International Journal of Engineering Research
Volume No.3, Issue No.11, pp : 632-636
ISSN:2319-6890)(online),2347-5013(print)
01 Nov. 2014

Elaboration of Tea Polyphenols-Chitosan Complexes with Antibacterial and Antioxidant Properties through Adsorption

H. Ben Ali[1], W. Tahri[2], N. Rahmouni[1], S.Besbes- Hentati[1]
[1]Laboratory of Materials Chemistry, Faculty of science Bizerte, Tunisia
[2]Laboratory of Biochemistry and Molecular Biology, Faculty of science Bizerta, Tunisia
Corresponding Email: salma.hentati@fsb.rnu.tn

Abstract*— *Antibacterial and antioxidant polyphenols-chitosan agents have been elaborated through the adsorption of green tea and black tea extracts into chitosan. Their incorporation leads to an enhancement of the inhibitory effect of the biopolymer against the gram-negative and gram-positive bacteria. Voltammetry at a rotating disk electrode reveals a difficulty of the reduction of oxygen in the presence these complexes.

Keywords— Adsorption; Chitosan-polyphenols complexes; Antibacterial activity; Antioxidant.

I. Introduction

Polyphenolic compounds are well known for having antimicrobial activities toward a large number of pathogenic bacteria [1–4]. Tea extracts have been associated to the inhibition of various Gram–positive and Gram–negative bacteria including Salmonella typhimurium and Vibrio cholerae [3,5–10]. This of green tea inhibits Staphylococcus aureus, Shigella disenteriae, Vibrio cholerae, Campylobacter jejuni and Listeria monocytogenes of the food [5]. The antimicrobial activities of some polyphenols extracted from the green tea, including the epigallocatechin and the epicatechin gallate, as well as a theaflavin mixture of black tea against Salmonella, Escherichia coli and some species of the genus Vibrio have been also revealed [9]. To explain this benefic effect, an inhibition of DNA and RNA synthesis [11], this of cytoplasmic membrane function [12] and an interfering with energy metabolisms, of bacterial cells by green tea polyphenols, have been proposed [13].

In view to contour the instability of these powerful antimicrobials polymers [14], a large number of works are focused on their extraction [15]. Entrapment of polyphenols by chitosan, which results from deacetylation of the crustaceans shell chitin, has been used to protect catechin from interactions with the food matrix and to produce drug delivery systems [16]. Besides, it has been reported that the incorporation of the green tea extract into the active film of chitosan [17], enhances its corresponding antibacterial properties. The biological activity of this polysaccharide that consists of glucosamine and N–acetylglucosamine copolymers is generally attributed to the interaction between its ammonium cation and the negatively charged macromolecules of microbial cell [18].

Although there was some number of reports on the adsorption of tea polyphenols into polymeric materials [19–21], there appears that the study of the adsorption process of the tea polyphenols into chitosan, in points of view equilibrium isotherm, kinetic and physico-chemical characterization, has never been undertaken, whereas it could provide valuable information about the interaction between these two biopolymers. Beyond these mechanistic questions, their adjunction should be important for the elaboration of new antibacterial agent.

The objective of this work relates to the elaboration of new polyphenols/chitosan complexes by means of the adsorption of green tea (GT) and black tea (BT) polyphenols (Fig. 1a) into chitosan (CH, Fig. 1b). In a first part, we'll report the investigation of this process, by using equilibrium isotherm and kinetic study. Then, we will focus on the characterization of the two obtained complexes. From there, we'll turn to their antibacterial activities against Staphylococcus aureus (*S. aureus*), Escherichia coli (*E. coli*), Pseudomonas aeruginosa (*P. aeruginosa*) and Salmonella typhimurium, (*S. typhimurium*).

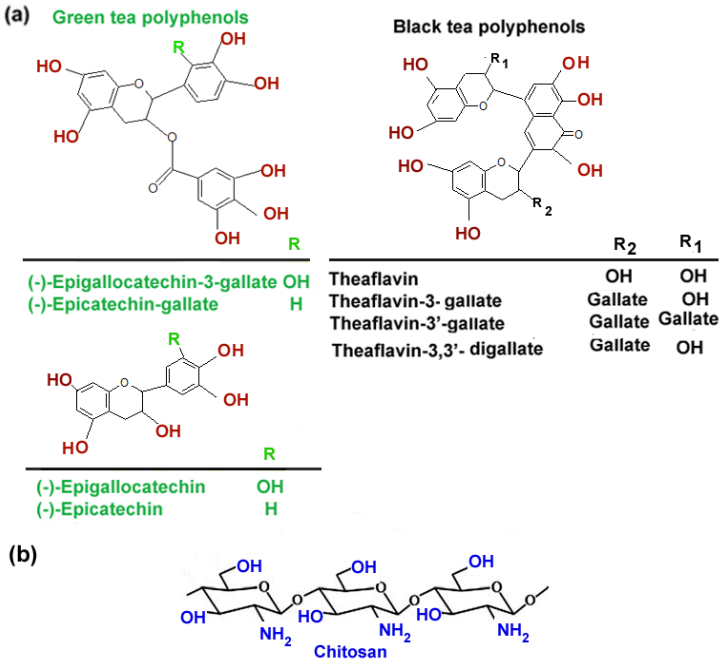

Figure 1: Structures of GT and BT tea polyphenols (a) and chitosan (b).

II. Material and Methodology

GT and BT Lipton Tazza CTC leaf tea powder (Hindustan Lever Ltd, Bombay) were used. CH was prepared by purification and deacetylation of deep-pink shrimp shell chitin (Parapenaeus Longirostris) [22]. Its degree of deacetylation was about 82 %

ISSN:2319-6890)(online),2347-5013(print)
01 Nov. 2014

[23]. Its average particle sizes were < 600 µm. Its BET surface area, determined by the nitrogen adsorption method was of 6.5972 $m^2\ g^{-1}$.

The infusions of tea (0.30–2.50 g in 100 cm^3 of distilled water) were stabilized for 24 Hours at 25°C. Their pH was adjusted by H_2SO_4 and NaOH solutions and CH (0.02–0.30 g) was added. After an agitation for 8 Hours in a thermostatic rotary shaker (T = 30 °C, 150 rpm), 5 cm^3 of the bath solution was extracted, at pre-determined time intervals. The analysis of the samples was performed by UV-visible, at a maximum absorption band of 270 nm. Uptakes were determined as the difference between the initial tea concentration and the one of the supernatant.

CH/GT and CH/BT were prepared at the optimum conditions, by mixing 0.1 g of chitosan with 100 cm^3 of the tea extracts (2g dm^{-3}, pH 4).

Antimicrobial susceptibilities of *S. aureus* and *E. coli* (JW 1772), *P. aeruginosa* and *S. typhimurium* (ATCC14028) were performed using the Kirby-Bauer Disk diffusion method. These pathogenic organisms are grown on Mueller-Hinton agar in the presence of CH, CH/GT and CH/BT (30 mg dm^{-3}). 2.4 % (w/v) of these materials is dissolved in 1.0 % (v/v) acetic acid, before being added to Nutrient Broth (Boikar diagnostics). The obtained mixtures were autoclaved at 120 ºC for 15 min. Colonies of bacteria were inoculated to 5 cm^3 of nutrient broth until an optical density was adjusted to 0.5 OD unit at 570 nm (C UF = 10^6 cm^{-3}). After an incubation for 3 h at 37 °C, 0.1 cm^3 of these suspensions were spread over the agar surface. Excess slurry is removed and the box is placed in an oven for 20 min at 37 °C. Sterile Whatman papers (N° 3), placed on the agar surface, are soaked with 0.03 cm^3 of the solutions. Chloramphenicol (0.03 cm^3) was used as a positive control. An acetic acid solution (1 %) was also tested. The Petri dishes were placed at 4 °C for 2 hours, and then incubated at 37 °C for 24 h [24].

UV-visible analyses were made on a Shimadzu UV–2101 PC spectrophotometer. FTIR characterizations were performed with a Brucker IFS 66 NS spectrometer. Samples of chitosan and its complexes were coated with a thin layer of gold and examined by a JEOL scanning electron microscopy (Model JSM – 6390LV). Their thermal stabilities (5 mg) were studied by Metler Toledo DSC–1 Star System, at a heating rate of 10 °C min^{-1}.

The electrochemical set-up was a Tacussel (PGP 201) potentiostat. A three-electrode cell consisting of a rotating platinum disc (φ = 2 mm), as the working electrode (EDI type radiometer) and a platinum wire as the counter electrode. The reference electrode was AgCl/Ag.

III. Results and Discussion

Adsorption process

The equilibrium uptakes of tea extracts present optimum values for pH 4 [25,26], 0.1 g of CH [27] and tea extracts concentration of 2 g dm^{-3} (Fig. 2a-c).

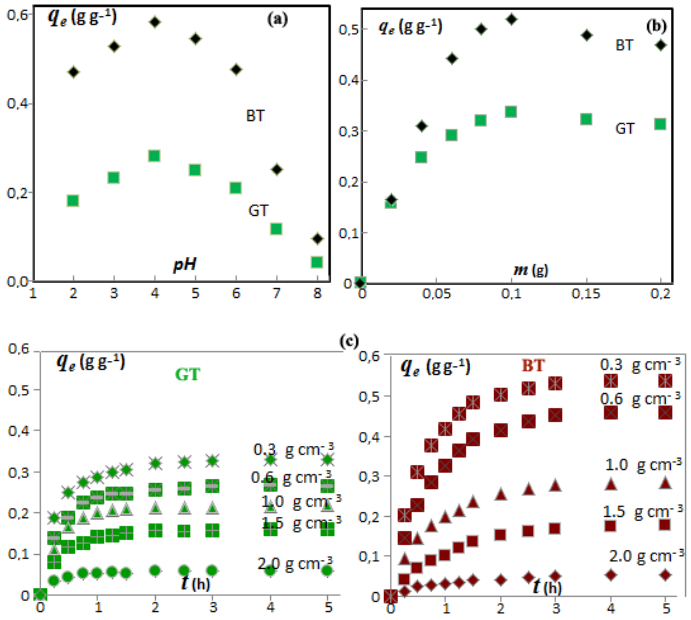

Figure.2:. Effects of the experimental conditions on the efficiency of GT and BT adsorption into CH at T= 30°C, (a) the pH of the tea extracts (C_0 = 2 g dm^{-3}, 0.1 g of chitosan), (b) the chitosan dose (pH=4, C_0 = 2 g dm^{-3}) and (c) the initial concentration of tea extracts (pH= 4, 0.1 g of CH).

For the adsorption isotherms (Fig.3), the highest values of the correlation coefficient (R^2), which measures the difference between the experimental and the theoretical data of the linear equations of Langmuir and Freundlich [28,29], are obtained for the later model (Eq 1, Table 1).

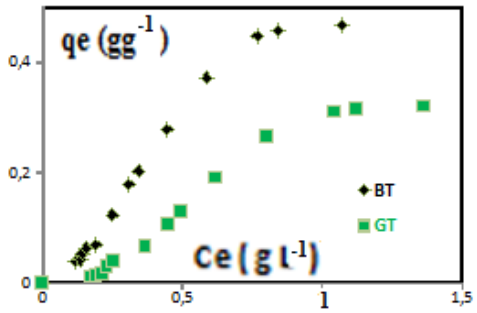

Figure. 3: Isotherm equilibrium of GT and BT adsorption into CH at T= 30°C (pH= 4, C_0 = 2 g dm^{-3} and 0.1 g of CH).

The linear expression of **Freundlich** equation is:

$$\ln q_e = \ln K_F + \frac{1}{n} \ln C_e \qquad (1)$$

K_F and n are the binding energy constant and the Freundlich exponent related to the adsorption intensity (dimensionless), respectively.

ISSN:2319-6890)(online),2347-5013(print)
01 Nov. 2014

The obtained values of n > 1, give an indication of the favorability and the capacity of the adsorption [31].

Table 1. Langmuir and Freundlich isotherm constants of GT and BT polyphenols adsorption into CH.

Langmuir isotherm constants			
	q_m (g g^{-1})	K_L (L g^{-1})	R^2
GT	0.343	0.912	0.9067
BT	0,663	1.094	0.9631
Freundlich isotherm constants			
	n	K_F (L g^{-1})	R^2
GT	1.867	0.404	0.9743
BT	1.317	0.705	0.9803

Among the pseudo–first order and pseudo–second order models [32,33], the kinetic of the adsorption fit well with the later one (Eq. 2, Table 2), which predicts chemical adsorption [34].

$$\frac{t}{q} = \frac{1}{q_e^2 K_I} + \frac{1}{q_e} t \qquad (2)$$

In Equations (2), q (g g^{-1}), q_e (g g^{-1}) and K_I (g mg^{-1} min^{-1}) are the amounts of the adsorbat, at the equilibrium and at each time contact (min) and the pseudo–second order kinetic rate constant, respectively.

Table 2: The pseudo-second order rate constants of the adsorption processes of GT and BT polyphenols into CH.

	C_0 (g L^{-1})	$K_I \times 10^4$ (g mg^{-1} min)	Qe (g g^{-1})	R^2
GT	0.3	9.447	0,060	0,9981
	0.6	2.178	0,166	0,9981
	1	2.207	0,223	0,9986
	1.5	1.468	0,275	0,9984
	2	1.21	0,341	0,9984
BT	0.3	7.61	0,066	0,9961
	0.6	48.586	0,168	0,9974
	1	28.233	0,250	0,9992
	1.5	4.662	0,362	0,9997
	2	1.249	0,513	0,9993

Noticeable modifications of the characteristics of chitosan, in the two elaborated complexes at the optimum conditions, have been highlighted by DSC, FTIR, SEM and ^{1}H NMR (Fig.4).

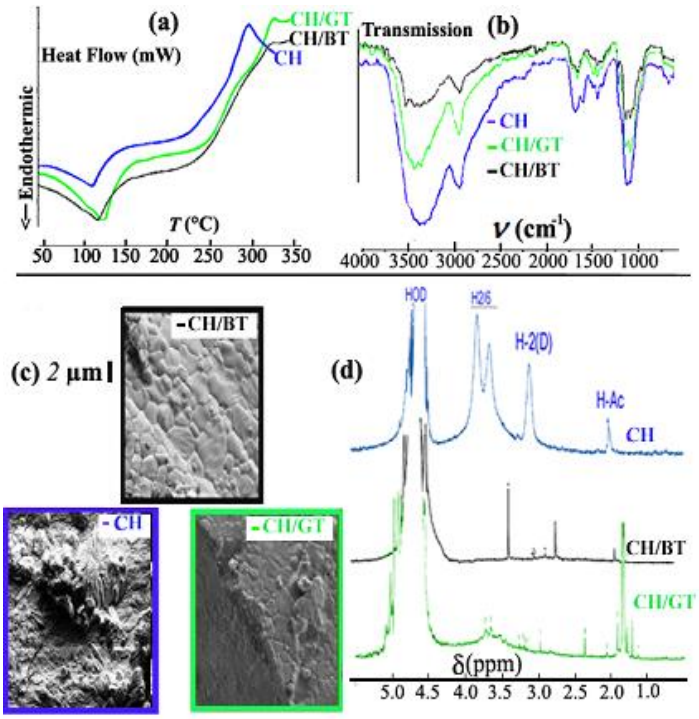

Figure 4: (a) DSC thermograms, (b) FTIR spectra, (c) scanning electron micrographs and (d) ^{1}HNMR spectra of CH and its complexes (CH /GT and CH /BT).

A remarkable characteristic in the thermograms of CH/GT and CH/BT is the appearances of two exothermic processes follow one after another steps with a small time lag in the same temperature range as those of tea, while the exothermic step of degradation of chitosan is not clearly evidenced [35,36]. The overlapped events may be attributed to decompositions of the newly complexes.

SEM characterization shows a densely and homogeneously adhesion of tea polyphenols on CH.

In FTIR spectra, an important decreasing of the band between 3500 and 3000 cm^{-1} (vibration of free OH, asymmetric and symmetric stretching of the NH bonds is observed. In addition, the strong band at 1530 cm^{-1} (OH in-plane bending) disappears completely. Finally, new peaks are observed at 1700 cm^{-1} (CO stretching) and 1640 cm^{-1} (CC stretching). These funding are in good agreement with the formation of new functional groups [37].

1H NMR spectra indicate changing of the characteristic peaks of CH [38,39], in addition to the appearance of new signals.

At 25 °C, desorption curves show two periods (Fig. 5), which are insensible to pH. Between 0 and 20 min, rapid deliverance of

ISSN:2319-6890)(online),2347-5013(print)
01 Nov. 2014

GT and BT polyphenols reach only 6.5 and 8 % of the incorporated ones, respectively. After 20 min, the releases become independent to time.

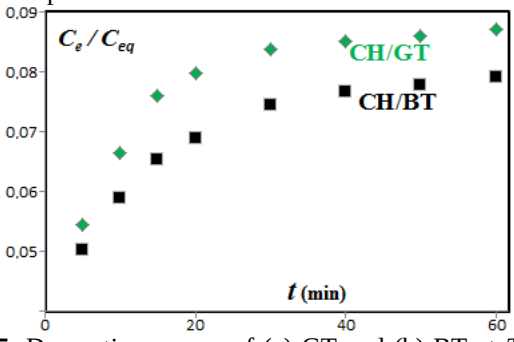

Figure 5: Desorption curves of (a) GT and (b) BT at T = 30°C and pH = 4.00, from complexes of CH /GT and CH /BT, respectively.

Antibacterial effect

Excepting of S. typhimurium, the antibacterial activity of chitosan is enhanced by the adjunction of the tea polyphenols (Table 3).

Table.3. Diameters of inhibitory zone of CH, CH/BT, CH/GT against *S. aureus*, *E. coli*, *P. aeruginosa* and *S. typhimurium*

	Inhibition zone diameter (mm)				
Microorganims	CH	CH/BT	CH/GT	Positive control	Negative control
S.aureus	11±0.199	13±0.156	15±0.125	34	-
E.Coli	10±0.156	11±0.241	13±0.177	28	-
P.aeruginosa	9±0.123	11±0 .125	12±0.222	30	-
S.typhimurium	-	-	-	10	-

Besides, CH/GT is the most effective material against *E. coli*, *P. aeruginosa and S. aureus* (Fig.6), with inhibition zone of 13, 12, 15 mm, respectively. The later bacterium presents the highest sensitivity to all antimicrobial disks. The greatest inhibition is observed against the gram–positive bacterium [40-42].

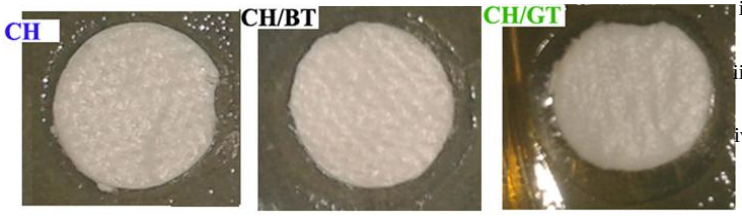

Figure.6: The diameter of the inhibition zone against *S. aureus.*

Antioxidant effect

In the presence of the two complexes (Fig. 7), a decreasing of the cathodic reduction of oxygen, which arises at more cathodic potential, is observed. These funding indicate a diminution of the concentration of the dissolved oxygen as well as a biggest difficulty of its reduction. Here again a greater antioxidant effect occurs with the first complex.

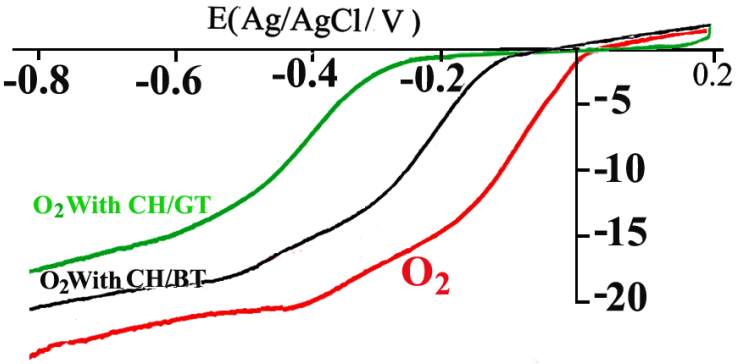

Figure 7: Voltammograms of O_2 in the absence and the presence of the complexes, at Platinum Rotating disk (ϕ = 2 mm, ω = 1000 rev min^{-1}), in acetate buffer solution (pH = 4.00).

IV. CONCLUSION

The incorporation of the polyphenols in GT, as well as those of BT within the CH, through this adsorption process, can be used to modify the properties of chitosan and strengthen its inhibitory as well as its antioxidant action. These newly elaborated assemblies could be regarded as quite interesting biomaterials for human and animal health.

.

Acknowledgement

This work was supported by the Ministry of Scientific Research Technology and Competence Development of Tunisia.

References

i. Ignat, I., Volf, I. and Popa, V. I, A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables, Food Chem., 2011, 12,1821-1835.

ii. Cowan, M. M., Plants products as antimicrobial agents, Clin. Microbiol. Rev., 12 (4), 1999, 564-582.

iii. Hamilton-Miller, J. M. T., Antimicrobial properties of tea (Camellia sinensis L), Antimicrob. agents ch., 39 (11), 1995, 2375-2377.

iv. Diker, K. S., Akan, M.G., Hascelik and Yurdakok, M., "The bactericidal activity of tea against *Campylobacter jejuni* and *Campylobacter coli*, Lett. Appl. Microbiol, 12, 1991, 34-35.

v. Almajano, M. P., Carbó, R., Jiménez J. A. L., and Gordon, M. H., Antioxidant and antimicrobial activities of tea infusions, Food Res. Int., 43, 2010,1379-1382.

vi. Blanco, A. R., Roccaro, A. S., Spoto, G.C., Nostro, A. and Rusciano, D., Epigallocatechin gallate inhibits biofilm formation by ocular staphylococcal isolates, Antimicrob. Agents Ch. 49, 2005, 4339-4343.

ISSN:2319-6890)(online),2347-5013(print)
01 Nov. 2014

vii. Ikigai, H., Nakae, T., Hara, Y. and Shimamura, T., Bactericidal catechins damage the lipid bilayer, Biochim. Biophys. Acta, 1147, 1993, 132-136.

viii. Zhao, W. H., Hu, Z. Q., Hara, Y. and Shimamura, T., Inhibition of penicillinase by epigallocatechin gallate resulting in restoration of antibacterial activity of penicillin against penicillinase-producing Staphylococcus aureus, Antimicrob. Agents. Ch, 46, 2002, 2266-2268.

ix. Taguri, T. Tanaka, T. and Kouno, I., Antimicrobial activity of 10 different plant polyphenols against bacteria causing food-borne disease, Biol. Pharm. Bull., 27 (12), 2004, 1965-1969.

x. Ubonrat, S. and Bruce, R.H., Physical properties and antioxidantactivity of an active film from chitosan incorporated with green tea extract, Food Hydrocolloid, 24, 2010, 770-775.

xi. Ubonrat, S. and Suparat, N., Active film from chitosan incorporating green tea extract for shelf life extension of pork sausages, Food Hydrocolloid, 27, 2012, 102-108.

xii. Farkas, V., Fungal cell walls: Their structure, biosynthesis and biotechnological aspects, Acta Biotechnol., 10, 1990, 225-238.

xiii. Badawy, M. E. I., Rabea, E. I., Rogge, T. M., Stevens, C. V., Smagghe, G., Steurbaut, W. and Höfte, M., Synthesis and fungicidal activity of new N,O-acyl chitosan derivatives. Biomacromolecules, 5, 2004, 589-595.

xiv. Rabea, E. I., Badawy, M. E. T., Stevens, C. V., Smagghe, G. and Steurbaut, W., Chitosan as antimicrobial agent: applications and mode of action, Biomacromolecules, 4, 2003, 1457-1465.

xv. Young, D. H., Kohle H. and Kauss, H., Effect of Chitosan on Membrane Permeability of Suspension-Cultured Glycine max and Phaseolus vulgaris Cells, Plant. Physiol., 70 (5), 1982, 1449-1454.

xvi. Yang, E. J., Kim, J. G., Kim, J. Y., Kim, S. and Lee, N., Anti-inflammatory effect of chitosan oligosaccharides in RAW 264.7 cells, Cent. Eur. J. Biol., 5, 2010, 95-102.

xvii. Chung, Y. C., Yeh, J. Y., and Tsai, C. F., Antibacterial Characteristics and Activity of Water-Soluble Chitosan Derivatives Prepared by the Maillard Reaction, Molecules, 16, 2011, 8504-8514.

xviii. Huang, J., Huang, K., Liu, S., Luo, Q. and Xu, M., Adsorption properties of tea polyphenols onto three polymeric adsorbents with amide group, J. Colloid Interf. Sci., 315, 2007, 407-414.

xix. Li, H.T., Xu, M. C., Shi, Z. Q. and He, B. L., Molecular imprinted polymers-Novel polymer adsorbents, Chin. J. React. Polym., 10, 2001, 84-94.

xx. Spencer, C. M., Cai, Ya., Martin, R., Gaffney, S. H., Goulding, P. N., Magnolato, D., Lilley T. H,. and Haslam, E., Polyphenol complexation-some thoughs and observations, Phytochemistry, 27, 1998, 2397-2409.

xxi. Tolaimate, A. Desbrieres, J., Rhazi, M. and Alagui, A., Contribution to the preparation of chitins and chitosan with controlled physico-chemical properties, Polymer, 44, 2003, 7939-7952.

xxii. Domszy, J. G. and Roberts, G. A. F., Evaluation of infrared spectroscopic techniques for analyzing chitosan, Macromol. Chem., 186, 1985, 1671-1677.

xxiii. Cox, S. D ., Man, C.M., Markham, J.L. Bell, H.C., Gustafson, J.E., Warmington, J. R. and Wyllie, S.G., The mode of antimicrobial action of the essential oil of Melaleuca alternifolia (tea tree oil), J. Appl. Microbiol., 88, 2000, 170-175.

xxiv. Liu, F., Luo, X., and Lin, X., Adsorption of tannin from aqueous solution by deacetylated konjac glucomannan, J. Hazard. Mater., 178, 2010, 844-850.

xxv. An, J. H. and Dulz, S., Adsorption of tannic acid on chitosan montmorillonite as a function of pH and surface charge properties, Appl. Clay Sci., 36, 2007, 256-264.

xxvi. Crini, G., Martel, B. and Torri, G., Adsorption of C. I. Basic Blue 9 on Chitosan based materials, Int. J. Environ. Pollut., 34, 2008, 451-465.

xxvii. Langmuir, I., The adsorption of gases on plane surfaces of glass, mica and platinum, J. Am. Chem. Soc., 40, 1918, 1361-1368.

xxviii. Freundlich, H., Over the*adsorption in solution*, *Z. Phys. Chem., 57, 1906, 384*–470.

xxix. Uzun, İ. and Güzel, F., Kinetics and thermodynamics of the adsorption of some dyestuffs and p-nitrophenol by chitosan and MCM-chitosan from aqueous solution, J. Colloid Interf. Sci., 274, 2004, 398-412.

xxx. Kumar, K.V., Sivanesan, S. and Ramamurthi, V., Adsorption of malachite green onto Pithophora sp., a fresh water algae, Process Biochem., 40, 2005, 2865-2872.

xxxi. Ho, Y. S., Review of second-order models for adsorption systems, J. Hazard. Mater., 136, 2006, 681-689.

xxxii. Ng, J. C. Y., Chung, W. H. and Mckay, G., Equilibrium studies of the sorption of Cu (II) ions onto chitosan, J. Colloid. Interf. Sci., 255, 2002, 64-74.

xxxiii. Ho, Y. S. and McKay, G., Pseudo-second order model for sorption processes; Process Biochem., 34, 1999, 451-465.

xxxiv. Harish Prashanth, K. V., Kittur, F. S. and Tharaathan, R. N., Solid state structure of chitosan prepared under different N-deacetylating conditions, Carbohydr. Polym., 50, 2002, 27-33.

xxxv. Guinesi, L. S. and Cavalheiro, E. T. G., The use of DSC curves to determine the acetylation degree of chitin/ chitosan samples, Thermochim. Acta, 444, 2006, 128-133.

xxxvi. Curcio, M., Puoci, F., Iemma, F., Parisi, O. I., Cirillo, G., Spizzirri, U. G. and Picci, N., Covalent insertion of antioxidant molecules on chitosan by a free radical grafting procedur J. Agric. Food Chem., 57, 2009, 5933-5938.

xxxvii. Duarte, M.L., Ferreira, M.C., Marvao, M.R., and J. Rocha, Determination of the degree of acetylation of chitin materials by 13C CP/MAS NMR spectroscopy, Int. J. Biol. Macromol., 28, 2001, 359-363.

xxxviii. Hirai, A., Odani, H. and Nakajima, A., Determination of degree of deacetylation of chitosan by ^{1}H NMR spectroscopy, Polym. Bull., 26, 1991, 87-94.

xxxix. No, H. K., Park, N. Y., Lee, S. H. and Meyers, S. P., Antibacterial activity of chitosans and chitosan oligomers with different molecular weights, Int. J. Food Microbiol., 74, 2002, 65-72.

xl. Takahashi, T., Imai, M., Suzuki, I. and Sawai, J., Growth inhibitory effect on bacteria of chitosan membranes regulated with deacetylation degree, Biochem. Eng. J., 40, 2008, 485–491.

xli. Coma, V., Deschamps, A. and Martial-Gros A., Bioactive Packaging Materials from Edible Chitosan Polymer-Antimicrobial Activity Assessment on Dairy Related Contaminants, J. Food Sci., 68, 2003, 2788-2792.