LWT - Food Science and Technology 71 (2016) 25–32

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Effect of physical and physicochemical characteristics of chitosan on fat-binding capacities under *in vitro* gastrointestinal conditions

Nootchanartch Panith [a], Jetsada Wichaphon [b], Sittiwat Lertsiri [a], Nuttawee Niamsiri [a, *]

[a] Department of Biotechnology, Faculty of Science, Mahidol University, Bangkok, 10400, Thailand
[b] Department of Agro-Industry, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Phitsanulok, 65000, Thailand

ARTICLE INFO

Article history:
Received 11 November 2015
Received in revised form
7 March 2016
Accepted 8 March 2016
Available online 10 March 2016

Keywords:
Chitosan
Physicochemical characteristics
Fat binding
Cholesterol binding
Bile acid binding

ABSTRACT

The correlation of physical and physicochemical characteristics of five different chitosan on their fat-binding capacities was studied using *in vitro* model of gastrointestinal conditions. Increasing molecular weight (Mw) and tap density of chitosan could significantly increase their fat-binding ability. Higher Mw chitosan (2100 and 890 kDa) show significantly higher fat binding capacity than 30 kDa. When tested at chitosan to fat 1:40, the ratio often used as dietary supplement, higher tap density chitosan showed an improved fat-binding capacity at around 2-fold increase. Interestingly, 2100 kDa high density (HD1P) could maintain the highest oil entrapment, ranging between 0.77 and 27.50 g oil/g chitosan depending on the interaction ratios. In addition, HD1P already maximized its fat-binding capacity since 30 min under *in vitro* gastric condition. *In vitro* cholesterol- and bile acids-binding experiments were also performed with HD1P and found to bind cholesterol at 820.9 ± 21.43 mg/g chitosan, deoxycholic acid at 17.50 ± 0.18 mg/g chitosan, and cholic acid at 5.28 ± 0.57 mg/g chitosan. Therefore, this study shows that the ability of HD1P for binding high amount of fat at short dissolution time might be due to the combination characteristics of having high Mw and being in a high density form.

1. Introduction

Chitosan is a natural cationic polysaccharide composed of β-[1-4]-D-glucosamine and N-acetyl-β-[1-4]-D-glucosamine units. It is produced by deacetylation of chitin. Because of its desirable properties such as biodegradability, biocompatibility and low toxicity, chitosan has been attracted a lot of attention in many applications, especially in medical and pharmaceutical areas. Medical applications of chitosan include artificial skin, absorbable surgical suture and wound healing accelerator. Moreover, chitosan possesses antitumor activity (Jeon & Kim, 2002), immuno-enhancing effect (Zaharoff, Rogers, Hance, Schlom, & Greiner, 2008), antifungal activity (Hirano & Nagao, 1989), and antimicrobial activity (Zheng & Zhu, 2003). Chitosan can also be defined as a dietary fiber as it cannot be digested by human digestive enzyme. An interesting property of chitosan is the ability to bind fat and cholesterol in the digestive tract (Sugano, Watanabe, Kishi, Izume, & Ohtakara, 1988). Recent studies demonstrated a hypocholesterolemic effect of chitosan where usage of chitosan claimed to control obesity and to lower serum cholesterol level (Gallaher et al., 2002; Jing, Li, Ji, Takiguchi, & Yamaguchi, 1997; Wuolijoki, Hirvelä, & Ylitalo, 1999). In addition, some studies also showed that chitosan could provide ability to inhibit the absorption and enterohepatic circulation of bile acids, leading to a decrease of plasma cholesterol level (Thongngam & McClements, 2005). These properties of chitosan could be explained by the fact that chitosan is a polycationic molecule, in which its positive charges on the chitosan molecule give the ability to bind with negatively charged lipids, fats and bile acids. In general, chitosan can be solubilized in aqueous acid solutions, while it often precipitates upon rising the alkaline pH due to the deprotonation of amino groups. Therefore, after migrating into stomach (pH 2), the solubilized chitosan can attract negatively charged droplets of triglycerides, fatty acids, bile acids cholesterol and other sterols, forming complexes and micelles. Chitosan would bind to fat droplets in the stomach and then precipitate before they are adsorbed into blood stream (Czechowska-Biskup, Rokita, Ulanski, & Rosiak, 2005). Fat binding capacity of chitosan is related to chemical and physical characteristics of chitosan including degree of deacetylation, molecular weight (Mw) and viscosity (Vahouny, Satchithanandam, Cassidy, Lightfoot, & Furda, 1983). It is reported that chitosan with high Mw and high degree

* Corresponding author.
E-mail address: nuttawee.nia@mahidol.ac.th (N. Niamsiri).

http://dx.doi.org/10.1016/j.lwt.2016.03.013
0023-6438/

of deacetylation (i.e. >90%) provides a better fat binding capacity than low Mw and low degree of deacetylation chitosan (Deuchi, Kanauchi, Imasato, & Kobayashi, 1995).

Although effects of Mw and degree of deacetylation of chitosan on fat binding capacity have been studied, only few attempts have been made to evaluate the effect of physical (i.e. melting temperature, tap density, particulate morphology) and physicochemical characteristics when dispersed in aqueous medium (i.e. viscosity). With respect to the use of chitosan as a dietary supplement, high density chitosan powder is more desirable to deliver the dose of fat-binding capacity with smaller volume comparing to low density chitosan powder. There are different processing methods to generate high density chitosan particulates within a range from 0.5 to 0.8 g/mL (Hugerth, Caram-Lelham, & Sundelöf, 1997). Therefore, the main objective of our study was to evaluate the effect of physical and physicochemical characteristics of commercial chitosan products on fat-binding capacities. In addition, the most desirable one was also tested for other binding abilities with cholesterol and bile acids under *in vitro* conditions mimicking human gastrointestinal system.

2. Materials and methods

2.1. Materials

In this study, five commercially available chitosan samples (designated 1–5) were obtained from Marine Bio Resources Co., LTD (Thailand) with molecular weight, degree of deacetylation, and tap density shown in Table 1. All chitosan particulates were white-colored and acid soluble derived from shrimp shell with sizes less than 150 μm. All samples had moisture content between 8 and 9 g/100 g of chitosan and were always kept in a desiccator.

Cholesterol, cholic acid (>98%), deoxycholic acid (>99%) and fluorescein isothiocyanate (FITC) used in this study were purchased from Sigma–Aldrich (Singapore), while refined soybean oil was purchased from a local supermarket. Other reagents and solvent that were used for the experiment were analytical grade.

2.2. Physical and physicochemical characterizations of chitosan products

Morphology of chitosan particulates was observed under a scanning electron microscope (HITACHI SEM S-2500, Japan). The specimens were first dried in a desiccator, and then the chitosan sample was scattered on carbon tape. The specimens were coated with gold and observed under an acceleration voltage of 15 kV.

The tapped density of chitosan powders was determined by pouring a known mass of powder (i.e. 25 g) under gravity into a pre-weighed graduated cylinder with 0.5 mL markings. The tap volume was measured using mechanical tapping for 200 times by AS-100 Tap Density Volumeter (AimSizer Scientific Ltd., Dandong, China) with no further change in the powder volume observed. The tap density was then computed as g/mL of the sample.

Melting temperature (Tm) of chitosan samples were analyzed by Differential Scanning Calorimetry (DSC) (DSC STAR^e 1 model, Metller Toledo, US). Briefly, 10 mg of each chitosan powered samples was sealed in an aluminum pans and heated at a scanning range rate of 20 °C/min under nitrogen atmosphere from 30 to 250 °C. STAR^e software (Metller Toledo, US) was used for data analysis to obtain Tm.

The viscosity of the chitosan sample with different Mw was analyzed by Brookfield viscometer, Model LVDV-II+ (Brookfield Engineering Laboratories, Stoughton, MA). Chitosan sol was prepared according to the previous work (Youn, No, & Prinyawiwatkul, 2009). Briefly, each chitosan sample (0.5 g) was dispersed in 100 mL of 0.1 mol/L HCl and continuously stirred. Viscosity measurement of each chitosan sol (8 mL) was performed at 30 min after mixing and in triplicate using a small sample adapter SC4-18 spindle at a fixed shear rate of 66 s^{-1} at room temperature with values reported in mPa·s unit.

2.3. Fat-binding capacity of each chitosan and the effect of amount of soybean oil and interaction time

In fat-binding experiments, the *in vitro* digestive model experiments were performed based on previously established protocols (Wang & Kinsella, 1976). Briefly, 0.1 g of chitosan was dispersed in 20 mL of 0.1 mol/L HCl and incubated at 37 °C for 30 min with shaking 200 rpm to mimic the gastric environment. For investigating the effect of mass ratio between chitosan to fat (1:1, 1:10, 1:20 and 1:40), different amounts of soybean oil were added at a fixed chitosan amount in order to form chitosan-soybean oil emulsion under stirring condition at 200 rpm. After 30 min, the emulsion formed under the acidic medium were then brought to pH 6.8 with 15 g/L of $NaHCO_3$ under stirring condition at 200 rpm at 37 °C, in which this particular pH of 6.8 was mimicked to the physiological pH of the duodenum. After 30 min, the resulting "oil-bound chitosan particulates" were centrifuged at 4024×g for 20 min. The gel-like "oil-bound chitosan particulates" was collected using muslin cloth and then subjected to a gentle purging process with pure N_2 gas in order to separate unbound oil at the gel surface. After 1 h, the entrapped oil in the chitosan gel was extracted by 10 mL diethyl ether for 3 times. Then, the extracting solvent was eliminated by a rotary evaporator, and the total entrapped oil in the chitosan gel was gravimetrically measured. All tests were conducted in triplicate. The fat-binding capacity of each chitosan sample was determined as fat entrapment as a following calculation: Fat entrapment (g of oil/g of chitosan) = [oil bound (g)/chitosan sample weight (g)].

Besides the effect of amount of soybean oil on the fat-binding capacity of chitosan, we also investigated interaction time between each chitosan and oil at a specific ratio. The selected chitosan samples were emulsified with 1 g soybean oil (the chitosan: oil mass ratio at 1:40), the chitosan-fat emulsion mixtures were left stand various binding time points; 30, 60, 120 min under stomach condition, then the emulsion were brought to pH 6.8 with 15 g/L of $NaHCO_3$ pH 6.8 under stirring condition at 200 rpm at 37 °C (i.e. mimicking the duodenum). After 30 min, the resulting "oil-bound

Table 1
Characteristics of chitosan products.

Chitosan samples	Mw (kDa)	Tap density (g/mL)	Tm (°C)	Viscosity (mPa·s) at 30 min[a]
1	30	0.386	148.67	5.77 ± 0.47
2	30	0.814	167.33	1.51 ± 0.44
3	890	0.376	153.00	23.20 ± 0.66
4	2100	0.351	157.00	41.90 ± 0.72
5	2100	0.811	154.67	27.87 ± 0.95

[a] Mean ± standard deviation of triplicate determinations. Viscosity of 0.5 g/100 mL chitosan sol (in 0.1 mol/L HCl).