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Role of Aluminum Monostearate on Heat Treated-Chitosan Sponges Properties

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Abstract

Heat treatment can be employed to modify properties such as biocompatibility and degradation rate of chitosan-based materials. However, heat treatment generally has effect on worsening of physical properties of the biomaterials. Our previous study indicated that fatty additive; aluminum monostearate (Alst), could improve thermal stability of chitosan sponges. Aim of this study is to investigate an effect of amount of Alst on physicochemical properties of chitosan sponges treated by different heat treatment methods. Two methods of heat treatment including moist heat (autoclave at 121°C, 15 psi for 5 minutes) and dry heat (vacuum heat at 110°C for 24 hours) were employed to treat chitosan sponges fabricated by lyophilization technique. Lactic acid solution (2% w/v) was used as solvent of chitosan. Alteration of functional group interaction of the prepared chitosan sponges including untreated, moist heat treated and dry heat treated systems was characterized by using FT-IR spectroscopy. Other physical properties, e.g. water sorption and erosion, morphology and mechanical property of the sponges were evaluated. FT-IR spectra analysis indicated that dry heat treatment created a formation of amide bond between carboxylate groups of side chain molecules; lactate or stearate, and ammonium groups of chitosan. The similar result was observed from moist heat treatment but some peak that indicated hydrolyzed stearic acid was found at 1700 cm^{-1} in the IR spectra. Increment of Alst had influence on decrement of water sorption and erosion of the prepared sponges both untreated and heat treated systems. Moist heat treated sponges had more rigidity than dry heat treated sponges since moist heat treatment evoked shrinkage of sponge structure whereas dry heat treatment was not clearly affected alteration of sponge structure. Changes of physical appearance of the sponges treated by moist heat treatment was lesser observed in the system containing higher amount of Alst. This result indicated that Alst could improve thermal stability of chitosan sponge during moist heat treatment. Both heat treatment methods led to reduction of aqueous solubility of the prepared sponges. Dry heat treated sponges significantly had higher percent of water sorption and erosion than moist heat treated sponges. In conclusion, Alst could reduce physical changes of chitosan sponges from heat treatment. Moist heat treatment had higher effect than dry heat treatment on changes of the physical properties of the chitosan sponges.

Keyword: Chitosan, Aluminum monostearate, Moist heat treatment, Dry heat treatment

Introduction

In the preparation process of chitosan-based biomaterials, some cross-linking agents or neutralization have been employed in order to eradicate acid that used for dissolving chitosan and to modify degradation rate, swelling property, aqueous solubility and to improve mechanical strength of the biomaterials.(1-4) However, these approaches normally consume time and several processes for preparing and neutralizing the final products and had effect on structural deformation. Moreover, toxicity of the cross-linking agent can be occurred. To avoid using cross-linking agents and neutralization process, heat treatment is an interesting alternative approach. Moreover, heat treatment also has an important role in sterilizing process of biomaterials. However, heating process has influence on physicochemical properties of various polymers including chitosan.(5) Some researcher applied heat treatment to cure chitosan-based materials and found that some properties of the materials had been changed, for example, biocompatibility,(6) swelling property(7) and aqueous solubility and water permeability(8) but the treating process generally worsen their mechanical properties and cause shrinkage. To improve mechanical properties of chitosan-based materials, various additives or other polymers have been combined with chitosan.(9-11) Some pevious studies reported that shrinkage of the chitosan-based material after heat treatment could sustain drug release from the system. (7, 12-13)

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However, high swelling of the system when exposed to aqueous medium or severe shrinkage and deformation after heat treatment due to its high active hydrogen bonding functional groups (–OH, –NH_2) would limit its application as 3D structure in tissue engineering field.(14) Although polymer blend could improve mechanical properties of the final material, the problem of shrinkage and deformation after drying was still observed owing to its high hydrophilicity. Therefore, hydrophobic additives may be another choice to solve this problem.

Aim of this study is to investigate an effect of amount of fatty additive; aluminum monostearate (Alst), on physicochemical properties of chitosan sponges treated by different heat treatment methods. Two different heat treatment methods including moist heat treatment (autoclave at 121°C, 15 psi for 5 minutes) and dry heat treatment (vacuum heat at 110°C for 24 hours) were employed to treat chitosan sponges. Lactic acid solution (2% w/v) was used as solvent of chitosan. Changes of functional group interaction of the prepared chitosan sponges including untreated, moist heat treated and dry heat treated systems were characterized by using FT-IR spectroscopy. Other physical properties, e.g. water sorption and erosion and mechanical property of the sponges were evaluated.

Materials and Experimental Procedures

Materesls

Chitosan (Aqua premier, Chonburi, Thailand) having degree of deacetylation of 97% with molecular weight of 70 kDa was sieved through sieve No. 80 mesh before used. Aluminum monostearate (Fluka, Sigma-Aldrich.Co, Missouri, USA), lactic acid, di-sodium hydrogen orthophosphate, potassium hydrogen phosphate, sodium chloride and potassium chloride (Ajax finechem, New South Wales, Australia) were purchased and were analytical grade.

Methods

Chitosan Sponges Preparation

Chitosan powder was dissolved in 2% lactic acid solution overnight with 500 rpm stirring rate to obtain chitosan-lactate (CL) solution containing 4% w/w chitosan. CL solution was then filtered via cellulose fabric to remove insoluble residue. Chitosan-aluminum monostearate (CLALst) composite dispersion was prepared by dispersing various amounts of aluminum monostearate (0.5, 2.5 and 5.0% w/w) in CL solution for 24 hours with 1000 rpm stirring rate. Thereafter, the CL solution and CLAlst dispersion were filled into tablet mold strip (cylindrical shape with 1 cm-diameter and 0.5 cm-depth) before being frozen at -30°C and dried by using lyophilization technique.

Two different heat treatments were employed to treat the prepared chitosan sponges, CL and CLAlst. Moist heat treatment was performed using autoclave sterilization method at 121°C, 15 psi for 5 minutes and the sponges were then dried again in hot air oven at 50°C whereas dry heat treatment was performed using vacuum drying at 110°C for 24 hours.

FT-IR Spectroscopy

Functional groups interaction of the samples (three groups of the chitosan sponges including untreated, moist heat treated and dry heat treated) and single substances (including chitosan and aluminum monostearate) was analyzed using FT-IR spectroscopy (Nicolet 4700, Becthai, USA). The samples were cut into small pieces before grinding with KBr powder in the ratio of approximately 2:100 of sample to KBr. The powder mixture was then pressed into pellet using KBr die kit. The IR spectra were run in the wave number ranges of 4000-400 cm^{-1} with 32 scan at 4 cm^{-1} resolution.

Morphology of the Chitosan Sponges

Morphology of the chitosan sponges was observed using scanning electron microscope (SEM) (Maxim 200 Cam scan, Cambridge, England) at 50x and 200x magnifications in BEI (backscattered electron image) mode. The operation was performed at an accelerating voltage of 15 KeV. The sponges were cut horizontally at the middle and adhered on metal stub using double carbon adhesive and sputter-coated with gold before test.

Water Sorption and Erosion

Chitosan sponges were accurately weighed (W1) and immersed in phosphate buffer pH 7.4 overnight. Subsequently, the wet sponges were removed from buffer solution and wiped off the excess liquid before weighing (W2). Thereafter, the sponges were dried again using hot air oven at 50°C and weighed (W3). Water sorption (% w/w) and erosion (%w/w) of the chitosan sponges were calculated using the following equations.

Water sorption (% w/w) = ((W2-W3)/W3)x100 ……. eq. 1
Erosion (%w/w) = ((W1-W3)/W1)x100 ……..eq. 2

Mechanical Property of the Chitosan Sponges

Chitosan sponges were cut horizontally at the top to obtain a flat surface. Mechanical strength of the sponges was analyzed using texture analyzer (Charpa Techcenter, Godalming, Stable micro Systems Ltd., UK). The test was run in compression mode with target of 6% strain. The instrument was set up with 5 kg load cell. Small cylindrical probe (P/2, 2.0 mm diameter, stainless) was used. Pretest and test speed were set at 2.0 mm/sec and 1.0 mm/sec, respectively. Three sponges of each sample were analyzed.

Results and Discussion

Sponge Physical Appearance

After heat treatment, color of the chitosan sponges was intensified. For moist heat treated group, their color was altered into brown whereas the intensity of their color was lower in the system containing higher amount of Alst. Color of the sponges treated by dry heat was slightly changed into yellow which the color of the system containing different amounts of Alst was not apparently different. Moist heat caused shrinkage of chitosan sponges structure where the shrinkage was less observed in the system containing higher amount of Alst whereas dry heat did not affect the structural changes of the sponges. Upon drying, molecules of chitosan could form intermolecular hydrogen bond causing shrinkage of its structure.(15) In case of moist heat treatment, the sponges exposed to high amount of water molecules that could form numbers of hydrogen bonds with –OH and $-NH_2$ group of chitosan molecules. Thereafter, the sponges were dried in hot air oven where evaporation of water molecules rapidly occurred. Therefore, intermolecular hydrogen bonding of chitosan molecules was formed instead at the position that water molecules evaporated. For dry heat, even it also caused evaporation of water molecules but it did not cause much effect on the sponge shrinkage because the sponges before treating by dry heat were the lyophilized sponges that contained lower amount of water comparing to moist heat treated sponges. Minor shrinkage of moist heat treated sponges was observed from the system containing higher amount of Alst. This might be a result of stearic effect and hydrophobic manner of stearate side chain that obstructed hydrogen bond formation between water molecules and chitosan molecules, therefore, intermolecular hydrogen bonding of chitosan during drying was lessen.

FT-IR Spectroscopy

FT-IR spectra of the single substances were shown in Figure 1. Spectra of chitosan exhibited –C=O stretching peak (amide I) at 1644 cm^{-1} and –N-H bending of amine band at 1597 cm^{-1}.(16) Spectra of Alst exhibited –C=O stretching of carboxylate group ($-COO^-$) of metal stearate at 1589 cm^{-1} and 1470 cm^{-1}).(17) FT-IR spectra of the chitosan sponges in the wave number ranges of 2000-500 cm^{-1} were demonstrated in Figure 2. FT-IR spectra of untreated CL exhibited absorption peak of –C=O stretching of free carboxyl group of lactic acid at 1732 cm^{-1} and carboxylate salt ($-COO^-$) of lactate side chain that bound to amino group ($-NH^{3+}$) of chitosan at 1590 cm^{-1} via electrostatic interaction. For untreated CLAlst, there were two side chain molecules that bound electrostatically to amino group of chitosan chain including lactate and stearate molecules. Moreover, non-dissociated Alst were still in the CLAlst system. Therefore, as the amount of Alst was increased, the –C=O stretching peaks of metal stearate at 1587 cm^{-1} and 1470 cm^{-1} were sharper whereas intensity of the –C=O stretching of carboxyl group of lactic acid at 1732 cm^{-1} was decreased. After heat treatment, minor change of CL spectra was found. The –C=O stretching peak of carboxyl group of free lactic acid at 1730 cm^{-1} was found more intensified in the moist heat treated group comparing to that of untreated and dry heat treated group. This result was concurrent with the previous study. The previous studies reported that for chitosan salt film which the corresponding acid molecule composed of carbon not more than three atoms (such as formic, acetic and propionic acid), there was possibility of amide bond creation between $-NH^{3+}$ of chitosan and $-COO^-$ of the corresponding acid during heat treatment.(18-19) However, the system containing the corresponding acid composed of carbon more than three atoms, the acid could be hydrolyzed during moist heat treatment.(18)

Whereas in CLAlst system, changes of the spectra were found in the system containing 0.25 and 5.0% w/w Alst. Different heat treatments had different effects on changes of their spectra. Comparing to that of IR spectra of the untreated CLALst, dry heat treated-CLAlst exhibited absorption peak around 1635 cm^{-1} which corresponding to –C=O stretching (amide I) peak that overlapped with –C=O stretching of carboxylate group of non-dissociated Alst at 1587 cm^{-1}. The amide I peak could identify that amide bond between amino group of chitosan and carboxyl group of stearate molecule was created during dry heat treatment. This result was concurrent with the previous research work that reported the effect of rigorous dry heat treatment on chitosan-acetate scaffolds. The result indicted that heat treatment (performing at 110°C in vacuum dryer for 1-3 days) evoked formation of amide bound between amino group of chitosan and carbonyl group of acetic acid.(6) Previous study explained that during dry heat treatment, water molecules in some part of ionic bond between amino group of chitosan and carboxylate group of the side chain molecules (−NH^{3+}−$^{-}$OOC−) were cleaved and the amide bond were left instead.(20)

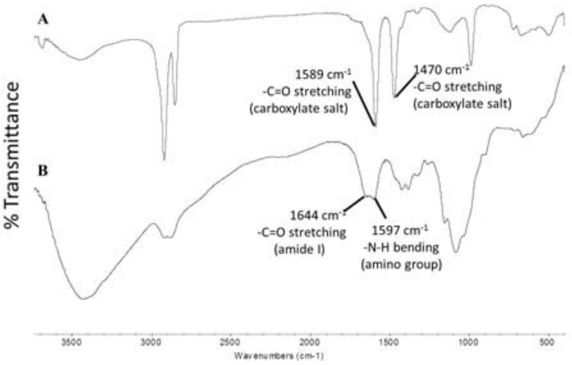

Figure 1. FT-IR spectra of aluminum monostearate (A) and chitosan (B)

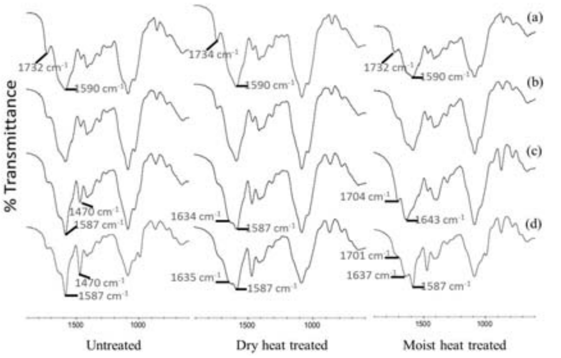

Figure 2. FT-IR spectra of chitosan sponges; untreated, dry heat treated and moist heat treated where (a)=CL, (b)=CLAlst0.5%, (c)=CLAlst2.5% and (d)=CLAlst5.0%

IR spectra of the moist heat treated-CLAlst sponges were different from that of dry heat treated system. In the system containing Alst at 2.5 and 5.0 % w/w, the absorption peak at 1637 cm^{-1} assigning to amide I was intensified. Whereas the –C=O stretching peak of metal stearate at 1587 cm^{-1} was disappeared in the system containing 2.5% w/w Alst but less intensified in the system containing 5.0% w/w Alst. The absorption peak at 1463 cm^{-1} of metal stearate was also decreased. New absorption peak at 1704 cm^{-1} was found in these two spectra which assigned to –C=O stretching of free carboxyl group of stearic acid that some part might be hydrolyzed from non-dissociated Alst and remainder might be cleaved from that of bound on chitosan chain under accelerating condition of acidic environment and high temperature involving with moisture during autoclave process.

Morphology

SEM images of the untreated, moist heat treated and dry heat treated-chitosan sponges were demonstrated in Figure 3. Morphology of almost system exhibited homogeneous porous pattern with irregular pore shape excepted for that of CLAlst containing 5.0% w/w ALst that did not exhibit porous but sheet-like structure. Pore's wall of CL was rather smooth whereas that of CLAlst was partly covered with cluster of non-dissociated Alst/ sterates in system containing Alst not more than 2.5% w/w. For CLAlst containing 5.0% w/w Alst, surface of its wall was completely covered with Alst/stearates. Pore size of CL was approximately in the ranges of 50-100 μm. Pore size of the sponges was larger to the ranges of 150-250 μm in the Alst contained-sponges with declining of the pore regularity. Metal stearates had been reported about their dissociation into metal and stearic acid under acidic environment.(17) In this study, Alst could partly dissociate when immersed in chitosan-lactate solution. Therefore, the dissociated stearate ions could bind to amino group of chitosan to form chitosan-stearate salt complex. Furthermore, the complex cluster could be enlarged to a bigger size by aggregation via hydrophobic interaction of the salt complex and the excess Alst/stearic acid. This result was consistent with the previous report that the higher ratio of fatty acid to chitosan, the bigger size of the complex was.(21) This complex cluster could interrupt 3D network formation of chitosan chain, therefore, larger pore size was exhibited in the system containing higher Alst amount. Heat treatments were not apparently effect on pore size of the sponges but slightly affected on character of the Alst/stearate clusters on chitosan structure.

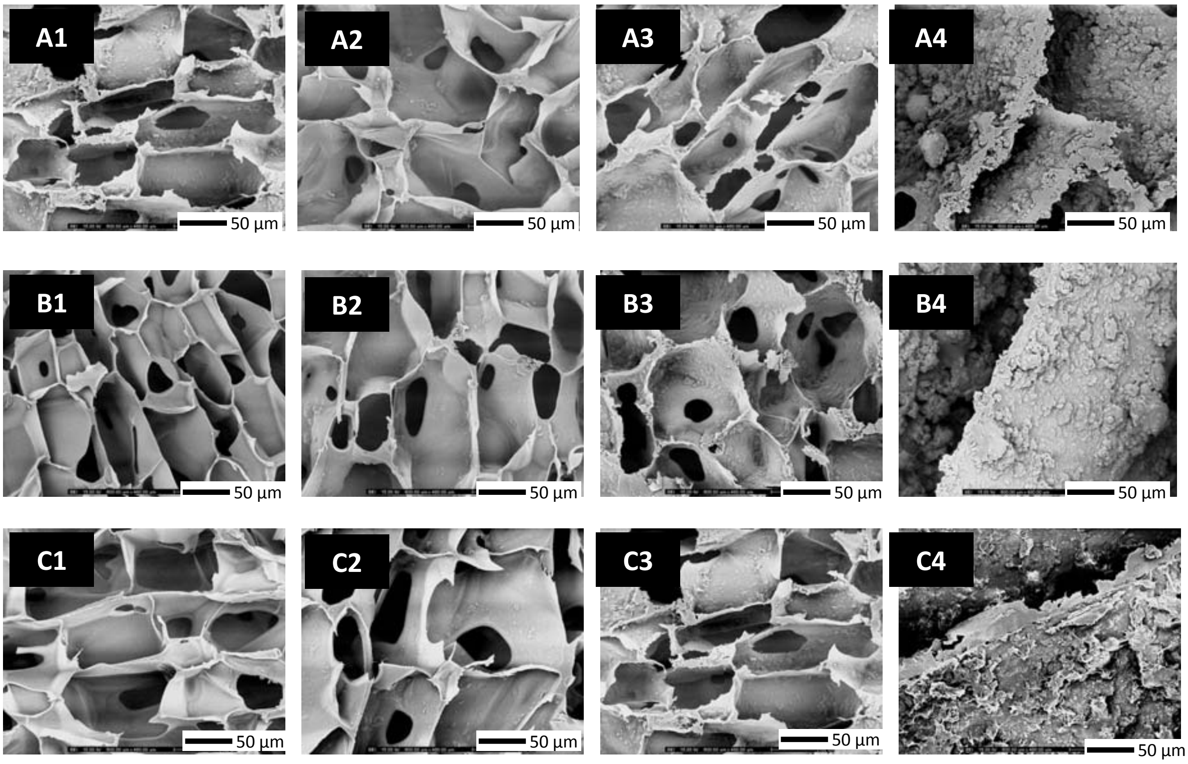

Figure 3. SEM images of chitosan sponges at 200x magnification; A= untreated, B=moist heat treated and C= dry heat treated which 1=CL, 2=CLAlst0.5%, 3=CLALst2.5% and 4=CLAlst5.0%

Water Sorption and Erosion

Untreated CL sponges was rapidly dissolved after soaking in phosphate buffer pH 7.4 and CLAlst0.5 was also completely dissolved but with slower rate than that of CL, whereas CLALst2.5 and CLAlst5.0 were still left some residue after soaking overnight. Since CL and CLAlst0.5 were completely dissolved, their water sorption was assumed as 0 % as shown in Figure 4(A). Water sorption (%) of CLAlst2.5 and CLAlst5.0 were approximately 1036 and 692 %, respectively. After heat treatment, the chitosan sponges both CL and CLAlst systems was changed to insoluble but still could swell in aqueous. Comparing between heat treated sponges, dry heat treated sponge exhibited higher water sorption than that of moist heat treated sponge. Dry heat treated CL sponge was swelled and exhibited gel-like structure with transparent yellow color after immersed in phosphate buffer pH 7.4 whereas the moist heat treated CL was swelled and its structure was like rigid fibrous sponge where its structure was unaltered from original shape. Mean percent of water sorption of CLAlst0.5 sponges treated by different heat treatments were the same at approximately 1692 % w/w whereas for that of sponges containing higher amount of Alst, higher percent of water sorption was observed in dry heat treated system. Higher percent of water sorption of dry heat treated sponge might be due to lower rigidity of the sponge structure comparing to that of moist heat treated group which there were shrinkage of the sponges.

Lower percent of erosion of the prepared sponges was exhibited in the system containing Alst comparing to that of CL sponge as shown in Figure 4(B). As the amount of Alst increased, lower percent of erosion was demonstrated. However, for heat treated sponges, at highest amount of Alst; 5% w/w, percent of erosion of the sponge was higher which might be due to low strength of its structure. Comparing between heat treatment methods, higher percent of erosion was observed from dry heat treated sponges which their structure was less compact than that of moist heat treated group.

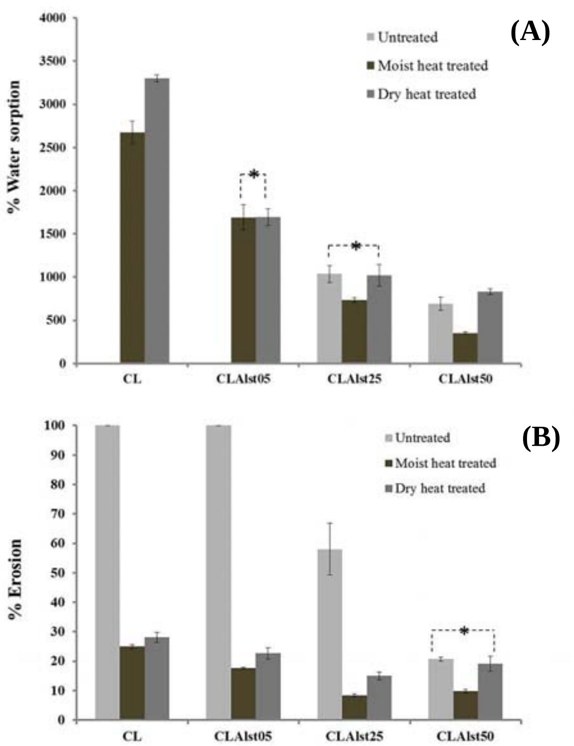

Figure 4. Percent of water sorption (A) and erosion (B) of chitosan sponges; untreated, moist heat treated and dry heat treated group (symbol [--*--] in the diagrams mean "not significantly different", statistical data was analyzed by one way ANOVA with p value < 0.05 using SPSS)

Texture Analysis

Adding Alst into chitosan system had influence on an increment of mechanical strength of the sponges. However, as amount of Alst was increased, mechanical strength of the prepared sponges was decreased especially for that of CLAlst5.0 system as shown in Figure 5. This was a result of loosening structure of the sponge containing high amount of Alst as seen from SEM images. Moist heat treatment resulted more rigidity of the sponges structure that evoked an increment of their mechanical strength comparing to that of untreated sponges. Mechanical strength of dry heat treated CL and CLAlst0.5 sponges was higher than that of untreated sponges but lower than that of moist heat treated systems, however, effect of dry heat treatment on mechanical strength was not clearly different from untreated sponges in the system containing Alst more than 0.5 % w/w. This result was concurrent with previous studies that hydrophobic additives could cause reduction of mechanical strength of chitosan-based film.(11, 21-22)

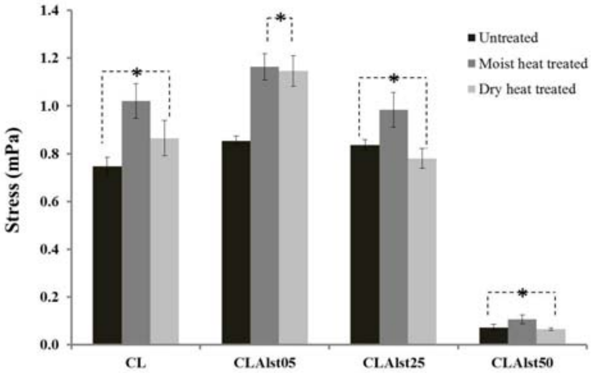

Figure 5. Compressive stress value at 6% strain of dried chitosan sponges; untreated, moist heat treated and dry heat treated group (symbol [--*--] in the diagram mean "not significantly different", statistical data was analyzed by one way ANOVA with p value < 0.05 using SPSS)

Conclusion

Different heat treatment methods had different effects on physicochemical properties of the chitosan sponges. Moist heat led to more shrinkage and color intensification than that of dry heat treatment. Adding Alst could reduce shrinkage and color intensification of the sponges after heat treatment. Heat treated sponges were insoluble in phosphate buffer pH 7.4 but could swell. Lesser percent of water sorption and erosion was exhibited in moist heat treated group comparing to that of dry heat treated group. Adding Alst caused reduction of percent of water sorption and erosion of the sponges. Alst could improve strength of the sponges, however, strength of the system was reduced at high amount of Alst due to loosening of the sponges structure.

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References

1. Welsh, E.R., Schauer, C.L., Qadri, S.B. and Price, R.R. (2002). Chitosan cross-linking with a water-soluble, blocked diisocyanate. 1. solid state. *Biomacromolecules*. **3** : 1370-1374.

2. Silva, R.M., Silva G.A., Coutinho, O.P., Mano, J.F. and Reis, R.L. (2004). Preparation and characterisation in simulated body conditions of glutaraldehyde crosslinked chitosan membranes. *J. Mater. Sci. -Mater. Med.* **15** : 1105-1112.

3. New, N., Furuike, T. and Tamura, H. (2009). The mechanical and biological properties of chitosan scaffolds for tissue regeneration templates are significantly enhanced by chitosan from *Gongronella butleri. Materials.* **2** : 374-398.

4. Moura, M.J., Faneca, H., Lima, M.P., Gil, M.H. and Figueiredo, M.M. (2011). *In situ* forming chitosan hydrogels prepared via ionic/ covalent co-cross-linking. *Biomacromolecules.* **12** : 3275-3284.

5. Pielichowski, K. and Njuguna, J. (2005). Natural polymer. In : *Thermal degradation of polymeric materials*. Shropshire, : Rapra Technology : 129-151.

6. Kim, C.H., Park, H.S., Gin, Y.J., Son, Y.S., Lim, S.H., Choi, Y.J., Park, K.S. and Park, C.W. (2004). Improvement of the biocompatibility of chitosan dermal scaffold by rigorous dry heat treatment. *Macromol. Res.* **12(4) :** 367-373.

7. Cruz, A.B., Surdi, M., Cenci, M.M.P. and Rodrigues, C.A. (2008). Tetracycline release from chitosan films. *Lat. Am. J. Pharm.* **27(3) :** 360-3.

8. Rivero, S., García, M.A. and Pinotti, A. (2012). Heat treatment to modify the structural and physical properties of chitosan-based films. *J. Agr. Food Chem.* 60 : 492 – 499.

9. Xu, Y.X., Kim, K.M., Hanna, M.A. and Nag, D. (2005). Chitosan–starch composite film: preparation and characterization. *Ind. Crop. Prod.* **21** : 185–192.

10. Rotta, J., Minatti, E. and Barreto, P.L.M. (2011). Determination of structural and mechanical properties, diffractometry, and thermal analysis of chitosan and hydroxypropylmethylcellulose (HPMC) films plasticized with sorbitol. *Ciênc. Tecnol. Aliment.* 31(2) : 450-455.

11. Cerqueira, M.A., Souza, B.W.S., Teixeira, J.A. and Vicente, A.A. (2012). Effect of glycerol and corn oil on physicochemical properties of polysaccharide films: a comparative study. *Food Hydrocolloid.* **27** : 175-184.

12. Ritthidej, G.C., Phaechamud, T. and Koizumi, T. (2000). Additives in moist- heat-treated chitosan acetate films: a method to extend the release of propranolol hydrochloride-coated tablets. *S.T.P. Pharma Sci.* **10(1)** : 112-119.

13. Hsieh, W.C., Chang, C.P. and Gao, Y.L. (2006). Controlled release properties of chitosan encapsulated volatile citronella oil microcapsules by thermal treatments. *Colloid Surf. B.* **53** : 209–214.

14. Cestari, A.R., Vieira, E.F.S., Pinto, A.A. and Lopes, E.C.N. (2005). Multistep adsorption of anionic dyes on silica/chitosan hybrid 1. Comparative kinetic data from liquid- and solid-phase models. *J. Colloid Interface Sci.* **292** : 363–372.

15. Yamaguchi, I., Itoh, S., Suzuki, M., Osaka, A. and Tanaka, J. (2003). The chitosan prepared from crab tendons: II. The chitosan/apatite composites and their application to nerve regeneration. *Biomaterials.* **24** : 3285–3292.

16. Yahya, M.Z.A. and Arof, A.K. (2003). Effect of oleic acid plasticizer on chitosan–lithium acetate solid polymer electrolytes. *Eur. Polym. J.* **39** : 897–902.

17. Phaechamud, T., Charoenteeraboon, J. and Mahadlek, J. (2009). Characterization and in-vitro drug release of a chitosan-magnesium stearate monolithic matrix system. *Asian J. Pharm. Sci.* **4(5)** : 265-276.

18. Ritthide, j. G.C., Phaechamud, T. and Koizumi, T. (2002). Moist heat treatment on physicochemical change of chitosan salt films. *Int. J. Pharm.* **232(1-2)** : 11-22.

19. Zotkin, M.A. , Vikhoreva, G.A. , Smotrina, T.V. and Derbenev, M.A. (2004). Thermal modification and study of the structure of chitosan films. *Fibre Chem.* **36(1)** : 16-20.

20. Mudarisova, R.Kh., Kulish, E.I., Kuzina, L.G., Kolesov, S.V. and Monakov, Yu.B. (2009). Thermal modification of chitosan films as a way to control their transport properties. *Russ. J. Appl. Chem*. 82(8) : 1479 − 1482.

21. Vargas, M., Albors, A., Chiralt, A. and González-Martínez, C. (2009). Characterization of chitosan–oleic acid composite films. *Food Hydrocolloid*. **23** : 536 – 547.

22. Martins, J.T., Cerqueira, M.A . and Vicente, A.A. (2012). Influence of α-tocopherol on physicochemical properties of chitosan-based films. *Food Hydrocolloid*. **27** : 220-227.