging from 1100 kDa to 100 kDa, and deacetylation ranged from 67.3 to 95.7% by NaOH alkaline treatment. Furthermore, the rates of molar weight decrease and deacetylation degree increase gradually decreased with an increase of time.

Molar weight and deacetylation degree values found in the experiments (Figs. 1 and 2) were similar to values reported in the literature [8,9,23–26].

Experimental conditions studied by Chang et al. [23], varied from 10 to 60% NaOH, at 70 to 150 °C, and 5 to 45 mL of alkaline solution per gram of chitin. In conditions that have high NaOH concentration (51.9 or 60%) and high temperature (133.8 or 150 °C), the deacetylation proceeded extremely fast. Tolaimate et al. [6] and Methacanon et al. [25], studied conditions for β-chitin extraction from squid chitin and different deacetylation processes.

Figs. 3 and 4 show the fit of the neperian graphs of viscosity average molar weight versus time and the neperian logarithm of the

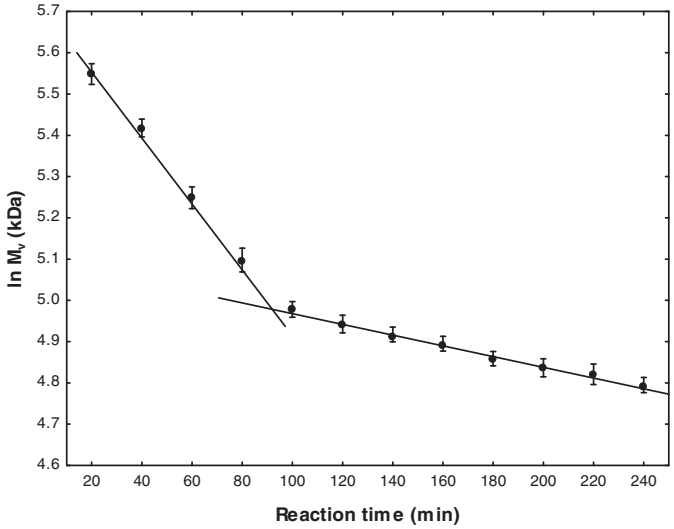

Fig. 3. Graph of neperian logarithm viscosity average molar weight versus deacetylation reaction time.

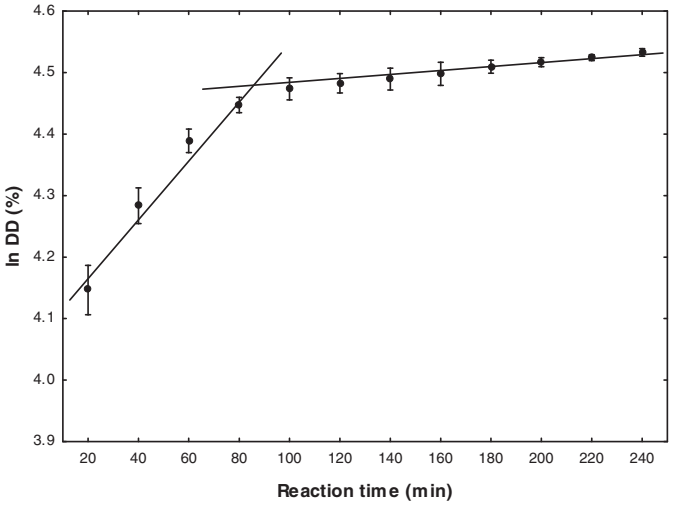

Fig. 4. Graph of neperian logarithm deacetylation degree versus deacetylation reaction time.

deacetylation degree of chitosan versus reaction time by pseudo-first equation, respectively. The values of the kinetic constants, for both responses, were found for the first 90 min and for the time after 90 min, these values are shown in Table 1.

In Fig. 3, the pseudo-first-order kinetic behavior can also be observed for the deacetylation reaction, as cited by other authors, who studied not only shrimp wastes (α-chitin) but also squid wastes (β-chitin), and noted the same kinetic behavior. Galed et al. [9] found that the deacetylation reactions of chitin/chitosan from three crustacean species under the same conditions follow a pseudo-first order and their apparent rate constants are very similar.

In the fit of the $\ln M_v$ as a function of deacetylation reaction time, was found the value for the degradation rate constant for first period (Table 1) which was in the same order of the value found by Chang et al. [23], of approximately 5.86×10^{-3} min^{-1}, during chitin deacetylation from shrimp under different conditions. Tolaimate et al. [6], reported that the degradation rate constant was of 1.58×10^{-3} min^{-1}, who worked with conditions for β-chitin extraction from squid chitin and different deacetylation processes. According to Rhazi et al. [24], in their work with β-chitin, a first-order kinetics reaction may be assumed with rate of 1.6×10^{-3} min^{-1}.

This shows that even when using different sources of raw materials in the deacetylation reaction rate, chitin degradation has the same behavior (Figs. 3 and 4). According to Roberts et al. [12], this behavior may occur due to the decrease of N-acetyl-D-glucosamine residues and a consequent increase of D-glucosamine residues, gradually increasing the probability of degradation reactions which lead to a deacetylation dependence. Also according to these authors, heterogeneous N-deacetylation occurs preferably at the amorphous region of chitin, from the contour to the interior of the crystalline region, influencing the reaction rate. Thus it is well known that chitin degradation occurs during N-deacetylation.

In the presence of alkali, polysaccharide chains were found to undergo degradation because of the high concentration of reagents and prolonged reaction times required to obtain complete deacetylation. The low reactivity of chitin in the deacetylation reaction was ascribed to the trans arrangement of acetamido groups in the monomeric unit with respect to the hydroxyl group OH-3 [24].

Conventionally, the presence of 50% amine groups is a boundary between chitin and chitosan, i.e. chitin has DD value <50% and chitosan has DD value >50% [27]. Chitosan paste samples, with low and high deacetylation degrees, obtained after 20, 90 and 240 min of reaction were used to produce biofilms.

Molar weight and deacetylation degree changes would provide changes in biofilm hydrophilicity and cristalinity [28–30], and consequently, changes in water vapor permeability and mechanical properties of the biofilm obtained from chitosan.

Table 2 shows that the biofilm characteristics (mechanical properties and water vapor permeability) showed significance difference ($p < 0.05$). It was observed that films with low deacetylation degree showed lower water vapor permeability (WVP), tensile strength (TS) and elongation (E) when compared to films with a high deacetylation degree. That may have occurred due to the fact that there was a relatively little protonation of the amide group in the low deacetylation degree of chitosan. The WVP values of chitosan films observed in this study were not directly comparable to those of Kim et al. [28] because chitosan solutions can be affected by various factors such as deacetylation degree, molar weight, and measuring conditions such as RH gradient, temperature and film thickness. Higher TS values were found for high than low deacetylation degree of chitosan films because they had more active ionic sites in their molecules. The elongation (E) value was not very affected by the chitosan both of high and low deacetylation degree. Similar results were reported by Moreno-Osório et al. [29] in chitosan films containing polygodial.

Table 1
Values of kinetic constant (k') and coefficient of determination (R^2) to viscosity average molar weight and deacetylation degree.

Parameter	Period	k'[a] (min^{-1}) × 10^3	R^2
M_v	First	3.40 ± 0.14	0.99
	Second	0.19 ± 0.06	0.97
DD	First	5.00 ± 0.20	0.90
	Second	0.42 ± 0.03	0.99

[a] Mean values ± standard error (in triplicate).

Table 2
Biofilms characteristics.

Characteristics	20 min	90 min	240 min
Water vapor permeability (g mm m^{-2} kPa^{-1} d^{-1})	1.96 ± 0.05[a]	2.95 ± 0.05[b]	3.95 ± 0.05[c]
Tensile strength (MPa)	23.2 ± 1.5[a]	42.3 ± 1.5[b]	46.2 ± 1.5[c]
Elongation (%)	27.7 ± 0.5[a]	28.8 ± 0.5[b]	29.9 ± 0.5[c]

Mean values ± standard error for three analyses. Equal letters in same line ($p > 0.05$). Different letters in same line ($p < 0.05$).

4. Conclusion

Through the results found during the four hours of chitin deacetylation reaction, it can be concluded that the chitosan showed a decreasing molecular weight behavior for the reaction condition (NaOH of 421 g L^{-1} and temperature of 130 °C), reaching a molar weight of 120 kDa. In terms of deacetylation degree, there was an increase with reaction time, reaching a maximum value of 93%. The molar weight and deacetylation degree presented a pseudo-first-order kinetic behavior by deacetylation reaction, similar to the one reported in the literature.

Chitosan paste obtained was used to produce biofilm. Chitosan biofilm obtained from high deacetylation degree showed upper changes in relation to tensile strength and water vapor permeability when compared with the biofilms from chitosan of low deacetylation degree, however the elongation did not show larger changes between biofilms.

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References

[1] E.S. Abdou, K.S.A. Nagy, M.Z. Elsabee, Extration and characterization of chitin and chitosan from local sources, Bioresour. Technol. 99 (2008) 1359–1367.
[2] Y. Wan, K.A.M. Creber, B. Peppley, V.T. Bui, Ionic conductivity of chitosan membranes, Polymer 44 (2003) 1057–1065.
[3] D. Zeng, J. Wu, J.F. Kennedy, Application of a chitosan flocculant to water treatment, Carbohydr. Polym. 71 (2008) 135–139.
[4] P.R. Rege, R.J. Garmise, L.H. Block, Spray-dried chitinosans. Part I. Preparation and characterization, Int. J. Pharm. 252 (2003) 41–51.
[5] M.R. Ksaai, Various methods for determination of the degree of N-acetylation of chitin and chitosan: a review, J. Agric. Food Chem. 57 (2009) 1667–1676.
[6] A. Tolaimate, J. Desbrières, M. Rhazi, A. Alagui, M. Vincendon, P. Vottero, On the influence of deacetylation process on the physicochemical characteristics of chitosan from squid chitin, Polymer 41 (2000) 2463–2469.
[7] D.K. Youn, H.K. No, W. Prinyawiwatkul, Physical characteristics of decolorized chitosan as affected by sun drying during chitosan preparation, Carbohydr. Polym. 69 (2007) 707–712.
[8] A. Tolaimate, J. Desbrières, M. Rhazi, A. Alagui, Contribution to the preparation of chitins and chitosans with controlled physic–chemical properties, Polymer 44 (2003) 7939–7952.
[9] G. Galed, B. Miralles, I. Panõs, A. Santiago, A. Heras, N-deacetylation and depolymerization reactions of chitin/chitosan: influence of the source of chitin, Carbohyd. Polym. 62 (2005) 316–320.
[10] A. Martinou, D. Kafetzopoulos, V. Bouriotis, Chitin deacetylation by enzymatic means: monitoring of deacetylation processes, Carbohyd. Res. 273 (1995) 235–242.
[11] R.F. Weska, J.M. Moura, L.M. Batista, J. Rizzi, L.A.A. Pinto, Optimization of deacetylation in the production of chitosan from shrimp wastes: use of response surface methodology, J. Food Eng. 80 (2007) 749–753.
[12] G.A.F. Roberts, J.G. Domszy, Determination of the viscosimetric constants for chitosan, Int. J. Biol. Macromol. 4 (1982) 374–377.
[13] S.C. Tan, E. Khor, T.K. Tan, S.M. Wong, The degree of deacetylation of chitosan: advocating the first derivative UV-spectrophotometry method of determination, Talanta 45 (1998) 713–719.
[14] X. Jiang, L. Chen, W. Zhong, A new linear potentiometric titration method for the determination of deacetylation degree of chitosan, Carbohydr. Polym. 54 (2003) 457–463.
[15] I.A. Alsarra, S.S. Betigeri, H. Zhang, B.A. Evans, S.H. Neau, Molecular weight and degree of deacetylation effects on lipase-loaded chitosan bead characteristics, Biomaterials 23 (2002) 3637–3644.
[16] M.N.V.R. Kumar, A review of chitin and chitosan applications, React. Funct. Polym. 46 (2000) 1–27.
[17] M.F. Cervera, J. Heinämäki, M. Räsänen, S.L. Maunu, M. Karjalainen, O.M.N. Costa, A.I. Colarte, J. Yliruusi, Solid-state characterization of chitosans derived from lobster chitin, Carbohydr. Polym. 58 (2004) 401–408.
[18] C.O. Ferreira, C.A. Nunes, I. Delgadillo, J.A. Lopes da Silva, Characterization of chitosan whey-protein films at acid pH, Food Res. Int. 42 (2009) 807–813.
[19] American society for testing and materials, 1995, Standard methods of water vapor transmission E96-95, Annual book of ASTM, Philadelphia, PA.
[20] American society for testing and materials, 1995, Standard methods for tensile proprieties on thin plastic sheeting D882, Annual book of ASTM, Philadelphia, PA.
[21] S. Mima, M. Miya, R. Iwamoto, S. Yoshikawa, Highly deacetylated chitosan and its properties, J. App. Polym. Sci. 28 (1983) 1909–1917.
[22] K.T. Hwang, S.T. Jung, G.D. Lee, M.S. Chinnan, Y.S. Park, H.J. Park, Controlling molecular weight and degree of deacetylation of chitosan by response surface methodology, J. Agric. Food Chem. 50 (2002) 1876–1882.
[23] K.L.B. Chang, G. Tsai, J. Lee, W. Fu, Heterogeneous N-deacetylation of chitin in alkaline solution, Carbohydr. Res. 303 (1997) 327–332.
[24] M. Rhazi, J. Desbrières, A. Tolaimate, A. Alagui, P. Vottero, Investigation of different natural sources of chitin: influence of the source and deacetylation process on the physicochemical characteristics of chitosan, Polym. Int. 49 (2000) (2000) 337–344.
[25] P. Methacanon, M. Prasitsilp, T. Pothsree, J. Pattaraarchachai, Heterogeneous N-deacetylation of squid chitin in alkaline solution, Carbohydr. Polym. 52 (2003) 119–123.
[26] C.J. Jiang, M.Q. Xu, Kinetics of heterogeneous deacetylation of β-chitin, Chem. Eng. Technol. 29 (2006) 511–516.
[27] S.M. Taghizadeh, G. Davari, Preparation, characterization, and swelling behavior of N-acetylated and deacetylated chitosans, Carboydr. Polym. 64 (2006) 9–15.
[28] K.M. Kim, J.W. Son, S.K. Kim, C.L. Weller, M.A. Hanna, Properties of chitosan films as a function of pH and solvent type, J. Food Sci. 71 (2006) 119–124.
[29] L. Moreno-Osório, M. Garcia, R. Villalobos-Carvajal, Effect of polygodial on mechanical, optical and barrier properties of chitosan films, J. Food Process. Preserv. 34 (2010) 219–234.
[30] T. Bourtoom, M.S. Chinnan, Preparation and properties of rice starch-chitosan blend biodegradable film, LWT-Food Sci. Technol. 41 (2008) 1633–1641.