Study of Mechanism of Bile Acids Binding in chitosan Cross-linked Banana Fruit Dietary Fiber

Paweena Jansuk[1], Noppadon Cheamsawat[2], Naphaporn Chiewchan[3] and Khanok Ratanakhanokchai[4]

Abstract

This research studied the dietary fiber in banana fruit whose pectin and starch had been removed. The fiber was hydrolyzed with sulfuric acid (0.4% w/v) for 12 hours and was heated with saturated steam at a temperature of 200 $^{\circ}C$ for 4 minutes in a steam explosion system. This method is considered as chemical and physical treatments to the fiber. The analysis of chemical compositions of the fiber was conducted, both before and after treatment. The structure and morphology of the fiber were tested by scanning electron microscopy (SEM), both before and after cross-linkage treatment with chitosan, which was confirmed by checking the polymer loading (PL). The results revealed that the PL of the treated fiber was higher than the untreated elucidate. The analysis of the fiber cross-linked with chitosan was measured by Ninhydrin method to analyze the crystal structure of cellulose after bonding. The test of the structure and properties of fiber chitosan cross-linked was conducted by SEM; cross-function was analyzed by Fourier transform infrared spectroscopy (FT-IR). The cellulose pretreatment with acid hydrolysis has more matrix void than that with steam explosion process, which operates by expanding dietary fiber.

Keywords: bile acid cellulose dietary fiber chitosan steam explosion process acid hydrolysis process.

[1,2] Department of Chemical Engineering, Faculty of Engineering, King Mongkut's University of Technology Thonburi, Bangkok 10140, Thailand.

[3] Department of Food Engineering, Faculty of Engineering, King Mongkut's University of Technology Thonburi, Bangkok 10140, Thailand.

[4] Department of Biochemical Technology, Faculty of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok 10140, Thailand.

Introduction

This research is a part of development in the main research of “Banana Innovative products”. Banana is the good source of nutrients, which contain protein, mineral and dietary fiber. The projects related to this research have been developed since 2002 including 1) concentrate fructose extraction process 2) production of RS3 contained flour from banana and 3) the development of chitosan cross-linked dietary fiber. The fiber content of 1-3% which is 10-30 kilogram from banana 1,000 kilogram, the by-product of the concentrate fructose extraction from banana will be in further process for supplementary food for GI (Gastrointestinal) track system.

Nowadays, a practice to control cholesterol is the use of Bile acid sequestrant namely Cholestyramine, a strong anion exchanger polymer resin with quaternary ammonium functional group attached to the inert styrene-divinylbenzene copolymer. This can catch bile acid in intestine, which is there released as waste interrupting the normal enterohepatic circulation, and helps to reduce the cholesterol in blood. Since this medicine is a chemical polymer that does not dissolve in the water but can absorb the water well, it causes difficulty in swallowing of the material or event block the GI track in some cases.

It is therefore interesting to develop an alternative product from nature, such as cellulose dietary fiber in this case, which possesses the hypocholesterol property, while enhancing other aspects of GI functioning.

Objectives

1. To study the pretreatment by both acid hydrolysis and steam explosion process to increase chitosan cross-linking.
2. To study bile acid binding of acid hydrolysis and steam explosion with and without crosslinking in vitro, using Cholestyramine as a control.
3. To develop the mechanisms of bile acids binding in dietary fiber from banana fruit with chitosan cross-linking.

Research Methodology

Characterization is normally the first step in production of fiber that has chitosan cross-linked in lab scale level, the raw material is ripe banana fiber, which is rinsed by tap water filtered by carbon (non chlorine and Conductivity is not over 200 microS/cm) and digested by α-amylase enzyme and pectinase after that cleaned by water. Fiber from previous preparation cross-linked with chitosan and then studies more about situation in both chemical and physical by testing cross-link reaction in qualitative by FTIR, SEM and testing cross-link in quantitative by measuring Polymer Loading method and Ninhydrin method. After that testing products (pretreated and non pretreated fiber, cross-linked and non cross-linked fiber) binding with bile acids in in vitro and oxbile, comparing with Bile acid sequestrant in Cholestyramine. The focus of this paper are to explan binding mechanisms by 1) trapping by matrix of fiber that has no ion involving. 2) Ion exchange at chitosan position NH^{3+} 3) The competition of binding between bile acid and other cation such as Ca^{2+}.

Result from the related researches

1. Fiber and ability to bind with Bile acid

The word Dietary fiber has broad meaning. Previous definitions include: (1) non starch polysaccharide (NSP) that contain the cellulose (2) Hydrocolloids (non-fermentable portion) for example, arabinoxylan and algal hydrocolloids (3) Resistant starch (RS) and (4) Viscosity and Gel formation for example, Pectin, Guar gum and Locust bean gum. Dietary fiber in general has the qualification to increase water absorb in colon to make waste bulky, reduce transit time, able to catch Bile acids (Primary and Secondary) and the RS part can be Prebiotic too.

In this project, the word Dietary Fiber may refer to the fiber that is edible from fruit cellulose, which is NSP. Shown in Figure1.

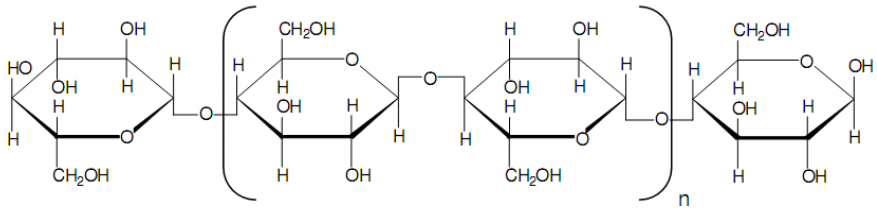


Figure1 Structure of Cellulose Molecule

The research compares ability of 7 fruits fibre (fresh fruit) in Bile acids absorption. The absorption ability of bile acid in the following order: banana > peach > pineapple > grape > pear > apricot > nectarine (Kahlon, T.S., & Smith, G.E. 2007). For ability to reduce cholesterol, in practice, clinic in general have confirmed that fiber from fruits can reduce cholesterol but the results are varied. One important system in Bile acids reduction is trapping in fiber matrix, which is physical properties, and still need to be studied.

2. Chitosan

Chitosan is the biopolymer from Chitin Deacetylation of shrimp shell and crab shell. Its structure is share in Figure2. The cation can be formed at the position of amine, NH_2, which is the qualification to be as “Bile acids sequestrant” in two ways: (1) by binding anion of the Bile acids so that chitosan can be used to reduce LDL in cholesterol or (2) by trapping bile acids in a group of chitosan fibre.

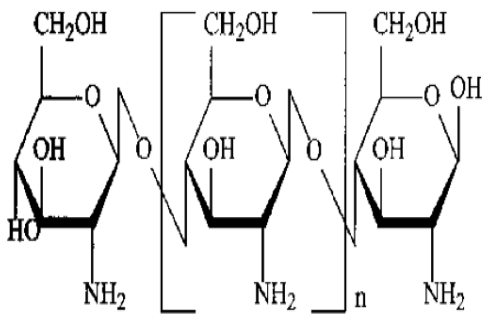


Figure2 Chitosan molecular structure

Chitosan is a cationic biopolymer that has been used extensively in dietary supplements to reduce fat absorption in the fight against obesity. The mechanism of fat binding of chitosan is still not fully understood and has been the subject of controversy (Helgason et al., 2008). The result has shown in Figure3.

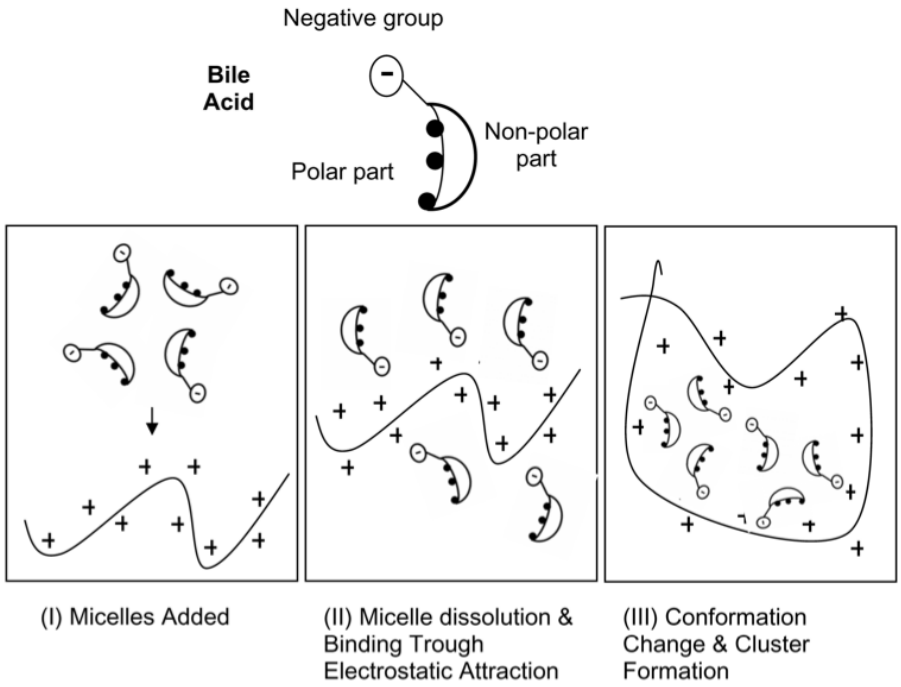


Figure3 Schematic diagram of the physicochemical processes occurring when bile Salts are added to a solution containing a positively charged polymer in the regionwhere the bile salts bind to the polymer (Helgason et al., 2008).

3. Cellulose-Chitosan Cross-linking: Innovative dietary fiber

It is reported by cellulose can cross-linked with chitosan, in citric acid (NaH_2PO_4 or $NaH_2PO_4^+$ UV to increase reaction conversion). It still has NH_2 active site of Chitosan. The cross linking position is confirmed by Fourier Transform Infrared spectrum at 1723.50 cm^{-1}. The amount of attached chitosan is measured by the PL (Polymer Loading) value of approximate between 1-3% (10-30 mg Chitosan/g product).

Patinya Chunthong has developed and clearly verified by using FTIR and found that there was crosslinking as informed before. Fiber pretreatment was also found to be

very important. The cellulose and alpha cellulose has arranged in layers of fibril lignocellulose, which are binded between cellulose fibril and alpha cellulose, with lignin in between. Steam explosion process increased alpha cellulose from 5.06 to 12.12. After doing crystallinity index analysis and fiber Diameter of cellulose by X-ray diffraction (XRD) analysis, the researcher found that percentage of the crystallinity pretreated by cellulose has increased from 10.81-68.29% and fiber diameter was smaller. The possible reason is that amorphous structure may be broken crosswise Hydroxyl (OH) bonding broken longitudinally. These OH bonding is significant to increase chitosan loading as shown in Figure4.

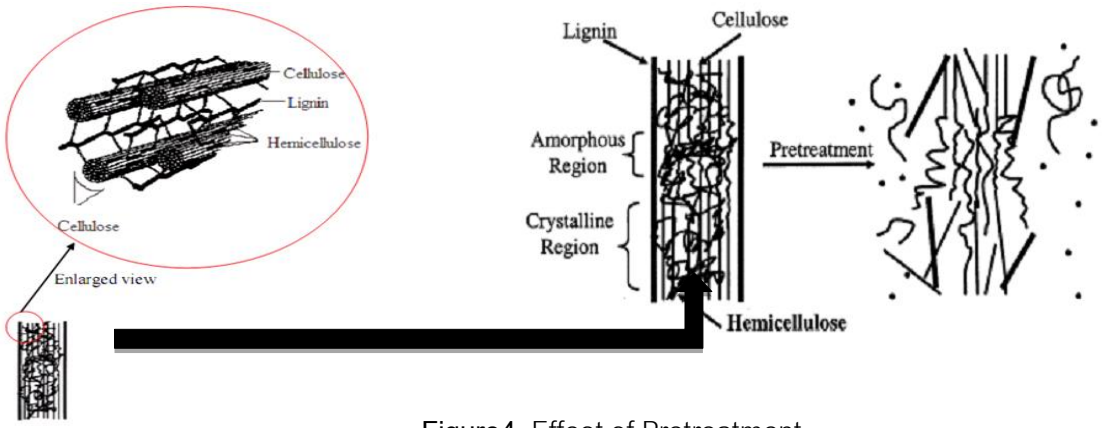


Figure4 Effect of Pretreatment

After doing steam explosion, the researcher found that there was more linkage with bile acid. From the theory, it is possible that binding occurs in 3 positions (1) cation position at NH_2 of chitosan by ion exchange, which may bind calcium or others that has cation. (2) Fiber matrix trapping of which more detail was investigated in this research. (3) Anion reaction with NH_2.

3.1. Cholesterol and Bile Acids

Cholesterol in human being will change to Bile acid which is called as primary bile acids of 2 types; chloric acid and chenodeosxycholic acid (Figure5) that may conjugate with Glycine and Taurine for better dissolving in water. These bile acid will be stocked in Gall bladder and released to intestine when the body receives foods. They function as emulsifier and build micelle when taking lipids, fatty acids, monoglycerides

and cholesterol including the vitamins that dissolve in fat like A, D, E and K for absorbing back to intestine and blood circulation system.

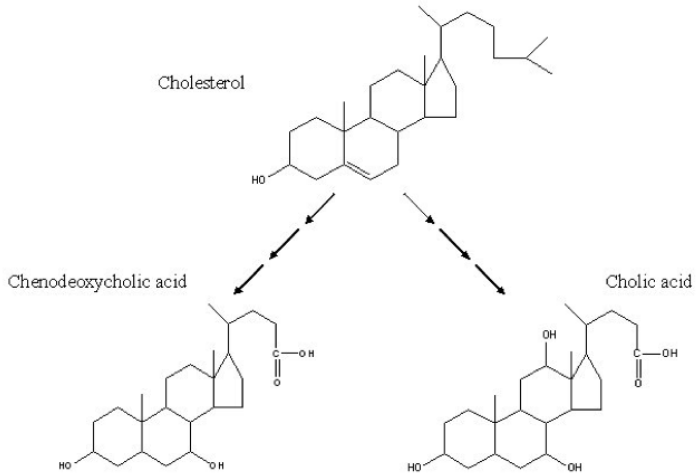


Figure5 Cholesterol and Bile Acids

Approximately 95% of Bile acid (in the salt form) is taken back to the body. The process is called "Enterohepatic Circulation" (Figure5) which accumulates Cholesterol in blood stream. Consequently, if we can remove some of bile acid from the body, the system of closed entherohepatic circulation will be interrupted and cholesterol will be wasted as bile acid and removed from the body. The substance that binds bile acid is called Bile acid sequestrant

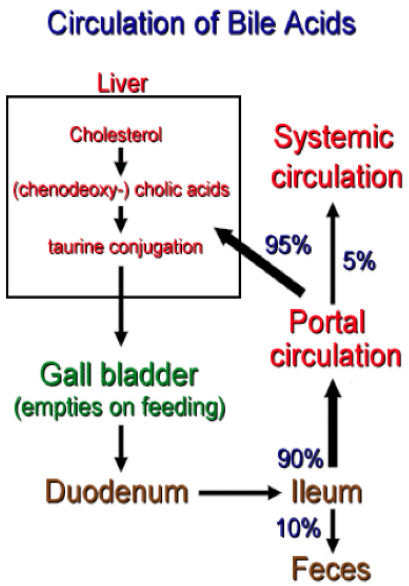


Figure6 Enterohepatic Circulation

3.2 Bile Acid Sequestering Agent

At present, the better way for treat high cholesterol patient is by using Bile Acid Sequestering Agents to remove bile acid from “Enterohepatic Cycle”. These substances are Anion Exchange Resins (Mullner, S. 1992). Its duty is to exchange (anions) with bile acid in small intestine. The Resin is not absorbed to GI tract cell but will be removed as waste.

The substance for binding bile acid is “CHOLESTYLRAMINE RESIN” (Trade name: Cholestylramin, Dowex 1-X2-Cl, Cuemid, Quantalan and Questran). It’s the synthesis polymer type “Strongly basic anion-exchange resin” in Chloride (Cl^- is the exchange ion) and attached to Styrene-divinyl-benzene which has the structure shown in Figure7 (Zarras, P., & Vogl, O. 1999).

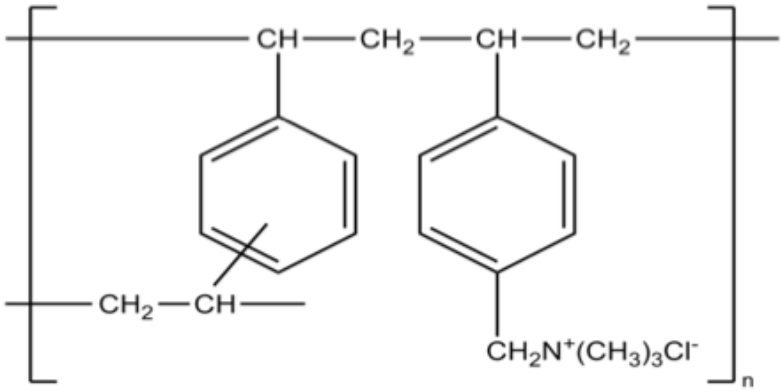


Figure7 Main structure of Cholestylramine Resin

This Cholestyramine Resin is “Hydrophilic” but it’s not dissolved in the water and is not decayed by GI tract enzyme. The substance is taken as medicine to control cholesterol. It is non-toxic and able to use for children and pregnant women but will have side effect e.g. throat irritation, stomach and cause compilation, upset, indigestion (EPA). The medicine is in first step to control the cholesterol because it’s effective and easy to use but may cause blocking of the GI track.

There have been researched about “Effectiveness” and “Toxicity” in animals for example, dog, cockerel bird, hamster and monkey. The dosage is found to be about 10-16 gram per day, which can reduce LDL Cholesterol by 15-30 %.

3.3 Cellulose-Chitosan Crosslinking Reaction

Alonso, D. et.al. found that they can bring Cellulose to crosslink reaction with Chitosan in Citric acid (NaH_2PO_4 or $NaH_2PO_4^+$) by UV which will increase the conversion but still retain NH_2. The position of Crossing is confirmed by FTIR (Fourier Transform Infrared) spectrum at 1723.50 cm^{-1} and the number of Polymer loading is approximately 1-3% (10-30 mg Chitosan/g product).

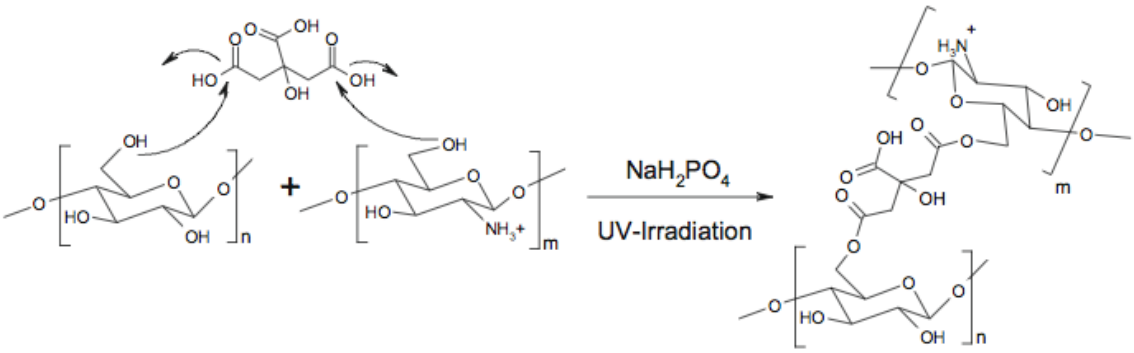


Figure8 Crosslink reaction between Cellulose and Chitosan

3.4 Bile Acid binding with Chitosan

Bile acids have anion part that will bind with cation of Chitosan (NH_2) which is part of the cross-linked fibre. The additional bile acid binding is expected to be by physical trapping in the matrix.

Zhou, K., et.al. has studied that Chitosan can react with Bile acids but depends on types of Bile acids and chemical - physic qualification of each Chitosan. Bile acid binding capacity was found to be 0.2-0.61 micro-mol/g of Chitosan. Moreover, that Mullner, S. has compared 3 types of Bile acid sequestrant with Cholestyramine (which is possible by doctors nowadays).

Helgason, T., et.al. proposed device that Micelle forming and Conformation Change Cluster Forming for Chitosan in binding Bile acids.

For Cellulose – Chitosan Cross link, the result of the study by Alonso, D., et. al. and Patinya Jantong are important guidelines to develop this project.

For Toxicity of chitosan, which is of concern for food product, the report of EPA

(Environmental Protection Agency) USA (Chitin and Chitosan Summary Document, Registration Review: Initial Docket September 2007) report that chitosan is non-toxic to be used in Food Ingredient (Deuchi K., et. al., 1995)

The side effect of chitosan is reducing the vitamins that must dissolve in fat like A, D, E and K. And because general chitosan is made from shrimp hull and crab hull, it will cause allergy in the people who is seafood allergic.

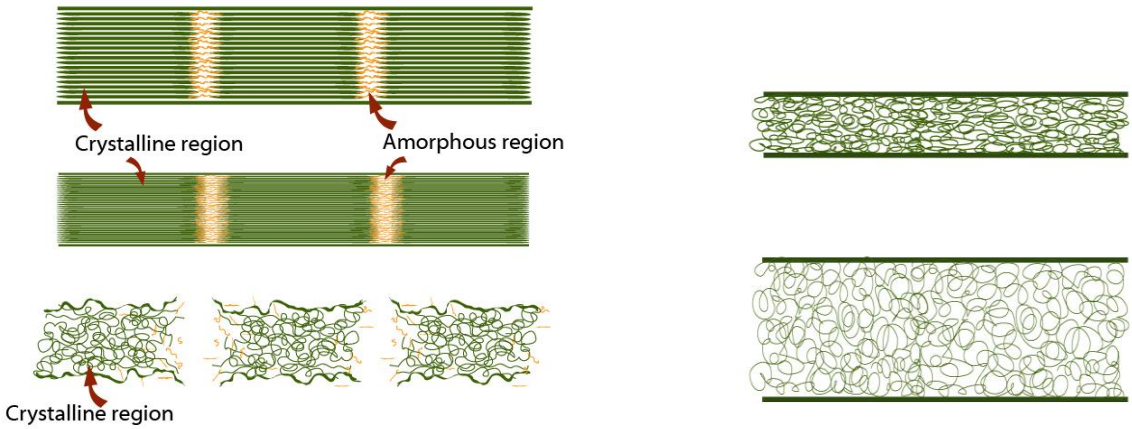
Deuchi K. and et. al. has done the experiment with rat and found that giving chitosan to the rat of the high dose and for a prolonged time will reduce the ability to absorb calcium in bone mass. Therefor the consumer should get more Calcium supplementary together with chitosan.

The basic information from laboratory by Supatra Modtong and her team investigating Cross-link between Fibre from banana and Chitosan by FTIR has confirmed the linkage of fiber cellulose of banana chitosan and chitosan by COO^- at wave number 1740 cm^{-1}. They found the value of PL (Polymer Loading) at 1.2-1.4%.

B. Deepa and team has studied structure, morphology and special appearance of Fiber heat in banana Nano level by steam explosion. The result is that cellulose has increase from 64% to 95% by adding acid and base. The removing of Hemicellulose and lignin that happen during the steam explosion and morphology research by SEM, AFM, TGA technique and DSC show the fiber development at nano level.

Summary

According to an analysis by both engaging researches and references we can infer that the cellulose pretreatment with acid hydrolysis will have more matrix void than steam explosion process, because acid react at amorphous region and then dietary fiber breaks down to small pieces and reforms to the “Matrix ball”** but steam explosion process reacts by expanding dietary fiber as shown in Figure9.



Acid hydrolysis Steam explosion

Figure9 Comparison of acid hydrolysis and steam explosion pretreatment

Note ** The author word, the better word to define their structure shall be later provided.

Modeling: at the conceptual level

(1) Matrix forming

Given **E** as matrix void (space in matrix)

$E = E_1 + E_2$

E_1 = Void before pretreatment

E_2= Void after pretreatment

E_2 depend on pretreatment classified in to 2 types of E_2

Namely E_2, steam explosion and E_2, acid hydrolysis

Proof experiment binding bile acid before cross-linking will provide qualitative data for verification of the understanding mechanism.

(2) Matrix forming after crosslinking and pretreatment

$$E = E_1 + E_2^{crosslinked}$$

$E_2^{crosslinked}$ α pretreatment

$E_2^{crosslinked}$, $E_2^{crosslinked}$

steam explosion acid hydrolysis

Proof experiment on acid binding will provide qualitative on matrix effect, regarding that NH^{3+} was terminated, may be by higher affinity ion such as Ca^{2+} (Shall be further studies) with assume affinity effect of Ca^{2+} in the matrix.

(3) NH_2 active site bind bile acid binding capacity α NH_2, E

When E effect has been clarified in (1)+(2) the pure ion exchange effect can be separated.

Reference

ปฏิญญา จันทร์ทอง. (2555). กลไกของปฏิกิริยาการเชื่อมข้ามระหว่างเซลลูโลสกับไคโตซาน และปฏิกิริยากับกรดน้ำดี. วิทยานิพนธ์ปริญญาวิศวกรรมศาสตรมหาบัณฑิต ภาควิชาวิศวกรรมเคมี. กรุงเทพมหานคร: บัณฑิตวิทยาลัย มหาวิทยาลัยเทคโนโลยีพระจอมเกล้าธนบุรี.

สุภัทรา มดทอง, อภิรักษ์ สรีชัยสันติสุข, คณาศักดิ์ สายบุญลี, ฐิติวุฒิ จูจันทร์, (2552). การพัฒนากระบวนการผลิตใยอาหารจากกล้วย. วิทยานิพนธ์ปริญญาวิศวกรรมศาสตรมหาบัณฑิต ภาควิชาวิศวกรรมเคมี, กรุงเทพมหานคร: บัณฑิตวิทยาลัย มหาวิทยาลัยเทคโนโลยีพระจอมเกล้าธนบุรี. 38-46.

Alonso, D., Gimeno, M., Olayo, R., Torrres, H.V., Jose D., Sanchez, S., & Shirai, K. (2009). Cross-linking chitosan into UV-irradiated cellulose fibres for the preparation of antimicrobial-finished textiles. Carbohydrate Polymers, (77), 536-543.

David, H., Young & Kauss, H. (1983). Release of Calcium from Suspension-Cultured Glycine max Cells by Chitosan, Other Polycations, and Polyamines in Relation to Effects on Membrane Permeation. Plant Physiology, (73), 698-702.

Deepa, B., Abraham, E., Cherian, B.M., Bismarck, A., Jonny J. B., Lary, A., Pothan, Leao, A.L., Souza, S.F., & Kottaisamy, M. (2011). Structure, morphology and thermal characteristic of banana nano fibres obtained by steam explosion. Bioresource Technology, (102), 1988-1997.

Deuchi K., Kanauchi, O., Shizykuishi, M., & Kobayashi, E. (1995). Continuous and massive intake of chitosan affects mineral and fat-soluble vitamin status in rats fed on a high-fat diet. Biosci. Biotechnol. Biochem., 59 (7), 1211-1216.

EPA, Chitin and Chitosan Summary Document, Registration Review: Initial Docket, September 2007

Helgason T, Weiss J, McClements D.J., Gislason J, Einarsson J.M., Thormodsson F.R., & Kristbergsson K. (2008). Examination of the Interaction of Chitosan and Oil-in-Water Emulsions Under Conditions Simulating the Digestive System Using Confocal Microscopy. Journal of Aquatic Food Product Technology, 17(3), 216-233.

Kahlon, T.S., & Smith, G.E. (2007). In vitro binding of bile acids by bananas, peaches, pineapples, grapes, pears, apricots and nectarines. Food Chemistry, (101), 1046-1051.

Mullner, S. (1992). Evaluation of binding characteristics of bile acid sequestrants using ox bile. Fresenius J anal Chem, 343(82).

Zarras, P., & Vogl, O. (1999). Polycationic Salts as Bile Acid Sequestering Agents. Prog. Polym. Sci, (24), 485-516.

Zhou, K., Xia, W., Zhang, C., & Yu, L. (2006). In vitro binding of bile acids and triglycerides by selected chitosan preparations and their physico-chemical properties. Food Science and Technology, 39(10), 1087-1092.